AS PER PCI REGULATIONS
SECOND YEAR B. PHARM. | SEMESTER-III

PHYSICAL PHARMACEUTICS - I

Dr. ASHOK A. HAJARE



NIRALI PRAKASHAN
ADVANCEMENT OF KNOWLEDGE

A Text Book Of

PHYSICAL PHARMACEUTICS - I

As Per PCI Regulations

SECOND YEAR B. PHARM.

Semester III

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NIRALI PRAKASHAN
ADVANCEMENT OF KNOWLEDGE

N3952

Physical Pharmaceutics - I **ISBN 978-93-88194-17-4**

First Edition : July 2018

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Published By :
NIRALI PRAKASHAN
Abhyudaya Pragati, 1312, Shivaji Nagar,
Off J.M. Road, PUNE – 411005
Tel - (020) 25512336/37/39, Fax - (020) 25511379
Email : niralipune@pragationline.com

Polyplate

Printed By :
YOGIRAJ PRINTERS AND BINDERS
Works: Sr. No. 10\1,Ghule Industrial Estate,
Nanded Village Road,
TAL-HAVELI, DIT-PUNE 411041.
Mobile - 9850046517, 9404225254

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Preface

It is indeed a matter of great pride for us that, the *Pharmacy Council of India (PCI), New Delhi has framed Bachelor of Pharmacy (B. Pharm.)* course regulations 2014. The Physical Pharmaceutics-I is a very important subject at Second year of the course. It gives us a great pleasure to present this book in the hand of readers. The book is strictly written as per syllabus framed by PCI under Section 6, 7 & 8 of Regulation 2014 and we had made an attempt to make it simple and understandable to the readers. The sequence of syllabus content is designed unit wise imparting better understanding of subject matter. In this book wherever needed full forms and abbreviations, pictorial diagrams, tabular data, examples of formulation including marketed products and manufacturers are given. Model questions are given at the end of every subunit to exercise on contents studied.

We owe a great debt of gratitude to Hon. Dr. Patangraoji Kadam, Founder, Bharati Vidyapeeth Pune, for their encouragement. We are indeed very grateful to Prof. Dr. Shivajirao Kadam, Pro-Chancellor, Bharati Vidyapeeth University, Pune for his consistent and cheerful support and Dr. Vishwajit Kadam, Secretary Bharati Vidyapeeth, Pune for encouragement and motivation. We honestly extend our gratitude to Dr. H. N. More, Principal, Bharati Vidyapeeth College of Pharmacy, Kolhapur for freedom to work and timely help. We are thankful to Mrs. Snehal Patil Librarian for her co-operation during literature work.

We thank Mrs. Suvarna and Mrs. Shital, Digvijay and Aarush and our parents from bottom of heart for sustained support, encouragement and for their forbearance.

We are thankful to Mr. Jignesh Furia, Ilyas Shaikh, Anagha Medhekar, Manasi Pingle of Nirali Prakashan, Pune and Staff of Nirali Prakashan for bringing out nicely printed book.

Dr. A. A. Hajare

Syllabus

UNIT I (10 Hours)

Solubility of Drugs: Solubility expressions, mechanisms of solute solvent interactions. ideal solubility parameters, solvation and association, quantitative approach to the factors influencing solubility of drugs, diffusion principles in biological systems. Solubility of gas in liquids, solubility of liquids in liquids, (Binary solutions, ideal solutions) Raoult's law, real solutions, partially miscible liquids, Critical solution temperature and applications. Distribution law, its limitations and applications.

UNIT II (10 Hours)

State of matter and properties of a matter changes in the state of matter, latent heats, vapour pressure, sublimation critical point, eutectic mixtures, gases, aerosols - inhalers, relative humidity, liquid complexes, liquid crystals, glassy states, solid-crystalline, amorphous and polymorphism.

Physicochemical properties of drug molecules: Refractive index, optical rotation, dielectric constant, dipole moment, dissociation constant, determinations and applications.

UNIT III (08 Hours)

Surface and Interfacial Phenomenon: Liquid interface, surface and interfacial tensions, surface free energy, measurement of surface and interfacial tensions, spreading coefficient, adsorption at liquid interfaces, surface active agents, HLD scale, solubilization, detergency, adsorption at solid interface.

UNIT IV

Complexation and Protein Hinding: Introduction, Classification of Complexation, Applications, Methods of analysis, Protein binding, Complexation and drug action, Crystalline structures of complexes and thermodynamic treatment of stability constants.

UNIT V (07 Hours)

pH, Buffers and Isotonic Solutions: Sorensen's pH scale, pH determination (electrometric and calorimetric), applications of buffers, buffer equation, buffer capacity, buffers in pharmaceutical and biological systems, buffered isotonic solutions.



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Unit ...**1**

SOLUBILITY OF DRUGS

♦ OBJECTIVES ♦

Solubility is the physical property of substances that varies with temperature and pressure as well as the nature of the solute and the solvent. In the view of making formulations bioavailable and stable the knowledge of basic concepts of solubility is must. After knowing the importance of studying and understanding the phenomenon of solubility the student should be able to:

- Identify the descriptive terms for solubility, their meaning, and various types of solutions.
- Understand the terms and concepts of miscibility.
- Understand the factors controlling the solubility of drugs.
- Understand partition coefficient and its importance in pharmaceutical systems.
- Overcome problems arising during preparation of pharmaceutical solutions.
- Calculate the partition coefficients for different types of solutes in aqueous/organic solvent systems.

1.1 INTRODUCTION

Solubility is the ability of one substance to fully dissolve in another substance under specified conditions. The word soluble comes from the fourteenth century, from the Latin word '*solvere*' meaning to dissolve. The concentration of a solution is usually quoted in terms of mass of solute dissolved in a particular volume of solvent. The solubility is generally expressed in gram per litre. Therefore, solubility of a solute in a solvent at a particular temperature is the number of grams of the solute necessary to saturate 100 grams or mL of the solvent at that temperature. Most commonly encountered solutions are solids dissolved in liquids. The solid that dissolve in a liquid is the *solute* and the liquid in which it dissolves is *solvent*. A solute is the dissolved agent usually the less abundant part of solution whereas solvent is more abundant part of solution. If a solid can dissolve in a liquid, it is said to be *soluble* in that liquid, if not it is said to be *insoluble*. As we add more solids to a liquid the solution becomes more concentrated. The greater the solubility of a substance the more concentrated it is possible to make the solution. Solubility is measured after solute of interest has had sufficient contact time (however long it takes) with the solvent. There are two types of solubility: one is called intrinsic solubility and the other one is apparent solubility. *Intrinsic*

solubility is defined as the maximum concentration to which a solution can be prepared with a specific solute and solvent. It is often derived from calculation, and is a single numeric number (for example, 0.5 µg/mL) that is independent of the environmental factors. The *apparent solubility* is dependent on the environmental factor such as pH and ionic strength and is obtained from the experimental measurements. The rate of solubility is affected by many factors such as type of solvent, size and amount of solute particles, stirring speed and temperature. The concept of solubility is very important because it governs the preparation of solutions as dosage forms and a drug must be in solution before it can be absorbed by the body or have any biological activity. Since activity of drug depends on solubility, it is equally important to control environmental conditions which impact various types of solution.

1.2 SOLUBILITY EXPRESSIONS

The solubility of a drug or other substance in a solvent can be expressed quantitatively in numerous terms *viz*. percent by mass, percent by volume, molality (m), molarity (M), mole fraction (*x*), and parts per million (ppm), etc. The particular terminology we use depends largely on the use to which we will put it. Solubility of substance is defined as the amount of solute dissolved in a specific amount of solvent at specific temperature. The British Pharmacopoeia and other official chemical and pharmaceutical compendia frequently use the term parts per parts of solvent (for example, parts per million, ppm). The expressions 'insoluble', 'very highly soluble' and 'soluble' also can be used to express solubility of solutes but being inaccurate often not found to be helpful. For quantitative work specific concentration terms must be used. Most substances have at least some degree of solubility in water and while they may appear to be 'insoluble' by a qualitative test, their solubility can be measured and quoted precisely. In aqueous media at pH 10, chlorpromazine base has a solubility of 8×10^{-6} mol/dm^3. It is very slightly soluble and it might be considered as 'insoluble' upon visual inspection due to lack of disappearance of solid.

In many solutions the concentration has a maximum limit that depends on various factors, such as temperature, pressure, and the nature of the solvent. Relative concentrations of a solute/solvent system can often be expressed by the terms dilute and concentrated, or by the terms unsaturated, saturated, and supersaturated. Solutes in water are often categorized as either strong electrolytes, if completely ionized in water or weak electrolytes, if only partially ionized or non-electrolytes when non-ionized. In regard to solubility, general terms can be used when describing whether a compound is soluble or not. These terms are given in Table 1.1, and are based on the part of solvent needed to dissolve 1 part of the solute for example, testosterone is considered insoluble in water but soluble in alcohol, ether or other organic solvents. Fortunately, when injected to body, insoluble testosterone is diluted and the larger volume of body water permits testosterone to go into solution.

Table 1.1: General Terms of Solubility

Term	Parts of solvent required per part of solute
Very soluble	Less than 1 part
Freely soluble	1 - 10
Soluble	10 - 30
Sparingly soluble	30 - 100
Slightly soluble	100 - 1000
Very insoluble	1000 - 10,000
Insoluble	More than 10,000

Saturated Solution

A solution in which dissolved solute is in equilibrium with the undissolved solute or solid phase is known as saturated solution. It is when no more of the solid will dissolve into the solution. When we add solute to a solvent a point is reached where no more solute dissolve under specified condition. The solution is saturated. The concentration of the solute in a saturated solution is the solubility of the solute in that solvent at that temperature. Saturation of solution also can be defined as the point where the solution is in equilibrium with undissolved solute. In a saturated solution containing undissolved solid solute, the rate at which the molecules or ions leave the solid surface is equal to the rate which the solvated molecules return to the solid.

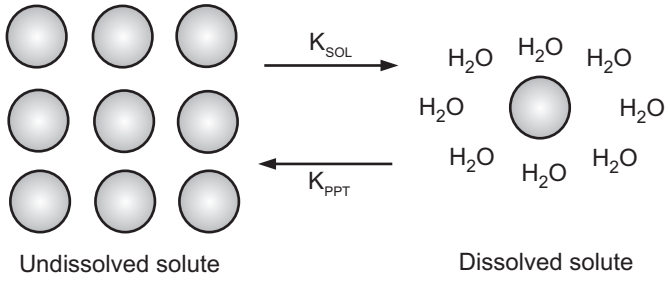


Figure 1.1: Saturated solutions

In Fig. 1.1, K_{SOL} is the rate constant at which solid is solvated and K_{PPT} is the rate constant at which the solvated molecule is returned to the solid. The solubility of substance is ratio of these rate constants at equilibrium in a given solution. At equilibrium the rate of a solute precipitating out of solution is equal to the rate in which the solute goes into solution.

Unsaturated Solution:

An unsaturated solution is a solution containing the dissolved solute in a concentration less than a saturated solution. If less solute is added to the solvent, then the solution is said to be unsaturated. Most pharmaceutical solutions are considered to be unsaturated.

Supersaturated Solution:

A solution which contains more concentration of solute than saturated solution is known as *supersaturated* solution. It requires an increase in temperature to make it possible to dissolve more solute into solvent than is required to produce a saturated solution. This yields a supersaturated solution. These solutions can be prepared by heating the saturated solutions at higher temperatures. The solute is dissolved into the solvent at a high temperature and then the solution is slowly cooled, such solution is unstable and the addition of small amount of solute cause all of the excess dissolved solute to crystallize out of the solution.

A saturated potassium chloride solution at 10^{o}C will have 31 grams of this substance dissolved in 100 grams of water. If there are 40 grams of potassium chloride in the container, then there will be 9 grams of undissolved potassium chloride remaining in the solution. Raising the temperature of the mixture to 30^{o}C will increase the amount of dissolved potassium chloride to 37 grams and there will be only 3 grams of solid undissolved. The entire 40 grams can be dissolved if the temperature is raised above 40^{o}C. Cooling the hot solution (40^{o}C) will reverse the process. When the temperature decreased to 20^{o}C the solubility will eventually be decreased to 34 grams. There is a time delay before the extra 6 grams of dissolved potassium chloride crystallizes. This solution is "supersaturated" and is a temporary condition. The "extra" solute will come out of solution when the randomly moving solute particles can form the crystal pattern of the solid. A "seed" crystal is sometimes needed to provide the surface for solute particles to crystallize on and establish equilibrium.

Concentration Units:

A wide range of units is commonly used to express solution concentration, and confusion often arises in the inter-conversion of one set of units to another. Wherever possible throughout this book we have used the SI system of units. Although this is the currently recommended system of units in Great Britain, other more traditional systems are still widely used and these are also used in latter sections.

Weight Concentration:

Concentration is often expressed as a weight of solute in a unit volume of solution; for example, g/dm^3, or % w/v, which is the number of grams of solute in 100 cm^3 of solution. This is not an exact method when working at a range of temperatures, since the volume of the solution is temperature dependent and hence the weight concentration also changes with temperature. Whenever a hydrated compound is used, it is important to use the correct state of hydration in the calculation of weight concentration. Thus, 10% w/v $CaCl_2$ (anhydrous) is approximately equivalent to 20% w/v $CaCl_2 \cdot 6H_2O$ and consequently the use of the vague statement '10% calcium chloride' could result in gross error. The SI unit of weight concentration is kg/m^3 which is numerically equal to g/dm^3.

Molarity and Molality:

Molarity and molality are similar-sounding terms and must not be confused. The *molarity* of a solution is the number of moles (gram molecular weight) of solute in 1 litre (1 dm^3 or

1000 mL) of *solution*. The *molality* is the number of moles of solute in 1 kg of *solvent*. Molality has the unit, mol/kg, which is an accepted SI unit. Molarity may be converted to SI units using the relationship 1 mol/L = 1 mol/dm^3 = 1M= 1000 mol/m^3.

Interconversion between molarity and molality requires knowledge of the density of the solution. Of the two units, molality is preferable for a precise expression of concentration because it does not depend on the solution temperature as does molarity; also, the molality of a component in a solution remains unaltered by the addition of a second solute, whereas the molarity of this component decreases because the total volume of solution increases upon the addition of the second solute.

Milliequivalents:

The unit milliequivalent (mEq) is commonly used clinically in expressing the concentration of an ion in solution. The term 'equivalent', or gram equivalent weight, is analogous to the mole or gram molecular weight. When monovalent ions are considered, these two terms are identical. A 1 molar solution of sodium bicarbonate, $NaHCO_3$, contains 1 molar 1 Eq of Na^+ and 1 mol or 1 Eq of HCO_3 per litre (dm^3) of solution. With multivalent ions, attention must be paid to the valency of each ion; for example, 10% w/v $CaCl_2 \cdot 2H_2O$ contains 6.8 mmol or 13.6 mEq of Ca_2 in 10 cm^3.

The Pharmaceutical Codex gives a table of milliequivalents for various ions and also a simple formula for the calculation of milli equivalents per litre. In analytical chemistry a solution which contains 1 Eq/dm^3 is referred to as a *normal* solution. Unfortunately the term 'normal' is also used to mean physiologically normal with reference to saline solution. In this usage, a physiologically normal saline solution contains 0.9 g NaCl in 100 cm^3 aqueous solutions and *not* 1 equivalent (58.44 g) per litre.

1.3 MECHANISMS OF SOLUTE SOLVENT INTERACTIONS

A solute dissolves in a solvent when it forms favourable interactions with the solvent. This dissolving process all depends upon the free energy changes of both solute and solvent. The free energy of solvation is a combination of several factors. The process can be considered in three stages:

(i) A solute (drug) molecule is 'removed' from its crystal.

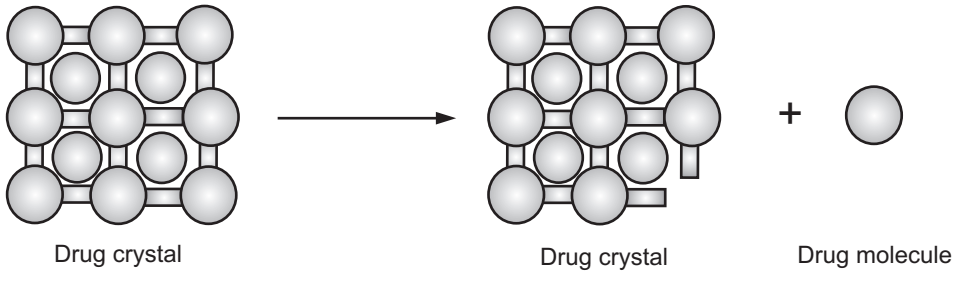


Figure 1.2 (a) : Removal of solute molecule

The solute must separate out from the bulk solute. This is enthalpically unfavourable as solute-solute interactions are breaking but is entropically favourable.

(ii) A cavity for the drug molecule is created in the solvent.

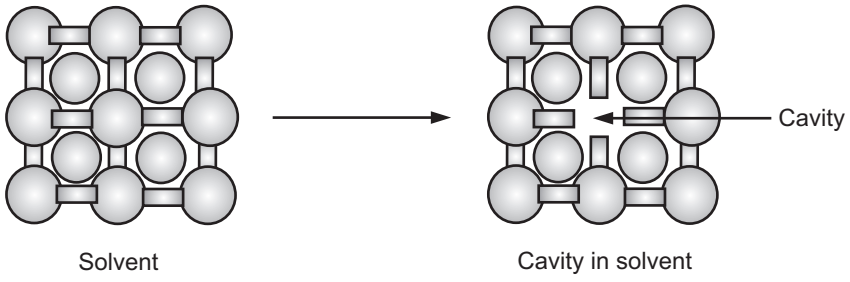


Figure 1.2 (b) : Creation of cavity

A cavity must be created in the solvent. The creation of the cavity will be entropically and enthalpically unfavourable as the ordered structure of the solvent decreases and there are fewer solvent-solvent interactions.

(iii) The solute (drug) molecule is inserted into this cavity.

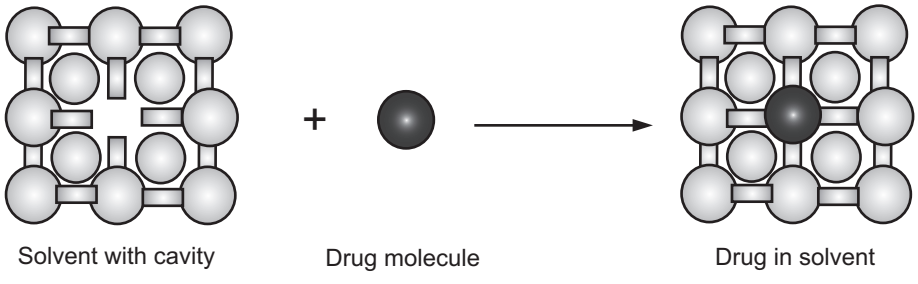


Figure 1.2 (c) : Insertion of solute

The solute must occupy the cavity created in the solvent. Placing the solute molecule in the solvent cavity requires a number of solute–solvent contacts; the larger the solute molecule, the more contacts are created. If the surface area of the solute molecule is A, and the solute–solvent interface increases by γ_{12} A, where γ_{12} is the interfacial tension between the solvent$_1$ and the solute$_2$ then it leads to favourable solute-solvent interactions. This is entropically favourable as the mixture is more disordered than when the solute and solvent are not mixed.

Dissolution often occurs when the solute-solvent interactions are similar to the solvent-solvent interactions, signified by the term '*Like dissolves Like*'. Hence, polar solutes dissolve in polar solvents, whereas non-polar solutes dissolve in non-polar solvents. Dissimilar nature of solute and solvent makes solute insoluble in the solvent. Substances dissolve when solvent-solute attraction is greater than solvent-solvent attraction and solute-solute attraction.

1.4 IDEAL SOLUBILITY PARAMETERS

Regular solution theory characterises non-polar solvents in terms of solubility parameter, δ_1, which is defined as

$$\delta_1 = \left(\frac{\Delta U}{V}\right)^{1/2} = \left(\frac{\Delta H - RT}{V}\right)^{1/2} \quad \ldots (1.1)$$

Where, ΔU is the molar energy and ΔH is the molar heat of vapourization of the solvent. The ΔH is determined by calorimetry at temperatures below the boiling point at constant volume and V is the molar volume of the solvent. The solubility parameter is thus a measure of the intermolecular forces within the solvent and gives us information on the ability of the liquid to act as a solvent. The ratio ΔU/V is the liquid's cohesive energy density, a measure of the attraction of a molecule from its own liquid, which is the energy required to remove it from the liquid and is equal to the energy of vapourization per unit volume. As cavities have to be formed in a solvent by separating other solvent molecules to accommodate solute molecules the solubility parameter δ_1 enables predictions of solubility to be made in a semi-quantitative manner, especially in relation to the solubility parameter of the solute, δ_2. By itself the solubility parameter can explain the behaviour of only a relatively small group of solvents – those with little or no polarity and those unable to participate in hydrogen bonding interactions. The difference between the solubility parameters expressed as $(\delta_1-\delta_2)$ will give an indication of solubility relationships. For solid solutes a hypothetical value of δ_2 can be calculated from $(U/V)^{1/2}$, where U is the lattice energy of the crystal. In a study of the solubility of ion pairs in organic solvents it has been found that the logarithm of the solubility (log S) correlates well with $(\delta_1/\delta_2)^2$.

1.5 SOLVATION

The process of solvation is sometimes called dissolution. Solvation is a kinetic process and is quantified by its rate. It is the attraction and association of molecules of a solvent with molecules or ions of a solute. When a solute is soluble in a certain solvent, the solute's molecules or ions spreads out and became surrounded by solvent molecules. A complex formed of molecule or ion of solute in a solvent is known as a solvation complex. Solvation is the process of rearranging solvent and solute molecules into solvation complexes to distribute solute molecules evenly within the solvent. Solvation process is affected by hydrogen bonding and van der Waals forces (which consist of dipole-dipole, dipole-induced dipole, and induced dipole-induced dipole interactions). Which of these forces are at play depends on the molecular structure and properties of the solvent and solute. Insoluble solute molecules interact with other solute molecules rather than break apart and become solvated by the solvent, for example, solvation of functional groups on a surface of ion-exchange resin. In fact solvation is an interaction of a solute with the solvent, which leads to stabilization of the solute species in the solution. Solvation of a solute by water is called *hydration*.

Solvation is, in concept, distinct from solubility. Solubility quantifies the dynamic equilibrium state achieved when the rate of dissolution equals the rate of precipitation. The consideration of the units makes the distinction clearer. The typical unit for dissolution rate is mol/sec. The units for solubility express a concentration as mass per volume (mg/mL), molarity (mol/L) etc. The similarity between solvent and solute determines how well a solute can be solvated by a solvent.

1.6 ASSOCIATION

Association or ion association is a chemical reaction wherein ions of opposite electrical charge come together in solution to form a distinct chemical entity. Ion associates are classified, according to the number of ions that associate with each other, as ion pairs, ion triplets etc. Ion pairs are also classified according to the nature of the interaction as contact, solvent-shared or solvent-separated. The most important factor that determines the extent of ion association is the dielectric constant of the solvent. Ion associates have been characterized by means of vibrational spectroscopy.

Ion pairs are formed when a cation and anion come together:

$$A^{n+} + B^{m-} \rightleftharpoons AB^{(n-m)+}$$

There are three distinct types of ion pairs depending on the extent of solvation of the two ions:

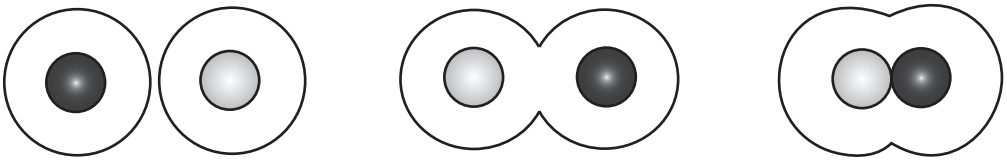


Figure 1.3: Schematic of types of ion pair

In the above schematic representation, the circles represent spheres. The sizes are arbitrary and not necessarily similar as shown in Fig. 1.3, the cation is coloured dark and the anion is coloured grey. The area surrounding ions represents solvent molecules in a primary solvation shell; secondary solvation is ignored. When both ions have a complete primary solvation sphere, the ion pair may be termed fully solvated. When there is about one solvent molecule between cation and anion, the ion pair may be termed solvent-shared. Lastly, when the ions are in contact with each other, the ion pair is termed a *contact* ion pair. In contact ion pair the ions retain most of their solvation shell and the nature of this solvation shell is generally not known. In aqueous solution and in other donor solvents, metal cations are surrounded by between 4 and 9 solvent molecules in the primary solvation shell, but the nature of solvation of anions is mostly unknown.

Another name for a solvent-shared ion pair is an outer-sphere complex. Usage of outer-sphere complex is common in co-ordination chemistry and denotes a complex between a solvated metal cation and an anion. Similarly, a contact ion pair may be termed an inner-sphere complex. The major difference between these three types is the closeness with which the ions approach each other: The order of closeness is prevented as Fully solvated > Solvent-shared > Contact. With fully solvated and solvent-shared ion pairs the interaction is primarily electrostatic, but in a contact ion pair some covalent character in the bond between cation and anion is also present.

An ion triplet may be formed from one cation and two anions or from one anion and two cations. Higher aggregates, such as a tetramer $(AB)_4$, may be formed. Ternary ion associates involve the association of three species. Another type, named intrusion ion pair, has also been characterized.

1.7 QUANTITATVE APPROACH TO THE FACTORS INFLUENCING SOLUBILITY OF DRUGS

The solubility of most solid solutes is significantly affected by temperature. When some solid dissolves in a liquid a change in the physical state of the solid analogues (melting) takes place. Heat is required to break the bonds holding the molecules in the solid together. At the same time, heat is given off during the formation of new solute-solvent bonds. The typical solubility data for some common inorganic compounds at respective temperatures is given in Table 1.2.

Table 1.2: Solubility of Common Inorganic Compounds in g/100 mL of Water

Substance	0°C	10°C	20°C	30°C	40°C	50°C
Potassiumiodide	127.5	136	144	152	160	168
Potassium chloride	27.6	31.0	34.0	37.0	40.0	42.6
Sodium chloride	35.7	35.8	36.0	36.3	36.6	37.0
Sodium bicarbonate	6.9	8.15	9.6	11.1	12.7	14.45
Sodium hydroxide	–	–	109	119	145	174
Epsom salts, magnesium sulfate heptahydrate	–	23.6	26.2	29	31.3	–

These values are the amount of solute that will dissolve and form a saturated solution at the temperatures listed. The solubility can be increased if the temperature is increased. The solubility of solute usually increases with increasing temperature but there are exceptions such as $Ce_2(SO_4)_3$ as shown in Fig. 1.4.

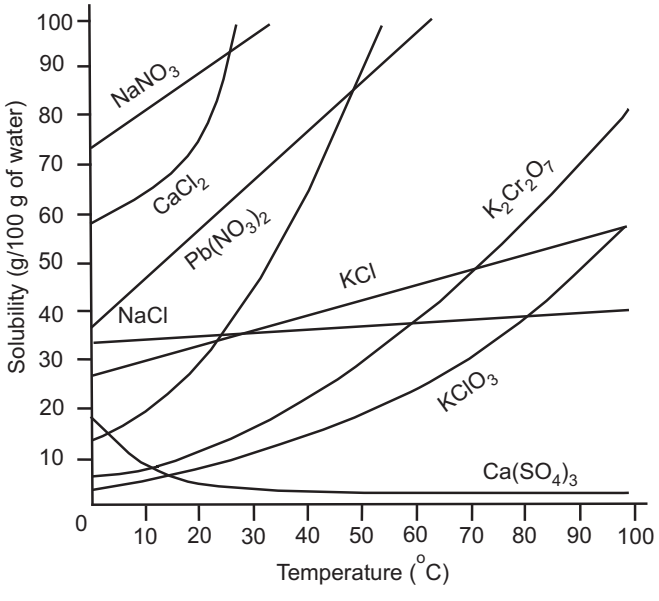


Figure 1.4: Solubility of common inorganic compounds

Generally, increase in temperature increases solubility of solids in solvent. Although in many cases solubility increases with the rise in temperature and decreases with the fall of temperature, it is not necessary in all cases. It means there are exceptions that solubility decreases with increase in temperature.

CASE I: Increase in Solubility with Temperature

In endothermic processes solubility increases with the increase in temperature and vice versa. For example, solubility of potassium nitrate increases with the increase in temperature. If the heat given off in the dissolving reaction is less than the heat required to break apart the solid, the net dissolving reaction is endothermic (energy required). Therefore, the heat is drawn from the surroundings. The addition of more heat facilitates the dissolving reaction by providing energy to break bonds in the solid. This is the most common situation where an increase in temperature produces an increase in solubility for solids.

CASE II: Decrease in Solubility with Temperature

In exothermic processes solubility decrease with the increase in temperature. For example, solubility of calcium oxide decreases with the increase in temperature. Gases are more soluble in cold solvent than in hot solvent. If the heat given off in the dissolving process is greater than the heat required to break apart the solid, the net dissolving reaction is exothermic (energy given off). The addition of more heat (increases temperature) inhibits the dissolving reaction since excess heat is already being produced by the reaction. This situation where an increase in temperature produces a decrease in solubility is not very common, for example, calcium hydroxide is more soluble at cold temperatures than at warm. When we dissolve a substance we must separate the intermolecular forces which surround the molecules. Separation of molecules requires a certain amount of energy which, in this case, can be provided in the terms of heat. There is also the possibility that compound will form a bond with the solvent resulting in energy release. However, care must be taken while supplying heat that may destroy a drug or cause other changes in the solution. For example, sucrose solution when we heat in presence of acid results in formation of invert sugar. The energy is supplied in the form of heat, providing a cooling effect. On the other hand, there is possibility of interaction between solute and solvent with formation of dipole-dipole type bond and this interaction will tend to give off heat. Based on which of these interactions are greater, we can get increase or decrease in temperature. A good example is mixture of chloroform and acetone. There exists a strong interaction between acetone and chloroform molecules. The heat produced by solute-solvent interaction is so much higher than the heat necessary to separate the molecules of acetone and chloroform, that the excess heat can be detected as rise in temperature of the liquid.

Solubility Curves:

Solids are usually more soluble at higher temperatures; more salt will dissolve in warm water than in an equal amount of cold water. A graph showing the solubility of different solids as a function of temperature are very useful in chemical analysis. A curve drawn between solubility and temperature is called solubility curve. It indicates the effect of

temperature on solubility of substances. Substances such as calcium acetate and calcium chromate show decreased solubility with increase in temperature while sodium nitrate and lead nitrate show increase in solubility with increase in temperature. The solubility curve of sodium chloride shows very minute rise with increase of temperature. There are two types of solubility curves as shown in Fig. 1.5.

Continuous Solubility Curve:

Solubility curve of substance such as calcium salts of fatty acids, potassium chlorates, lead nitrate and sodium chloride are continuous solubility curves. They show no sharp break in the curves anywhere. The solubility curve of hydrated calcium sulphate shows a rise and then fall but it remains continuous at maximum point.

Discontinuous Solubility Curve:

The solubility curve which shows sudden change in direction is called as discontinuous solubility curve. For example, sodium sulphate, calcium chloride, ammonium nitrate etc. At the break a new solid phase appears and another solubility curve of that new phase starts. The break in a solubility curve shows with sharp point where two different curves meet each other.

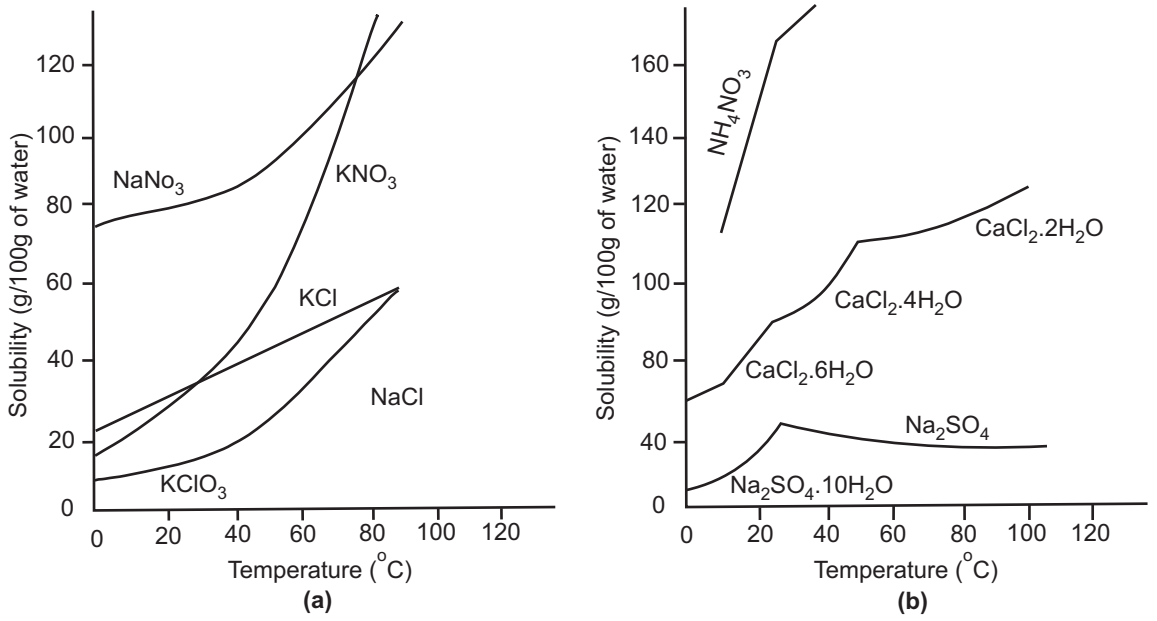


Figure 1.5: Solubility Curves (A) Continuous and (B) Discontinuous

1.8 DIFFUSION PRINCIPLES IN BIOLOGICAL SYSTEMS

Matter moves by diffusion along energy gradients from areas of high concentration to areas of lower concentration. The rate of diffusion depends on temperature, size of the particles, and the size of the concentration gradient. In biology, the selectively permeable cell membrane creates two special forms of diffusion namely: osmosis for the diffusion of water, and dialysis for the diffusion of solutes.

Diffusion is one principle method of movement of substances within cells, as well as for essential small molecules to cross the cell membrane. Cell membranes act as barriers to most, but not all, molecules. A cell membrane that could allow some materials to pass while prevent the movement of other molecules is a major step in the development of the cell. The cell membrane functions as a semi-permeable barrier, allowing a very few molecules across it while holding majority of chemicals inside the cell. Cell membranes separate the inner cellular environment from the outer cellular (or external) environment. Most of the molecules move from higher to lower concentration, although there will be some molecules that move from low to high. The overall movement is thus from high to low concentration. If there is no energy input into the system, the molecules reaches a state of equilibrium and gets uniformly distributed throughout the system.

A cell membrane is composed of phospholipids and proteins. Absorption of drugs across the stomach lining/mucosa and the blood/brain barrier are two representative examples of transport phenomenon. Skin is another great example of a membrane for the entry of drugs. The transport of drug molecules through a non-porous membrane occurs by diffusion. Transport through porous cell membranes occurs by diffusion and convection. The rate of diffusion is expressed by equation (1.2).

$$\frac{dM}{dt} = DSK\frac{(C_1 - C_2)}{h} \quad \ldots (12)$$

Where, M is amount of drug dissolved, t is time, D is diffusion coefficient of the drug, S is surface area of membrane, K is oil/water partition coefficient, h is thickness of the liquid film, C_1 is the concentration of drug at donor side of membrane and C_2 is the concentration of drug at receptor side and $C_1 - C_2$ is concentration gradient. However, C_1 and C_2 are not measured since these are values varies within the membrane.

Typically, the gradient is measured as $C_d - C_r$, representing the partition at each phase, namely $K_{o/w} = C_1/C_d$ and $K_{o/w} = C_2/C_r$. The rate of drug transport into diffusional system is predominantly dependent upon the magnitude of the concentration gradient considering the other parameters constant.

Water, carbon dioxide, and oxygen are among the few simple molecules that can cross the cell membrane by diffusion (or a type of diffusion known as osmosis). Gas exchange in lungs operates by diffusion process. All cells because of cellular metabolic processes produce carbon dioxide. Since the source is inside the cell, the concentration gradient is constantly being replenished/re-elevated; leading to net flow of CO_2 out of the cell. Metabolic processes in animals and plants usually require oxygen, which is in lower concentration inside the cell, have the net flow of oxygen into the cell through diffusion.

1.9 SOLUBILITY OF GAS IN LIQUIDS

Solubility of gas in liquids is the concentration of dissolved gas in the liquid when it is in equilibrium with the pure gas above the solution. The example of gas in liquid includes effervescent preparations containing dissolved carbon dioxide, ammonia water and hydrochloride gas. Aerosol products containing nitrogen or carbon dioxide as propellant are also considered to be solution of gases in liquids.

Factors Affecting Solubility of Gas in Liquids:

The solubility of gas in liquids depends on pressure, temperature, salt present, chemical reaction and micellar solubilization.

Pressure:

Liquids and solids exhibit practically no change of solubility with changes in pressure. When considering solubility of gases in liquids, the pressure of the gas in contact with the liquid is important. At higher gas pressure, more gas is dissolved in liquids, Fig 1.6. For example, the soda bottle is packed at high pressure of carbon dioxide before sealing. When the cap of bottle is opened, the pressure above the liquid is reduced to 1 atm and the soda fizzes. This fizzing is just carbon dioxide that was dissolved in soda, is getting released. Therefore, if lower is the pressure less carbon dioxide is soluble.

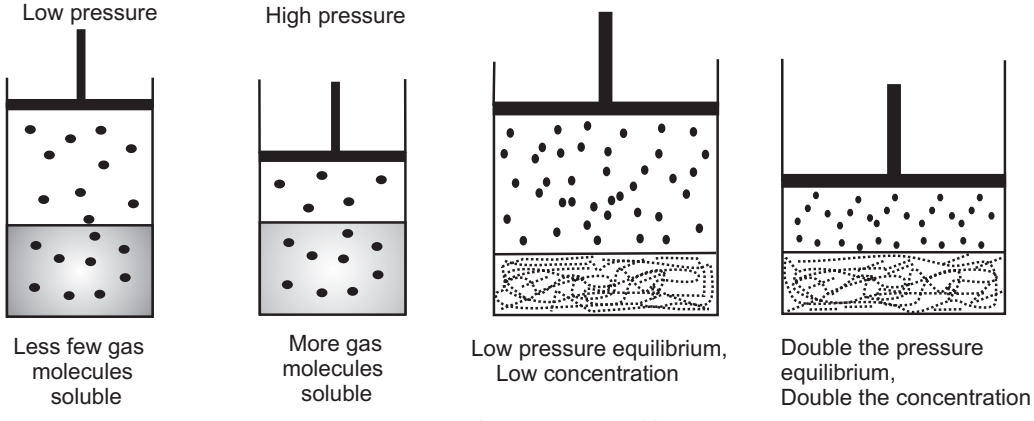


Figure 1.6: Solubility of Gases at Different Pressures

The effect of pressure on the solubility of gas is given Henry's law which states that in dilute solution the mass of gas which dissolves in each volume of liquid solvent at constant temperature is directly proportional to partial pressure of gas. Mathematically it is expressed as

$$S_g = K_H P_g \quad \ldots (1.3)$$

Where, S_g is solubility of gas, expressed as mol/L; K_H is Henry law constant which is different for each solute-solvent system and P_g is partial pressure of the gas in mmHg. The amount of undissolved gas above the solution is obtained by subtracting the vapour pressure of the pure liquid from the total pressure of the solution.

Example 1.2: The solubility of a pure gas in water at 25 °C and at 1 atm pressure is 1.5×10^{-3} mol/L. What will be the concentration of the gas at same temperature at 0.5 atm?

Solution: Given that: Pressure = 1 atm = 101.3 kPa

Concentration = 1.5×10^{-3} mol/L

Solubility (S_g) = ?

$S_g = K_H P_g$

$1.5 \times 10^{-3} = K_H \times 101.3$

$K_H = 1.519 \times 10^{-5}$

Now, at P = 0.5 atm = 0.5×101.3 kPa

$$S_g = K_H P_g$$
$$= 1.519 \times 10^{-5} \times 0.5 \times 101.3$$
$$= 7.693 \times 10^{-4} \text{ mole/L}$$

The concentration of gas at 25°C and at 0.5 atm pressure will be 7.693×10^{-4} mole/L.

1.10 SOLUBILITY OF LIQUIDS IN LIQUIDS

1.10.1 Binary Solutions

It is very common for two or more liquids to be mixed together to make a solution. Therefore, we need to know what liquids can be mixed together without precipitation. Examples of pharmaceutical solutions of liquid dissolved in liquids are hydroalcoholic solutions, aromatic waters, spirits, elixirs, lotions, sprays and some medicated oils that contain mixture of two or more miscible oils. When two or more liquids mixed together they can be completely miscible, partially miscible or practically immiscible. Completely miscible liquids mix uniformly in all proportions and hence do not get separated. Partially miscible liquids form two immiscible liquid layers, each of which is saturated solution of one liquid in the other. Such liquid pairs are called as conjugated liquid pairs.

The mutual solubility of partially miscible liquids, being temperature specific, is affected by changes in temperature. For binary phase systems, such as phenol-water system, the mutual solubility of two conjugate liquid phase increases with increase in temperature called as conjugate temperature, where as above this temperature they are soluble in any proportions. Other examples of partial miscibility include conjugate liquid pair of nicotine and water, ether and water, and triethnolamine and water. Immiscibility refers to those systems which do not mix with each other at all such as water and liquid paraffin or water and oil. The dielectric constant of a substance also affects the solubility of substance, Fig. 1.7.

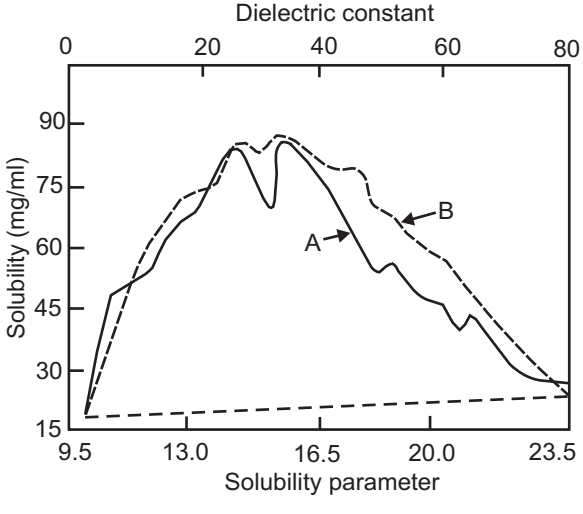


Figure 1.7: Effect of Dielectric Constant on Solubility

It is known fact that the polarity of solvent is dependent on the dielectric constant. Also, remember that LIKE DISSOLVES LIKE. The influence of a foreign substance on a liquid-liquid system is like the idea of three component system in the phase rule. Ternary systems are produced by addition of third component to a pair of partially miscible liquids to produce a solution. If added component is soluble in only one of the two components or if its solubility in the two liquids is markedly different, the mutual solubility of the liquid pair is decreased. If added solute is roughly soluble in both the liquids approximately to the same extent, then the mutual solubility of the liquid pair is increased. This is called blending. An example of this is when succinic acid is added to the phenol-water mixture. The succinic acid is soluble or completely miscible in each phenol and water therefore it causes a blending of the liquids making the mixture one phase.

1.10.2 Ideal Solutions

Dilute solutions consists of negligible amount of solute compared to pure solvents. These solutions are referred as ideal solutions. An *ideal* solution is one in which there is no change in the properties of the components other than dilution when they are mixed to form the solution. No heat is evolved or absorbed during the solution formation. The final volume of real solution is an additive property of the individual component. In another way it can be stated as a solution which shows no shrinkage or expansion when components are mixed to form solution. Ideal solutions are formed by mixing different substances having similar properties and therefore there is complete uniformity of attractive intermolecular forces. For example, when equal amounts of methanol and ethanol are mixed together, the final volume of the solution is the sum of the volumes of the methanol and ethanol.

Solutions used in pharmacy consist of wide variety of solutes and solution. The basis of solubility and solution theory is based on ideal solution. In ideal solution there is a complete absence of attractive or repulsive forces and therefore the solvent does not affect solubility. The solubility in this case depends on temperature, the melting point of solute and the molar heat of fusion (ΔH_f). In ideal solution heat of solution is equal to ΔH_f. Therefore solubility in an ideal solution can be expressed by,

$$-\log X_2^i = \frac{\Delta H_f}{2.303R}\left(\frac{T_o - T}{T_o T}\right) \quad \ldots (1.4)$$

Where, X_2^i is the ideal solubility in terms of mole fraction, R is gas constant; T is the temperature of solution and T_o is the temperature (Kelvin) of solute. The equation (1.4) can be used to calculate molar heat of fusion by plotting the log solubility versus reciprocal of absolute temperature which results in a slope of $-\Delta H_f/2.303R$. Unfortunately most of the solutions are non-ideal (real) because there may be interaction between solute and solvent. In these solutions mixing of solute and solvent can release or absorb heat into or from surroundings, respectively. While describing non-ideal solution, activity of solute must be considered. Activity of solute is defined as concentration of solute multiplied by the activity coefficient (X_2). The activity coefficient is proportional to the volume of solute and to

the fraction of the total volume occupied by the solvent. On substitution these values in equation (1.4) we get;

$$-\log X_2 = \left[\frac{\Delta H_f}{2.303R}\right]\left[\frac{T_o - T}{T_o}\right] + \log(\mu_2) \quad \ldots (1.5)$$

As activity approaches unity, the solution becomes more ideal. For example, as a solution become more dilute the activity increases and the solution becomes ideal. The log of activity coefficient ($\log X_2$) is the term that considers the work of solubilization, volume of solute and the volume of solvent. The work of solubilization includes the intermolecular forces of attraction removing molecule from the solid and integrating into the solvent. One more term solubility parameter (γ_2) which is a measure of cohesive forces between like molecules is considered for solubility. It is expressed by following equation.

$$-\log \gamma_2 = (\rho_1 - \rho_2)\frac{V_2\varphi_1^2}{2.303} \quad \ldots (1.6)$$

$$P = \left[\frac{(\Delta H_v - RT)}{V_1}\right]^{1/2} \quad \ldots (1.7)$$

Where, ΔH_v is heat of vapourization of solute, V_1 is volume/mole of solute as a liquid, V_2 is the molar volume of solute and φ_1^2 is the volume fraction of solvent, T is temperature (Kelvin) and R is gas constant.

Example 10.2: The molar heat of fusion and melting point of benzoic acid is 4139 cal/mole and 122°C, respectively. Calculate ideal mole fraction solubility of benzoic acid at 25°C. Given: Gas constant = 8.134 J/K mole.

Solution: Given that:

$T_o = 122\ °C = 273 + 122 = 395\ K$

$T = 25\ °C = 273 + 25 = 298\ K$

$R = 8.134\ J/K.mole$

$\Delta H_f = 4139\ cal/mole = 4139 \times 4.184 = 17317.58\ J/mole$

$$-\log X_2^i = \left[\frac{\Delta H_f}{(2.303R)}\right]\left[\frac{(T_o - T)}{(T_oT)}\right]$$

$$= \left[\frac{17317.58}{(2.303 \times 8.314)}\right]\left[\frac{(395 - 298)}{(395 \times 298)}\right]$$

$$= \left(\frac{17317.58}{19.1471}\right)\left(\frac{97}{117710}\right)$$

$$= 0.7453$$

$$X_2^i = \text{antilog}(-0.7453)$$

$$= 0.1798$$

The ideal mole fraction solubility of benzoic acid is 0.1798.

1.11 RAOULT'S LAW

In an ideal solution volume changes are negligible. Dilute solutions show colligative properties. These properties are the factors that determine how properties of a bulk solution change depending upon the concentration of the solute in it. Colligative properties are properties of a solution that depend mainly on the relative numbers of particles of solvent and solute molecules and not on the chemical properties of the molecules themselves. These can almost be referred as statistical properties because they can be understood solely based on relative number of different particles in a solution. There are four types of colligative properties namely:

1. Lowering of vapour pressure.
2. Elevation of boiling point.
3. Depression of freezing point.
4. Osmotic pressure..

Colligative properties of non-electrolyte solutions are regular. The values of colligative properties are approximately equal for equimolar concentration of drugs. It is possible to determine the number of solute particles present in the solution by measuring these properties and comparing them with the corresponding properties of the pure solvent. If mass of solute present in known, the number average molecular weight can be calculated by dividing the mass of solute by number of particles present to obtain the average mass of particles. Osmotic pressure is the most important colligative property since it is related with physiological compatibility of parentral, ophthalmic and nasal solution. It is difficult and inconvenient to measure osmotic pressure and therefore other colligative properties are determined and related to osmotic pressure.

In the following section equations for colligative properties of ideal solution are derived and are validated for this type of solutions. These equations can be applied to real solutions with respect to limit of small concentrations. While using these equations for real (non-ideal) solutions it requires correction to be made to these ideal equations because in real solutions there exist intermolecular interactions.

Lowering of Vapour Pressure:

Lowering of vapour pressure is the simplest of the colligative properties and easiest to understand based on physical model. The pressure brought by vapour in equilibrium with its liquid at constant temperature is known as vapour pressure. It increases with temperature. The vapour pressure of solvent is due to its escaping tendency. Temperature at which the vapour pressure of the liquid is equal to the atmospheric pressure is called as normal boiling point. The vapour pressure of pure liquid solvent depends upon the rate of escape of molecule from the surface known as escaping tendency. Solvents with greater escaping tendencies have greater vapour pressure.

The added solute is generally non-volatile which does not contribute directly to the vapour pressure of the solution. The solute interferes and prevents solvent molecules from

escaping into the atmosphere. Therefore, the vapour pressure of solution is lower than that of pure solvent. The lowering of vapour pressure is proportional to the number of solute particles or ions. The effect of non-volatile solute on the vapour pressure may be determined in dilute solutions by applying Raoult's law. It states that in an ideal solution the partial vapour pressure of each volatile constituent is equal to the vapour pressures of pure constituent at that temperature multiplied by its mole fraction in the solution. In equation form for two volatile constituent A and B, it can be expressed as

$$P_A = \overset{\circ}{P_A} X_A \quad \ldots (1.8)$$

$$P_B = \overset{\circ}{P_B} X_B \quad \ldots (1.9)$$

where, P_A and P_B are partial vapour pressures, $\overset{\circ}{P_A}$ and $\overset{\circ}{P_B}$ are vapour pressures of pure constituents and X_A and X_B are mole fractions of the constituent A and B, respectively. The total vapour pressure of solution is sum of partial vapour pressure of each volatile constituent. Therefore,

$$P = P_A + P_B \quad \ldots (1.10)$$

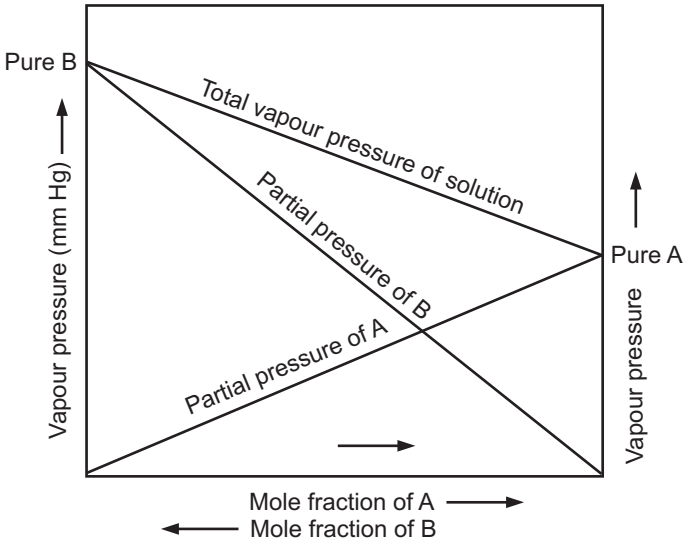


Figure 1.8: Partial Vapour Pressures of Volatile Constituents A and B and the Total Vapour Pressure of their Solution at Different Mole Fraction

The partial vapour pressure of A and B and the total vapour pressure of solution is shown in Fig. 1.8. There are two ways to explain Raoult's law. First, the simple visual way and the second one is a more sophisticated way based upon entropy. To describe using a simple way, consider that equilibrium is set-up where the number of molecules of solvent breaking and escaping away from the surface and some of them are sticking on to the surface again as shown in Fig. 1.9. An added solute molecule to the solvent replaces some of the solvent molecules present at the surface causing reduction in surface area.

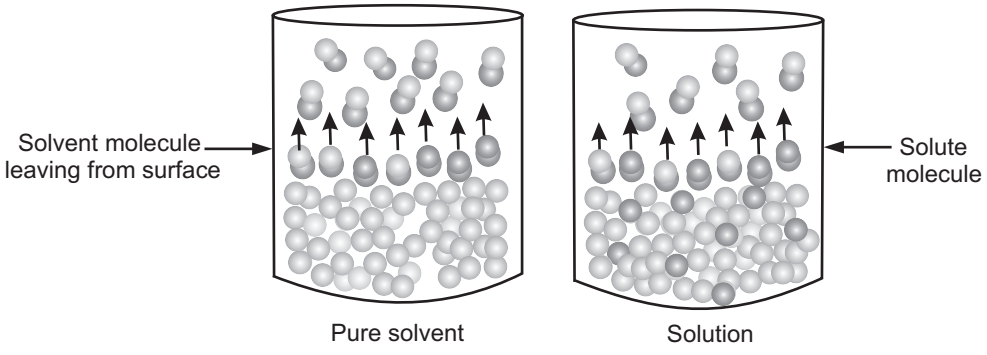


Figure 1.9: Lowering of Vapour Pressure on Addition of Non-volatile Solute

A certain fraction of the solvent molecules has enough energy to escape from the surface. If these molecules are decreased as added solute replaces some of them causing reduction in the number of molecules escaping from the surface. The net result of this reduction in number is that the vapour pressure of the solvent is reduced.

The composition of the solution in terms of mole fraction can be expressed as

$$X_A + X_B = 1 \quad \ldots (1.11)$$

$$\therefore \quad X_A = 1 - X_B \quad \ldots (1.12)$$

Substituting equation (1.12) in equation (1.8) gives

$$P_A = P_A^{\circ}(1 - X_B) \quad \ldots (1.13)$$

Simplifying equation (1.13) we get

$$X_B = \frac{(P_A^{\circ} - P_A)}{P_A^{\circ}} \quad \ldots (1.14)$$

Substituting terms for mole fraction in equation (1.14) gives

$$\frac{(P_A^{\circ} - P_A)}{P_A^{\circ}} = \frac{nB}{(nA + nB)} \quad \ldots (1.15)$$

where, *n*A and *n*B are number of moles of solute and solvent. Above equations (1.14) and (1.15) shows that relative lowering of vapour pressure of the solution is equal to the mole fraction of the solute. The mole fraction and vapour pressure in equation (1.14) and (1.15) has no units because these are relative expressions. Hence any units consistent with the system can be used.

Deviations from Raoult's Law:

In real solutions, there is no complete uniformity of intermolecular attractive forces. There are many such liquid pairs that show greater cohesive forces than the attractive forces and greater attractive forces than the cohesive forces. It can be observed even when liquids are completely miscible in all proportions. Such mixtures of liquid pairs are real or non-ideal

solutions. They do not adhere to the Raoult's law over the entire range of concentrations and are represented as deviations. This behaviour shown by liquid mixtures are called as positive deviation, Fig. 1.10 (a) and negative deviation, Fig. 1.10 (b).

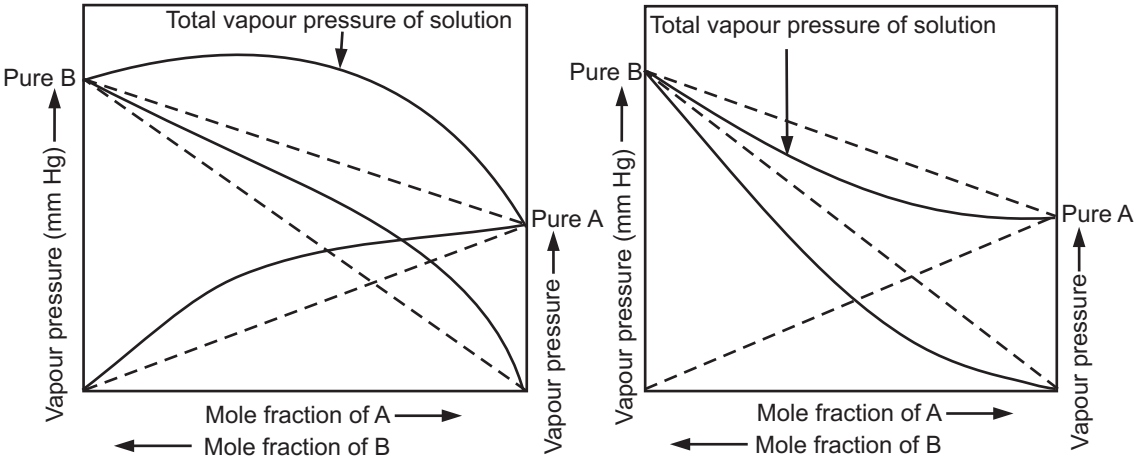


Figure 1.10: Deviations from Raoult's Law

Limitations of Raoult's Law:

Raoult's law work only for ideal solutions over entire range of concentrations. An ideal solution obeys Raoult's law. While applying this law to real solutions it has following limitations.

Real Solutions:

In real solution, the concentration of solute is high and thus intermolecular forces between solute-solute and solute-solvent are predominant that slows down the escaping of solvent molecules from the surface. This causes deviation from Raoult's law because it is applicable only to dilute solutions where the forces between solute and solvent are exactly same as those between solvent-solvent molecules.

Nature of the Solute:

Raoult's law is applicable only for solutes which are non-volatile in nature. Volatile solutes can contribute for vapour pressure above the solution which may cause the deviation from Raoult's law. Raoult's law does not apply if the added solute associates or dissociates in solvent. If association takes place the number of particles or molecules decreases causing reduction in lowering of vapour pressure. On the contrary, if solute gets dissociated more number of particles or ions are formed. For example, when 1 mole of solid sodium chloride is added to water it dissociates to produce two moles of ions as Na^+ and Cl^-.

$$Na^+ Cl^-_{(solid)} \longrightarrow Na^+_{(aq)} + Cl^-_{(aq)}$$

If 0.1 mole of sodium chloride is added to water its dissociation takes place to form 0.2 moles of particles in solution. Thus, it increases lowering of vapour pressure of solution.

1.12 REAL SOLUTIONS

Real solutions show change in the total volume of the solution upon mixing its different components together. Also, there is absorption or evolution of heat during mixing and solution formation. For example, at room temperature when 100 mL of sulfuric acid is mixed with 100 mL of water, the total volume of solution becomes 180 mL rather than 200 mL. During mixing of acid and water considerable heat is evolved causing reduction in total volume of the solution.

1.13 PARTIALLY MISCIBLE LIQUIDS

Although three types of liquid/liquid systems are commonly encountered liquid-liquid systems are mainly divided into two categories depending on the solubility of one substance in the other. The categories are complete miscibility and partial miscibility. Miscibility is the common solubilities of the components in liquid-liquid systems. Partial miscibility is when the substances only mix partially. When mixed, there are two layers formed each layer containing some of both liquids. Of these two mixed layers, each layer contains some of both the liquids for example, phenol and water. Some liquids are practically immiscible (for example, water and mercury), whilst others (for example, water and ethyl alcohol or acetone) mix with one another in all proportions.

The mutual solubility or miscibility of two liquids is a function of temperature and composition. When two liquids (liquid A and liquid B) are partially soluble in each other, two liquid phases can be observed. At equilibrium, each phase contains liquid A and liquid B in amounts that reflect their mutual solubility. Some systems are totally miscible (i.e. they form a one-phase liquid) at high temperatures, but separate into two liquid phases at lower temperatures. These systems have an upper consolute temperature, T_{UCT}, in a plot of temperature versus mole fraction. Other systems are totally miscible at low temperatures but separate into two phases at higher temperatures giving rise to a lower consolute temperature, T_{LCT}.

Oil and water don't mix. Pouring 10 mL of olive oil into 10 mL of water results in two distinct layers, clearly separated by a curved meniscus. Each layer has the same volume and essentially the same composition as the original liquids. Because very little mixing occurs apparently, the liquids are called "immiscible". For example, pouring grain alcohol into the water results in a single liquid phase. No meniscus forms between the alcohol and the water, and the two liquids are considered "miscible". Nearly any pair of liquids is miscible if only a trace amount of one of the liquids is present.

Many liquid mixtures fall between these two extremes. Two liquids are "partially miscible" if shaking equal volumes of the liquids together results in a meniscus visible between two layers of liquid, but the volumes of the layers are not identical to the volumes of the liquids originally mixed. For example, shaking water with certain organic acids results in two clearly separate layers, but each layer contains water and acid (with one layer mostly water and the other, rich in acid.) Liquids tend to be immiscible when attractions between like molecules

are much stronger than attractions between mixed pairs. Many examples are known, however, in which the liquids are partially miscible with one another. If, for example, water be added to ether or if ether be added to water and the mixture shaken, solution will form up to a certain point; beyond this point further addition of water on the one hand, or of ether on the other, will result in the formation of two liquid layers, one consisting of a saturated solution of water in ether and the other a saturated solution of ether in water. Two such mutually saturated solutions in equilibrium at a temperature are called conjugate solutions. A conjugate system has two partially miscible liquids in contact with each other. The proportionate quantities of these liquids are responsible for their existence as two liquids in contact with. Under this condition a saturated solution of one liquid in other or vice-versa is formed. The miscibility of such solution mixture can be increased by increasing temperature. For example, phenol – water, nicotine – water, triethanolamine – water etc.

Phenol-water solution is characterized by increasing mutual solubility with rise of temperature. Thus, when phenol is added to water at the ordinary temperature, a homogeneous liquid is produced. When the concentration of the phenol in the solution has risen to about 8 %, the addition of more phenol results in the formation of a second liquid phase, which may be regarded as a solution of water in phenol. If now the temperature is raised, the second liquid phase will disappear and more phenol must be added to produce a separation of the liquid into two layers. By increasing the amount of phenol in this way and observing the temperature at which the two layers disappear, the so-called solubility curve of phenol in water may be determined. In a similar manner, the solubility curve of water in liquid phenol may be obtained, and it is found that the solubility also increases with rise of temperature. Since with rise of temperature the concentration of water in the phenol layer and of phenol in the water layer increases. The compositions of the two conjugate solutions become more and more nearly the same and at a certain temperature the two solutions become identical in composition. The temperature at which the two layers become identical in composition and are in fact one layer is known as the critical solution temperature or the consolute temperature of the system. Above this temperature, the two liquids are miscible in all proportions. If the resulting mixture is represented by a point in the area enclosed by the solubility curve, separation into two layers will take place, whereas if the total composition of the mixture and the temperature is expressed by a point lying outside the solubility curve a clear homogeneous solution will result.

1.14 CRITICAL SOLUTION TEMPERATURE AND ITS APPLICATIONS

A phase diagram is a plot describing conditions of temperature and pressure under which two or more physical states coexist in dynamic equilibrium. It means *phase diagram* is a graphical representation of chemical equilibrium. This diagram is also called as Pressure – Temperature graph.

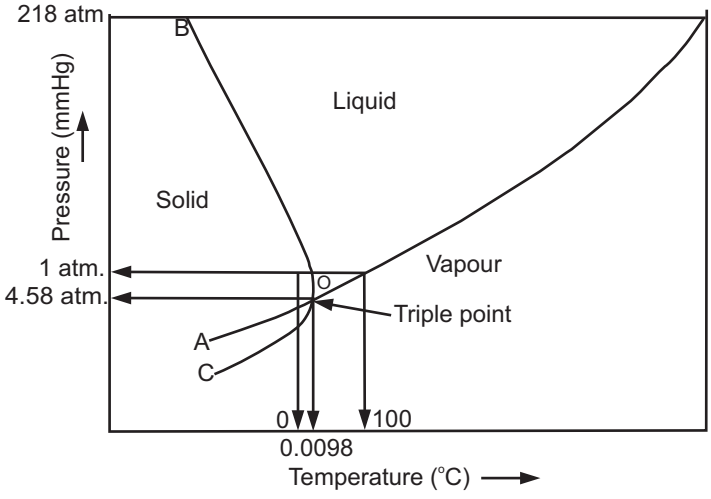


Figure 1.11: Phase Diagram of Water System

In phase diagram of water there are three lines or curves that separate the area of each phase. Adjacent to each line there exist a different single phase of water. At any point on line there exist equilibrium between two phases shown by area i.e. solid/liquid, liquid/vapour and solid/vapour. The line OA, OB and OC represents equilibrium between liquid and vapour, solid and liquid and solid and vapour phases, respectively. The line OA represents vapourization curve and OC represent sublimation curve. For example, above line OA the liquid-water exist and below it water vapour exists. The liquid – vapour equilibrium curve has a top limit labeled as C in the phase diagram. This is known as critical point. Water has a critical point of 374°C. The temperature and pressure corresponding to this point is known as the critical temperature and critical pressure, respectively. The solid – liquid equilibrium line (m. p. line) slopes backwards (negative slope) rather than forward (positive slope). It means in case of water; the melting point gets lower at higher pressures. At solid – liquid equilibrium the ice is less dense than liquid water formed as it melts, and the water formed occupies a smaller space. At this equilibrium if pressure is increased the equilibrium move to reduce the pressure again. That means it moves to the side with smaller volume. To make the liquid water freeze again at this high pressure, we need to reduce the temperature. Higher pressure means lower melting point.

The transition temperature (T_{UCT}) of a system helps to determine percent purity of substances. The change in T_{UCT} is proportional to the concentration of substance added. For example, in phenol-water system addition of sodium chloride or potassium chloride changes its T_{UCT} depending upon concentration of these substances. If known different concentration solutions of sodium chloride are prepared and added separately to phenol-water mixtures having composition say 50:50, then T_{UCT} of the system is determined by plotting a phase diagram by taking concentrations of sodium chloride on *x*-axis and UCT on *y*-axis. An unknown solution of sodium chloride is then added to phenol – water (50:50) system and

again T_{UCT} is determined. It is plotted on curve to obtain its concentration by extrapolating on *x*-axis. The T_{UCT} is mostly used as criterion to test the purity of substances that form conjugate system with some other liquid.

Phenol USP is a necrotic agent having freezing point 17ºC. Thus, at room temperature it exists in solid crystalline form. The corrosive characteristic and solid nature of phenol makes it difficult to handle. The Liquefied Phenol BP contains 80% w/w of phenol in water. The presence of other substance or impurities solidifies phenol approximately at about 10°C. The miscibility curve of phenol-water system suggest that 76% w/w of phenol should be used in the preparation. At this concentration freezing point of phenol is 3.5°C. Such preparations remain in liquid form that can be handled easily. In India, we have wide variety of climatic conditions with diverse temperatures ranging from 10 – 40°C during different seasons. Hence, a preparation which is in dry powder state in winter or rainy season would become pasty during summer. The T_{UCT} can also be used to determine percent compositions of each component in unknown mixtures. The temperature below which when system containing partially miscible liquids exist only as a single phase is known as *lower consolute temperature* (T_{LCT}). For example, triethanolamine (TEA) - water system has T_{LCT} of about 18.5°C at 13% w/w of TEA. The temperature – concentration plot of this system is shown in Fig. 1.12. Above 18.5°C mixture of these liquids forms two layers. The left upward curve shows decrease in miscibility of TEA in water whereas right upward curve shows decrease in miscibility of water in TEA with increase in temperature of system, respectively. At 50% by weight of TEA in water at 18.5°C forms single phase. This temperature is called T_{LCT} of TEA – water system. The region outside the curve shows mutual solubility of TEA and water in each other. Other examples of liquid pairs that shows T_{LCT} are dimethylamine – water (43°C, 13% w/w weight of dimethylamine), 1-methyl piperidine – water (48°C, 5% w/w of piperidine), polyethylene glycol – water, paraldehyde – normal saline, water – Tween 80, etc.

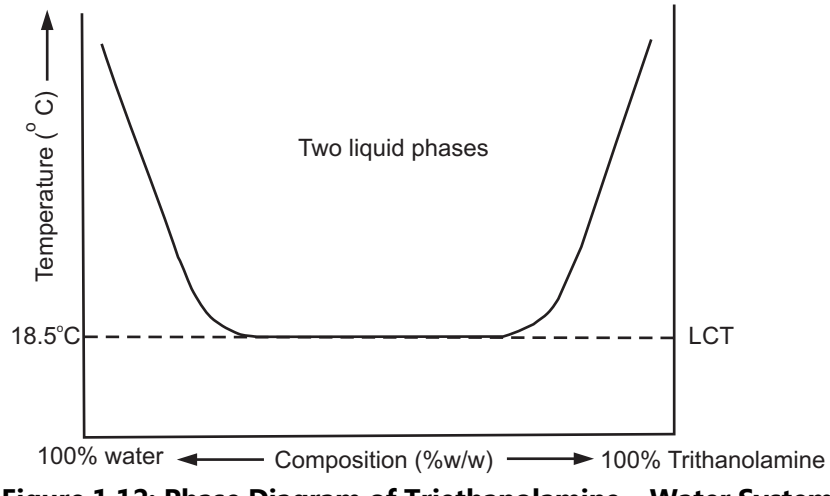


Figure 1.12: Phase Diagram of Triethanolamine – Water System

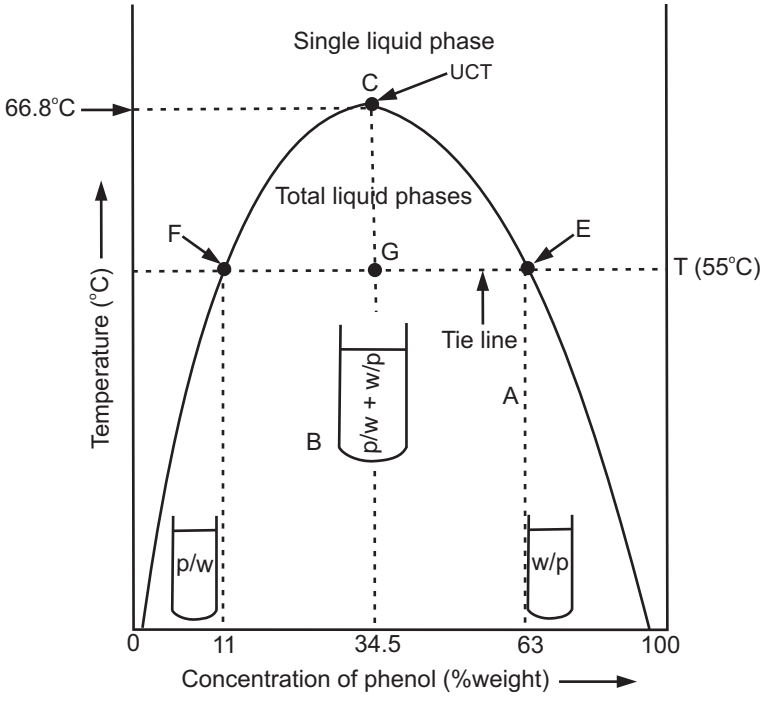


Figure 1.13: Phase Diagram of Phenol – Water System

Phenol and water are partially miscible liquids at room temperature. In this system, addition of small amount of phenol to water or water to phenol significantly changes relative volumes of two layers but not their compositions.

If temperature is increased by keeping composition constant the mutual solubility of both the liquids increases and at a specific temperature they become completely miscible and two layers becomes one. Thus, at a specific temperature the composition of both the components are fixed and both the liquids are miscible in all proportions with each other. The temperature at which two partially miscible liquids are in the state of one phase is known as critical solution temperature (CST) or upper consolute temperature (T_{UCT}). This behaviour of critical solution temperature is shown by phenol-water system as represented in Fig. 1.13. At any temperature (say T°C) the points F and C represents the composition of two layers in equilibrium with each other. The two solutions A (phenol in water) and B (water in phenol) are in equilibrium at a temperature is known as conjugate solution temperature. At this temperature two solutions of different concentrations exist in equilibrium with each other. The line in phase diagram of phenol-water joining the points F and E is called as tie line. It is defined as the line which connects the compositions of the two layers in equilibrium on the phase diagrams of the system. At point C, the top of the dome shaped curve, two layers become identical, resulting in disappearance of second layer to form a single phase. The temperature at which this happens is called as T_{UCT} of phenol – water system. At any temperature above T_{UCT} both the liquids are completely miscible; whereas below this temperature they exist in separate layers as individual entities.

Applications:

Basically, CST allows the temperature limits for some reactions to be determined if it requires that two liquids are miscible. An important application of the CST is to determine the water content in substances such as methyl and ethyl alcohols. Here the system is usually the alcohol and a hydrocarbon, such as -hexane or dicyclohexyl. The water is, insoluble in the hydrocarbon. Thus, the methyl alcohol-cyclohexane system has a CST 45 - 50 °C and even presence of 0.1 % water produces a rise of 0-15 °C in the CST. The concept of T_{LCT} is helpful in preparation of paradeladehyde in saline. If during preparation solution of paraldehyde is cooled, rapid solution formation takes place. At room temperature nicotine and water are miscible in all proportions with each other. As temperature is raised the mutual solubility of these liquids decreases. Further increase in temperature again at some higher temperature their mutual solubility increases. Nicotine - water system possess both T_{UCT} and T_{LCT} of 208 °C at 32 % w/w and 61 °C at 22% w/w of nicotine, respectively. Above T_{UCT} and below T_{LCT} system exist as single phase where as at any temperature between them it exists as two phase system (partial miscibility). The solubility curves of these two liquids are shown as closed curve. Pressure has effect on nicotine – water system that increase in pressure increases T_{LCT} and decreases T_{UCT}. At a specific pressure and above, these partially miscible liquids are completely miscible in all proportions with each other at all the temperatures. Other systems that show T_{UCT} as well as T_{LCT} are glycerin and m-toludine and β-picoline and water. The CST is affected by pressure and by the presence of impurities. Hence the CST may be taken as a criterion for the purity of a substance.

1.15 DISTRIBUTION LAW

In pharmaceutical practice, often a single substance is dissolved in two immiscible phases' i.e. two liquid phases in contact that do no not mix, such as chloroform and water. When an excess amount of solute is added to two immiscible liquid phases, it distributes itself between these phases until saturation, if mixed by shaking vigorously. If insufficient amount of solute is added it distributes in a definite ratio. The term partition coefficient is commonly refers to the equilibrium distribution of single substance between two solvent phases separated by a boundary. If third substance dissolves to some extent in both phases, the partition coefficient is the ratio of the amounts of the third substance dissolved in two phases. Partition coefficients' are sometimes called distribution coefficient. The partition coefficient is a measure of drugs lipophilicity and is an indication of its ability to cross cell membranes. It is commonly determined using an oil phase of octanol or chloroform and water.

If there is possible confusion with the extraction factor or mass distribution ratio the term concentration distribution ratio should be used. The terms distribution coefficient, extraction coefficient and, wherever appropriate, scrubbing coefficient, stripping coefficient are widely used as alternatives but are not recommended. If they must be used in a given situation the term ratio is preferable to coefficient. In equations relating to aqueous/organic systems the organic phase concentration is, by convention, the numerator and the aqueous phase

concentration the denominator. In the case of stripping ratio, the opposite convention is sometimes used but should then be clearly specified. In the past, there has been much confusion between the distribution ratio as defined above, the value of which varies with experimental conditions, for example, pH, presence of complexing agents, extent of achievement of equilibrium etc. and the true partition coefficient which is by definition invariable or the partition coefficient or distribution constant which apply to a chemical species under specified conditions. For this reason, the terms distribution constant, partition constant, partition coefficient, partition ratio and extraction constant should not be used in this context. The use of the ratio of light phase concentration to heavy phase concentration is ambiguous and is not recommended. The distribution ratio is an experimental parameter and its value does not necessarily imply that distribution equilibrium between the phases has been achieved.

Thermodynamic Deduction of Distribution Law:

Chemical potential of solute is at equilibrium in both aqueous and organic immiscible solvents. The chemical potential (μ_w) of solute in aqueous phase is expressed as:

$$\mu_w = \mu_w^\circ + RT \, ln \, C_w \quad \ldots (1.16)$$

where, μ_w° standard chemical potential of solute in aqueous phase, C_w is concentration of solute in aqueous phase, R is gas constant and T is absolute temperature. Similarly, the chemical potential of solute in organic phase is expressed as

$$\mu_{org} = \mu_{org}^\circ + RT \, ln \, C_{org} \quad \ldots (1.17)$$

where, C_{org} is concentration of solute in organic phase and μ_{org}° is standard chemical potential of solute in organic phase. At the equilibrium upon distribution:

$$\mu_w = \mu_{org} \quad \ldots (1.18)$$

$$\mu_w^\circ + RT \, ln \, C_w = \mu_{org}^\circ + RT \, ln \, C_{org}$$

$$RT \, ln \, (C_w/C_{org}) = \mu_w - \mu_{org} \quad \ldots (1.19)$$

At given temperature, standard chemical potentials of solute in aqueous and organic phases are constant.

$$RT \, ln \, (C_w/C_{org}) = \text{Constant} \quad \ldots (1.20)$$

Therefore, ratio C_w/C_{org} is constant at given temperature. The ratio constant is called as distribution coefficient or partition coefficient and it depends upon amount of solute added. The equation (1.20) represents the Nernst distribution law.

The ability of a drug to dissolve in a lipid phase when an aqueous phase is also present often referred to as lipophilicity, can be best characterized by a partition coefficient. The true or intrinsic partition coefficient can be defined as ratio of unionized drug distributed between the organic and aqueous phases at equilibrium. It is expressed for unionizable molecules as

$$K_{o/w} = C_o/C_w \quad \ldots (1.21)$$

where, C_o is concentration of unionized drug in organic phase and C_w concentration of unionized drug in aqueous phase. For ionizable molecules (acids, bases, salts) it is expressed as

$$K_{o/w} = \frac{C_o}{(1 - \alpha)C_w} \quad \ldots (1.22)$$

In equation (1.22) the term α is the degree of ionization in aqueous solution. Since partition coefficients are difficult to measure in living systems, they are usually determined in vitro using *n*-octanol as the lipid phase and a phosphate buffer of pH 7.4 as the aqueous phase. This permits standardized measurements of partition coefficients'. The $K_{o/w}$ is expressed in the form of log $K_{o/w}$ as the measure of lipophilicity. If added solute has equal molecular weight in both the phases, then ratio of the concentration of solute in both phases is found to be constant. These concentrations of solute in aqueous and organic phases are expressed in g/liter or gram equivalent/liter. Being the ratio of two concentrations the constant, partition coefficient is dimensionless value. The value of $K_{o/w}$ is unit less. It is necessary to specify in which of these two ways the partition coefficient is being expressed. No convention has been established with regard to whether the concentration in aqueous phase or in the organic phase should be placed in the numerator. Therefore, partition coefficient may be expressed as

$$K_{o/w} = C_w/C_o$$

or
$$K = C_o/C_w \quad (1.23)$$

Partition coefficient is measured using low solute concentration, where K or $K_{o/w}$ is a very weak function of solute concentration. Extensive data for the partitioning of drugs between octanol and water has been tabulated through the years, Table 1.3. For drugs having values of K much greater than 1 are classified as lipophilic where as those with K smaller than 1 indicates a hydrophilic.

Table 1.3: Partition Coefficients of Some Drugs

Drugs	Liquid-pair	$K_{o/w}$
Barbital	Chloroform/water	0.7
Benzoic acid	Peanut oil/water	5.33
Diazepam	Diethyl ether/water	4.0
Iodine	Carbon tetrachloride/water	85.0
Phenobarbitone	Chloroform/water	4.5
Phenol	Amyl alcohol/water	16.0
Secobarbital	Chloroform/water	0.125
Succinic acid	Ether/Water	3.98
Codeine	Octanol/water	3.98
Boric acid	Amyl alcohol/water	0.266

1.15.1 Limitations of Distribution Law

(i) The selected solvent liquid pair must immiscible with each other. Any mutual solubility must not affect distribution of solute if left aside for enough time to separate.

(ii) The experimental temperature must be maintained constant. As temperature has effect on solubility of solute, any change in temperature during determinations may change the findings.

(iii) The solute in question should be in same molecular state in both the solvents. If any chemical change is observed the concentration of species common to both solvents only should be considered.

(iv) Solute must present in both the solvent at low concentrations. At high concentrations of solutes Nernst's distribution law does not hold good.

(v) Samples should be withdrawn for analysis only after achievement of equilibrium. Early equilibrium attainment can be possible by vigorous shaking.

1.15.2 Applications of Distribution Law

(i) Partition coefficient first finds applications in medicinal chemistry and drug design.

(ii) It has proved useful in other related areas such as drug absorption, bioavailability, toxicity, bioaccumulation and metabolism. Although it appears that the partition coefficient may be the best predictor of absorption rate, the effect of dissolution rate, pKa and solubility on absorption must not be neglected.

(iii) Partition coefficient values are helpful in knowing the hydrophobic drug receptor interactions.

(iv) Partition coefficient help to understand the mechanism of preservative action of weak acids and determination of its optimum concentration for the effectiveness of action.

(v) From the partition coefficient general idea about the drugs solubility in solvent can be judged. It can be further useful in drugs solubility enhancement.

(vi) For series of compounds, the partition coefficient can provide an empiric handle in screening for biologic properties. For drug delivery, the lipophilic/hydrophilic balance has been shown to be a contributing factor for the rate and extent of drug absorption.

(vii) Although partition coefficient data alone does not provide understanding of in-vivo absorption, it does provide a means of characterizing the lipophilic/hydrophilic nature of the drug. Since biological membranes are lipoidal in nature the rate of drug transfer for passively absorbed drugs is directly related to the lipophilicity of the molecule.

(viii) Partition coefficient values are helpful in the extraction of drugs from mixtures such as blood, urine and crude plant extracts. Drugs depending upon its partition coefficient values extracts in organic or aqueous solvents. Efficient extraction is carried out by repeating steps several times.

(ix) Partition coefficient has applications in drug separation by partition chromatography. This technique comprises of silica column soaked in water to which a mixture containing drugs is applied. A water immiscible solvent such as hexane is allowed to flow through column. The drugs in mixture partitions into hexane in order of their partition coefficient. The drug having high partition coefficient will partition first followed by other drugs in mixture with lower partition coefficient values. Each drug can be collected separately.

(x) Partition coefficient values help to study structure activity relationship for series of compounds.

(xi) To study release of drug from gels, ointments and creams partition coefficient is a very important consideration.

EXERCISE

1. Define solubility and give general principles of solubility.
2. Define following terms.
   (a) Solubility parameter
   (b) Tie line
   (c) Insolubility
   (d) Saturated solution
   (e) Solute
   (f) Solvent
   (g) Supersaturated solution
   (h) Saturated solution
   (i) Dilute solution
   (j) Concentrated solution
   (k) Apparent solubility
   (*l*) Conjugate temperature
   (m) Blending
   (n) Intrinsic solubility
   (o) True partition coefficient
   (p) Critical solution temperature
   (q) Ideal solution
   (r) Real solution.
3. What is a solution? List the multitude of solution types that exist. Give some examples of pharmaceutical solutions.
4. Classify solutions on the basis of concentration of solutes.
5. Distinguish between solutes, solvents, and solutions.
6. Differentiate between solutions and colloids.
7. Explain solubility in ideal and real solutions.
8. Explain principle of solubility with example using dissolution process.
9. Describe in detail energetics of solubility.
10. Explain mechanisms of solvent actions for solubility.
11. Write notes on
    (a) Miscibility of liquids
    (c) Nernst distribution law
    (b) Partition coefficient
    (d) Solubility of electrolytes

12. Explain factors affecting solubility of gases in liquids.
13. Explain the temperature dependence of gas solubility in liquid solutions.
14. Describe relationship between dielectric constant and solubility.
15. Describe preparation of saturated solution for determination of solubility.
16. Describe analysis of saturated solution to determine solubility of solids.
17. The pharmacist must take precautionary measures to avoid the inappropriate findings while determining solubility of solids in liquids'. Explain this statement.
18. Enlist factors affecting solubility of solids in liquids.
19. 'Although in many cases solubility increases with the rise in temperature and decreases with the fall of temperature but it is not necessary in all cases'. Explain with suitable examples.
20. What is solubility curve? Explain continuous and discontinuous solubility curves with suitable examples.
21. What is importance of solubility enhancement? Explain different methods for the same.
22. Explain Raoult's law. Give its limitations.
23 Solubility of majority of the drugs in water is influenced by the pH of the system. Explain with suitable example.
24. Altering chemical structure of the molecule changes solubility of solute in the same solvent. Explain.
25. Define partition coefficient. Deduce partition law thermodynamically. Give some examples or partition coefficient of drugs with respective solvent systems.
26. Explain partition coefficient of ionizable solute in solvent system.
27. What are limitations of distribution law?
28. Enlist conditions essential for partition coefficient.
29. How partition coefficient help to determine equilibrium constant of a chemical reaction?
30. Enlist and explain in brief pharmaceutical applications of partition coefficient.
31. A 100 mg of a non-polar drug X (mol. weight = 510) was shaken with 100 mL of 1 : 1 v/v of octanol/water mixture for a K-value determination. The concentration of the drug in the aqueous layers was found to be 5.2×10^{-4} M. Calculate,
    (a) The partition coefficient P of the drug.
    (b) log K
    (c) K'
    (d) Will the partition coefficient change if the pH of the aqueous layer is changed?
32. The log K value of the Sulindac was experimentally determined to be 3.34. Calculate K' of the drug at pH 4.9 assuming that pKa = 3.88.

33. The octanol-water distribution studies summarized in the Fig. 1.14 below are for Codeine which is a monovalent tertiary amine. Calculate the partition coefficient P, and the Kb of the drug.

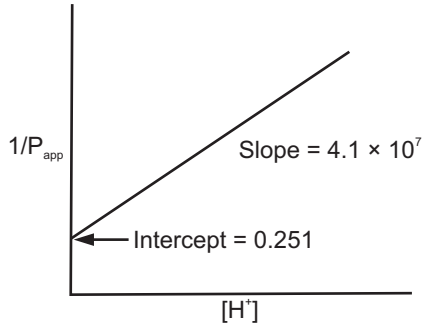


Figure 1.14: Octanol-Water Distribution Data for Codeine

34. Calculate the total concentration of benzoic acid that must be added to preserve an emulsion composed of equal volumes of oil and water and the aqueous phase is buffered at pH 4.4. The minimum effective concentration for benzoic acid is 0.25 mg/mL. (Given that: Ka = 6.3×10^{-5} and K = 6).

35. Calculate the fraction of sorbic acid that remains undissociated in the aqueous phase of a concentrated peppermint emulsion (2% per volume, o/w) if the initial concentration of sorbic acid added to the aqueous layer that was buffered at pH 4.2 was 0.45% w/v. The log K of the preservative is 1.10 and the dissociation constant of the sorbic acid is 1.8×10^{-5}.



Unit ...2

STATES AND PROPERTIES OF MATTER AND PHYSICOCHEMICAL PROPERTIES OF DRUG MOLECULES

♦ OBJECTIVES ♦

Physical science, includes chemistry and physics, and is usually thought of as the study of the nature and properties of matter and energy in non-living systems. Matter is the "stuff" of the universe — the atoms, molecules and ions that make-up all physical substances. Matter is anything that has mass and takes up space. There are five known phases, or states, of matter: solids, liquids, gases, and plasma and Bose-Einstein condensates. The main difference in the structures of each state is in the densities of the particles. Adding energy to matter causes a physical change causing matter to move from one state to another. For example, adding thermal energy (heat) to liquid water causes it to become steam or vapour (a gas). Taking away energy also causes physical change, such as when liquid water becomes ice (a solid) when heat is removed. These changes in states of matter and their inherent properties are studied and are applied in various area of pharmacy. Thus the objective of studying this chapter is to :

- Understand characteristics of states of matter.
- Understand the different physical properties of each state of matter.
- Study applications of states of matter in synthesis of drugs, analysis and design and development of dosage forms.
- Apply physical properties in various field of pharmacy such as raw material testing, preformulation, formulation characterization and stability studies etc.

2.1 STATES OF MATTER AND PROPERTIES OF MATTER

2.1.1 States of Matter

Matter can be defined as anything that has mass and occupies space. Based on its composition and properties, matter can be classified as elements, pure compounds, pure substances and mixtures, Fig. 2.1.

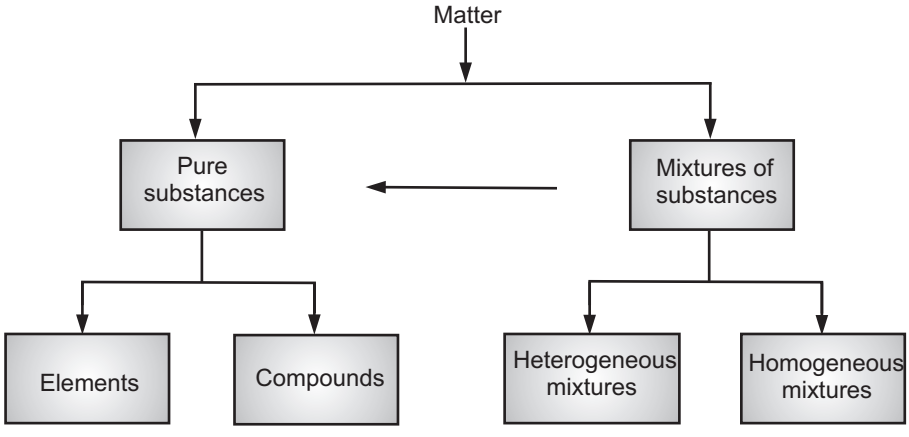


Figure 2.1: Types of Matter

A substance is a form of matter that has a constant composition. Physicochemical properties of a substance are dependent on the organizational arrangement of its constituent atoms. For example, *n*-butane has the same chemical formula as *iso*-butane, C_4H_{10}. Physical properties namely; boiling point, melting point and relative density of both these compounds are given in Table 2.1. Vapour pressures of these compounds at a temperature and their chemical properties like reactivity differ due to different arrangement of the same atoms in each molecule. They have different structural formulas as *n*-butane: CH_3-CH_2-CH_2-CH_3 and *iso*-butane: $CH_3-CH-(CH_3)-CH_3$ and thus the physicochemical property of substance vary with structural arrangement.

Table 2.1: Physical Properties for *n*-butane and *iso*-butane

Physical Properties	*n*-butane	*iso*-butane
Boiling point	0 °C	0 °C
Melting point	– 138 °C	– 159 °C
Relative density at –20°C	0.622 g/mL	0.604 g/mL

Solid, liquid and gas represents the three basic states of matter as shown in Fig. 2.2, however plasma and Bose Einstein condensate are considered as other states of matter. In pharmaceutical view point, basic three states are significant while other two states has limited applications in pharmacy but they has major applications in physics. The plasma state is not related to blood plasma but it represents an ionized gas at very high temperatures. There is no sharp distinction between solid, liquid and gaseous states because they may exist in any state depending upon intensity of intermolecular forces and physical forces like temperature and pressure. For a molecule to exist in aggregate as compound there must be some intramolecular binding force. Knowledge of these forces is important to understand the properties of solids, liquids and gases as well as solutions, suspensions, emulsions and powders etc. The Table 2.2, summaries properties of solids, liquids and gases that identify their microscopic behaviour responsible for each property.

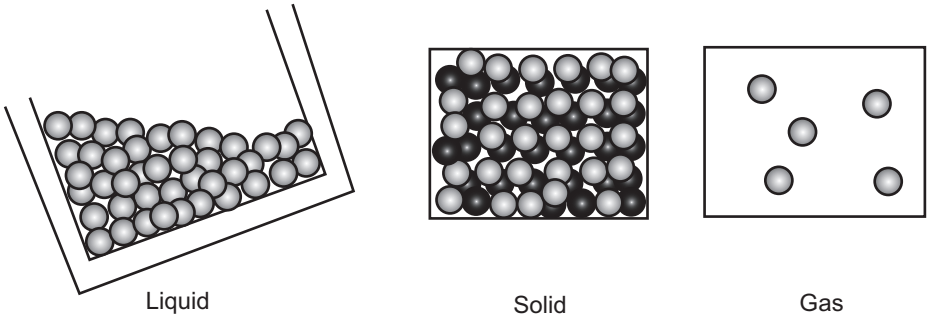


Figure 2.2: Three States of Matter

Table 2.2: Properties of Solid, Liquid and Gaseous State

Solid	Liquid	Gas
Retains volume and shape.	Assumes the shape of part of the container it occupies.	Assumes the shape and volume of container.
Particles are rigid and locked into place.	Particles can move/slide past one another.	Particles (molecules, atoms, ions) can move past one another.
A little free space exists between molecules.	A little free space exists between molecules.	Lots of free space exists between molecules.
Do not flow easily.	Flows easily.	Flows easily.
Not easily compressible.	Not easily compressible.	Easily compressible.

An element cannot be further divided by chemical means where as *compound* is a form of substance in which two or more atoms are linked chemically. Molecular compounds can be broken down to pure elements by chemical means and are defined by its atomic number. Some elements have isotopes, radioactive ^{125}I, for example, frequently used in thyroid cancer treatment is an isotope of the stable ^{127}I. All isotopes have the same atomic number but they have different mass number (i.e. different number of neutrons). Pharmacist frequently uses radioisotopes as a means to study *in-vivo* fate of biologically active macromolecules and synthetic drug compounds. Radioisotopes are also used in diagnostic applications. A combination of two or more substances is known as mixture, which may or may not retain original physicochemical properties of its constituent components. There are two types of mixture namely; homogeneous mixture and heterogeneous mixture.

Homogenous Mixture:

In homogenous mixture of solid and liquid the chemical and physical properties of individual components cannot be determined by any single instrumental method of analysis. Depending upon temperature substances exist in different states. Aspirin, for example, as shown in Fig. 2.3, indicate that below 135 °C it exists in solid crystalline form whereas above this temperature it exists in liquid form.

Dissolving aspirin crystals in water makes aqueous aspirin solution. Water destroys the intermolecular forces between the aspirin molecules that exist as crystalline arrangement during the process of solution formation. In the formation of a molecular dispersion there must be some mutual interaction between solute and solvent. Thus, the properties of the individual components of the mixture get changed. All the physical properties of aspirin are changed upon interaction with water. Similarly, the properties of water are also get changed by the presence of the aspirin. Another physical property called absorption of electromagnetic radiation is changed due to homogeneous mixing. Halothane, for example, shows different absorption of light in the visible and ultraviolet region as pure liquid and as a solution in organic solvents. The homogeneous mixtures of liquids and solids and mixtures of gases are always homogeneous.

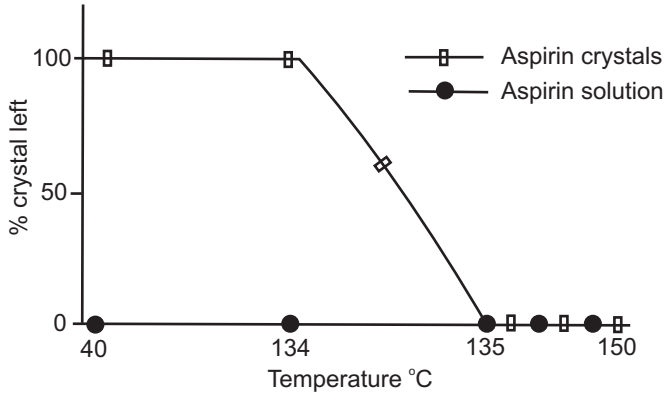


Figure 2.3: A Plot Showing Melting of Aspirin Crystals (determined by DSC)

The chemical composition of a homogeneous mixture is always same throughout. Some examples of solid, liquid and gas pharmaceutical homogeneous mixtures, respectively, are: suppositories composed of a mixture of polyethylene glycols (PEG 8000 = 40 % and PEG400 = 60 %) prepared by the melting and congealing at room temperature, Simple Syrup (85 % w/w or 66.8 % w/v) prepared by dissolving sucrose in water and general anaesthesia prepared as mixture of nitrous oxide gas with oxygen (80:20 v/v).

Heterogeneous Mixture:

A heterogeneous mixture is one in which the individual components of the mixture retains their original physicochemical properties. The composition of a heterogeneous mixture may or may not be uniform throughout. The commonest example of heterogeneous liquid mixture is pharmaceutical suspension. Suspensions are liquids in which the insoluble drugs are present in the fine state and are somewhat uniformly dispersed in aqueous media. Kinetic forces exerted by the water molecules on the suspended drug molecules are primarily responsible for their suspension in solvent. The larger particles are more difficult to keep uniformly suspended in the water. Since the drug solubility is less, the physicochemical properties of drug and water in pharmaceutical suspensions remain practically intact.

By means of physical methods components of homogeneous and heterogeneous mixtures can be separated and recovered as pure substances. However, for homogenous mixtures great care need to be taken to recover pure components. For example, water present in simple syrup can be removed by boiling syrup and condensing generated vapours to get back pure water leaving behind the pure dry sugar powder. The sugar is recovered in a pure form, but not in its original, crystalline state. A tablet prepared by direct compression of a drug and other excipients such as lactose, polyvinyl pyrrolidone (PVP) and magnesium stearate is an example of a heterogeneous solid mixture. Lactose powder tries to remain as a separate entity from the magnesium stearate and the solid drug. For the excipients to exert its effect in the tablet, they must retain their distinct identity along with their physicochemical properties within the powder mixture. PVP is the disintegrant and its swelling property facilitates disintegration of tablet in dissolution media. Interaction of PVP with the drug or with any of the other excipients may change or even neutralize its disintegration property. Similarly, interaction of magnesium stearate, a lubricant, with other excipients may eliminate its lubricant properties. But most importantly, active drug-excipient interactions that are not expected could lead to product instability, ineffective therapy or sometimes toxicity. The carbonate salts, for example, is commonly used in effervescent tablets that may cause hydrolysis of an ester drug in the presence of moisture. Similarly, interaction of the drug with excipients may lead to complex formation, which may have reduced solubility that may affect drug performance.

2.1.2 Changes in the State of Matter

In the solid-state particles are held near by intermolecular, interatomic or ionic forces therefore the particles of solid oscillate about fixed position. As the temperature of solid is increased, the particles acquire enough energy to breakdown the ordered arrangement of the lattice and pass in to the liquid form. On further application of energy by increasing temperature, liquid molecules pass in to the gaseous state. The transition between different states of matter and the processes involved in these transitions is shown in Fig. 2.4.

The examples of the substances that exist in different physical states are nitrogen (gas), water (liquid) and glucose (solid) under the normal temperature (22 °C) and pressure (1 atm) conditions. Ice water, liquid water and vapour water, is classic example of a substance that exist in three different states. Some solids with high vapour pressure like iodine and camphor can pass directly in to gaseous state without melting called as sublimation. A change in which gas state directly changes to solid state is called condensation. A substance may co-exist in two or three states simultaneously at temperature and pressure conditions. For example, ice in liquid water at temperature very close to freezing point or coexistence of ice, liquid and water vapour at triple point of water.

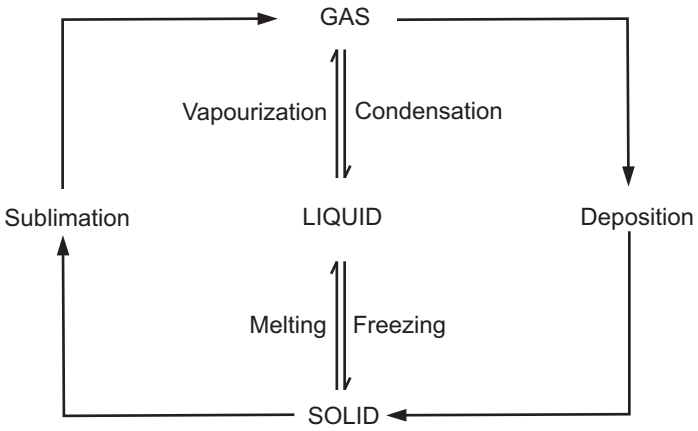


Figure 2.4: Transitions in State of Matter

The changes in the physical states of a substance are reversible in nature. These are due to rearrangement of the molecules in a substance, while on other hand; chemical changes are due to change in specific orientation or arrangement of the atoms and groups of the substance. Chemical changes may be irreversible or completely or partially reversible. Chemical changes always result in a formation of a new compound having different properties. An example of an irreversible chemical change is decomposition of water causing the molecules to breakdown in to new substances hydrogen and oxygen. An example of reversible chemical change is esterification of salicylic acid with malonic anhydride to form aspirin.

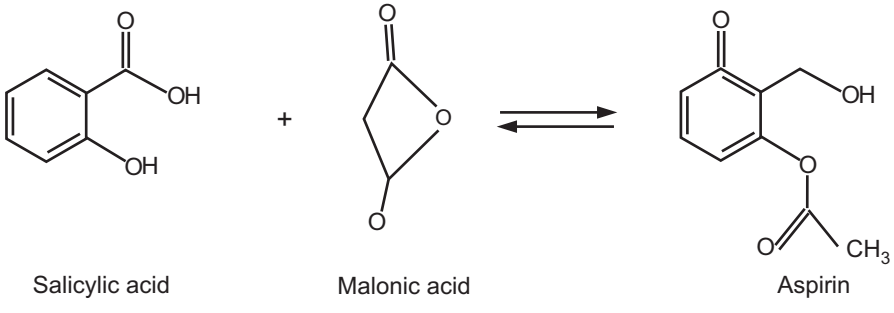


Figure 2.5: Reversible chemical change

2.1.3 Latent heat

The amount of heat required to raise the temperature of one gram of the solid is called the heat capacity. The temperature of solid continuously increases until it reaches to its melting point. At melting point the temperature will hold steady for a while, even though heat is added to the solid. It will hold steady until the solid completely melts. The temperature rising stops because melting requires energy. All the energy added to a crystalline solid at its melting point goes into melting, and none of it goes into raising the temperature. Then again, the temperature of the solid will begin to increase. This heat is called the latent heat of melting. Once the solid get melted, the temperature begins to rise but at a slower rate. The molten solid (liquid) has a higher heat capacity than the solid crystalline state therefore it absorbs more heat with a smaller increase in temperature. Hence,

when a crystalline solid melt it absorbs a certain amount of heat, the latent heat of melting, and it undergoes a change in its heat capacity. Any change like melting, freezing, boiling or condensation brought about by heat which has a change in heat capacity and a latent heat involved, is called a first order transition. But when an amorphous solid is heated to its Tg, the temperature increases. It increases at a rate determined by the solid's heat capacity. There is no latent heat of glass transition. At Tg, the temperature does not stop rising. The temperature keeps upon increasing above Tg but at different rate than below Tg. The solid does undergo an increase in its heat capacity when it undergoes the glass transition due to change in heat capacity. Any change brought about by heat, which has a change in heat capacity, but a latent heat is not involved, is called a second order transition. In first order transition melting is observed with crystalline solid, and in second order transition the glass transition is observed with amorphous solid.

2.1.4 Vapour pressure

Physical properties of liquids are controlled by strength and nature of intermolecular attractive forces. The most important properties are vapour pressure, viscosity, surface tension and light absorption and refraction. A liquid placed in a container partially evaporates to establish a pressure of vapour above the liquid. The established pressure depends on the nature of the liquid, and at equilibrium it becomes constant at any given temperature. This constant vapour pressure is the saturated vapour pressure of liquid at that temperature. Until the vapour pressure is maintained, no further evaporation observes. As shown in Fig. 2.6, at lower pressures a liquid evaporates into the vapour phase while at higher pressure the vapour tend to condensate till equilibrium establishes. During vaporization heat is absorbed by liquid. At any given temperature, the amount of heat required per gram of liquid is definite quantity called as heat of vaporization of liquid (ΔH_v). It is difference in enthalpies of vapour (H_v) and liquid (H_l), respectively. Therefore,

$$\Delta H_v = H_v - H_l \quad \ldots (2.1)$$

During evaporation ΔH_v is always positive while during condensation it becomes always negative. As per definition of change of enthalpy, ΔH_v is the difference in internal energy of vapour and liquid.

$$\Delta H_v = \Delta E_v + P\,\Delta V_v \quad \ldots (2.2)$$

where, P is vapour pressure and ΔH_v is change in volume during vapour to liquid transition.

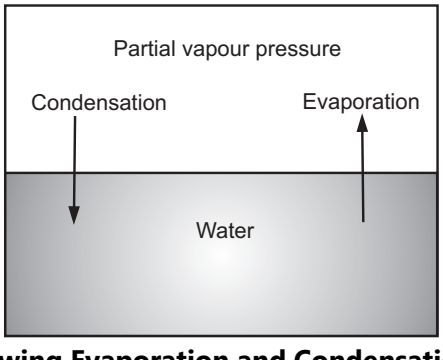


Figure 2.6: Schematic Showing Evaporation and Condensation in Liquids with Change in Temperature

The temperature of a substance depends on the average kinetic energy of its molecules. Average kinetic energy is considered because there is an enormous range of kinetic energies for these molecules. Even at temperatures well below the boiling point of a liquid, some of the particles are moving fast enough to escape from the liquid. During this process the average kinetic energy of the liquid decreases. As a result, the liquid becomes cooler. It therefore absorbs energy from its surroundings until it returns to thermal equilibrium. But as soon as this happens, some of the water molecules once again have enough energy to escape from the liquid.

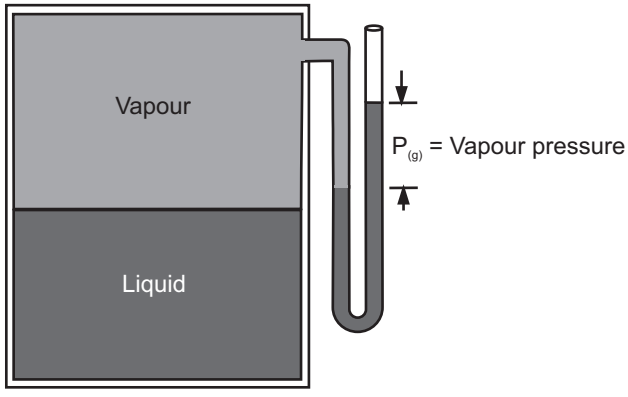


Figure 2.7: Closed Container Showing Vapour Pressure of Liquid at Given Temperature

In an open container, this process continues until all the water evaporates. In a closed container, some of the molecules escape from the surface of the liquid to form a vapour. Eventually, the rate at which the liquid evaporates to form a gas becomes equal to the rate at which the vapour condenses to form the liquid. At this point, the system is said to be in equilibrium. As shown in Fig. 2.7, the space above the liquid is saturated with water vapour, and no more water evaporates. The pressure of the water vapour in a closed container at equilibrium is called the vapour pressure.

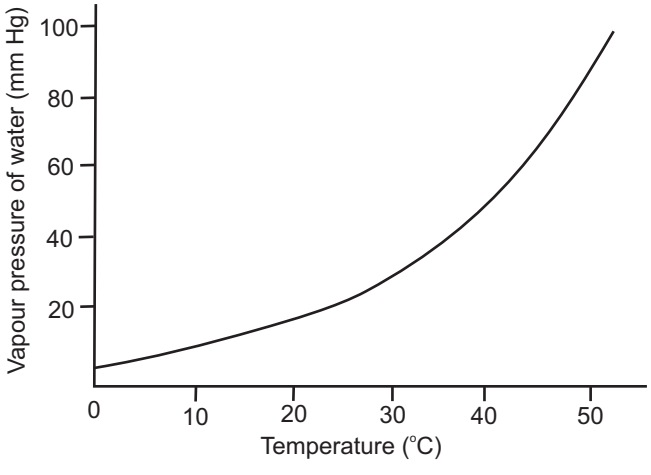


Figure 2.8: Plot of Vapour Pressure versus Temperature of Water

The Fig. 2.8 shows that the relationship between vapour pressure and temperature is not linear. The vapour pressure of water increases more rapidly than the temperature of the system.

Measurement of Vapour Pressure:

Vapour pressures of liquids are measured by static and dynamic methods.

Static Method:

Vapour pressure of liquid is generally measured by the isoteniscopic method, which is precise, flexible and convenient over a range of temperatures. A simple apparatus is shown in Fig. 2.9. It consist essentially an isoteniscopic bulb of 2 cm diameter.

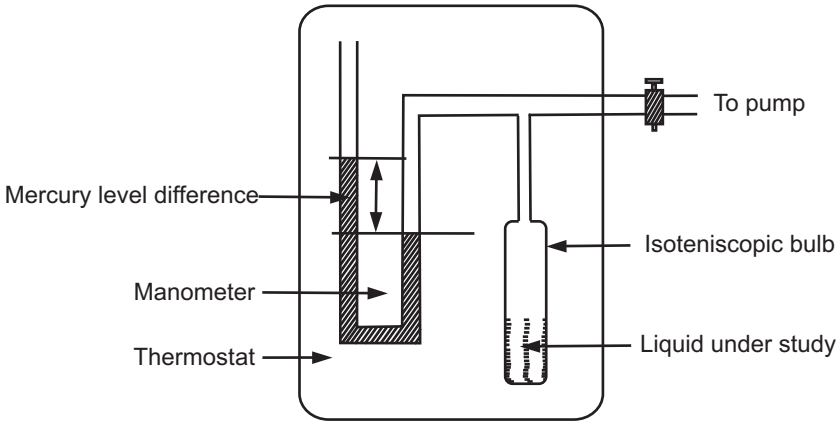


Figure 2.9: Schematic of Isoteniscopic Method

A liquid under test is filled in bulb-up to half level mark, which is connected to mercury manometer and a pump. The air inside the bulb is removed by application of vacuum. Now there is no air present in the bulb. To maintain equilibrium, part of liquid evaporates. The system is maintained at constant temperature so that the equilibrium between liquid and vapour attains. The generated vapours exert pressure on mercury present in column. The difference in height of mercury in column is determined which is equal to vapour pressure of that liquid. By maintaining the system at any other temperature, it is possible to determine vapour pressure at that temperature. This method is used for liquids having vapour pressures on higher sides close to one atmosphere.

Dynamic Method:

This method is proposed by Walker and is useful especially in determinations of very low vapour pressure of liquid mixtures. Great care is required to obtain excellent results. An illustrative apparatus is shown in Fig. 2.10. An inert gas such as nitrogen is passed through the given liquid at constant temperature. The inert gas is saturated with the vapours of liquid under test and leaves the flask at exit of the tube. If P is total vapour pressure in the apparatus at saturation, n is the moles of gas passed through and n_v is number of moles of vapour collected. The n_v is given as

$$n_v = \frac{W_v}{M_v} \quad \ldots (2.3)$$

where, W_v is loss in weight of liquid and M_v is molecular weight of liquid. The partial pressure of vapour, P' is same as vapour pressure of liquid at saturation and can be given as

$$P' = \left(\frac{n}{n + n_v}\right) \quad \ldots (2.4)$$

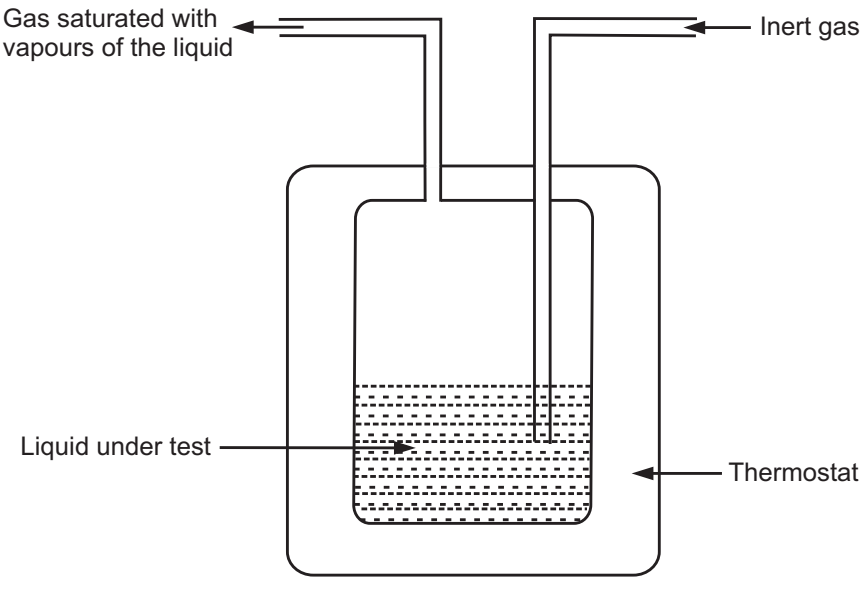


Figure 2.10: Schematic of Dynamic Walker's Method

In the other form equation (2.4) can be written as

$$P = \frac{m}{MV} \times RT \quad \ldots (2.5)$$

where, m is loss in weight of liquid as vapour, V is volume of gas passed through, M is molecular weight of liquid and R is gas constant.

Boiling Point of Liquids:

The boiling point is temperature at which vapour pressure of liquid equals the 760 mmHg pressure. However, increasing temperature can boil liquids at any temperature from its freezing point to critical temperature either or decreasing applied external pressure. Hence, boiling point of liquid is temperature at which vapour pressure of liquid is equal to pressure acting on its surface. Boiling is characterized by formation of bubbles within it and release from the surface. The change in boiling point with pressure is calculated if molar heat of vaporization (ΔH_v) for the liquid is known. If T_1 is the boiling point at pressure P_1 and T_2 is boiling point at pressure T_2 then using equation (2.5) boiling point of liquid at given pressure is obtained.

$$\log \frac{P_1}{P_2} = \frac{\Delta H_v}{2.303\ R} \frac{T_2 - T_1}{T_1 T_2} \quad \ldots (2.6)$$

However, when ΔH_v is not known its value is estimated from Trouton's rule which states that

$$\frac{\Delta H_v}{T_b} = \text{Constant} \quad \ldots (2.7)$$

where, T_b is normal boiling point of liquid on absolute temperature scale. Boiling points of some liquids at one atmospheric pressure are given in Table 2.3.

Table 2.3: Boiling Points of Some Liquids at One Atmospheric Pressure

Liquid	Boiling point (K)	Liquid	Boiling point (K)
Acetic acid	391.3	Chloroform	334.4
Acetone	329.4	Ethyl alcohol	351.6
Ammonia	339.8	Ethyl ether	307.8
Benzene	353.3	Formic acid	374.0
Water	373.15	–	–

Boiling Point and Vapour Pressure:

Bubbles are formed on heating liquid, which rises to liquid surface and bursts. When liquid vapourizes, the molecule in vapour state remain together as tiny bubbles with the vapour pressure within it. This vapour pressure in bubbles within the liquids is different than that of atmospheric pressure. When a bubble rises to surface, it burst to have equal vapour pressure that of atmosphere. Therefore, boiling point of a liquid is a temperature at which vapour pressure of liquid is equal to atmospheric pressure. Reducing external pressure can reduce the boiling point and at low temperature it is equal to external pressure. Similarly, increasing external pressure increases boiling point of liquid and at high temperature it is equal to external pressure.

2.1.5 Sublimation

Sublimation is another form of phase transitions. Here solid turns directly into a gas. As a sublimating material changes from a solid to a gas, it never passes through the liquid state. As we know water exists in its three forms namely ice, water, and steam. Sublimation is just one of the ways water or another substance can change between its potential phases. Substances such as water and carbon dioxide (CO_2) can be plotted on as pressure vs. temperature to understand their state of matter (solid, liquid, or gas) at a given temperature and pressure. At a typical atmospheric pressure, water is a solid at temperatures below 0° C, a liquid from 0 to 100° C, and a gas at higher temperatures. But atmospheric pressure, however, can change, particularly with altitude. Higher altitudes yield lower atmospheric pressures. Water doesn't always change phase at the same temperatures. For example, with lower pressures, liquid water changes to a gas at temperatures lower than 100° C. If the pressure is dropped low enough, water reaches what's known as a triple point. At pressure and temperature of triple point a substance can exist in solid, liquid, and gaseous forms.

Below this point, solid water sublimes, changing directly into a gas with a rise in temperature and never pass through the liquid phase. The CO_2 has a triple point at a pressure higher than 1 atmospheric pressure, meaning that at Earth's standard atmospheric pressure, CO_2 will sublime as it heats and is converted from solid to a gas.

2.1.6 Critical point

A liquid need not always have to be heated to its boiling point before it changes to a gas. The kinetic energy of the molecules is proportional to the absolute temperature of the gas. Due to high kinetic energy gas molecules are in the state of constant motion. In liquids, only few molecules have lower or higher kinetic energy. It is illustrated in Fig. 2.11. At low temperature, the number of molecules having high kinetic energy is less as shown by ABCD while at high temperature the number of molecules having higher kinetic energy increases as shown by FBCE. The molecules with high kinetic energy are important to escape from liquid state to vapour state. Upon cooling, kinetic energy gradually decreases. Since the temperature being decreased a stage is attained at which gas molecules loses their energy that they are unable to overcome forces of attraction between them. This situation brings the gas molecules near to have contact with each other achieving more condensed liquid state. This state also can be possible to achieve by increasing pressure of the gas but it has a limitation that pressure is effective only below specific temperature. This temperature is called as critical temperature. It is defined as the temperature above which gas cannot be liquefied, even if very high pressure is applied.

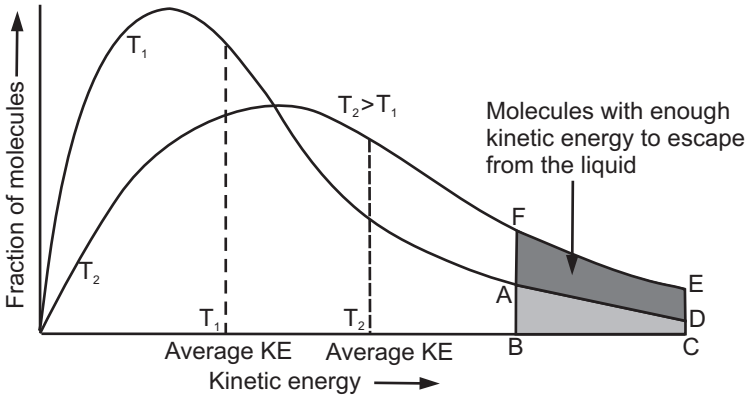


Figure 2.11: Energy Distribution of Molecules in Liquid

The critical temperature of water is 374 °C or 647 K and its critical pressure is 218 atm. If liquid such as water is sealed in evacuated tube, a specific amount of it evaporates to produce vapour at constant temperature. Like gas, water vapour exerts pressure and maintains equilibrium between liquid and vapour phases. Exerted vapour pressure is characteristic of every liquid and is constant at any given temperature.

The vapour pressure of water at 25 °C is 23.76 mmHg while at 10 °C it is 760 mmHg and therefore it is clear that vapour pressure increases continuously with temperature. As water is heated further, it evaporates to more amount resulting in increased vapour pressure.

When temperature reaches 374 °C the water meniscus becomes invisible. At critical temperature, physical properties of liquid and vapour become identical and no distinction can be made between the two. This point is also called as *critical point*. The temperature, saturated vapour pressure and molar volume corresponding to this point are designated as critical temperature (T_c), critical pressure (P_c) and critical volume (V_c) respectively. For water these critical constants are; T_c = 374 K, P_c = 219.5 atm and V_c = 58.7 mL/mole. The critical points for different gases are given in Table 2.4.

Table 2.4: Critical Temperatures, Pressures and Boiling Points of Common Gases

Gas	Critical temperature (°C)	Critical pressure (atm)	Boiling point (°C)
He	−267.96	2.261	−268.94
H_2	−240.17	12.77	−252.76
Ne	−228.71	26.86	−246.1
N_2	−146.89	33.54	−195.81
CO	−140.23	34.53	−191.49
Ar	−122.44	48.00	−185.87
O_2	−188.38	50.34	−182.96
CH_4	−82.60	45.44	−161.49
CO_3	31.04	72.85	−78.44
NH_3	132.4	111.3	−33.42
Cl_2	144.0	78.1	−34.03

2.1.7 Eutectic mixtures

A two-component system containing a solid and liquid in which the two components are completely miscible in the liquid states and are completely immiscible in the solid state. This is because the solid phase consists of pure component. This mixture is known as eutectic mixture. The temperature at which such system exists in liquid phase is known as eutectic temperature. Above this temperature, the components are liquid and below this temperature they are solids. Physically eutectic systems are solid dispersions. Some examples of this type are thymol – salol, thymol – camphor, menthol – camphor etc.

In Fig. 2.12, the melting temperature of two substances A and B are plotted against mixture compositions. The curves separating the regions of A + Liquid and B + Liquid from regions of liquid AB are termed liquidus curves. The horizontal line separating the fields of A + Liquid and B + Liquid from A + B all solid, is termed the solidus. Upon addition of B to A or A to B, their melting points are reduced. The point, E, where the liquidus curves and solidus intersect, is termed the eutectic point. At the eutectic point in this two-component system, all three phases, that is Liquid, crystals of A and crystals of B, all exist in equilibrium. The eutectic

point represents a composition (eutectic mixture composition) at which any mixture of A and B has the lowest melting point. Note that the eutectic is the only point on the diagram where this is true. At the eutectic point the maximum numbers of allowable phases are in equilibrium. When this point is reached, the temperature must remain constant until one of the phases disappears. A eutectic is an invariant point. Below eutectic temperature no liquid phase exists.

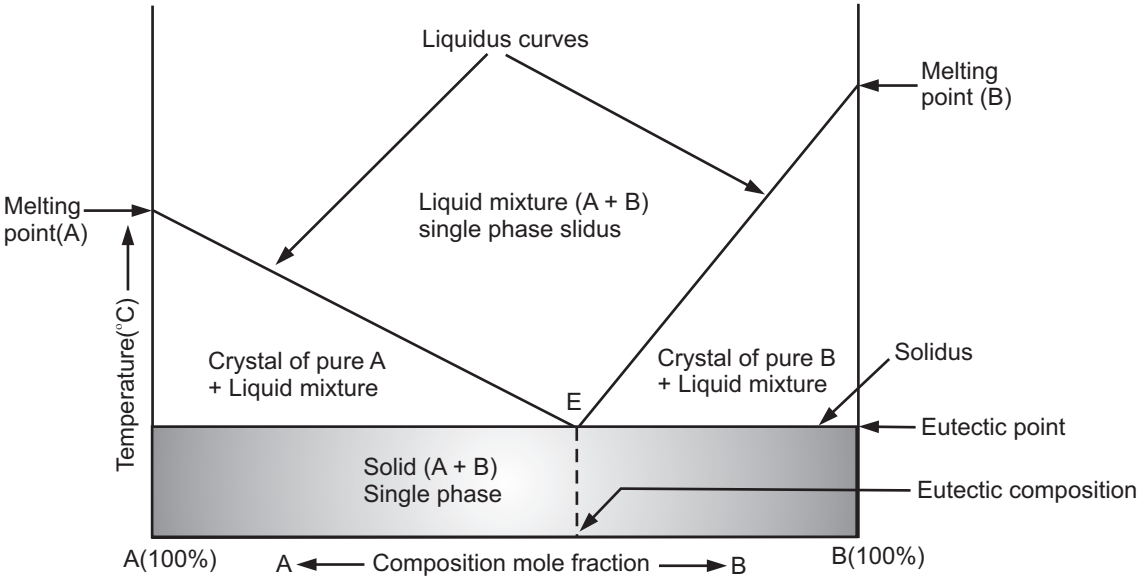


Figure 2.12: Eutectic Mixture

If we cool solution of A and B which is richer in A than the eutectic mixture, then the crystal of pure A will appear. As the solution is cooled further, more and more of A get crystallize out and the solution becomes richer in B. When the eutectic point is reached, the remaining solution crystallizes out forming a microcrystalline mixture of pure A and pure B. If salol – thymol combinations is to be dispensed as dry powder, it is necessary that the ambient temperature should be below its eutectic point of 13 ^{o}C. Above this temperature, it exists in liquefied form. At eutectic point their contribution with respect to composition is 34 % thymol and 66 % salol.

2.1.8 Gases

The gaseous state is the simplest state amongst the three states of matter. A microscopic representation of gaseous state is shown in Fig. 2.2. The molecules in gas are wide apart in empty space and are free to move in any direction in the container they are contained in. The gas molecules exert pressure on the walls of the container in all directions. Gases have indefinite expansion ability to fill the entire container. If movable piston is fitted into container containing gas, then on application of pressure by piston they get easily compressed. When two or more gases placed together they rapidly diffuse throughout each other and form a homogenous mixture. Upon heating gas in the container inside pressure increases and if container is fitted with piston under this condition its volume increases.

Chemical properties of gases vary significantly whereas Physical properties are simpler to understand. Gaseous state can be described by considering small scale action of individual molecules or by large action of the gas. By studying these properties, we can understand the behaviour of gases. The model called as kinetic molecular theory can easily describe the properties.

Kinetic Molecular Theory of Ideal Gases:

The statements made in this theory are only for what is called an ideal gas. They cannot all be rigorously applied to real gases, but can be used to explain their observed behaviour qualitatively. The kinetic molecular theory is based upon the following postulates;

1. All matter is composed of tiny discrete particles (molecules or atoms).
2. Ideal gases consist of small particles (molecules or atoms) that are far apart in comparison to their own size.
3. These particles are dimensionless points, which occupy zero volume.
4. These particles are in rapid, random and constant straight-line motion. Well-defined and established laws of motion can describe this motion.
5. There are no attractive forces between gas molecules or between molecules and the sides of the container with which they collide.
6. Molecules collide with one another and the sides of the container.
7. Energy can be transferred in collisions among molecules.
8. Energy is conserved in these collisions, although one molecule may gain energy at the expense of the other.
9. Energy is distributed among the molecules in a fashion known as the Maxwell-Boltzmann Distribution.
10. At any instant, the molecules in each sample of gas do not at all possess the same amount of energy. The average kinetic energy of all the molecules is proportional to the absolute temperature.

Above mentioned postulates are meant for ideal gas only and are only approximately valid for real gases.

Characteristics of Gases:

The volume (V), pressure (P), temperature (T) and the number of moles (n) in the container are measurable characteristic properties of the gas.

Volume:

The volume of container is the volume of gas sample and is expressed in unit liter (L) or milliliter (mL).

Pressure:

Atmospheric pressure is measured using a barometer, Fig. 2.13. If a tube, completely filled with mercury (Hg), is inverted into a dish of mercury, mercury will flow out of the tube until the pressure of the column of mercury equals the pressure of the atmosphere on the

surface of the mercury in the dish. The height of the mercury in the tube is 760 mm for 1 atm of pressure. Column of mercury is used to measure pressure of a gas closed in a container. The height 'h' of mercury column of manometer, Fig. 2.14, indicate how much higher the pressure of gas is in the container than outside.

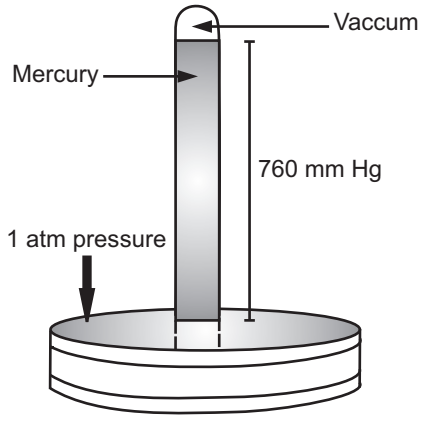


Figure 2.13: Barometer

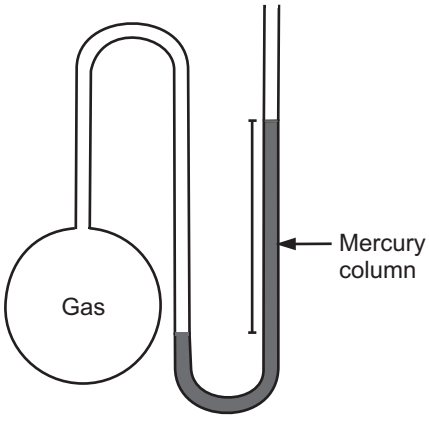


Figure 2.14: Manometer

Pressure of a gas is proportional to average force per unit area that gas molecules exert on the walls of the container. The greater the number of gas molecules in each container, the higher is the pressure as the greater average number of collisions occurring with the wall of the container. If the volume of the container is reduced, the average number of collisions will increase. Pressure is directly proportional to the kinetic energy of the gas molecules therefore higher the temperature the greater is the kinetic energy and greater the pressure of the gas.

Temperature:

Temperature of gas is measured in Kelvin temperature scale. The product of pressure and volume per mole is proportional to the average molecular kinetic energy. The average kinetic energy is proportional to the absolute temperature.

Number of Moles of Gas:

The concentration of gas in a container can be obtained as ration of mass 'm' of the gas sample to the molar mass, M.

$$\text{Moles of gas} = \frac{\text{Mass (m)}}{\text{Molar mass (M) of the gas}} \quad \ldots (2.8)$$

Gases are classified into two type namely ideal gases and non-ideal (real) gases. An ideal gas is one that obeys certain laws while real gases are those, which obey these laws only at low pressures.

In ideal gases, the volume occupied by its molecules is negligible compared to total volume at all temperatures and pressures and at these conditions the intermolecular attraction is extremely small. In case of real gases both these parameters are appreciable and magnitude depends on nature, temperature and pressure of gas. Ideal gas is hypothetical

gas and real gas contains molecules that have definite volume and intermolecular attraction between each other. When influence of these parameters is negligible gas is considered as ideal gas. This is practically observed at low pressures and high temperatures when the free space between gas molecules is large that very little or negligible attractive forces exist between the molecules.

Gas Laws:

Physical laws describing the behaviour of gas under various conditions of pressures, volumes and temperature is known as gas laws. These laws are described below.

Boyle's Law:

Robert Boyle, in 1662, formulated a generalization that the volume of any definite quantity of gas at constant temperature is inversely proportional to its pressure. Mathematically it is expressed as;

$$V \propto \frac{1}{P}$$

or $$V = \frac{k}{P} \quad \text{(when temperature is held constant)} \ldots (2.9)$$

where, V is the volume and P is pressure of the gas whereas k is proportionality constant. This constant is dependent of temperature, weight of gas, its nature and the PV units. The equation (2.9) is the mathematical expression of Boyle's law; at constant temperature, the volume occupied by a fixed weight of a gas is inversely proportional to the pressure exerted on it.

Boyle's law describes the behavior of an ideal gas and approximates the behaviour of a real gas. The approximation is very poor at high pressures and low temperatures.

If in certain condition, as shown in Fig. 2.15, pressure and volume of gas are P_1V_1 and at any other condition they are P_2V_2, then at constant temperature, this can be expressed as,

$$P_1V_1 = k = P_2V_2 \quad \ldots (2.10)$$

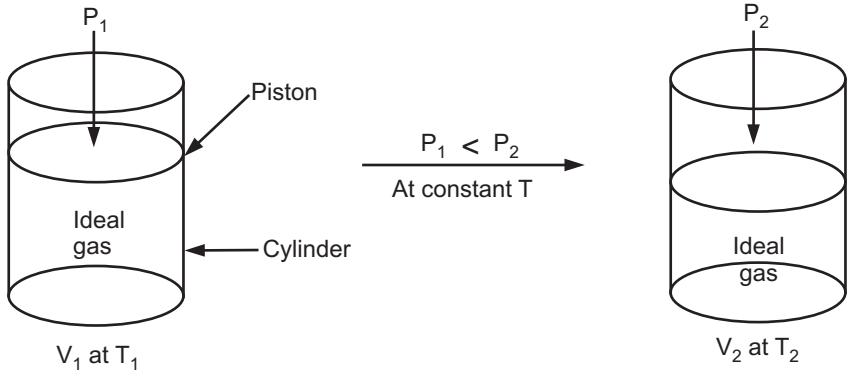


Figure 2.15: Effect of Pressure on Volume of Ideal Gas at Constant Temperature

A plot of the volumes at various pressures is given in Fig. 2.16 below.

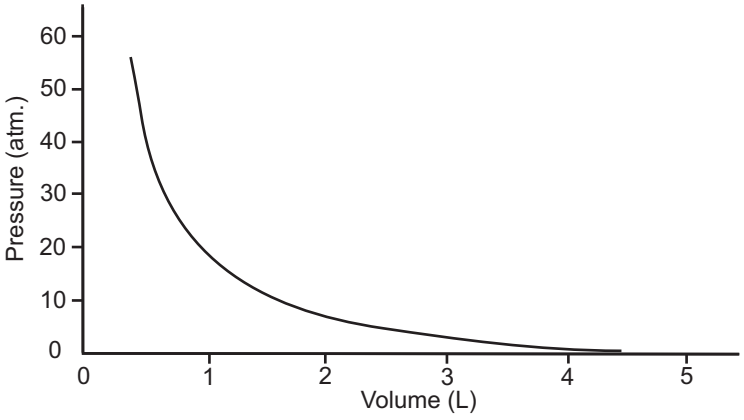


Figure 2.16: A plot of Pressure versus Volume of Ideal Gas at Constant Temperature

Example 2.1: If 6 g sample of a gas occupies 10.3 L at 300 torr, what volume will the gas occupy at the same temperature and 500 torr?

Solution: Since *n* and temperature are held fixed,

$P_1V_1 = P_2V_2$ = constant and $V_1 = 10.3$ L, $P_1 = 300$ torr, $P_2 = 500$ torr, $V_2 = ?$

Substituting values;

$$300 \text{ torr} \times 10.3 \text{ L} = 500 \text{ torr} \times V_2$$

$$V_2 = 6.18 \text{ L}$$

Charles's Law:

Charles in 1787 investigated that gases such as hydrogen, carbon dioxide and oxygen expand to an equal amount upon heating from 0 °C to 80 °C at constant pressure. However, Gay-Lussac in 1802 showed that volume of all gases increases with each 1 °C increase in temperature and was approximately equal to 1/273.15 volume of gas at 0 °C.

Consider the change in volume of one mole of an ideal gas with the change in temperature when the pressure is held constant as shown in Fig. 2.17.

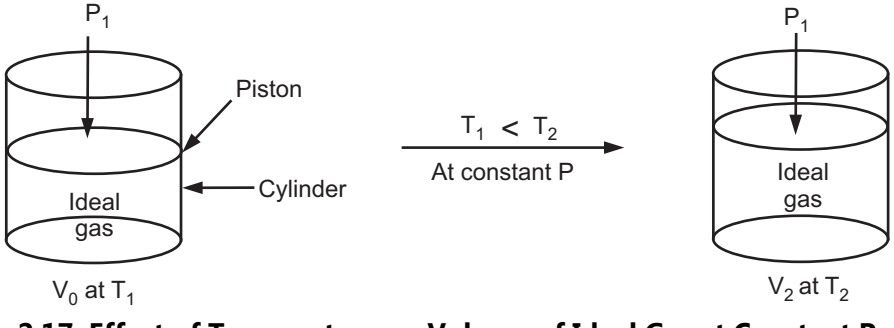


Figure 2.17: Effect of Temperature on Volume of Ideal Gas at Constant Pressure

If V_1 is the volume at any temperature t, then;

$$V_2 = V_1 + \left(\frac{t}{273.15}\right) V_1 \quad \ldots (2.11)$$

On simplifying equation (2.11);

$$V_2 = V_1\left[\frac{(273.15 + t)}{273.15}\right] \quad \ldots (2.12)$$

If, 273.15 + t, is designated as T_2 and 273.15 as T_1 then equation (2.12) becomes;

$$\frac{V_2}{V_1} = \frac{T_2}{T_1} \quad \ldots (2.13)$$

Therefore, this law is stated as the volume of definite quantity of gas at constant pressure is directly proportional to absolute temperature. It is expressed as;

$$V \propto T \quad \ldots (2.14)$$

$$V = kT \text{ (when pressure is held fixed)} \quad \ldots (2.15)$$

A plot of the volumes at various temperatures is given in Fig. 2.18.

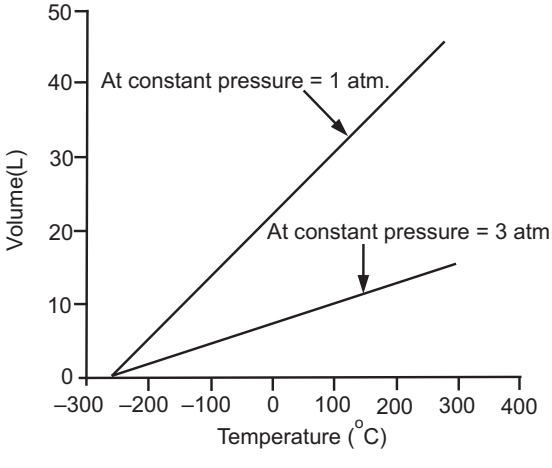


Figure 2.18: A Plot of Volume vs Temperature (°C) Ideal Gas at Constant Pressure

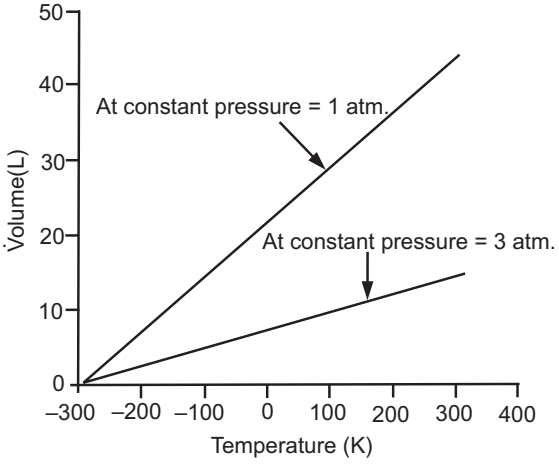


Figure 2.19: A Plot of Volume vs Temperature (K) of Ideal Gas at Constant Pressure

The volume is a linear function of temperature (°C) with V = 0 at −273.15 °C. On defining temperature in absolute or Kelvin scale as,

$$T\,(K) = (t\,°C) + 273.15 \quad \ldots (2.16)$$

Then the plot of volume versus temperature (K) yields Fig. 2.19, in which the volume is directly proportional to the absolute temperature.

Charles' law describes the behaviour of an ideal gas and approximates the behaviour of a real gas. The approximation is very poor at high pressures and low temperatures.

Example 2.2: If 10.3 g sample of a gas occupies 10.3 L at 650 torr and 400 K, what volume will be gas occupies at the same pressure and 25 °C?

Solution: Since *n* and P are held constant, and V_1 = 10.3 L, T_1 = 400 K, T_2 = 25 °C + 273 = 298 K, V_2 =?

On substituting given values;

$$\frac{V_1}{T_1} = \frac{V_2}{T_2} = \text{constant}$$

$$\frac{10.3\text{L}}{400\text{ K}} = \frac{V_2}{298\text{ K}}$$

$$V = 7.67\text{ L}$$

Avogadro's Law:

It states that at constant pressure and temperature the volume occupied by a gas is directly proportional to the number of moles of the gas. Mathematically it is expressed as;

$$V = n \times \text{constant (when P and T are held fixed)} \quad \ldots (2.17)$$

If V_1 and V_2 are volumes and n_1 and n_2 are number of moles of gas at constant temperature and pressure, then;

$$\frac{V_1}{n_1} = \frac{V_2}{n_2} \quad \ldots (2.18)$$

The 1 mole of an ideal gas at 1 atm and 0 °C (Standard Temperature and Pressure, STP) occupies 22.414 L (or dm^3).

Ideal Gas Law:

The ideal gas law relates to the volume and pressure of a gas at a constant temperature. On combining Boyle's law, Charles's and Gay-Lussac law and Avogadro's law we find that the volume of gas depends on pressure, temperature and number of moles of gas in the container.

Summary:

Boyle's Law:	$V \propto \frac{1}{P}$	(when *n* and T are held constant)
Charles's Law:	$V \propto T$	(when *n* and P are held constant)
Avogadro's Law:	$V \propto n$	(when P and T are held constant)

Therefore, volume should be proportional to the product of these three terms as;

$$V \propto \frac{1}{P} \times T \times n \quad \ldots (2.19)$$

Replacing proportionality symbol (∝) with equal to symbol (=) and adding the proportionality constant, (R), we get;

$$V = R \times \frac{1}{P} \times T \times n \quad \ldots (2.20)$$

$$\therefore \quad PV = nRT \quad \ldots (2.21)$$

where, P represents the pressure of the gas, V stands for the volume of the gas, *n* represents the number of moles of the gas, R stands for the molar gas constant which is always 0.08205 L atm/K.mol and T represents the temperature of the gas. The equation (2.21) is

known as ideal gas equation. As can be understood from the above equation, the pressure and the volume are inversely proportional. As the pressure increases the volume decreases, and as the volume increases the pressure decreases. But the volume and temperature are directly proportional. As the volume increases the temperature also increases. The ideal gas law can be very useful when one needs to find the approximate molecular weight of a gas. The *n* is replaced by g/M, which is grams of the gas divided by molecular weight.

Applications of the Ideal Gas Law:

The ideal gas law PV = *n*RT has four parameters and a constant, R. This equation can be rearranged to give an expression for each of P, V, *n* or T. For example, P = nRT/V and P = (*n*R/V) T. These equations are Boyle's law and Charles law, respectively. Similar expressions can be derived for V, *n* and T in terms of other variables. Thus, ideal gas law has many applications; however, it is important to use proper numerical value for the gas constant R as per the units we have for the parameters. Furthermore, *n*/V is number of moles per unit volume and this quantity has the same units as the concentration. The concentration is a function of pressure and temperature as given in equation below.

$$C = \frac{P}{RT} \qquad \ldots (2.22)$$

At 1 atm pressure and room temperature of 298 K the concentration of an ideal gas is 0.041 mol/L. The Avogadro's law can be further applied to correlate gas density ρ (weight per unit volume or *n*M/V) and molecular mass, M, of a gas. The following equation is easily derived from the ideal gas law:

$$PM = \frac{nM}{V} RT \qquad \ldots (2.23)$$

Thus, we have

$$PM = \rho RT \qquad \left(\because \rho = \frac{nM}{V}\right)$$

$$\rho = \frac{PM}{RT}$$

$$M = \frac{\rho RT}{P} \qquad \ldots (2.24)$$

Example 2.3: An air sample containing only nitrogen and oxygen gases has a density of 1.3390 g/L at STP. Find the weight and mole percentages of nitrogen and oxygen in the sample.

Solution: From the density (ρ), we can evaluate an average molecular weight (also called molar mass).

$$PM = \rho RT$$

$$M = 22.4 \times \rho \qquad \left(\because \frac{RT}{P} = 22.4 \text{ L/mol}\right)$$

$$= 22.4 \text{ L/mol} \times 1.3390 \text{ g/L}$$

$$= 30.01 \text{ g/mol}$$

Assume that we have 1 mol of gas, and x mol of which is nitrogen, then $(1 - x)$ is the amount of oxygen. The average molar mass is the mole weighted average, and thus,

$$28.0\ x + 32.0\ (1 - x) = 30.01$$
$$-4x = -1.99$$
$$x = 0.497 \text{ mol of } N_2, \text{ and}$$
$$1.0 - 0.497 = 0.503 \text{ mol of } O_2$$

Now, to determine weight percentages we need to find the amounts of nitrogen and oxygen in 1.0 mol (30 g) of the mixture.

$$\text{Mass of 0.497 mol nitrogen} = 0.497 \times 28.0 = 13.916 \text{ g}$$
$$\text{Mass of 0.503 mol oxygen} = 0.503 \times 32.0 = 16.096 \text{ g}$$
$$\text{Percentage of nitrogen} = 100 \times (13.916/30) = 46.38\ \%$$
$$\text{Percentage of oxygen} = 100 \times (16.096/30) = 53.65\ \%$$
$$= 100 - 46.38 = 53.62\ \%$$

2.1.9 Aerosols

Gases can be liquefied by increasing pressure, provided we work below the critical temperature. When the pressure is reduced, the molecules expand and the liquid reverts to a gas. This reversible change of state is the basic principle involved in the preparation of pharmaceutical aerosols. In such products, the drug is dissolved or suspended in a 'propellant', a material that is liquid under the pressure conditions existing inside the container and forms a gas under normal atmospheric conditions. Chlorofluorocarbons and hydro fluorocarbons have traditionally been utilized as propellants in these products because of their physicochemical properties. However, in the face of increasing environmental concerns (ozone depletion) their use is tightly regulated which has led to the increased use of other gases such as nitrogen and carbon dioxide.

2.1.10 Inhalers

The delivery of drugs by inhalation is a critical issue in obstructive airway diseases such as bronchial asthma and chronic obstructive pulmonary disease. The inhaled drugs are targeting the lungs directly and being a lower dose with a quick onset of action, and better therapeutic index. Now day's inhalers are a major component of patient's therapeutic management. Several effective molecules have been developed till date but their true effectiveness in real life can be affected and modulated substantially by the device used for inhalation. An increasing number of inhalation devices have been engineered, either for single or combined molecules. However, it was assumed since long ago that the ideal device should be:

1. Effective: such as, able to consent the inhalation of a sufficient fraction of drug with a particle size ≤ 6 μ, independently of the patient's inspiratory flow.
2. Reproducible: such as, able to always consent the inhalation of the same drug amount, also in terms of its respirable fraction.

3. Precise: such as, able to consent to know at any moment the amount (or the number of doses) of the drug remaining in the device, and whether or not the inhalation was correctly performed: thus the need for providing dry powder inhalers (DPIs) of a "dose counter" and of a "double-dosing protection counter", in order to avoid a further inhalation if the patient is unaware or not sure of having taken the previous one.
4. Stable: such as, able to protect the drug(s) contained from the effects of temperature and/or humidity changes.
5. Comfortable: such as, easy to use in different circumstances (particularly in critical conditions), and possibly containing several doses of the drug(s) for a long-term use.
6. Versatile: such as, it should consent the use of other drugs by inhalation.
7. Environmentally compatible, such as not containing chemical contaminants.
8. Affordable: such as, of acceptable cost, and possibly rechargeable.

The DPIs' family independently of wet nebulizers, pocket devices can be basically grouped in three major classes:

(a) Metered Dose Inhalers (MDIs), still largely used for single and combined molecules, and which need a propellant for the dose delivery;
(b) Dry Powder Inhalers (DPIs), which do not require any propellant, and are increasingly prescribed for single and combined molecules;
(c) Soft Mist Inhalers (SMIs), at present consisting in only one device for only one molecule (Respimat for Tiotropium bromide).

DPIs are available in wide variety of design and represent a substantial improvement in the inhalation therapy. They fit majority of the above mentioned requirements. Mainly, they eliminate the use of propellants; simplify the inhalation technique; reduce the patient's co-operation and improve the patient's compliance to treatment; favor a higher deposition of drugs within the lungs; reduce the variability of the inhaled dose; reduce the incidence of both local and systemic side effects, and finally ameliorate the consistency of the dose and then the outcomes substantially. The most advanced DPIs also fitted the most sophisticated patients' requirements in terms of minimization of the number of actions needed for preparing the actuation.

Table 2.5: Classification of DPIs based on their intrinsic resistance with pressure drop across the device

DPI	Pressure drop across the device	Examples
Low resistance DPIs	< 5 Mbar 1/2 L/min	HandyHaler, Easyhaler and Twishaler.
Medium resistance DPIs	5 - 10 Mbar 1/2 L/min	Turbohaler, Accuhaler/Diskus, Ellipta, Novolizer and Genuair.
High resistance DPIs	> 10 Mbar 1/2 L/min	Aerolizer and Breezhaler.

The performance of each DPI can be affected by the inspiratory flow generated by the patient, and the turbulence produced inside the device, which uniquely depends upon its

original technical characteristics. These factors affect the disaggregation of the powdered drug dose, diameter of the particles to inhale, the consistency and the variability of the dose. The inspiratory airflow generated by the patient represents the only active force able to produce the micro-dispersion of the powdered drug to inhale. The extent of the patient's inspiratory airflow depends on the patient's airway and lung conditions, and, partially, on the intrinsic resistive regimen of the device. During an inspiratory movement, the right balance between these two forces represents the critical factor which decides the true effectiveness of the "molecule-device". The higher the airflow the higher is the powder dispersion generating a fine particles. A high airflow leads to a higher impaction losses in the proximal airways and, consequently, to a lower dose reaching peripheral airways. Whereas, a lower airflow consents a deeper lung deposition of the powdered drug, even if a too low airflow can limit deposition by affecting powder distribution and dispersion. Changes in these two forces can be achieved only by changing the airflow characteristics or the original DPI design. When using a medium-resistance DPI, both the distribution and the micro-dispersion of the powdered drug are relatively independent of the patient's inspiratory airflow. This is because the driving force depending on the intrinsic resistance of the DPI itself and is able to produce per sè the turbulence required for an effective drug micro dispersion. In these cases, the speed of the particulate is lower, the distribution of the drug is much better within the lung, and the variability of the effective inhaled dose is quite lower, thus leading to a drug delivery which is more fitting to the corresponding original claim.

In case of a low-resistance DPI, the only driving force for the distribution and the micro dispersion of the drug to inhale is the patient's inhalation airflow rate which depends on the patient's airflow limitation and disease severity. The role of the resistance-induced turbulence is obviously negligible in these cases. Therefore, the required regimen of turbulence is achieved only by increasing the inhalation airflow. It frequently represents the main critical limitation for airway obstructive patients. Under these conditions, the variability in the dose consistency is higher and the effective inhaled dose can be far from the original claim. This also is due to the higher oropharyngeal impact of the powdered drug. In correct sense, the "low resistance DPIs" should not be mandatory associated to the concept of "the most effective DPIs" because just in these cases patients are required for a higher inspiratory performance, which frequently cannot be achieved by patients affected by a disease-induced airflow limitation.

2.1.11 Relative humidity

Relative humidity is the ratio of the partial pressure of water vapour to the equilibrium vapour pressure of water at a given temperature. Relative humidity depends on temperature and the pressure of the system of interest. It requires less water vapour to attain high relative humidity at low temperatures; more water vapour is required to attain high relative humidity in warm or hot air.

The relative humidity (RH or ϕ) of an air–water mixture is defined as the ratio of the partial pressure of water vapour (P_{H_2O}) in the mixture to the equilibrium vapour pressure of water ($p^*_{H_2O}$) over a flat surface of pure water at a given temperature:

$$RH \text{ or } \phi = \frac{P_{H_2O}}{P^*_{H_2O}} \quad \ldots (2.25)$$

Relative humidity is normally expressed as a percentage; a higher percentage means that the air–water mixture is more humid.

Vapour Concentration (Absolute Humidity)

The vapour concentration or absolute humidity of a mixture of water vapour and dry air is defined as the ratio of the mass of water vapour (M_w) to the volume (V) occupied by the mixture. $D_v = M_w / V$, expressed in grams/m^3 or in grains/cu ft. The value of D_v can be derived from the equation PV = *n* RT.

Relative humidity is the ratio of two pressures;

$$\%RH = \frac{P}{P_s} \times 100 \quad \ldots (2.26)$$

where, P is the actual partial pressure of the water vapour present in the ambient and P_s the saturation pressure of water at the temperature of the ambient. Relative humidity sensors are usually calibrated at normal room temperature (well above freezing). Consequently, it generally accepted that this type of sensor indicates relative humidity with respect to water at all temperatures (including below freezing). As already noted ice produces a lower vapour pressure than the liquid water. Therefore, when ice is present, saturation occurs at a relative humidity of less than 100 %. For instance, a humidity reading of 75 % RH at a temperature of −30 °C corresponds to saturation above ice.

Method of Calibration:

A frequent method of calibrating a relative humidity instrument is to place the humidity sensor in a closed container. By putting a known solution of water and another substance inside the container, a known humidity is established at equilibrium. This humidity value is used to provide a reference against which the instrument can be adjusted or calibrated.

Temperature stability:

Obtaining equilibrium conditions is one of the most critical requirements of the method. This means that there should be no difference of temperature between the humidity sensor, the solution and the head space above the solution. Unstable temperature during calibration will not permit this. A temperature stability of 0.02°C/min or better is required during the calibration process for the method to be accurate.

Temperature of calibration:

The relative humidity values generated by the different solutions used for the purpose of calibration are affected by temperature. Therefore, a correction must be made for the temperature of calibration. However, no correction is required for the effect of temperature on the total pressure inside the calibration container. The temperature of calibration may also be restricted by the design of the instrument. For instance, an instrument that provides a compensation for the effect of temperature on the humidity sensor does so by assuming that the temperature of calibration is always the same. In that case, the manufacturer provides a

recommendation as to the range of calibration temperature that result in the best overall accuracy for the instrument.

Significance of RH:

Climate control:

Climate control refers to the control of temperature and relative humidity in buildings, vehicles and other enclosed spaces for the purpose of providing for human comfort, health and safety, and of meeting environmental requirements of machines, sensitive materials (for example, labile pharmaceuticals) and technical processes.

Human discomfort:

Humans are sensitive to high humidity because the human body uses evaporative cooling, enabled by perspiration, as the primary mechanism to get rid of waste heat. Perspiration evaporates from the skin more slowly under humid conditions than under arid. Because humans perceive a low rate of heat transfer from the body to be equivalent to a higher air temperature, the body experiences greater distress of waste heat burden at high humidity than at lower humidity, given equal temperatures. For example, if the air temperature is 24 °C (75 °F) and the relative humidity is zero percent, then the air temperature feels like 21 °C (69 °F). If the relative humidity is 100% at the same air temperature, then it feels like 27 °C (80 °F). In other words, if the air is 24 °C (75 °F) and contains saturated water vapour, then the human body cools itself at the same rate as it would if it were 27 °C (80 °F) and at 20% relative humidity (an unstated baseline used in the heat index). The heat index and the humidex are indices that reflect the combined effect of temperature and humidity on the cooling effect of the atmosphere on the human body.

In cold climates, the outdoors temperature causes lower capacity for water vapour to flow about. Thus although it may be snowing and at high humidity relative to its temperature outdoors, once that air comes into a building and heats up, its new relative humidity is very low, making the air very dry, which can cause discomfort and can lead to ill health, although, dry air is good for those suffering from some lung disorders.

Effect on skin:

Low humidity causes tissue lining nasal passages to dry, crack and become more susceptible to penetration of Rhinovirus cold viruses. Low humidity is a common cause of nosebleeds. The use of a humidifier in homes, especially bedrooms, can help with these symptoms. Indoor relative humidities should be kept above 30% to reduce the likelihood of the occupant's nasal passages drying out. Humans can be comfortable within a wide range of humidities depending on the temperature from 30% to 70% but ideally between 50% and 60%. Very low humidity can create discomfort, respiratory problems, and aggravate allergies in some individuals. In the winter, it is advisable to maintain relative humidity at 30 percent or above. Extremely low (below 20%) relative humidities may also cause eye irritation.

Buildings:

For climate control in buildings using HVAC systems, the key is to maintain the RH at a comfortable range low enough to be comfortable but high enough to avoid problems

associated with very dry air. When the temperature is high and the relative humidity is low, evaporation of water is rapid; soil dries, wet clothes hung on a line or rack dry quickly, and perspiration readily evaporates from the skin. Wooden furniture can shrink, causing the paint that covers these surfaces to fracture. When the temperature is low and the relative humidity is high, evaporation of water is slow. When relative humidity approaches 100%, condensation can occur on surfaces, leading to problems such as mold growth, corrosion, decay, and other moisture-related deterioration. Condensation can pose a safety risk as it can promote the growth of mold and wood rot as well as possibly freezing emergency exits shut. Certain production and technical processes and treatments in factories, laboratories, hospitals, and other facilities require specific relative humidity levels to be maintained using humidifiers, dehumidifiers and associated control systems.

Water vapour is independent of air:

The notion of air "holding" water vapour or being "saturated" by it is often mentioned in connection with the concept of relative humidity. This, however, is misleading because the amount of water vapour that enters a given space at a given temperature is independent of the amount of air that is present. Indeed, a vacuum has the same equilibrium capacity to hold water vapour as the same volume filled with air; both are given by the equilibrium vapour pressure of water at the given temperature.

Pressure dependence:

The relative humidity of an air–water system is dependent not only on the temperature but also on the absolute pressure of the system of interest. This dependence is demonstrated by considering the air–water system shown in Fig. 2.20. The system is closed (i.e., no matter enters or leaves the system).

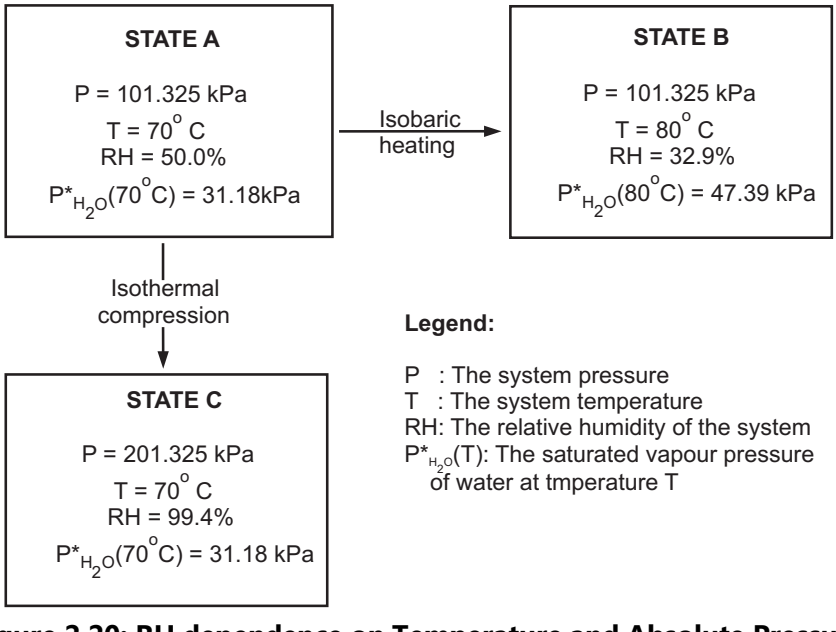


Figure 2.20: RH dependence on Temperature and Absolute Pressure

If the system at State A is isobarically heated (constant pressure) the RH of the system decreases because the equilibrium vapour pressure of water increases with increasing temperature. This is shown in State B. If the system at State A is isothermally compressed (constant temperature) the relative humidity of the system increases because the partial pressure of water in the system increases with the volume reduction. This is shown in State C. At above 202.64 kPa the RH would exceed 100% and water may begin to condense. If the pressure of State A is changed by simply adding more dry air, without changing the volume, the relative humidity would not change. Therefore, a change in relative humidity can be explained by a change in system temperature, a change in the volume of the system, or change in both of these system properties.

Enhancement factor:

The enhancement factor (f_w) is defined as the ratio of the saturated vapour pressure of water in moist air (e'_w) to the saturated vapour pressure of pure water (e^*_w).

$$f_w = e'_w / e^*_w \quad \ldots (2.27)$$

The enhancement factor is equal to unity for ideal gas systems. However, in real systems the interaction effects between gas molecules result in a small increase of the equilibrium vapour pressure of water in air relative to equilibrium vapour pressure of pure water vapour. Therefore, the enhancement factor is normally slightly greater than unity for real systems.

The enhancement factor is commonly used to correct the equilibrium vapour pressure of water vapour when empirical relationships, such as those developed by Wexler, Goff, and Gratch, are used to estimate the properties of psychrometric systems. Buck has reported that, at sea level, the vapour pressure of water in saturated moist air amounts to an increase of approximately 0.5% over the equilibrium vapour pressure of pure water.

The term relative humidity is reserved for systems of water vapour in air. The term relative saturation is used to describe the analogous property for systems consisting of a condensable phase other than water in a non-condensable phase other than air.

Measurement:

A device used to measure humidity is called a hygrometer; one used to regulate it is called a humidistat, or sometimes hygrostat. The humidity of an air–water vapour mixture is determined through the use of psychrometric charts if both the dry bulb temperature (T) and the wet bulb temperature (T_w) of the mixture are known. These quantities are readily estimated by using a sling psychrometer. There are several empirical formulas that can be used to estimate the equilibrium vapour pressure of water vapour as a function of temperature. The Antoine equation is among the least complex of these, having only three parameters (A, B, and C). Other formulas, such as the Goff-Gratch equation and the Magnus-Tetens approximation, are more complicated but yield better accuracy.

The formula presented by Buck is commonly encountered in literature:

$$e^*_w = (1.0007 + 3.46 \times 10^{-6} P) \times (6.1121) e^{(17.502T/240.97+T)} \quad \ldots (2.28)$$

where T is the dry bulb temperature expressed in °C, P is the absolute pressure expressed in millibars, and e^*_w is the equilibrium vapour pressure expressed in millibars. Buck has reported

that the maximum relative error is less than 0.20% between −20 °C and +50 °C when this particular form of the generalized formula is used to estimate the equilibrium vapour pressure of water.

2.1.12 Liquid Complexes

Liquid complexes are binary mixtures that have coexistence between two phases: solid–liquid (suspensions or solutions of macromolecules such as polymers), solid–gas (granular), liquid–gas (foams) or liquid–liquid (emulsions). They exhibit unusual mechanical responses to applied stress or strain due to the geometrical constraints that the phase coexistence imposes. The mechanical response includes transitions between solid-like and fluid-like behavior as well as fluctuations. Their mechanical properties can be attributed to characteristics such as high disorder, caging, and clustering on multiple length scales.

Complex systems are distinguished by their behaviour as determined by competing processes of self-organization (ordering) and self disorganization (disordering) creating a hierarchical adaptive structure. A notion of complexity is also used in amorphous materials exhibiting slow and non-exponential relaxation, in particular in glass-forming liquids and glasses. However in liquid complexes, complexity is not yet a quantifiable but rather a qualitative characteristic. Numerous experimental and theoretical studies and, more recently, computer simulations revealed important macro-and mesoscopic details associated with materials complexity such as dramatic slowing-down of structure changes on cooling, wide spectrum of relaxation times and stretched-exponential (KWW) relaxation kinetics and dynamic heterogeneity on microscopic length-scales. These features and the sometime observed power law correlations are often used as practical but rather qualitative criteria of complexity in materials. In the Literature, the assumed physical cause of materials complexity is the dynamic competition between aggregation of particles into preferred structures, and factors preventing crystallization. Understanding the origins of complexity and the dynamics of structure in complex materials is most important but hardest problems in condensed matter.

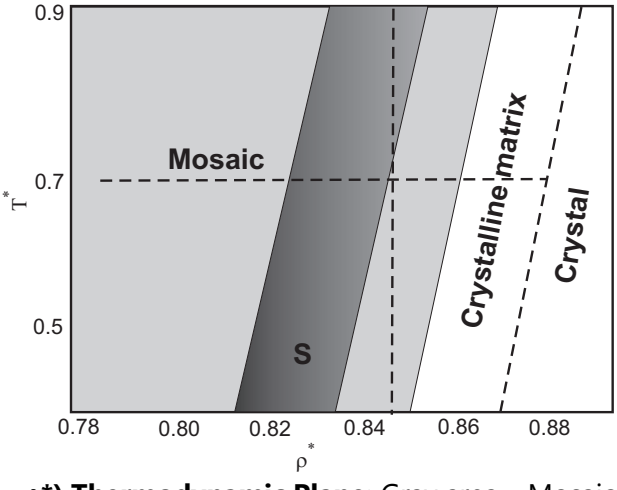


Figure 2.21: The (T* − ρ*) Thermodynamic Plane: Gray area – Mosaic states (15% − 80% of particles in crystallites), Dark grey (S) – Crystallites percolate, the stretching exponent is below the 0.65. The isotherm T* = 0.70 and isochore ρ* = 0.84

Not every liquid becomes complex on cooling. Three-dimensional (3D) liquids with simple two-particle interactions (molten metal's and salts, liquefied noble gases, Morse particles) aggressively crystallize on cooling before they show any significant signs of complexity. Classical 3D complex liquids have complicated and competing interactions and special supercooling regimes are necessary to avoid crystallization on supercooling. Two-dimensional (2D) liquids with simple interactions have a continuous or almost continuous crossover from simple liquid state to crystal. At crossover temperatures, Fig. 2.21, particles in these equilibrium liquids aggregate to form a dynamic mosaic of crystalline-ordered regions (crystallites) and less-ordered clusters. At the high-temperature end of the mosaic states, crystallites are small and separated island of order in a disordered (amorphous) matrix. Crystallites fraction of the system increases at lower temperatures where crystallinity percolates. At even lower temperatures, crystallites merge into a multiconnected crystalline matrix with expected algebraic decay of orientation order (hexatic liquid) or long range order. The mosaic is a feature observed at temperatures where the correlation length for orientations is finite and the 2D liquid is in normal (not hexatic) state.

2.1.13 Liquid Crystals

The three distinct states of matter as solid, liquid, and gas have been discussed so far. However, there is a state of matter, which does not meet the necessary requirements of any of these three categories. For example, a substance like cholesterol or mayonnaise is somewhere between a liquid and a solid. This is not quite liquid or quite solid, but is a phase of matter whose order is intermediate between that of a liquid and crystal. It is often called a mesomorphic state which is state of matter in which the degree of molecular order is intermediate between the perfect three dimensional, long-range positional and orientational order found in solid crystals and the absence of long-range order found in isotropic liquids, gases, and amorphous solids. It is also called as meso intermediate. Physically, they are observed to flow like liquids showing some properties of crystalline solids. Hence this state is considered to be the next (fourth) state of matter known as liquid crystal (LC) state. The LC state is also known as *mesophase* and can be defined as the condensed matter that exhibit intermediate thermodynamic phase between the crystalline solid and simple liquid state. LC's can be considered to be crystals, which have lost some or all of their positional order while maintaining full orientational order. They are free to move, but like to line up in about the same direction. The degree of mobility of the molecules in the LC's is less than that of a liquid.

The liquid crystals are of thermotropic and lyotropic types. The lyotropic liquid crystals are induced by the presence of solvent. Thermotropic liquid crystals are induced by a change in temperature and are essentially free of solvent. Liquid crystals are liquids featuring a

certain level of orientational order. Specifically, molecules in LCs tend to point to a certain direction, while they still have translational (positional) freedom. Although they are best known for their application in displays, liquid crystals are also an essential part of all life forms. Lyotropic liquid crystals are essential organic substances, DNA, lipids of cellular membranes and proteins are some examples of well known liquid crystals. In liquid crystals drug delivery crystalline solids exhibit short as well as long-range order with regard to both position and orientation of the molecules. Whereas liquids are amorphous in general but may show short-range order with regard to position and/or orientation. Liquid crystals show at least orientational long-range order and may show short-range order, whereas positional long range order disappears.

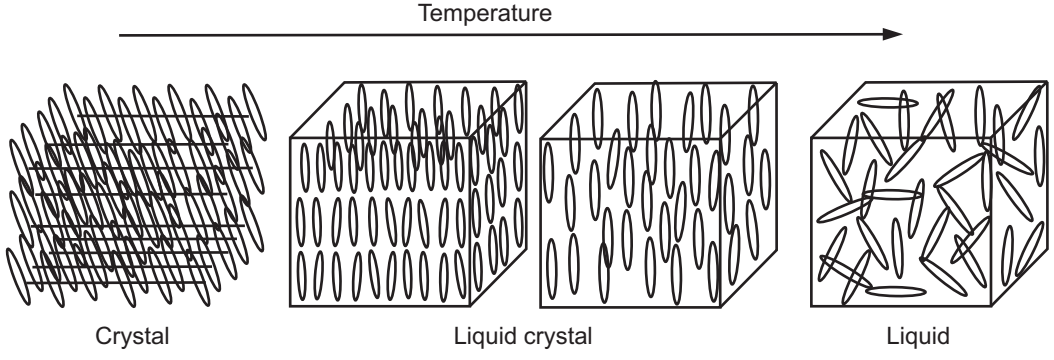


Figure 2.22: Diagrammatic Presentation Thermotropic Liquid Crystal

The LC state is widespread in nature such as lipoidal forms found in nerves, brain tissue and blood vessels. LC's may also be associated with arthrosclerosis and formation of gallstones. They are believed to have structures similar to those of cell membranes. In general most molecules that form a liquid crystalline state are organic, elongated, rectilinear, rigid, and found to have strong dipoles and easily polarizable groups. The existence of liquid crystalline state may be because of heating of solids or from the action of certain solvents on solids. Cholesterol acetate, a liquid, which exhibit optical properties, is first of its kind known LC's. Since, this state of matter possesses orientational or weak positional order; they display some physical properties of crystals but flow like liquids. When transition between the phases is temperature dependent, as shown in Fig. 2.22, they are called thermotropic and when transitions are dependent of different components these LC's are called lyotropic.

Thermotropics are mostly used in technical applications, while lytropics are important for biological systems such as membranes. Liquid crystals due to anisotropic intermolecular forces usually consist of steric rod or disc like organic molecules aligning themselves with long-range order. There are three types of liquid crystals as shown in Fig. 2.23.

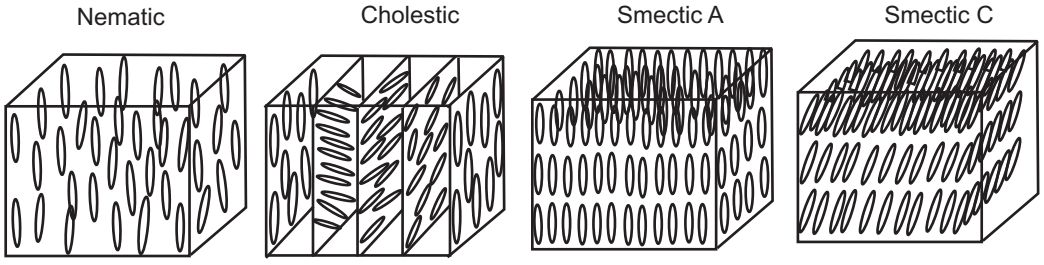


Figure 2.23: Liquid Crystalline Phases

Types of Liquid Crystals:

Nematic Crystal:

In the simple liquid crystalline state the molecules possess only orientational but no positional order are called nematic crystal phase. In the nematic phase the molecules can rotate about one axis (i.e. uniaxial) and are mobile in three directions. They are polarizable thread or rod like organic molecules on the order of 25 Å in lengths and 5 Å in height, Fig. 2.24. The order of nematic crystal is a function of temperature.

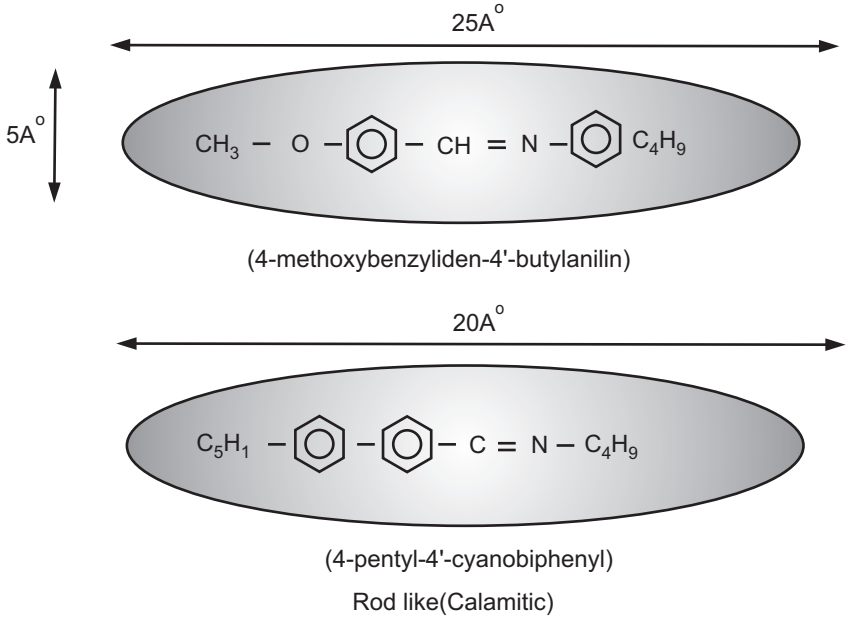


Figure 2.24: Twisted Nematic Crystal Phase with Director L

The name nematic has been given with respect to thread-like textures as observed under polarizing microscope. A unit vector called nematic director can describe the direction of considered alignment. Because of their tendency to organize themselves in a parallel fashion they demonstrate interesting and useful optical properties. As nematics are characterized by orientational order of the constituent molecules, the molecular orientation and hence the material's optical properties, can be controlled with applied electric fields.

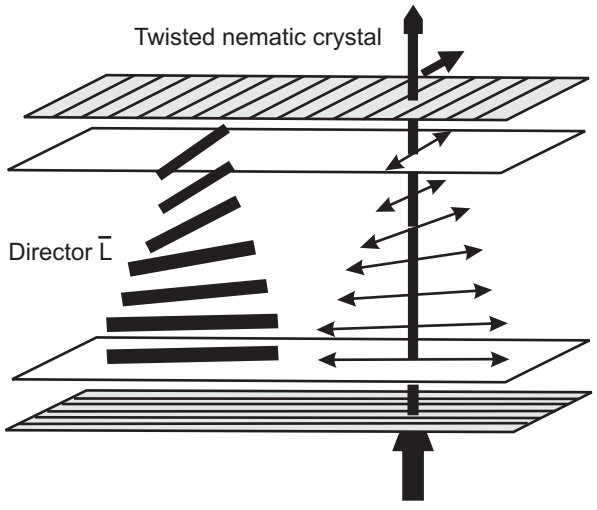
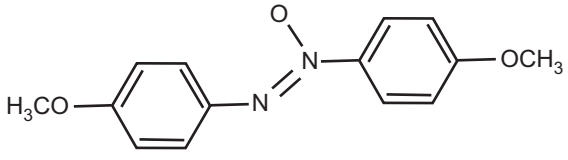


Figure 2.25: Twisted Nematic Crystal Phase with Director L

Nematics are the most commonly used phase in liquid crystal displays with many such devices using the twisted nematic geometry. The schematic presentation of twisted nematic crystal phase is shown in Fig. 2.25. Smectic liquid crystals are characterized by one more additional degree of positional order than nematics that the molecules can only rotate around one axis and mobile in only two directions, for example, *p*-ozoxyanisole Fig. 2.26. The molecules are arranged in layers and can be considered as single dimensional density waves. The molecular orientation is perpendicular to the layers, whereas the director is tilted. The molecular orientation and director in the smectic crystal show no positional order within the layers and therefore considered as two-dimensional liquids. The distance perpendicular to the layer through which the direction of alignment shifts is 360° which is order of wavelength of visible light. The smectic phases are found at lower temperatures than the nematics and form well defined layers that can slide over one another like soap. The smectic phase of chiral molecules may form a helical structure. Other smectic phases are of either weak cubic or hexagonal positional order within the layers. There are several different categories to describe smectics. The two best known of these are Smectic A, in which the molecules align perpendicular to the layer planes, and Smectic C, where the alignment of the molecules is at some arbitrary angle to the normal. The smectic phase is most pharmaceutically significant and is usually used to form ternary mixtures containing a surfactant, water, and a weakly non-polar additive.



P-Ozoxyanisole

Figure 2.26: Structure of p-Ozoxyanisole

Cholesteric crystal:

LC's when made of chiral (asymmetric) molecules that differ from their mirror image acholesteric liquid crystal e.g. cholesterol acetate, is obtained. Cholesteric can be similar to nematics, but differ in the considered orientation that it forms a helical structure with the helical axis perpendicular to the director.

Physical Properties:

Physical properties of liquid crystals are anisotropic due to orientational order. These properties are the heat of diffusion, the magnetic susceptibility, the dielectric permittivity or the optical birefringence. Liquid crystals are sensitive to electrical fields, a property that has been used in display systems. Liquid crystals are mobile and found to show flow properties of liquids like rotational viscosity acting on dynamic director deformations, respectively.

Pharmaceuticals and Cosmetic Applications of LCs:

(a) Liquid Crystal Emulsion:

A large part of cosmetic products are made in the form of emulsions, a form that allows the simultaneous use of lipophilic and hydrophilic ingredients in the required dosages. A product in the form of an emulsion also has the advantage of having the most convenient appearance and texture that also facilitates its application. They can be formulated to be liquid, milk type emulsions of variable consistency, creams, or even super liquid sprayable emulsions. It is well known fact that an emulsion is the best carrier for active ingredients and functional substances. The theory of stabilizing an emulsion through the formation of a network of liquid crystals is different than the HLB theory. The gelification of the water phase obtainable with hydrosolvatable polymers or with emulsifiers that are able to form a reticular organised structure in liquid crystal form, eliminates the need to use waxy components in large quantities and consistency factors that are no longer in harmony with the modern conception of light and easy to spread emulsions. LCs (mesophases) provides the following advantages to emulsion.

1. Stability: Emulsion stability of the multilayers around the oil droplets act as a barrier to coalescence. If oil droplets coalesce emulsion breaks. This barrier for coalescence acts as increased stability property of the emulsion

2. Prolonged hydration: Lamellar liquid crystalline and gel network contain water layer, which shows that 50% of the water of oil in water (o/w) emulsion can be bound to such structures. Such water is less prone to evaporation when applied to the skin and permits a long lasting moisturisation / hydrating effect, necessary for drug entry.

3. Controlled Drug delivery: Liquid crystals prevent the fast release of the drug dissolved in the oil phase of an emulsion. This is attributed to the lamellar liquid crystalline multilayer, which reduces the interfacial transport of a drug dissolved within the oil droplets. Microscopic observations under polarized light show the exceptional thickness of liquid crystalline lamellar layer around the oil droplets.

Function and Properties of LCs Emulsion System

LCs, when present at the oil/water interface, the liquid crystals help to give the system rigidity and, by limiting the fluctuation of the components at the interface give great stability to the emulsion. Furthermore, the liquid crystal system enhances the moisturizing ability of

the emulsion. The quantity of inter-lamellar water can be extremely high and become immediately available when the cream is applied to the skin. For these reasons these emulsions have a shinny surface, a fresh and original feel and they leave a light and pleasant sensation on the skin. In recent years, the moisturizing effect of creams and lotions has become increasingly more important and cosmetic chemists are constantly searching for better methods of retaining water in the superior layers of the skin. The evaporation of the bonding water in emulsions containing anisotropic lamellar phases is slower and permits a hydro retentive action that prolongs the moisturizing effect. The associations that are formed because of the excess water are particularly interesting; in these cases the ability of the crystalline phase to swell is strictly linked to the stability and the behaviour of the emulsion because, in a liquid crystal system, the quantity of inter-lamellar water and of hydrophile elements can amount to 70% of the total external phase.

(b) Controlled Release of Bioactive Materials:

The release of the active substance from liquid crystalline delivery systems is often controlled by diffusion, and some systems using the photo induced or thermal phase transition of the liquid crystals as the release for bioactive materials.

(c) Drug Loading:

According to the nature of the drug, it can be added in both the aqueous as well as oil phase. Loading totally depends on solubility of active constituents and their partition between existing phases. For example, cefazolin, cefuroxime, clomethiazole, clindamycin phosphate, 4-phenylbutylamine, prilocaine, oestriol, isosorbide mononitrate, insulin, indomethacin, clotrimazole, gramicidin, nitroglycerin, lidocaine hydrochloride etc.

(d) Other Applications:

Lyotropic LC's include organic substances that are essential for life. Examples of lyotropic liquid crystals include DNA, proteins, cholesterol etc. LC pharmaceuticals are a unique class of lyotropic LC's that represent novel drug candidates for the treatment of a wide range of diseases. LC's are useful in cosmetic and pharmaceutical compositions as well as methods comprising delivery systems for the controlled release and enhanced penetration of biologically active materials (for example, vitamin A) to the skin. The delivery systems comprises cholesteric liquid crystals wherein the active material is retained within the lamellar molecular structure (i.e., between the molecular sheets) of the cholesteric LC. Another example of LC is new investigational antitumor drug called Tolecine™, a compound that also has antiviral and antibacterial applications. LC's are also used in solubilization of water insoluble substances. LC's have its applications in most areas due to its remarkable features of anisotropic optical properties. As a result of strong Bragg's reflection of light cholesteric LC's have vivid iridescent colours. In some of the LC's the pitch of spiral and reflected colour changes with temperature therefore can be used to measure temperature of the skin and other surfaces. This can be useful in detecting elevated temperatures under the skin as in certain disease states.

2.1.14 Glassy State

The rapid cooling of a liquid below its melting point (Tm) leads to an amorphous state with structural characteristics of a liquid but with a much greater viscosity as shown in Fig. 2.27 (a) and (b).

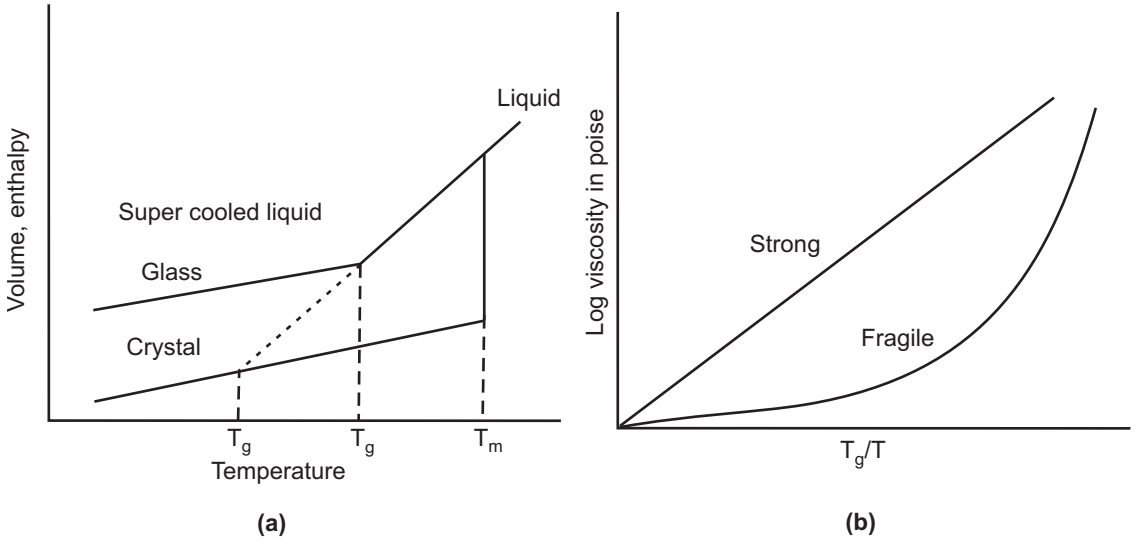


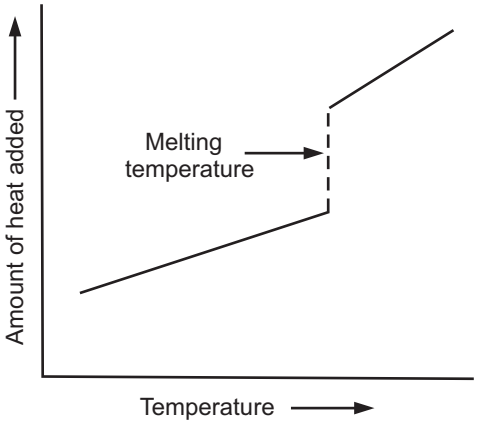
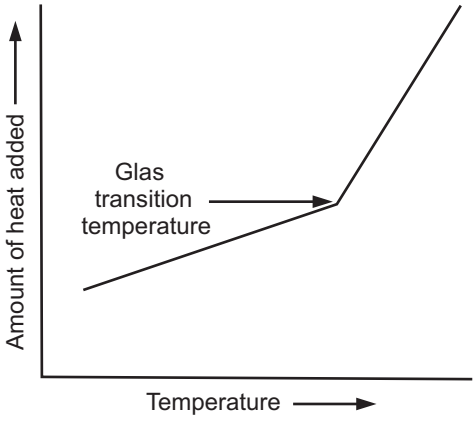
Figure 2.27: Schematic of (a) Enthalpy Change with Temperature and (b) Molecular Mobility Change as Function of Temperature above Tg of Amorphous Material

The enthalpy and volume changes immediately below Tm exhibit no discontinuity with those observed above Tm, so the amorphous state is considered to be equilibrium super cooled state. The amorphous state is also called as rubbery state because of the macroscopic properties of amorphous solids in this region. The 3-D long-range order that normally exists in a crystalline material does not exist in the amorphous state and position of molecule relative to another molecule is more random as in the liquid state. Therefore they are considered as super cooled liquids. Typically, an amorphous solid exhibit short-range order over a few molecular dimensions and has physical properties quite different than the crystalline solids.

Amorphous state can also be characterized by rate and extent of molecular motions. The molecular motions in the supercooled liquids are usually less than 100 s and viscosity is between 10^{-3} to 10^{12} Pa.s and both properties are strongly temperature dependent. Further cooling of supercooled liquid reduce molecular mobility of a liquid to a point where material is unable to attain equilibrium in time scale as it loses its thermal energy leading to change in temperature dependence of the enthalpy and volume. The temperature at which this occurs is called as glass transition temperature (Tg). Below Tg the material is kinetically frozen into thermodynamically unstable glassy state with respect to equilibrium liquid and crystalline state. At Tg physical properties like hardness, volume, and percent elongation-to-break and Young's modulus undergo change.

Melting is observed in case of crystalline solids, while the glass transition happens only to amorphous solids. A given sample may often have both amorphous and crystalline domains within it, so the same sample can show a Tm and a Tg. But the chains that melt are not the chains that undergo the glass transition. When crystalline solids are heated at a constant rate, the temperature increases at a steadily.

Table 2.6: Difference between Melting Temperature and Glass Transition temperature

Melting temperature (Tm)	Glass transition temperature (Tg)
1. It happens to crystalline material.	1. It happens to amorphous material.
2. It is first order transition reaction.	2. It is second order transition reaction.
3. When a crystalline solid melts, it absorbs a certain amount of heat, the latent heat of melting and it undergoes a change in its heat capacity.	3. When an amorphous material melts it undergo an increase in its heat capacity when it undergoes the glass transition due to change in heat capacity.
4. When plotted as given below shows following type of characteristics.	4. When plotted as given below shows following type of characteristics.
 **Figure 2.28 : Plot of 100% crystalline solid**	 **Figure 2.29 : Plot of 100% amorphous solid**
5. The transition curve is discontinuous showing break at the melting point.	5. When amorphous solid is heated, shows no break in the transition curve.
6. At this break, a lot of heat is added without any temperature increase at all (i.e. the latent heat of melting).	6. The only change at the glass transition temperature is an increase in slope that shows increase in heat capacity. A change in heat capacity at the Tg, but no break, and no latent heat involved with the glass transition.
7. Slope is steeper on the high side of the break.	7. The slope of plot is equal to the heat capacity, and increase in steepness of slope corresponds to increase in heat capacity above the melting point.

2.1.15 Solids

The state, in which a substance has no tendency to flow under stress, resists forces that tend to deform it, and remain in definite size and shape is called as solid state. In solid state the molecules are closely bound to one another. A solid hold its shape. The volume of solid is fixed by the shape of solid. There are two types of solids namely; crystalline solids and amorphous solids. They differ from one another by the way their particles are arranged and their melting points.

2.1.15.1 *Crystalline Solids*

Atoms, molecules or ions are the units that constitute crystalline system. The structural units of crystalline solids such as ice, menthol or sodium chloride are arranged in a fixed geometric pattern or lattices. Crystalline solids have definite shape and its units have an orderly arrangement as well as they are practically in compressible. Crystalline solid have definite melting points and so they pass sharply from solid to liquid state. The binding force between the crystals is electrostatic attraction of the oppositely charged ions. In case of organic compounds hydrogen bonding and van der Waals forces are responsible for holding the molecules in crystals whereas in graphite and diamond, the carbon molecules are covalently bond together. Depending upon the nature of units which occupy the lattice points, crystals are classified as follows.

Types of Crystals

Molecular Crystal:

Molecular crystal consists of specific molecules, which do not carry charge. Dipole-dipole and van der Waal's forces hold the molecules of molecular crystal. It has less binding energy due to low heat of vaporization which is energy required to separate the molecules form one another. Also it has low heat of fusion, which is heat required to increase the interatomic and intermolecular distances in crystals. The increase in distances between atoms and molecules allows melting to occur. These types of crystals are bound by weak forces and therefore, generally have low melting and boiling points and are volatile in nature. They are soft and easily compressible as well as can easily distort. As no charge is present in them they are bad conductors of electricity in solid as well as in the liquid state.

Covalent Crystal:

The lattice of covalent crystal consists of atoms joined together by covalent bonds. The examples of these crystals are diamond, graphite, silicone and most organic crystals. The bond strength and mutual orientation are the most typical atomic features of covalent materials. Iodine is also a covalent crystal because it has 10 times higher lattice energy.

Metallic Crystal:

Metallic crystals consist of positively charged ion in the field of free flowing electrons. The force that binds metal ions (kelmel) to a number of electrons within its sphere of

influence is nothing but the net metallic bond. The force of attraction between metallic crystals is very strong and therefore they are compact and solid in nature. Major characteristics of these crystals are that they are good conductors of heat and electricity, hard and tough, malleable and ductile, exhibit luster when freshly cut, have high melting and boiling points with exception of alkali metals, possess elasticity and have high tensile strength.

Ionic Crystal:

The unit of ionic crystals consists of positive and negative ions, for example, Na^+Cl^-. Coulombic forces of attraction between all ions of opposite charge hold the Na^+ and Cl^- ions. These forces are strong and therefore require high-energy input to separate them from one another. They have high heats of vaporization, low vapour pressure, high melting and boiling points and are hard and brittle. They are insulators in solid state and good conductors of electricity when dissolved in water. They dissolve in all polar solvents.

Characteristics of Crystals

Crystal Lattice:

The particles in the crystals are highly organized such that their arrangement extends in all direction. This ordered arrangement is termed as crystal lattice, space lattice or just lattice. Actually crystals are collection of large number of unit cells. The unit cell may be atom, ion or molecule. Inorganic substances have ionic lattice. The crystal lattice of substance is represented by position of structural unit cell in space as shown in Fig. 2.30.

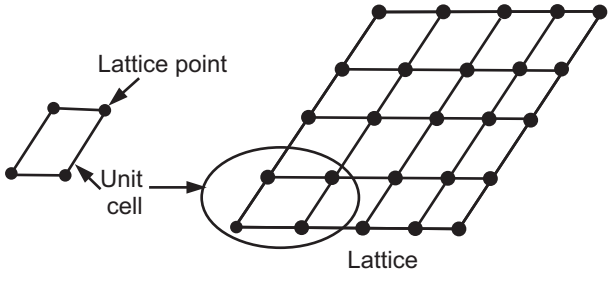


Figure 2.30: 2-D Depiction of Crystal Unit Cell and Lattice

The positions shown by bold dots are termed as lattice points. The unit cell determines overall shape and structure of crystal system. A unit cell has one atom, ion or molecule at each corner of the lattice but they may present at the faces and inside the unit called as body crystal units. A crystal, which does not contain any unit in the interior, is called primitive cell. It means in primitive cell atoms or ions are present only at the corner of the cell.

Crystal habit and interfacial angle:

Crystal habit is nothing but an external shape or morphology of the crystal. Each plane surface of the crystal is called its face. Angle between the faces of crystal is referred as interfacial angle. Every crystalline substance has a constant interfacial angle, which is its

characteristic. Crystal habit of same substance depends on the rate of development of its various faces. It may vary with change in conditions during the growth of crystals. An interfacial angle despite of differences in their habits helps to identify the crystal. Presence of impurities affects the growth rate of crystal faces, which may give rise to many faces. For example, cubic crystals are formed when sodium chloride is crystallized from supersaturated solution but octahedral habit is formed if urea is added as an impurity.

Table 2.7: Various Crystal Units: Examples with Relative Axial Lengths and Angles

Crystal units	Example	Relative axial lengths	Angles	Minimum elements of symmetry
Cubic	Sodium chloride, Calcium oxide, Cesium chloride, Potassium chloride, Zinc sulphide, Diamond etc.	a = b = c	$\alpha = \beta = g = 90°$	9 planes of symmetry 13 axes of symmetry
Tetragonal	Titanium oxide, Urea, Tin etc.	a = b ± c	$\alpha = \beta = \gamma = 90°$	5 planes of symmetry 5 axes of symmetry
Orthorhombic	Potassium sulfate, Potassium nitrate, Barium sulfate, Calcium carbonate, Iodine (I_2) etc.	a ± b ± c	$\alpha = \beta = \gamma = 90°$	3 planes of symmetry 3 axes of symmetry
Trigonal	Quartz, Sodium nitrate, Calcite, Calamine etc.	a = b = c	$\alpha = \beta = \gamma \pm 90°$	7 planes of symmetry 7 axes of symmetry
Hexagonal	Silver iodide, Mercuric sulphide, Ice, Graphite, Iodoform (I) etc.	a = b ± c	$\alpha = \beta = 90°$, $\gamma = 120°$	7 planes of symmetry 7 axes of symmetry
Monoclinic	Calcium sulfate dehydrate, Potassium chlorate, Potassium ferric cyanide, Sucrose etc.	a ± b ± c	$\alpha = \beta = 90°$, $\gamma \pm 90°$	1 plane of symmetry 1 axis of symmetry
Triclinic	Copper sulfate pentahydide, Potassium dichromate, boric acid etc.	a ± b ± c	$\alpha \pm \beta + \gamma + 90°$	1 plane of symmetry 0 axes of symmetry

Crystal Structure:

Scientist Bavis classified the crystal lattices into seven types. These are characterized by parameters like relative lengths of edges along the three axes and three angles between the edges as shown in Fig. 2.31.

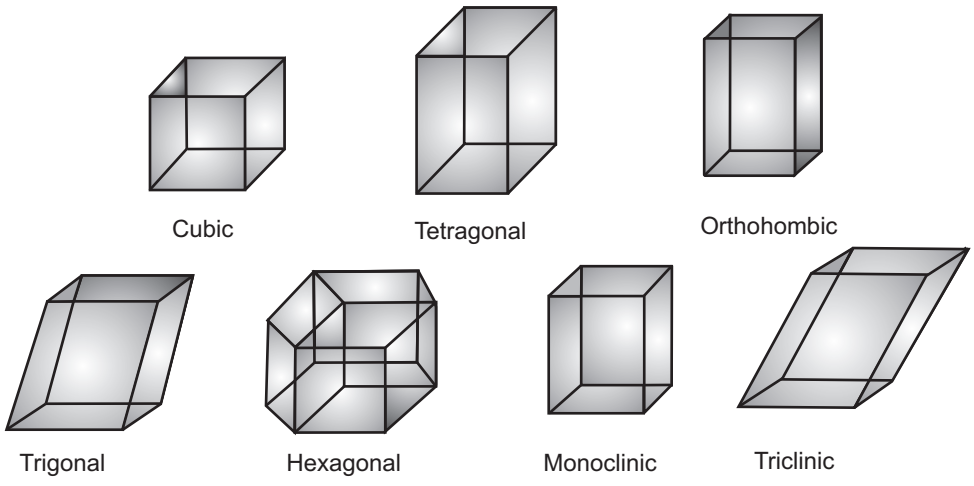


Figure 2.31: Seven Shapes of Crystal as Proposed by Bavis

They are cubic, tetragonal, rhombic (orthorhombic), monoclinic, triclinic, trigonal (rhombohedral) and hexagonal. Various crystal units with their examples, relative axial lengths, and angles are listed in Table 2.7.

Anisotropy:

Anisotropy is defined as directional differences in the properties of the substances. Crystalline substances show property anisotropy. The magnitude of physical properties such as coefficient of thermal expansion and velocity of light (double refraction) of crystalline solids varies with direction in which it is measured.

Crystal Symmetry:

Symmetry is another important property of crystalline substances in addition to interfacial angle. Three types of symmetry namely; plane, axes and centre are associated with the crystals and are termed as *elements of symmetry*. Elements of symmetry in cubic crystal are shown in Fig. 2.32.

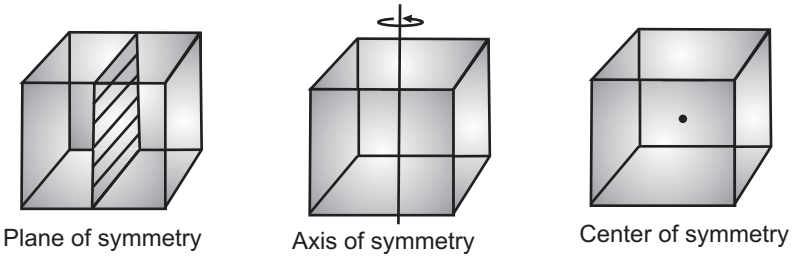


Figure 2.32: Elements of Symmetry Observed in Cubic Crystal

Plane of Symmetry:

A plane of symmetry is one, which divides the crystal into two identical, equal or mirror image halves by an imaginary plane.

Axis of Symmetry:

Axis of symmetry is an imaginary line about which the crystal may be rotated in such a way that it presents exactly the same appearance more than once in the due course of its rotation through 360°. If axis of symmetry appears at 180° it will appear twice in one rotation, called as two-fold symmetry. Similarly, if it appears at 120°, 90°, 60°... of rotations, it will repeat for 3, 4, 6 ... times respectively, termed as 3-fold, 4-fold, 6-fold symmetry and so on. The 5-fold symmetry do not exists.

Center or Point of Symmetry:

It is a point at centre of the crystal through which a line drawn meets at the opposite parallel surfaces of the crystal at equal distances on either side. A crystal may have number of planes of symmetry or axes of symmetry but it can have only one centre of symmetry. All axes of symmetry must pass through centre of symmetry. All crystal may not have centre of symmetry and so the axes of symmetry.

Miller Indices:

Crystal units in its lattice are arranged in parallel planes therefore each crystal plane lies parallel to crystal face. These planes cut the three axes along the crystallographic axes. If the intercept of the unit plane ABC is a, b and c then any other plane say LMN in the crystal will intercept at *l*a, *m*b and *n*c, respectively, is known as law of rational indices. The reciprocal of these intercepts are simple integers like 1, 2, 3 etc. The number *l, m* and *n* are called Miller indices.

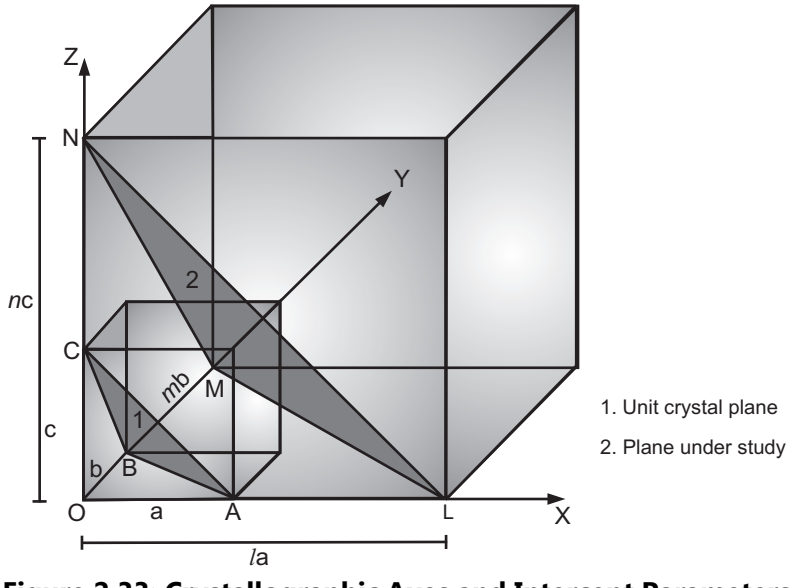


Figure 2.33: Crystallographic Axes and Intercept Parameters

Miller indices of a plane may be defined as the reciprocals of the intercepts, which the plane makes with the axes. For illustration let us consider Fig. 2.33, which represents the axes OX, OY and OZ for crystal planes ABC and LMN. The intercepts of unit plane ABC have lengths a, b and c respectively. The plane LMN have lengths *l*, *m* and *n* and are expressed in multiples

of a, b and c as *l*a, *m*b and *n*c, respectively. The terms *l*, *m* and *n* are either integral whole numbers or fractions of whole numbers. The reciprocals of these numbers are written together in bracket to give Miller indices of the plane under study. For example, a lattice plane when intercepts along axes at 2a, 4b, and 2c, where unit cell intercepts are namely a, b and c while intercepts of given plane are 2a, 4b and 2c. The lengths of intercepts in terms of unit cell intercept are 2, 4 and 2 and their reciprocals are ½, ¼ and ½, respectively. Whole numbers of these fractions can be obtained by multiplying each fraction by 4. Hence, the Miller indices of the given plane are (2, 1, 2).

Types of Cubic Unit Cells:

Total elements of symmetry for a regular cube are obtained as follows:

Planes of symmetry = 3 + 6 = 9 elements

Axes of symmetry = 3 + 4 + 6 = 13 elements

Centre of symmetry = 1 element

Thus, total number of elements of symmetry in cubic crystal is 9 + 13 + 1 = 23.

The three types in which the cubic unit cell can be arranged are namely; primitive or simple cube, body-centered cube and face-centered cube as shown in Fig. 2.34 below.

Primitive or Simple Cubic Unit Cell:

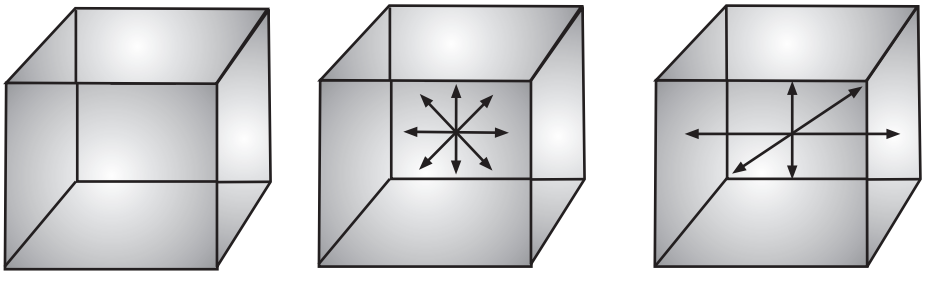


Figure 2.34: Types of Cubic Unit Cell

The simple cubic unit cell has one atom or ion called unit at each corner of the cube. As cube has 8 corners therefore, total 8 units are there in each unit cubic cell.

Body-centered cube unit cell:

Similar to the simple cube unit cell, body-centered cubic unit cell also has eight units at each corner. In addition to these 8 units it has one extra unit present at the centre of the cube. So it has total 9 units in a unit cell.

Face-centered cube unit cell:

The face-centered cubic unit cell contains one unit at its each face. Cube has in all six faces and therefore it has 6 face-centered units along with one unit at every corner of the cube. So, total 14 units are present in face-centered cubic unit cell. In addition to types discussed above there may be some cases in which units present at corners or faces of the cubic cell are shared with the adjacent cubic cells. Hence total numbers of units in such cells

are required to be calculated. For example, if one corner unit is shared with adjacent unit cells, then total numbers of units in such cases are calculated as follows.

1. Consider the case of sharing one corner unit by eight other units of other cube cells, as shown in Fig. 2.35. It means that the simple unit cell has the equivalent of one atom (i.e.1/8^{th} of atom) and therefore at 8 corners there will be (1/8) × 8 = 1 atom.
2. Every face-centered atom is shared by two unit cells. The face-centered cubic unit cell therefore contains the equivalent of four atoms: as at 8 corners the equivalent is one atom, and of 6 face-centered positions of each atom equal to 3 atoms. Therefore, total equivalents atoms are sum of equivalent atoms of 8 corners and the 6 faces; in this case it becomes 1 + 3 = 4 atoms.
3. The body centered cubic unit cell contains a central unshared atom and in addition it has one atom equivalent of 1/8, each. Therefore, body centered unit cell contain the equivalent of two atoms.

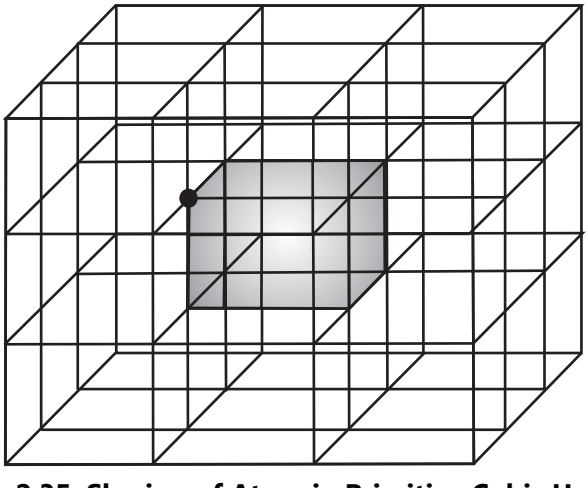


Figure 2.35: Sharing of Atom in Primitive Cubic Unit Cell

Co-ordination Number of Crystal:

Crystal structure is characterized by coordination number which is number of atoms, ions or molecules that are adjacent to every other atom, ion or molecule in the crystal.

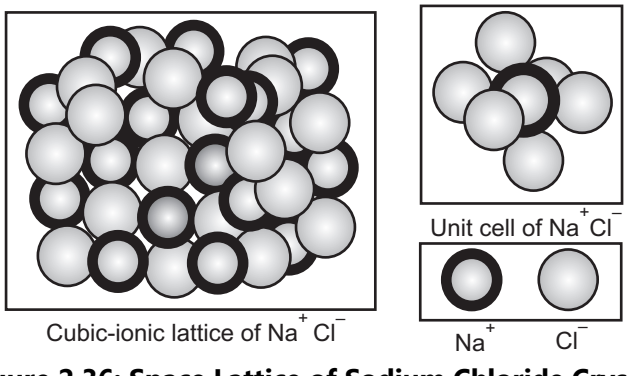


Figure 2.36: Space Lattice of Sodium Chloride Crystal

Consider an example of sodium chloride crystal as shown in Fig. 2.36. The structural units of sodium chloride are arranged in fixed geometric pattern. The binding force of the crystal is the electrostatic attraction of the oppositely charged ions. Sodium chloride crystal is cubic-ionic type consisting face centered cubic lattice of Na^+ ions linked with similar lattice of Cl^- ions. The unit cell repeats itself in three dimensions within the entire crystal. The gray circles represent Cl^- ions where as pink circles represents Na^+ ions. Sodium chloride exists in ion lattice as Na^+ Cl^- and as such no molecule of NaCl exists. A unit cell of sodium chloride consists of 13 Na^+ ions and 14 Cl^- ions. Each Na^+ ion is surrounded by 6 Cl^- ions and similarly, each Cl^- ion is surrounded by 6 Na^+ ions and therefore co-ordination number is 6. Co-ordination numbers of body centered and face centered cubic lattice are 8 and 12, respectively.

Crystal Defects:

Up till now we studied about ideal or perfect crystals, which have specific types of unit cells that contain same lattice point uniformly distributed throughout the entire crystal system. A defect in ideal crystal is another area that has significant importance as they affect the physical and chemical properties of crystalline solids. Most of the crystals prepared in the laboratory or found in nature are called real crystals that contain defects while forming its lattice. These crystal defects are also called as crystal imperfections and are defined as any variation in the ideally perfect crystal from its regular and specific arrangement of its units. On the basis of improper alignment of atoms, ions or molecules, crystal defects are classified into two basic types namely; point defects and line defects while one more type called impurity defect also found in some crystals.

Point Defects:

Point defect in the crystal is a condition in which unit cell contain an extra unit or miss any unit or even have dislocated unit. This type of defect is observed either due to improper packing or higher thermal energies of the atoms of the crystal lattice. Point defects are net result of creation of empty spaces within the crystal that may lower crystal density, lattice energy and cause partial or complete collapse leading to decreased stability. There are four types of point defects.

Schottky defect:

When any of the unit of the crystal is removed from the crystal, which leaves the point unoccupied, the defect is called *Schottky* defect or *vacancy* defect. The unoccupied points are called as *lattice vacancies*. This defect is most commonly observed with ionic crystals that have positive and negative ions of equivalent size. The common examples of this type of defect are sodium chloride and caesium chloride. Schottky defect observed in sodium chloride is shown in Fig. 2.37, which indicates that one atom each of Na^+ and Cl^- is missing in the lattice resulting into neutral crystal. On the contrary, they carry electric current to some extent by ionic mechanism; it means it acts typically as semiconductor.

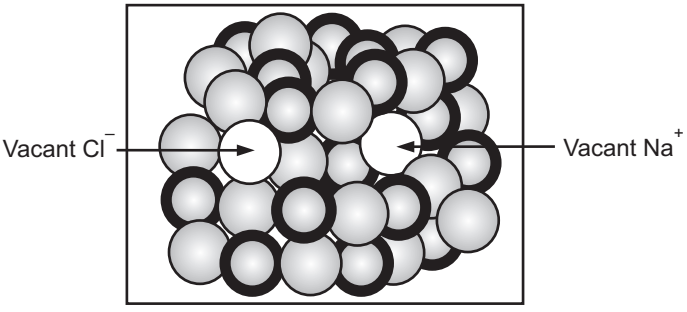


Figure 2.37: Vacancy Defect in Sodium Chloride Crystal

Frenkel defect:

Frenkel defect is also called as interstitial defect. It is observed when an ion leaves its original position to occupy an interstitial space between the lattice points. Frenkel defects are found in crystal that contains larger negative ions and smaller positive ions. This defect imparts electrical conduction property to some extent to the crystal in which it appears. The common example of Frenkel defect is $Ag^+ Br^-$.

Metal deficiency defect:

Metal deficiency defect is generally observed in the transition state metallic crystals like FeO and FeS, when one of the positive ions is missing from its lattice position. The extra negative charge is balanced by other metal ion making crystal neutral, so the system has two charges.

Metal excess defect:

Metal excess defect is related with the addition of extra metal ion when exposed to same metal vapour. It results in to formation of non-stoichometric compound. For example, when sodium chloride crystal is exposed to vapours of sodium metal, Na^+ ion gets doped into sodium chloride crystal imparting yellow colour due to slight excess of Na^+ ions. Another example of this defect is formation of magenta colored non-stoichometric compound of potassium chloride when exposed to vapours of potassium metal. Colour formation is due to excess of K^+ ions. The vacancy positions are filled by electron generated in the ionization of extra K^+ metal ion.

Line Defects:

When specific arrangement of crystals in lattice is distorted along the certain axis or direction the resulting condition of crystal is referred to as the line defect. Dislocation is the common type of line defect responsible for easy deformation of the crystal. The two types of line defects are edge dislocation and screw dislocation. Edge dislocation is net result of introduction of an extra row or column of atoms in the units of crystal where as when crystal lattice is dislocated in such a way that it cut from one end and job is created at other end resulting the deformation look like a spiral ramp called a screw distortion. This defect is useful for easy crystal growth in the process of crystallization.

Impurity Defects:

Impurity defect appear in perfect crystal lattice by incorporation of external atom or ion as an impurity near the edge located area causing the atoms of crystal to push together above the edge and pull apart below the edge. Impurity may be incorporated as substitution or trapped in vacant sites. Larger atoms of impurity concentrate below the edge while smaller atoms above the edge. Metal alloy, a mixture of two or more metals, is considered as one metal (as impurity) in other metal. Binding force in the metal alloy is very strong and hence for permanent deformation it requires more shearing force.

X-Ray Diffraction:

The temperature at which a solid state changes to the liquid state is known as the *melting point* where as the temperature at which liquid state changes to solid state is known as *freezing point* and is identical to the melting point. Both these temperatures are considered to be same when the solid and liquid exist in equilibrium at an external pressure of 1 atmosphere. The heat absorbed when a gram of a solid melts or the heat liberated when it freezes is called as the latent heat of fusion. The heat of fusion in crystals permits melting to occur. A crystal that is bound together by weak forces generally has a low heat of fusion and a low melting point, whereas the one that is bound by strong atomic forces has high heat of fusion and a high melting point.

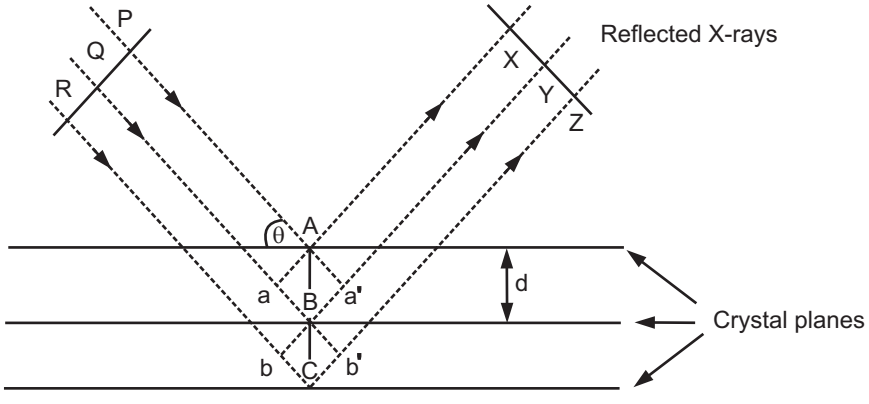


Figure 2.38: X-Ray Reflections from Different Planes of Crystal Lattice

Perfect crystal lattice is made-up of regular stack of planes or layers of atoms separated by equal distances. The spacing of lattice planes in crystal is of order of the wavelength of X-rays. Therefore, X-rays can be used to determine the spacing (inter-atomic distances) between the planes. In crystal, electrons scatter X-rays and reflected monochromatic radiations that occur at certain angles are determined by wavelength of X-rays and distances between adjacent planes. The relationship between these variables is called as Bragg's equation. Considering Fig. 2.38 may derive this equation.

The horizontal lines represent different planes in the stack of crystal lattice separated by distance d. The plane PQR is perpendicular to the incident beam of parallel monochromatic X-ray, and the plane XYZ is perpendicular to the reflected rays of the beam. As the angle of

incidence θ is changed a reflection is obtained only when the waves are in plane at XYZ. It means the difference in distance between PQR and XYZ. The reflection is measured along rays reflected from the different planes as whole number multiple of wavelength. This occurs when;

$$aB + Ba' = n\lambda \quad \ldots (2.29)$$

Since, $$\sin\theta = \frac{aB}{d}$$

$$2d \sin\theta = n\lambda \quad \ldots (2.30)$$

The equation (2.30) is known as Bragg's equation, where, θ is referred as angle of reflection, while n is order of Bragg's reflection. Bragg's diffractometer is used for these determinations. When the reflection corresponding to n = 1, it is called first order of reflection and when n = 2, called as second order reflection and so on.

2.1.15.2 *Amorphous Solids*

A state of substance that consists of disordered arrangement of molecules or that do not posses distinguishable crystal lattice, but just strewn in any old fashion is called as amorphous state. Amorphous substances do not have characteristic melting points but they soften over wide temperature range, generally, lower than melting point of crystalline forms of same compounds. The common examples of amorphous solids are glass and plastics. Amorphous character is also common with polymeric molecules used as excipient and large peptides and proteins used as therapeutics agents. In addition, it also occurs with small organic and inorganic molecules.

For most substances, the amorphous form is unstable, returning to more stable crystalline form in a few minutes or hours. In pharmaceutical viewpoint, the beauty of amorphous forms is that they have a higher dissolution rates and solubilities than the crystalline forms. The reason behind this is the energy required for molecule of a drug to escape from a crystal form is much greater than required for amorphous form. However, very few drugs are naturally amorphous.

The examples of amorphous drugs are accupril/accuretic used to treat high blood pressure and intraconazole used as an acne medication. When drug should not dissolve in water it should not be then in amorphous form. Solubility and oral bioavailability of a poorly water soluble drug can be improved by different techniques so that it can exist in an amorphous state in the product even after storing at a stressed condition. There are four main ways by which amorphous character is induced in a solid. These are condensation from vapour state, supercooling of melt, mechanical activation of crystalline mass (during milling) and rapid precipitation from solution during freeze-drying or spray drying. Solid dispersions of drug in polymers are widely used to obtain the amorphous state of materials. However amorphous state is unstable and may create possibility that during processing or storage the amorphous state may spontaneously convert back to the crystalline state. An estimation method for the physical stability of amorphous drug and a clarification of the effect of

polymer on crystallization of amorphous drug in solid dispersion are primarily required. The difference between amorphous and crystalline solids is important in synthetic procedure of drug design.

Characterization of Amorphous Solids:

The molecular motions are depends upon temperature that determines important physical properties of amorphous materials such as location of glass transition temperature and ease of glass formation. Depending upon the magnitude and activation energy for molecular motions near and above Tg in supercooled liquids amorphous solids are classified as strong or fragile. Effect of pressure on amorphous materials are significant with respect to molecular packing modifying glass transition temperature, thermal expansion behaviour and the strength or fragility of supercooled liquids. As pharmaceutical solids rarely exist in pure crystalline or amorphous form, the coexistence of two thermodynamically different state of material probably results in significant and measurable structural heterogeneities and batch-to-batch variations in physical properties. The presence of crystalline form in amorphous form has found to alter the Tg of amorphous form. Upon passing into the supercooled state or through the glass to rubber transition it is possible to observe changes in common physical properties of material including density, viscosity, heat capacity, X-ray diffraction and diffusion behaviour.

The techniques, which measure these properties directly or indirectly, can be used to detect presence or absence amorphous material. Some of them are used to quantify amount of molecular order or disorder in a system.

X-ray Diffraction Techniques:

As there is no long-range three-dimensional molecular order associated with the amorphous state, the X-ray diffraction of electromagnetic radiation is irregular compared to crystalline state as shown in Fig. 2.39.

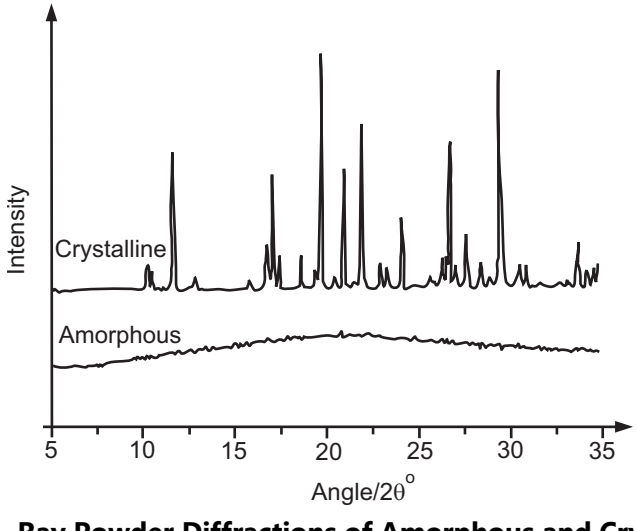


Figure 2.39: X- Ray Powder Diffractions of Amorphous and Crystalline Solids

Diffraction techniques such as small angle and wide-angle diffraction are most definitive methods of detecting and quantifying molecular orders. Conventional X-ray diffractions are used to quantify non-crystalline material down to level of 5%.

Gas and Liquid Displacement Methods:

Accurate measurements of density or volume of amorphous state substances are difficult to measure because this state consists of irregularly arranged molecules that are spaced apart resulting into greater volume and the less density. Gas displacement pycnometry is used for quantifying amorphous content in the given sample. Liquid displacement method has also been used to determine amorphous nature of the several samples. Precise dilatometry techniques are also used but being time consuming and difficult to perform are not used for routine determinations.

Viscosity:

Viscosity being the most characteristic mechanical property of amorphous solids is used to characterize amorphous state. Methods used are quite specialized and include bending of rods and curved fibers below Tg and the torsion pendulum and falling sphere methods above T_g. Diffusion controlled processes such as gas transport, self diffusion and some chemical reactions which are closely related to the viscosity of amorphous matrix used to determine amorphous state.

Spectroscopy Techniques:

Use of molecular probe, such fluorescent or phosphorescent, for determination of properties of amorphous material is quite common. Spectroscopic techniques like NMR, Raman, IR and electron spin resonance (ESR) are used because of their high structural resolution.

Thermal Methods:

A thermal analytical method such as differential scanning calorimeter (DSC) is used to determine Tg of amorphous materials. Thermal analytical methods determine fundamental thermodynamic properties such as heat capacity and enthalpy changes. Samples ranging from simple powders or solutions to entire dosage forms can be studied using these non-destructive techniques. Dielectric relaxation and dynamic mechanical spectroscopy has also been used to study amorphous materials. Thermomechanical analysis (TMA) method is used to determine relaxation times of the molecules of amorphous materials.

Water Sorption Techniques:

Typically, crystalline materials adsorb water vapours in small quantities at their surfaces or take up large quantities to form solvates. In contrast, amorphous materials absorb vapours include vacuum microbalance and desiccator/saturated salt solution gravimetric methods.

Other Methods:

Other methods includes volumetric and potentiometric methods to determine amorphous nature of the substance. Microcalorimeter can be used to detect Tg events and secondary transitions in amorphous pharmaceutical solids. Isothermal solution calorimetry

has been successfully used to identify and quantify the degree of crystallinity. This method has advantage of great thermal sensitivity, which can be very useful for studying weak secondary transitions in amorphous solids. From the methods described above it clarifies that there are many precise and accurate methods suitable for studying and characterizing amorphous pharmaceutical materials in all their configurations including final forms are available. The major difference in these methods is the ability to quantify the amount of order and disorder in partially amorphous systems.

Pharmaceutical Significance:

Processes such as milling, lyophilisation, granulating, drying etc., may introduce certain level of amorphous characteristic structure to highly crystalline materials. The amorphous state may also be deliberately introduced to enhance the biopharmaceutical properties of the products. For example, for a crystalline drug with poor aqueous solubility the formation of co-amorphous mixture with a water-soluble additive can provide an opportunity to enhance dissolution and bioavailability. Some excipients are fully or partially amorphous where as some are purposefully made amorphous to enhance functionality. Small amounts of water absorbed can plasticize amorphous solids. Relative humidity is important factor influencing the solid-state properties of amorphous systems. Transport properties of amorphous pharmaceutical materials are important and can be used to control drug release in modified release dosage forms such as transdermal patches. While studying solid-state properties of amorphous pharmaceuticals; crystallization, chemical degradation and mechanical responses are three important areas that need serious consideration.

Crystallization of amorphous solids:

Since molecules in amorphous state are thermodynamically metastable relative to crystalline state, the potential for crystallization during handling and storage is always present. Such changes are responsible for phenomenon like post compression, hardening of tablets, lyophilized cake collapse and particle aggregation in dry powders.

Chemical degradation:

Chemical degradation of drugs in solid state, particularly at elevated temperatures and relative humidities is a common incident with drugs exhibiting susceptibility for degradation when in solution. In amorphous state the reacting molecules have sufficient free volume and molecular mobility to react. A comparison of reaction rates of amorphous and crystalline forms under identical conditions shows that greater rates are with amorphous forms. But with respect to positional specificity, crystalline solids are very susceptible for degradation. Insulin, for example, some pathways require amorphous state while positional degradation requires crystalline state.

Mechanical properties:

In the processing and handling of solid pharmaceuticals there are number of situations where rheological or mechanical properties are very critical for product manufacturing, stability and performance. Typically most crystalline materials tend to exhibits high level of elasticity and tend to exhibit varying degree of viscoelasticity, depending on their

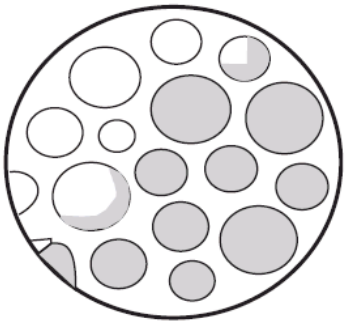
temperature relative to Tg. Such viscoelastic behaviour provides solids with ability to flow under conditions of mechanical stress and to provide number of important excipient functions. Relief of mechanical stresses through flow would appear to be important in creating tablet bonds after compression of powders and preventing mechanical failure of polymeric film coats on tablets as result of stress relaxation.

The amorphous state is critical in determining the behaviour and properties of many pharmaceutical formulations. In cases where amorphous character is desired in a pharmaceutical formulation, it may be stabilized using strategies based on understanding of the thermodynamic and kinetic properties of amorphous systems. When amorphous character is undesirable, available approaches can be used to minimize disorder and prevent conversion of amorphous material to most stable crystalline state.

The Crystalline and Amorphous State:

The amorphous solids are formed in the process of crystallization. An antibiotic, chloramphenicol, exists in three crystalline forms and an amorphous form while novobiocin exist in amorphous form. The former antibiotic is inactive in crystalline form whereas latter shows rapid absorption in GIT with good therapeutic response. With respect to stability, crystalline forms have better stability over amorphous forms. The crystalline form of penicillin G sodium or potassium salt is more stable than its corresponding amorphous form. The crystal lattice of drug has to be disrupted by solvent before the drug can dissolve. In crystal form molecules are hold tightly therefore driving force for the drug to dissolve is low. Hence crystalline forms have lower intrinsic solubilities compared to amorphous forms. The crystalline and amorphous solids are differentiated for various properties in Table 2.8.

Table 2.8: Difference between Crystalline and Amorphous Solids

Crystalline Solids	Amorphous Solids
1. Crystalline solids are arranged in neat and orderly fashion as fixed 3D crystal lattice or geometric patterns. Examples are ice, methanol, penicillin G and sodium chloride.	1. Amorphous solids are just strewn in any old fashion with random unoriented molecules. Examples are glass, plastic, penicillin G, and novobiocin.
	
Figure 2.40 (a): A 100% Crystalline Solid	**Figure 2.40 (b): A 100% Amorphous Solid**

… (Contd.)

Crystalline Solids	Amorphous Solids
2. Practically incompressible.	2. Practically compressible.
3. Crystalline solids show definite melting point so they pass sharply from solid to liquid state..	3. Amorphous solids do not show definite melting point so transition from solid to liquid takes place at wide temperature range.
4. Higher energy is required for molecule to escape from a crystal form.	4. Low energy is required for molecule to escape from an amorphous form.
5. Take less time to remove solvent through the space between crystals	5. Take comparatively more time to remove solvent and is removed by diffusion.
6. Handling quality of crystalline materials is poor.	6. Handling quality of amorphous materials is better.
7. Shows poor aqueous solubility because more energy required by orderly arranged molecules for dissolution.	7. Shows good aqueous solubility because minimal energy required by randomly arranged molecules for dissolution.
8. In crystalline solids melting happens.	8. In amorphous form glass transition happens.
9. When crystalline solid is heated at a constant rate, the temperature increases at a constant rate.	9. When crystalline solid is heated at a constant rate, the temperature increases at different rates.
10. They show poor absorption and low bioavailability.	10. They are rapidly absorbed and show higher bioavailability.
12. These solids are stable than amorphous solids.	11. They are less stable than crystalline solids.

2.1.15.3 Polymorphism

Pharmaceutical solids rarely exist as 100% crystalline or 100% amorphous forms. Many substances due to differences in their intermolecular forces exist in more than one crystalline or amorphous form. These forms are called as polymorphs and substances are called polymorphic. Polymorphism is the ability of a molecule to crystallize into more than one different crystal structure. The term allotropy used for elements is synonymous to the polymorphism. That means, polymorphs have same molecular composition but have different crystalline forms. Substance in two different forms is called dimorphic while in three forms called trimorphic and so on. Polymorphs are chemically same but are different with respect to physicochemical properties. The different forms have different thermodynamic properties such as lattice energy, melting point, and x-ray diffraction pattern; vapour pressure, intrinsic solubility, and the biological activity. The difference between polymorphs is variation in packing, shape of crystal and conformation of the molecules. Different

crystallization processes by different solvents, different rate of cooling and different pressures obtain different polymorphs during crystallization.

Almost all long-chain organic compounds exhibit polymorphism. Many drugs such as steroids (cortisone, testosterone, and prednesolone), barbiturates and sulphonamides show property of polymorphism. Sulphanilamide exist in four different α, β, γ and δ polymorphic forms. First three polymorphs are of monoclinic crystal type while fourth one is different than previous ones. Mebendazole has three polymorphic forms namely; Form A, B and C. Anthelmintic activity of one form is more than other. Other examples of drugs that show polymorphism are oxytetracycline, mefenamic acid, phenyl butazone, terfenadine etc. Genetic variation i.e. variation in DNA is also a kind of polymorphism.

Types of Polymorphs:

The two polymorphs cannot be converted into one another without undergoing a phase transition.

Monotropic:

When polymorphic change is not reversible the system is called monotropic. It occurs when one form is stable while other is metastable. Metastable form may be converted to stable form over the time. The vapour pressure of both form are different therefore no transition temperature exists, for example, phosphorus. The transition point is above the melting points of both polymorphs.

Enantiotropic:

If the change from one polymorph to another is reversible, the system is called enantiotropic. At definite temperature one form is converted to other form. Both forms have different vapour pressures. For example rhombic α form of sulphur is converted to other monoclinic β form upon heating at 95.6 °C and cooling at same temperature again it exist in its original form and therefore they are enantiotropic.

Transition Temperature:

When heated gradually one polymorphic form is converted into another polymorph at a fixed temperature is known as transition temperature. For example, transition temperature of mebendazole polymorph B is 137 °C and polymorph C is 107 °C. Both B and C are converted into stable polymorph A. The transition temperature in polymorphism is important because it helps to characterize the system and determine more stable forms at low temperatures.

Determination of Transition Temperature:

Transition temperature is determined from changes in physical properties of polymorphic substances such as colour, density and solubility.

Colour:

When substance is heated, recording temperature at which its colour changes is the transition temperature of that polymorphic substance. For example, red mercury (II) iodide when heated in boiling test tube it becomes yellow at a specific temperature is its transition temperature.

Density:

One polymorph when changes to other on heating or cooling, its density changes. Change in density is obtained from change in volume at different temperatures. Dilatometer is used to determine specific volumes of polymorphic substance at different temperatures. The mean of specific volume of heating and cooling curves is its transition temperature.

Solubility:

Two polymorphic forms have different solubilities at different temperatures but at transition temperatures they have same solubilities. Therefore by determining solubilities at different temperatures and plotting them on a graph the point at which the line meets is transition temperature.

Cooling Curve Method:

When polymorphic substance changes from one form to another form there is always either absorption or evolution of heat, for example, if Form A changes to Form B. On plotting heat evolved or absorbed against the temperature the inflection in the curve is indication of transition temperature. This method is used to determine transition temperatures of hydrates and anhydrous salts.

Solvates and Polymorphs:

Solvates are the crystalline adducts containing molecule of solvent incorporated within the crystal lattice. Solvates are sometimes called pseudo polymorphs. If solvent is water it is called as hydrates for example, caffeine hydrate, theophylline hydrate, ampicillin trihydrate, ampicillin monohydrate etc. The anhydrous form is preferred in formulations because it has higher energies and show rapid dissolution rates than hydrate forms. Ampicillin anhydrous show faster dissolution rate and greater extent of absorption than trihydrate form. Organic solvates shows property opposite to hydrates. They have high internal free energy and therefore show better dissolution, for example, succinyl sulfathiazole solvate of n-naphthol dissolves rapidly than non-solvated form.

Importance of Polymorphism:

Polymorphism is pharmaceutically most important because different polymorphs exhibit different physicochemical properties. It affects mechanical strength and other formulation aspects like compressibility, flowability, hardness and binding strength etc. Unstable polymorphs are not suitable in design of dosage forms because they get converted to stable polymorphs. Metastable polymorphs have higher energy level than the stable form. Metastable forms exhibit greater dissolution rates, better bioavailability and superior therapeutic activity.

Melting point:

As mentioned earlier, polymorphs can vary in melting point. Theobroma oil, a polymorphic natural fat because it consist mainly a single glyceride, it melts over a narrow temperature range of 34 °C – 36 °C. The theobroma oil exist in four different polymorphic forms; α, β (prime), β' (stable) and γ having melting points 20 °C, 28 °C, 34.5 °C and 18 °C,

respectively, of which only one form is stable. This is an important consideration in the preparation of theobroma suppositories. If the oil is heated to a point where it is completely liquefied (about 35 °C), the crystals of the stable polymorph are destroyed and the mass does not crystallize until it is cooled to 15 °C. The crystals formed are unstable and the suppositories melt at 24 °C. Theobroma suppositories must, therefore, be prepared below 35 °C. When the liquid formed is subsequently cooled, the obtained solid is more stable and melts at 34 °C.

Solubility:

The melting point of the solid is closely related to solubility and therefore polymorphs are most likely to have different solubilities. Since the solubilities vary between polymorphs, some polymorphs of drugs work better than others. The solubility can affect the biological availability of the drug. Chloramphenical palmitate, an antibacterial drug, when logarithm of intrinsic solubility is plotted against reciprocal of absolute temperature, two intersecting lines occurs at transition temperature. It can be represented by another example of two polymorphs of sulfathiazole, Form-I and Form-II in 95% ethanol as shown in Fig. 2.41.

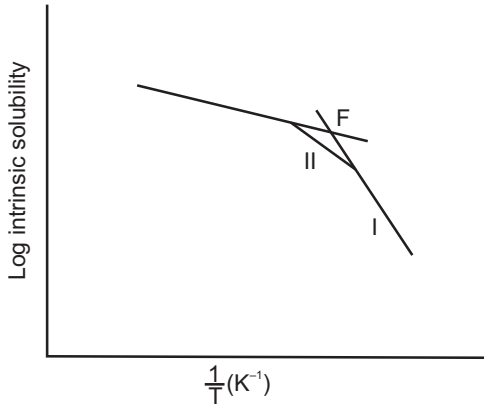


Figure 2.41: Transition temperature of sulfathiazole polymorphs

Metastable form is used in solid dosage forms because it has higher solubility than stable form and therefore dissolution is higher. The solubility of different polymorphs is determined at various temperatures. The heat of solution of polymorph can be determined using Van't Hoff equation (2.30);

$$\frac{\Delta \log P}{\Delta (1/T)} = \frac{-\Delta H}{R} \quad \ldots (2.30)$$

where, P, is molal solubility, T is absolute temperature, R is gas constant and ΔH is heat of solution. Slope of the graph gives heat of solution as

$$\Delta H = - \text{Slope} \times R \quad \ldots (2.31)$$

Amorphous form of novobiocin has greater solubility than the crystalline form. If the wrong polymorph is chosen during the formulation process, the metastable (i.e., thermodynamically unstable form) form can convert to the stable form, which can result in changes in solubility. Three forms of terfenadine also show different solubilities.

Compression behaviour:

A change in polymorphic and disordered structure of the drug is net result of application of higher compression force causes cracking of tablets. The Form B of the drug tolbutamide creates problems in tableting.

Complex formation:

Iodine, depending upon pH of buffer system, the complex formed with zinc in solution may be amorphous or crystalline. Amorphous complex is rapidly absorbed while crystalline complex is slowly absorbed exerting action for longer time. Sometimes combination of amorphous and crystalline forms in proportions such as 50% : 50% or 30% : 70% can be used to exert intermediate action in terms of time.

Dissolution rate:

High energy level molecular forms such as metastable crystalline forms, amorphous forms, anhydrates and solvates have rapid dissolution rates as well as greater extent of absorption. Different polymorphs exhibit different dissolution rates and ultimately variation in biological activity. Examples of drug polymorphs that show different dissolution rates are aspirin, chloramphenicol palmitate, novobiocin, ibuprofen, and tetracycline. Methyl prednesolone exist in two polymorphic forms namely Form-I and Form-II, showing different dissolution rates. Form-I is stable while Form-II is metastable with better dissolution rate than the stable one.

Biological activity:

Different polymorphs can have different rates of absorption in the body leading to lower or higher biological activity than desired. In extreme cases an undesired polymorph can even be toxic. The example of polymorphism that may affect drug behaviour is chloramphenicol-3-palmitate (CAPP). CAPP is a broad-spectrum antibiotic known to crystallize in at least three polymorphic forms and one amorphous form with the most stable Form A. The difference in biological activity between Form A and Form B is a factor of 8, creating the danger of fatal dosages when the unwanted polymorph is unwittingly administered due to alterations in process and/or storage conditions. The HIV protease inhibitor drug ritonavir exist as Form-I and Form II. Form-I is poorly absorbed while Form-II is precipitated upon storage decreasing solubility to 50% with reduction in dissolution rates affecting the bioavailability and ultimately biological activity.

Caking:

Polymorphism is also an important factor in suspension technology. Cortisone acetate, for example, has 5 polymorphs of which four are unstable in water, but only one form is stable in suspension. Other Forms produces caking on storage. Heating, grinding and suspension in water are all factors that affect the interconversion of the different cortisone forms.

Chemical stability:

Different polymorphs have different arrangements of atoms within the unit cell, and this can have a profound effect on the properties of the final crystallized compound. The colour

of dyes can be affected by the polymorph of the pigment. Tamoxifen citrate an antiestrogenic and antineoplastic drug used in the treatment of breast cancer. It exist in A and B polymorphic forms. Form A is more stable in its molecular configuration due to formation of hydrogen bonding. The metastable Form B converts to Form A in ethanolic solution. The stable polymorph is more resistant to chemical degradation and has low solubility and hence can be formulated as suspensions.

Storage stability:

A completely different situation arises when the crystalline state of a drug changes to less stable (higher energy) crystalline state or to the least stable amorphous state due to drug excipient interactions. Another common case is the chocolate that softens at slightly higher temperatures than desired storage temperature.

Colour change:

Quinacridone is the parent compound of one of the most important classes of organic pigments and is known to exhibit three polymorphic forms each with a different shade of red. One of the forms of quinacridone shows superior performance due to its outstanding light fastness, weather resistance, and thermal stability.

Identification of Polymorphous:

The potential effects of polymorphism on biological activity and the strong competition in the drug sector makes it vital to analyze identify and patent every polymorph of a new drug molecule. However, finding all possible polymorphs is not as easy as it sounds. Traditionally, different polymorphic structures can only be found by creating them in the laboratories. Different polymorphs may be produced under different conditions, and the pharmacist must try to vary conditions in order to achieve as many different polymorphs as possible. Polymorphs can be studied by different techniques such as hot stage microscopy, electron microscopy, IR spectrophotometry, X-ray crystallography (x-ray diffractometer) and dilatometry.

X-ray Diffraction:

The crystalline structures can be analyzed using x-ray diffraction. If a pure single crystal is grown, single crystal x-ray diffraction is the best way to get high quality data about the crystalline structure. However, it is often difficult and time-consuming to grow crystals large enough to be examined using single crystal x-ray diffraction. In such cases only a powder can be crystallized, and the resulting x-ray powder diffraction pattern are subjected to interpretation. However, this whole process is difficult and time-consuming. Success depends not only on the skill of the pharmacists in interpreting the x-ray diffraction patterns, but also on whether they have happened to crystallize all of the polymorphic forms of the molecule. The structures are ranked in order of stability to have the lowest-energy (most stable) structures be identified as potential polymorphs.

The 4-amidino indanone guanyl hydrazone is a potential anti-cancer drug as a selective inhibitor of S-adenosyl methionine decarboxylase. Two anhydrous polymorphs of this compound were known to exist, but only one crystal structure had been determined

experimentally because suitable single crystals of the other polymorphic form could not be grown. It is possible to determine the unknown polymorphic form using low-quality powder diffraction data. Furthermore, the physical organization (such as double-helical, or the complex three-dimensional arrangement of folded proteins) is readily available by x-ray diffraction. The polymorphism in a glycan such as cellulose or starch is usually a function of the material origin.

Dilatometry:

Dilatometer as shown in Fig. 2.42 (a) helps to study polymorphs melting behaviour as function of temperature. It measures specific volume in mL/g of substance. For example, two samples A and B of theobroma oil, one sample A is obtained by rapid cooling and other B by slow cooling. Gradually samples are heated in sample tube and specific volume is recorded from the height of mercury column of the dilatometer. The confirmation of metastable form of theobroma oil is the contraction in the temperature range of 20 °C – 25 °C as shown in dilatometric curve given below, Fig. 2.42 (b).

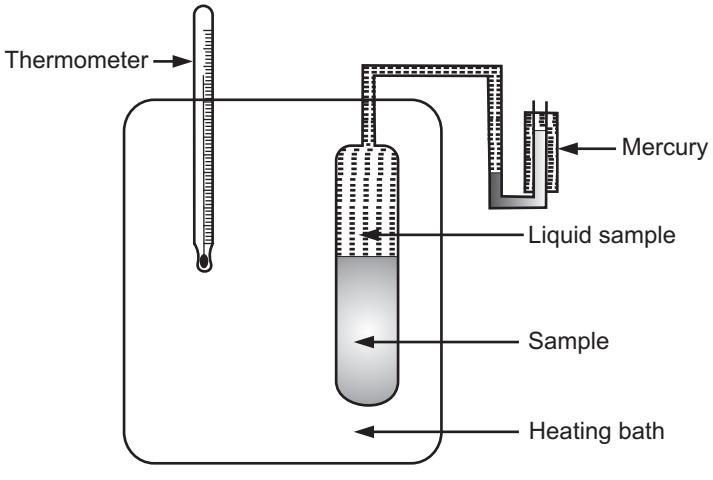


Figure 2.42 (a): Schematic of Dilatometer

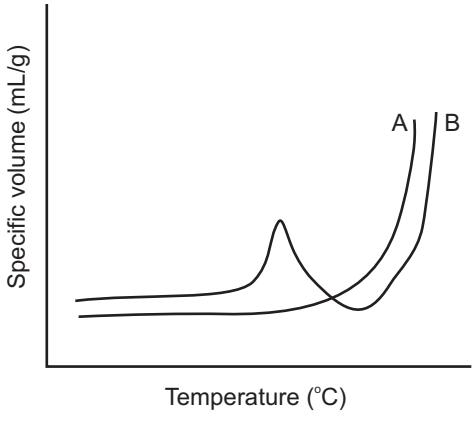


Figure 2.42 (b): Dilatometric Curves of Theobroma Oil

2.2 PHYSICOCHEMICAL PROPERTIES OF DRUG MOLECULES: DETERMINATIONS AND APPLICATIONS

The molecular structure of the compound uniquely defines all its physical, chemical and biological properties. It is generally recognized that physicochemical properties play an important role in product development including studies on biological performance of drugs. A study of the physical properties of drug molecules is a prerequisite for product preformulation, formulation development and optimizing storage and usage conditions. It often leads to a better understanding of the relationship between molecular structure and drug action. The most important physical properties related to product formulation and biological performance is summarized below:

Classification:

Physical properties of substances may be classified in to three types;

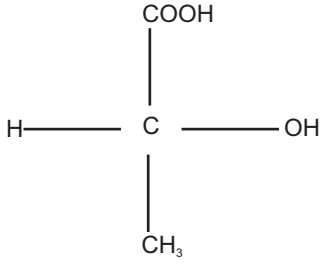
(i) Additive Properties:

Additive properties are derived from sum of the properties of individual properties of atoms or functional groups present within the molecule. The examples of this type are mass or molecular weight, volume etc. Consider the case of acetic acid (CH_3COOH). Obtaining molecular weight of acetic acid involves addition of molecular weights of individual atoms that makes it. Acetic acid contains; C = 2, H = 4 and O = 2. So the molecular weight is calculated as;

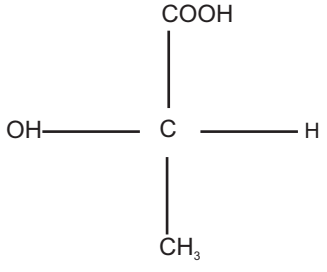
$$\begin{aligned}\text{Molecular weight of acetic acid} &= C \times 2 + H \times 4 + O \times 2 \\ &= 12 \times 2 + 1 \times 4 + 16 \times 2 \\ &= 60 \text{ g/mol}\end{aligned}$$

(ii) Constitutive Properties:

These properties are depending on the structural arrangement of atoms and functional groups as well as bond structure that exists within the molecules. The examples of this type are optical activity, surface tension, viscosity etc. Consider the case of lactic acid. It exists in two forms namely *d*-lactic acid and *l*-lactic acid. The specific rotation of *d*-lactic acid is +3.8° while *l*-lactic acid shows it as -3.8°.



d-lactic acid (+3.8°)



l-lactic acid (–3.8°)

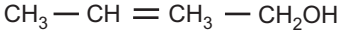
(iii) Combined Additive-Constitutive Properties:

Many physical properties are constitutive and yet to have some measure of additivity is called as additive-constitutive properties. The example of this type is molar refraction.

The standard contributions of atoms, groups and structural unit's to the molar refractions are listed in Table 2.9. Molar refractions of ethyl methyl ketone and 2, 3-butanol is obtained as follows.



Ethyl methyl ketone



2,3 butanol

Table 2.9: Atoms, Groups and Structural Unit's Contributions to Molar Refractions

Atoms and groups	Contribution	Structure	Contribution
H	1.027	C (single bond)	1.67
O (in OH)	1.527	C (double bond)	4.16
O (C = O)	2.180	C (triple bond)	1.97
O (in ester)	1.65	Three member ring	0.17
Cl	5.849	Four member ring	0.317
Br	8.84	Five member ring	− 0.10
I	13.9	Six member ring	− 0.15
C	2.590	–	–

The molar refraction of both these compounds is obtained as sum by substituting values of their contributions for groups and bond structures as given in Table 2.10. According to definition of additive property, molar refractions of both these molecules are sum of atoms and groups that makes these molecules. Therefore molar refraction is an additive property. Although the number and types of atoms in both these molecules are same their arrangements in molecules is different and they show different molar refraction values as ethyl methyl ketone has 20 while 2, 3 - butanol has 18.60. Therefore, molar refraction is additive as well as constitutive property.

Table 2.10: The molar refraction of ethyl methyl ketone and 2, 3-butanol

Compounds	Atom	Number of atoms	Contribution	Molar refraction	Total R_M
	H	8	1.1	8.800	
	C (single bond)	3	2.418	7.254	
Ethyl methyl ketone	C (double bond)	1	1.733	1.722	19.998 ≈ 20.00
	O (C = O, i.e. ketone)	1	2.211	2.211	
	H	8	1.1	8.800	
	C (single bond)	2	2.418	7.254	
2, 3 butanol	C (double bond)	2	1.733	1.722	18.627 ≈ 18.60
	O(O-H, i.e. hydroxyl)	1	1.525	1.525	

Colligative Properties:

Colligative properties are defined as the properties which depend upon the total number of non-volatile solute particles present in the solution. Dilute solutions which contain negligibly small amount of non-volatile solute exhibit colligative properties. The examples of these properties are lowering of vapour pressure, freezing point depression, boiling point elevation and osmotic pressure. These properties are used to determine molecular weights of compounds.

In this chapter various physical properties discussed are refractivity, optical activity, dielectric constant and induced polarization, dipole moment and dissociation constant, magnetic properties, molecular and Raman spectra, nuclear magnetic resonance and x-ray diffraction.

2.2.1 Refractive index

In 1621, a Dutch physicist named Willebrord Snell derived the relationship between the different angles of light as it passes from one transparent medium to another. When light passes from one transparent medium to another, it bends according to Snell's law which states:

$$Ni \times Sin\ (Ai) \ - \ Nr \times Sin\ (Ar) \qquad \ldots (2.32)$$

where, Ni is the refractive index of the medium the light is leaving, Ai is the incident angle between the light ray and the normal to the medium to medium interface, Nr is the refractive index of the medium the light is entering; Ar is the refractive angle between the light ray and the normal to the medium to medium interface. In other words refractive index of substance is the ratio of velocity of light in vacuum or air to that in the substance.

$$n = \frac{\text{Velocity of light in substance}}{\text{Velocity of light in vacuum or air}} \qquad \ldots (2.33)$$

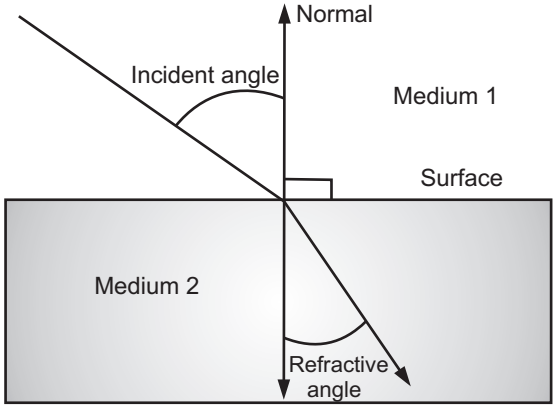


Figure 2.43: Angles of Incidence and Refraction (2D)

When monochromatic light passes through a less dense medium such as air or vacuum and enters a denser medium, the advancing waves at interface are modified and brought closer together, Fig. 2.43. This leads to decrease in speed and shortening of wavelength. When light passes the denser medium, a part of wave slows down more quickly as it passes

through interface and makes it bend towards the interface. This phenomenon is called as refraction. If light passes from denser medium to less denser medium then it is refracted away from the interface. This effect observed between mediums is expressed as refractive index (n). The refractive index is a constant for a given pair of materials under specified conditions. It can be defined as ratio of speed of light in material 1 to the speed of light in material 2. This is usually written 1n2 and is the refractive index of material 2 relative to material 1. The incident light is in material 1 and the refracted light is in material 2. When the incident light is in a vacuum this value is called the absolute refractive index of material.

Refractive indices of most substance are more than air because the velocity of light in air is greater than in the substance for example, absolute refractive index of water is 1.330, soda lime glass 1.510. By definition the refractive index of a vacuum is 1. In practice, air makes little difference to the refraction of light with an absolute refractive index of 1.0008. The refractive indices of some liquids are given in Table 2.11.

Table 2.11: Refractive Indices of Some Materials

Material	Refractive Index	Material	Refractive Index
Air	1.00029	Crystal	2.00
Water	1.330	Diamond	2.417
Glass, soda-lime	1.510	Ethyl Alcohol	1.36
Vacuum	1.000000 (exactly)	Glass	1.5
Air (STP)	1.00029	Ice	1.309
Acetone	1.36	Iodine Crystal	3.34
Alcohol	1.329	Sodium Chloride	1.544
Crown Glass	1.52	Sugar Solution (30%)	1.38
Sugar Solution (80%)	1.49	Water (20°C)	1.333

Bending light:

The bending of light rays is due to the refraction. As light passes from one transparent medium to another, it changes speed, and bends. How much this happens depends on the refractive index of the mediums and the angle between the light ray and the line perpendicular (normal) to the surface separating the two mediums, Fig. 2.43. Each medium has a different refractive index. The angle between the light ray and the normal as it leaves a medium is called the angle of incidence. The angle between the light ray and the normal as it enters a medium is called the angle of refraction.

Critical angle:

If the angle of incidence is increased there is an increase in angle of refraction. The maximum angle of incidence that can be achieved is 90^{o}.

$$\sin i = \frac{\sin 90}{\sin r} = \frac{1}{\sin r} \quad \ldots (2.34)$$

The r in equation (2.34) is called as critical angle.

Refraction simulator is used to know how light bends toward the normal when the light enters a medium of greater refractive index, and away from the normal when entering a medium of lesser refractive index. When the light is moved to an angle close to 90° or − 90° in the medium with a higher refractive index we approach the critical angle and the refracted light approaches 90° or − 90°. At critical angle the angle of refractions becomes 90° or − 90° and the light is no longer transmitted across the $medium_1/medium_2$ interface. For angle with greater in absolute value than the critical angle, all the light is reflected. This is called total reflection.

Specific Refraction:

Initially, it was not possible to draw any conclusion regarding the nature of the substance from the refractive index. In 1880, scientist Lorentz showed the property specific refraction which was found to be more useful in characterization of substance independent of temperature. The specific refraction is mathematically expressed as

$$RS = \frac{(n^2 - 1)}{(n^2 + 2)} \times \frac{1}{\rho} \text{ mL/g} \qquad \ldots (2.35)$$

where, RS is specific refraction in mL/g, n is refractive index of substance and ρ is density of substance at the temperature at which refractive index is determined.

Molar Refraction:

Molar refraction is defined as molecular weight times the specific refraction of substance. It is more useful property than specific refraction as it is characteristic of the substance and useful in structural studies like finding nature of bonding in molecules and in determination of dipole moment. It is expressed as

$$RM = \frac{(n^2 - 1)}{(n^2 + 2)} \times \frac{M}{\rho} \text{ mL/mol} \qquad \ldots (2.36)$$

where, RM is molar refraction in mL/mol and M is molecular weight of the substance under study. The experimentally determined values of molar refractions are compared with the theoretical values giving their contribution of atoms, groups and structural units to the molar refraction.

Example 2.4: The refractive index of ethyl alcohol is 1.329 for D-line of sodium at 20 °C. Calculate its molar refraction if the density is 0.931 g/mL.

Solution: Substituting values in equation (2.36)

$$RM = \frac{(n^2 - 1)}{(n^2 + 2)} \times \frac{M}{\rho} \text{ mL/mol}$$

$$= \frac{(1.329)^2 - 1}{(1.329)^2 + 2} \times \frac{46}{0.931}$$

$$= \frac{(1.329)^2 - 1}{(1.329)^2 + 2} \times (49.40)$$

$$= 10.04 \text{ mL/mol}$$

Measurement of Refractive Index:

Refractive index is determined by using instrument called refractometer. Abbes refractometer, immersion refractometer and Pulfrich refractometer are used for this purpose. Abbes refractometer is commonly used at laboratory scale because of its advantages over other refractometers. It is most convenient, reliable and simple instrument with small sample size requirement suitable for range of substances. Ordinary light source, easy maintenance and economy and easy determinations are some of the other advantages of this instrument. The components of Abbes refractometers include light reflection mirror, dispersion compensator, telescope, and index arm and prism box. The schematic of abbes refractometer is shown in Fig. 2.44. Abbes refractometer may be calibrated with anyone of the liquid specified in Table 2.12 at temperatures below 25 °C using D-line of sodium.

Table 2.12: Reference liquids for calibration of Abbes' refractometer

Reference liquids	Refractive index (n_d^{25})	Temperature coefficient
Water	1.3325	–
Carbon tetrachloride	1.4969	0.00057
Toluene	1.4969	– 0.00056
α-methylnaphthalene	1.6176	– 0.00048

The refractive index varies with varies with temperature and wavelength of light used and hence it is not a constant property.

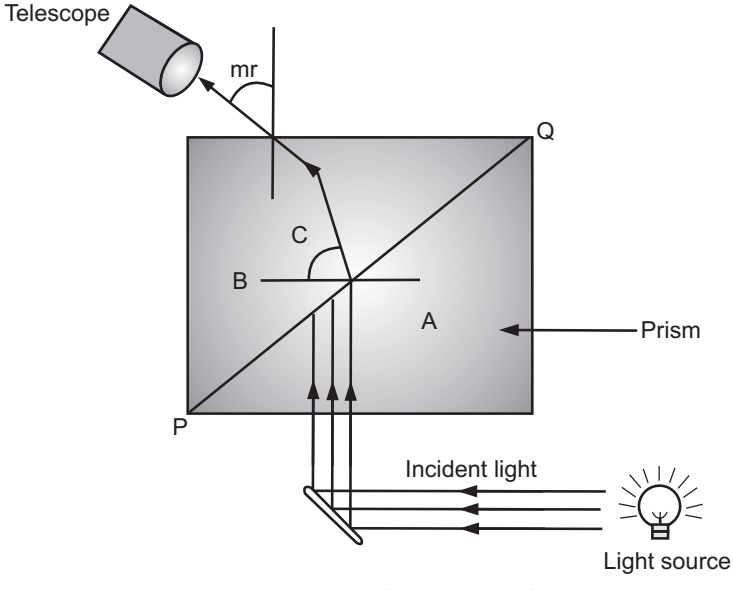


Figure 2.44: Schematic of Abbe's refractometer

Recent research has demonstrated the existence of negative refractive index. Not thought to occur naturally, this can be achieved with so called metamaterials and offers the possibility of perfect lenses and other exotic phenomena such as a reversal of Snell's law. The

real and imaginary parts of the complex refractive index can be determined as a function of wavelength from an absorption spectrum of the material. The refractive index of certain media may be different depending on the polarization and direction of propagation of the light through the medium. This is known as birefringence or anisotropy. The strong electric field of high intensity light for example, a laser, may cause a medium's refractive index to vary as the light passes through it, giving rise to non-linear optics. If the index varies quadratically with the field it is called the optical Kerr effect and causes phenomena such as self-focusing and self phase modulation. If the index varies linearly with the field it is known as the Pockels effect. If the refractive index of a medium is not constant, but varies gradually with position, the material is known as a gradient index medium.

Applications:

Since refractive index is a fundamental physical property of a substance it is often used to analyze and identify a particular substance, confirm its purity, or measure its concentration. Refractive index values are useful in determination of molecular weights and structures of organic compounds from their molar refraction values. Refractive index is used to measure refraction characteristics of solids, liquids, and gases. Most commonly it is used to measure the concentration of a solute in an aqueous solution. For a solution of sugar, the refractive index can be used to determine the sugar content. Similarly alcohol content in bioproduction is also determined from the refractometry. Dielectric constant and molar polarizibility values can be obtained from the refractive index. Refractive index of a material is the most important property of any optical system that uses refraction for example, lenses and prisms.

2.2.2 Optical Rotation

Ordinary light consists of vibrations, which are evenly distributed in all directions in a plane perpendicular to the direction of propagation, called as unpolarised light, Fig. 2.45 (a). When the vibrations of light are restricted to only one plane, the light is said to be polarized light, Fig. 2.45 (b). Some substances rotates the plane of polarized light are called as optically active substances. This property of optically active substance is measured as angle of rotation. The property in which rotation of plane polarized light is observed is known as optical activity. Optically active substances include organic molecules with a central carbon atom to which four different groups are attached, making the molecule very asymmetric (chiral carbon).

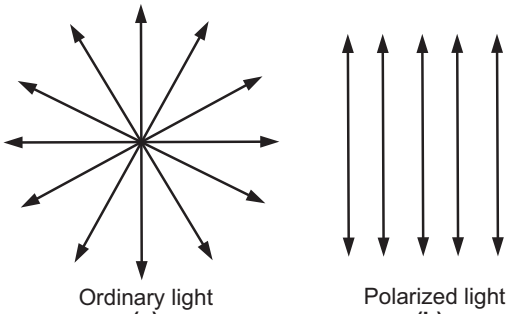


Figure 2.45: Ordinary and polarized light

Similarly, laevulose, more commonly known as fructose causes the plane of polarization to rotate to the left. Fructose is even more strongly leavo rotatory than the glucose. The substance which rotates the plane of polarized light to the right or clockwise when viewed in the direction of light propagation is called dextro rotatory (*d*) or (+) substance.

The use of one name for glucose, dextrose, refers to the fact that it causes linearly polarized light to rotate to the right or dexter side. Those optically active substances that rotate plane polarized light to the left or counter clockwise are known as leavo rotatory (*l*) or (−) substance. Laevulose, more commonly known as fructose causes the plane of polarization to the left. Fructose is even more strongly leavo rotatory than the glucose. Other examples of optically active substances are lactic acid, tartaric acid, 2-methyl -1-butanol etc. Optical rotation occurs because of optically active substances have different refractive indices for left and right polarized light. Another way to make this statement is that left and right polarized light travel through an optically active substance at different velocities. Optical activity is considered to be due to the interaction of plane polarized radiations with electrons in molecules which shows electronic polarization. This interaction rotates the direction of vibration of radiation by altering electric field.

Optically active substances can be categorized in to two types:

1. Those which are optically active only in the crystal state due to their characteristic crystal structure and becomes optically inactive in the fused or dissolved state, for example, sodium chlorate, quartz crystal etc and,
2. Those which shows optical activity in all states *viz*. Crystalline (solid), fused (liquid) and gaseous state, due to their structural configurations.

Specific Rotation:

When a polarized light passes through an optically active substance, all the molecules in the path of light rotates plane of polarization by some constant amount which is a characteristic of that substance. The total rotation in the emergent light beam is proportional to the path length (*l*) and density (ρ) of the substance. This relation is mathematically expressed as

$$\theta \propto \rho \qquad \ldots (2.37)$$

$$\therefore \quad \theta = [\alpha]\, \rho \qquad \ldots (2.38)$$

where, α, is proportionality constant called as specific rotation. The amount of optical rotation depends on the number optically active species through which the light passes and thus depends on both the sample path length and analyte concentration. Specific rotation provides a normalize quantity to correct for this dependence, and is defined as;

$$[\alpha]_{\lambda}^{T} = \frac{\theta}{l \times \rho} \qquad \ldots (2.39)$$

where, θ, is measured optical angle of rotation in deg cm^2/g or degrees, *l* is sample path length in decimeters (dm) and ρ is density if the substance is pure liquid, λ is the wavelength of light used for observation, usually 589 nm, the D line of a sodium lamp unless otherwise specified and T is the temperature in °C.

Example 2.5: A sample of pure (S)-2-butanol was placed in 10 cm polarimeter tube, using the D-line of sodium lamp; the observed angle of rotation at 20 °C was +104°. The density of this compound is 0.805 g/mL. What is the specific rotation of this compound?

Solution: Substituting the given values in equation

$$[\alpha]_{\lambda}^{T} = \frac{\theta}{l \times \rho}$$

$$[\alpha]_{\lambda}^{T} = \frac{104}{l \times 0.805}$$

$$= 129°$$

Example 2.6: Calculate the observed angle of rotation of solution of 0.5245 g of (S)-1-amino-1-phenyl ethane diluted to have a volume of 10 ml with methanol at 20 °C, using D-line of sodium lamp and a 1.0 dm tube. Specific rotation of the substance is −30°.

Solution:

$$\theta = [\alpha]_{\lambda}^{T} \times [l \times \rho]$$

$$= (-30) \times 1 \times \left(\frac{0.5245}{10}\right)$$

$$= -1.57°$$

For solids which are in solution form the term ρ in equation (2.39) is replaced by concentration, g/100 mL. Therefore it is expressed as

$$[\alpha]_{\lambda}^{T} = \frac{\theta \times 100}{l \times C} \quad \ldots (2.40)$$

The angle of rotation changes with change in concentration of optically active substance, as concentration decreases the angle of rotation also decreases. For example, sucrose solutions of strength 10, 20 and 30 g/100 mL at 20 °C in 2 dm length sample tube shows 13.33, 26.61 and 39.86° angle of rotation, respectively.

Example 2.7: Calculate specific rotation of tartaric acid on the following observations: A 0.856g of sample of pure tartaric acid was diluted to 20 mL with water and placed in a 1 dm sample cell. The observed rotation using the 589 nm line of sodium lamp at 20 °C was 1.5°.

Solution:

$$[\alpha]_{\lambda}^{T} = \frac{20 \times \theta}{l \times C}$$

$$= \frac{20 \times 1.5}{1 \times 0.856}$$

$$= \frac{30}{0.856}$$

$$= 35°$$

Example 2.8: What is expected observed angle of rotation of 1×10^{-4} M methanolic solution of potent anticancer drug paclitaxel? Given: $[\alpha]_{\lambda}^{T} = -49°$, l = 10 dm and molecular weight of paclitaxel = 853.93 g/mol.

Solution:

$$\theta = [\alpha] \times [l \times C]$$

$$= [-49] \times \left[\frac{1 \times 0.8593}{100}\right]$$

$$= -0.004179°$$

Example 2.9: A certain compound has a specific rotation of −43.2° at concentration 5 g/mL determined using sample tube of 1 dm length. What is the observed angle of rotation of a same compound of concentration 1 g/mL in the same solvent and sample tube?

Solution:

$$\theta = [\alpha] \times [l \times C]$$

$$= [-43.2] \times 1 \times \left[\frac{1}{5}\right]$$

$$= -8°$$

The angle of rotation changes with change in wavelength of light used. Therefore the specific rotation also changes with the wavelength of light used. The graph of specific rotation versus wavelength shows an inflection and then passes through zero at the wavelength of maximum absorption of polarized light. This change in specific rotation is called as cotton effect. Substances which show maximum rotation before passing through zero due to smaller wavelength of polarized light are said to show positive cotton effect. If specific rotation shows maximum value after passing through zero, the substance shows negative cotton effect. Cotton effects are helpful in characterizing enantiomers, especially in structural elucidation of organic compounds. The variation in angle of rotation with wavelength of light is called the optical rotatory dispersion. It is recorded using spectropolarimeter, which has a tungsten lamp and as canning monochomator as a light source. A motorized mount rotates the analyzer to maintain a minimum signal at the detector. Usually a modulation is introduced in to polarization angle of light beam so that DC signals to the analyzer motor.

Molar Rotation:

Molar rotation is characteristic property of optically active substances. It is obtained from multiplication of specific rotation and molecular weight of the compound as

$$\mu = M[\alpha] \times 100 \quad \ldots (2.41)$$

where, μ is molar rotation, M is molecular weight and $[\alpha]$ is specific rotation.

Example 2.10: The specific rotation of 10% solution of a substance having molecular weight 60 g/mol is 50°; calculate its molar rotation.

Solution: Substituting values in equation (2.40)

$$\mu = M \times [\alpha] \times 100$$

$$\mu = 60\ [50] \times 100$$

$$= 3 \times 10^{4}$$

Enantiomeric Purity:

The molecules that are non-superimposable mirror images are called enantiomers. In case of optically active substances if only one enantiomer is present then the substance is considered to be optically pure, while if it consists of mixtures of two enantiomers (a racemic mixture), it will not rotate plane of polarized light and is optically inactive. A mixture that contains one enantiomer in excess displays a net plane of polarization which is characteristic of the enantiomer that is in excess. The optical purity or the enantiomeric excess (%ee) of a sample can be determined as follows:

$$\begin{aligned} \text{Optical purity} &= \% \text{ enantiomeric excess} \\ &= \% \text{ enantiomer}_1 - \% \text{ enantiomer}_2 \\ &= \frac{10 \times [a] \text{ of mixture}}{[a] \text{ of pure sample}} \end{aligned}$$

$$\%e = 100 \frac{([R] - [S])}{([R] + [S])} \quad \ldots (2.42)$$

where, [R] and [S] are the concentrations of R and S isomers, respectively.

Measurement of Optical Activity:

Measurement of orientation of plane polarized light is called polarimetry, and the instrument used is called a polarimeter. The simplest polarimeter, Fig. 2.46, consists of monochromatic light source, a polarizer, a sample cell, a second polarizer which is called the analyzer and a light detector. Polariser and analyzer are made up of Nicol prisms. When analyzer is oriented 90° to the polarizer no light reaches to the detector. The polarizer is placed near to the light source while analyzer is placed between sample cell and the detector. The sample cell of suitable size and capacity with outward projection at the centre, to trap the air bubble is usually used. When an optically active substance is placed in the sample cell and beam of light is passed through, it rotates the polarization of the light reaching the analyzer so that there is a component that reaches the detector. The angle that the analyzer must be rotated from the original position is the optical rotation.

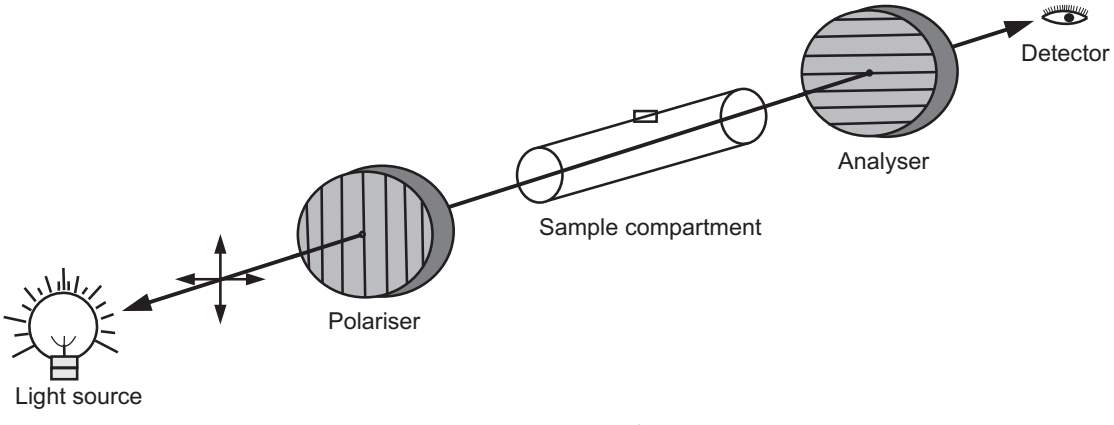


Figure 2.46: Schematic of Polarimeter

For a pure substance in solution, if the colour and the path length are fixed and the specific rotation is known, then observed rotation can be used to calculate the concentration. Optical activity is useful in studying the structure of anisotropic materials, and for checking the purity and identifying chiral mixtures. Adulterations in the optically active substances can be determined from the optical rotation. For example, optical rotation of honey is opposite to that of sugar due to the presence of fructose and glucose and hence can be determined from the optical rotation. Chemical kinetic studies are also carried out by determining concentration at different time intervals as in case of sugar inversion. Polarimetry is used in the analysis of various drugs and pharmaceutical formulations such as Adrenaline Bitartarate, anticoagulant Citrate Dextrose Solution, Dextran 40 Injection, Dextrose Injection, Sodium Chloride and Dextrose injection etc.

2.2.3 Dielectric Constant

A polar molecule can sustain a separation of electric charge either through the induction by an external electric field or by a permanent charge separation within a molecule. The separation of charge can be best understood from the concept called dielectric constant. Consider the example of parallel plate condenser, Fig. 2.47.

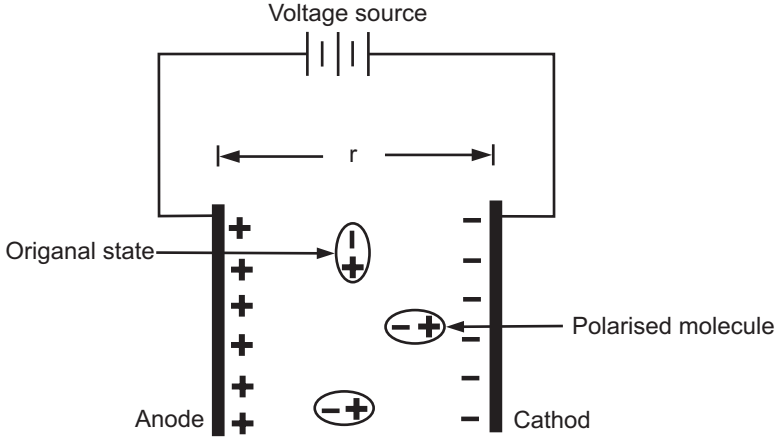


Figure 2.47: Parallel Plate Condenser

The parallel plates are separated by some medium across a distance r and connected to voltage supply source. The electricity will flow across the plates from left to right through the battery until potential difference of the plates equals that of the battery which is supplying the initial potential difference. The capacitance, C, is equal to the amount of electric charge, q, stored on the plates, divided by V, the potential difference, between the plates.

$$C = \frac{q}{V} \qquad \ldots (2.43)$$

The capacitance of condenser depends on the type of thickness of the condenser separating the plates. The C_o is used as capacitance reference medium on which to compare other mediums. The C_o is the capacitance between the plates when a vacuum fills the space between the plates. The ratio of capacitance of test material (C_x) divided by the capacitance of reference material is termed as dielectric constant.

$$\varepsilon = \frac{C_x}{C_o} \quad \ldots (2.44)$$

where, ε is dielectric constant and since it is ration of capacitance it is unit less quantity. The dielectric constants of some liquids are given in Table 2.13. The polarity of the solvent depends on the dielectric constant as more is the polar solvent greater is the dielectric constant. Therefore dielectric constant of a substance affects the solubility of that substance. The highest solubility of caffeine at 25 °C in dioxane – water mixture was found in the dielectric constant range of 20 to 40.

If the polar molecules are placed between plates of charged capacitor, the molecules can undergo an induced polarization. This occurs because of the separation of the electric charge within the molecules as it is placed in the electric field between the plates. This polarization is usually temporary and is independent on the ease with which the molecules can be polarized. This temporary induced polarization is proportional to field strength of capacitor and induced polarizibility, αp, which is characteristic property of the particular molecules. The ease with which a molecule is polarized by any external force (electric field, light or any other molecule) is known as polarizibility. The dipole moment and polarizability of some solvents are given in Table 2.13.

Table 2.13: Dielectric Constants of Some Liquid at 20 °C

Liquids	Dielectric constants	Liquids	Dielectric constants
Acetone	21.4	Formaldehyde	22.0
Benzene	2.28	hexane	5.0
CCl_4	2.24	Glycols	50.0
Chloroform	4.8	Methanol	33.7
Ethanol	25.7	Mineral oil	0
Ethyl acetate	6.4	N-Methylformamide	190
Ethyl ether	4.34	Phenol	9.7
Ether petroleum	4.35	Vegetable oil	0
Fixed oil	0	Water	80.4
Octanol	10	Cyclohexane	2.0

Table 2.14: Dipole Moment and Polarizibility of Some Gases

Gas	Dipole moment	Polarizibility
C_6H_5	0	11.6
HCl	3.6	2.93
$CHCl_3$	3.37	9.46
CH_3OH	5.70	3.59
H_2O	6.17	1.65
NH_3	4.90	2.47

The relation between concentration, dielectric constant and polarizibility is given by *Clausius-Mossotti* equation as;

$$\left[\frac{(\varepsilon - 1)}{(\varepsilon + 2)}\right] = [4/3]\,[\pi n \alpha p] \quad \ldots (2.45)$$

In equation (2.45) n is the number of molecules per unit volume. The total polarization is the sum of induced molar polarization and temporary polarization.

$$P = Pi + P_o \quad \ldots (2.46)$$

Since $\pi = 0$, P_o is zero. To obtain an induced molar polarization (Pi) equation (2.46) can be multiplied by the M/ρ on both sides.

Therefore,

$$\left[\frac{(\varepsilon - 1)}{(\varepsilon + 2)}\right] = \frac{4}{3} \times \frac{\pi n M \alpha P}{\rho}$$

$$= \frac{4}{3} \times \pi n \alpha P$$

$$= Pi \quad \ldots (2.47)$$

A condition in which electric field strength of condenser (V/m) is unity, π represents the induced molar polarization.

Example 2.11: Density and dielectric constant of benzene is 0.878 g/mL and 2.27 respectively; calculate its induced molar polarizability.

Solution: Substituting values in equation (2.46), we get

$$Pi = \left[\frac{(\varepsilon - 1)}{(\varepsilon + 2)}\right] \times \left[\frac{M}{\rho}\right]$$

$$= \left[\frac{(2.27 - 1)}{(2.27 + 2)}\right] \times \left(\frac{78}{0.878}\right)$$

$$= 26.38 \text{ mL/mol}$$

Example 2.12: Calculate dielectric constant of mixture of ethyl alcohol and water having 50 : 50 ratio.

Solution: Dielectric constant of mixture is calculated as

$$\varepsilon_{Mixture} = \varepsilon_{alcohol} + \varepsilon_{water}$$

$$= 0.5 \times 30 + 0.5 \times 80$$

$$= 55$$

The dielectric constant of 50:50 mixtures of ethyl alcohol and water is 55.

2.2.4 Dipole moment

Dipole is a pair of separated opposite electric charges. Electric dipole is an assemblage of atoms or subatomic particles having equal electric charges of opposite sign separated by a finite distance. Dipoles are characterized by their dipole moment, a vector quantity with a magnitude equal to the product of charge or magnetic strength of one of the poles and the distance separating the two poles.

$$\mu = q \times r \quad \ldots (2.48)$$

where, μ is dipole moment, q is charge on atom and *r* is distance of separation of charge.

The direction of the dipole moment corresponds for electric dipoles, to the direction from the negative to the positive charge. The direction of an electric field is defined as the direction of the force on a positive charge, electric field lines away from a positive charge and toward a negative charge.

Molecular Dipoles:

Many molecules have dipole moments due to non-uniform distributions of positive and negative charges on its various atoms. In the case of HCl, the bonding electron pair is not shared equally rather is attracted towards the more electronegative chlorine atom due to its higher electro-negativity which pulls the electrons towards it. It leads to development of positive charge to H atom and negative charge to chlorine atom.

A molecule having positive and negative charges at either terminal is referred as electric dipoles or just dipole. Dipole moments are often stated in Debyes; The SI unit is the coulomb meter.

Molecular dipoles are of three types;

1. **Permanent dipoles**: These occur when two atoms in a molecule have substantially different electro-negativity with one atom attracting electrons more than another becoming more electronegative, while other atom becomes more electropositive.
2. **Instantaneous dipoles**: These occur due to chance when electrons happen to be more concentrated in one place than another in a molecule, creating a temporary dipole.
3. **Induced dipole**: These occur when one molecule with a permanent dipole repels another molecule's electrons, inducing a dipole moment in that molecule.

In a diatomic molecule, the dipole moment is a measure of the polar nature of the bond; i.e. the extent to which the average electron charges is displaced towards one atom. In a polyatomic molecule, the dipole moment is the vector sum of the dipole moments of the individual bonds. In a symmetrical molecule, such as tetrafluoromethane (CF4), there is no overall dipole momental though the individual C-F bonds are polar.

Molecular dipole moments:

In most molecules even though the total charge is zero, the nature of chemical bond is such that the positive and negative charges do not overlap. These molecules are said to be polar because they possess a permanent dipole moment. The example of this type is water molecule. The molecules with mirror symmetry like oxygen, nitrogen carbon dioxide and carbon tetrachloride have no permanent dipole moments. Even if there is no permanent dipole moment, it is possible to induce a dipole moment by the application of an external electric field and is called as polarization. The magnitude of the dipole moment induced in the molecules is a measure of the polarizability of that molecular species.

Permanent dipole moment:

The permanent dipole moment differs from induced polarization in the sense that it is a permanent separation and it happens only to be in the polar but not in the non-polar molecules. These charges that separate balance out each other and therefore have a net charge of zero. The water is example of permanent dipole moment. The permanent dipole moment is defined as the vector sum of the individual charge moments within the molecules.

Applications:

The structure of the molecule can be confirmed from the dipole moment values, for example, chlorobenzene, benzene, carbon dioxide etc. The *cis* and *trans* isomers can be differentiated form dipole moment values, for example, *cis* and *trans* dichloroethylene. Dipole moments can be used to determine percent ionic characteristic of bond of the molecule, e.g. H-Cl a covalent bond, ionic characteristic is 17%. Permanent dipole moments can be correlated with the biological activities to obtain information about the physical parameters of molecules. The more soluble the molecule the easier it passes the lipoidal membrane of insects and attacks the insect's nervous system. Therefore, the lower is the dipole moment the greater is the insecticidal action. For example, *p*, *m* and *o* isomers of DDT show different insecticidal activities due to their differences in permanent dipole moment as *p*- isomer shows μ=1.1 and has predominant toxicity, o-isomer shows μ = 1.5 with intermediate toxicity while m-isomer shows μ = 1.9 with least toxicity. The variations in activities of different isomers are due to the greater solubilities in non-polar solvents.

2.2.5 Dissociation Constant

Dissociation is the process by which a chemical compound breaks-up into simpler constituents as a result of either added energy (dissociation by heat), or the effect of a solvent on a dissolved polar compound (electrolytic dissociation). It may occur in the gaseous, solid, or liquid state, or in solution. An example of dissociation is the reversible reaction of hydrogen iodide at high temperatures

$$2HI_{(g)} \rightleftharpoons H_{2(g)} + I_{2(g)}$$

The term dissociation is also applied to ionization reactions of acids and bases in water. For example,

$$HCN + H_2O \rightleftharpoons H_2O^+ + CN^-$$

This is often regarded as a straight forward dissociation into ions.

$$HCN \rightleftharpoons H^+ + CN^-$$

Dissociation constant is a constant whose numerical value depends on the equilibrium between the undissociated and dissociated forms of a molecule. A higher value indicates greater dissociation. The equilibrium constant of such a dissociation is called the acid dissociation constant or acidity constant, given by

$$K_a = \frac{[H^+] \cdot [CN^-]}{[HCN]} \quad \ldots (2.49)$$

The concentration of water [H_2O] can be taken as constant. Similarly, for a base, the equilibrium in following reaction is also dissociation;

$$NH_3 \rightleftharpoons NH_4^+ + OH^-$$

The base dissociation constant or basicity constant, given by

$$K_b = \frac{[NH_4^+] \cdot [OH^-]}{[NH_4]} \quad \ldots (2.50)$$

where, K_a or K_b is the measures of the strength of the acid (base).

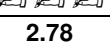
The acid-base dissociation constant, is a measure of the tendency of a molecule or ion to keep a proton (H^+) at its ionization center(s), and is related to the ionization ability of chemical species. Since water is a very polar solvent (ε = 80), ionization will increase the likelihood of a species to be taken-up into aqueous solution. If a molecule does not readily ionize, it will tend to stay in a non-polar solvent such as cyclohexane (ε = 2) or octanol (ε =10). Dissociation constant is the core property of substance that defines its chemical and biological behaviour. In biological terms, dissociation constant is important in determining whether a molecule will be taken-up by aqueous tissue components or lipid membranes. The scientists require an understanding of dissociation constant because it impacts the choice of techniques used to identify and isolate the compound of interest. Dissociation constant is also closely related to the concepts of pH (the acidity of solution) and log *P* (the partition coefficient of a neutral compound between immiscible liquids).

EXERCISE

1. What are homogeneous and homogeneous mixtures? Give four pharmaceutical examples each.
2. How is a chemical change different from a physical change?
3. What is the difference between a homogeneous and a heterogeneous mixture?
4. Explain how transition takes place between states of matter.
5. What do you understand from the terms sublimation and condensation?
6. What is vapour pressure of liquid? Enlist and explain methods to determine it.
7. How boiling point of liquid is determined if molar heat of vapourization is known?
8. Describe the terms critical temperature and critical pressure with reference to water.
9. With the help of graph explain energy distribution in the molecules of liquid at two different temperatures.
10. Write the difference between a gas and a vapour.
11. Write note on characteristics of gaseous state.
12. What is ideal gas and real gas?
13. What is gas law? Write statements of Boyles's law, Charles law and Avogadro's law.
14. Derive an equation of ideal gas law.
15. Express gas constant in three different energy units.
16. Explain the significance to the development of the kinetic molecular model of the observation that the ideal gas law works well only at low pressure.
17. Give a brief molecular explanation for the observation that the pressure of a gas at fixed temperature increases proportionally with the density of the gas.

18. Give a brief molecular explanation for the observation that the pressure of a gas confined to a fixed volume increases proportionally with the temperature of the gas.
19. Give a brief molecular explanation for the observation that the volume of a balloon increases roughly proportionally with the temperature of the gas inside the balloon.
20. Explain why there is a correlation between high boiling point and strong deviation from the ideal gas law.
21. Which parameters of real gases differ from ideal gases?
22. Obtain relationship between van der Waals constants and critical constants in a van der Waals equation.
23. Discuss applications of ideal gas law.
24. Calculate the number of moles of a gas present in a container of 0.0432 m^3 volume at temperature and pressure 21 °C and 15.4 atm.
25. A 40 L cylinder contains 30.5 g of nitrogen gas at 21 °C. What is the pressure inside the cylinder expressed in psi units?
26. Calculate the volume occupied by 60 g of oxygen gas at a temperature and pressure of 25 °C and 24 atm, respectively.
27. What are aerosol and inhalers? Give some pharmaceutical examples of each of them.
28. What are liquid crystals? Classify them and write about its pharmaceutical applications.
29. Define the terms:
    (a) Matter
    (b) Substance
    (c) Element
    (d) Latent heat of vapourization
    (e) Latent heat of fusion
    (f) Boiling point
    (g) Melting point
    (h) Freezing point
    (i) Isotropy
    (j) Crystal lattice
    (k) Anisotropy
    (*l*) Crystalline solid
    (m) Amorphous solid.
30. Explain theory of Bragg's method of crystal analysis.
31. What are point groups and space groups in crystal units?
32. Write note on Bravis lattice.
33. What are minimum numbers of atoms per unit cell of sodium chloride?
34. Explain different elements of symmetry of cubic unit cell.
35. Give difference between:
    (a) Primitive unit cell and non-primitive unit cell.
    (b) Plane of symmetry and axes of symmetry.
36. Calculate co-ordination number in a cubic body centered and face centered crystals.
37. Derive the relationship; $n\lambda = 2d \sin \theta$.
38. Enlist and explain various imperfections observed in crystals.
39. Write characteristics of crystals.
40. What do you understand from the term glass transition temperature?
41. Write about physical properties of amorphous solids.
42. Differentiate between melting and glass transition temperatures.

43. Describe in detail characterization of amorphous solids.
44. Write note on significance of amorphous state in pharmaceuticals.
45. Differentiate between crystalline and amorphous solids.
46. What do you mean by polymorph? Classify them and describe methods to identify polymorphs.
47. Explain significance of polymorphism in pharmaceuticals with some examples.
48. What is transition temperature? Describe methods to determine it.
49. Differentiate between solvates and polymorphs.
50. Write about physical properties of liquids.
51. What is liquid crystalline state? Write reasons for existence of the same.
52. What are liquid complexes? Discuss its applications in pharmacy.
53. What are types of liquid crystals? Explain them.
54. Write pharmaceutical applications of liquid crystals.
55. Define the terms
    (a) Additive property (b) Constitutive property
    (c) Colligative property (d) Refractive index
    (e) Molar refraction (f) Optical activity
    (g) Specific rotation (h) Dipole moment
    (i) Dielectric constant (j) Diamagnetic substance
56. How dipole moment is helpful in elucidation of molecular structure?
57. Explain the terms specific and molar refractivity.
58. Write about induced and orientation polarization.
59. Explain polarimetric measurements.
60. Prove that molar refraction is additive as well as constitutive property.
61. Write note on
    (a) Molar refraction (b) Refractive index
    (c) Dipole moment (d) Optical rotation
    (e) Polarimeter (f) Refractometer
62. Explain the statement "Refractive index decreases with rise in temperature."
63. What do you mean by the terms - plain polarized light, optically active substance, angle of rotation and molar rotation?
64. Enlist and explain factors on which magnitude of rotation depends for optically active substance.
65. What is difference between polar and non-polar molecule? How the dipole moment of the molecule can be determined?
66. Draw well labelled diagrams of polarimeter and Refractometer.
67. Write applications of following properties in pharmaceutical field.
    (a) Refractivity (b) Optical activity
    (c) Dipole moment (d) Dielectric constant
    (e) X-ray diffraction (f) Light absorption
    (g) Dissociation constant



Unit ...3

SURFACE AND INTERFACIAL PHENOMENON

♦ OBJECTIVES ♦

Surface tension occurs whenever there is an interface between a liquid, a solid or a gas. Surface tension of water is an important property in situations where small volumes of liquid occur, or the liquid is in contact with small diameter tubes or porous media. The behaviour of molecules at boundaries between two immiscible phases is different from their behavior in the bulk of the phases, which has implications for the physiology of the human body as well as for pharmacy. Interfacial phenomena affect drug delivery systems. For example, solubilization and dispersion of drugs, suspension or emulsion stability, and adsorption of drugs on different substrates are all affected by the interfacial properties of drugs and their environment.

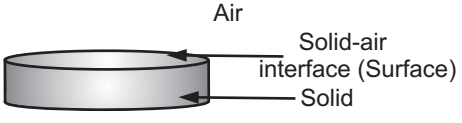
After studying the contents of the chapter, students are expected to:

- Understand types of interfaces and describe relevant examples.
- Understand the terms surface tension and interfacial tension and their application in pharmaceutical sciences.
- Understand the concept of surface and interface tensions, surface free energy, its changes, work of cohesion and adhesion, and spreading and methods of their measurements.
- Understand the mechanisms of adsorption on liquid and solid interfaces.
- Differentiate between different types of mono-layers and recognize basic methods for their characterization.

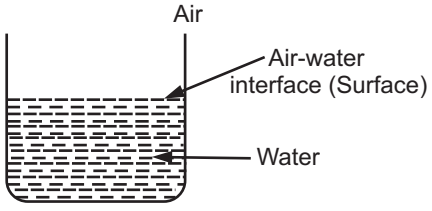
Many natural and biotechnological processes involve different phases (gases, liquids and/or solids), which come together at some stage to form an interface for example, emulsification, flotation, coating, detergency, lubrication, dispersion of powders etc. Applications range from coatings and films to foam. In particular, deposition of liquid on a solid surface as in case of tablet coating, and formation of films as in case of dispersed systems are of significant pharmaceutical importance.

3.1 LIQUID INTERFACE

The term surface is used to represent the boundary between solid-gas and liquid-gas phases. The two words surface and interface often used synonymously, although interface is preferred for the boundary between two condensed phases i.e. liquid-liquid. The cases where the two phases are formed explicitly for example, solid-gas and liquid-gas interface, the term surface is used as illustrated in Fig. 3.1 (a) and (b).



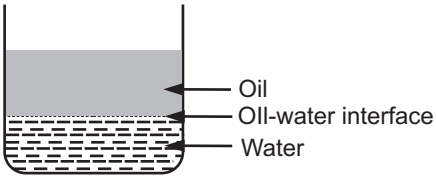
(a) Solid Surface, for example, Tablet



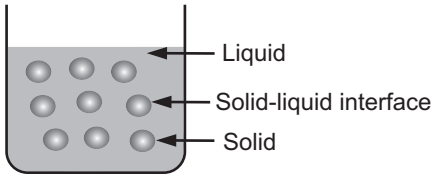
(b) Liquid Surface, for example, Water in Beaker

Figure 3.1: Types of Surfaces

The boundary that exists between two immiscible phases is called as interface. Several types of interface are possible depending on whether the two adjacent phases are in the solid, liquid or gaseous state as shown in Fig. 3.2 (a) and (b). The interface is further divided into solid interface and liquid interface. Solid interface is associated with solid and gas phases, solid and liquid phases or solid and solid, while liquid interface deals with association of liquid-gas phase or liquid-liquid phase, Table 3.1. The word surface is used to designate the limit between a condensed phase and a gas phase, whereas the term interface is used for the boundary between two condensed phases.



(a) Liquid/Liquid Interface, for example, Oil on Water Surface



(b) Solid-liquid Interface, for example, Suspension

Figure 3.2: Types of Interfaces

The interface has applications such as adhesion between particles or granules, manufacturing of multilayer tablets, application of powders to body, flow of materials, and adsorption of colours etc. Whereas, solid-liquid interface has applications in the biopharmaceutical study, filtration processes, chemical interaction, adsorption studies, preparation of dispersed systems like colloids, emulsions, suspensions, wetting of solids etc.

Table 3.1: Classification of Surface/Interface of Systems

Phases	Type	Example
Gas/gas	No interface possible	Air
Gas/liquid	Liquid surface	Water exposed to air
Gas/solid	Solid surface	Bench top
Liquid/liquid	Liquid-liquid interface	Oil on water surface
Liquid/solid	Liquid-solid interface	Suspension
Solid/solid	Solid-solid interface	Powder mixture

3.2 SURFACE TENSION

The tension that exists between solid-gas phase and liquid-gas phase is known as surface tension. The origin of surface tension in a liquid is the cohesive force of attraction between the molecules that make-up the liquid. In the absence of other forces, this mutual force of attraction of the molecules causes the liquid to coalesce in accordance with the LaPlace law. In the bulk of liquid each molecule is pulled equally in all direction by neighbouring liquid molecule resulting in a net force of zero, Fig. 3.3.

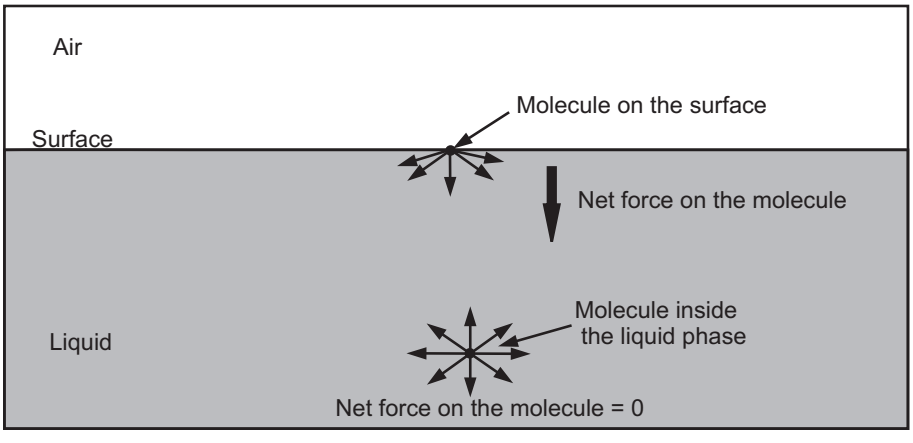


Figure 3.3: Tension at the Surface of Liquid

The molecules at the deep inside the bulk of the liquid pulls the molecules present at surface inwards, but there are no liquid molecules on the outside to balance these forces. There may be a small outward adhesive force of attraction caused by air molecule, but as air is much less dense than the liquid, this force is negligible. All of the molecules at the surface are therefore subject an inward force of cohesive molecular attraction leading to squeezing of liquid together until it has the lowest surface area possible. This force is the surface tension, defined as the magnitude of the force acting perpendicular to a unit length of a line at the surface. According to definition, surface tension is expressed as:

$$\gamma = \frac{F}{L} \qquad \ldots (3.1)$$

Where, the symbol γ represent surface tension and *F* is the force perpendicular to the length *l*. Surface tension is represented by different symbols like γ, τ or σ. A few examples of liquids with their surface tensions and interfacial tensions against water are given in Table 3.2.

SPHERICAL DROP:

As seen in previous section liquids has tendency to reduce its exposed surface to the smallest possible area and hence a drop of liquid tends to assume the shape of sphere. This phenomenon is attributed to cohesion, i.e. stronger attractive force acting between the molecules of the liquid, Fig. 3.4.

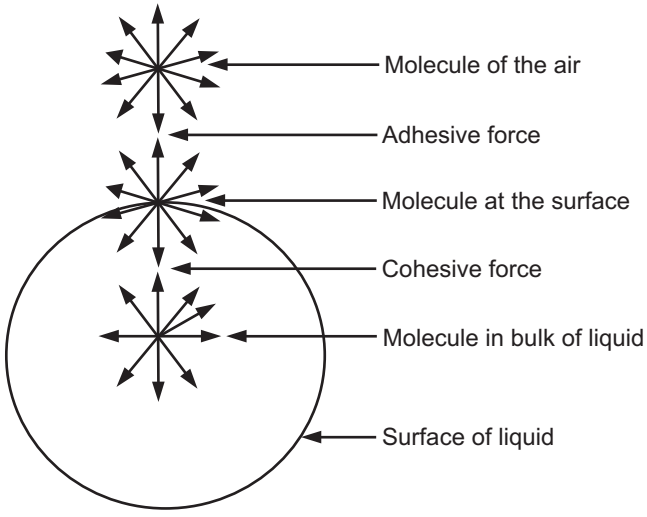


Figure 3.4: Forces Acting in the Formation of liquid Drop

The molecules within the liquid are attracted equally from all sides, but those near the surface experience unequal attractions and thus are drawn toward the centre of the liquid mass by this net force. The surface then appears to act like an extremely thin membrane, and the small volume of water that makes-up a drop assumes the shape of sphere. The spherical shape held constant with equilibrium between the internal pressures due to surface tension.

Unit of Surface Tension:

The CGS unit of surface tension is dyne/cm and SI unit is N/m. The relation between these units is as N/m is equal to 1×10^3 dyne/cm or dyne/cm is equal to m N/m.

3.3 INTERFACIAL TENSION

When two miscible liquids combined together no interface exist between them for example, ethyl alcohol and water mixture. Wherever, if two immiscible liquids combined there exists an interface between them. The tension exerted at the interface between them is due to difference in forces acting on molecules of immiscible liquids for example, chloroform and water, olive oil and water etc. Interfacial tension is defined as the force per unit length acting at right angle over the interface between two immiscible liquids. Interfacial tension

represents the strength of adhesive forces at the boundary between two immiscible liquids. Interfacial tension is useful in analyzing fluid reforming, spreading, emulsification, washability and other liquid characteristics. The surface tensions of some liquids and their interfacial tensions against water at 20 °C are given in Table 3.2.

Unit of Surface and Interfacial Tension:

Interfacial tension has units that of surface tension, that is dyne/cm or N/m.

Table 3.2: Surface and Interfacial Tensions (dyne/cm or mN/m) of Some Liquids at 20°C

Liquid	Surface tension	Interfacial tension against water
Water	72.75	–
n-Octanol	27.50	8.5
Carbon tetrachloride	26.8	45.0
Chloroform	27.10	32.8
Olive oil	35.8	22.9
n-hexane	18.4	51.1
Mercury	470.0	375.0
Oleic acid	32.5	15.6
Benzene	28.88	35.0
Ethyl ether	17.0	10.7
Glycol	47.7	
Ethyl alcohol	22.4	
Isopropyl alcohol	21.7	

3.4 SURFACE FREE ENERGY

The situation shown in Fig. 3.5 describe that free energy is present in the form of tension at the surface. Tension at the surface is helpful in maintaining the minimum surface possible. This energy is called as surface free energy. It can be defined, as the work required in increasing the area by one cm^2. The surface free energy can be derived from the following illustration.

ABCD is a three-sided frame with a movable bar CD of length L. A soap film is formed over the area ABCD. Applying a force F to movable bar, the film stretches to the downward.

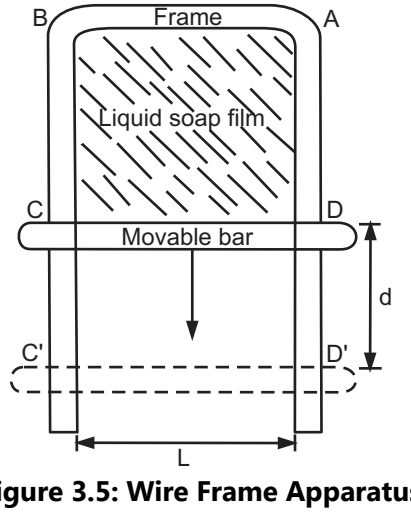


Figure 3.5: Wire Frame Apparatus

To break the film some force is required. If the applied force is less than what is required to break the film then the film retract due to surface tension. If the force F is applied on a movable bar CD, it shifts by a distance *d* to C'D'. The work done W is expressed as;

$$W = F \times d \quad \ldots (3.2)$$

While stretching of the film the force acts against the surface tension of the liquid as it try to contract the liquid. The soap film has liquid–gas interface. The total length of contact of the film is equal to double length of the bar because film has two surfaces on either side. Therefore, force acting on surface is expressed mathematically as

$$F = \gamma \times 2L \quad \ldots (3.3)$$

Substituting values of downward force F, in equation (3.2), gives;

$$W = \gamma \times 2L \times d \quad \ldots (3.4)$$

The quantity 2L is equal to increased surface area ΔA produced by extending the film. Then the equation (3.4) changes to:

$$W = \gamma \times \Delta A \text{ or } \Delta G = \gamma \times \Delta A \quad \ldots (3.5)$$

where, W is work done or increased surface energy expressed in ergs.

In the thermodynamic sense any form of energy can be split into two factors namely, intensity factor and capacity factor. In film stretching surface tension is the capacity factor. The equation (3.5) is applied in gas adsorption studies on the solid surfaces, in studying physical instability of suspensions and thermodynamic instability of emulsions. The dimensional analysis of work energy theorem shows that the unit of surface tension (N/m or dyne/cm) is equivalent to J/m^2. This means surface tension also can be considered as surface free energy.

Surface Free Energy Measurement:

Following are some methods used to measure free energy of solid materials.

Dyne Pen Method:

This method involves use of set of commercially available felt-tip pens containing a range of inks of known surface tension. One of the pens is used to apply a thin film of ink over area of test surface may be solid or liquid. If the ink film breaks-up into droplets in less than two seconds, the process is repeated using a pen with ink having a lower surface tension. This procedure is used to establish the lowest surface tension ink that yield a film that remains intact for at least two seconds. The value of the surface tension of the ink is taken as the surface free energy of the substrate.

Contact Angle Method:

In this method, a drop of liquid of known surface tension is placed on the test surface and then observed through a movable eyepiece. The eyepiece is connected to an electronic protractor, which displays the viewed angle. The construction of the angle is such that while viewing, angle equals the contact angle; the illumination viewed through the eyepiece is maximized. The contact angle and the surface tension of the liquid can then be used to calculate the surface energy of the test surface.

Interfacial Tensiometer Method:

The method involves use of tensiometer in which the solid is dipped into and retracted from a liquid of known surface tension. The variation of contact angle with immersion depth is measured and these values are used to calculate surface free energy of the solid. This method is used for the solids where all exposed faces have same composition.

Example 3.1: If the length of bar is 5 cm and the force required to break a soap film is 0.4 g. What is surface tension of soap solution? What is the work required to pull the wire by 1 cm?

Solution:

$$\gamma = \frac{\text{Force}}{2 \times L}$$

$$= \frac{0.4 \times 980.655}{2 \times 5}$$

$$= 39.226 \text{ dyne/cm}$$

$$\text{Work} = \gamma \times \Delta A$$

$$= 39.226 \times (2 \times 5)$$

$$= 392.26 \text{ ergs.}$$

Example 3.2: A surfactant solution having surface tension 37.3 dyne/cm is applied to metal frame bar of 5 cm. Calculate work required to pull down wire by 0.5 cm.

Solution:

$$\text{Work} = \gamma \times 2L \times d$$

$$= 37.3 \times 5 \times 5 \times 0.5$$

$$= 186 \text{ ergs}$$

3.5 CLASSIFICATION OF METHODS

Table 3.3: Classification of Methods to Determine Surface and Interfacial Tensions of Liquids and Solids

Liquids	Static methods	Surface tension	▪ Wilhelmy plate method
		Interfacial tension	▪ Spinning drop method
		Surface and Interfacial tension	▪ DuNouy ring method ▪ Pendant drop method
	Dynamic methods	Surface tension	▪ Capillary rise method ▪ Bubble pressure method ▪ Drop weight method
		Interfacial tension	▪ Drop volume method
		Surface and Interfacial tension	▪ Number drop method
Solids		Surface tension	▪ Sessile drop method ▪ Dynamic Wilhelmy method ▪ Single fiber Wilhelmy method ▪ Powder contact angle method

Measuring Techniques for Liquids

Static methods:

1. **DuNouy ring method:** The traditional method used to measure surface or interfacial tension. Wetting properties of the surface or interface have little influence on this measuring technique. Maximum pull exerted on the ring by the surface is measured.
2. **Wilhelmy plate method:** A universal method especially suited to check surface tension over long time intervals. A vertical plate of known perimeter is attached to a balance, and the force due to wetting is measured.
3. **Spinning drop method:** This technique is ideal for measuring low interfacial tensions. The diameter of a drop within a heavy phase is measured while both are rotated.
4. **Pendant drop method:** Surface and interfacial tension can be measured by this technique, even at elevated temperatures and pressures. Geometry of a drop is analyzed optically.

Dynamic methods:

1. **Capillary rise method:** A method for estimation of surface tension based on fact that most liquids when brought in contact with the fine glass capillary tube rises in tube above a level of the liquid outside the tube.
2. **Bubble pressure method:** A measurement technique for determining surface tension at short surface ages. Maximum pressure of each bubble is measured.
3. **Drop volume method:** A method for determining interfacial tension as a function of interface age. Liquid of one density is pumped into a second liquid of a different density and time between drops produced is measured.
4. **Drop weight method:** The process of drop formation by liquids is, in part, controlled by the surface tension of the fluid. To determine surface tension, the stalagmometer used. In a drop weight method average drop weight of specified volume of liquid are compared to those from a reference liquid.
5. **Number drop method:** In case of number drop method numbers of drops formed of specified volume of liquid are compared to those from a reference liquid. This is used to determine surface tensions as well as interfacial tensions.

Measuring Techniques for Solids:

1. *Sessile drop method*: Sessile drop method is an optical contact angle method. This method is used to estimate wetting properties of a localized region on a solid surface. Angle between the baseline of the drop and the tangent at the drop boundary is measured. It is ideal for curved samples, where one side of the sample has different properties than the other.

2. *Dynamic Wilhelmy method*: A method for calculating average advancing and receding contact angles on solids of uniform geometry. Both sides of the solid must have the same properties. Wetting force on the solid is measured as the solid is immersed in or withdrawn from a liquid of known surface tension.
3. *Single fiber Wilhelmy method*: Dynamic Wilhelmy method applied to single fibers to measure advancing and receding contact angles.
4. *Powder contact angle method*: Enables measurement of average contact angle and sorption speed for powders and other porous materials. Change of weight as a function of time is measured.

3.6 MEASUREMENT OF SURFACE TENSION

Capillary Rise Method:

This is a good method because the parallel walls of the test tube allow better viewing of the two meniscuses that need to be seen. Consider the simple situation as depicted in Fig. 3.6, in which the end of a capillary tube of radius r, is immersed in a liquid of density ρ. For sufficiently small capillaries, one observes a substantial rise of liquid up to height h, in the capillary as the force exerted on the liquid due to surface tension. The balance point can be used to measure surface tension. The surface tension acting along the inner circumference of the tube exactly counterbalances the weight of the liquid. The surface tension at surface of the meniscuses is due to the force acting per unit length at a tangent. If θ is the angle between capillary wall and the tangent, then the upward vertical component of the surface tension is $\gamma \cos \theta$. The total surface tension along the circular contact of meniscus is $2\pi r$ times $\gamma \cos \theta$. Therefore,

$$\text{Upward force} = (2\pi r\gamma) \cos \theta \quad \ldots (3.6)$$

Since, for most liquids θ is equal to zero, then $\cos \theta = 1$, and upward component reduces to $2\pi r\gamma$. The liquid is pulled downward by the weight of the liquid column. Thus,

$$\text{Downward force} = \text{Weight} = \text{Mass} \times g$$

$$= h\pi r^2 \rho g \quad \ldots (3.7)$$

At balance point, upward force is equal to downward force,

$$\text{Upward force} = \text{Downward force}$$

Substituting values of equation (3.6) and (3.7), we get,

$$(2\pi r\gamma) \cos \theta = h\pi r^2 \rho g \quad \ldots (3.8)$$

where, r is radius of capillary, h is the capillary rise, ρ is liquid density, g is acceleration due to gravity and γ is the surface tension of the liquid. Rearrangement of equation (3.8) gives a simple expression for surface tension:

$$\gamma = \frac{\rho\, grh}{2} \quad \ldots (3.9)$$

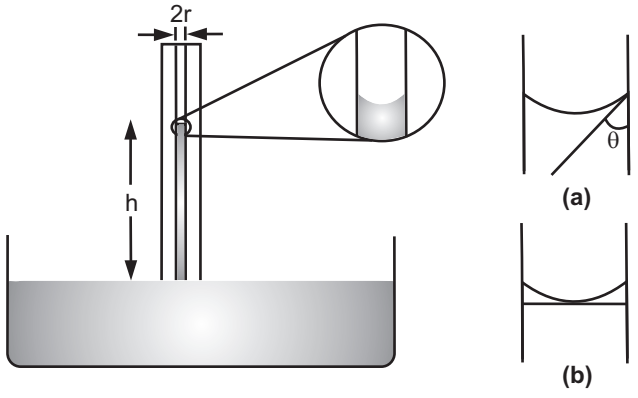


Figure 3.6: Rise in a Capillary Tube due to Surface Tension: (a) Contact Angle between Surface of Liquid and Capillary Wall (b) Mass of Liquid above Meniscus

A careful look at Fig. 3.6 (a) and (b), the meniscus boundary shows that the liquid surface in the tube is not perfectly flat. Instead it curves-up (or sometimes down, for example, mercury) at the wall to form a meniscus. The material in this region also contributes to the force of gravity, so one often finds correction to equation (3.9) to yield

$$\gamma = \frac{\rho g r \left(h + \frac{r}{3}\right)}{2} \quad \ldots (3.10)$$

where, the contact angle (the angle between the surface of the liquid and the inner wall of the glass of capillary) has been assumed to be zero.

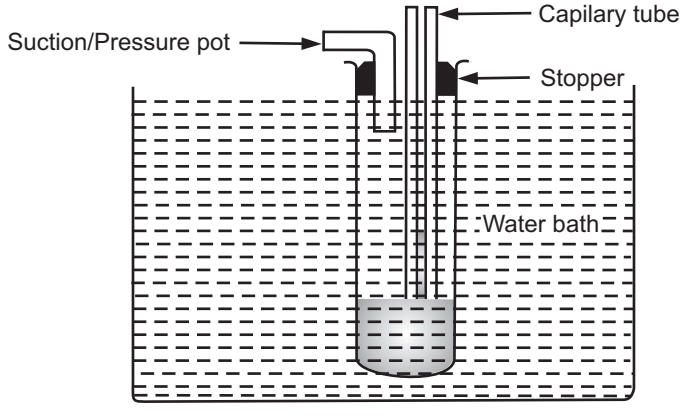


Figure 3.7: Schematic of the Device for Measuring Capillary Rise

By this method surface tension against the air is determined. The liquid in the capillary must be raised and lowered several times before making the first reading. To get good results the cleaned capillary should be soaked in nitric acid for several minutes, following by washing with deionized water. When not in use, the capillary should be stored in polyethylene bottle containing deionized water. The apparatus is shown in Fig. 3.7. A test tube is fitted with a two-hole stopper. Through one hole the capillary tube is fitted. The tube is fitted through a glass slave and held in place by a piece of rubber tubing. In the second

hole another tube is fitted through which pressure or suction can be applied. This whole apparatus is immersed in a water bath to allow control of temperature as change in temperature causes rapid disturbance in the liquid level. The apparatus is calibrated by determining capillary rise of deionized water, for which the temperature dependent surface tension is well known.

Example 3.3: The radius of a given capillary is 0.105 mm. A liquid whose density is 0.8 g/mL rises in this capillary to height of 6.25 cm; calculate the surface tension of the liquid.

Solution: The formula for calculation of surface tension by capillary rise method is

$$\gamma = \frac{1}{2} \rho g r h$$

$$\gamma = \frac{1}{2} (0.8 \times 0.0105 \times 6.25 \times 980.655) \quad [\therefore 0.105 \text{ mm} = 0.015 \text{ cm}]$$

$$\gamma = 25.74 \text{ dyne/cm}$$

The surface tension of liquid by capillary rise method is 25.74 dyne/cm.

Tensiometer:

Tensiometers are used to determine surface or interfacial tension with the help of an optimally wettable probe suspended from a precision balance. The probe is either a ring or a plate. A height adjustable sample carrier is used to bring the liquid to be measured into contact with the probe. A force acts on the balance as soon as the probe touches the surface. If the length of the plate or circumference of the ring is known, the force measured can be used to calculate the surface or interfacial tension. The probe must have a very high surface energy. The ring is made of platinum iridium alloy and plate is made of platinum.

DuNouy Ring Tensiometer:

Historically the ring method was the first to be developed; hence many of the values for interfacial and surface tension given in the literature are the results of the ring method. In this method, the liquid is raised until contact with the surface is observed. The sample is then lowered again so that the liquid film produced beneath the ring is stretched as shown in Fig. 3.8.

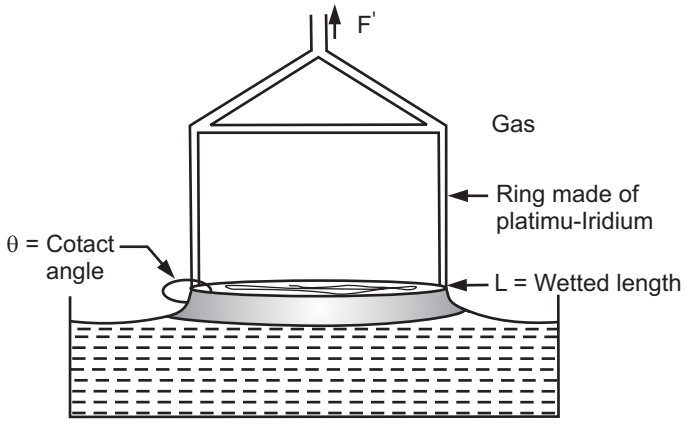


Figure 3.8: Schematic Diagram of the Ring Method

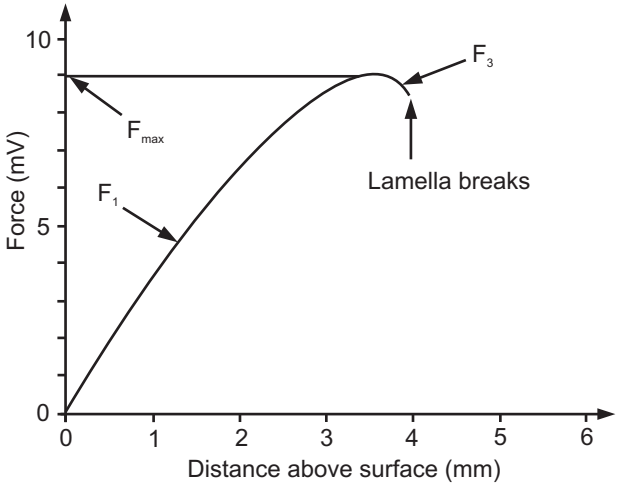


Figure 3.9: Change of Force with Ring Distance

As the film is stretched, a maximum force is experienced; this is recorded in the measurement. At the maximum, the force vector is exactly parallel to the direction of motion; at this moment, the contact angle θ is zero. The illustration in Fig. 3.9 shows the force change as the function of distance of ring from the surface of liquid. In practice the distance is first increased until the area of maximum force has been passed through. The sample trough containing the liquid is then moved back so that the maximum point is passed through a second time. The maximum force is only determined exactly on this return movement and used to calculate the surface tension. The following equation (3.11) is used for the calculation;

$$\gamma = \frac{[F_{max} - F_v]}{[L \times \cos \theta]} \quad \ldots (3.11)$$

where, γ is surface or interfacial tension, F_{max} is maximum force, F_v is weight of volume of liquid lifted, L is wetted length and θ is contact angle. The contact angle decreases as the extension increases and has the value zero degree at the point of maximum force, this means that the term cos θ has the value equal to 1.

Correction for the ring method:

The weight of the volume of the liquid lifted beneath the ring, expressed by the term Fv, must be subtracted from measured maximum force (F_{max}) as it also affects the balance. The curve of the film is greater at the inside of the ring than at outside. This means that maximum force (at contact of angle = 0°) is reached at different ring distances for the inside and outside of the ring; thus, the measured maximum force does not agree exactly with the actual value. Harkins and Jordan, have a drawn-up tables of correction values by determining different surface tensions of standard liquid with rings of different diameters. Zuidema and Waters scientists also obtained correction values for small interfacial tensions by extrapolating data given by Harkins and Jordan to cover the range of tensions accurately.

Advantages:

1. Many values in the literature have been obtained with the ring method; this means that in many cases the ring method should be preferred for comparison purposes.
2. As the wetted length of the ring is high it leads to a higher force on the balances so there has a better accuracy.
3. Small interfacial tensions can be obtained more accurately.
4. Cationic surfactants, which show poor wetting properties on platinum, the surface line between ring and liquid is more than that of plate.

Disadvantages:

1. Corrections are required for volume of liquid lifted beneath the ring.
2. Densities of the liquids are to be known.

Wilhelmy Plate Method:

In the Wilhelmy plate method the liquid is raised until the contact between the surface and the plate is observed. The maximum tension acts on the balance at this instance; this means that the sample does not need to be moved again during the measurement. Fig. 3.10 shows the illustrative diagram of Wilhelmy plate. Following equation (3.12) makes the surface tension calculation

$$\gamma = \frac{F}{[L \cos \theta]} \quad \ldots (3.12)$$

where, γ is surface or interfacial tension, F is force acting on the balance, L is the wetted length and θ is contact angle. The plate is made of roughened platinum and is optimally wetted so that contact angle is virtually a 0°. This means the term cos θ has a value of approximately the measured force and the length of plate need to be taken into consideration. Correction calculations are not necessary with plate method.

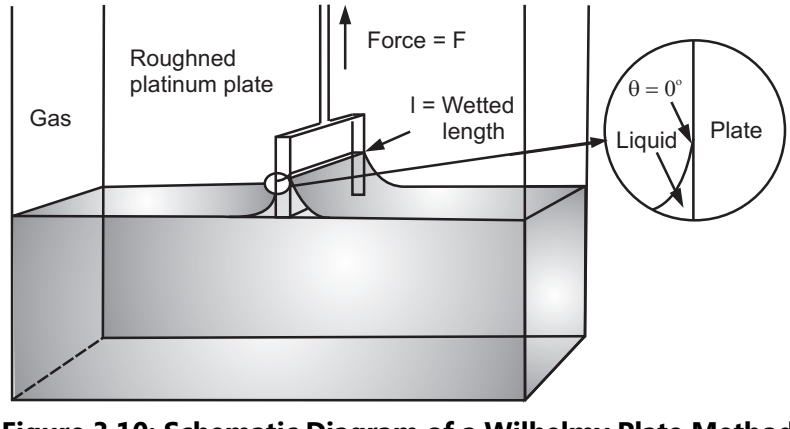


Figure 3.10: Schematic Diagram of a Wilhelmy Plate Method

Advantages:

1. No correction is required for measured values obtained by this method.
2. The densities of the liquids don't have to be known.

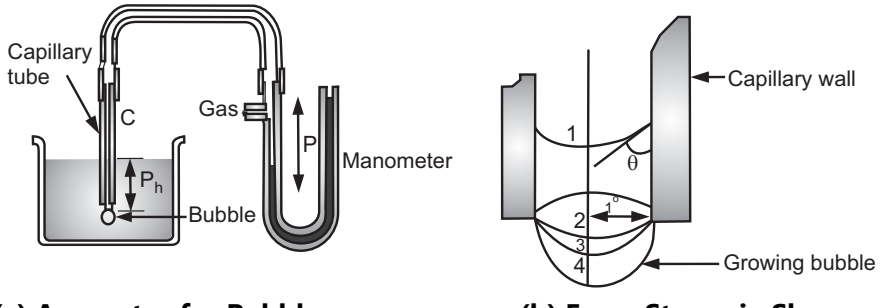
3. In an interfacial tension measurement, the surface is only touched and not pressed into or pulled out of the other phase; this avoids the phases becoming mixed.
4. This method is used for static measurement i.e. the plate does not move after the surface or interface has been detached. The surface or interface renewal and ultimately measurement failure is avoided.

Disadvantages:

1. The wetted length surface is small, so small force is required leading to variation in results.
2. Not suitable for cationic surfactants as platinum has poor wetting properties.

Maximum Bubble Pressure Method:

This is an easy method also called Jaegers method, for determining dynamic surface tensions of the liquids at short surface edges. The air pressure is applied slowly through a capillary tube immersed in the test liquid as shown in Fig. 3.11 (a). The gas bubble enters the liquid through a capillary whose radius is known. As pressure is applied gas bubble is formed at exactly defined rate at the end of the capillary. Initially the pressure is below maximum pressure (Pmax) the radius of curvature of the air bubble is larger than the radius of the capillary. When the pressure inside the tube is increased the pressure, curve passes through maximum and it is recorded by manometer attached to capillary tube.



(a) Apparatus for Bubble Pressure Method **(b) Force Stages in Slow Formation of Bubble**

Figure 3.11

At this stage, Fig. 3.11 (b), the air bubble radius is same as that of capillary, and it is of an exact hemisphere. At this point the force due to maximum pressure is equal to that of opposing forces the hydrostatic pressure (Ph) and the surface tension (γ) at the circumference (2πr) of the capillary. This relation between two opposing forces is expressed as:

$$P_{max}\,\pi r^2 = P_h + 2\pi r\,\gamma \quad \ldots (3.13)$$

$$P_{max} = P_h + \frac{2\gamma}{r} \quad \ldots (3.14)$$

$$P_{max} = h\,\rho\,g + \frac{2\gamma}{r} \quad \ldots (3.15)$$

where, r is radius of capillary tube, ρ is density of the liquid and h is the length of capillary tube immersed in liquid. After the maximum pressure, the 'dead time' of measurement starts.

The pressure decreases again and the radius of air bubble becomes larger, Fig. 3.12. The bubble finally escapes from the capillary. The cycle begins again with the formation of new bubble. Knowing the values of P_{max}, h, ρ and r, surface tension of the liquid can be obtained.

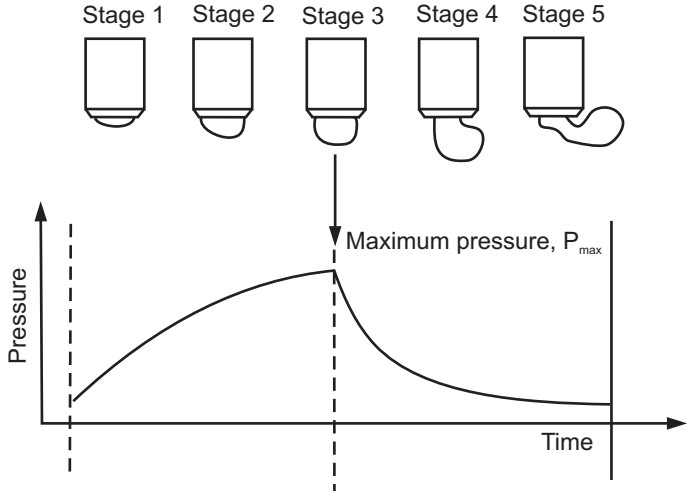


Figure 3.12: Graphical Stagewise Schematic Showing Maximum Pressure (P_{max})

Pendant Drop Shape Method:

The shape of a drop of liquid hanging from a syringe tip in immiscible liquid of different density is determined from surface tension of that liquid. The surface or interfacial tension at the liquid interface can be related to the drop shape through the following equation:

$$\gamma = \frac{\Delta\rho\, g\, r^2}{\beta} \quad \ldots (3.16)$$

where, γ is surface tension, Δρ is difference in density between liquids at interface, g is gravitational constant, r^2 is radius of drop curvature at apex and β is shape factor. The shape factor can be defined through the Young-LaPlace equation expressed as three dimensionless first order equations as shown in the equation (3.17) below.

$$\frac{d\theta}{ds} = 2 + \beta z - \frac{\sin\theta}{x} \quad \ldots (3.17)$$

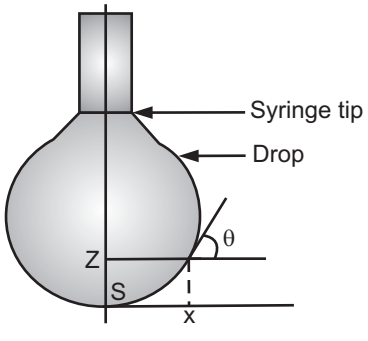


Figure 3.13: Force Acting on a Hanging Drop

Modern computational methods with interactive approximations use the Young-LaPlace equation to determine β. Thus, for any pendant drop where the densities of the two liquids in contact are known, the surface tension may be measured based upon the Young-LaPlace equation.

Advantages:

1. Easy and accurate compare to traditional methods.
2. Able to use very small volumes.
3. Measures low interfacial tensions.
4. Measures surface tensions of molten materials easily.
5. High quality surface and interfacial measurements can be made.

Drop Weight Method:

The apparatus used in this method is stalagmometer. It is pipette-having capillary below and above the bulb as shown in Fig. 3.14. About twenty drops of test liquid are collected from the stalagmometer in a weighing bottle and weighed to determine average weight of a drop. Similar type of determinations is carried out for the reference liquid after properly cleaning the apparatus. When the drop is formed at the tip of the stalagmometer, it is supported in upward direction by force of surface tension (γ) acting at the outer circumference ($2\pi r$) of the stalagmometer tip, while the downward force acting on the drop is its weight ($m \times g$).

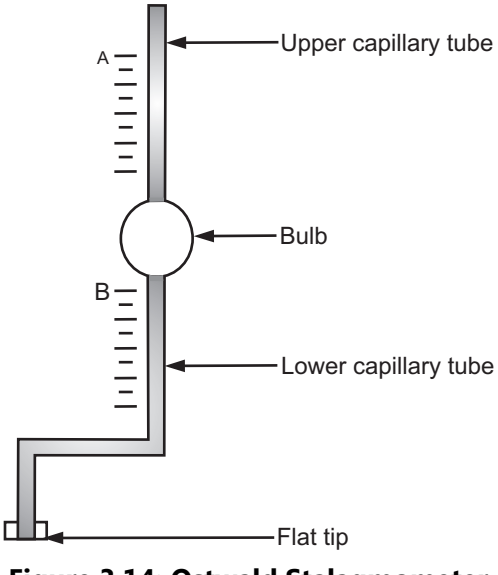


Figure 3.14: Ostwald Stalagmometer

When upward and downward forces are equal the drop breaks from the surface and at the point of breaking this situation is expressed as:

$$\text{Upward force} = \text{Downward force}$$

$$2\pi r \gamma = m \times g \quad \ldots (3.18)$$

where, r is outer radius of the stalagmometer, γ is surface tension of the liquid, m is mass of the drop and g is acceleration due to gravity. From equation (3.18), for liquid$_1$ and liquid$_2$, we get

$$2\pi r \gamma_1 = m_1 g \quad \ldots (3.19)$$

$$2\pi r \gamma_2 = m_2 g \quad \ldots (3.20)$$

Dividing equation (3.19) by (3.20)

$$\frac{\gamma_1}{\gamma_2} = \frac{m_1}{m_2} \quad \ldots (3.21)$$

Substituting the values of average weights per drop of liquids and surface tension of reference liquid, surface tension of liquid under test can be calculated.

Harkin's and Brown have shown that the surface tension of the liquid may be determined from the weight (W) of the falling drop and the radius (r) of the capillary tip by the relation

$$\gamma = \frac{Wg}{2\pi r\varphi} \quad \ldots (3.22)$$

where, the term φ is the function of ($r/V^{1/3}$) with V the volume of drop.

Example 3.4: In measuring the surface tension of a liquid by the drop weight method, 12 drops of the liquid falling from the tip whose diameter is 0.8 cm are found to weigh 0.971g. If φ = 0.6 under these conditions, what is surface tension of the liquid?

Solution:

$$\gamma = \frac{Wg}{2\pi r\varphi}$$

$$\gamma = \frac{0.08 \times 980.655}{2 \times 3.14 \times 0.4 \times 0.6} \qquad \left[\therefore \frac{0.971}{12} = 0.08 \text{ g}\right]$$

$$\gamma = 52.30 \text{ dyne/cm}$$

The surface tension of liquid by weight drop method is 52.30 dyne/cm.

Example 3.5: Using stalagmometer 10 mL each of water and test liquid formed 35 and 46 drops, respectively. If density of liquid is 0.913 g/mL and surface tension of water is 72.75 dyne/cm, calculate surface tension of test liquid.

Solution:

$$\gamma_2 = \left[\frac{\eta_1\rho_2}{\eta_2\rho_1}\right] \times \gamma_1$$

$$= \left[\frac{35 \times 0.913}{46 \times 1}\right] \times 72.75$$

$$= 50.53 \text{ dyne/cm}$$

Number Drop Method:

In this method number of drops of some fixed volume of reference liquid and test liquid are determined by using Stalagmometer. If V is the volume of liquid between two marks A and B as shown in Fig. 3.14, ρ_1 and ρ_2 are densities and n_1 and n_2 are number of drops of

reference liquid and test liquid, respectively, the volume of one drop of liquid is V/n and the mass is equal to (V/n) ρ. Thus, as per equation (3.21)

For reference liquid$_1$ $2\pi r \gamma_1 = (V/n_1) \rho_1 g$ … (3.23)

And for the test liquid$_2$ $2\pi r \gamma_2 = (V/n_2) \rho_2 g$ … (3.24)

Dividing equation (3.23) by (3.24) and on simplification we get

$$\gamma_2 = \left[\frac{n_1 \rho_2}{n_2 \rho_1}\right] \gamma_1 \quad \text{… (3.25)}$$

where, γ_1 and γ_2 are surface tensions of the liquid$_1$ and liquid$_2$, respectively. If surface tension of one liquid is known the surface tension of other liquid can be calculated by equation (3.25).

3.7 MEASUREMENT OF INTERFACIAL TENSION

In addition to the DuNouy ring, pendant drop and number drop methods used for determining surface tension and interfacial tensions, the drop volume method and the spinning drop method are exclusively used for interfacial tension determination.

Drop Volume Method:

A drop volume tensiometer is an instrument for determining the dynamic interfacial tension. Drops of a liquid are produced in a vertical capillary in a surrounding second liquid. The volume at which the drops detach from the tip of the capillary is measured. The dynamic surface tension can also be measured if measurements are made in air as the bulk phase. In the drop volume method, a liquid is introduced into a bulk phase through a capillary. A drop, which tries to move upwards due to buoyancy, forms at the tip of the capillary. The reverse arrangement, in which drops of the heavy phase drop from the tip of the capillary, is also possible.

Because of the interfacial tension (γ) the drop tries to keep the interface with the bulk phase as small as possible. As a new interface comes into being when the drop detaches from the capillary outlet, it is necessary to overcome the corresponding interfacial tension. The drop does not detach until the lifting force or weight compensates for the force resulting from the interfacial tension on the wetted length of the capillary, the circumference. The formula for this relationship is:

$$\sigma = \frac{V \Delta \rho g}{\pi d} \quad \text{… (3.26)}$$

g = Acceleration due to gravity,

V = Drop volume,

d = Inside diameter of capillary,

Δρ = Difference in density between the phases.

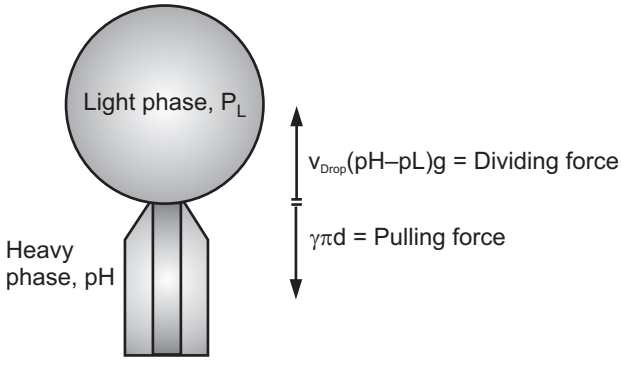


Figure 3.15: Measuring Principle of a Drop Volume Tensiometer

Spinning Drop Method:

A spinning drop tensiometer is an instrument for determining the interfacial tension. Here, a horizontally arranged capillary filled with a bulk phase and a specifically lighter drop phase is set in rotation. The diameter of the drop which is elongated by centrifugal force correlates with the interfacial tension.

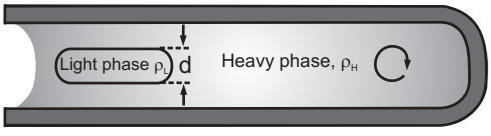


Figure 3.16: Schematic Diagram of the Spinning Drop Method

When a heavy bulk phase and a light drop phase is situated in a horizontal, rotating capillary, the drop radius perpendicular to the axis of rotation depends on the interfacial tension γ between the phases, the angular frequency ω of the rotation and the density difference $\Delta\rho$. Thus, with a given speed of rotation and with known densities of the two phases, the interfacial tension can be calculated from the measured drop diameter d (= 2r) in accordance with Vonnegut's equation:

$$\sigma = \frac{r^3 \omega^2 \Delta\rho}{4} \quad \ldots (3.27)$$

The drop diameter is determined from the video image of the drop by means of drop shape analysis. The length of the drop along the axis of rotation must be at least four times the diameter of the drop to minimize the error due to the curvature of the interface. Extremely low interfacial tensions can be measured with a spinning drop tensiometer. The spinning drop method is frequently used when the conditions for forming a micro-emulsion are to be investigated, e.g. with surfactant flooding in enhanced oil recovery (EOR) or in solvent-free degreasing.

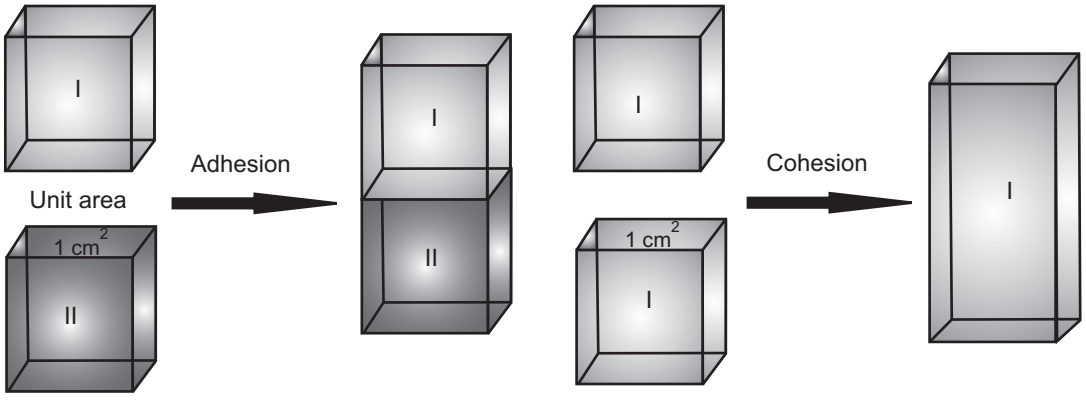
Comparison of Methods:

Of the several methods exist for surface and interfacial tension determinations there is no method available which suits all types of systems. Basically, choice depends on accuracy, sample size, whether surface or interfacial tension or effect of time on surface tension is to be determined.

3.8 SPREADING COEFFICIENT

Spreading can be observed by adding one liquid to surface of other liquid. The supporting liquid for instance is designated with 'II' while the liquid being added to the top by 'I', since it initially forms a lens. There are two possibilities, first, the liquid can spread over the surface of sublayer liquid or second, the added liquid will contract into a small lens on the surface of sublayer liquid. We can predict what will happen to the system by determining net loss in free energy. The spreading of liquid is controlled by surface tensions of pure immiscible liquids and interfacial tension between them.

The concept of work of cohesion and work of adhesion help to understand the spreading of one liquid on another and predict whether it would spread spontaneously or not. There exists a mathematical relationship, which can be used to forecast the outcome of the situation called as spreading coefficient, denoted by S.



(a) Work of Cohesion **(b) Work of Adhesion**

Figure 3.17: Schematic Presentation of Spreading of a Liquid

Work of Cohesion:

Let's consider a column of some liquid as shown in Fig. 3.17(a) , who's cross-sectional area is 1 cm^2. On application of force to the liquid it separates with formation of two new surfaces each of 1 cm^2 area. The work done in separation of liquids is work of cohesion. It is defined, as a work required in separating the molecules of the spreading liquid so that it can flow over the sub-layer liquid. When the liquid alone is considered, no interfacial tension exists as cohesive forces are operating. Since two new surfaces are created the area becomes 2 cm^2. The work of cohesion is denoted by Wc and is equal to surface tension times the amount of new area created. As per definition the work of cohesion is written as;

$$W_c = 2\gamma_1 \quad \ldots (3.28)$$

where, γ_L is surface tension of liquid.

Work of Adhesion:

Work of adhesion, W_a, is the work done to destroy the adhesion between unlike molecules. Let's imagine the situation as shown in Fig 3.17 (b), where the column of liquid is

made-up of two immiscible liquids like oil and water. If a force is applied along the liquid column to cause the liquids to separate in to two parts, the work done is work of adhesion. Here by destroying 1 cm^2 interface between sublayer and spreading liquid we have created a 1 cm^2 surface of sublayer liquid and 1 cm^2 surface of spreading liquid. The work of adhesion is then expressed as;

$$W_a = \gamma_1 + \gamma_2 - \gamma_{12} \quad \ldots (3.29)$$

where, γ_L is surface tension of spreading liquid, γ_2 is surface tension of sub-layer liquid and γ_{12} is interfacial tension between them.

Spreading Coefficient:

The spreading coefficient is obtained by following equation

$$S = W_a - W_c \quad \ldots (3.30)$$

Substituting values from equation (3.28) and (3.29) we get

$$S = (\gamma_1 + \gamma_2 - \gamma_{12}) - 2\gamma_1 \quad \ldots (3.31)$$

The coefficient of each of the term is one because we either created or destroyed 1 cm^2 surface or interface respectively. On simplifying equation (3.31) we get

$$S = \gamma_2 - \gamma_1 - \gamma_{12}$$

$$S = \gamma_2 - (\gamma_1 + \gamma_{12}) \quad \ldots (3.32)$$

A positive value of S means that the liquid will spread and negative means it will not. Water has surface tension of 72.8 dyne/cm, benzene has surface tension of 28.9 dyne/cm, and interfacial tension between them is 35 dyne/cm. If benzene is added to water surface, there exists two possibilities. First, the benzene can spread over the surface of water if S is positive or second, it will contract into small lens on surface of water if S is negative. On substituting these values of surface and interfacial tension of water and benzene in equation (3.32),

$$S = 72.8 - (28.9 + 35)$$

$$S = 8.9$$

As the value of spreading coefficient is positive benzene will spread on the surface of water. In spreading of organic liquids on surface of water, the initial spreading coefficient may be positive or negative, but the final spreading coefficient is negative. On addition of benzene to water, even though polar groups are absent in benzene, it spreads over the water due to stronger adhesive forces over the cohesive forces. With time benzene begins to saturate the water and surface tension of water saturated with benzene decreases to 62.0 dyne/cm.

Now substituting values in equation (3.32) we get

$$S = 62.2 - (28.9 + 35)$$

$$S = -1.7$$

Since value of S is negative, benzene contract on the surface of water and forms a lens. The spreading coefficient of substances depends on their structures, especially presence of

polar functional groups and non-polar carbon chain length. Polar substances such as acids and alcohols spread more freely compared to non-polar hydrocarbons like benzene, octane and liquid petroleum due to presence of polar groups.

Table 3.4: Spreading Coefficients of Some Liquids on Water

Spreading Liquid	S
Benzene	8.10
Ethyl ether	45.44
Ethanol	50.00
Liquid petroleum	– 13.41
Oleic acid	24.21
Propionic acid	45.80
Octane	– 0.22

The phenomenon of spreading coefficient is useful in improving bioavailability of drugs in dosage forms like creams and lotions by addition of surfactant to increase polarity as well as spreadability. Spreading coefficient values of blend of surfactants help to select proper combination of blend which improves stability of emulsions. Spreading of liquid on solid surface is also useful tool in designing quality pharmaceutical suspension by improving wettability. Some values of spreading coefficients of liquids are listed in Table 3.4.

Example 3.6: At 20 °C the surface tension of water and chloroform are 72.75 and 27.10 dyne/cm, respectively while the interfacial tension between the two is 32.8 dyne/cm. Calculate (a) work of cohesion (b) work of adhesion and (c) the spreading coefficient of chloroform on water. Will chloroform spread on water?

Solution: (a) Work of cohesion:

$$\begin{aligned} Wc &= \gamma_1 + \gamma_2 + \gamma_{12} \\ &= 27.10 + 72.75 - 32.8 \\ &= 99.85 - 32.8 \\ &= 67.05 \end{aligned}$$

(b) Work of adhesion:

$$\begin{aligned} Wa &= 2\,\gamma_1 \\ &= 2 \times 27.10 \\ &= 54.20 \end{aligned}$$

(c) Spreading coefficient:

$$\begin{aligned} S &= \gamma_2 - \gamma_1 - \gamma_{12} \\ &= 72.75 - 27.10 - 32.8 \\ &= 12.85 \end{aligned}$$

Since, the value of spreading coefficient is positive, chloroform will spread on the surface of water.

3.9 ADSORPTION AT LIQUID INTERFACES

Like surface tension, adsorption is a consequence of surface energy. The molecules from bulk of liquid are brought to the interface. In bulk of liquid, all the bonding requirements, such as ionic or covalent of the constituent atoms of the molecule are fulfilled, but atoms at the clean surface experience a bond deficiency, because they are not wholly surrounded by similar other atoms. Thus, it is energetically favorable for them to bond with whatever happens to be available. The exact nature of bonding depends on the species involved. According to this principle greater the molecules and ions that are dispersed in liquid, they move towards the interface decreasing their concentration in the bulk and accumulates at the interface, this leads to reduction in surface free energy of the system. This phenomenon is known as adsorption. Adsorption is a process that occurs when added molecules partitioned to surface forming a molecular or atomic film. More specifically it is regarded as positive adsorption. It is different from absorption, where added molecules diffuse into a liquid to form solution also called negative adsorption or reverse adsorption. The term sorption encompasses both process namely adsorption and absorption.

Amphiphiles:

Paul Winsor coined the word amphiphile 60 years ago. It comes from Greek roots *amphi* which means, "double", "from both sides", "around", as in amphitheater or amphibian and *philos* that expresses friendship or affinity, as in "philanthropist" (the friend of man), "hydrophilic" (compatible with water), or "philosopher" (the friend of wisdom or science). An amphiphilic substance exhibits a double affinity, which can be defined from the physico-chemical point of view as a polar/non-polar duality. A typical amphiphilic molecule consists of two parts: on the one hand a polar group which contains heteroatoms such as O, S, P, or N, included in functional groups such as alcohol, thiol, ether, ester, acid, sulfate, sulfonate, phosphate, amine, amide etc. On the other hand, an essentially non-polar group, which is in general a hydrocarbon chain of the alkyl or alkylbenzene type sometimes with halogen atoms and even a few non-ionized oxygen atoms.

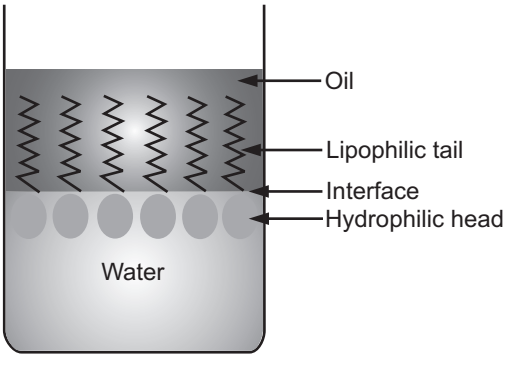


Figure 3.18: Orientation of Amphiphile in Oil-water System

An important case of adsorption on liquid surfaces is that of surface-active molecules. The polar portion exhibits a strong affinity for polar solvents, particularly water, and it is often

called hydrophilic part or hydrophile, Fig. 3.18. The non-polar part is called hydrophobe or lipophile, from Greek roots Phobos (fear) and Lipos (grease). The following structure is an example of amphiphilic molecule commonly used in shampoos.

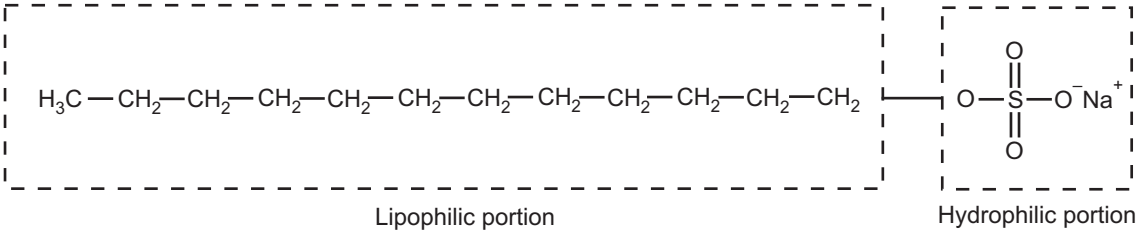


Figure 3.19: Sodium Dodecyl (ester) Sulfate an Amphiphile

3.10 SURFACE ACTIVE AGENTS

Because of dual affinity of an amphiphilic molecule it does not feel "at ease" in any solvent, be it polar or non-polar, since there is always one of the groups which "does not like" the solvent environment. Therefore, amphiphilic molecules exhibit a very strong tendency to migrate to interfaces or surfaces and to orientate so that the polar group lies in water and the non-polar group is placed out of it, and eventually in oil. In English, the term surfactant (surface-active-agent) designates a substance, which exhibits some superficial or interfacial activity. Only the amphiphiles with equilibrated hydrophilic and lipophilic tendencies are likely to migrate to the surface or interface. It does not happen if the amphiphilic molecule is too hydrophilic or too hydrophobic, in which case it stays in one of the phases.

In other languages, such as French, German or Spanish the word "surfactant" does not exist, and the actual term used to describe these substances is based on their properties to lower the surface or interface tension, for example, tensioactif (French), tenside (German), tensioactivo (Spanish). This would imply that surface activity is strictly equivalent to tension lowering, which is not general, although it is true in many cases. Amphiphiles exhibit other properties than tension lowering and therefore they are often labelled as per their main use such as: soap, detergent, wetting agent dispersant, emulsifier, foaming agent, bactericide, corrosion inhibitor, antistatic agent etc. In some cases, they are known from the name of the structure they can build, i.e. membrane, microemulsion, liquid crystal, liposome, vesicle or gel.

CLASSIFICATION OF SURFACTANTS

From the commercial point of view surfactants are often classified as per their use. The most accepted and scientifically sound classification of surfactants is based on their dissociation in water.

Anionic Surfactants:

Anionic Surfactants are dissociated in water in an amphiphilic anion, and a cation, which is in general an alkaline metal (Na^+, K^+) or a quaternary ammonium. They are the most commonly used surfactants. They include alkylbenzene sulfonates (detergents), (fatty acid) soaps, lauryl sulfate (foaming agent), di-alkyl sulfosuccinate (wetting agent), lignosulfonates (dispersants) etc.

Non-ionic Surfactants:

Nonionic Surfactants do not ionize in aqueous solution, because their hydrophilic group is of a non-dissociable type, such as alcohol, phenol, ether, ester, or amide. A large proportion of these nonionic surfactants are made of hydrophilic portion (by the presence of a polyethylene glycol chain) and lipophilic portion (alkyl or alkylbenzene)

Cationic Surfactants:

Cationic Surfactants are dissociated in water into an amphiphilic cation and an anion, most often of the halogen type. A very large proportion of this class corresponds to nitrogen compounds such as fatty amine salts and quaternary ammoniums, with one or several long chains of the alkyl type, often coming from natural fatty acids. They are used as bactericide and as positively charged substance, which can adsorb on negatively charged substrates to produce antistatic and hydrophobant effect. When a surfactant molecules exhibit both anionic and cationic dissociations it is called amphoteric or zwitterionic, for example, betaines or sulfobetaines and natural substances such as amino acids and phospholipids.

Polymeric Surfactants:

Polymeric surfactants are often not accounted as surfactants. Their importance is growing however; because they enter in many formulated products as dispersants, emulsifiers, foam boosters, viscosity modifiers, etc. Some of them commonly used are polyEO-PolyPO block copolymers, ethoxylated or sulfonated resins, carboxymethyl cellulose and other polysaccharide derivatives, polyacrylates, xanthane etc.

3.11 HLB SCALE

The hydrophilic lipophilic balance (HLB) system is based on the concept that some molecules of surfactants are having hydrophilic groups; other molecules have lipophilic groups and some have both hydrophilic and lipophilic groups called amphiphilic molecules. Hydrophilic and lipophilic portions dissolve in aqueous and oily phase. It is useful to correlate and measure these characteristics of the surfactants by some means for their applications in various fields such as to formulate various dispersed systems like lotions and emulsions. A common system, which is used to express the amphiphilic nature as a balance between hydrophilic and lipophilic portion of the molecule is called as HLB system.

Weight percentage of each type of group in a surfactant molecule or in a mixture of surfactants predicts what behaviour the surfactant molecular structure will exhibit. Griffin in 1949 and its latter development in 1954 introduced the HLB system, a semi-empirical method. It is the number on scale of 1 to 40, as shown in Fig. 3.20. The HLB value for a given surfactant is the relative degree to which the surfactant is water-soluble or oil soluble. An emulsifier having a low HLB number indicates that the number of hydrophilic groups present in the molecule is less and it has a lipophilic character. For example, spans generally have low HLB number and they are also oil soluble. Because of their oil soluble character, spans cause the oil phase to predominate and form a w/o emulsion.

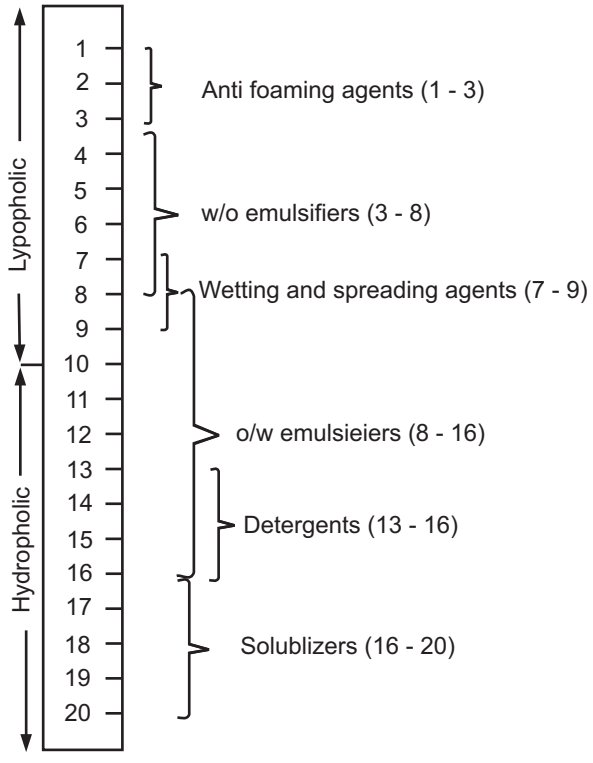


Figure 3.20: HLB Scale Showing Functions of Surfactants along with Their HLB Range

A higher HLB number indicate that the emulsifier has a large number of hydrophilic groups on the molecule and therefore is more hydrophilic in character. Tweens have higher HLB numbers and they are also water soluble. Because of their water-soluble character, Tweens will cause the water phase to predominate and form an o/w emulsion.

The usual HLB range is from 1 to 20, while there is one exception to this range as shown in Table 3.5 at the bottom. Sodium lauryl sulphate, a surfactant dissolves in water very well and is common additive to most of the heterogeneous systems and to almost all common detergents. As HLB value is additive, the blending of surfactants with known HLB values to get a desired one is very easy. The appropriate HLB values are calculated by various methods.

Table 3.5: HLB Values of Some Surfactants

Use	Example	HLB
Antifoaming agent	Oleic acid	1
	Sorbitan tristearate	2
	Glyceryl monostearate	3
Emulsifying agent (w/o)	Sorbitan mon-oleate (Span 80)	4
	Glyceryl monostearate	5
	Diethylene glycol monolaurate	6

... (Contd.)

Use	Example	HLB
Emulsifying (w/o), wetting and spreading agents	(None)	7
	Sorbitan monolaurate (span 20)	8
	Polyethylene lauryl ether (Brij 30)	9
Emulsifying agents (w/o)	Methyl cellulose (Methocel 15 cps)	10
	Polyxyethylene monostearate (Myrj 45)	11
	Triethanolamine oleate	12
Emulsifying agents (o/w) and detergents	Polyethylene glycol 400 monolaurate	13
	None	14
	Polyxyethylene sorbitan mon-oleate (Tween 80)	15
Emulsifying (o/w), solubilizing agent, detergents	Polyxyethylene sorbitan monolaurate (Tween 80)	16
Solubilizing agents	Polyxylene lauryl ether (Brij 35)	17
	Sodium oleate	18
	None	19
	Potassium oleate	20
Everything	Sodium lauryl sulfate	40

Methods to Determine HLB:

Method I - Alligation or Algebraic manipulations:

If *a* and *b* are the HLB values of surfactant A and B, respectively, and the *c* is desired HLB value then proportional parts required of A and B surfactants are x and y, respectively.

$$\frac{x}{y} = \frac{(c - a)}{(a - c)} \quad \ldots (3.33)$$

Or $$HLB_{Blend} = f\,HLB_A + (1 - f)\,HLB_B \quad \ldots (3.34)$$

where, *f* is fraction of surfactant A and (1 − *f*) is fraction of surfactant B in the surfactant blend.

Method II - Water dispersibility:

Approximation of HLB for those surfactants and not described by Griffin can be made either from characterization of their water dispersibility, Table 3.6.

Method III - Experimental estimation:

From experimental estimations blends of unknown surfactants in varying ratio with an emulsifier of known HLB are used to emulsify oil. The blend that performs best is assumed to have a value approximately equal to the required HLB of the oil.

Table 3.6: Estimation of HLB of Surfactants based on Water Dispersibility

HLB range	Water dispersibility
1 – 4	Not dispensible
3 – 6	Poorly dispersible
6 – 8	Milky dispersion only on vigorous agitation
8 – 10	Stable milky dispersion
10 – 13	Translucent to clear dispersion
≥ 13	Clear solution

Method IV – Group contribution method:

Davis and Rideal suggested an empirical calculation of HLB based upon the positive and negative contribution of various functional groups to the overall hydrophilicity of a surfactant. Substituting values given in Table 3.7 for various group numbers in equation 3.35 gives HLB of a surfactant.

$$HLB = \sum (\text{Hydrophilic group number}) - \sum (\text{Lipophilic group number}) + 7 \quad \ldots (3.35)$$

Table 3.7: HLB Contribution of Hydrophilic and Lipophilic Groups

Hydrophilic groups	Group number	Lipophilic groups	Group number
$-SO_4^- Na^+$	38.7	−CH =	0.475
$-COO^- K^+$	21.1	$-CH_2-$	0.475
$-COO^-Na^+$	19.1	$-CH_3-$	0.475
$SO_3^-Na^+$	11.0	$-CH_4$	0.475
R_2N	9.4	$-CF_2-$	0.870
−COOH	2.1	$-CF_3$	0.870
−OH (free)	1.9		
−O−	1.3		
$-(OCH_2CH_2)-$	0.5		
$-(OCH_2CH)-$	0.33		
−OH (sorbitan ring)	0.15		
Ester (sorbitan ring)	6.8		
Ester (free)	2.4		

Experimental estimations of HLB values of lanolin derivatives like bees wax and wool fat cannot be obtained easily. Each atom or group has assigned a constant and used in calculation of HLB. For example, if surfactant contains Polyoxyethylene chains, the HLB is calculated by equation;

$$HLB = \frac{E + P}{5} \quad \ldots (3.36)$$

where, E and P are percent by weight of oxyethylene chains and polyhydric alcoholic groups, respectively in the surfactant molecule. When the molecule contains only oxyethylene groups then, HLB is calculated by equation:

$$HLB = \frac{E}{5} \quad \ldots (3.37)$$

HLB values of surfactants such as glyceryl monostearate that contain fatty acid esters and polyhydric alcohols is calculated by equation:

$$HLB = 20\left(1 - \frac{S}{A}\right) \quad \ldots (3.38)$$

where, S is saponification number and A is acid number.

Saponification number is defined as the number of milligrams of potassium hydroxide required to neutralize the acid formed during saponification of one gram of sample. Acid number is the number of milligrams of potassium hydroxide required to neutralize the free acid in one gram of sample. One part of structure of glyceryl monostearate contains a fatty acid stearic acid, which is lipophilic in nature while other part is alcohol, which is hydrophilic in nature. Therefore, analysis of these parts by saponification gives HLB estimates.

Factors Affecting HLB Value:

1. Nature of immiscible phase
2. Presence of additive
3. Concentration of surfactant
4. Phase volume
5. Temperature

Drawbacks of HLB system:

HLB system provides only information about the hydrophilic and lipophilic nature of the surfactants but concentration of these surfactants is not considered. For optimum stability and therapeutic safety concentrations of the surfactant are equally important. It does not consider the effect of temperature as well as the presence of other additives.

Example 3.7: Calculate overall HLB value of a mixture of 30% Span 80 and 70% Tween 80.

Solution: HLB value of span 80 is 4.3 and that of Tween 80 is 15. Therefore,

$$HLB = (0.3 \times 4.3) + (0.7 \times 15)$$

$$HLB = 11.8$$

The overall HLB of surfactant mixture is 11.8.

Example 3.8: Surfactant A has HLB of 16 and surfactant B has HLB of 4. What would be the HLB of a surfactant made when 1 part of surfactant A is added to 3 parts of surfactant B?

Solution:

$$HLB_{mixture} = f\,HLB_A + (1 - f)\,HLB_B$$

$$= \frac{1}{4}(16) + \left(1 - \frac{1}{4}\right)4$$

$$= 4 + \left(\frac{3}{4}\right)4$$

$$= 7$$

The HLB of surfactant mixture is 7.

Example 3.9: Surfactant A has HLB of 15 and surfactant B has HLB of 6. What fraction of surfactant. A would be used to produce surfactant of HLB 9?

Solution:

$$HLB_{mixture} = f\,HLB_A + (1 - f)\,HLB_B$$

$$9 = f\,(15) + (1 - f)\,6$$

$$= 15f + 6 - 6f$$

Since, f = 1/3, therefore 1/3 of surfactant A and 2/3 of surfactant B produces HLB of 9.

Example 3.10: What would be HLB value of blend of equal amounts of Polysorbate 80 and Sorbitan monooleate 80?

Solution: The HLB value of a blend of equal amounts of Polysorbate 80 (HLB 15.0) and Sorbitan monooleate 80 (HLB 4.3) is calculated as

$$HLB = 15\left(\frac{1}{2}\right) + 4.3\left(\frac{1}{2}\right)$$

$$HLB = 9.65$$

3.12 SOLUBILIZATION

When drugs are in development, one property that is essential to its success is its solubility. Although water is widely used, most drugs being organic will not go into an aqueous solution easily. Strongly ionized substances are likely to be freely soluble in water over a wide pH range and cause no problem. Similarly, weakly acidic and weakly basic drugs should be sufficiently soluble at favourable pH. Sometimes soluble but concentration of the solute is very close to its limit of solubility, and get precipitated on cooling or evaporation of solvent. This section will briefly discuss ways to enhance solubility of unionized drugs and weak electrolytes. There are several different ways to enhance solubility but the method of choice depends on the nature of the solute and the degree of solubilization needed.

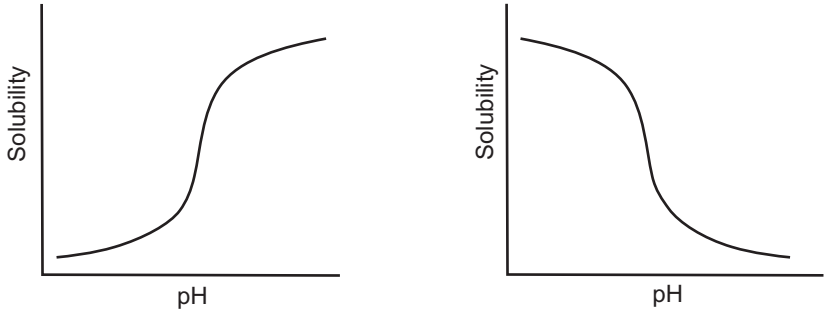
Use of Cosolvents:

The cosolvency concept is used for increasing solubility of electrolytes and non-electrolytes in water. This can be achieved by addition cosolvent that is miscible with water and in which the substance in question is soluble. The cosolvents work by modifying affinity of solvent for solutes by decrease in interfacial tension between solute and solvent or

by changing dielectric constant. The expected dielectric constant values for the solvent and cosolvent blend should be in the range of 25 to 80. Choice of such solvents for the pharmaceutical use is limited due to toxicity and irritancy characteristics. Ethanol (for paracetamol), isopropylalcohol (betamethasone valetrate), glycerin and propylene glycol (for co-trimazole) are some of the examples of cosolvents used for solubilization of drugs mentioned in brackets. Other examples of cosolvents are glycerin, polyethylene glycol, sorbitol, mannitol, etc. and are used for increasing solubilities of electrolytes and non-electrolytes.

pH Control:

Majority of the drugs are either weak acids or bases, and therefore their solubilities in water can be influenced by the pH of the system. There is a little or no effect of pH on solubility of non-ionizable substances with few exceptions. For ionizable solutes such as carboxylic acid (HA) solubility is function of pH, Fig. 3.21. The solubility of weak acid is increased by an increasing pH where as solubility of weak base increased by decreasing pH.



(a) Weak Acid **(b) Weak Base**

Fig. 3.21: The Effect of pH on Solubility

The pH of solute is related to its pKa and concentration of the ionized and unionized form of the solute by equation

$$pH = pKa + \log \frac{[A^-]}{[HA]} \quad \ldots (3.39)$$

If the solute is brought outside its pKa by changing the pH value where half portion is ionized and half portion remains unionized, then the solubility will be changed. This is due to introduction of intermolecular forces, mainly ionic force of attraction. For example, carboxylic acid groups (−COOH) have pKa around pH 4 and if the pH is increased above 4 the −COOH is changed to $-COO^-$. The negative charge introduced is free to have introduction with a partial positive charges of the hydrogen of water. The effect of the pH on solubility of weak electrolytes is described by equation

$$pHp = pKa + \log\left[\frac{S - S_o}{S_o}\right] \quad \ldots (3.40)$$

where, pHp is the pH below which the drug precipitates from solution as the undissociated acid, S is the total solubility and S_o is the molar solubility of the undissociated acid. We often

consider that ionize form is freely soluble but is not always true. For example, carboxylic acids have pKa ~ 4. For the administration of methyl prednesolone hemisuccinate (solubility <1 mg/ml) if base such as sodium hydroxide is added the carboxylic acid becomes deprotonated and solubility increases to more than 200 mg/ml. The same can be observed for base, therefore,

$$pHp = pKw + pKb + \log\left[\frac{S}{S - S_o}\right] \quad \ldots (3.41)$$

where, pKw is dissociation constant of water, pKb is dissociation constant of base and pHp is the pH above which the free base precipitates out of solution.

Solubility of weak electrolytes in buffer solution can be changed by addition of cosolvents. The undissociated species get dissolved by modifying polarity of solvent to a more favourable value. In improving solubility of drugs by pH control it must be ensured that the selected pH does not change the other requirement of the product such as chemical stability that may also depend on pH. Non-ionizable, hydrophobic solutes can have improved solubility by changing the dielectric constant of solvent by use of cosolvent. The maximum solubility must be best achieved by appropriate balance between pH and concentration of cosolvent. The solubilities of the non-electrolytes are not much affected by the pH changes therefore other methods can be tried for their solubility enhancement.

Example 3.11: The solubility and pKa of phenobarbital sodium in 15% alcohol solution is 0.22% and 7.6, respectively. What is pH of 2% phenobarbital sodium hydroalcoholic solution?

Solution: Given that: $S = 2$; $S_o = 0.22$; $pKa = 7.6$

$$pHp = pKa + \log\left[\frac{S - S_o}{S_o}\right]$$

$$= 7.6 + \log\left[\frac{2 - 0.22}{0.22}\right]$$

$$= 7.6 + \log 8.09$$

$$= 8.508$$

The pH of hydroalcoholic phenobarbital sodium solution is 8.508.

Surfactants in Solubilization:

Surfactants play a vital role in many processes of interest. One important property of surfactants is the formation of colloidal-sized clusters in solutions, known as micelles, which have significance in pharmacy because of their ability to increase the solubility of sparingly soluble substances in water. The solubility of drugs that are insoluble or poorly soluble in water can be improved by incorporation of surfactants above its critical micelle concentration (CMC). This phenomenon is widely used for the solubilization of poorly soluble drugs. Micelles are known to have an anisotropic water distribution within their structure. In other words, the water concentration decreases from the surface towards the core of the micelle, with a completely hydrophobic (water-excluded) core. Consequently, the spatial entrapment of a solubilized drug in a micelle depends on its polarity. The non-polar molecules will be

solubilized in the micellar core, and substances with intermediate polarity will be distributed along the surfactant molecules in certain intermediate positions. Numerous drug delivery and drug targeting systems have been studied to minimize drug degradation and loss, to prevent harmful side effects, and to increase drug bioavailability. Within this context, the utilization of micelles as drug carriers presents some advantages when compared to other alternatives such as soluble polymers and liposomes. Micellar systems can solubilize poorly soluble drugs and thus increase their bioavailability, they can stay in the body (blood) long enough to provide gradual accumulation in the required area, and their sizes permit them to accumulate in areas with leaky vasculature.

In general, surfactants play an important role in contemporary pharmaceuticals, since they are largely utilized in various drug dosage forms to control wetting, stability, bioavailability, among other properties. It is important to notice that lyophobic colloids, such as polymers, require certain energy to be applied for their formation, are quite unstable from the thermodynamic point of view, and frequently form large aggregates. Association colloids such as micelles, on the other hand, can form spontaneously under certain conditions (self-assembling systems), and are thermodynamically more stable towards both dissociation and aggregation. Surfactants and their role in pharmacy are of paramount importance, especially with respect to their ability of solubilizing hydrophobic drugs.

The hydrophilic surfactants having HLB value above 15 such as sodium lauryl sulfate, polysorbates, polyoxyl stearate, polyethylene glycol, and castor oils are used for micellar solubilization. The fat soluble vitamin phytomendione is solubilized by use of polysorbates. The solubility of amiodarone hydrochloride can also be enhanced similarly. Macrogol ethers have been found to improve solubility of iodine by producing iodophores. Polyoxyethylated castor oil is used to increase solubility of an immuno expressing drug cyclosporine and anticancer drug paclitaxel. Cetomacrogol has been found to show improved solubility of chloramphenicol. Solubility of volatile and essential oils can be improved by use of lanolin derivatives. Chloroxylenol which normally has solubility of 0.03% in water can be improved by use of soaps. Vitamin A, D, E, and K, griseofulvin, aspirin and phenacetin, etc. are poorly soluble drug that are solubilized by micellar solubilization.

Surfactants are amphiphilic molecules composed of a hydrophilic or polar moiety known as head and a hydrophobic or non-polar moiety known as tail. The surfactant head can be charged (anionic or cationic), dipolar (zwitterionic), or non-charged (non-ionic). Sodium dodecyl sulfate (SDS), dodecyl tri-methyl ammonium bromide (DTAB), n-dodecyl tetra (ethylene oxide) ($C_{12}E_4$) and dioctanoyl phosphatidylcholine (C_8-lecithin) are typical examples of anionic, cationic, non-ionic and zwitterionic surfactants, respectively. The surfactant tail is usually a long chain hydrocarbon residue and less often a halogenated or oxygenated hydrocarbon or siloxane chain, Fig. 3.22.

A surfactant, when present at low concentrations in a system, adsorbs onto surfaces or interfaces significantly changing the surface or interfacial free energy. Surfactants usually act to reduce the interfacial free energy, although there are occasions when they are used to

increase it. When surfactant molecules are dissolved in water at concentrations above the CMC, they form aggregates known as micelles. In a micelle, the hydrophobic tails flock to the interior to minimize their contact with water, and the hydrophilic heads remain on the outer surface to maximize their contact with water, Fig. 3.23. The micellization process in water results form a delicate balance of intermolecular forces such as hydrophobic, steric, electrostatic, hydrogen bonding, and van der Waals interactions.

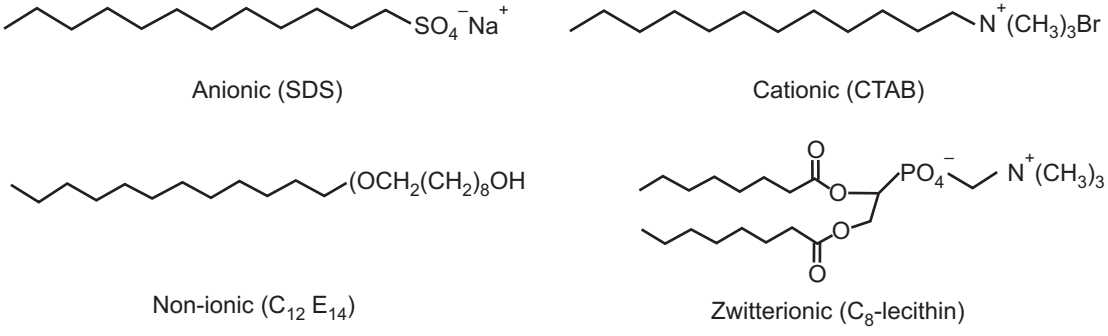


Figure 3.22: Examples of surfactants

The main attractive force results from the hydrophobic effect associated with the non-polar surfactant tails, and the main opposing repulsive force results from steric interactions and electrostatic interactions between the surfactant polar heads. Whether micellization occurs and, if so, at what concentration of monomeric surfactant, depends on the balance of the forces promoting micellization and those opposing it.

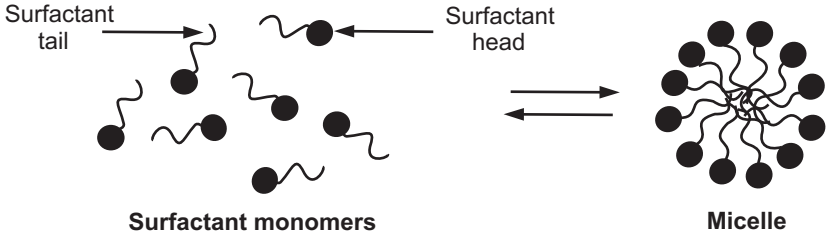


Figure 3.23: Schematic of the Reversible Monomer-Micelle Thermodynamic Equilibrium

The dark circles represent the surfactant heads (hydrophilic part) and the black curved lines represent the surfactant tails (hydrophobic part).

Micelles are labile entities formed by the non-covalent aggregation of individual surfactant monomers. Therefore, they can be spherical, cylindrical, or planar (discs or bilayers). Micelle shape and size can be controlled by changing the surfactant chemical structure as well as by varying solution conditions such as temperature, overall surfactant concentration, surfactant composition (in the case of mixed surfactant systems), ionic strength and pH. Depending on the surfactant type and on the solution conditions, spherical micelles can grow one-dimensionally into cylindrical micelles or two-dimensionally into bilayers or discoidal micelles. Micelle growth is controlled primarily by the surfactant heads,

since both one-dimensional and two-dimensional growth require bringing the surfactant heads closer to each other to reduce the available area per surfactant molecule at the micelle surface, and hence the curvature of the micelle surface.

A combination of solubilization and cosolvency can be used to increase solubility of chloroxylenol. An alternative to use of surfactants as solubilizing agents polymers such as cyclodextrins have been found to show improvement in the solubility of poorly soluble drugs such as itraconazole and corticosteroids.

Complex Formation:

The apparent solubility of some substance in given solvent may be increased or decreased by incorporation of complex forming substances. The degree of complex formation decides the apparent change in solubility of original solute. For example, complex formation between iodine and povidone increases solubility of iodine. Similarly, complex between iodine and potassium iodide to form polyiodides increases solubility of iodine. The interaction of salicylates and benzoates with theophylline or caffeine also increases solubility of these drugs. Other examples of complex forming substances that increases solubility of drugs are nicotinamide and β-cyclodextrins.

Drug Derivatization:

One method to increase solubility of a drug is to alter the chemical structure of the molecule. The addition of polar groups like carboxylic acids, ketones and amines can increase solubility by increasing hydrogen bonding and the interaction with water. Another structure modification can be to reduce intramolecular forces. An example of structure modification to enhance solubility by the latter method is methyl dopa, solubility ~10 mg/ml, and methyl dopate (aprodrug of methyldopa), solubility 10-300 mg/ml depending on pH, Fig. 3.24. The addition of the ethyl ester to methyldopa reduces the intramolecular hydrogen bond between the carboxylic acid and primary amine. Therefore, this addition reduces the melting point and increases solubility. Other examples of chemical modifications for the solubility enhancement include; sodium phosphate salt of hydrocortisone, prednesolone and beta methasone.

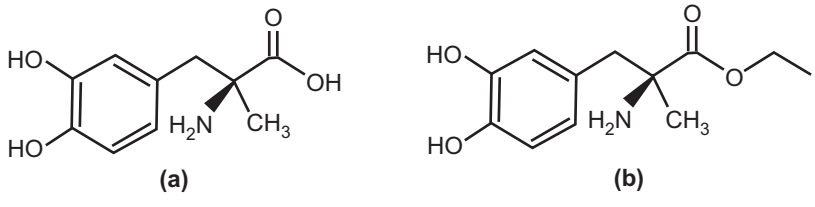


Figure 3.24: Structure of (a) Methyldopa and (b) Methyl Dopate

Solid State Manipulation:

The size and shape of particle have significant effect of solubilities. Increase in surface area by decrease in particle size provides more area for interaction between solvent and solute causing higher solubilities.

3.13 DETERGENCY

Detergency is a complex process involving the removal of foreign matter from surfaces. Surfactants are used for the removal of dirt through the detergency effect. Initial wetting of the dirt and of the surface to be cleaned is carried out by deflocculation and suspension or emulsification or solubilization of the dirt particles. It also involves foaming of the agent for entertainment and washing away of the particles. A wetting agent that when dissolved in water, lowers the advancing contact angle, aids in displacing an air phase at the surface and replacing it with a liquid phase. Wetting agents are useful in

1. Displacement of air from sulfur, charcoal and other powders for dispersing these drugs in liquid vehicles
2. Displacement of air from the matrix of cotton pads and bandages so that medicinal solutions may be absorbed for application to various body areas.
3. Displacement of dirt and debris using detergents in the washing of wounds.
4. The application of medicinal lotions and sprays to the surface of the skin and mucous membrane.

Solid surfaces adsorb dissolved or undissolved substances from the solutions. Common example is adsorption of acetic acid on activated charcoal. Fraction of added acid is adsorbed by activated charcoal and the concentration of acid in solution decreases. Other example of adsorption by activated charcoal are removal of solutes from solutions such as ammonia from ammonium hydroxide, phenolphthalein from solutions of acids and bases, high molecular weight non-electrolyte substances from their solutions.

Adsorption from solution follows general principle laid down for adsorption of gases and is subject to same factors. Adsorbent is more effective in attracting certain substances to their surface than others. Temperature decreases the extent of adsorption while surface area has opposite effect to that of temperature as increase in surface area adsorption increases. Adsorption from solutions involves equilibrium between amount adsorbed on to surface and amount present in bulk solution. The effect of concentration of adsorbate on extent of adsorption is represented by Freudlich's isotherm equation as in the following equation

$$y = kC^{1/b} \quad \ldots (3.42)$$

where y is mass of adsorbate per unit mass of adsorbent, C is equilibrium concentration of adsorbate being adsorbed, while k and b are empirical constants. By taking logarithms of both the sides of the equation (3.42) we obtain

$$\log y = \log k + \frac{1}{b} \log C \quad \ldots (3.43)$$

The plot of log y versus log C is linear with slope equal to 1/b and intercept equal to log k.

Clarification of sugar liquors by charcoal, recovery of dyes from solvents, recovery and concentration of vitamins and other biological substances, wetting and detergency are some of the applications of the adsorption at solid liquid interfaces. One of the uses of adsorption

at solid/liquid interface is to remove poisonous levels of drugs and other toxins from the body. Very often activated charcoal is used as an antidote to poisons. This powder is not wet by water but has a high affinity for some types of drugs. As an example, the sulfonylurea such as tolbutamide will concentrate on the surface of the activated charcoal. Another example is the common OTC analgesic acetaminophen. An overdose of this drug can cause severe liver complications leading to death. A dose of 15 g can kill an adult. By administering activated charcoal, we can reduce the amount of the dose that is absorbed into the body and some researches have shown that some of the drug will cross from the blood supply into the gut.

3.14 ADSORPTION AT SOLID INTERFACE

The substance in adsorbed state is called adsorbate, while that present in one or other (or both) of the bulk phases and capable of being adsorbed may be distinguished as adsorptive. When adsorption occurs at the interface between liquid and solid, the solid is usually called the adsorbent; for gas-liquid interfaces sometimes the liquid is called adsorbent. The adsorption process is generally classified as physisorption or chemisorption.

Adsorption of gases has wide applications as removal of objectionable odors from food, rooms, characterization of powders, adsorption chromatography, prevention of obnoxious gases entering body by gas masks, production of high vacuum, moisture removal etc. Adsorption of gas on solid is like that of adsorption at liquid surfaces, where the surface free energy is reduced. While comparing solids and liquids with respect to adsorption the surface tension determinations are easier for liquids as they are more mobile than the solids. The average lifetime of molecule at liquid surface is very low i.e. 1 sec compared to atoms at the surface of non-volatile metallic surface.

Solid-Gas Adsorption:

It is probable that all solids adsorb gases to certain extent, but the phenomenon is not prominent unless adsorbent possess large surface area. The adsorption of gas on to a solid surface is of mainly of two types.

Physisorption:

Physisorption is adsorption in which the forces involved are intermolecular forces (van der Waals forces) of the same kind as those responsible for imperfection of real gases, condensation of vapors and which do not cause a significant change in electronic orbital patterns of species involved. The term van der Waals adsorption is synonymous with physical adsorption but its use is not recommended.

Characteristics of Physisorption:

1. It is a general phenomenon and occurs in any solid/fluid systems.
2. Minimum change in electronic state of adsorbate and adsorbent is observed.
3. Adsorbed species are chemically identical with those in the chemical adsorbent, so the chemical nature of the adsorbent is not changed by adsorption and subsequent desorption.

4. Energy of interaction between the molecules of adsorbate and adsorbent is of same order of magnitude.
5. Elementary step in adsorption of gas does not involve activation energy.
6. Equilibrium is established with increase in pressure and usually decreases with temperature.
7. Under appropriate condition of temperature and pressure, molecules of gas can be adsorbed more than those in direct contact with surface.

Chemisorption:

Chemical adsorption or chemisorption is a process in which valance forces of some kind, operating in the formation of chemical compounds are involved. The difference between chemisorption and physisorption is same as that of difference between physical and chemical interaction in general.

Characteristics of Chemisorption:

1. The phenomenon is characterized by chemical specificity.
2. Change in electronic state may be detectable by suitable physical means (e.g. UV, IR, microwave spectroscopy, conductivity etc.)
3. The chemical nature of the adsorptive may be altered by surface reaction in such a way that on desorption the original surfaces cannot be recovered.
4. Like chemical reactions, chemisorption is either exothermic or endothermic and magnitude of energy changes may vary from small to very large.
5. The elementary step in chemisorption involves activation energy.
6. The rate of chemisorption increases with increase in temperature and when activation energy of adsorption is small, removal of chemisorbed species from the surface may be possible under extreme conditions of temperature and pressure or by some suitable chemical treatment of the surface.
7. Adsorbed molecules are linked to the surface by valence bonds that occupy certain adsorption sites on surface forming monolayer.

Factors Affecting Adsorption:

Surface area of adsorbent:

Being surface phenomena extent of adsorption depends on available surface area of adsorbent. Finely divided materials since has large surface area, more adsorption is observed on their surfaces.

Nature of adsorbate:

The amount of adsorbate adsorbed on solids depends on its nature; easily liquefiable gases adsorbed to greater extent.

Temperature:

As seen under the characteristics of physical adsorption, it decreases with increase in temperature, while chemical adsorption increases with increase in temperature.

Pressure:

Applying LeChatelier's principle, dynamic equilibrium exists between adsorbed gas molecules and molecules in contact with adsorbate. In fact, it is observed that increase in pressure increases adsorption.

Process characteristics:

As physical adsorption, inversely proportional and chemical adsorption is directly proportional to temperature, reversing this process condition adsorption can be decreased.

Thickness of adsorbed layer:

Langmuir from his studies of isotherms showed that at low pressures physically adsorbed gas forms only one layer one molecule thick while at higher pressures forms multilayers with increased extent of adsorption.

ADSORPTION ISOTHERMS

Adsorption isotherm is the relation between the quantity of adsorbate adsorbed and the partial pressure in the gas phase (or composition of bulk phase, in adsorptions from liquids) under equilibrium conditions at constant temperature.

Freudlich's Adsorption Isotherm:

The scientist Freudlich's studied adsorption of gas on solid and from the experimental data; he gave empirical equation called equation of Freudlich's adsorption isotherm,

$$y = \frac{w}{m} = kP^{1/b} \quad \ldots (3.44)$$

where, y is amount (w) of adsorbate adsorbed by m gram of adsorbent at equilibrium pressure P and are determined from the experiment at constant temperature. The constants k and b depends on nature of adsorbate and adsorbent as well as on temperature.

In equation (3.44), b > 1 therefore the amount of adsorbed gas increases less rapidly than the pressure. This equation holds good only for medium pressures of gas. If w/m is plotted against pressure, a curve results of which first part is linear and over this range at low pressures $x/m \propto p$. At higher pressures a limiting value x/m is reduced and curve is parabolic in shape as shown in Fig. 3.25. Equation (3.44) is known as Freudlich's adsorption isotherm.

Taking logarithm on both sides of equation (3.44)

$$\log\left(\frac{w}{m}\right) = \left(\frac{1}{b}\right) \log P + \log K \quad \ldots (3.45)$$

This equation is valid at a given temperature. If adsorption is on the surface on solid, then equation (3.45) becomes

$$\log\left(\frac{w}{m}\right) = \left(\frac{1}{b}\right) \log C + \log K \quad \ldots (3.46)$$

Extrapolating the line from the any point on line, the intercept on Y-axis is log (w/m) and on X-axis is log C and slope of the line is 1/b.

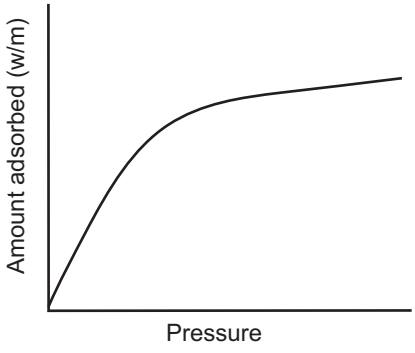


Figure 3.25: Adsorption of Gas on a Solid

Langmuir Adsorption Isotherm:

In 1916, scientist Irving Langmuir (1916) published a new isotherm for gases adsorbed on solids, which retained his name. It is an empirical isotherm derived from assumptions of his extensive study.

1. The surface of a solid is made-up of elementary spaces and each space can adsorb one gas molecule.
2. All the elementary spaces are identical in their capacity for adsorbing a gas molecule.
3. The adsorption of a gas molecule in one element of space does not affect the properties of neighboring spaces.
4. It is possible that the adsorption layers are just of a single molecule thickness because intra-molecular forces fall off rapidly at distance beyond it.
5. Due to thermal kinetic energy of some the adsorbed molecule they get detached and pass back into space. Therefore, adsorption can be considered as consisting of two opposing processes in equilibrium (i.e. condensation and evaporation).
6. Initially rate of adsorption is high but as the surface area of adsorbent is covered with adsorbate molecules the rate of removal of adsorbed molecules goes on increasing. (i.e. rate of adsorption and evaporation are equal).

Langmuir had developed an equation based on the theory that the molecules or atoms of gas are adsorbed on active sites of the solid to form a layer one molecule thick. If fraction of active centers occupied on surface of adsorbent by gas molecules at pressure P is expressed as θ then the fraction of sites unoccupied is $1 - \theta$. The rate of adsorption (R_1) is proportional to unoccupied spots and the pressure P and the rate of evaporation (R_2) of molecule bound on surface is proportional to the fraction of surface occupied, θ.

$$R_1 \propto \text{fraction of sites unoccupied} \times \text{Pressure}$$

$$R_1 = k_1 (1 - \theta) P \quad \ldots (3.47)$$

$$R_2 \propto \text{Fraction of sites occupied}$$

$$R_2 = k_2 \theta \quad \ldots (3.48)$$

At equilibrium, $R_1 = R_2$

$$k_1(1 - \theta)\, P = k_2\, \theta \qquad \ldots (3.49)$$

After rearranging equation (3.49) he obtains

$$\theta = \frac{k_1 P}{k_2 + k_1 P}$$

$$\theta = \frac{\left(\frac{k_1}{k_2}\right) P}{1 + \left(\frac{k_1}{k_2}\right) P} \qquad \ldots (3.50)$$

Replacing θ by y/y_m and k_1/k_2 by b, where y is mass of gas adsorbed per gram of adsorbent at pressure P and at constant temperature and y_m is mass of gas that adsorbed on 1 gram of adsorbent to form complete monolayer. On substituting the values for θ and k_1/k_2 the following equation is obtained

$$\frac{y}{y_m} = \frac{bP}{1 + bP} \qquad \ldots (3.51)$$

$$\therefore \quad y = \frac{y_m bP}{1 + bP} \qquad \ldots (3.52)$$

The equation (3.52) is known as Langmuir adsorption isotherm equation and it can also be written in the following form.

$$\frac{P}{y} = \frac{1}{y_m b} + \frac{P}{y_m} \qquad \ldots (3.53)$$

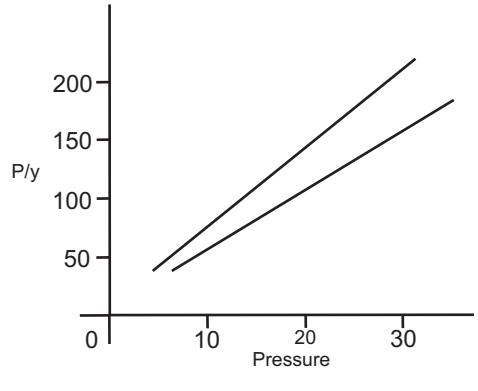


Figure 3.26: Langmuir Adsorption Isotherm

By plotting a graph of P/y against P, Fig. 3.26, we get a straight line with slope equal to y_m and intercept as b.

Types of Isotherms:

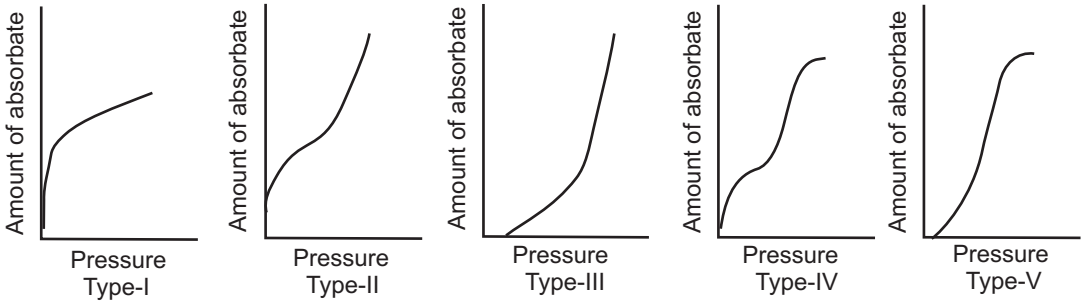


Fig. 3.27

Type-I:

Langmuir and Freundlich isotherms are of Type-I, Fig. 3.27, where adsorption takes place on non-porous solids. It represents behaviour of nitrogen or oxygen on charcoal. Total surface area can be determined from this isotherm by multiplying the total number of molecules in the volume of gas adsorbed by the cross-sectional area of the molecule.

Type-II:

In this type of isotherm gases are physically adsorbed on a non-porous solid forming monolayer followed by multilayer formation. The first inflection in the curve represents formation of monolayer and subsequent increase in pressure shows multilayer adsorption. This isotherm is explained by BET (Branauer, Emmett and Teller) equation

$$\left[\frac{P}{y}(P_0 - P)\right] = \left[\frac{1}{y_m b}\right] + \left[\frac{(b-1)}{y_m b}\right] \times \left[\frac{P}{P_0}\right] \quad \ldots (3.54)$$

where, P is pressure of the adsorbate, y is mass of vapour per gram of adsorbent; P_0 is vapor pressure at saturation of adsorbent by adsorbate, y_m is amount of vapour adsorbed per unit mass of adsorbent when the surface is covered with monomolecular layer and b is constant equal to difference between heat of adsorption in the first layer and latent heat of condensation in the next layers. This isotherm is sigmoid in shape and observed with adsorption of nitrogen on iron catalysts, on silica gel and other surfaces.

Type-III:

This isotherm is rarely observed for example, bromine and iodine on silica gel, where heat of adsorption in the first layer is less than the latent heat of condensation in the next layers. The constant b of the BET equation is less than two.

Type-IV:

This isotherm is typical of adsorption onto porous solids where if the first point is extrapolated to zero pressure represents the amount of gas required in forming monolayer on solid surface. Condensation within the capillaries is responsible for the further adsorption. The example of this type is adsorption of benzene on ferric oxide and silica gel.

Type-V:

It is like type-III adsorption as capillary condensation is observed on the porous solids for example, adsorption of water vapor on charcoal at 100°C.

EXERCISE

1. What are surface, interface and surface?
2. What are units of surface tension and interfacial tension?
3. Explain the terms surface excess and surface pressure.
4. Enlist methods to determine surface tension of liquids and solids.
5. Describe capillary rise method for determination of surface tension of liquids.
6. Describe drop method to determine surface tension of liquid.
7. Why drop of liquid hanging in air is spherical in shape?
8. What is effect of surfactant concentration and solute on surface tension of liquids?
9. Write about first tensiometer developed and used to determine surface tension.
10. Explain bubble pressure method to determine surface tension.
11. Classify surfactants based on their HLB values.
12. Write note on HLB system and its applications.
13. A polyhydric fatty acid ester has saponification number 48 and acid number 280. What will be HLB value of ester?
14. Draw HLB scale stating different HLB value ranges for surfactant for their application.
15. Enlist factors affecting HLB value of surfactant. Write on drawbacks of HLB.
16. What is surface free energy? Explain methods to determine it.
17. Elaborate the statement 'Surface tension decreases with increase in temperature'.
18. At 20°C the same volume of water and oil produced 20 and 60 drops using Stalagmometer. If surface tension of water is 72.8 dynes/cm at same temperature, at which density of oil is 0.872 g/mL; calculate surface tension of oil.
19. The surface excess of long chain amphiphile in water was 3×10^{-9} mol/cm^2; calculate area occupied by each molecule at the surface.
20. The surface excess of amphiphile is 5.49×10^{-9} mol/cm^2 at a bulk concentration of 3×10^{-3} mol/L; calculate area occupied by each amphiphile molecule at the surface. ($N = 6.02 \times 10^{23}$)
21. What are wetting agents? Explain their mechanism of action.
22. What is critical micelle concentration? Explain the changes observed on properties of surfactant solutions at CMC.
23. What are pharmaceutical applications of critical micelle concentration?
24. Enlist methods other than surface tension to determine CMC of surfactant solution.

25. Write note on Gibb's adsorption isotherm.
26. What is adsorption? Differentiate between physisorption and chemisorption.
27. Write characteristics of physisorption and chemisorption.
28. What are assumptions of Langmuir's adsorption study?
29. Describe Langmuir's adsorption isotherm to determine the constants 'log k' and 'b' in the isotherm equation.
30. Explain Freundlich adsorption isotherm.
31. Write short on Langmuir adsorption isotherm.
32. What is spreading coefficient? Obtain expression for the same.
33. Derive an equation of spreading coefficient. What is its significance in pharmacy?
34. How knowledge of surface tension does helps in understanding of spreading coefficient?
35. Addition of solid particles in to a liquid vehicle is critical step in the preparation of pharmaceutical dispersions. Explain this statement with spreading wetting.
36. When two immiscible liquids are mixed together they fail to remain mixed. Explain.
37. Surface tension of water is 77.8 dyne/cm and that of benzene is 27.1 dyne/cm while interfacial tension between them is 35 dynes/cm then what was the initial spreading coefficient? After establishment of equilibrium, surface tension of water reduces to 62.2 dynes/cm and that of benzene it becomes 27 dynes/cm. What was the final spreading coefficient?
38. Draw different type of adsorption curves and discussion their applications.
39. How you will determine cross sectional area per molecule form adsorption studies?
40. Draw schematic of film balance. Explain the concept of surface pressure.
41. A 5 mL of an oil having molecular weight 300 and density 0.9 g/mL is placed on half an acre (2×10^7 cm^2) of pond; calculate length and cross sectional area of the oil molecule.
42. Explain phenomenon of wetting and spreading with the help of suitable contact angle measurement.
43. Explain mechanism of Cosolvents in improving solubility of solutes with suitable examples.
44. Solubility of majority of the drugs in water is influenced by the pH of the system. Explain with suitable example.
45. Describe use of surfactant to solubilize insoluble solutes.
46. Altering chemical structure of the molecule changes solubility of solute in the same solvent. Explain.



Unit ...4

COMPLEXATION AND PROTEIN BINDING

♦ OBJECTIVES ♦

The term complexation is used to characterize the covalent or non-covalent interactions between two or more compounds capable of independent existence. The ligand, a molecule, interacts with substrate the molecule to form a complex. Drug molecules can form complexes with other small molecules or with macromolecules. Once complexation occurs, the solubility, stability, partitioning, energy absorption and emission, and conductance of the drugs are changed. Drug complexation, therefore, can lead to beneficial properties such as enhanced aqueous solubility and stability. Complexation can also be useful in the optimization of delivery systems and affect the distribution in the body after systemic administration due to protein binding. The drug-protein binding in this unit is covered in depth in the later part. Contrary, complexation can also lead to poor solubility or decreased absorption of drugs in the body. For certain drugs, complexation with certain hydrophilic compounds can enhance excretion. Overall, complexes can alter the pharmacologic activity of drugs.

After studying the contents of the chapter, students are expected to:

- Understand the significance of complexation in pharmaceutical products.
- Understand the fundamental forces that are related to the formation of drug complexes.
- Differentiate between different complexation types and understand the mechanism of complex formation.
- Relate the formation of complexes with improvements in the physicochemical properties and bioavailability of drugs.
- Identify the significance of protein-ligand interactions in drug action.
- Understand properties of plasma proteins and its mechanism of interactions with drugs.
- Understand the techniques of in vitro analysis and factors affecting complexation and protein binding.

4.1 INTRODUCTION

Complexes or co-ordination compounds result from a donor–acceptor mechanism or Lewis acid–base reaction between two or more different chemical components. The term complexation is used to characterize the covalent or non-covalent interactions between two or more compounds capable of independent existence. The ligand, a molecule, interacts with substrate, the molecule, to form a complex. Drug molecules can form complexes with other small molecules or with macromolecules. Once complexation occurs, the solubility, stability, partitioning, energy absorption and emission, and conductance of the drugs are changed. Drug complexation, therefore, can lead to beneficial properties such as enhanced aqueous solubility and stability. Complexation can also be useful in the optimization of delivery systems and affect the distribution of drug in the body after systemic administration due to protein binding. Contrary, complexation can lead to poor solubility or decreased absorption of drugs in the body. For certain drugs, complexation with certain hydrophilic compounds can enhance excretion. Overall, complexes can alter the pharmacologic activity of drugs.

Complexes can be divided broadly into two classes depending on whether the acceptor component is a metal ion or an organic molecule; these are classified according to one possible arrangement. Another class, the inclusion/occlusion compounds, involves the entrapment of one compound in the molecular framework of another. Intermolecular forces involved in the formation of complexes are the van der Waals forces of dispersion, dipolar, and induced dipolar types. Hydrogen bonding provides a significant force in some molecular complexes, and co-ordinate covalence is important in metal complexes. Many drugs bind to plasma proteins which has significant influence on duration of drug action. Some drugs in body exist only in a bound form and proper distribution of such drugs into extra vascular part is governed by the process of dissociation of drugs from the complex. The fraction of drug that can be in free form can vary but may be as low as 1%. The other fraction remains in associated form as a complex with the protein. The free form of drug is pharmacologically active and is responsible for action on body. Thus, the protein binding features of the drug plays significant role in its therapeutic actions.

4.2 CLASSIFICATION OF COMPLEXATION

Based upon type of interaction, ligand-substrate complexes are classified as follows.

(I) Metal ion or co-ordination complexes :
- (a) Inorganic type
- (b) Chelates
- (c) Olefin type
- (d) Aromatic type
  - (i) Pi (π) complexes
  - (ii) Sigma (σ) complexes
  - (iii) Sandwich compounds

(II) Organic molecular complexes :
- (a) Quinhydrone type
- (b) Picric acid type
- (c) Caffeine and other drug complexes
- (d) Polymer type

(III) Inclusion or occlusion compounds :
- (a) Channel lattice type
- (b) Layer type
- (c) Clathrates
- (d) Monomolecular type
- (e) Macromolecular type

(I) Metal Ion or Co-ordination Complexes :

A satisfactory understanding of metal ion complexation is based upon a familiarity with atomic structure and molecular forces, and electronic structure as well as hybridization. The co-ordination complex or metal complex is a structure made-up of a central metal atom or ion (cation) surrounded by a number of negatively charged ions or neutral molecules possessing lone pairs. The ions surrounding the metal are known as ligands. The number of bonds formed between the metal ion and ligand is called as co-ordination number.

(a) Inorganic Complexes : Ligands are generally bound to a metal ion by a covalent bond and hence called to be co-ordinated to the ion. The interaction between metal ion and the ligand is known as a Lewis acid-base reaction wherein the ligand (base) donates a pair of electron (to the metal ion, an acid) to form the co-ordinate covalent bond. For example, the ammonia molecules in hexamine cobalt (III) chloride, as the compound $[Co(NH_3)_6]^{3+} \cdot Cl_3$ is called as the ligands and are said to be co-ordinated to the cobalt ion. The co-ordination number of the cobalt ion, or number of ammonia groups co-ordinated to the metal ions, is six. Other complex ions belonging to the inorganic group include $[Ag(NH_3)_2]^+$, $[Fe(CN)_6]^{4-}$, and $[Cr(H_2O)_6]^{3+}$.

Each ligand donates a pair of electrons to form a co-ordinate covalent bond between itself and the central ion having an incomplete electron shell. For example,

$$Co^{3+} + 6{:}NH_3 = [Co(NH_3)_6]^{3+}$$

Hybridization plays an important part in co-ordination compounds in which sufficient bonding orbitals are not ordinarily available in the metal ion. The understanding about hybridization can be acquainted using the example of the quadric valence of carbon. It will be recalled that the ground-state configuration of carbon is

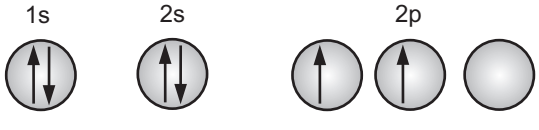


Figure 4.1: Hybridization of Carbon

This cannot be the bonding configuration of carbon, however, because it normally has four rather than two valence electrons. Pauling suggested the possibility of hybridization to account for the quadric valence. As per this mixing process, one of the 2s electrons is promoted to the available 2p orbital to yield four equivalent bonding orbitals.

Another example is interaction between silver and ammonia;

$$\underset{\text{Silver ion}}{Ag^+} + \underset{\text{Ammonia}}{2(:NH_3)} = \underset{\text{Silver-ammonia coordiniate complex}}{[Ag(NH_3)_2]^+}$$

In this case silver metal ion interacts with ammonia to form silver-ammonia co-ordinate complex. Electron pair donating ligands such as H_2O:, NC:, Cl: etc neutralizes co-ordinate complexes. The $[Ag(NH_3)_2]^+$ complex is neutralized with Cl as $[Ag(NH_3)_2]Cl$. The co-ordination compounds through bonds with central metal atom and surrounding ligands plays important role in controlling the structure and functions of various enzymes in our body.

Co-ordinating a metal to a drug in a non-aqueous system favours the formation of a co-ordination complex that the resultant co-ordination complex exhibits a surprising and unexpected buffering effect. Due to buffering effect, drug can remain soluble in water at physiological pH for a period sufficient for the preparation of a safe and convenient parenteral formulation and for delivering the drug to its targets in the body. Thus, the co-ordination complexes resolve the problems associated with drugs having poor water solubilities that could not safely be converted to injectable forms or that show declined bioavailability due to their inabilities to migrate to their target sites in the predetermined time. The additional co-ordination of a buffering ligand or adjuvant to a metal complexed with a drug provides additional buffering capacity and lowers the pH and/or increases the solubility of the entire metal co-ordination complex.

(b) Chelates : A substance containing two or more donor groups may combine with a metal to form a special type of complex known as a chelate. Some of the bonds in a chelate may be ionic or of the primary covalent type, whereas others are co-ordinate covalent links. When the ligand provides one group for attachment to the central ion, the chelate is called monodentate. For example, pilocarpine behaves as a monodentate ligand toward Co(II), Ni(II), and Zn(II) to form chelates of pseudo tetrahedral geometry.

Chelation holds stringent steric requirements on both metal and ligands. Ions such as Cu(II) and Ni(II), which form square planar complexes, and Fe(III) and Co(III), which form octahedral complexes and can exist in either of two geometric forms. Because of this isomerism, only cis-co-ordinated ligands (ligands adjacent on a molecule) is readily replaced by reaction with a chelating agent. Vitamin B_{12} and the hemoproteins are incapable of reacting with chelating agents because their metal is already co-ordinated in such a way that only the *trans*-co-ordination positions of the metal are available for complexation. In contrast, the metal ion in certain enzymes, such as alcohol dehydrogenase, which contains zinc, can undergo chelation, suggesting that the metal is bound in such a way as to leave two *cis*-positions available for chelation.

Applications of chelation:

Chlorophyll and hemoglobin, two extremely important compounds, are naturally occurring chelates involved in the life processes of plants and animals. Albumin is the main carrier of various metal ions and small molecules in the blood serum. The amino-terminal portion of human serum albumin binds to Cu(II) and Ni(II) with higher affinity than that of dog serum albumin. This fact partly explains why humans are less susceptible to copper poisoning than are dogs. The binding of copper to serum albumin is important because this metal is possibly involved in several pathologic conditions. The synthetic chelating agent ethylene diamine tetra acetic acid (EDTA) has been used to tie-up or sequester iron and copper ions so that they cannot catalyze the oxidative degradation of ascorbic acid in fruit juices and in drug preparations. In the process of sequestration, the chelating agent and metal ion form a water-soluble compound. EDTA is widely used to sequester and remove calcium ions from hard water.

(c) Olefin Type:

Olefins belong to a family of organic compounds called hydrocarbons. They consist of different molecular combinations of the two elements, carbon and hydrogen. Another name for an olefin is an alkene. Alkenes contain one or more double bonds between the carbon atoms of the molecule. Olefins form different compounds based on their structure. Some have short chains with only two, three or four carbons, such as ethylene. Others form long chains or closed ring structures. Some have a combination of both. Alkenes are insoluble and exist in all three states of matter. Some short chain alkenes are gases at room temperature and pressure. More complicated structures exist as liquids and solids.

Olefin ligands are common in organotransition metal chemistry. The first organotransition metal complex, Zeise's salt ($K[PtCl_3(C_2H_4]\cdot H_2O$) was an olefin complex. The bonding of an olefin to a transition metal can activate the ligand to electrophilic or nucleophilic attack depending on the nature and charge of the metal center. For example, if there is a high formal charge on the metal center then the olefin is subject to attack by nucleophiles at the face opposite the metal (giving trans addition). Likewise, electron rich metal centers in low oxidation states are activated for attack by electrophiles at the C-C bond.

(d) Aromatic Type :

(i) Pi (π) complexes : The example of Pi complex is interaction of local anesthetic bupivacaine and its structural analogs such as 2,6-dimethylaniline, and N-methyl-2, 6-dimethylacetanilide, and cocaine, with several electron deficient aromatic moieties. In solution, the anesthetic, its analogs and cocaine are electron donors and form π-π charge transfer complexes with strong aromatic acceptors. The concentrations of free bupivacaine, its analogs and of cocaine are reduced from solution via binding to aromatic-functionalized silica.

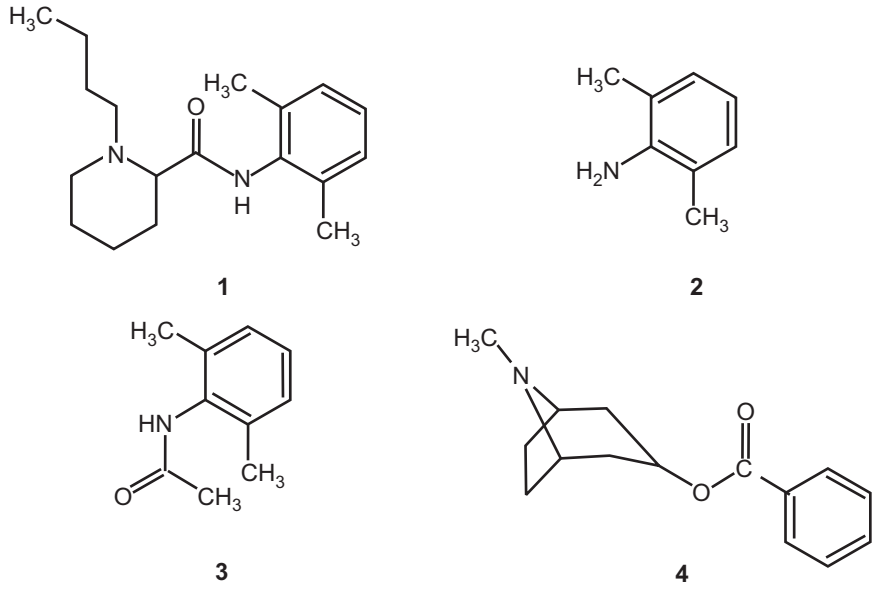


Figure 4.2: Pi Complex Interaction in Bupivacaine and its Structural Analogs

The rapid binding of bupivacaine (1) and its analogs 2, 6-dimethylaniline (2) and 2, 6-dimethylacetanilde (3), respectively, and of cocaine (4), by the acceptor molecules. The structures 1, 2, 3 and 4 show that the molecules are lipophilic in nature, a characteristic common to toxic molecules. 1, 2 and 3 include a benzene ring with two methyl and a nitrogen electron-donating groups, making this portion of the molecules π-electron rich, and hence strong π-donors. The aromatic ring of cocaine, 4, also has weak π-donor capability when complexed with a strong π-acceptor. The selective removal of excess bupivacaine and cocaine from solution is charge transfer complex formation of the π-π type through aromatic-aromatic interaction, based on the assumption that dinitrobenzoyl groups possessing less π-electron density would not only bind with the more π-electron rich bupivacaine and cocaine but would also reduce their toxic effects. The LD_{50} of bupivacaine is 7.8 mg/kg subcutaneously. The effectiveness of this approach is based on the fact that only free, unbound molecules in the blood possess toxicity and that they lose toxicity once bound to or conjugated with another moiety.

(ii) Sigma (σ) complexes : An arenium ion is a cyclohexadienyl cation that appears as a reactive intermediate in electrophilic aromatic substitution. This complex is also called a Wheland intermediate or a sigma complex or σ-complex. The smallest arenium ion is the benzenium ion ($C_6H_7^+$), which is protonated benzene.

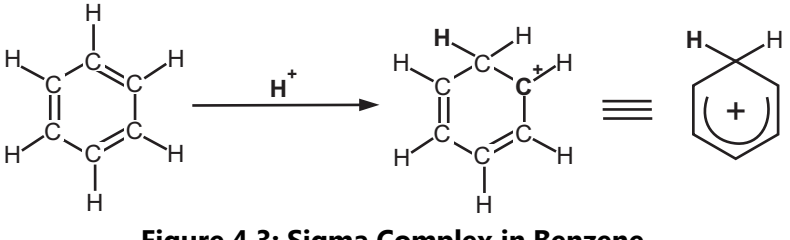


Figure 4.3: Sigma Complex in Benzene

Two hydrogen atoms bonded to one carbon lie in a plane perpendicular to the benzene ring. The arenium ion is no longer an aromatic species; however it is relatively stable due to delocalization. The positive charge is delocalized over 3 carbon atoms via the Pi system, as depicted in resonance structures, Fig. 4.4.

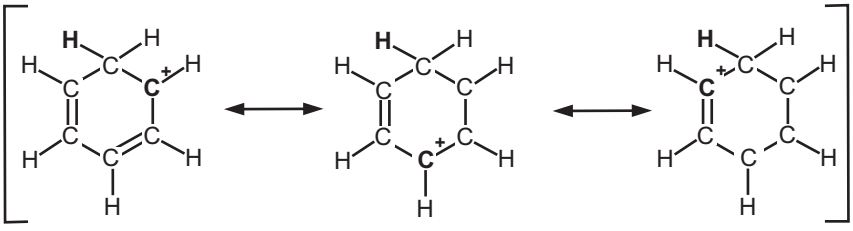


Figure 4.4: Charge Localization via Pi System

A complexed electrophile can contribute to the stability of arenium ions. A benzenium ion can be isolated as a stable compound when it is protonated by the carborane superacid $H(CB_{11}H(CH_3)_5Br_6)$. The benzenium salt is crystalline with thermal stability up to 150 °C. Bond lengths deduced from X-ray crystallography are consistent with a cyclohexadienyl cation structure.

Methylene arenium ion stabilization by metal complexation is another example of σ-complex. In the reaction sequence the R-Pd(II)-Br starting complex is stabilized by tetra-methylethylene diamine (TMEDA) which is converted by 1,2-Bis(diphenylphosphino) ethane (DPPE) to metal complex. Electrophilic attack of methyl triflate forms methylene arenium ion with positive charge located in aromatic para position and with the methylene group at 6° out of the plane of the ring. Reaction first with water and then with triethylamine hydrolyzes the ether group.

(iii) Sandwich compounds : A sandwich compound is a metal bound by haptic covalent bonds to two arene ligands. The arenes have the formula C_nH_n, substituted derivatives (for example $C_n(CH_3)_n$) and heterocyclic derivatives (for example BC_nH_{n+1}). Because the metal is usually situated between the two rings, it is said to be "sandwiched". Special classes of sandwich complexes are the metallocenes. Metallocenes including just one facially-bound planar organic ligand instead of two gives rise to a still larger family of half-sandwich compounds. The most famous example is probably methylcyclopentadienyl manganese tricarbonyl. Compounds such as the cyclopentadienyl iron dicarbonyl dimmer and cyclopentadienyl molybdenum tricarbonyl dimer can be considered a special case of half-sandwiches, except that they are dimetallic.

(II) Organic Molecular Complexes :

An organic molecular complex consists of constituents held together. The forces involved are of donor and acceptor type or by hydrogen bonds. There is a difference between complexation and the formation of organic compounds. For example, dimethyl aniline and 2,4,6-trinitroanisole react at low temperature to give a molecular complex. The dotted line in the complex, Fig. 4.5, indicates that the two molecules are held together by a weak secondary valence force. It is not to be considered as a clearly defined bond but rather as an overall

attraction between the two aromatic molecules. The type of bonding existing in molecular complexes in which hydrogen bonding plays no part is not fully understood, but it may be considered for the present as involving an electron donor–acceptor mechanism corresponding to that in metal complexes but ordinarily much weaker.

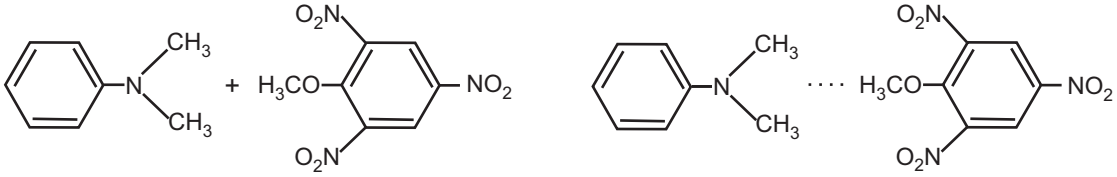


Figure 4.5: Molecular Complex Formation Through Weak Secondary Valence Force

These two compounds react at a higher temperature to form a salt wherein the constituent molecules in products are held together by primary valence bonds, Fig. 4.6.

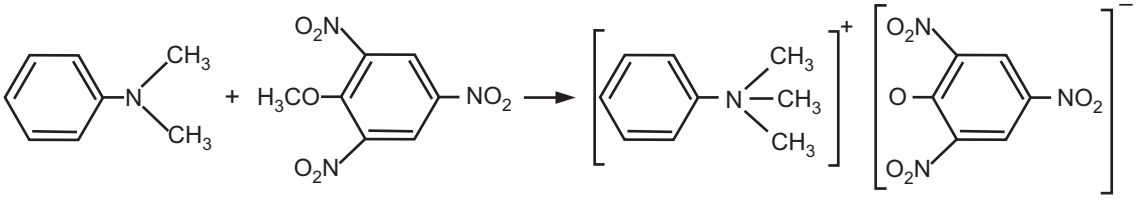


Figure 4.6: Salt Formation Through Primary Valence Bonds

Some of the organic complexes are too weak and cannot be separated as definite compounds. They are even difficult to detect by any chemical and physical means. The energy of attraction between the constituents is approximately <5 kcal/moles and the bond distance is usually greater than 3Å. One molecule of complex polarizes the other to form ionic interaction or charge transfer. Such molecular complexes are referred as charge transfer complexes. For example, the polar nitro groups of trinitrobenzene induce a dipole in the readily polarizable benzene molecule. The net electrostatic interaction results into complex formation as shown in Fig. 4.7.

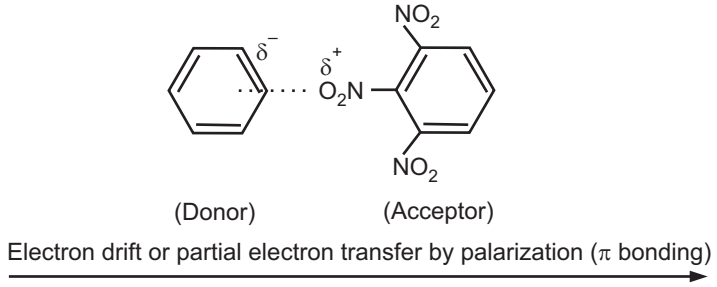


Figure 4.7: Charge Transfer Complex Formation

The drug used against alcohol addiction (disulfiram), a sedative–hypnotic and anticonvulsant (clomethiazole), and an antifungal agent (tolnaftate), each of these drugs possesses a nitrogen–carbon–sulfur moiety. A complex may form from the transfer of charge from the pair of free electrons on the nitrogen and/or sulfur atoms of these drugs to the

antibonding orbital of the iodine atom. The tying up iodine by the molecules containing the N−C=S moiety inhibits thyroid action in the body.

Drug Complexes :

In the formation of drug complex degree of interaction depends upon certain effects. For example, the complexing of caffeine with several acidic drugs. The interaction between caffeine and sulfonamide or barbiturate is a dipole–dipole force or hydrogen bonding between the polarized carbonyl groups of caffeine and the hydrogen atom of the acid. The secondary interaction occurs between the non-polar parts of the molecules and the resultant complex is "squeezed out" of the aqueous phase due to the great internal pressure of water.

The complexes formed between esters and amines, phenols, ethers, and ketones have been attributed to the hydrogen bonding between a nucleophilic carbonyl oxygen and an active hydrogen. There are no activated hydrogens on caffeine; the hydrogen at the number 8 position is very weak (Ka = 1×10^{-14}) and is not likely to enter complexation, Fig. 4.8. The complexation occurs due to dipole–dipole interaction between the nucleophilic carboxyl oxygen of benzocaine and the electrophilic nitrogen of caffeine.

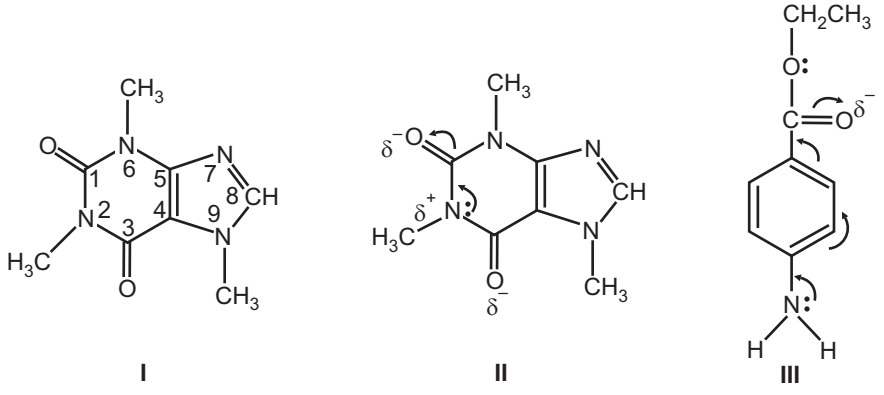


Figure 4.8: Complexing Sites in Caffeine (I and II) and Benzocaine (III)

Caffeine forms complexes with organic acid anions that are more soluble than the pure xanthine, but the complexes formed with organic acids, such as gentisic acid, are less soluble than caffeine alone. Such insoluble complexes provide caffeine in a form that masks its normal bitter taste and serve as a suitable state for chewable tablets. Salicylates form molecular complexes with benzocaine. Complexation between benzocaine and salicylates improve or impair drug absorption and bioavailability. The presence of sodium salicylate significantly influence release of benzocaine, depending on the type of vehicle involved.

Polymer Complexes :

The polymers containing nucleophilic oxygens such as polyethylene glycols, polystyrene, carboxymethylcellulose and similar can form complexes with various drugs. The examples of this type include incompatibilities of carbowaxes, pluronics, and tweens with tannic acid, salicylic acid, and phenol. The interactions may occur in suspensions, emulsions, ointments, and suppositories and are manifested as a precipitate, flocculate, delayed biologic absorption, loss of preservative action, or other undesirable physical, chemical, and

pharmacological effects. The interaction of povidone (PVP) with ionic and neutral aromatic compounds is affected by several factors that affect the binding to PVP of substituted benzoic acid and nicotine derivatives. Ionic strength has no influence but the binding increases in phosphate buffer solutions and decreases as the temperature is raised. Crospovidone, a cross-linked insoluble PVP, can bind drugs owing to its dipolar character and porous structure. There exits an interaction of crospovidone with acetaminophen, benzocaine, benzoic acid, caffeine, tannic acid, and papaverine hydrochloride. This interaction is mainly due to any phenolic groups on the drug. Hexyl resorcinol shows exceptionally strong binding.

Solutes in parenteral formulations may migrate from the solution and interact with the wall of a polymeric container. The ability of a polyolefin container to interact with drugs depends linearly on the octanol–water partition coefficient of the drug. For parabens and drugs that exhibit significant hydrogen bond donor properties, a correction term related to hydrogen-bond formation is needed. Polymer–drug container interactions may result in loss of the active component in liquid dosage forms. Such complexes are used to modify biopharmaceutical parameters of drugs; the dissolution rate of ajmaline is enhanced by complexation with PVP. The interaction is due to the aromatic ring of ajmaline and the amide groups of PVP to yield a dipole–dipole-induced complex. Some molecular organic complexes of interest to the pharmacist are given in Table 4.1.

Table 4.1: Pharmaceutical examples of molecular organic complexes

Agent	Drugs forming Complex
Polyethylene glycols	Salicylic acid, o-phthalic acid, acetyl salicylic acid, resorcinol, catechol, phenol, phenobarbital
Polyvinyl-pyrrolidone	Benzoic acid, salicylic acid, sodium salicylate, mandelic acid, sulfathiazole, chloramphenicol, phenobarbital
Sodium carboxy methyl cellulose	Quinine, benadryl, procaine, pyribenzamine
Oxytetracycline andtetracycline	γ-butyrolactone, sodium salicylate, sodium saccharin, caffeine

Inclusion Compounds :

The inclusion or occlusion compounds results from the architecture of molecules. One of the constituents of the complex is trapped in the open lattice or cage like crystal structure of the other to yield a stable arrangement.

Channel Lattice Type :

The bile acids especially cholic acids form a complex of deoxycholic acid in combination with paraffin, organic acids, esters, ketones, and aromatic compounds and with solvents such as ether, alcohol, and dioxane. The crystals of deoxycholic acid are arranged to form a channel into which the complexing molecule can fit. Camphor has been partially resolved by

complexation with deoxycholic acid, and dl-terpineol using digitonin, which occludes certain molecules in a manner like that of deoxycholic acid. Urea and thiourea also crystallize in a channel-like structure permitting enclosure of unbranched paraffin, alcohols, ketones, organic acids, and other compounds. The well-known starch–iodine solution is another example of channel-type complex consisting of iodine molecules entrapped within spirals of the glucose residues. Monostearin, an interfering substance in the assay of dienestrol, could be extracted easily from dermatologic creams by channel-type inclusion in urea. Urea inclusion might become a general approach for separation of long-chain compounds in assay methods.

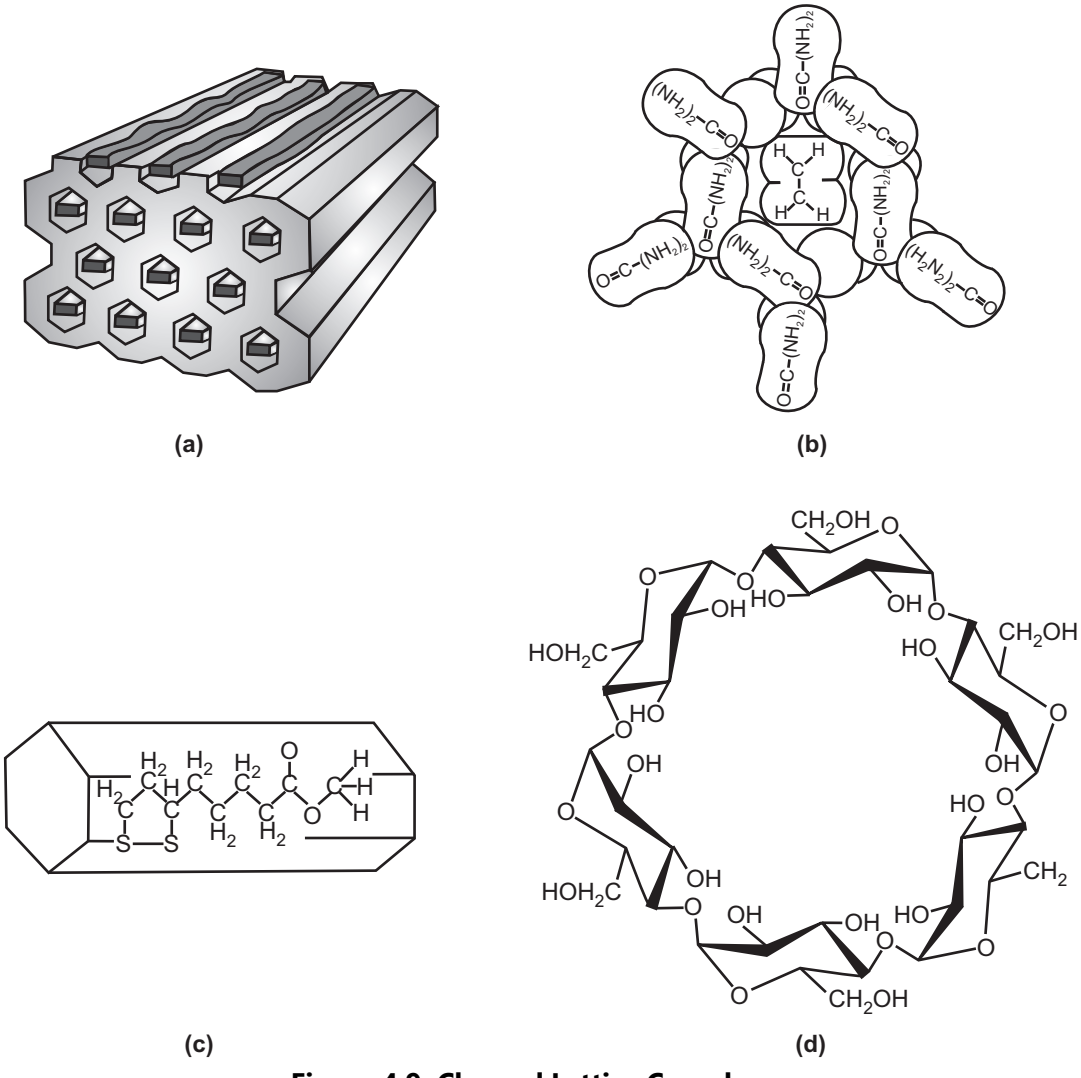


Figure 4.9: Channel Lattice Complexes

In the Fig. 4.9 a channel complex formed with urea molecules as the host. (a) These molecules are packed in an orderly manner and held together by hydrogen bonds between

nitrogen and oxygen atoms. (b) The hexagonal channels, approximately 5 Å in diameter, provide room for guest molecules such as long-chain hydrocarbons. A hexagonal channel complex (adduct) of methyl α-lipoate and 15 g urea in methanol (c) is prepared with gentle heating. Needle crystals of adduct separated overnight at room temperature. This inclusion compound or adduct begins to decompose at 63 °C and melts at 163 °C. Thiourea may also be used to form the channel complex. Cyclodextrin (d) is another example of this type.

Layer Type :

Some other examples includes clay montmorillonite, the principal constituent of bentonite, can trap hydrocarbons, alcohols, and glycols between the layers of their lattices. Graphite can also intercalate compounds between its layers.

Clathrates :

The clathrates crystallize in the form of a cage like lattice in which the co-ordinating compound is entrapped. Chemical bonds are not involved in these complexes, and only the molecular size of the encaged component is of importance. The stability of a clathrate is due to the strength of the structure. The highly toxic agent hydroquinone (quinol) crystallizes in a cage like hydrogen-bonded structure. The holes have a diameter of 4.2Å and permit the entrapment of one small molecule to about every two quinol molecules. Small molecules such as methyl alcohol, CO_2, and HCl may be trapped in these cages, but smaller molecules such as H_2 and larger molecules such as ethanol cannot be accommodated. It is possible that clathrates may be used to resolve optical isomers and to bring about other processes of molecular separation. The warfarin sodium USP, is a clathrate of water, isopropylalcohol, and sodium warfarin in the form of a white crystalline powder.

(III) Monomolecular Inclusion Compounds: Cyclodextrins

Inclusion compounds are of channel - and cage-type (clathrate) and mono- and macro molecular type. Monomolecular inclusion compounds involve the entrapment of a single guest molecule in the cavity of one host molecule. Monomolecular host structures are represented by the cyclodextrins (CD). These compounds are cyclic oligosaccharides containing a minimum of six *dextro*-glucopyranose units attached by α-1,4 linkages produced by the action on starch of Bacillus macerans amylase. The natural α−, β−, and γ− cyclodextrins consist of six, seven, and eight units of glucose, respectively.

Cyclodextrins are cyclic oligomers of glucose that can form water-soluble inclusion complexes with small molecules and portions of large compounds. These complexes are biocompatible and do not elicit any immune responses and have low toxicities in animals and humans. Some examples of cyclodextrins used in therapeutics along with their method of preparation are listed in Table 4.2.

Cyclodextrins has wide pharmaceutical applications such as improvement in the bioavailability of drugs of specific interest and delivery of nucleic acids. The CD has ability to form inclusion compounds in aqueous solution due to the typical arrangement of the glucose units. The cyclodextrin structure forms a doughnut ring. The molecule exists as a

truncated cone that it can accommodate molecules such as mitomycin C to form inclusion compounds. The interior of the cavity is relatively hydrophobic because of the CH_2 groups, whereas the cavity entrances are hydrophilic due to the presence of the primary and secondary hydroxyl groups. The α-CD has the smallest cavity (id 5Å), β-CD and γ-CD has larger cavity size (id 6Å and 8Å, respectively) and are the most useful for pharmaceuticals. Water inside the cavity tends to be squeezed out and to be replaced by more hydrophobic species. Thus, molecules of appropriate size and stereochemistry can be included in the cyclodextrin cavity by hydrophobic interactions. Complexation does not ordinarily involve the formation of covalent bonds. Some drugs may be too large to be accommodated totally in the cavity. Mitomycin C interacts with γ-CD at one side of the torus. Thus, the aziridine ring of mitomycin C is protected from degradation in acidic solution. The inclusion of indomethacin with β-CD is detected using a $_1$H-NMR technique. The *p*-chloro benzoyl part of indomethacin enters the β-CD ring, whereas the substituted indole moiety is too large for inclusion and rests against the entrance of the CD cavity.

Table 4.2: Examples of cyclodextrins used in the therapeutics and methods of complexation

Drug	Type CD	Method	Application
Celecoxib	βCD	Kneading evaporation and freeze drying	Improvement of aqueous solubility and dissolution rate
Celecoxib	HPβCD	Physical mixing on grinding, kneading and evaporation	Fast dissolution
Rofecoxib	SBE7βCD	Kneading	Better solubility enhancement with SBE7BCD than βCD
Valdecoxib	HPβCD and SBE7βCD	Kneading and co-evaporation	Enhanced solubility, dissolution rate and similar in vivo absorption rate with both CDs
Captopril	HPβCD and perbutanoyl βCD (TBβCD)	Kneading	Binary HPβCD gives faster release rate than binary TBβCD. Ternary captopril, TBβCD and HPβCD system shows better plasma profile.
Flurbiprofen	βCD, MβCD and hydroxyl ethyl βCD	Physical mixing kneading, sealed heating, co-evaporation and co-lyophilization	Solubility enhancement depending on cyclodextrin type and the preparation method
Eflucimibe	γCD	Kneading	Solubility enhancement

Complex formation has been used to alter the physicochemical and biopharmaceutical properties of drug. Complex drug may have altered stability, solubility, molecular size,

partition coefficient and diffusion coefficient. It is used in the various types of poisonings as well as in enhancing drug absorption and bioavailability from various dosage form. Cyclodextrins are used to trap, stabilize, and solubilize sulfonamides, tetracyclines, morphine, aspirin, benzocaine, ephedrine, reserpine, and testosterone.

4.3 APPLICATIONS OF COMPLEXATION

(i) Solubility enhancement : The aqueous solubility of retinoic acid (0.5 mg/L), a drug used topically in the treatment of acne, is increased to 160 mg/L by complexation with β-CD. Derivatives of the natural crystalline CD have been developed to improve aqueous solubility and to avoid toxicity. Partial methylation (alkylation) of some of the OH groups in CD reduces the intermolecular hydrogen bonding, leaving some OH groups free to interact with water, thus increasing the aqueous solubility of CD. A low degree of alkyl substitution is preferable. Derivatives with a high degree of substitution lower the surface tension of water, and this has been correlated with the hemolytic activity observed in some CD derivatives. Amorphous derivatives of β-CD and γ-CD are more effective as solubilizing agents for sex hormones than the parent cyclodextrins. The relatively low aqueous solubility of the CD is due to the formation of intramolecular hydrogen bonds between the hydroxyl groups, which prevent their interaction with water molecules.

(ii) Bioavailability enhancement : Dissolution rate plays an important role in bioavailability of drugs, fast dissolution usually favours absorption. The dissolution rates of famotidine (used in the treatment of gastric and duodenal ulcers) and that of tolbutamide (oral antidiabetic drug) is increased by complexation with β-CD. The testosterone complex with amorphous hydroxypropyl β-CD allow an efficient transport of hormone into the circulation upon sublingual administration. This route avoids metabolism in the intestines and first-pass decomposition in the liver and thus improves bioavailability.

(iii) Modifying reactivity : Cyclodextrins may increase or decrease the reactivity of the guest molecule depending on the nature of the reaction and the orientation of the molecule within the CD cavity. For example, α-cyclodextrin favours pH-dependent hydrolysis of indomethacin in aqueous solution, whereas β-CD inhibits it. The water solubility of β-CD (1.8 g/100 mL at 25°C) is insufficient to stabilize drugs at therapeutic doses. It is associated with nephrotoxicity when CD is administered by parenteral routes.

(iv) Modifying drug release : The hydrophobic forms of β-CD have been found useful as sustained-release drug carriers. The release rate of diltiazem (water-soluble calcium antagonist) was significantly decreased by complexation with ethylated β-CD. The release rate was controlled by mixing hydrophobic and hydrophilic derivatives of CD at several ratios. Ethylated β-CD has also been used to retard the delivery of isosorbide dinitrate, a vasodilator.

(v) Taste masking : Cyclodextrins may improve the organoleptic characteristics of oral liquid formulations. The bitter taste of suspensions of femoxetine (antidepressant) is greatly suppressed by complexation of the drug with β-CD.

(vi) Administration of therapeutic agents : Some therapeutic agents administered only as complexes due to physicochemical limitations. For example, iron complex with ferrous sulphate and carbonate and insulin complex with Zn and Vitamin-B12. These complexes reduce the GIT irritation, increase the absorption after oral administration and causes less irritation at the site of injection.

(vii) Use of ion exchange : Cholestyramine resin (quaternary ammonium anion exchange resin) is used to relief pruritus, the resin exchange chloride ion from bile result in increased elimination of bile through faeces.

(viii) In diagnosis : Technetium 90 (a radionuclide) is prepared in the form of citrate complex and this complex is used in diagnosis of kidney function and glomular filtration rate. Squibb (complex of a dye Azure A with carbacrylic cation exchange resin) is used for detection of achlorhydria due to carcinoma and pernicious anemia.

(ix) Complexation as a therapeutic tool : Complexing agents are used for variety of uses. Many of them are related to chelation of metal ion. One of the important uses is preservation of blood. EDTA and citrates are used for in-vitro to prevent clotting. For example, anticoagulant acid citrate dextrose solution and anticoagulant sodium citrate solution. Citrates act by chelating calcium ion in blood as it depletes body calcium.

(x) Treatment of poisoning : Therapeutic procedure involves complexation to minimize poisoning. It is possible by two pathways. First by absorption of toxicants from GIT using complexing and adsorbing agent and second by inactivation of toxic material systemically and enhanced elimination of toxic substance through use of dialysis. In case of heavy metal poisoning the basic step involve in detoxification wherein inactivation of metal present in body is carried out through chelation (metal chelates) and the water-soluble constituents are readily eliminated from body via kidney.

(a) Arsenic and mercury poisoning: The most effective agent is BAL (Dimercaprol). The arsenical dimercaprol is shown as: $CH_2SCHSAs\text{-}RCH_2OH$. Two sulphahydryl groups chelate with metal and a free OH group promotes water solubility. BAL is effective in treatment of poisoning from gold, bismuth, cadmium and polonium.

(b) Lead poisoning : Treatment of choice for acute/chronic lead poisoning is i.v. administration of calcium or disodium complex of EDTA. This complex chelates ions which exhibit a higher affinity of EDTA than do the calcium. The route of administration of complex is important and is given only by slow i.v. drip in isotonic NaCl or Sterile 5% dextrose solution. Oral administration promotes absorption of lead from GIT and increase body levels of lead.

(c) Radioactive materials : Poisoning with radioactive materials particularly with long biological half-life encounters problems that metal has toxic effect and body suffer from radiation damage. Uranium and plutonium exposure have been successfully treated with CaNaEDTA. Plutonium get deposited and chelate in bone so, prompt treatment is necessary.

(d) Dialysis and complexation in poisoning : Removal of poisons from systemic circulation can be done by artificial kidney or by peritoneal dialysis. Dialyzing fluid is injected into peritoneal cavity continually and circulated into and out of the cavity. The toxic material diffuses through the wall of the blood vessel into the fluid present in the cavity. The efficiency of this procedure is improved by using principle of complexation. If the toxicant is complexed with some high molecular weight non-diffusible component, the rate of dialysis of the toxicant is increased and complexed toxicant is prevented from returning into the circulation. It is useful in humans and animals. In the treatment of intoxication due to salicylates and barbiturates serum albumin is commonly used.

4.4 METHODS OF ANALYSIS

A determination of the stoichiometric ratio of ligand to metal or donor to acceptor and a quantitative expression of the stability constant for complex formation are important in the study and application of co-ordination compounds. A limited number of the more important methods for obtaining these quantities are described below.

Method of Continuous Variation :

The use of an additive property such as the spectrophotometric extinction coefficient such as dielectric constant or the square of the refractive index may also be used for the measurement of complexation. If the property for two species is sufficiently different and if no interaction occurs when the components are mixed, then the value of the property is the weighted mean of the values of the separate species in the mixture. This means that if the additive property, say dielectric constant, is plotted against the mole fraction from 0 to 1 for one of the components of a mixture where no complexation occurs, a linear relationship is observed.

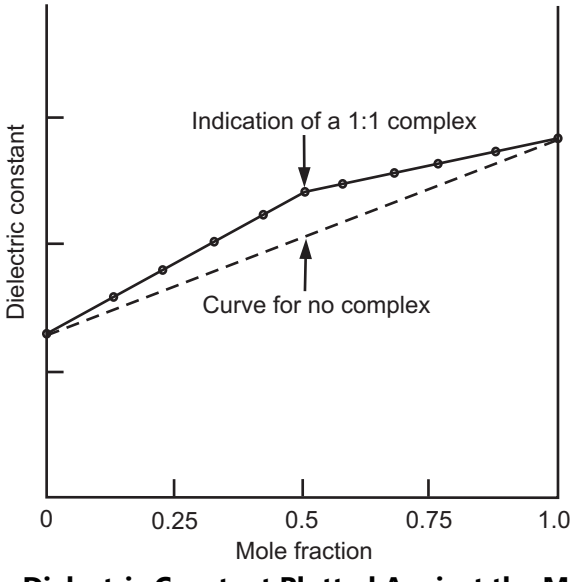


Figure 4.10 : Dielectric Constant Plotted Against the Mole Fraction

If solutions of two species A and B of equal molar concentration (and hence of a fixed total concentration of the species) are mixed and if a complex form between the two species, the value of the additive property will pass through a maximum (or minimum) as shown by the upper curve in Fig. 4.10. For a constant total concentration of A and B, the complex is at its greatest concentration at a point where the species A and B are combined in the ratio in which they occur in the complex. The line therefore shows a break or a change in slope at the mole fraction corresponding to the complex. The change in slope occurs at a mole fraction of 0.5 indicating a complex of the 1:1 type.

When spectrophotometric absorbance is used as the physical property, the observed values obtained at various mole fractions when complexation occurs are usually subtracted from the corresponding values that would have been expected had no complex resulted. This difference, D, is when plotted against mole fraction, as shown in Fig. 4.11 the molar ratio of the complex is readily obtained from such a curve.

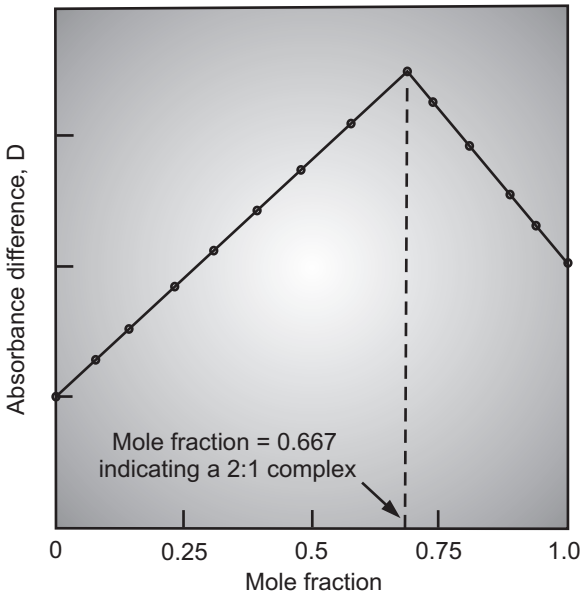


Figure 4.11 : A Plot of Absorbance Difference Against Mole Fraction

By means of a calculation involving the concentration and the property being measured, the stability constant of the complex formation can be determined by a method described by Bent and French. If the magnitude of the measured property, such as absorbance, is proportional only to the concentration of the complex MA_n, the molar ratio of ligand A to metal M and the stability constant can be readily determined. The equation for complexation can be written as

$$M + nA = MA_n \quad \ldots (4.1)$$

and the stability constant as

$$K = \frac{[MA_n]}{[M]\,[A]^n} \quad \ldots (4.2)$$

or, in logarithmic form,

$$\log [MA_n] = \log K + \log [M] + n \log [A] \quad \ldots (4.3)$$

where, $[MA_n]$ is the concentration of the complex, [M] is the concentration of the uncomplexed metal, [A] is the concentration of the uncomplexed ligand, n is the number of moles of ligand combined with 1 mole of metal ion, and K is the equilibrium or stability constant for the complex. The concentration of a metal ion is held constant while the concentration of ligand is varied, and the corresponding concentration, $[MA_n]$, of complex formed is obtained from the spectrophotometric analysis. Now, according to equation (4.3), if log $[MA_n]$ is plotted against log [A], the slope of the line yields the stoichiometric ratio or the number n of ligand molecules co-ordinated to the metal ion, and the intercept on the vertical axis allows one to obtain the stability constant, K, because [M] is a known quantity.

pH Titration Method :

This is most reliable method and used whenever the complexation is attended by a change in pH. The chelation of the cupric ion by glycine is represented as

$$Cu_2 + 2NH_3 + CH_2COO^- = Cu(NH_2CH_2COO)_2 + 2H^+ \quad \ldots (4.4)$$

In the reaction of equation since two protons are formed (equation 4.4) the addition of glycine to a solution containing cupric ions should result in a decrease in pH. The potentiometric titration curves are obtained from the results of data obtained by adding a strong base to a solution of glycine and to another solution containing glycine and a copper salt. The pH against the equivalents of base added is plotted as shown in Fig. 4.12. The curve for the metal–glycine mixture is well below that for the glycine alone, and the decrease in pH shows that complexation is occurring throughout most of the neutralization range. Similar results are obtained with other zwitterions and weak acids (or bases), such as N, N′-diacetyl ethylene diamine diacetic acid.

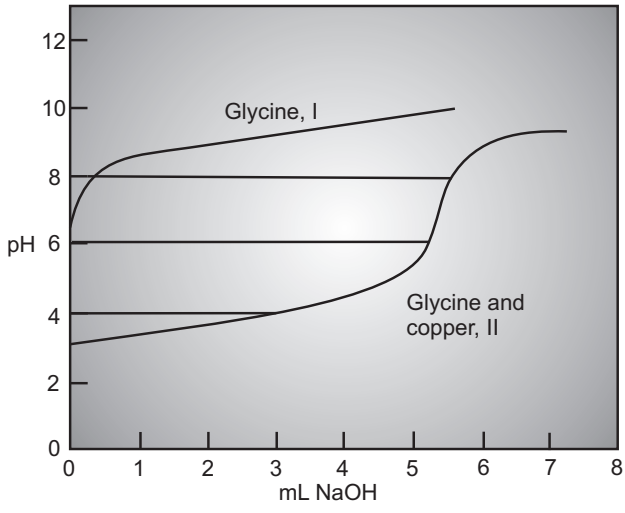


Figure 4.12 : Titration of Glycine and of Glycine in the Presence of Cupric Ions.

The difference in pH for a given quantity of base added indicates the occurrence of a complex.

The results are treated quantitatively to obtain stability constants for the complex. The two successive or stepwise equilibria between the copper ion or metal (M), and glycine or the ligand, (A), can be written in general as

$$M + A = MA;\ K_1 = \frac{[MA]}{[M][A]} \quad \ldots(4.5)$$

$$M + A = MA_2;\ K_2 = \frac{[MA_2]}{[M][A]} \quad \ldots (4.6)$$

and the overall reaction, (4.5) and (4.6), is

$$M + 2A = MA_2;\ \beta = K_1K_2 = \frac{[MA_2]}{[M][A]^2} \quad \ldots (4.7)$$

The terms K_1 and K_2 are formation constants, and the term β is equilibrium constant for the overall reaction and is known as the stability constant. A quantity *n* is the number of ligand molecules bound to a metal ion. The average number of ligand groups bound per metal ion present is therefore designated $\bar{n}$ (n bar) and is written as

$$\bar{n} = \frac{\text{Total concentration of ligand bound}}{\text{Total concentration of metal ion}} \quad \ldots (4.8)$$

$$\bar{n} = \frac{[MA] + 2[MA_2]}{[M] + [MA] + [MA_2]} \quad \ldots (4.9)$$

Although *n* has a definite value for each species of complex (1 or 2 in this case), it may have any value between 0 and the largest number of ligand molecules bound, i.e. 2 in this case. The numerator of equation 4.9 gives the total concentration of ligand species bound. The second term in the numerator is multiplied by 2 as two molecules of ligand are contained in each molecule of MA_2. The denominator gives the total concentration of metal present in all forms, both bound and free. For the special case in which $\bar{n} = 1$, equation (4.9) becomes

$$[MA] + 2[MA_2] = [M] + [MA] + [MA_2]$$

$$[MA_2] = [M] \quad \ldots (4.10)$$

Employing the results in equations (4.7) and (4.10), we obtain the following relation:

$$\beta = K_1K_2 = \frac{1}{[A]^2}$$

$$\log \beta = -2 \log[A]$$

and finally

$$p[A] = \frac{1}{2} \log \beta \text{ at } \bar{n} = 1 \quad \ldots (4.11)$$

where p[A] is written for –log [A]. Bjerrum also showed that, to a first approximation,

$$p[A] = \log K_1 \text{ at } \bar{n} = \frac{1}{2}$$

$$p[A] = \log K_2 \text{ at } \bar{n} = \frac{3}{2}$$

It should now be possible to obtain the individual complex formation constants, K_1 and K_2, and the overall stability constant, β, if one knows two values: $[\bar{n}]$ and p[A].

Equation (4.8) shows that the concentration of bound ligand must be determined before ñ can be evaluated. The horizontal distances represented by the lines in Fig. 4.12 between the titration curve for glycine alone (curve I) and for glycine in the presence of Cu^{2+} (curve II) give the amount of alkali used up in the following reactions:

This quantity of alkali is exactly equal to the concentration of ligand bound at any pH, and, according to equation (4.8), when divided by the total concentration of metal ion, gives the value of $[\bar{n}]$.

The concentration of free glycine [A] as the "base" $NH_2CH_2COO^-$ at any pH is obtained from the acid dissociation expression for glycine:

$$NH_3^+CH_2COO^- + H_2O = H_3O^+ + NH_2CH_2COO^-$$

$$K_a = \frac{[H_3O^+]\,[NH_2CH_2COO^-]}{[NH_3{+}CH_2COO^-]}$$

Or
$$[NH_2CH_2COO^-] = [A] = \frac{K_a[HA]}{[H_3O^+]} \quad \text{.... (4.12)}$$

The concentration $[NH_3^+CH_2COO^-]$, or [HA], of the acid species at any pH is taken as the difference between the initial concentration, $[HA]_{init}$, of glycine and the concentration, [NaOH], of alkali added. Then

$$[A] = K_a \frac{[HA]_0 - [NaOH]}{[H_3O^+]}$$

Or
$$-\log [A] = p[A] = pKa - pH - \log ([HA]_0 - [NaOH]) \quad \text{... (4.13)}$$

where, [A] is the concentration of the ligand glycine.

Distribution Method :

The method of distributing a solute between two immiscible solvents can be used to determine the stability constant for certain complexes. The complexation of iodine by potassium iodide may be used as an example to illustrate the method. The equilibrium reaction in its simplest form is

$$I_2 + I^- \rightleftharpoons I_3^-$$

Additional steps also occur in polyiodide formation; for example, $2I^- + 2I_2 \rightleftharpoons I_6^{2-}$ may occur at higher concentrations, but it need not be considered here. Higuchi investigated the complexing action of caffeine, polyvinylpyrrolidone, and polyethylene glycols on many acidic drugs, using the partition or distribution method. According to a study, the reaction between caffeine and benzoic acid to form the benzoic acid–caffeine complex is

$$\text{Benzoic acid} + \text{Caffeine} = (\text{Benzoic acid} - \text{Caffeine}) \quad \text{... (4.14)}$$

and the stability constant for the reactions at 0 °C is

$$K = \frac{[\text{Benzoic acid} - \text{Caffeine}]}{[\text{Benzoic acid}] - [\text{Caffeine}]}$$

$$= 37.5$$

Although the results varied, the value 37.5 being an average stability constant. It was reported that caffeine exists in aqueous solution primarily as a monomer, dimer, and tetramer, which would account in part for the variation in *K*.

Solubility Method :

According to the solubility method, excess quantities of the drug are placed in well-stoppered containers, together with a solution of the complexing agent in various concentrations, and the bottles are agitated in a constant-temperature bath until equilibrium is attained. Aliquot portions of the supernatant liquid are removed and analyzed.

The solubility method was used to investigate the complexation of *p*-amino benzoic acid (PABA) by caffeine. The results of the study are plotted as shown in Fig. 4.13. The point A at which the line crosses the vertical axis is the solubility of the drug in water. With the addition of caffeine, the solubility of PABA rises linearly owing to complexation. At point B, the solution is saturated with respect to the complex and to the drug itself. The complex continues to form and to precipitate from the saturated system as more caffeine is added. At point C, all the excess solid PABA has passed into solution and has been converted to the complex. Although the solid drug is exhausted and the solution is no longer saturated, some of the PABA remains uncomplexed in solution, and it combines further with caffeine to form higher complexes such as (PABA-2 caffeine) as shown by the curve at the right of the diagram.

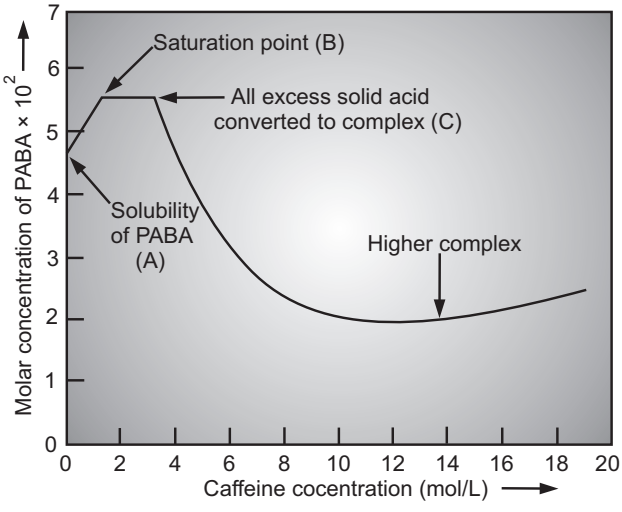


Figure 4.13: The Solubility of *Para*-Aminobenzoic Acid (PABA) in the Presence of Caffeine

The stability constants for many caffeine complexes obtained principally by the distribution and the solubility methods. Other example of water-soluble complexes of various ligands using the solubility method is an antiviral drug acyclovir.

Spectroscopy and Change Transfer Complexation Method :

Absorption spectroscopy in the visible and ultraviolet regions of the spectrum is commonly used to investigate electron donor–acceptor or charge transfer complexation. When iodine is analyzed in a non-complexing solvent such as CCl_4, a curve is obtained with a single peak at about 520 nm. The solution is violet. A solution of iodine in benzene exhibits a maximum shift to 475 nm, and a new peak of considerably higher intensity for the charge-shifted band appears at 300 nm. A solution of iodine in diethyl ether shows a still greater shift to lower wavelength and the appearance of a new maximum. These solutions are red to brown. These curves are shown in Fig. 4.14.

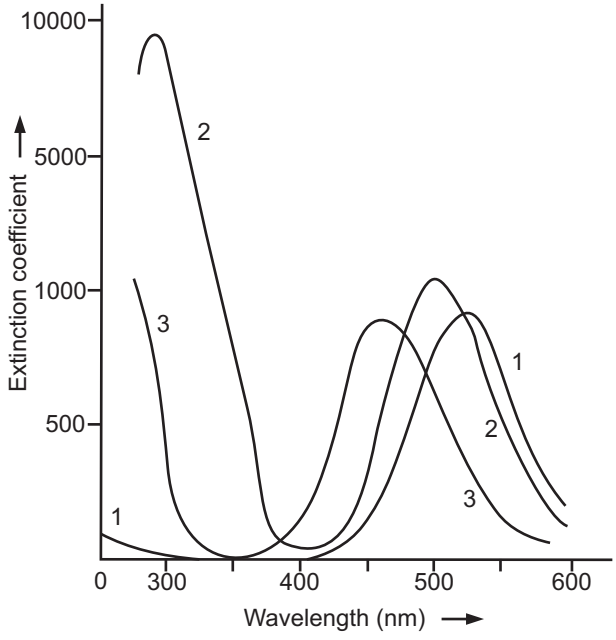


Figure 4.14 : Absorption Curves of Iodine in the Non-complexing Solvent : (1) CCl_4 and the complexing solvents (2) benzene and (3) diethyl ether.

In benzene and ether, iodine is the electron acceptor and the organic solvent is the donor; in CCl_4, no complex is formed. The shift toward the ultraviolet region becomes greater as the electron donor solvent becomes a stronger electron-releasing agent. These spectra arise from the transfer of an electron from the donor to the acceptor in close contact in the excited state of the complex. The more easily a donor such as benzene or diethyl ether releases its electron, as measured by its ionization potential, the stronger it is as a donor. Ionization potentials of a series of donors produce a straight line when plotted against the frequency maximum or charge transfer energies (1 nm = 18.63 cal/mole) for solutions of iodine in the donor solvents.

The complexation constant, *K*, can be obtained by use of visible and ultraviolet spectroscopy. The association between the donor *D* and acceptor *A* is represented as

$$D + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} DA \qquad \ldots (4.15)$$

where, $K = k_1/k_{-1}$ is the equilibrium constant for complexation (stability constant) and k_1 and k_{-1} are the interaction rate constants. When two molecules associate according to this scheme and the absorbance A of the charge transfer band is measured at a definite wavelength, K is readily obtained from the *Benesi–Hildebrand equation*:

$$\frac{A_o}{A} = \frac{1}{\varepsilon} + \frac{1}{K\varepsilon}\frac{1}{D_o} \quad \ldots (4.16)$$

A_o and D_o are initial concentrations of the acceptor and donor species, respectively, in mole/liter, ε is the molar absorptivity of the charge transfer complex at its wavelength, and K, the stability constant, is given in liter/mole or M^{-1}. A plot of A_o/A versus $1/D_o$ results in a straight line with a slope of $1/(K_\varepsilon)$ and an intercept of $1/\varepsilon$.

There has been reports about the interaction of nucleic acid bases (electron acceptors) with catechol, epinephrine, and isoproterenol (electron donors). Catechols have low ionization potentials and hence a tendency to donate electrons. Charge transfer complexation was evident as demonstrated by ultraviolet absorption measurements. With the assumption of 1:1 complexes, the equilibrium constant, K, for charge transfer interaction was obtained from Benesi–Hildebrand plots at three or four temperatures, and $\Delta H°$ was obtained at these same temperatures from the slope of the line.

Other Methods :

Many other methods are available for studying the complexation of metal and organic molecular complexes. They include NMR and infrared spectroscopy, polarography, circular dichroism, kinetics, X-ray diffraction, and electron diffraction. Several of these will be discussed briefly in this section.

(a) ^{1}H-NMR method: Complexation of caffeine with L-tryptophan in aqueous solution was investigated by using ^{1}H-NMR spectroscopy. Caffeine interacts with L-tryptophan at a molar ratio of 1:1 by parallel stacking. Complexation is a result of polarization and π - π interactions of the aromatic rings. The tryptophan, which is presumed to be the binding site in serum albumin for certain drugs, can interact with caffeine even as free amino acid. However, caffeine does not interact with other aromatic amino acids such as L-valine or L-leucine.

(b) Circular dichroism: The coil–helix transition of polyadenylic acid induced by the binding of the catecholamines norepinephrine and isoproterenol, using circular dichroism. Most mRNA molecules contain regions of polyadenylic acid, which are thought to increase the stability of mRNA and to favor genetic code translation. The change of the circular dichroism spectrum of polyadenylic acid was interpreted as being due to intercalative binding of catecholamines between the stacked adenine bases. Catecholamines may exert a control mechanism through induction of the coil-to-helix transition of polyadenylic acid, which influences genetic code translation.

(c) Infrared spectroscopy: The infrared spectroscopy was also used to investigate the hydrogen-bonded complexes involving polyfunctional bases such as proton donors. This is a very precise technique for determining the thermodynamic parameters involved in

hydrogen-bond formation and for characterizing the interaction sites when the molecule has several groups available to form hydrogen-bonded. Caffeine forms hydrogen-bonded complexes with various proton donors: phenol, phenol derivatives, aliphatic alcohols, and water. From the infrared technique, the preferred hydrogen-bonding sites are the carbonyl functions of caffeine. Seventy percent of the complexes are formed at the C=O group at position 6 and 30% of the complexes at the C=O group at position 2 of caffeine. Conductometric and infrared methods has also been used to characterize 1:1 complexes between uranyl acetate and tetracycline.

4.5 PROTEIN BINDING

A complete analysis of protein binding, including the multiple equilibria below.

We write the interaction between a group or free receptor *P* in a protein and a drug molecule *D* as

$$P + D \rightleftharpoons PD$$

The equilibrium constant, disregarding the difference between activities and concentrations, is

$$K = \frac{[PD]}{[P]\,[D_f]}$$

Or
$$K\,[P]\,[D_f] = [PD] \quad \ldots (4.17)$$

Where, *K* is the association constant, [*P*] is the concentration of the protein in terms of free binding sites, [D_f] is the concentration of free drug (in moles), sometimes called the ligand, and [*PD*] is the concentration of the protein–drug complex. The value of *K* varies with temperature and would be better represented as $K_{(T)}$; [*PD*], is bound drug and is sometimes written as [D_b] or [*D*], the free drug, as [D_f]. If the total protein concentration is designated as [P_t], we can write

$$[P_t] = [P] + [PD]$$

Or
$$[P] = [P_t] - [PD] \quad \ldots (4.18)$$

Substituting the expression for [*P*] from equation (4.18) into (4.17) gives

$$[PD] = K\,[D_f]\,([P_t - [PD]) \quad \ldots (4.19)$$

$$[PD] = K\,[D_f\,]\,[PD] \quad \ldots (4.20)$$

$$= K\,[D_f\,]\,[Pt]$$

$$\frac{[PD]}{Pt} = \frac{K\,[D_f]}{(1 + K\,[D_f])} \quad \ldots (4.21)$$

Let *r* be the number of moles of drug bound, [*PD*], per mole of total protein, [P_t]; then

$$r = \frac{[PD]}{[P_t]}, \text{ or}$$

$$r = \frac{K\,[D_f]}{(1 + K\,[D_f])} \quad \ldots (4.22)$$

The ratio *r* can also be expressed in other units, such as milligrams of drug bound, *x*, per gram of protein (*m*). Although equation (4.22) is one form of the Langmuir adsorption isotherm and is quite useful for expressing protein-binding data, it must not be it must not necessarily be that protein binding be an adsorption phenomenon. The equation (4.22) can be converted to a linear form, convenient for plotting, by inverting it:

$$\frac{1}{r} = \left(\frac{1}{K[D_f]}\right) + 1 \quad \ldots (4.23)$$

If *v* independent binding sites are available, the expression for *r*, equation (4.23), is simply *v* times that for a single site, or

$$r = v\left(\frac{K[D_f]}{1 + K[D_f]}\right) \quad \ldots (4.24)$$

and equation (4.24) becomes,

$$\frac{1}{r} = \left(\frac{1}{v\,K}\right)\left(\frac{1}{[D_f]}\right) + \frac{1}{v} \quad \ldots (4.25)$$

The equation (4.25) is called a *Klotz reciprocal plot*. An alternative manner of writing equation (4.25) is to rearrange it first to

$$r + rK\,[D_f] = \nu\,K\,[D_f] \quad \ldots (4.26)$$

$$r/[D_f] = \nu\,K - r\,K \quad \ldots (4.27)$$

Data presented according to equation (4.20) are known as a *Scatchard plot*.

The binding of bishydroxycoumarin to human serum albumin and the graphical treatment of data using equation (4.27) heavily weights those experimental points obtained at low concentrations of free drug, *D*, and may therefore lead to misinterpretations regarding the protein binding behavior at high concentrations of free drug. The equation (4.27) does not have this disadvantage and is the method of choice for plotting data. Curvature in these plots usually indicates the existence of more than one type of binding site. The equation 19 and 20 cannot be used for the analysis of data if the nature and the amount of protein in the experimental system are unknown. For these situations, Sandberg recommended the use of a slightly modified form of equation (4.27):

$$\frac{[D_b]}{[D_f]} = -K[D_b] + \nu\,K\,[P_t] \quad \ldots (4.28)$$

where $[D_b]$ is the concentration of bound drug. The equation (4.28) is plotted as the ratio $[D_b]/[D_f]$ versus $[D_b]$, and in this way *K* is determined from the slope and $vK[P_t]$ is determined from the intercept.

The Scatchard plot yields a straight line when only one class of binding sites is present. Frequently in drug-binding studies, *n* classes of sites exist, each class *i* having *vi* sites with a unique association constant *Ki*. In such a case, the plot of $r/[D_f]$ versus *r* is not linear but exhibits a curvature that suggests the presence of more than one class of binding sites. The data in Fig. 4.15 is analyzed in terms of one class of sites for simplification. The plots at 20 °C

and 40 °C clearly show that multiple sites are involved. Blanchard reviewed the case of multiple classes of sites. The equation (4.24) is then written as

$$r = \frac{v_1K_1[D_f]}{1 + K_1[D_f]} + \frac{v_2K_2[D_f]}{1 + K_2[D_f]} + \cdots \frac{v_nK_n[D_f]}{1 + K_n[D_f]} \quad \ldots (4.29)$$

or

$$r = \sum_{i=1}^{n} \frac{v_iK_i[D_f]}{1 + K_i[D_f]} \quad \ldots (4.30)$$

As mentioned earlier, only *v* and *K* need to be evaluated when the sites are all of one class. When *n* classes of sites exist, equation (4.29) and equation (4.30) can be written as

$$r = \sum_{i=1}^{n-1} \frac{v_iK_i[D_f]}{1 + K_i[D_f]} + v_nK_n[D_f] \quad \ldots (4.31)$$

The binding constant, K_n, in the term on the right is small, indicating extremely weak affinity of the drug for the sites, but this class may have many sites and so be considered unsaturable.

4.6 COMPLEXATION AND DRUG ACTION

The Fig. 4.16 depicts transfer of Drug (D), Complex (DC), and complexing agent (C) across biological membrane. With subsequent dissociation of complex after transfer.

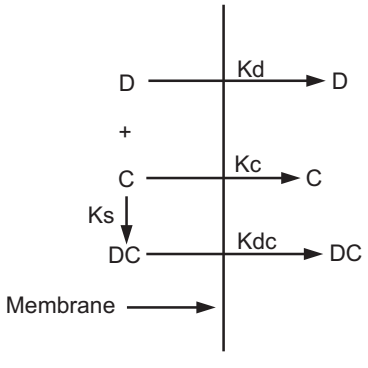


Figure 4.15 : Transfer of Moieties Across Biological Membrane

The rate of transfer of total drug on the right side of the membrane is a function of rate of transport of drug in its free and complex form. If transport rate of complex is more than drug, the diffusion will be aided by complex formation. If complexing agent is not diffusible rate of appearance of drug will be a function of transfer of free (uncomplexed) drug. If the complex is not transported, diffusion is retarded by complexation. The mechanism by which complex formation can affect the passage compound include alteration of o/w partition coefficient, apparent solubility, effective size of drug, change in the charge of the drug and alteration in diffusion of drug.

One of the best example of this class is the interference of calcium ions with the intestinal absorption of tetracycline. Earlier tetracycline preparations were made with calcium diphosphate to treat gastric irritation that was administered with milk but it was observed

that poor absorption was due to the formation of relative insoluble complex of tetracycline and calcium. Other examples wherein absorption was decreased due to formation of complex include oral administration of neomycin and kanamycin with bile salt. Complexing agent EDTA depress the absorption of strychnine alcohol and sulfanilamide in animals. EDTA is thought to be related to their interaction with metal ion in the G.I.T. On the contrary, enhanced drug absorption through complex formation was also observed in some cases. Thus, complex formation was proved to be an effective means of enhancing the absorption of poorly absorbed drug. For example, improvement in intestinal absorption of tetracycline's with the addition of citric acid, glucosamine or sodium hexametaphosphate or use of tetracycline phosphate complex. Other examples where drug absorption includes heparin whose absorption is increased in G.I.T. in the presence of EDTA or SLS or dioctyl sodium sulfosuccinate. The intestinal absorption of various quaternary ammonium compounds, organic acids and some neutral molecules such as mannitol and inulin is also found to be increased in presence of EDTA.

Other examples of drugs where complex has significant effect on drug action is through enhancing solubility and drug stability. For example, adrenochrome monosemicarbazone is complexed with sodium salicylate. Adrenochrome (active) was found to be unstable in solution and semicarbazone has only limited solubility at the pH at which it is stable. However, the stable product can be prepared by the addition of sodium salicylate which complexes with adrenochrome, and thus increases its apparent solubility by about 10 folds. The injectable caffeine and sodium benzoate is used as stimulant and diuretic. The complexation of caffeine by sodium benzoate increases solubility of caffeine.

The problem of stabilization of the ingredient present in the preparation against hydrolysis, oxidation etc. is another instance where complexation formation has been used. The interaction of labile functional groups of a drug with complexing agent may protect the drug from the attack of other species or the interaction may alter the usual electronic properties of the drug that result into either increase or decrease in stability. For example, local anesthetic esters have been stabilized against hydrolysis by complexation with caffeine. The half-life for procaine in the solution has been observed to increase from 26 h in the absence of caffeine to about 46 h in the presence of 2% caffeine and to about 71 h in the presence of 5% caffeine. The stabilization of certain compound can be done by incorporation within the crystal lattice of a solid or with in the voids formed by the arrangement of large polymeric molecules in solution.

4.7 CRYSTALLINE STRUCTURES OF COMPLEXES

Complex or co-ordination compounds cover the range from quite simple inorganic salts to elaborate metal-organic hybrid materials and intricate bioactive metalloproteins. Their present uses and their potential applications are diverse due to their compositions, their molecular and crystal structures and their chemical and physical properties. Besides their use as chemical reactants, complex compounds are considered for extraction processes and as active agent in remedies and for drug delivery.

4.8 THERMODYNAMIC TREATMENT OF STABILITY CONSTANTS

The thermodynamics of metal ion complex formation provides much significant information. In particular, it is useful in distinguishing between enthalpic and entropic effects. Enthalpic effects depend on bond strengths and entropic effects have to do with changes in the order/disorder of the solution as a whole. The chelate effect, below, is best explained in terms of thermodynamics.

Equilibrium constant is related to the standard Gibbs free energy change for the reaction

$$\Delta G^\circ = -2.303\ RT \log_{10} \beta \quad \ldots (4.32)$$

where, R is the gas constant and T is the absolute temperature.

At 25 °C, $\Delta G^\circ = -5.708\ kJ\ mol^{-1} \cdot \log \beta$.

The free energy is made-up of an enthalpy term and an entropy term.

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad \ldots (4.33)$$

The standard enthalpy change can be determined by calorimetry or by using the Van't Hoff equation, though the calorimetric method is preferable. When both the standard enthalpy change and stability constant have been determined, the standard entropy change is easily calculated from the equation above.

The fact that stepwise formation constants of complexes of the type ML_n decrease in magnitude as *n* increases may be partly explained in terms of the entropy factor. Take the case of the formation of octahedral complexes.

$$[M(H_2O)_mL_{n-1}] + L \rightleftharpoons [M(H_2O)_{m-1}L_n] \quad \ldots (4.33\ [a])$$

For the first step m = 6, n = 1 and the ligand can go into one of 6 sites. For the second step m = 5 and the second ligand can go into one of only 5 sites. This means that there is more randomness in the first step than the second one; ΔS° is more positive, so ΔG° is more negative and $\log K_1 > \log K_2$. The ratio of the stepwise stability constants can be calculated on this basis, but experimental ratios are not exactly the same because ΔH° is not necessarily the same for each step.

The entropy factor is also important in the chelate effect.

The thermodynamic equilibrium constant, K°, for the equilibrium $M + L \rightleftharpoons ML$ can be defined as

$$K^\circ = \frac{\{ML\}}{\{M\}\ \{L\}} \quad \ldots (4.34)$$

Where, {ML} is the activity of the chemical species ML, K° is dimensionless since activity is dimensionless. Activities of the products are placed in the numerator whereas activities of the reactants are placed in the denominator. Since activity is the product of concentration and activity coefficient (γ) the definition could also be written as

$$K^\circ = \frac{[ML]}{[M]\ [L]} \times \frac{\gamma_{ML}}{\gamma_M\ \gamma_L} = \frac{[ML]}{[M]\ [L]} \times \Gamma \quad \ldots (4.35)$$

Where, [ML] represents the concentration of ML and Γ is a quotient of activity coefficients. This expression can be generalized as

$$\beta^{\circ}_{pq\ldots} = \frac{[M_pL_q\ldots]}{[M]^p\,[L]^q\ldots} \times \Gamma \qquad \ldots (4.36)$$

For example, the stability constant in the formation of $[Cu(glycinate)]^+$ is dependent of ionic strength ($NaClO_4$).

To avoid the complications involved in using activities, stability constants are determined, where possible, in a medium consisting of a solution of a background electrolyte at high ionic strength, that is, under conditions in which Γ can be assumed to be always constant. For example, the medium might be a solution of 0.1 mol/dm^3 sodium nitrate or 3 mol/dm^3 sodium perchlorate. When Γ is constant it may be ignored and the general expression in theory, above, is obtained.

All published stability constant values refer to the specific ionic medium used in their determination and different values are obtained with different conditions, as illustrated for the complex CuL (L = glycinate). Furthermore, stability constant values depend on the specific electrolyte used as the value of Γ is different for different electrolytes, even at the same ionic strength. There does not need to be any chemical interaction between the species in equilibrium and the background electrolyte, but such interactions might occur in particular cases. For example, phosphates form weak complexes with alkali metals, so, when determining stability constants involving phosphates, such as ATP, the background electrolyte used will be, for example, a tetralkylammonium salt. Another example involves iron (III) which forms weak complexes with halide and other anions, but not with perchlorate ions.

All equilibrium constants vary with temperature according to the Van't Hoff equation

$$\frac{d(\ln K)}{dT} = \frac{\Delta H^{\ominus}_m}{RT^2} \qquad \ldots (4.37)$$

where, R is the gas constant and T is the thermodynamic temperature. Thus, for exothermic reactions, (the standard enthalpy change, $\Delta H°$, is negative) K decreases with temperature, but for endothermic reactions ($\Delta H°$ is positive) K increases with temperature.

EXERCISE

1. Understand the significance of complexation in pharmaceutical products.
2. Appreciate the fundamental forces that are related to the formation of drug complexes.
3. Differentiate between co-ordination and molecular complexation.
4. Understand the mechanism of co-ordinate bond formation leading to the formation of co-ordinate complexes.
5. Appreciate the biological and pharmaceutical roles of co-ordinate complexes.
6. Describe the mechanism of inclusion complex formation, with special emphasis on drug-cyclodextrin complexes.

7. Relate the formation of drug-cyclodextrin complexes with improvements in the physicochemical properties and bioavailability of drugs.
8. Determine the values of the association constant and the stoichiometry of association.
9. Understand the importance of the ion-exchange mechanism and its role in drug delivery and therapy.
10. Appreciate the significance of protein-ligand interactions.
11. Understand the significance of plasma protein binding for the distributive properties of drugs in the body.
12. Identify the important properties of plasma proteins and the mechanism of their interactions with drugs.
13. Appreciate equilibrium dialysis and other techniques for in vitro analysis of drug-protein binding.
14. Analyse protein-binding data by the double-reciprocal method and determine the values of the association constant and the number of binding sites.
15. Analyse protein-binding data by the Scatchard method and determine the values of the association constant and the number of binding sites.
16. Appreciate the advantages of the Scatchard method over the double-reciprocal method of analysis with respect to multiple binding affinities.
17. Define the three classes of complexes (co-ordination compounds) and identify pharmaceutically relevant examples.
18. Describe chelates, their physically properties, and what differentiates them from organic molecular complexes.
19. Describe the types of forces that hold together organic molecular complexes and give examples.
20. Describe the forces involved in polymer–drug complexes used for drug delivery and situations where reversible or irreversible complexes may be advantageous.
21. Discuss the uses and give examples of cyclodextrins in pharmaceutical applications.
22. Determine the stoichiometric ratio and stability constant for complex formation.
23. Describe the methods of analysis of complexes and their strengths and weaknesses.
24. Discuss the ways that protein binding can influence drug action.
25. Describe the equilibrium dialysis and ultrafiltration methods for determining protein binding.
26. Understand the factors affecting complexation and protein binding.
27. Understand the thermodynamic basis for the stability of complexes.



Unit ...5

pH, BUFFERS AND ISOTONIC SOLUTIONS

♦ OBJECTIVES ♦

Buffers are compounds that resist changes in pH upon the addition of limited amounts of acids or bases. Buffer systems are usually composed of a weak acid or base and its conjugate salt. The components act in such a way that addition of acid or base results in the formation of a salt causing only a small change in pH. Buffer capacity is a measure of the efficiency of a buffer in resisting changes in pH. In practice, smaller pH changes are measured and the buffer capacity is quantitatively expressed as the ratio of acid or base added to the change in pH produced. The buffer capacity depends on various factors. The addition of any compound to a solution affects the isotonicity. The osmotic pressure of a solution is affected not only by the drug but also by any of the buffer components that are included in the formulation. But even after these buffers have been added, it is still possible that the solution may not be isotonic. Thus, it may be necessary to add additional sodium chloride to bring the solution to isotonicity.

Upon studying this unit, students should be able to:

- Define and determine pH and pOH.
- Define buffers, buffer capacity, isotonicity, iso-osmoticity, osmotic pressure, hypertonicity, hypotonicity.
- Describe the uses of buffers in pharmaceutical solutions.
- Identify the range of solution pH considered safe for ophthalmic solutions.
- Formulate and analyze a buffer solution of desired pH and buffer capacity.
- Explain the importance of isotonicity in ophthalmic solutions.
- Formulate and prepare pharmaceutically and physiologically acceptable parentral solutions.

5.1 INTRODUCTION

Acids and bases contain ions of the element hydrogen. Acids are the substances that deliver hydrogen ion to the solution. The law of mass action can be applied to ionic reactions, such as dissociation of an acid into positively charged hydrogen ion and a

negatively charged anion. The hydrogen ion concentration and hydroxyl ion concentrations are used to characterize solutions. The dissociation constant of weak acid is measure of an acid's strength. Ions are atoms or molecules that have lost or gained electrons. If atoms lose one or more electrons they become positively charged ions (cations). If they gain one or more electrons, they become negatively charged ions (anions). Hydrogen and hydroxyl ion concentrations found in aqueous solutions can be written in molar units, and denoted as $[H^+]$, and $[OH^-]$, respectively. The concept of pH arose from a need to quantify the $[H^+]$ in aqueous solutions. Water has a nearly balanced concentration of positive (H^+) and negative (OH^-) ions.

5.2 SORENSEN'S pH SCALE

The hydrogen ion concentration in pure water at room temperature is about 1.0×10^{-7} M. Since every water solution contains hydrogen ions, their concentration is one of the most important parameters describing solution properties. The pH scale was defined because the enormous range of hydrogen ion concentrations found in aqueous solutions make using H^+ molarity awkward. For example, in a typical acid-base titration, $[H^+]$ may vary from about 0.01 M to 0.0000000000001 M. Such numbers are inconvenient to use but it is easier to write as "the pH varies from 2 to 13".

To simplify things Danish biochemist Soren Sorensen in 1909 developed the pH scale and introduced pH definition as minus (−) logarithm of $[H^+]$ to the base 10. A pH of 7 is considered as "neutral", because the concentration of hydrogen ions is exactly equal to the concentration of hydroxide (OH^-) ions produced by dissociation of the water. Increasing the concentration of hydrogen ions above 1.0×10^{-7} M produces a solution with a pH of less than 7, and the solution is considered as "acidic". On other hand decreasing the concentration of hydrogen ions below 1.0×10^{-7} M produces a solution with a pH above 7, and the solution is considered "alkaline" or "basic".

The pH is an indication of the degree of acidity or basicity (alkalinity) relative to the ionization of water. The term pH is an abbreviation for "pondushydrogenii" (translated as potential hydrogen) meaning hydrogen power, as acidity is caused by a predominance of hydrogen ions. Initially pH was written as PH. According to the Compact Oxford English Dictionary, the modern notation "PH" was first adopted in 1920 by W. M. Clark. The letter "p" in the term "pH" stands for the German word "potenz" (power), so pH is an abbreviation for "power of hydrogen". In simple terms pH is a logarithmic measure of hydrogen ion concentration.

$$pH = -\log [H^+] \quad \ldots (5.1)$$

where, log is a base −10 logarithm and $[H^+]$ is the concentration of hydrogen ions in moles per liter of solution.

With the progress and development of theory of chemical reactions, the definition of pH was reexamined. As the role and behaviour of electrical charge in chemical reactions became better understood, the definition of pH was changed to consider the active hydrogen ion

concentration. Debeye, Huckle and Lowry described a more detailed and theoretically more complete definition of pH.

$$pH = -\log aH^+ \quad \ldots (5.2)$$

where, aH^+ is the hydrogen ion activity meaning effective concentration of hydrogen ion.

There is a difference between concentration and activity for acids, but the same holds true for bases.

1. In dilute solutions (0.001 molar = 1mM) all anions and all cations are so far apart that they are capable to produce the maximum of the chemical energy, i.e. $[H^+] = aH^+$.
2. At higher acid or base concentrations, the physical spacing between cations and anions decreases, such that they begin to obstruct each other, and shield each other's charge. Therefore, the mobility of the any ion is impaired by interactions with other ions and their associated electrical fields. These local electric field interactions affect the extent to which the ions can participate in chemical reactions, and give an apparent concentration that is always smaller than the real concentration. In this case, the ion activity is "slowed down"; specifically, $[H^+] > aH^+$.
3. The difference between ion activity and concentration increases with the acid concentration. Therefore, for acid concentrations greater than 1mM it is generally advisable to use activities instead of concentrations to accurately predict pH.

The pOH

Not only H^+ ions are present in every water solution but OH^- ions are also always present, and their concentration can change in the very wide range. Thus, it is convenient to use similar definition to describe $[OH^-]$.

$$pOH = -\log [OH^-] \quad \ldots (5.3)$$

In real solutions ion activities rather than concentrations should be used for calculations. The definition of pH uses minus logarithm of activity and not the minus logarithm of concentration. In diluted solutions activity, for all practical purposes is identical to concentration. It means when the concentration goes higher activity starts first to be lower than the concentration and then once the concentration rises it becomes higher than the concentration. If the concentration of charged ions present in the solution is below 0.001M then don't concerned about activities and use classic pH definition. The relationship between pH, $[H^+]$ and $[OH^+]$ in moles/L at 25 °C is given in Table 5.1.

The presence of hydrogen ions in solutions allows us to measure the pH of a solution. The quantity of hydrogen ions (cations) or hydroxyl ions (anions) in a solution determines whether the solution is acidic or alkaline. The concentrations of hydrogen and hydroxyl ions in pure, acidic and alkaline aqueous solutions are used to find the following ionic concentrations:

Pure water:

$$[H^+] = 1 \times 10^{-7} \text{ moles/L and } [OH^-] = 1 \times 10^{-7} \text{ moles/L}$$

Acidic water (1 molar aqueous HCl):

$[H^+] = 1 \times 10°$ moles/L and $[OH^-] = 1 \times 10^{-14}$ moles/L

Alkaline water (1 molar aqueous NaOH):

$[H^+] = 1 \times 10^{-14}$ moles/L and $[OH^-] = 1 \times 10°$ moles/L

Concentration of H^+ ions have major effects on most of the chemical reactions. Depending on concentration hydrogen peroxide can behave as oxidizing or reducing agent, pepsin an enzyme used for digestion works best in strongly acidic conditions (which becomes inactive in neutral solutions) and tea changes its color on addition of a slice of lemon. Therefore, acidity of the solution is of such importance that it was convenient to create a pH scale for its measurements based upon use of Sorensen's pH definition.

Table 5.1: Relationship between pH, $[H^+]$ and $[OH^+]$ in Moles/L at 25° C

pH	$[H^+]$	$[OH^+]$
0	(10^0) 1	0.00000000000001 (10^{-14})
1	(10^{-1}) 0.1	0.0000000000001 (10^{-13})
2	(10^{-2}) 0.01	0.000000000001 (10^{-12})
3	(10^{-3}) 0.001	0.00000000001 (10^{-11})
4	(10^{-4}) 0.0001	0.0000000001 (10^{-10})
5	(10^{-5}) 0.00001	0.000000001 (10^{-9})
6	(10^{-6}) 0.000001	0.00000001 (10^{-8})
7	(10^{-7}) 0.0000001	0.0000001 (10^{-7})
8	(10^{-8}) 0.00000001	0.000001 (10^{-6})
9	(10^{-9}) 0.000000001	0.00001 (10^{-5})
10	(10^{-10}) 0.0000000001	0.0001 (10^{-4})
11	(10^{-11}) 0.00000000001	0.001 (10^{-3})
12	(10^{-12}) 0.000000000001	0.01 (10^{-2})
13	(10^{-13}) 0.0000000000001	0.1 (10^{-1})
14	(10^{-14}) 0.00000000000001	1 (10^0)

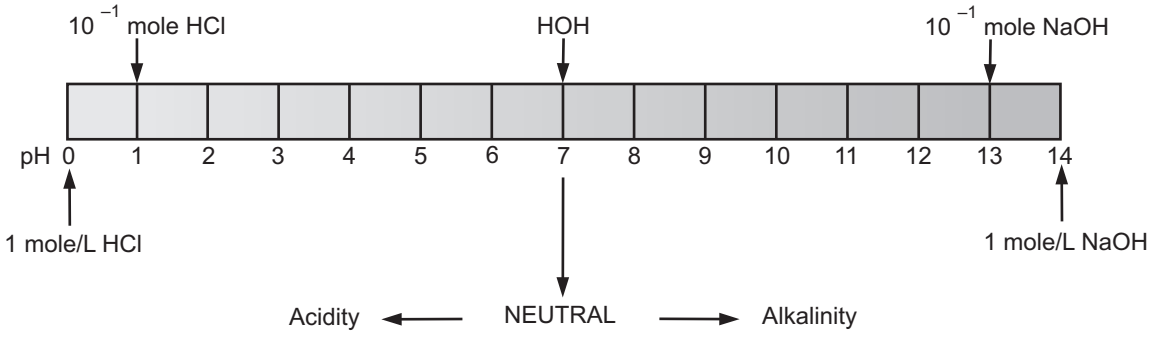


Figure 5.1: Comparison of pH Scale and $[H^+]$ and $[OH^-]$ Molar Concentrations

In aqueous solutions, the reversible reaction between hydrogen ions, hydroxyl ions and water molecules, namely $H^+ + OH^- \rightleftharpoons H_2O$. It leads to the following equation:

$$[H^+] \times [OH^-] = 10^{-14} \quad \ldots (5.4)$$

The pH scale shown in Fig. 5.1 indicates both $[H^+]$ and $[OH^-]$ through this relationship. The mid-point of 7 on the pH scale indicates ionic neutrality of the solution, when $[H^+] = [OH^-]$. The range of acid pH values extends from 0 to 7, while that of alkaline pH values from 7 to 14. The Fig. 5.2 illustrates these ranges, and provides some examples of common acidic and alkaline solutions.

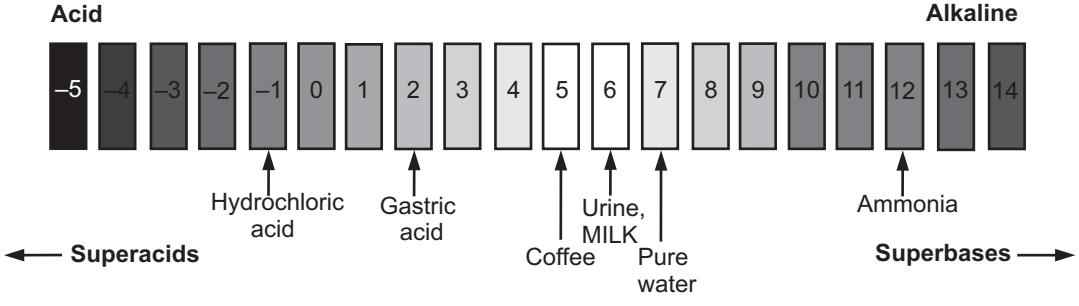


Figure 5.2: pH Scale Showing Some Examples of Acid and Alkaline Substances

On the pH scale, pure water has pH 7. Since air always contains small amounts of carbon dioxide, it dissolves in water to make it slightly acidic with pH of about 5.7. The lower the pH, the more acidic solution and solutions with pH above 7 are basic and hence higher the pH the more basic solution is. As the pH scale is logarithmic, it does not start at zero. The most acidic of liquids can have a pH as low as −5. The most alkaline solution has pH of 14. Measurement of extremely low pH values has various complications.

There are two important things about pH scale.

1. As pH scale is logarithmic, 1 unit pH change means tenfold change in the H^+ ion concentration.
2. Only solution with pH = 7 is strictly neutral and all solutions with pH in the range 4 to 10 have real concentration of H^+ and OH^- lower than 10^{-4} M which can be easily disturbed with small additions of acid and base.

The pH scale described earlier is sometimes called "concentration pH scale" because it considers H^+ ion concentration. The another one is called "thermodynamic pH scale" which considers the H^+ activity rather than the H^+ ion concentration. Using pH electrodes we measure just activity and not the concentration in the solution and thus it is a thermodynamic pH scale. It describes real solutions but not the concentration. Concentration pH scale is defined for pure substance and not for water solution whereas thermodynamic pH scale can be defined not only for water solutions, but also for some other solvents, like methanol, ammonia, acetic acid etc. Range of pH for such solvents depends on their ion product for example, pH for acetic acid ranges from 0 to 15.2 while pH for methanol ranges from 0 to 16.7.

5.3 ELECTROMETRIC pH DETERMINATION

The pH of the sample is determined electrometrically using either a glass electrode in combination with a reference potential or a combination electrode. The measuring device is calibrated using a series of standard solutions of known pH. A pH is commonly measured with a potentiometric glass electrode. A pH electrode consists of two half-cells; an indicating electrode and a reference electrode. This may, however, be combined into a single probe, called a "combination pH electrode". A pH electrode contains a bulb at the end covered with a thin glass membrane, Fig. 5.3. This membrane becomes hydrated in the presence of water. Hydrogen ions can enter the silicon-oxygen structure of the glass and alter the charge. This creates a change in electrical potential with respect to the silver/silver chloride reference. The free energy change is related to the change in hydrogen ion activity by equation (5.5).

$$G = -RT\, ln \frac{[H^+]_1}{[H^+]_2} \quad \ldots (5.5)$$

where $[H^+]_1$ and $[H^+]_2$ are hydrogen ion activities of unknown and reference.

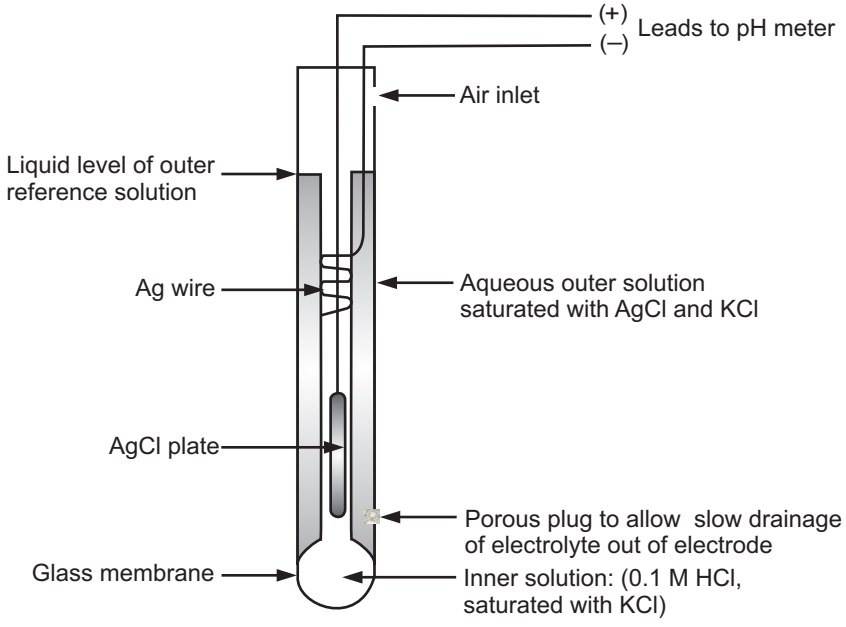


Figure 5.3: A Standard Glass Combination pH Electrode

Glass pH electrodes respond to hydrogen ion activity rather than concentration. Activity may be expressed as product of an activity coefficient (γ) and the hydrogen ion concentration.

$$[H^+] = \gamma\,[H^+] \quad \ldots (5.6)$$

where, the activity coefficient is a function of ionic strength and it has a value close to 1 in dilute solution. Glass electrodes respond to sodium ions to a slight extent causing errors under conditions of low hydrogen ion activity (i.e., high pH) and high sodium concentrations.

pH METER

pH meter is a precise voltameter connected to the pH electrode which is very selective to ions. pH meter can read small millivolt changes from the pH electrode system. Voltage produced by the pH electrode is proportional to logartithm of the H^+ activity. The pH meter display is scaled in such a way that the displayed results of measurement is just the pH of the solution.

pH measurement involves comparing the potential of solutions with unknown $[H^+]$ to a known reference potential. pH meters convert the voltage ratio between a reference half-cell and a sensing half-cell to pH values. The meter is seldom source of problems for pH measurements. A successful pH reading is dependent upon all components of the system being operational. Problems with any one of the three: electrode, meter or buffer yields poor readings. Over 90% of pH measurement problems are related to the improper use, storage or selection of electrodes. Most applications today use a combination electrode with both half cells in one body. Today pH meters have temperature compensation (either automatic or manual) to correct for variations in slope caused by changes in temperature. Microprocessor technology has created many new convenience features for pH measurement such as auto-buffer recognition, calculated slope and % efficiency and log tables for concentration of ions etc.

In acidic or alkaline solutions, the voltage on the outer membrane surface changes proportionally to changes in $[H^+]$. The pH meter detects the change in potential and determines $[H^+]$ of the unknown by the equation (5.7).

$$E = E_o + \frac{2.303\ RT}{n} \times F \times \log \frac{\text{unknown } [H^+]}{\text{internal } [H^+]} \qquad \ldots (5.7)$$

where, E is total potential difference (measured in mV), E_o is reference potential, R is gas constant, T is temperature in Kelvin, n is number of electrons, F is Faraday's constant and $[H^+]$ is the hydrogen ion concentration.

Temperature Compensation

The pH of any solution is a function of its temperature. Voltage output from the electrode changes linearly in relationship to changes in pH, and the temperature of the solution determines the slope of the graph. One pH unit corresponds to the standard voltage of 59.16 mV at 25 °C and temperature to which all calibrations are referenced. The electrode voltage decreases to 54.20 mV/pH units at 0 °C and increases to 74.04 mV/pH units at 100 °C. Since pH values are temperature dependent, pH applications require some form of temperature compensation to ensure standardized pH values. Meters and controllers with automatic temperature compensation (ATC) receive a continuous signal from a temperature element and automatically correct the pH value based on the temperature of the solution. Manual temperature compensation requires the user to enter the temperature of the solution to correct pH readings for temperature is more practical for most pH applications. Although there are some restrictions on the use of the electrodes and the way they are treated between measurements, pH meters are in most cases the best way to check pH of the

solution, as they are much more precise than indicators and pH papers. Using properly calibrated pH meter with a good electrode one may measure pH with ± 0.01-unit accuracy without any problem.

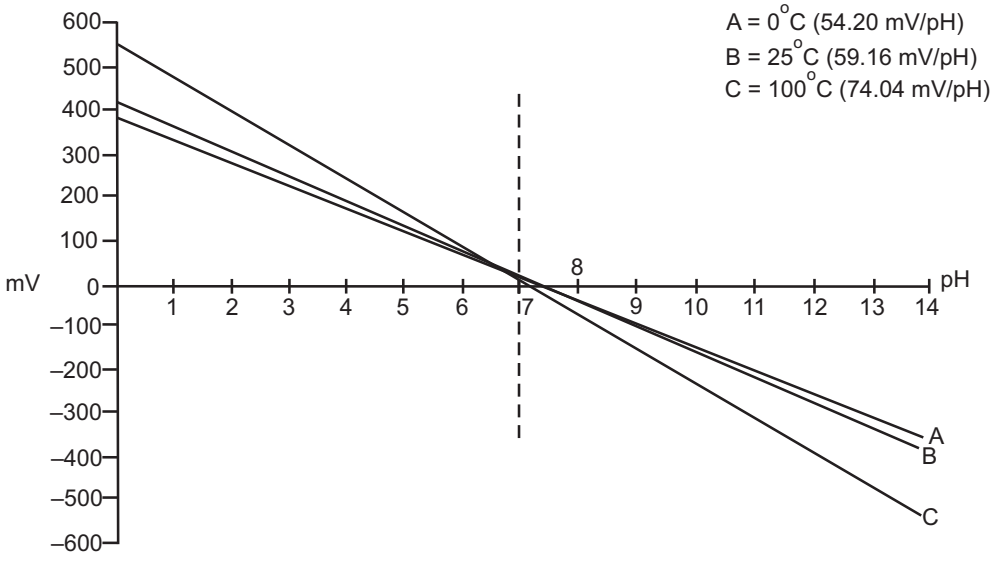


Figure 5.4: Typical pH Electrode Response as a Function of Temperature

Ionization of compounds and hydrogen ion activity in the solution may be temperature dependent. The actual pH of the sample changes with temperature due to change in the hydrogen ion activity in the solution. Temperature compensation does not correct for this and is not desirable because accurate pH measurement is desired at that specific temperature. Temperature compensation only corrects for the change in the output of the electrode, and not for the change in the actual pH solution. Temperature also affects the glass membrane's impedance (total effective resistance of an electric circuit). For each 8° below 25 °C, the specified impedance approximately doubles. Depending on the original impedance of the glass membrane, the meter must handle higher impedance at a lower temperature.

It is a fact that pH measurement determines only the concentration of active hydrogen ions in solution and is also responsible for the observed temperature dependence of measured pH values. For example, the pH of pure water at room temperature is 7.0. If the temperature increases, the dissociation of hydrogen and hydroxyl ions increases, and the pH decreases, even though the water is still charged neutral. Therefore, to predict the pH value of a solution at a desired temperature from a known pH reading at some other temperature, it is very important to know the relationship between the dissociation constant and temperature.

5.3.1 Colorimetric pH Determination

Colorimetric means to measure color. The colorimetric (photometric) pH determination method is based on the property of acid-base indicator dyes, which produce color depending on the pH of the sample. In the colorimetric method, chemicals are added to the

water sample and those chemicals react with the water to produce a color change. The color indicates the pH of the water. The color can be measured visually or electronically as an absorbance change spectrophotometrically. The colorimetric method does not work when the water is already colored because it contains dissolved organic matter or large amounts of algae. Colorimetric test kits are inexpensive and can cover a wide range of pH values.

5.4 APPLICATIONS OF BUFFERS

1. Buffers are used in chemical analysis and calibration of pH measurement system (an electrode and the meter). There can be small differences between the output of electrodes, as well as changes in the output over time. Therefore, the measurement system must be periodically calibrated. Most pH meters require calibration at several specific pH values. One calibration is usually performed near the isopotential point (the signal produced by an electrode at pH 7 is 0 mV at 25 °C), and a second is typically performed at either pH 4 or pH 10. It is best to select a buffer as close as possible to the actual pH value of the sample to be measured.
2. Buffers resistance to changes in pH makes these solutions very useful for chemical manufacturing and essential for many biochemical processes. The ideal buffer for a pH has a pKa equal to the pH desired, since a solution of this buffer would contain equal amounts of acid and base and be in the middle of the range of buffer capacity.
3. Buffer solutions are necessary to keep the correct pH for enzymes in many organisms to live. Many enzymes work only under very precise conditions; if the pH is too far, the enzymes slow or stop working and can denature, thus permanently disabling its catalytic activity. A buffer of carbonic acid (H_2CO_3) and bicarbonate (HCO_3^-) present in blood plasma, help to maintain a pH between 7.35 and 7.45. Pepsin is another example which shows maximum activity at pH 1.5.
4. Industrially, buffer solutions are used in fermentation processes.
5. Buffers can also be used to maintain the drug in its ionized as well as unionized form. The ionized form of a drug is more water soluble than the unionized form. Buffers can be used to maintain a drug in its ionized (salt) form for aqueous solutions. The unionized form of a drug is more lipid soluble than the ionized form. The unionized form therefore penetrates biological membranes much more efficiently than the ionized form.
6. Amphoteric compounds are least soluble at isoelectric points. Substances such as proteins are purified based on this fact. Buffers are useful in maintaining the isoelectric pH. For example, insulin gets precipitated in the pH range of 5 to 6 and hence buffers are used for its purification.
7. The pH can affect the stability of a drug in an aqueous solution. For example, ester drugs are very susceptible to hydrolytic reactions. Buffering formulations at low pH (pH 3-5) can reduce the rate of hydrolysis. Buffers also help to improve aspartame stability. Other examples are the alkaline instability of penicillin and ascorbic acid.

8. High or low pH can cause tissue irritation. The pH of formulation must match the pH of body fluids otherwise it may cause discomfort. Buffering a formulation to near neutral pH can reduce tissue irritation for example, ophthalmic products are least irritating at pH 7 to 9. Other examples of discomfort are blood (hemolysis) and abraded surfaces (burning sensation). An extremely acid or alkaline pH must be avoided to reduce tissue damage.
9. Solubility of compounds can be controlled by providing a medium of suitable pH. For example, many organic salts such as Fe^{+3}, phosphates, borates become soluble in acidic pH but precipitates in alkaline pH range.
10. Buffers help to maintain texture in gelled products by controlled gelling. Controlled gelling reduces reaction rates and minimizes variation in pH. They are also used to prevent color and flavour in food changes in the beverage systems. For example, red color of cherry and raspberry syrups has been maintained at acidic pH which becomes pale yellow to nearly colorless at alkaline pH.

5.5 BUFFER EQUATION

Buffers have properties that the pH of buffer solution remains constant, does not change with the dilution and on addition of small quantities of acids or bases as well as on storage for long period.

In case of moderate pH solutions addition of small amounts of acids or bases leads to absorption by buffer with only slight pH change. For solutions having extreme pH values, small amounts of solutions of strong acids or bases for example, in case of pH 1, acid concentration is relatively high (0.1 M) and small addition of acid or base doesn't change pH of such solution significantly. In most cases, we need to know the pKa of the weak acid to do these calculations. The pH of the buffer solution can be obtained by rearranging the equation (5.8) for dissociation constant:

$$[H_3O^+] = Ka \frac{[CH_3COOH]}{[CH_3COO^-]} \quad \ldots (5.8)$$

Since acetic acid ionizes very slightly, the concentration of acetic acid can be considered as total concentration of acid in the solution. The term $[CH_3COOH]$ can be replaced by the term [Acid] and the term $[CH_3COO^-]$ can also be replaced by [Salt]. Thus,

$$[H_3O^+] = Ka \frac{[Acid]}{[Salt]} \quad \ldots (5.9)$$

To calculate pH of buffer solution containing both acid and its conjugated base the dissociation constant equation can be rearranged and rewritten as follows:

$$[H^+] = Ka \frac{[HA]}{[A^-]} \quad \ldots (5.10)$$

where, [HA] is concentration of acid and $[A^-]$ is concentration of its conjugate base. Expressing equation (5.10) in logarithmic form it becomes,

$$pH = pKa + \log \frac{[A^-]}{[HA]} \quad \ldots (5.11)$$

i.e. $$pH = pKa + \log \frac{[Salt]}{[Acid]}$$

The equation (5.11) is called Henderson-Hasselbalch equation. It can be used for pH calculation of solutions containing pair of acid and conjugate base like HA/A^-, HA^-/A^{2-} or B^+/BOH.

The buffer equation for weak bases and their corresponding salts can be obtained like that of weak acid buffers. Thus,

$$[OH^-] = Kb \frac{[Base]}{[Salt]} \quad \ldots (5.12)$$

Since the ionic product of water (Kw) is $H_3O^+ \times OH^-$

$$OH^- = Kw/H_3O^+ \quad \ldots (5.13)$$

On substituting value for OH^- in equation (5.13) we get

$$Kw/H_3O^+ = Kb \frac{[Base]}{[Salt]} \quad \ldots (5.14)$$

In logarithmic form equation (5.14) can be expressed as,

$$Kw/H_3O^+ = pKb + \log \frac{[B^+]}{[BOH]} \quad \ldots (5.15)$$

i.e. $$= pKb + \log \frac{Salt}{Base}$$

or $$pH = pKw - pKb + \log \frac{Base}{Salt} \quad \ldots (5.16)$$

where, [salt], [acid] and [base] are the molar concentrations of salt, acid and base. Henderson-Hasselbalch equation is used mostly to calculate pH of solution prepared by mixing known amount of acid and conjugate base. For example, the pH of a solution prepared by mixing reagents so that it contains 0.1 M of acetic acid and 0.05 M NaOH, the pH is calculated by using Henderson-Hasselbalch equation. If half of the acid is neutralized, then the concentrations of acid and its conjugate base are identical. Thus quotient under logarithm is 1 which is equal to 0 and therefore, pH = pKa. Henderson-Hasselbalch equation is valid when it contains equilibrium concentrations of acid and conjugate base. In case of solutions containing not so weak acids (or not so weak bases) equilibrium concentrations can be far from concentrations of components added into solution. If acetic acid is replaced with dichloroacetic acid (pKa = 1.5) then the proper pH value is 1.78 because dichloroacetic acid is strong enough to dissociate on its own. Therefore, the equilibrium concentration of conjugate base is not 0.05 M but it is 0.0334 M.

It is important to remember that acids with pKa less than 2.5 dissociate too easily and the use of Henderson-Hasselbalch equation for pH prediction can give wrong results, especially in case of diluted solutions. For solutions above 10 mM and acids weaker than pKa ≥ 2.5, Henderson-Hasselbalch equation gives results with acceptable error. The same holds for bases with pKb ≥ 2.5. However, the same equation works perfectly regardless of the pKa value in calculating ratio of acid to conjugated base in the solution with known pH. Henderson-Hasselbalch equation also can be used for pH calculation of polyprotic acids, if the consecutive pKa values differ by at least 2. Thus it can be safely used in case of phosphoric buffers but not in case of citric acid buffers. The Henderson-Hasselbalch equation (also known as buffer equation) is adapted to consider acids and their conjugate bases leading to solutions that are resistant to pH change. This equation can be used for the following purposes.

1. To calculate pH of a buffer solution when the HA/A^- ratio is known.

$$pH = pKa + \log \frac{[Base]}{[Acid]} \quad \ldots (5.17)$$

2. The pKa of various dugs can be determined from the pH of the solutions.

$$pKa = pH + \log \frac{[Acid]}{[Base]} \quad \ldots (5.18)$$

3. To calculate A^-/HA ratio to give a buffer of definite pH.

$$pH - pKa = \log \frac{[Base]}{[Acid]} \quad \ldots (5.19)$$

4. To calculate the HA/A^- ratio required to give a buffer of a definite pH.

$$pKa - pH = \log \frac{[Acid]}{[Base]} \quad \ldots (5.20)$$

5. To calculate the pH changes due to addition of an acid or base to a buffer solution.
6. To calculate the percentage of the drug ionized or unionized in the solution.
7. It is useful in selection of suitable salt forming substance.
8. It helps to predict pH dependent solubility when intrinsic solubility and pKa are known.

Example 5.1: Calculate pH of a solution prepared by adding 25 mL of 0.1 M sodium hydroxide to 30 mL of 0.2 M of acetic acid. Dissociation constant of acetic acid is 1.8×10^{-5}.

Solution:

$$\begin{aligned} pKa &= -\log Ka \\ &= -\log 1.8 \times 10^{-5} \\ &= -\log 10^{-5} - \log 1.8 \\ &= 5 \log 10 - 0.225 \\ &= 5 - 0.225 \\ &= 4.76 \end{aligned}$$

Before reaction:

$$\text{Acetic acid} = 2.5\ \text{M} \times 30\ \text{mL} = 6\ \text{mM}$$

$$\text{Sodium hydroxide} = 0.1\ \text{M} \times 25\ \text{mL} = 2.5\ \text{mM}$$

After reaction:

$$\text{Sodium acetate} = 2.5\ \text{mM}$$

$$\text{Acetic acid} = (6 - 2.5)\ \text{mM}$$

$$= 3.5\ \text{mM}$$

$$\therefore \quad \text{pH} = 4.76 + \log \frac{2.5}{3.5}$$

$$\text{pH} = 4.61$$

Therefore, the pH of solution prepared by adding 25 mL of 0.1 M sodium hydroxide to 30 mL of 0.2 M of acetic acid is 4.61.

Example 5.2: Calculate pH of the buffer solution containing 0.2 M each of acetic acid and sodium acetate, respectively. (Given: pKa of acetic acid is 4.76).

Solution:

$$\text{pH} = \text{pKa} + \log \frac{[\text{Salt}]}{[\text{Acid}]}$$

$$= 4.76 + \log \left(\frac{0.2}{0.2}\right)$$

$$= 4.76$$

Thus, the pH of buffer solution containing 0.2 M each of acetic acid and sodium acetate is 4.76.

Example 5.3: Calculate the pH of solution containing 0.2 mole of a drug and 0.2 mole of its salt per 1000 mL of solution. The dissociation constant (pKb) of a drug is 4.25.

Solution:

$$\text{pH} = \text{pKw} - \text{pKb} + \log \frac{[\text{Base}]}{[\text{Salt}]}$$

$$= 14 - 4.25 + \log \frac{0.2}{0.2}$$

$$= 14 - 4.25 + 1$$

$$= 10.75$$

The pH of solution is 10.75.

5.6 BUFFER CAPACITY

Buffer capacity is a quantitative measure of the efficiency of a buffer in resisting changes in pH. Buffer capacity may be defined as "maximum amount of either strong acid or strong base that can be added before a significant change in the pH occurs. In simple terms, it is the ability of a buffer system to resist pH changes. It is indicated by the term buffer index (β).

Conventionally, the buffer capacity is expressed as the amount of strong acid or base, in gram-equivalents, that must be added to one liter of the solution to change its pH by one unit. Mathematically buffer capacity is expressed as:

$$\beta = \frac{\Delta B}{\Delta pH} \quad \ldots (5.21)$$

where, ΔB is gram equivalent of strong acid or base added to change pH of 1 liter of buffer solution and ΔpH is the pH change caused by the addition of strong acid or base. Practically it is possible to measure smaller pH changes. The buffer capacity is quantitatively expressed as the ratio of acid or base added to the change in pH produced.

Buffer capacity must be large enough to maintain the product pH for a reasonably long shelf-life. Changes in product pH may be due to interaction of solution components with one another or with the type of product package for example, glass, plastic, rubber closures etc. On the other hand, the buffer capacity of ophthalmic and parenteral products must be low enough to allow rapid readjustment of the product to the physiological pH upon administration. The pH, chemical nature, and volume of the solution to be administered must all be considered. Buffer capacities ranging from 0.01 – 0.1 are usually adequate for most pharmaceutical solutions.

Buffer capacity is always positive. It is expressed as the normal concentration (equivalents per liter) of strong acid or base that changes pH by 1.0. The greater the buffer capacity the smaller is the change in pH upon addition of a given amount of strong acid or base. The buffer index number is generally experimentally determined by titration. For example, when 0.03 mole of sodium hydroxide is added to 0.1 M acetate buffer system the pH increases from 4.76 to 5.03 with a change of 0.27 pH units, Table 5.2. Therefore, by substituting values in the equation (5.21) we have;

$$\beta = \frac{\Delta B}{\Delta pH} \qquad (\therefore \Delta pH = 5.03 - 4.76 = 0.27)$$

$$= \frac{0.03}{0.27}$$

$$= 0.11$$

Table 5.2: Buffer Capacity of Solutions (Under same concentrations of acetic acid and sodium acetate)

Moles of NaOH added	pH of solution	Buffer capacity
0	4.76	–
0.01	4.85	0.11
0.02	4.94	0.11
0.03	5.03	0.11

It is important to remember that buffer capacity is highest when the smallest number of moles of NaOH is added. Buffer capacity is increased by increasing the concentration of the buffer system components. By doubling the total molar concentration of the buffer system will double the buffer capacity at a given pH. Buffer capacity can also be increased by using equimolar concentrations of the acid (HA) and its conjugate base (A^-). The buffer has its greatest capacity, when ratio [salt]/[acid] are equal to 1, i.e. [HA] = [A^-]. Therefore, the buffer equation (5.21) can be written as

$$pH = pKa \quad \ldots (5.22)$$

Factors Affecting Capacity of Buffer

1. Ratio of [A^-]/[HA]

The buffer capacity depends essentially on ratio of the salt to the acid or base. The actual concentrations of A^- and HA influences the effectiveness of a buffer. The more is the A^- and HA molecules available, the less of an effect of addition of a strong acid or base on the pH of a system. For example, consider the addition of a strong acid such as HCl. Initially, the HCl donates its proton to the weak base (A^-) through the reaction

$$A^- + HCl \rightarrow HA + Cl^-$$

This changes the pH by lowering the ratio [A^-]/[HA], but if there is lot of A^- present, the change in pH will be small. But if we keep adding HCl, the weak base A^- will be removed. Once the A^- is depleted, any addition of HCl will donate its proton to water as shown in reaction below.

$$HCl + H_2O \rightarrow H_3O^+ + Cl^-$$

This drastic increase in the [H^+] leads to pH drop called as "breaking the buffer solution". The amount of acid a buffer can absorb before it breaks is called the "buffer capacity for addition of strong acid". A solution with weaker base, [A^-], has a higher buffer capacity for addition of strong acid. Similarly, a buffer can break when the amount of strong base added is so large that it consumes all the weak acid, through the reaction

$$HA + OH^- \rightarrow A^- + H_2O$$

A solution with more weak acid, [HA], has a higher buffer capacity for addition of strong base. The buffer capacity is optimal when the ratio is 1:1; that is, when pH = pKa.

2. Total Buffer Concentration:

Buffer capacity depends upon the total buffer concentration. For example, it will take more acid or base to deplete a 0.5 M buffer than a 0.05 M buffer. The relationship between buffer capacity and buffer concentrations is given by the Van Slyke equation:

$$\beta = 2.303\ C \left\{ \frac{Ka\ [H_3O^+]}{(Ka + [H_3O^+])^2} \right\} \quad \ldots (5.23)$$

where, C is the total buffer concentration (i.e. the sum of the molar concentrations of acid and salt). A buffer solution containing a weak acid and its salt has a maximum buffer capacity

(β_{max}) when pH = pKa i.e. $[H_3O^+]$ = Ka. Therefore, by substituting $[H_3O^+]$ for Ka in equation (5.23), we get

$$\beta_{max} = (2.303 \times C) \frac{[H_3O^+]^2}{2[H_3O^+]^2} \quad \ldots (5.24)$$

$$\beta_{max} = \frac{2.303\ C}{(2)^2}$$

$$\beta_{max} = 0.576\ C$$

Example 5.5: Calculate the buffer capacity for a mixture of 0.01 moles of acetic acid and 0.03 moles of CH_3COONa in 100 mL of total solution. (Given: pKa= 4.76)

Solution:

$$pH = pKa + \log \frac{[Acid]}{[Base]}$$

$$= 4.76 + \log \frac{(0.03)}{(0.01)}$$

$$= 5.24$$

$$pH = -\log [H^+]$$

$$4.4 = -\log [H^+]$$

$$-\log [H^+] = -5.24$$

$$[H^+] = \text{antilog } 5.24$$

$\therefore$ $$[H^+] = 5.75 \times 10^{-6}$$

Since, $$C = (0.01 + 0.03) \text{ moles/100 mL}$$

$$C = 0.4 \text{ moles/L}$$

We know, $$pKa = -\log Ka$$

Since, $$pKa = 4.76$$

$$4.76 = -\log Ka$$

$$\log Ka = -4.76$$

$$Ka = \text{antilog } (-4.76)$$

$\therefore$ $$Ka = 1.74 \times 10^{-5}$$

$$\beta = 2.303 \times C \frac{Ka\ [H^+]}{(Ka + [H^+])^2}$$

$$= 2.303 \times 0.4 \frac{1.74 \times 10^{-5} \times 5.75 \times 10^{-6}}{(1.74 \times 10^{-5} + 5.75 \times 10^{-6})^2}$$

$$= \frac{9.20 \times 10^{-11}}{7.49 \times 10^{-10}}$$

$$= 0.172$$

The buffer capacity of a given mixture is 0.172.

3. Temperature:

Buffers are commercially available with a wide range of pH values, and they come in both premixed liquid form or as convenient dry powder, capsules or tablets (to be added to distilled water). These solutions contain acids and bases whose equilibrium is dependent on temperature. Thus, the precise pH is also a function of temperature. The buffers whose pH varies with temperature are shown in Table 5.3. Since the pH values are dependent on temperature, buffers are required to be maintained at constant temperature. Any change in temperature of the buffer results in reduction in effectiveness of the buffer. Buffer containing base and its salt found to show greater changes in buffer capacity with temperature.

4. Ionic Strength:

Ionic strength is reduced by dilution. Change in ionic strength changes the pH of buffer solution resulting in decreased buffer capacity. So, whenever pH of buffer solution is mentioned ionic strength should be specified.

Table 5.3: Standard Buffers: Effect of Temperature on Buffer pH

Temperature (°C)	Actual pH		
	Phthalate buffer	Phosphate buffer	Borate buffer
0	4.01	7.12	–
10	4.00	7.06	10.15
20	4.00	7.02	10.06
25	4.00	7.00	10.00
30	4.01	–	9.96
40	4.03	6.97	9.97
50	–	–	9.80
60	4.09	6.98	9.73

Example 5.6: A buffer solution made by 0.1 M each of acetic acid and sodium acetate has a pH 4.76. If 0.02 moles of sodium hydroxide is added to this buffer the resultant pH was found to be 4.94. Calculate the buffer capacity.

Solution:

$$\Delta pH = 4.94 - 4.76$$

$$= 0.18$$

Since, the amount of NaOH added (ΔB) = 0.02 moles

Therefore,

$$\beta = \frac{\Delta B}{\Delta pH}$$

$$= \frac{0.02}{0.18}$$

$$= 0.11$$

The buffer capacity of a given buffer is 0.11.

5.7 BUFFERS IN PHARMACEUTICALS

Buffering Agents:

Buffering agents are the substances that adjust the pH of a solution. Buffering agents can be either the weak acids or weak bases that make a buffer solution. These substances are usually added to water to form buffer solutions and are responsible for the buffering action seen in these solutions. The objective of a buffer is to keep the pH of a solution within a narrow range. The function of a buffering agent is to drive an acidic or basic solution to a certain pH state and prevent a change in pH. For example, buffered aspirin has a buffering agent magnesium oxide that maintains the pH of the aspirin as it passes through the stomach of the patient. The monopotassium phosphate also is an example of buffering agent. Buffering agents are primarily used to lower the acidity of the stomach for example, antacid tablets. These agents have variable properties that they have wide differences in solubility and acidity characteristics. As pH controllers, they are important in medicine. The buffering agents work similar to buffer solutions. As we know to avoid the little change in the concentration of the acid and base the solution is buffered. A buffering agent upon addition by providing the corresponding conjugate acid or base sets-up such a concentration ratio that stabilizes the pH of that solution. The resulting pH of this combination can be calculated using the Henderson-Hasselbalch equation. Buffering agents are the main and active components of buffer solutions. They both regulate the pH of a solution as well as resist changes in pH.

A buffer solution maintains the pH for the whole system in which it is placed, whereas a buffering agent is added to an already acidic or basic solution, which is modified to maintain a new pH. Buffering agents and buffer solutions are similar except for a few differences that buffer solution maintains pH of a system by preventing large changes in it, whereas agents modify the pH of what they are placed into. Buffering agents in humans, functioning in acid base homeostasis, are extracellular agents for example, bicarbonate, ammonia as well as intracellular agents including proteins and phosphate.

Buffering agents (Buffer salts) and buffer solutions (buffer systems) have different applications that they improve stability (for example, aspartame), control gelling (for example, pectin-based products), reduce rate of reaction (for example, sucrose inversion) and reduce variation in pH. Therefore, the color, flavour (for example, foods and beverages) and texture (for example, gelled products) is maintained. A buffer can be made by partially neutralizing a weak acid like citric or malic acid with sodium hydroxide. However, sodium hydroxide, or caustic soda, is both hygroscopic and hazardous. Instead of using sodium hydroxide, salts of weak acids such as trisodium citrate, sodium lactate, trisodium phosphate, or sodium acetate are used to partially neutralize the acid. Since they contribute to the buffer capacity themselves, these salts are buffer salts.

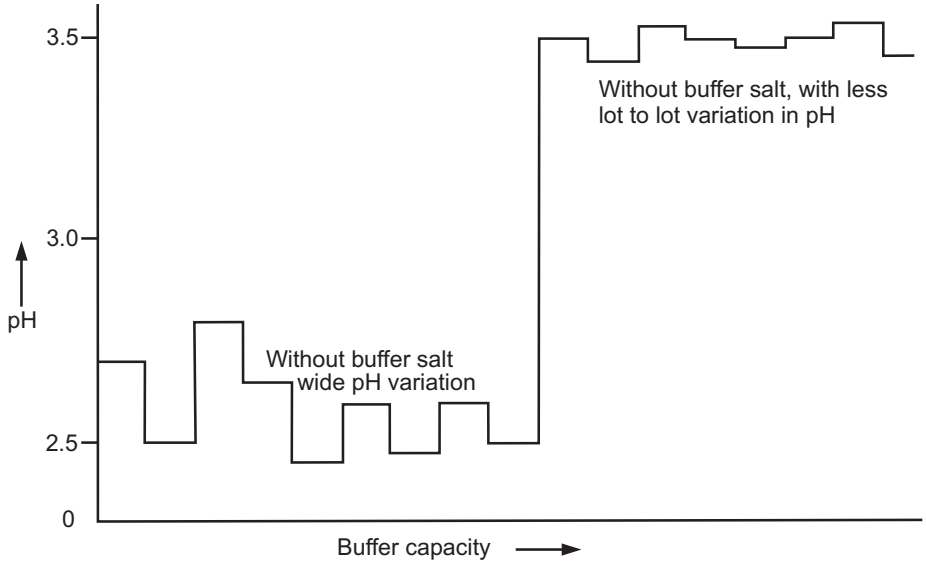


Figure 5.5: Effect of Salt on Buffer Capacity

As shown in Fig. 5.5, the variation in pH from lot to lot is reduced after the addition of a buffer salt. The buffer salts increase the buffer capacity of the buffer system and stabilize pH.

PREPARING BUFFER SOLUTIONS

The simplest way of preparing a buffer solution is dissolving known quantity of the salt of the weak acid (or base) in a solution of weak acid (or base) of known concentration. Another way is to neutralize an excess of weak acid (or weak base) with some strong base (or strong acid). The neutralization produces the salt of the weak acid (or base) 'in situ'. As the weak acid is in excess, there will still be some weak acid in the mixture. The resultant mixture contains both the salt of the weak acid and the weak acid itself.

Table 5.4: The Volumes of Citric Acid and Disodium Phosphate Solutions Mixed to Make Citric Acid - Phosphate Buffers of Specific pH

pH	0.2 M Disodium phosphate (mL)	0.1 M Citric acid (mL)
3.0	20.55	79.45
4.0	38.55	61.45
5.0	51.50	48.50
6.0	63.15	36.85
7.0	82.35	17.65
8.0	97.25	2.75

Weak acid and a salt of acids conjugate base in sufficient amounts are required to maintain the ability of buffer. Citric acid-phosphate buffer for example, is prepared by adding 0.1 M citric acid to 0.2 M disodium phosphate (Na_2HPO_4) solution followed by mixing to make 100 mL solution. The total amounts of these solutions of specific strength required to make buffer solution of particular pH are given in Table 5.4.

General considerations for preparing buffers:

1. Determine the optimal pH for the product, based on physical and chemical stability, therapeutic activity and patient comfort and safety (must consider chemical and physical nature of the active and other ingredients and the route of administration).
2. Select a weak acid with a pKa near the desired pH (must be non-toxic and physically/chemically compatible with other solution components).
3. Calculate the ratio of salt to acid required to produce the desired pH (use Henderson-Hasselbalch equation).
4. Determine desired buffer capacity of the product (consider stability of product, route of administration, volume of dose and chemical nature of product).
5. Calculate the total buffer concentration required to produce desired buffer capacity (Van Slyke equation).
6. Determine the pH and the buffer capacity of the prepared buffer solution by using suitable method.

There are four commonly used methods to prepare buffer solutions:

1. The Slow and Stupid Method:

A buffer composed of an acid and its salt is prepared by dissolving the buffering agent (acid form) in about 60% of the water required for the final solution volume. The pH is adjusted using a strong base, such as NaOH. To prepare a buffer composed of a base and its salt, start with the base form and adjust the pH with strong acid, such as HCl. When the pH is correct, dilute the solution to just under the final volume of solution. Check the pH and correct if necessary, then add water to make the final volume. This method is easy to understand but is slow and may require lots of base (or acid). If the base (or acid) is concentrated, it is easy to increase the pH. If the base (or acid) is dilute, it is easy to increase the volume. Adding a strong acid or base can result in temperature changes, which make pH readings inaccurate (due to its temperature dependence) unless the solution is brought back to its initial temperature.

2. The Mentally Taxing Method:

In this method using buffer pKa, the amounts (in moles) of acid/salt or base/salt present in the buffer at the desired pH is calculated. If both forms (i.e., the acid and the salt) are available, the amount required is converted from moles to grams, using the molecular weight of that component. The correct amounts of both forms are weighed and used. If only one form is available then the buffer is prepared by adding the entire buffer as one form, and then acid or base is added to convert some of the added buffer to the other form. Once the total concentration of buffer in the solution is decided, it is converted to amount (in moles) using the volume of solution, and then to grams, using the molecular weight of the buffer.

The amounts (in moles) of each form that will be present in the final solution are calculated using the buffer pKa and the desired pH. Then the amount of strong acid or base that must be added to give the correct amounts of each form at the pH of the final solution

is calculated. The buffer and strong acid or base is dissolved in slightly less water than is required for the final solution volume. The pH is checked and corrected if necessary. Water is added to make-up the final volume. It is a fast method and easy to prepare. This method requires the buffer pKa value. Additional pH adjustment is rarely necessary, and when needed, the adjustment is small.

3. The Two Solution Method:

The separate solutions of the acid form and base form of the buffer are prepared from solutions having the same buffer concentration. To obtain the desired pH, one solution is added to the other with continuous monitoring the pH. This method easy to do but requires both forms of buffer. The required solution volumes are proportional to the ratio of buffer components in the final solution at the desired final pH.

4. The Completely Mindless Method:

The correct amounts of acid or its salt or base or its salt required for different pH values are selected from the standard data value tables and the same amounts of the components are dissolved in the slightly less water than is required to make the final volume of solution. The pH is checked and corrected if required followed by adjusting the final volume by adding water. This method is easy to do because of use of suitable reference table. It is convenient method for frequently prepared buffers but it may be difficult to find such table. This method requires both forms of buffer. Components amounts from table need to be adjusted to produce the required buffer concentration and volume.

5. Alternative Method for Preparing Buffer Solutions:

This method is used rarely. In this method rather than mixing the weak acid with its salt a buffer solution is prepared by adding a limited amount of strong base to the weak acid to produce a solution of the weak acid (or base) and its conjugate base (or acid) which results in the weak acid and the salt of the weak acid.

Example 5.7: Calculate pH of a solution containing 0.5 M acetic acid and 0.5 M sodium acetate; both before and after enough SO_3 gas is dissolved to make the solution 0.1 M in sulfuric acid. The pKa of acetic acid is 4.75.

Solution: Before the acid is added, using buffer equation pH is calculated as follows,

$$pH = pKa + \log \frac{[Base]}{[Acid]}$$

$$pH = pKa + \log \frac{[0.5M]}{[0.5M]}$$

$$pH = 4.75 + \log 1$$

$$pH = 4.75 + 0$$

$$pH = 4.75$$

To calculate the pH after the acid is added, we assume that the acid reacts with the base in solution and that the reaction has a 100% yield. Therefore, it can be said that 0.1 moles/L of acetate ions reacts with 0.1 moles/L of sulfuric acid to produce 0.1 moles/L of acetic acid

and hydrogen sulfate. The second dissociation of sulfuric acid is ignored as it is minor in comparison to the first. So the final concentration of acetic acid is 0.6 M and acetate is 0.4 M. Substituting those values into the buffer equation gives a pH of 4.75. It is important to remember that 0.1 M solution of strong acid give a pH 1 but the buffer gives a pH of 4.75.

Example 5.8: Using acetic acid and sodium acetate prepare 500 mL of a buffer solution of pH 4.5 with a buffer capacity of 0.05. (Given: Molecular weight of acetic acid = 60; Ka = 1.75×10^{-5}; pKa = 4.76; density of glacial acetic acid = 1.05 g/mL; Molecular weight of sodium acetate = 82)

Solution:

$$pH = pKa + \log \frac{[salt]}{[acid]}$$

$$4.5 = 4.76 + \log \frac{[salt]}{[acid]}$$

$$\frac{[Salt]}{[acid]} = \text{Antilog } (4.5 - 4.76)$$

$$= 0.55$$

$$[Salt] = 0.55\ [acid]$$

Total Buffer Concentration

$$\beta = 2.3\ C \times \frac{Ka\ [H_3O^+]}{(Ka + [H_3O^+])^2}$$

$$pH = -\log [H^+]$$

$$[H_3O+] = \text{Antilog } (-pH)$$

$$= \text{Antilog } (-4.5)$$

$$0.05 = 2.3 \times C \times \frac{(1.75 \times 10^{-5})\ (3.16 \times 10^{-5})}{[(1.75 \times 10^{-5}) + (3.16 \times 10^{-5})]^2}$$

$$0.05 = 0.53C$$

$$C = 0.095M$$

Final Calculations:

$$C = [salt] + [acid]$$

$$C = 0.55\ [acid] + 1[acid]$$

$$= 1.55\ [acid]$$

Since,

$$C = 0.095\ M$$

$$1.55\ [Acid] = 0.095$$

$$[Acid] = \frac{0.095}{1.55}$$

$$= 0.061\ M \times 0.5\ L \times 60\ g/moles$$

$$= 1.83\ g \text{ acetic acid}$$

So,

$$\text{Glacial acetic acid} = 1.83\ g/1.05\ g/ml$$

$$= 1.74\ ml$$

$$[\text{Salt}] = 0.55\ [\text{acid}]$$
$$= 0.55 \times 0.061\ \text{M}$$
$$= 0.034\ \text{M} \times 0.5\ \text{L} \times 82\ \text{g/moles}$$
$$= 1.39\ \text{g sodium}$$

Therefore, 500 ml of a buffer solution of pH 4.5 and of buffer capacity 0.05 can be prepared by adding 1.39 g of sodium acetate to 1.83 g of acetic acid.

Standard Buffer Solutions:

The standard buffer solution of pH ranging from 1.2 to 10 are possible to prepare by appropriate combinations of 0.2 N HCl or 0.2 N NaOH or/and 0.2 M solutions of potassium hydrogen phthalate, potassium dihydrogen phosphate, boric acid-potassium chloride as given in pharmacopoeia. The pH range and the quantities of the ingredients used to make respective standard buffer at 25 °C are given in Table 5.5. Buffers have great use in biological research. Various criteria that can be applied while making buffers for this application are listed below.

1. Buffers must possess enough buffer capacity in the required pH range.
2. It must be available in highly purified form.
3. It must be highly water soluble and impermeable to biological membranes.
4. It must be stable especially with respect to hydrolysis and enzymatic action.
5. It must maintain pH which is influenced to a very small value by their concentration, temperature and ionic strength as well as salting out effect of the medium.
6. It must be non-toxic with no biological inhibition activity.
7. Buffers must not form complexes.
8. It must not absorb light in the visible or ultraviolet regions.
9. It must not precipitate in redox reactions.
10. It must not alter solubility of active ingredients.
11. It must be safe to use in biological systems and do not alter the pharmacological responses of the active ingredients.

Table 5.5: Standard Buffers with Their pH Range and Quantities of Ingredients

Buffer	pH	Method
Hydrochloric acid buffer	1.2 – 2.22	50 mL of 0.2 M KCl and sufficient amount of 0.2 N HCl. Final volume is made by water to make 200 mL solution.
Acid phthalate buffer	2.2 – 4.0	50 mL potassium hydrogen phthalate and sufficient volume of 0.2 N HCl. Final volume is made by water to make 200 mL solution.

… (Contd.)

Buffer	pH	Method
Neutralized phthalate buffer	4.2 – 5.8	50 mL potassium hydrogen phthalate and sufficient volume of 0.2 N NaOH. Final volume is made by water to make 200 mL solution.
Phosphate buffer	5.8 – 8.0	50 mL potassium dihydrogen phosphate and sufficient volume of 0.2 N HCl. Final volume is made by water to make 200 mL solution.
Alkaline borate buffer	8.0 – 10.0	50 mL boric acid - potassium chloride and sufficient volume of 0.2 N NaOH. Final volume is made by water to make 200 mL solution.

Pharmaceutical Buffers:

Generally, buffers are used in the pharmaceutical products for two purposes viz. to adjust the pH of product for maximum stability and to maintain the pH within the optimum physiological pH range. Pharmaceutical solutions generally have a low buffer capacity in order to prevent overwhelming the body's own buffer systems and significantly changing the pH of the body fluids. Buffers have concentrations in the range of 0.05 to 0.5 M and buffer capacities in the range of 0.01 to 0.1 which are usually sufficient for pharmaceutical solutions. The Table 5.6 gives some of the buffer systems used in the pharmaceutical formulations along with their pKa values. Most pharmaceutical buffers are composed of ingredients that are found in the body (for example, acetate, phosphate, citrate and borate). While selecting, the right pharmaceutical buffers choose a weak acid with pH > pKa. Carry out calculations using buffer equation to determination of acid/base needed to give required pH. Also, choose proper concentration needed to give suitable buffer capacity. The ingredients are selected from available ones considering their sterility, stability, cost, toxicity etc.

Table 5.6: Buffer Systems Used in the Pharmaceutical Formulations

Buffer System	pK_a	pH range
Acetic acid/Sodium acetate	4.76	3.8 - 5.6
Phsophate acid/Sodium phosphate (a) H_3PO_4 / NaH_2PO_4 (b) NaH_2PO_4 / Na_2HPO_4 (c) Na_2HPO_4 / Na_3PO_4	 2.1 (pKa_1) 7.2 (pKa_2) 12.3 (pKa_3)	 5 - 8
Citric acid / Sodium citrate	3.1 (pKa_1) 4.8 (pKa_2) 9.2 (pKa_3)	 1.2 - 6.6
Boric acid / Sodium borate	9.2	7.8 - 10.6

1. Solid dosage forms:

Buffers have been used widely in solid dosage forms such as tablets, capsules and powders for controlling the pH of the environment around the solid particles. This has practical application for the drugs that have dissolution rate limited absorption from unbuffered solutions. One of the special applications of buffers is to reduce the gastric irritation caused by the acidic drugs. For example, sodium bicarbonate, magnesium carbonate and sodium citrate antacids, used for reducing acidity.

2. Semisolid formulations:

Semisolid preparations such as creams and ointments undergo pH changes upon storage for long time resulting in its reduced stability. Hence buffers such as citric acid and sodium citrate or phosphoric acid/sodium phosphate are included in these preparations to maintain their stability.

3. Parenteral products:

Use of buffers is common in the parenteral products. Since the pH of blood is 7.4 these products are required to be adjusted to this pH. Change in pH to higher side (more than 10) may cause tissue necrosis while on lower side (below 3) it may cause pain at the site of action. As blood, itself function like buffer, adjustment of pH for small volume parenteral preparations is not required. Commonly used buffers include citrate, glutamate, phthalate and acetate. The pH optimization is generally carried out to have better solubility, stability and reducing irritancy of the product.

4. Ophthalmic products:

Many drugs, such as alkaloidal salts, are most effective at pH levels that favour the undissociated free bases. However, at such pH levels, the drug may be unstable Therefore such pH levels must be obtained by use of buffers. The purpose of buffering some ophthalmic solutions is to prevent an increase in pH caused by the slow release of hydroxyl ions by glass. Such a rise in pH can affect both the solubility and the stability of the drug. The decision whether buffering agents should be added in preparing an ophthalmic solution must be based on several considerations. Normal tears have a pH of about 7.4 and possess some buffer capacity.

The application of a solution to the eye stimulates the flow of tears and the rapid neutralization of any excess hydrogen or hydroxyl ions within the buffer capacity of the tears. Many ophthalmic drugs are weakly acidic and have only weak buffer capacity. Where only 1 or 2 drops of a solution containing them are added to the eye, the buffering action of the tears is usually adequate to raise the pH and prevent marked discomfort. In some cases, pH may vary between 3.5 and 8.5. Some drugs, notably pilocarpine hydrochloride and epinephrine bitartrate, are more acid and overtax the buffer capacity of the lachrymal fluid. Ideally, an ophthalmic solution should have the same pH, as well as the same isotonicity value, as lachrymal fluid. This is not usually possible since, at pH 7.4, many drugs are not appreciably soluble in water.

Most alkaloidal salts precipitate as these exists as free alkaloid at this pH. Additionally, many drugs are chemically unstable at pH levels approaching 7.4. This instability is more marked at the high temperatures employed in heat sterilization. For this reason, the buffer system should be selected that is nearest to the physiological pH of 7.4 and does not cause precipitation of the drug or its rapid deterioration.

An ophthalmic preparation with a buffer system approaching the physiological pH can be obtained by mixing a sterile solution of the drug with a sterile buffer solution using aseptic technique. Even so, the possibility of a shorter shelf-life at the higher pH must be taken into consideration, and attention must be directed toward the attainment and maintenance of sterility throughout the manipulations. Boric acid is often used to adjust isotonicity in ophthalmic solutions because of its buffering and anti-infective properties.

Many drugs, when buffered to a therapeutically acceptable pH, would not be stable in solution for long periods of time. Hence these products are lyophilized and are intended for reconstitution immediately before use, for example, Acetylcholine Chloride Ophthalmic Solution.

5.8 BUFFERS IN BIOLOGICAL SYSTEMS

Biochemical reactions are specifically sensitive to pH. Most biological molecules contain groups of atoms that may be charged or neutral depending on pH, and whether these groups are charged or neutral has a significant effect on the biological activity of the molecule. In all multicellular organisms, the fluid within the cell and the fluids surrounding the cells have a characteristic and nearly constant pH. There is great variation in the pH of fluids in the body and small variation is found within each system. For example, the pH of body fluid can vary from 8 in the pancreatic fluid to 1 in the stomach. The average pH of blood is 7.4, and of cells is in the range of 7.3 to 7.

This pH of body fluids is maintained through buffer systems. Body fluids contain buffering agents and buffer systems that maintain pH at or near 7.4. The kidneys and the lungs work together to help maintain a blood pH of 7.4 by affecting the components of the buffers in the blood. Proteins are the most important buffers in the body as their amino and carboxylic acid groups acts as proton donors or acceptors as H^+ ions are either added or taken out from the environment. Important endogenous (natural) buffer systems include carbonic acid/sodium bicarbonate and sodium phosphate in the plasma and hemoglobin, and potassium phosphate in the cells.

Two important biological buffer systems are the dihydrogen phosphate system and the carbonic acid system.

1. The Phosphate Buffer System:

The phosphate buffer system operates in the internal fluid of all cells. This buffer system consists of dihydrogen phosphate ions ($H_2PO_4^-$) as hydrogen ion donor (acid) and hydrogen phosphate ions (HPO_4^{2-}) as hydrogen-ion acceptor (base). These two ions are in equilibrium with each other as indicated by the chemical equation given below.

$$H_2PO^-_{4(aq)} \rightleftharpoons H^+_{(aq)} + HPO^{2-}_{4(aq)}$$

If additional hydrogen ions enter the cellular fluid, they are consumed in the reaction with HPO_4^{2-}, and the equilibrium shifts to the left. If additional hydroxide ions enter the cellular fluid, they react with $H_2PO_4^-$, producing HPO_4^{2-}, and shifting the equilibrium to the right. The equilibrium expression for this equilibrium is expressed as given below.

$$Ka = \frac{[H^+]\,[HPO_4^{2-}]}{[H_2PO_4^-]} \quad \ldots (5.24)$$

The value of Ka for this equilibrium is 6.23×10^{-8} at 25°C. From the equation (5.24), the relationship between the hydrogen ion concentration and the concentrations of the acid and base can be derived as follows.

$$[H^+] = Ka \frac{[H_2PO_4^-]}{[HPO_4^{2-}]} \quad \ldots (5.25)$$

Thus, when the concentrations of $H_2PO_4^-$ and HPO_4^{2-} are same, the value of the molar concentration of hydrogen ions is equal to the value of the equilibrium constant, and therefore;

$$pH = pKa\ (-\log Ka) \quad \ldots (5.26)$$

Buffer solutions are most effective in maintaining a pH near the value of the pKa. In mammals, cellular fluid has a pH in the range 6.9 to 7.4, and the phosphate buffer is effective in maintaining this pH range. The pKa for the phosphate buffer is 6.8, which allows this buffer to function within its optimal buffering range at physiological pH. The phosphate buffer only plays a minor role in the blood because H_3PO_4 and $H_2PO_4^-$ are found in very low concentration in the blood. Hemoglobin also acts as a pH buffer in the blood. Hemoglobin protein can reversibly bind either H^+ (to the protein) or O_2 (to the Fe of the heme group), but that when one of these substances is bound, the other is released. During exercise, hemoglobin helps to control the pH of the blood by binding some of the excess protons that are generated in the muscles. At the same time, molecular oxygen is released for use by the muscles.

2. The Carbonic Acid System:

Another biological fluid in which a buffer plays an important role in maintaining pH is blood plasma. In blood plasma, the carbonic acid and hydrogen carbonate ion equilibrium buffers the pH. In this buffer, carbonic acid (H_2CO_3) is the hydrogen ion donor (acid) and hydrogen carbonate ion (HCO_3^-) is hydrogen-ion acceptor (base). The simultaneous equilibrium reaction is shown below.

$$H_2CO_{3(aq)} \rightleftharpoons H^+_{(aq)} + HCO^-_{(3)\,(aq)}$$

This buffer functions in the same way as the phosphate buffer. Additional H^+ is consumed by HCO_3^- and additional OH^- is consumed by H_2CO_3. The value of Ka for this

equilibrium is 7.9×10^{-7}, and the pKa is 6.1 at body temperature. In blood plasma, the concentration of hydrogen carbonate ion is about twenty times the concentration of carbonic acid. The pH of arterial blood plasma is 7.4. If pH falls below this normal value, a condition called acidosis and when pH rises above the normal value, the condition is called alkalosis is observed

The concentrations of hydrogen carbonate ions and carbonic acid are controlled by two physiological systems. The concentration of hydrogen carbonate ions is controlled through the kidneys whereas excess hydrogen carbonate ions are excreted in the urine. The carbonic acid-hydrogen carbonate ion buffer works throughout the body to maintain the pH of blood plasma close to 7.4. Changes in hydrogen carbonate ion concentration, however, require hours through the relatively slow elimination through the kidneys. Carbonic acid concentration is controlled by respiration that is through the lungs. Carbonic acid is in equilibrium with dissolved carbon dioxide gas. An enzyme called carbonic anhydrase catalyzes the conversion of carbonic acid to dissolved carbon dioxide. In the lungs, excess dissolved carbon dioxide is exhaled as carbon dioxide gas.

$$H_2CO_{3(aq)} \rightleftharpoons CO_{2(aq)} + H_2O_{(l)}$$

$$CO_{2(aq)} \rightleftharpoons CO_{2(g)}$$

The body maintains the buffer by eliminating either the acid (carbonic acid) or the base (hydrogen carbonate ions). Changes in carbonic acid concentration bring about within seconds through increased or decreased respiration.

Lysis Buffer:

A lysis buffer is used for lysing cells for use in experiments that analyze the compounds of the cells (for example, western blot). There are many kinds of lysis buffers that one can apply; depending on what analysis the cell lysate will be used for example, RBC lysis buffer. In studies like DNA finger printing the lysis buffer is used for DNA isolation. Dish soap can be used in a pinch to break down the cell and nuclear membranes, allowing the DNA to be released.

5.9 BUFFERED ISOTONIC SOLUTIONS

Tonicity is a measure of effective osmolarity or effective osmolality in cell biology. Osmolality and osmolarity are properties of a solution, independent of any membrane. Osmolality is a concentration scale to express total concentration of solute particles and is directly related to any of the four colligative properties. It is derived from molality by factoring in the dissociation of electrolytic solutes.

Osmolality = Molecular weight $\times$ Number of particles/molecule

Tonicity is a property of a solution about a membrane, and is equal to the sum of the concentrations of the solutes which have the capacity to exert an osmotic force across that membrane. Tonicity depends on solute permeability. The permeable solutes do not affect tonicity but the impermeable solutes do affect tonicity. If a semi-permeable membrane is used to separate solutions of different solute concentrations, a phenomenon known as

osmosis occurs to establish concentration equilibrium. The pressure driving this movement is called osmotic pressure and is governed by the number of particles of solute in solution. If solute is a non-electrolyte, then number of particles is determined solely by the solute concentration. If the solute is an electrolyte, the number of particles is governed by both the concentration and degree of dissociation of the substance.

The distinction between the isosmotic and isotonic terms comes with the realization that red blood cell membranes are not perfect semipermeable membranes but allow passage of some solutes, such as alcohol, boric acid, ammonium chloride, glycerin, ascorbic acid, lactic acid, etc. A 2% solution of boric acid when physically measured found to be isosmotic (containing same number of particles) with blood and not isotonic (exerting equal pressure or tone) with blood but is isotonic with tears. This differentiation is not having any great significance and therefore isotonicity values are calculated based on the number of particles in solution is sufficient. The clinical significance of all this is to ensure that isotonic or isosmotic solutions do not damage tissue or produce pain when administered.

Tonicity is generally classified in three types; hypertonicity, hypotonicity and isotonicity. Hypertonic, isotonic and hypotonic solutions are defined in reference to a cell membrane by comparing the tonicity of the solution with the tonicity within the cell.

Hypertonicity:

A solution having higher osmotic pressure than the body fluids (or 0.9% NaCl solution) is known as hypertonic solution. These solutions draw water from the body tissues to dilute and establish equilibrium. An animal cell in a hypertonic environment is surrounded by a higher concentration of impermeable solute than exists in the inside of the cell. For example, if 2.0% NaCl solution is added to blood (defibrinated), osmotic pressure directs a net movement of water out of the cell causing it to shrink (the shape of the cell becomes distorted) and wrinkled (crenated), as water leaves the cell.. This movement is continued until the concentrations of salt on both sides of the membrane are identical. Hence, 2.0% NaCl solution is hypertonic with the blood, Fig. 5.6 (a).

Isotonicity:

The solution that have the same osmotic pressure as that of body fluids are said to be isotonic with the body fluid. Body fluids such as blood and tears have osmotic pressure corresponding to that of 0.9 % NaCl or 5% dextrose aqueous solution thus, a 0.9% NaCl or 5% dextrose solution is called as isosmotic or isotonic. The term isotonic means equal tone, and is used interchangeably with isosmotic regarding specific body fluids. Isosmotic is a physicochemical term that compares the osmotic pressure of two liquids that may or may not be body fluids. A cell in an isotonic environment is in a state of equilibrium with its surroundings with respect to osmotic pressure. When the amount of impermeable solute is same on the inside and outside of the cell, osmotic pressure becomes equal. When amount of impermeable solute is not same on the inside and outside of the cell, the force of water trying to exit or enter the cell to maintain the balance. This pressure drives hypertonic or hypotonic cells to become isotonic. For example, a 0.9% w/v solution of NaCl in water is isotonic in relation to RBC's and their semi-permeable membranes Fig. 5.6 (b).

Requirements of isotonic solutions are that they must not cause any contraction or swelling of the tissues. The product must not produce discomfort when instilled in the eye, nasal tract, blood, or other body tissue for example, isotonic NaCl. On addition of 0.9 g NaCl/100 mL (0.9%) into blood (defibrinated), the cells retain their normal size. Isotonic solution should be restricted to solutions having equal osmotic pressures with respect to a particular membrane.

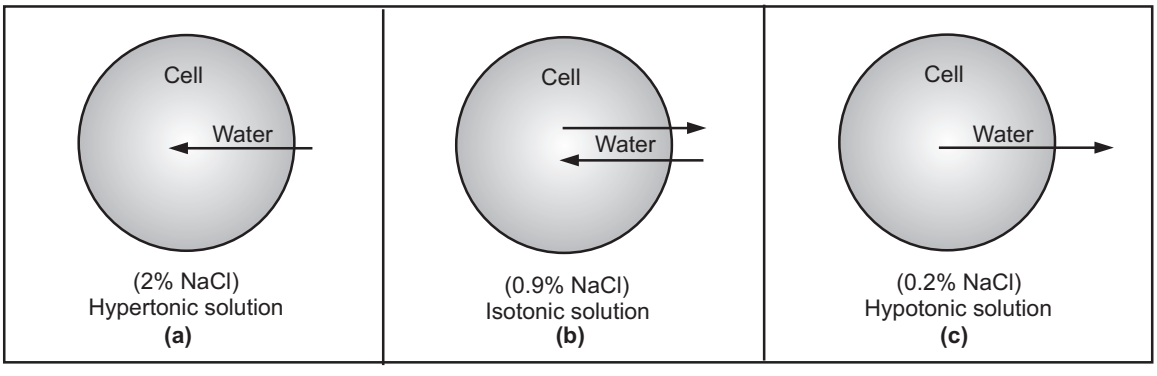


Figure 5.6: Osmotic Effects of Various Solutions on RBC

The addition of any compound to a solution affects its isotonicity, causing changes in osmotic pressure of a solution. It should not be affected only by drugs but also by any buffer compounds added in the formulation. Therefore, it is necessary to add additional NaCl to bring the solution to isotonicity. Adjustment of isotonicity is required for several dosage forms such as parenteral preparations for example, IV infusions, irrigating solutions; lotions for open wounds, subcutaneous injections, preparations meant for diagnostic applications, solutions meant for intrathecal injections, nasal drops and ophthalmic drops.

Hypotonicity:

A solution with low osmotic pressure than body fluids is known as hypotonic solution. Administration of a hypotonic solution produces shrinking of tissues (painful swelling) as water is pulled from the biological cells (tissues or blood cells) to dilute the hypertonic solution. The effects of administering a hypotonic solution are generally more severe than with hypertonic solutions, since ruptured cells can never be repaired. Hypotonic solutions show opposite effect compare to hypertonic solutions that the net movement of water is into the cell causing them to swell. If the cell contains more impermeable solute than its surroundings, water enters it. In the case of animal cells, they get swelled until burst; but this doesn't happen to plant cells i.e. they do not burst due to the reinforcement their cell wall provides. If 0.2% NaCl solution is added to blood (defibrinated), the cells get swelled and burst. Therefore, 0.2% NaCl solution is hypotonic with respect to the blood, Fig. 5.6 (c). A 2.0% solution of boric acid has the same osmotic pressure with blood; but it is hypotonic because boric acid passes freely through cell membrane regardless of concentration.

ISOTONICITY VALUE

Lachrymal fluid is isotonic with blood having an isotonicity value corresponding to that of a 0.9% NaCl solution. Ideally, an ophthalmic solution should have this isotonicity value; but the eye can tolerate isotonicity values as low as that of a 0.6 % NaCl solution and as high as that of a 2.0% NaCl solution without marked discomfort.

Some ophthalmic solutions are necessarily hypertonic to enhance absorption and to provide a concentration of the active ingredient(s) strong enough to exert a prompt and effective action. The amount of such solutions used is small because on administration the dilution with lachrymal fluid takes place rapidly with minimal discomfort from the hypertonicity which is only temporary. However, any adjustment toward isotonicity by dilution with tears is negligible where large volumes of hypertonic solutions are used as collyria to wash the eyes; it is, therefore, important that solutions used for this purpose be approximately isotonic.

Methods Used to Determine Tonicity Value:

Many chemicals and drugs are used in the pharmaceutical formulations. These substances contribute to the tonicity of the solution. Hence methods are needed to verify the tonicity and adjust isotonicity. Two of the methods used to determine tonicity value are described below.

(A) Hemolytic method:

Isotonicity value is calculated by using hemolytic method in which the effect of various solutions of drug is observed on the appearance of red blood cells suspended in solutions. In this method, RBC's are suspended in various solutions and the appearance of RBC's is observed for swelling, bursting, shrinking and wrinkling of the blood cells. In hypotonic solutions, oxyhemoglobin released is proportional to number of cells hemolyzed; in case of hypertonic solutions, the cells shrink and become wrinkled or crenated where as in case of isotonic solutions the cells do not change their morphology.

(B) Cryoscopic method:

Isotonicity values can be determined from the colligative properties of the solutions. For this purpose, freezing point depression property is most extensively used. The freezing point of water is 0 °C, and when any substance such as NaCl is added to it the freezing point of water decreases. The freezing point depression (ΔT_f) of blood is – 0.52 °C. Hence the ΔT_f value of the drug solution must be – 0.52 °C. This solution shows osmotic pressure equal to the blood and hence the RBC's morphology as well as functions found to be unchanged.

Methods of Adjusting Tonicity And pH:

Several methods are used to adjust isotonicity of pharmaceutical solutions. Isotonicity can also be calculated from the colligative properties of the drug solutions. If solutions are injected or introduced into the eyes and nose, these are to be made isotonic to avoid hemolysis of RBC's and to avoid pain and discomfort. This is possible for either manufactured or extemporaneously prepared solutions. By using the appropriate calculations based on

colligative properties of solutions, it is easy to determine the amount of adjusting agents to be added. It helps to overcome the side effects caused from administering solutions which contain adjusting agents less or more than isotonic solutions. The three frequently used methods to calculate isotonicity of the solutions are described below. If carried out correctly, these methods give closely comparable results with a little deviation.

Class I: NaCl or some other substance is added to the solution of the drug to lower the freezing point of the solution to − 0.52 °C and thus make the solution isotonic. Cryoscopic method and Sodium chloride equivalent method are the examples of this class.

Class II: Water is added to the drug in sufficient amount to make it isotonic and then the preparation is brought to its final volume with an isotonic or buffered isotonic solution. White –Vincent method is example of this type.

Class III: A freezing point depressions and L_{iso} values for number of drugs are estimated theoretically from the molecular weight of the drug and can be used to calculate the amount of adjusting substance to be added to make the solution isotonic for example, using reference tables for ΔT_f and L_{iso} values from different books.

A. Cryoscopic method:

In this method, the quantity of each substance required for an isotonic solution can be calculated from the freezing point depression values. A solution which is isotonic with blood has a ΔT_f of 0.52 °C. Therefore, the freezing point of drug solution must be adjusted to this value. Many pharmaceutical textbooks usually list the freezing point depression of many compounds and it is then easy to calculate the concentration needed to achieve isotonicity from these values. In case of drug solutions if it is not possible to adjust tonicity by altering the drug concentration then an adjusting substance is added to achieve desired tonicity.

The weight (in grams) of adjusting substance can be calculated as described below. For example, the drug concentration in 100 mL solution is 'a' grams, then:

$$\Delta T_f \text{ (for drug solution)} = a \times \Delta T_f \text{ of 1 \% drug solution}$$
$$= x$$

If w are the grams of the adjusting substance to be added to 100 mL of drug solution to make it isotonic then:

$$\Delta T_f \text{ (for adjusting solution)} = w \times \Delta T_f \text{ of 1 \% adjusting substance}$$
$$= w \times b$$

For making a solution isotonic:

$$x + wb = 0.52 \text{ or}$$
$$w = \frac{(0.52 - x)}{b} \quad \ldots (5.27)$$

If sodium chloride is used as adjusting substance whose ΔT_f of 1 % solution is 0.58 °C (≈ 0.576 °C), then

$$w = \frac{(0.52 - x)}{0.58} \quad \ldots (5.28)$$

Example 5.9: If 1 % solution of NaCl has freezing point depression of 0.576 °C; calculate concentration of NaCl required in making this solution isotonic.

Solution: Since the freezing point depression of blood is 0.52 °C the concentration of NaCl required to make this solution isotonic is calculated as:

$$\text{Concentration of NaCl} = \left(\frac{0.52}{0.576}\right) \times 1.0$$

$$= 0.9\ \%\ \text{w/v}$$

The concentration of NaCl required to this make isotonic is 0.9 % w/v.

Example 5.10: Calculate the amount of NaCl to be added to 250 mL of 0.5% w/v Lidocaine HCl solution to make it isotonic with blood. The freezing point depression of Lidocaine HCl is 0.13.

Solution:

$$b = \Delta T_f\ \text{NaCl} + \Delta T_f\ 1\%$$

$$b = 0.576 + 0.13$$

$$= 0.706$$

Therefore, the amount of NaCl to be added to 250 mL of solution is:

$$\text{Amount of NaCl} = \frac{0.706 \times 250}{100}$$

$$= 1.765\ \text{g}$$

Therefore, 1.765 g of NaCl must be added to 250 mL of a 0.5% w/v Lidocaine HCl solution to make it isotonic with blood.

Example 5.11: Calculate the amount of NaCl required in producing 100 mL solution of 1% apomorphine hydrochloride isotonic with blood serum? (Given: ΔT_f of apomorphine = 0.08)

Solution: The adjustment needed for $\Delta T_f = 0.52 - 0.08 = 0.44$.

Since, 1% NaCl solution has $\Delta T_f = 0.58$; amount of NaCl solution used to adjust the tonicity can be calculated as:

$$\text{To increase } \Delta T_f \text{ by 0.44, NaCl needed} = \frac{0.44}{0.58} = 0.75$$

Therefore, 0.75 g of NaCl must be dissolved in 100 mL of 1% solution of apomorphine hydrochloride isotonic with blood serum.

B. Sodium chloride equivalent method:

Addition of any buffering agent to a solution affects its isotonicity leading to change in osmotic pressure of a solution. It happens not only by drug but also by any buffer compounds that are added in the formulation. But on addition of these buffering agents the solution will not be isotonic and hence it is necessary to add additional NaCl to bring the solution to isotonicity. The most widely used method is the sodium chloride equivalent method. This method uses the NaCl equivalent to calculate the amount of an adjusting agent

needed to be added to a solution to bring it to isotonicity. The NaCl equivalent is the weight of the NaCl (in grams) that produces the same colligative properties (osmotic effect; based on number of particles) as that of 1 g of a drug. For example, if the E_{NaCl} of a drug is 0.20 this means that 0.20 g of NaCl will have identical osmotic pressure and freezing point depression as 1 g of the drug.

In this method, the amount of the drug is multiplied by the (E_{NaCl}) to obtain the amount of NaCl that will produce similar osmotic conditions to those of the drug in the solution. This value is then subtracted from the amount of NaCl needed to make an isotonic solution. If the adjusting solute is not NaCl, the amount of calculated NaCl is divided by the E_{NaCl} of the adjusting solute. This then represents the weight of the adjusting agent to be added to bring the solution to tonicity. The E_{NaCl} for many drugs are tabulated in Table 5.7.

Table 5.7: ΔT_f and E_{NaCl} values of some drugs and substances

Substances	$\Delta T_f^{1\%}$	E_{NaCl}	Substances	$\Delta T_f^{1\%}$	E_{NaCl}
Ammonium chloride	0.64	1.08	Glycerin	0.20	0.34
Apomorphine hydrochloride	− 0.08	0.14	Lidocaine hydrochloride	0.13	0.22
Atropine sulfate	0.07	0.13	Napazoline hydrochloride	0.16	0.27
Boric acid	0.29	0.52	Neomycin sulfate	0.06	0.11
Calcium gluconate	0.16	0.09	Oxymetazoline	0.11	0.20
Chlorobutanol	0.14	0.18	Phenol	0.20	0.35
Cocaine hydrochloride	0.09	0.16	Phenyleprine hydrochloride	0.18	0.32
Dextrose monohydrate	0.09	0.16	Pilocarpine nitrate	0.14	0.22
Ephedrine hydrochloride	0.18	0.30	Procaine hydrochloride	0.11	0.21
Ephedrine sulfate	0.14	0.23	Scopolamine hydrobromide	0.07	0.12
Epheneprine bitartrate	0.11	0.18	Silver nitrate	0.19	0.33
Epheneprine hydrochloride	0.17	0.29	Sodium chloride	0.58	1.00
Eucatropine hydrochloride	0.11	0.18	Sulphacetamide sodium	0.14	0.33
Fluorescein sodium	0.18	0.31	Tetracaine hydrochloride	0.11	0.18

Example 5.12: If the E_{NaCl} of Lidocaine HCl is 0.22, what will be osmotic equivalent of 0.5 g Lidocaine HCl in the solution? If dextrose is prescribed, what will be the amount of dextrose to be added as the adjusting solute?

Solution: The osmotic equivalent of 0.5 g Lidocaine HCl in the solution is $0.5 \times 0.22 = 0.11$ g NaCl.

The 0.5 % of Lidocaine HCl in the solution will have an osmotic pressure equivalent to 0.11 % of NaCl in solution.

Since an isotonic saline solution contains 0.9 g of NaCl per 100 mL of solution, then the amount of NaCl needed to be added in the above solution to make it isotonic will be:

$$0.9 - 0.11 = 0.79 \text{ g NaCl}$$

Therefore for 250 mL:

$$0.79 \times \frac{250}{100} = 1.975 \text{ g NaCl}$$

If dextrose was prescribed as the adjusting solute, then it becomes

$$= \frac{1.975}{0.16}$$

$$= 12.34 \text{ g}$$

C. The L_{iso}-method:

The E_{NaCl} value of tonicity adjusting substances also can be calculated from the L_{iso} value of the substances. The L_{iso} values of the tonicity adjusting substances are given in Table 5.8 and mentioned as constants in many references. In this method freezing point depression equation is used to calculate the amount of the isotonicity adjusting substance that must be added to hypotonic solution of drug to bring to tonicity. As the freezing point depression for solutions of electrolytes are greater than those calculated by the equation, $\Delta T_f = K_f m$, a new constant L_{iso} (= iK_f) is introduced to account for this deviation. The equation then becomes

$$\Delta T_f = L_{iso} C \quad \ldots (5.29)$$

where, $\Delta T_f = L_{iso}$ is molal freezing point depression of water considering the ionization of electrolyte (i.e. iK_f) and C is the concentration of the solution in molarity. In dilute solution, the molal concentrations are not much different from the molar concentration and can be used interchangeably.

The following equations help to calculate the E_{NaCl} value from L_{iso} value of these substances.

The ΔT_f of 1 g of drug per 1000 mL of solution is equal to $L_{iso}C$.

Therefore,

$$\Delta T_f = L_{iso} \frac{1 \text{ g}}{M}$$

$$= \frac{L_{iso}}{M} \quad \ldots (5.30)$$

where, M is molecular weight of the solute. Since, the L_{iso} value of NaCl is 3.4.

$$\Delta T_f = 3.4 \times \frac{E_{NaCl}}{58.45} \quad \ldots (5.31)$$

where, E_{NaCl} is the weight of NaCl with the same freezing point as 1 g of drug. Thus

$$L_{iso} = 3.4 \times \frac{E_{NaCl}}{58.45} \quad \ldots (5.32)$$

$$E_{NaCl} = 17 \times \frac{L_{iso}}{M} \quad \ldots (5.33)$$

In some cases instead of NaCl another isotonic agent such as mannitol, propylene glycol, or glycerin is used. Using E_{NaCl} values, isotonic solutions are prepared by just multiplying quantity of each drug in the formulation by its E_{NaCl} values and subtracting them from the 0.9 g/100 mL. Thus for '*x*' grams of drug the amount of NaCl required to obtain 100 mL solution isotonic is obtained as

$$\text{Amount of NaCl (Y)} = 0.9 - [x \times E_{NaCl}] \quad \ldots (5.34)$$

For using another isotonic agent its amount (X) required to make solution isotonic is obtained by

$$X = \frac{Y}{E_{NaCl}} \quad \ldots (5.35)$$

Table 5.8: L_{iso} Values of the Tonicity Adjusting Substances

Type of substance	Examples	L_{iso} values
Non-electrolytes	Sucrose, urea, glycerine, propylene glycol	1.9
Weak ekectrolytes	Boric acid, Phenobarbital	2.0
Di-divalent electrolytes	Zinc sulphate, magnesium sulphate	2.0
Uni-univalent electrolytes	Sodium chloride, amphetamine hydrochloride	3.4
Uni-divalent electrolytes	Sodium sulphate, atropine sulphate	4.3
Di-univalent electrolytes	Zinc chloride, calcium bromide	4.8
Uni-trivalent electrolytes	Sodium phosphate, sodium citrate	5.2
Tri-univalent electrolytes	Aluminium chloride, ferric iodide	6.0
Tetraborate electrolytes	Sodium borate, potassium borate	7.6

Example 5.13: Calculate E_{NaCl} of one of the amphetamine hydrochloride derivative (Molecular weight = 187).

Solution: Since the drug is univalent salt, the L_{iso} = 3.4

$$E_{NaCl} = \frac{(17 \times 3.4)}{187}$$

$$= 0.31$$

The E_{NaCl} value of amphetamine hydrochloride derivative is 0.31.

Example 5.14: A solution of ephedrine sulfate has concentration of 1 g/100 mL. Calculate quantity of NaCl that must be added to make the solution isotonic. How much dextrose would be required for this purpose?

Solution: From Table 5.7 the NaCl equivalent of ephedrine sulfate is 0.23. Therefore for 1 g of ephedrine sulfate the amount of NaCl required will be

$$1 \text{ g ephedrine sulfate} = 1.0 \times 0.23$$

$$= 0.23 \text{ g NaCl}$$

For isotonic solution 0.9 g of NaCl needed per 100 mL solution. Therefore,

$$\text{Amount of NaCl required} = 0.9 - 0.23$$
$$= 0.67 \text{ g}$$

Therefore, 0.67 g of NaCl is to be added to make the solution isotonic.

The amount of dextrose to substitute for NaCl is calculated as;

Since, E_{NaCl} of dextrose = 0.16 g NaCl. Therefore

$$\text{1 g dextrose/0.16 g NaCl} = \frac{X}{0.67} \text{ g NaCl}$$
$$X = 4.2 \text{ g of dextrose}$$

Thus, 4.2 g of dextrose would be required to make this solution isotonic.

Example 5.15: Prepare 200 mL of an isotonic aqueous solution of thimerosal (Molecular weight = 404.84) having concentration 0.2 g/liter. The compound is univalent drug having L_{iso} value 3.4. Also, if propylene glycol (Molecular weight = 76.09) is used to replace NaCl; how much of its quantity will be required to make solution isotonic? Given L_{iso} of propylene glycol is 1.9 (non-electrolyte).

Solution: The E_{NaCl} of thiomersal is calculated as

$$E_{NaCl} = \frac{(17 \times E_{NaCl})}{M}$$
$$= \frac{17 \times 3.4}{404.84}$$
$$= 0.143 \text{ g}$$

The amount of drug needed for 200 mL (x) = 200 mL × 0.2 g/1000 mL

$$= 0.04 \text{ g}$$
$$\text{Amount equivalent to NaCl} = x \times E_{NaCl}$$
$$= 0.04 \times 0.143$$
$$= 0.0057 \text{ g NaCl}$$

Since, isotonic solution has concentration of 0.9 g NaCl/100 mL therefore for 200 mL it will be 1.8 g NaCl/200 mL. The amount of NaCl needed (Y) is obtained as

$$Y = 1.8 \text{ g NaCl} - 0.0057 \text{ g NaCl}$$
$$= 1.794 \text{ g NaCl}$$

If propylene glycol is to be used to replace NaCl then its E_{NaCl} is obtained by equation

$$E_{NaCl} = \frac{17 \times L_{iso}}{M}$$

Since, L_{iso} of propylene glycol is 1.9, therefore;

$$E_{NaCl} = \frac{17 \times 1.9}{76.09}$$
$$= 0.42 \text{ g}$$

$$X = \frac{Y}{E_{NaCl}}$$

$$= \frac{1.794}{0.42}$$

$$= 4.3 \text{ g}$$

Therefore, to make 200 mL solution of thimerosal isotonic 4.3 g propylene glycol will be needed.

D. White-Vincent method:

This method involves use of addition of water to drug to make isotonic solution followed by final volume adjustment with addition of isotonic or isotonic buffered solution. White Vincent from their study of need of pH adjustment in addition to tonicity of ophthalmic solution developed an equation as given below.

For example, to make 40 mL of 1% solution of procaine hydrochloride isotonic with body fluid first the weight of drug (x) is multiplied by E_{NaCl}

$$X = x \times E_{NaCl} \quad \ldots (5.36)$$

$$= 0.4 \times 0.21$$

$$= 0.084 \text{ g}$$

The quantity 0.084 is amount of NaCl equivalent to 0.4 g of procaine hydrochloride. We know 0.9 g/100 mL solution is isotonic; therefore, the volume (V) of isotonic solution that can be prepared from (X) g of NaCl is obtained as

$$\frac{0.9}{100} = \frac{0.084}{V} \quad \ldots (5.37)$$

$$\therefore \quad V = 0.084 \frac{100}{0.9} \quad \ldots (5.38)$$

$$= 9.33 \text{ mL} \quad \ldots (5.39)$$

In equation (5.38) the 0.084 is equal to weight of drug (*x*) multiplied by E_{NaCl} as shown in equation (5.36). The ratio (100/0.9) can be written as 111.1. Therefore, the equation (5.38) can be written as

$$V = x \times E_{NaCl} \times 111.1 \quad \ldots (5.40)$$

where, V is volume in mL of isotonic solution prepared by mixing drug in water, x is grams of drug and E_{NaCl} is sodium chloride equivalent from Table 5.7. The constant 111.1 is volume in mL of isotonic solution prepared by dissolving 1 gram of sodium chloride in water.

The volume of isotonic solution prepared by dissolving drug in water is calculated as

$$V = 0.4 \times 0.21 \times 111.1$$

$$= 9.33 \text{ mL}$$

To make an isotonic solution sufficient sodium chloride solution or an isotonic buffered diluting solution is added to make 40 mL of final solution.

Example 5.16: Calculate weight of NaCl required to make 250 mL of 0.5% w/v solution of Lidocaine HCl (Molecular weight = 270.8) isotonic with blood. The L_{iso} values for both the drug and NaCl are 3.4.

Solution: The freezing point depression of the given solution is calculated as:

$$\Delta T_f = L_{iso}\, C$$

$$\Delta T_f = \frac{3.4 \times 5}{270.80}$$

$$= 0.063\ °C$$

Since the freezing point of body fluids is −0.52 °C, and since the above drug in the concentration of 0.018 mol/L reduces the freezing point by 0.063 °C, the concentration of NaCl to be added to bring the solution to isotonicity, i.e., to lower the freezing point by another 0.457 °C (0.52 °C − 0.063 °C) is:

$$0.457 = 3.4\ C$$

$$C = \frac{0.457}{3.4}$$

$$C = 0.134\ mol/L$$

Thus, the weight of NaCl to be added to make 250 mL of solution is:

$$\frac{0.134 \times 58.5 \times 250}{1000} = 1.96\ g$$

Example 5.17: Make 50 mL of a 1% solution of procaine hydrochloride isotonic with body fluid.

Solution:

1% procaine HCl = 1 g/100 mL i.e. 0.5 g/50 mL

E_{NaCl} of procaine HCl = 0.21

NaCl equivalence = 0.5×0.21

= 0.105 g NaCl

Since 0.9 g NaCl/100 mL is an isotonic solution therefore, the volume of water needed to make procaine HCl isotonic by itself is:

$$0.9\ x = 0.105 \times 100$$

$$\therefore \quad x = 11.66\ mL$$

Therefore 11.66 mL of 0.9% NaCl will make the solution isotonic with the blood.

Example 5.18: What is the freezing point (ΔT_f) lowering of a 1% solution of sodium propionate (Molecular weight = 96 g/mole)? Given: L_{iso} = 3.4

Solution: $\Delta T_f = L_{iso}\, C$

By relating the weight and volume to this equation, we get

$$C = \text{moles/Litre}$$

$$= \frac{m \times 1000}{M \times V}$$

where, m = weight of solute in g, M = molecular weight of solute and V = volume of solution in mL. Thus,

$$= \frac{1 \times 1000}{96 \times 100}$$

$$= 0.104$$

Therefore,

$$\Delta T_f = 3.4 \times 0.104$$

$$= 0.35\ °C$$

The freezing point (ΔT_f) lowering of a 1% solution of sodium propionate is 0.35 °C.

Example 5.19: Calculate the weight of NaCl required to make 40 mL of 2% atropine sulfate solution isotonic in water. Also calculate the amount of boric acid needed to replace the NaCl.

Solution: The amount of NaCl (x) required to make 40 mL of an isotonic solution is

$$\frac{0.9}{100} = \frac{x}{40\ mL}$$

$$x = 0.36\ g$$

The contribution of atropine sulfate to the NaCl equivalent is

$$\frac{40\ ml \times 2\ g}{100\ mL} = 0.8\ g \text{ atropine sulfate}$$

Since, E_{NaCl} of atropine sulfate is 0.13,

$$0.8\ g \times 0.13 = 0.140\ g$$

The amount of NaCl to be added to make the solution isotonic is obtained by subtraction as:

$$0.36\ g - 0.104\ g = 0.256\ g \text{ or } 256\ mg$$

Other substances in addition to or in place of NaCl may be used to render solutions isotonic. This is done by calculating the amount of the substance that is equivalent to the amount of NaCl.

Then the amount of boric acid (*x*) needed to replace the NaCl can be calculated as:

$$\frac{0.256\ g\ NaCl}{x\ g\ boric\ acid} = \frac{0.5\ g\ NaCl\ equivalent}{1\ g\ boric\ acid}$$

$$x = 0.512\ g$$

Or, more simply;

$$\frac{0.256\ g}{0.5} = 0.512\ g$$

Thus, 0.512 g or 512 mg of boric acid would be required to render the previous ophthalmic solution isotonic.

EXERCISE

1. Define the terms:
   (a) pH
   (b) pOH
   (c) Buffers
   (d) Buffering agent
   (e) Buffer capacity
   (f) Activity
   (g) Buffer action
   (h) Tonicity
   (i) Pharmaceutical buffers
   (j) Osmolality
   (k) Hypotonicity
   (*l*) Hypertonicity
   (m) Isotonicity
   (n) Isosmotic solution
   (o) Isotonicity value
2. Explain relation between pH and solubility.
3. Explain measurement of pH. Also add note on temperature compensation for the pH measurement.
4. Enlist different types of buffers. Add note on acidic buffers.
5. Enlist properties of buffers.
6. Describe the use of buffers in pharmaceutical preparations.
7. Identify pH range considered to be safe for ophthalmic solutions.
8. Formulate and analyze a buffer solution of desired pH and buffer capacity.
9. Explain the importance of isotonicity in ophthalmic solutions.
10. Calculate the pH of a buffer solution prepared by dissolving 242 mg of Tris in 10 mL of 0.170 M HCl and diluting to 100 mL with water. (Molecular weight of Tris is 121 g/mol and pKa for the its conjugate acid is 8.08)
11. Differentiate between buffered and unbuffered solutions. Explain how the pH of unbuffered solution changes when acid or base added to it.
12. What is Henderson-Hasselbalch equation? Give its applications in pharmaceutical sciences.
13. What is the importance of isotonic solutions in formulation development?
14. Differentiate between isosmotic and isotonic solutions.
15. Differentiate between buffering agent and buffer solution.
16. List out types of formulations that require the isotonicity adjustment.
17. Write on methods that determines tonicity.
18. Describe methods that are used to adjust pH and isotonicity.
19. Give some examples of pharmaceutical buffer solutions.
20. Write note on biological buffers.

21. Give applications of buffers in pharmacy.
22. Enlist and explain factors affecting buffer capacity.
23. Describe a method that estimates effectiveness of buffer.
24. Write note on:
    (a) Sorenson's pH scale.
    (b) Temperature compensation in pH meter.
    (c) Acidic and alkaline buffers.
    (d) Henderson-Hasselbalch equation.
    (e) Buffer capacity.
    (f) Preparation of buffer solution
    (g) Buffer salts.
25. Enlist commonly used methods to prepare buffer solutions.
26. Describe standard buffer solutions.
27. What do you mean by biological buffers? Explain any one buffer from this category.

