CHAPTER II

GENERAL INTRODUCTION (REVIEW OF THE EARLIER WORKS)

II.1. HOST-GUEST CHEMISTRY

In host-guest chemistry, an inclusion compound is a complex in which one chemical substance (host) having a cavity like character in which another molecules known as "guest" substance are encapsulated. The definition of inclusion compounds is very vast, host-guest chemistry illustrates complex that are composed of two or more molecules or ions that are bind together by interactions through noncovalent bonding (hydrogen bonds, ionic interaction, hydrophobic-hydrophobic interactions etc.) rather than covalent bonds. Inclusion complex, which makes use of molecules rather than atomic units, provides a new approach to the assembly of new substances on multiple length scales [1-9]. Encapsulation properties of "host-guest" relationship modify or improve the physical, chemical and biological charecteristics of the guest molecules. It has enormous applications in all sectors of industry such as: pharmacy, food, cosmetics, drug delivary, agriculture, biotechnology, textiles, etc [10]. The inclusion compounds in which the guest molecules is trapped as in a cage formed by host or by lattice of host molecules is known as a "clathrate" [11].

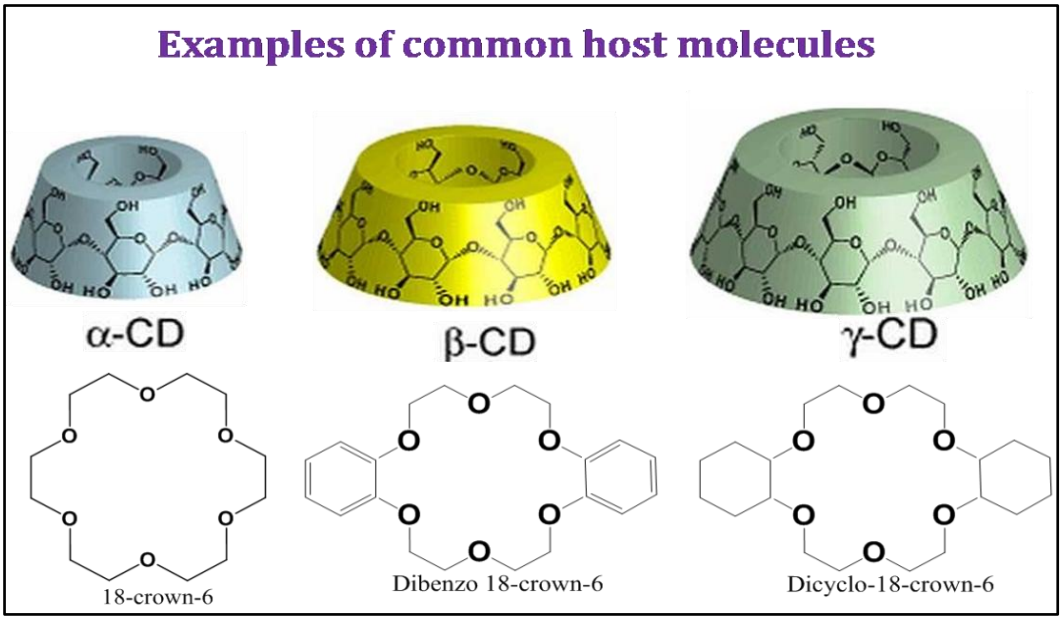
The common cyclic compounds such as cyclodextrins, crown ether, calixarenes, pillararenes, cucurbiturils, porphyrins, zeolites, cryptophanes etc can be used as the "host" molecules. In my research work, I have used cyclodextrins and crown ether as host molecules [12-15].

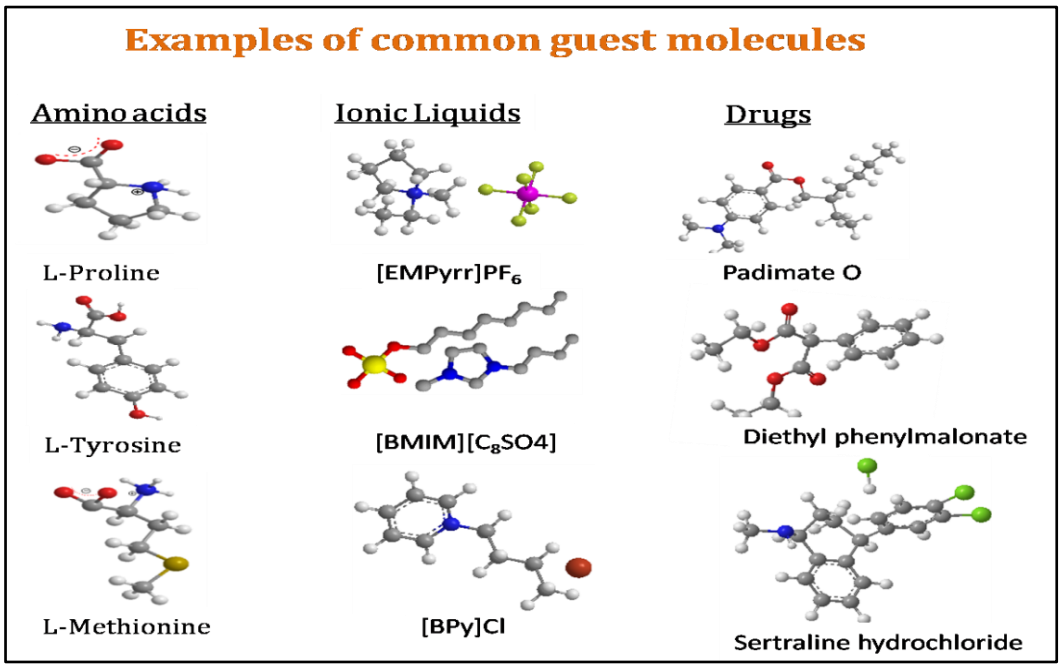
Cyclodextrins (cyds) are first isolated in 1891 as cyclic oligosaccharides [16]. Cyds are commercially accessible in the form of α, β and γ with varying the number of glucose units namely six, seven and eight respectively [17]. These glucopyranose units are bound by α-(1–4) linkages forming a truncated conical structure, which have a hydrophobic interior and hydrophilic rims having primary and secondary OH groups [18]. Because of having unique structure, they can assemble host-guest arrangement, i.e., it can accommodate entirely or at least partially the hydrophobic

moiety of a guest molecule into its hydrophobic cavity and the polar rims can stabilize the polar part of the guest, if any (e.g. amino acids, drugs, vitamins, ionic liquid etc.). Every cyd has its individual capacity to form inclusion complex with particular guests, which depends on the size-selective complexation between the guest molecule and hydrophobic core of cyd. The diameter of the hydrophobic part of guest should be smaller than the internal diameter of cyd to fom a stable inclusion complex [19]. Recently, different categories of cyd derivatives such as: hydrophilic, hydrophobic and ionic derivatives have been developed to extent physiocochemical properties and to improve inclusion capacity of cyds [20-22]. In the pharmaceutical industry cyclodextrins have mainly been used as complexing agents to increase aqueous solubility of poorly soluble guests, and to increase their bioavailability and stability against the effects of light, heat and oxidation. In addition, cyclodextrins can, for example, be used to reduce gastrointestinal drug irritation, convert liquid drugs into microcrystalline or amorphous powder, and prevent drug–drug and drug–excipient interactions. It can also reduce the volatility of guest molecules. A number of research works and review articles have been published on the pharmaceutical applications of cyclodextrins [23-26].

Crown ethers are cyclic chemical compounds that consist of a ring containing several ethers groups.The most common crown ethers are oligomers of ethylene oxide, the repeating unit being ethyleneoxy,i.e.,-CH_2CH_2O-. Crown ethers strongly attach certain cations, forming complexes. The oxygen atoms are in good positioned to coordinate with a cation situated at the interior of the ring, whereas the exterior of the ring is hydrophobic.The resulting cations often form salts that are soluble in nonpolar solvents, and because of this crown ethers are helpful in phase transfer catalysis. Crown ethers are the first generation of artificial macrocyclic hosts that suggest the birth of supramolecular chemistry. Host-guest interactions between a

crown-ether and a guest molecule where, complexation occurs due to weak non-covalent interactions such as: hydrogen bonding, π-stacking, cation- π stacking, charge transfer interaction and electrostatic interactions have been received as increasing attention recently [27-29]. Crown ether based host-guest complexations, which demonstrate excellent selectivity, high efficiency and reversibility, have been widely performed in recent years [30-33].





II.2. SOLVATION EFFECT

The branch of physical chemistry that studies the change in properties that arise when one component dissolves in another component is termed as solvation effect [34]. It investigates the solubility of components and how it is affected by the chemical nature of both solute and solvent. Thermophysical properties of solutions are very helpful to obtain information on the intermolecular interactions. Moreover, knowledge of the thermodynamic parameters is essential for industrial works and in theoretical and applied fields of research. In recent year, there are many interesting works has been reported based on the study of interactions and physicochemical properties of solvent-solvent and solute-solvent systems. There are three types of approaches by which solvation effect have been estimated. The first approach involves the studies of viscosity, conductance, etc., of electrolytes and the derivation of various factors associated with ionic salvation [35], the second is the thermodynamic approach by measuring the free energies, enthalpies and entropies of solvation of ions from which factors associated with solvation can be elucidated [36], and the third is to use spectroscopic measurements where the spectral solvent shifts or the chemical shifts determine their qualitative and quantitative nature [37]. Complete understanding of the phenomenon of solvation effect will develop into an authenticity only when solute-solute, solute-solvent and solvent-solvent interactions are revealed and thus the present research work is intimately related to the studies of solute-solute, and solvent-solvent interactions in some industrially important liquid systems.

II.3. FORCES OF ATTRACTION

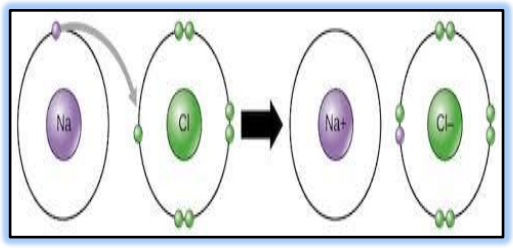
There are two broad categories of forces of attractions or interactions operate in molecules-(i) intramolecular: forces that exit within molecules and (ii) intermolecular: interactions exerted by one molecule of a molecular substance to another.

II.3.1. INTRAMOLECULAR INTERACTIONS

There are three types of intramolecular interactions

a) Ionic or Electrovalent Bond:

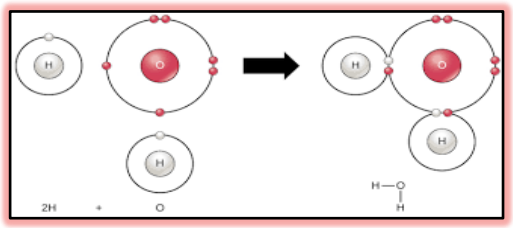
- Electrostatic force of attraction between oppositely charged ions, and is the primary interaction occurring in ionic compounds.
- Ions are formed from atoms due to electron tranfers fer from one atom to another.



- Formed as a result of large difference in electronegativity of atoms.

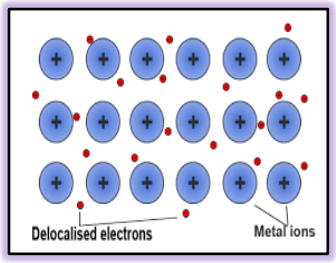
b) Covalent Bond:

- The attraction between the shared electrons and the nuclei that holds the molecule together.
- Formed by overlapping of atomic orbital.
- Formed between atoms with a small difference in electronegativity.



c) Metallic Bond:

- Attraction between valence electron and metal ions.



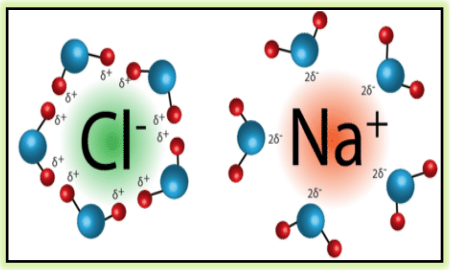
- Strong electrostatic force of attraction holds the system together.
- Positive ions are surrounded by a sea of delocalized electrons.

II.3.2. INTERMOLECULAR INTERACTIONS

Types of intermolecular interactions are as follows-

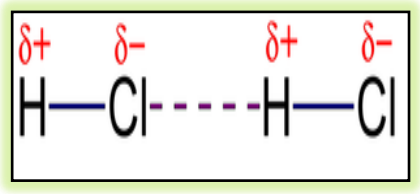
a) Ion-Dipole Interaction:

- Results from electrical interactions between an ion and the partial charges on a polar molecule (a substace with both positive and negative ends).
- In the presence of ions dipolar molecules orient themselves with posive end of dipole near the anion and negative end near cation.
- Magnetude of interaction depends on charge.



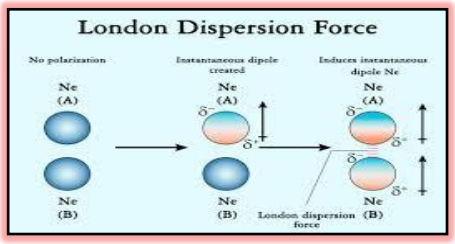
b) Dipole-Dipole Interaction:

- Intermolecular forces that operate between neutral molecules having molecular dipole moments.
- Results from interactions among dipoles on neighbouring molecules.
- The morw polar substance having the greater strength of dipole-dipole interaction.



c) London Dispertion Force or vander Waal's Force:

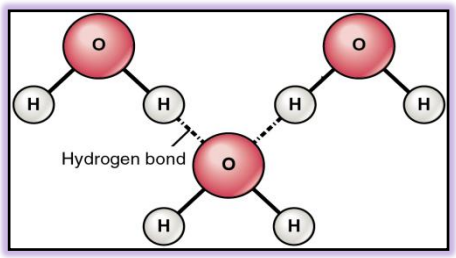
- It is distance dependent interaction between atoms and molecules and occurs between all types of molecules.
- These forces are always attractive but shorter ranged than electrostatic forces.



- If a charged molecule (ion) induces a dipole moment in a nearby neutral molecule, the two molecules will stick together, even though the neutral molecule was initially uncharged.

d) Hydrozen Bonding:

- A special kind of dipole-dipole force that occurs when a hydrogen atom is bonded to one of the very electronegative elements- F, O and N.



- The partial positive end of hydergen is attached to the partially negative end of the oxygen, nitrogen, or fluorine of another molecule.
- Hydrogen bonding is a relatively strong force of attraction between molecules, and considerable energy is required to break hydrogen bonds.

Intermolecular forces also play important roles in suppramolecular chemistry and in solutions chemistry. The majority of reactions occurring in solutions are of chemical or biological in nature. The importance of force of attractions has been realized after frequent demanding studies in host-guest inclusion complex and in solution chemistry that are performen in aqueous, non-aqueous and mixed solvents [38, 39]**.** Intermolecular forces are also important in determining the solubility, stability, bioavailability and other physical and chemical characters of a substance in another system. “Like” intermolecular forces for solute and solvent will make the solute soluble in the solvent. In this regard ΔH_{soln} is sometimes negative and sometimes positive. Furthermore, solubility is affected by (a) Energy of attraction

(due Ion-dipole force) affects the solubility. (b) Lattice energy (energy holding the ions together in the lattice. (c) Charge on ions: larger charge means higher lattice energy and (d) Size of the ion: large ions mean smaller lattice energy.

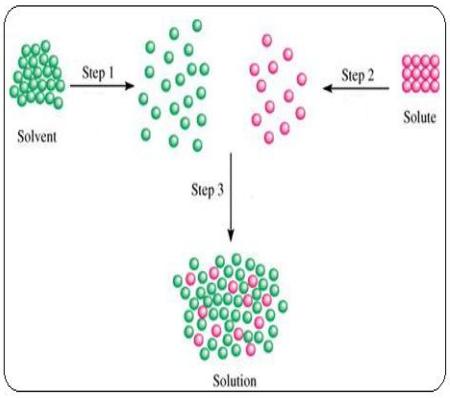
II.4. INTERACTIONS IN SOLUTION PHASE

Three types of interactions in the solution phase:

a) ***Solvent–solvent interactions****:* energy required to break weak bonds between solvent molecules.

b) ***Solute–solute interactions****:* energy required to break intermolecular bonds between the solute molecules.

c) ***Solute–solvent interactions****:* ΔH is negative since bonds are formed between them.



II.5. DENSITY

The physicochemical properties of liquid mixtures have attracted much attention from both theoretical and engineering applications points of view. Many engineering applications require quantitative data on the density of liquid mixtures. They also provide information about the nature and molecular interactions between liquid mixture components.

A material's density is defined as its mass per unit volume. It is basically, a measurement of how tightly matter is crammed together. The principal of density was discovered by the Greek scientist Archimedes [40].

$$Density(\rho) = mass(m) / volume(v)$$

One of the most common uses of density is in how different materials interact when they mixed together. It is a key concept in fluid mechanics, weather, geology, material science, engineering and other fields of physics. In fluid chemistry, the study of molecular attractions is determined by using various thermodynamic methods. Thermodynamic properties (enthalpy, entropy and Gibbs energy energy) are very expedient parameters for taking a concept about solute-solvent and solute-solute interactions in the solution phase. An interpretation of these thermodynamic properties in terms of molecular phenomena is usually not easy. Sometimes higher derivatives of these properties can be interpreted more effectively in terms of molecular interactions. The volumetric information may be of huge significance in this regard. A variety of concepts regarding molecular processes in solutions like electrostriction [41], hydrophobic hydration [42], micellization [43] and co-sphere overlap during solute-solvent interactions [44] have been derived and illustrated from the partial molar volume data of many compounds.

II.5.1. APPARENT AND PARTIAL MOLAR VOLUMES

By using density data, one can calculate the molar volume of a pure substance. However, the volume contributed to a solvent by the addition of one mole of an ion is difficult to determine, because, the volume of the solution is changed due to disintegration of the solvent structure near the ions and the compression of the solvent under the influence of the ion's electric field, i.e., electrostriction. Electrostriction is a general property of all dielectrics materials that causes them to chage their shape under the application of electric fields of the order of 10^{9}-10^{10} Vm^{-1}. Here, the compression of ions and molecules is likely to be significant. The effective volume of an ion in solution, the partial molar volume, can be determined

from a directly obtainable quantity- apparent molar volume (ϕ_V). ϕ_V, of the solutes can be expressed as (equation 1) [45].

$$\phi_V = \frac{M}{\rho_0} - \frac{1000(\rho - \rho_0)}{c\rho_0} \qquad (1)$$

Where, M= molar mass of the solute, c= molarity of the solution; ρ_o, ρ = densities of the solvent and the solution respectively. The partial molar volumes ϕ_{2v} can be determined from the equation 2 [46]

$$\phi_{2V} = \phi_V + \frac{(1000 - c\phi_V)}{2000 + c^{3/2}\left(\partial\phi_V / \partial\sqrt{c}\right)} c^{1/2}\left(\partial\phi_V / \partial\sqrt{c}\right) \qquad (2)$$

The extrapolation of the apparent molar volume of electrolyte to infinite dilution and the expression of the concentration dependence of the apparent molar volume have been made by four major equations over a period of years –(i) the Masson equation [47], (ii) the Redlich-Meyer equation [48], (iii) the Owen-Brinkley equation [49], and (iv) the Pitzer equation [50]. Masson found that the apparent molar volume of electrolyte, ϕ_V, vary with the square root of the molar concentration by the linear equation (3)

$$\phi_V = \phi_V^0 + S_V^* \sqrt{c} \qquad (3)$$

Where, ϕ_V^0 = limiting apparent molar volume (equal to the partial molar volume) at infinite dilution that implies Solute-Solvent interaction and S_V^* = Solute-Solute interaction (the experimental slope).

The majority of ϕ_V data in water [51, 52] and nearly all ϕ_V data in non-aqueous [36, 53-55] solvents have been extrapolated to infinite dilution through the use of equation (3).

The temperature dependence of ϕ_V^0 or different considered electrolytes in various solvents can be confirmed by the equation (4)

$$\phi_V^0 = a_0 + a_1 T + a_2 T^2 \qquad (4)$$

Where, a_0, a_1, a_2 signifies the coefficients of a particular electrolyte and T= temperature in Kelvin.

II.5.2. GROUP CONTRIBUTIONS OF LIMITING APPARENT MOLAR VOLUME

At each molality, limiting apparent molar volume, ϕ_V^0 values varies linearly with the number of carbon atoms in alkyl chain (R) of amino acids [56]. Similar correlations have been reported earlier by a number of workers [57-58]. The linear regression analysis of ϕ_V^0 values of amino acids versus the number of carbon atoms can be represented as [59]:

$$\phi_V^0 = \phi_V^0(NH_3^+, COO^-) + n_c \phi_V^0(CH_2) \qquad (5)$$

Where, n_c implies the number of carbon atoms in the alkyl chain of the amino acids; $\phi_V^0(NH_3^+, COO^-)$ and $\phi_V^0(CH_2)$ are the zwitterionic end group and methylene group contribution to ϕ_V^0, respectively. The values of $\phi_V^0(NH_3^+, COO^-)$ and $\phi_V^0(CH_2)$ have been calculated by a least- square regression analysis. It is well described in literature [60-61] that the values of $\phi_V^0(CH_2)$ obtained by this process characterizes the mean contribution of $\phi_V^0(CH_3)$ and $\phi_V^0(CH)$ values of amino acids. The

contribution of $\phi_V^0(CH_3)$ and $\phi_V^0(CH)$ alkyl chain of the amino acids have been calculated as follows [62]:

$$\phi_V^0(CH_3) = 1.5\phi_V^0(CH_2) \quad (6)$$

$$\phi_V^0(CH) = 0.5\ \phi_V^0(CH_2) \quad (7)$$

II.6. CONDUCTANCE

Conductivity of an electrolyte in various pure and mixed solvent systems is of a great interest of chemist. "Conductometric method" is popularly known to determine the extent of the dissociation constants of electrolytes in aqueous, mixed and non-aqueous solvents [63-65]. Ion association of electrolyte(s) in solution depends on the mode of salvation of its ions, which in turn depends on the nature of the sovenet or solvent mixture [66]. Conductance study, providing information on the mobility of ionic species in solution, is the most perfect technique offered at present to conclude the point to which ions associate in solution [67]. Behavior of electrolyte solutions can be obtained by studying their transport and thermodynamic properties. Because of its simplicity and versatility, the measurements of the conductivity of electrolyte solutions which can be carried out to a very high precision.

II.6.1. SPECIFIC CONDUCTANCE AND MOLAR CONDUCTIVITY

The reciprocal of specific registance (ρ) is termed as specific conductance (κ). Thus,

$$\kappa = 1/\rho \quad (8)$$

Specific conductance is also called conductivity.

Furthermore, $\rho = \frac{\mathrm{A} \times R}{l}$

$$\Rightarrow \frac{1}{\rho} = \frac{l}{\mathrm{A}} \times \frac{1}{\mathrm{R}}, \Rightarrow \kappa = \frac{l}{\mathrm{A}} \times G$$ [l= conductor length, A= cross-sectional]

Or, Specific conductance= Conductance x cell constant.

In the case of electrolyte solutions, the specific conductance is defined as the conductance of solution of definite dilution enclosed in a cell having two electrodes of unit area separated by unit area separated by one centimeter apart.

The conductance of that volume of solution containing one mole of an electrolyte is termed as molar conductivity (Λ_m). It is related to specific conductance (κ), with equation (9)

$$\Lambda_m = \kappa \times 1000/c \qquad (9)$$

c= molarity of the electrolyte solution.

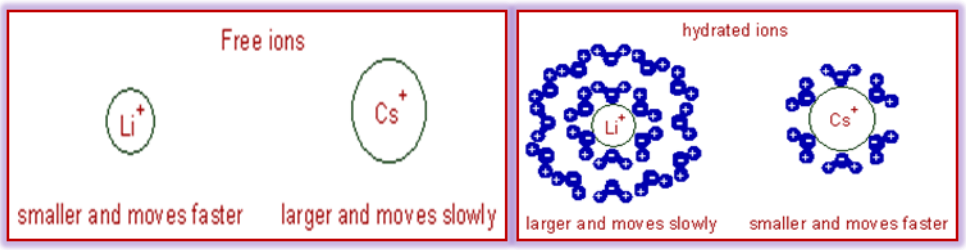
The factors affecting the conductance of electrolyte solutions are as follows:

- The conductance of an electrolyte solution increases with increase in tempetature due to increase in the extent of ionization.
- The specific conductance increases with increase in concentration of solution as the number of ions per unit volume increase. Whereas, molar conductance increase with decrease in concentration (i.e. upon dilution) since the extent of ionization increases. This is because, since the concentration decreases, one can expect decrease in equivalent conductivity due to decrease in available number of ions per unit volume. However the increase in volume (V) factor more than compensates this effect. The volume must be increased in order to get one

equivalent of electrolyte since the concentration is decreased. Hence the net effect is increase in equivalent conductivity.

- The strong electrolytes undergo complete ionization and hence show higher conductivities since they furnish more number of ions.
- Whereas weak electrolytes undergo partial ionization and hence show comparatively low conductivities in their solutions.
- The ionic mobility decreases with increase in its size and hence conductivity also decreases. However, in aqueous solutions the extent of hydration affects the mobility of the ion, which in turn affects the conductivity. Heavily hydrated ions show low conductance values due to larger size.

e.g. as the size of Li^+ ion is smaller than that of Cs^+ ion the conductivities of lithium salts are greater than Cs^+ in moltan state. But in aqueous solutions Li^+ ion with high charge density is heavily hydrated than Cs^+ ion with low charge density. Hence hydrated Li^+ bigger than hydrated Cs^+, as a result, lithium salts show lower conductivities compared to those of cesium salts in water.



II.7. MOLECULAR ASSOCIATION

Various types of interactions exist between the ions in solutions. These interactions result in the orientation of the solvent molecules towards the ion which

affect on their transport properties and also help to form a complex with the binding partners. Amino acids are building-block of protines (vital bio-molecules) and the mixture of them with some mixed aqueous solutions provides us key information about the effect of additives on them. For this purpose the physicochemical properties of amino acids in aqueous mixed solutions is very interesting. Recently, many relavant good articles have been published by many researchers in recent years [68-75].

Conductance measurements help us to determine the values of the formation constant, K_f for the process. The following mathematical treatment to estimate the formation constant is based on Evans et al. [76]. The inclution complex with 1:1 stoichiometry between host and guest molecules can be expressed as

$$M^{+} + C \rightleftarrows M^{+} \cdots C \tag{10}$$

The corresponding equilibrium constant (K_f) is given by,

$$K_f = \frac{[M^{+}C]f(M^{+}C)}{[M][C]f(M)f(C)} \tag{11}$$

Where, [MC^{+}], [M^{+}], [C] and f signifies equilibrium molar concentration of the complex, free guest molecule, free host molecules (or ligand) and the activity coefficients of the species respectively. Since the experiment has been done by dilution method, the activity coefficient of free host $f(C)$ can be considered as unity [77]. On the basis of Debye- Hückel limiting law [78], the activity coefficient of $f(MC^{+}) \simeq f(M^{+})$, thus the ratio $f(MC^{+})/\, f(M^{+})$, is considered as unity. Thus in equation (11), the formation constant in terms of the molar conductance (Λ_m), can be articulated as [77, 79]

$$K_f = \frac{[M^{+}C]}{[M][C]} = \frac{(\Lambda_M - \Lambda_{obs})}{(\Lambda_{obs} - \Lambda_{MC})[C]} \tag{12}$$

Where,
$$[C] = C_C - \frac{C_M(\Lambda_M - \Lambda_{obs})}{(\Lambda_M - \Lambda_{MC})} \quad (13)$$

Here, Λ_M stands for molar conductivity of the guest before addition of ligand; Λ_{MC} means molar conductivity of complexed ion; Λ_{obs} signify molar conductivity of the experimental solution during titration; C_C the analytical concentration of the macrocycle added and C_M the analytical concentration of the salt. The formation constant, K_f, and the molar conductance of the complex, Λ_{MC}, have been evaluated by using equations (12) and (13).

Molecular association (binding constant/association constant/ formation conatant) at equilibrium of a reaction can also be evaluated by using UV-Vis and fluorescence spectral data. The association constant (K_a) of the host-guest complexation can be calculated by using double reciprocal plots of Benesi-Hildebrand equation for the one-to-one (1:1 stoichiometry ratio) association [80-81]. For UV-Vis studies the equation is (equation 14)

$$\frac{1}{A - A_0} = \frac{1}{\Delta\varepsilon Ka[Guest]} \times \frac{1}{[Cyd]} + \frac{1}{\Delta\varepsilon[Guest]} \quad (14)$$

When the changes of absorbance with the addition of Cyd are very small then equation is modified as [82]

$$\frac{1}{I - I_o} = \frac{1}{Ka(I'' - I_o)} \cdot \frac{1}{[Cyd]} + \frac{1}{(I'' - I_o)} \quad (15)$$

In case of fluorescence studied this equation can be written as

$$\frac{1}{I - I_o} = \frac{1}{Ka(I'' - I_o)} \cdot \frac{1}{[Cyd]} + \frac{1}{(I'' - I_o)} \quad (16)$$

Where, the symbols have their usual significance.

II.8. THERMODYNAMIC PARAMETERS

The Gibbs energy change (ΔG) is a very important parameter that has been easily calculated from association constant (K_a) by using the following equation (17)

$$\Delta G = -RT \ln Ka \quad (17)$$

The negative ΔG value for the two binding partners indicates that the complexation procedure is preceded spontaneously.

Again, with the help of the van't Hoff equation (18) other thermodynamic parameters enthalpy (ΔH°) and entropy (ΔS°) can be evaluated from the temperature dependence of values as follows,

$$\ln Ka = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (18)$$

Plots of $\ln K_a$ Vs.1/T is a linear and the values of enthalpy (ΔH°) and entropy (ΔS°) have been determined from the intercept and slope of the plot respectively.

II.9. SURFACE TENSION

The surface tension experiments (accuracy is within ±0.1 mN·m−1) have done by platinum ring detachment method using a Tensiometer (K9, KRŰSS; Germany) at the experimental temperature. Temperature of the system has been maintained by circulating auto-thermostated water through a double-wall glass vessel containing the solution. The concentrations at which the inclusion occurred (the break point of the surface tension) have been calculated by solving the equation of two straight lines [84-85].

II.10. FTIR SPECTROSCOPY

Fourier Transfer Infrared Spectroscopy (FTIR) is one of the most general and broadly used imaging spectroscopic methods. The chemical and physical properties

and structure analysis of a variety of substances, including both organic and inorganic compounds can be elucided from such images. It can also be used for both qualitative and quantitative analysis of complex mixtures. The use of infrared spectroscopy began in the 1950's by Wilbur Kaye. There have been many advances in the field of IR spectroscopy; the most notable is the application of Fourier Transformations to this technique thus creating an IR method that had higher resolution and a decrease in noise. The year this method became accepted in the field was in the late 1960's [86]. Absorbing groups in the infrared region absorbe within a certain wavelength region and provides sharper absorption peaks. Different functional group absorbs different particular frequency of IR radiation. In this method, polychromatic light (light having different frequencies) is passed through a sample and the intensity of the transmitted light is measured at each frequency. When molecules absorb IR radiation, transitions occur from a ground vibrational state to an excited vibrational state. Thus, IR spectroscopy can be especially sensitive to identify of functional groups within a sample. A molecule has been determined by comparing its absorption peak to a data bank of spectra. FTIR spectrometers are generally used for measurements in the mid and near IR regions.

For a molecule to be IR active there must be a change in dipole moment as a result of the vibration that occurs when IR radiation is absorbed. Dipole moment is a vector quantity and depends on the orientation of the molecule and the photon electric vector. The dipole moment changes as the bond expands and contracts. Dipole moment in a heteronuclear diatomic molecule can be described as uneven distribution of electron density between the atoms. One atom is more electronegative than the other and has a net negative charge.

The dipole moment can be represented mathematically as

$$\mu = er \quad (19)$$

The relationship between IR intensity and dipole moment can be expressed as

$$I_{IR} \propto (d\mu/dQ)^2 \quad (20)$$

Where μ is the dipole moment and Q is the vibrational coordinate.

II.11. UV-VISIBLE SPECTROSCOPY

UV-Vis spectroscopy is an essential tool in analytical chemistry. It involves the poromotion of electrions from the ground state to higher energy or excited state. The ultraviolet region falls in the range of 190-380 nm, the visible region fall between 380-750 nm. Many molecules absorb ultraviolet or visible light. The absorbance of a solution increases as attenuation of the beam increases. Absorbance (A) is directly proportional to the path length, *l*, and the concentration, *c*, of the absorbing species. Beer's Law states that

$$A = \log(I_0 / I) = \varepsilon cl \quad (21)$$

Where, ε is a constant of proportionality, called the *absorbtivity*, I_0 is the intensity of incident light and I is the intensity of leaving light.

Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule. For example, the absorption that is observed in the UV region for the carbonyl group in acetone is of the same wavelength as the absorption from the carbonyl group in diethyl ketone.

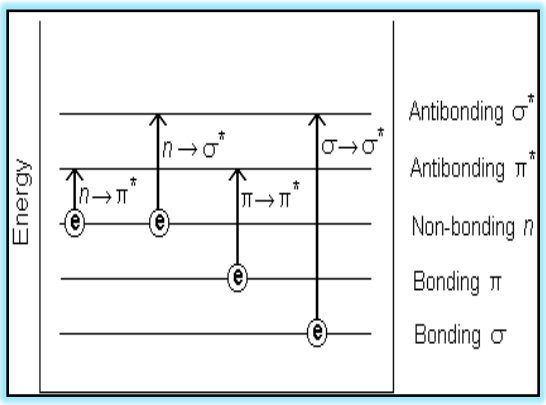
The absorption of UV or visible radiation corresponds to the excitation of outer electrons. There are three types of electronic transition which can be considered;

1. Transitions involving p, s, and *n* electrons
2. Transitions involving charge-transfer electrons (not included in this Unit)
3. Transitions involving *d* and *f* electrons (not included in this Unit)

When an atom or molecule absorbs energy, electrons are promoted from their ground state to an excited state. In a molecule, the atoms can rotate and vibrate with respect to each other. These vibrations and rotations also have discrete energy levels, which can be considered as being packed on top of each electronic level.

Absorbing species containing p, s, and n electrons:

Absorption of ultraviolet and visible radiation in organic molecules is restricted to c ertain functional groups (chromophores) that contain valence electrons of low excitation energy. The spectrum of a molecule containing these chromophores is complex. This is because the superposition of rotational and vibrational transitions on the electronic transitions gives a combination of overlapping lines. This appears as a continuous absorption band. Possible electronic transitions of p, s and n electrons are:



σ→ σ Transitions*

An electron in a bonding "s" orbital is excited to the corresponding antibonding orbital. It needs larger amount of energy. For example, methane (which has only C-H bonds, and can only undergo σ→ σ* transitions) shows an absorbance maximum at 125 nm. Absorption maxima due to σ→ σ* transitions are not seen in typical UV-Vis. spectra (200 - 700 nm).

n → σ Transitions*

Saturated compounds having atoms with lone pairs (non-bonding electrons) are proficient of *n* →σ* transitions. These transitions usually require less energy than σ →σ* transitions. They can be initiated by light whose wavelength is in the range 150 - 250 nm.

n →π and p→ π* Transitions*

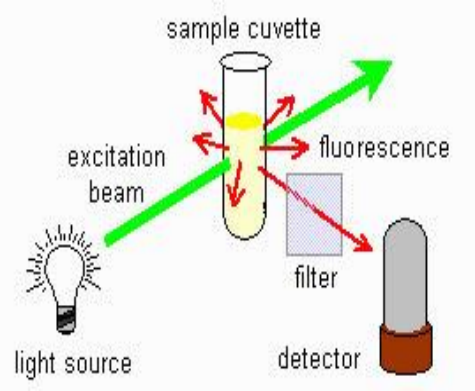
Most absorption spectroscopy of organic compounds is based on transitions of *n* or p electrons to the π* excited state. They can be initiated by light whose wavelength fall in the range of 200 -700 nm. These transitions require an unsaturated group in the molecule to provide the p electrons.

The solvent in which the absorbing species is dissolved also has an effect on the spectrum of the species. With increasing solvent polarity, resulting Peaks of *n* →π* transitions are shifted to shorter wavelengths (blue shift) and the reverse (red shift) is seen for π→π* transitions (often, but not always). This arises due to increasing solvation of the lone pair, which lowers the energy of the *n* orbital. This is caused by attractive polarisation forces between the solvent and the absorber, which lower the energy levels of both the excited and unexcited states. This effect is greater for the excited state, and so the energy difference between the excited and unexcited states is slightly reduced - resulting in a small red shift. This effect also influences *n* →π* transitions but is overshadowed by the blue shift resulting from solvation of lone pairs.

II.12. FLUORESCENCE SPECTROSCOPY

Fluorescence spectroscopy is a quick and easy method to confirm the concentration of an analyte in solution based on its fluorescent properties. It has

been used for comparatively simple analyses, where the type of compound to be analyzed ('analyte') is known, to do a quantitative analysis to determine the concentration of the analytes. Fluorescence is applied mainly for measuring compounds in solution.



In fluorescence spectroscopy, a beam within a wavelength range 180 to ~800 nm undergoes through a solution in a cuvette. We then measure – from an angle - the light that is emitted by the sample. In fluorescence spectrometry both the excitation spectrum (the light absorbed by the sample) and the emission spectrum (the light emitted by the sample) have been measured. The concentration of the analyte is directly proportional with the intensity of the emission.

There are some factors influencing the intensity and shape of the spectra. The factors are as follows:

- Excitation wavelength
- Concentration of the analyte solvent
- Path length of the cuvette
- Self-absorption of the sample

We have studied the Steady-State Fluorescence Spectroscopy which investigates the long-term average fluorescence of a sample when irradiated with UV, Visible or near-IR Light.

Edinburgh Instruments offers a range of research-grade and analytical Steady State Spectro-fluorometers. These vary in a number of ways but can be compact, benchtop or modular, and fully customisable for any type of fluorescence measurements that will meet the most demanding research requirements. The fluorescence spectrum is a plot of fluorescence intensity vs. the registered wavelength (energy and frequency) at one excitation wavelength. The fluorescence intensity measurements (spectral measurements) permit the resolve of the existence of fluorophores and their concentrations.

II.13. NMR SPECTROSCOPY

Nuclear magnetic resonance (NMR) spectroscopy is powerful analytical tool where, the experiment is performed on the nuclei of atoms, not on the electrons. The chemical environment of specific nuclei is deduced from information obtained about the nuclei. Nuclear magnetic resonance is defined as a condition when the frequency of the rotating magnetic field becomes equal to the frequency of the processing nucleus. If ratio frequency energy and a, magnetic field are simultaneously applied to the nucleus, a condition as given by the equation $v = \gamma H_0/2\pi$ is met. The system at this condition is said to be in resonance [v= frequency of radiation associated with transition from one state to the other; γ= proportionality constant and H_0= magnetic field].

1HNMR is the function of nuclear magnetic resonance in NMR spectroscopy with respect to hydrogen-1 nuclei within the molecules of a given sample, in order to establish the structure of its molecules. The protons have been regarded as a spinning positively charged unit and so it will generate a tiny magnetic field H ' along its spinning axis (as shown in figure 1). Now if this nucleus is placed in an external magnetic field H_0, it will naturally line up either parallel A or antiparallel B to the

direction of external field. The A will be more stable, being of lower energy. The energy difference AE between two states will be absorbed or emitted as the nucleus flips from one orientation to the other.

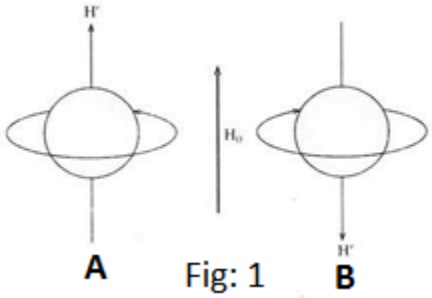


Fig: 1

Then, AE = hv (22)

Where, v signifies radiation frequency and h is Planck's constant.If correct frequency is applied to the sample containing hydrogen nuclie and sample is placed in the external magnetic field, then low energy nuclie A will absorb AE = hv, and flips to B. Thus on flipping back down, they remit hv as a radiation signal which is picked up by the instrument as chemical shift.

2D ROESY (Two Dimentional Rotating Frame Nuclear Overhauser Effect spectroscopy) is a set of NMR techniques that provide data plotted in a space defined by two frequency axes rather than one. ROESY is also known as "cross relaxation appropriate for minimolecules emulated by locked spins" (CAMELSPIN) [87]. It gives significant information about the special proximity between two molecules via observations of the intermolecular dipolar cross relation [88]**.** It is useful for determining the signals of any two protons in a molecule that are close to each other locating at a distance of 0.4 nm) in space even if they are not bonded. A ROESY spectrum yields through space correlation via spin-spin relaxation. It can also detect chemical and conformational exchange. A ROESY spectrum having a diagonal and cross peaks sgnals.The diagonal consists of the 1D spectrum. The cross peaks develop due to the presence of protons that are close to each other. It is further often used for the structural determination of the small molecules.

^{13}C NMR (Carbon-13 nuclear magnetic resonance) is the application of NMR spectroscopy to carbon. It is analogous to proton NMR (1HNMR) and permits the detection of carbon atoms in an organic molecule just as proton NMR identifies

hydrogen atoms. ^{13}C NMR is an essential tool in chemical structure elucidation in organic chemistry. ^{13}C spectra are more complex than for proton NMR. This is primarily because of the low isotopic abundance of ^{13}C (1.1%) in nature. The magnetic resonance of ^{13}C is much weaker. Moreover, gyromagnectic ratio of ^{13}C being only $1/4^{th}$ that of proton, so the resonance frequency of ^{13}C is $1/4^{th}$ of proton NMR. The most abundant isotope of carbon ^{12}C (99.1%) is has nuclear magnetic moment and thus it is NMR inactive. Since the abundance of ^{13}C is so small, spectra take much longer to obtain, although their usefulness is determining the carbon framework of a molecule is exceptionally helpful, not only do we get information about the number of carbon atoms in a sample, we also get information on how those atoms are arranged. Thus it presents information about the backbone of a molecule rather than the periphery. The normal mode of ^{13}C operation is with proton noise-decoupled mode. This experiment provides a single sharp peak for each type of carbon in the molecule.

II.14. POWDER X-RAY DIFFRACTION

X-ray powder diffraction (XRD) is a quick analytical method mostly used for phase identification of a crystalline material and can give information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined.

Max von Laue, in 1912 [89], discovered that crystalline materials act as three dimensional diffraction grating for X-ray wavelengths comparable to the spacing of planes in a crystal lattice. X-ray diffraction is now a common method for the study of crystal structures and atomic spacing. Diffraction arises when light is scattered by a periodic array with long range order, generating constructive interference at specific angles. The wavelengths of X-ray are related to the distance between two atoms;

powder X-ray diffraction (PXRD) methods apply this principle to explain the crystalline character of substances. The interaction of the incident rays with the sample makes constructive interference (and a diffracted ray) when it satisfys the conditions of Bragg's Law:

$$n\lambda = 2d\sin\theta \tag{23}$$

Where, the symbols have their usual significance.

This law states the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. All diffraction methods are based on generation of X-rays in an X-ray tube. These X-rays have been directed at the sample and diffracted X-rays are then identified, processed and counted. By examining the sample throughout a range of 2θ angles, all probable diffraction directions of the lattice have been achieved due to the arbitrary orientation of the powdered material. Switching of the diffraction peaks to *d*-spacings permits detection of the material because each material has a unique set of *d*-spacings. Typically, this is achieved by comparison of *d*-spacings with standard reference patterns [14-15, 90-91].

II.15. SCANNING ELECTRON MICROSCOPY (SEM)

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons in order to generate a high resolution image of a given sample. When the accelerated primary electrons strike the sample, it creates secondary electrons (SE). These SE are collected by a positive charged electron detector which in turn gives 3D image of the sample [92]. An account of the early history of SEM has been presented by McMullan [93]. The signals that derive from electron-sample

interactions make known information about the sample along with the external morphology (texture), chemical composition, and crystalline structure and orientation of substances making up the sample. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques. The SEM is also capable of performing analyses of selected point locations on the sample; this approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions (using energy-dispersive X-ray spectroscopy), crystalline structure, and crystal orientations (using electron backscatter diffraction EBSD).

II.16. MASS ANALYSIS

Mass spectrometry (MS) is the most precise analytical method for formative the molecular mass of the compound and its elemental composition. MS has been used in many different fields and is applied to pure as well as complex mixture [59, 90]. In this method, molecules have been bombarded with a beam of energetic electrons. The molecules are ionized and broken up into many ions. Each kind of ion has a specific mass to charge ratio (i.e. m/z ratio). For most ions, the charge is one and thus, m/z ratio is simply the molecular mass of the ion.

A parent ion forms when one electron is removed from the parent molecule of the sample

$$M + e \text{ ----------> } M^{+} + 2e$$

The m/z value of the parent ion is equal to the molecular mass of the compound. In a few cases, the parent ion peak may be the base peak and can be easily recognized. In most of the cases, parent ion peak is not the base peak and is often of very small abundance. Many elements occur naturally as isotopes; out of these the lightest one

greatly predominates. A mass spectrometer should always perform the following basic functions:

a) Produce ions from the sample in the ionization source.

$$M^+ \longrightarrow m_1^+ + m_2$$
$$\text{or} \qquad\quad m_1 + m_2^+$$

b) Separate ions according to their m/z ratio in the mass analyser.

c) Finally, fragment the selected ions and analyze the fragments in a second analyser.

d) Detect the ions emerging from the last analyser and measure their abundance with the detector that converts the ions into electrical signals.

e) The signals are transmitted to the computer and control the instrument through feedback.

II.17. THERMOGRAVIMETRIC ANALYSIS

Thermogravimetric analysis (TGA) is a method that determines the change in the mass of a given substance over a range of temperature. This technique provides information about physical and chemical phenomena of a sample [94]. The fundamental principle of TGA method is that as a sample is heated, its mass changes. This change has been used to conclude the composition of a sample and its thermal stability [95]. Generally, a sample loses weight when it is heated due to decomposition, reduction or evaporation but it could also gain weight because of oxidation or absorption. The change in weight of sample has been tracked by a microgram balance and temperature is monitored by thermocouple. It can also track

change in weight as a function of time. The graphed data are produced as weight percentage or time versus temperature.

II.18. REFRENCES

Refrences of CHAPTER II are listed in BIBLIOGRAPHY (Page: 220-225).