CHAPTER I

NECESSITY OF THE RESEARCH WORK

1.1 OBJECT, SCOPE AND APPLICATIONS

Modern science start to prosper in the early modern period, or more precisely, in the scientific revolution of 16th and 17th century in Europe. [1] The English word *scientist* is relatively young, first coined in the 19th century by William Whewell . [2] The then investigators of nature proudly entitled themselves as "natural philosophers". The primitive practical knowledge of chemistry was mainly concerned with metallurgy, dyes and pottery; these crafts were bloomed with notable skill, but there was lack of understanding of the principles associated, as early as 3500 BC in Mesopotamia and Egypt. Modern chemistry also start flourishing from the sixteenth through the eighteenth centuries through the material practices and theories promoted by alchemy, medicine, manufacturing and mining. [4] This era witnessed the salient evolvement of science, often aquainted as a progressive attainment of knowledge, in which false beliefs were thrown away by true theories.[3] Indeed, chemistry began to emerge as distinct from the pseudoscience of alchemy, after the Oxford Chemists (Robert Hooke, Robert Boyle, and John Mayow).

Research mean to carefully analyze the problems or to do the detailed study of the specific problems, by making use of special scientific methods. The main motive of the research is to get deep into the subject so that something adjuvant can churn out, which can be helpful for all. It helps us in many ways and provides a complete solution to the problems faced by humans. Now, when we humans, satisfied without any problems, this results in the advancement of the society. So, research undoubtedly helps in the development and betterment of society. The life we enjoy now or the things that we do in a minute, which looked impossible earlier, are all simply because of the research. We are accustomed to adapt new things, as our desires increases day by day. As our demands rise, the requirement of research also increases. Hence, it would not be an exaggeration if we say that “research is simply what that makes life easier”.

Being the most intelligent and wise among living beings, man has made his position above of all. Every research work is being continued for the interest of human being that they be alive comfortably. Research plays a significant role in our day to day life.

This particular branch of science known as chemistry in many way is related to human life, as well as to other sciences. It forms an indispensable part of any philosophy of nature. Chemistry is a knowledge which deals with the formation, composition, structure and characteristics of substances and with the transformations that they undergo. Most importantly, it holds the key which alone can unlock the gate to really fundamental knowledge of the hidden causes of health and disease. This is one of the most vital as well as precious ways in which any branch of science can serve humanity in the years to come.

In keeping with the increasing appreciation of the value of scientific research to humanity, there exists today among scientific men the effort to relate each particular science to every other, and to implicate all together in an indiscrete whole, without losing sight of the needed accuracy of each part. The existence of such amalgamated study as physical chemistry, biochemistry, physiological botany, and so forth, are an indication of the broader outlook. Some of the utmost and modern scientific advancements are being made along the border lines between the various sciences. We assume nature to be a unit, and our categorizations of her closely related occurences into special topics are also partly arbitrary. For example, biochemistry is truly both, life science and chemical science, exploring the chemistry of living organisms and the molecular basis relevant to the changes occurring in living cells. Biochemistry came out as a distinctive discipline around the beginning of the 20th century when scientists combine physics, chemistry, molecular biology and immunology to study the structural aspects and behaviour of the complex molecules found in biological entities and the ways these molecules undergo interaction to form cells, tissues and whole organisms. Biochemists are interested, e.g., in mechanisms of brain function, cellular multiplication and differentiation, communication within and between cells and organs, and the chemical bases of inheritance and the causes of many diseases in living beings. The biochemists seek to determine how specific molecules such as proteins, nucleic acids, lipids, vitamins and hormones function in such processes, emphasising on the regulation of chemical reactions in living cells. The methods innovated by biochemists are applied to in every fields of medicine, in agriculture and also, in many chemical and health related industries.

Nowadays, the close relationship between chemistry and medicine is clear to everyone. Our bodies, on the whole, are built up of chemical constituents, and the multifarious functions of the living organism depend, at least in part, upon chemical reactions. Chemical processes enable us to digest our food, keep us warm, supply us with mascular energy. It is likely that even the impressions of our senses, the thoughts of our brains, moreover, the mode of their transmission through the nerves, are all concerned more or less intimately with chemical reactions. The human body is a brilliantly intricate chemical machine, and its health and illness, its life and death, are fundamentally connected with the coordination of diverse complex chemical changes.

Virtually, 99% of the mass of the human body is made up of only six elements: oxygen, carbon, hydrogen, nitrogen, calcium and phosphorous. While, about 0.85% is composed of five more elements: sodium, potassium, sulphur, chlorine, and magnesium. All 11 are necessary for life. The remaining elements are trace elements, of which more than a dozen are thought on the basis of good evidence to be necessary for life. [5] While, water makes up about 60% of the body by weight. It is practically impossible to imagine life without water. Carbon (18%) is synonymous with life. Its central role is due to the fact that it has four binding sites that allow for the building of long, complex chains of molecules. Nitrogen (3%) has been found in various organic molecules including the amino acids that build up proteins, and the nucleic acids that make up DNA. Calcium (1.5%) is the most common mineral in the human body found mostly in bones and teeth. Elements like magnesium, iron, zinc, fluorine, copper, iodine etc. play significant roles in various biochemical processes. Potassium, sodium are two important electrolyte i.e, they carries charge in solution.

A solution is a homogenous mixture composed of two or more substances with variable composition. The substance constituting in the major part is called the solvent, whereas, the substance present in lesser proportion is called the solute. It is not that the solute always have to be in the same physical state as the solvent, but the physical state of the solvent generally defines the state of the solution. As long as the solute combines with the solvent to result in a homogeneous solution, the solute is said to be soluble in the solvent. It is also possible to get solutions having several solutes.

Water is definitely the most plentiful solvent in the body. Different amounts of water are, too, present in our vital organs. The brain, lungs, heart, liver and kidneys contain a lot of water – between 71% and 84%, depending on the organ. [6,7] Even our bones are 31% water! This water remains both within the cells, and between the cells that make up tissues and organs. Its numerous roles make water crucial to human functioning. The body requires water to carry out all the functions like regulating body temperature, moistening tissues in the mouth, eyes, and nose, lubricating joints, helping to dissolve nutrients and making them accessible in the bloodstream, transporting nutrients and oxygen to cells, and finally, for flushing out the waste products from the body. Water protects cells and organs from physical trauma, cushioning the brain within the skull, for example, water act as a shield to protect the delicate nerve tissue of the eyes. Water revives a fetus developing in the mother's womb as well.

Due to the inherent polarity of water molecules, with regions of positive and negative electrical charge, water can readily dissolve ionic compounds and polar covalent compounds. In order to survive, the cells in the body must be kept moist in a water-based liquid called a solution. The blood, which is a water-based solution, help carrying molecules to the necessary locations. The role of water as a solvent assists the transport of molecules like oxygen for respiration. This has a great impact on the capability of drugs to reach their targets in the body. Saline, also known as saline solution, is a mixture of sodium chloride in water and has a number of uses in medicine. By injection into a vein it is used to treat dehydration such as from gastroenteritis and diabetic ketoacidosis.[8] It is also used to dilute other medications to be given by injection.[9]

Chemistry can't be imagined without solution. Most chemical reactions are occurring in solution. The axiom that has been passed on from the days of alchemy, in Latin "corpora non agunt nisi soluta" and is translated in English as "substances do not interact with each other if they are not dissolved". We are bounded by solutions such as the air and waters (in lakes, rivers, and oceans). The air we breathe, the liquids, beverages we drink, and the fluids in our body are all solutions. Thus "Life" can be imagined as the sum of a series of complex processes taking place in solution. Aside from regulating life processes occurring in human body, solutions also play a key role in many biological processes in other organisms, and, in numerous laboratory and industrial applications of chemistry. In this research work, an attempt has been made to ascertain the outcome of solvent systems and investigate various interactions predominantly in solid and liquid phases.

Molecular interactions are often known as non-covalent interactions. [10-13] In the dominion of chemistry, interactions are the attractive or repulsive forces between molecules and between non-bonded atoms. Molecular interactions play significant role for various systems to exist. They have significant role in different areas of drug designing and its liberation systems, protein folding, material science, nanotechnology, separation and origins of life.

Molecules are formed when a set of atoms bind powerfully with each other. The binding may be as strong as to overcome different effects exerted by the environment which the molecule is exposed to. Intermolecular forces are weaker compare to intramolecular forces - the forces holding molecules together. The covalent bonds, involving sharing of electron pairs between atoms, is no doubt much stronger than the forces acting between neighbouring molecules. While the bond enthalpies are in the order of 100 kcal/mole, the enthalpy of a given molecular interaction, between a pair of non-bonded atoms, is 1-10 kcal/mole. Ideally, all molecular interactions are disrupted in processes like the melting of ice, boiling of water, vaporization, disassembling of membrane, unfolding of proteins and RNA, the covalent bonds remain intact. So, these are not chemical reactions as no bonds break or form, but all of them involve changes in molecular interactions. Even though non-covalent interactions are weak individually, cumulatively the energies of molecular interactions are significant.

A good qualitative indication of strengths of molecular interactions in the liquid phase is obtained from the differences in boiling temperatures. Liquids with high boiling point generally have strong molecular interactions. The reason why water (H_2O) has a boiling point hundreds of degrees greater than the same of nitrogen (N_2) is simply the stronger molecular interactions in H_2O (liq.) than in N_2 (liq.). This implies that forces acting between the molecules in H_2O (liq.) are much greater than those in N_2 (liq). During unfolding of a protein or an RNA (denaturation) or the separation of two strands of DNA by melting, or disassembling the ribosome, the interior sites become exposed to the surroundings, mostly water plus ions. Thus the molecular interactions amid the native state or assembly has been replaced by molecular interactions with aqueous environs.

Molecular interactions in solution phases can be best understood by studying various excess thermodynamic properties. Excess thermodynamic functions which are the difference between the thermodynamic functions of real solutions and the respective functions of ideal solutions, provide noteworthy information about the nature and strength of various intermolecular forces operating among mixed components. For an example, if a liquid mixture present an excess molar volume greater than 0, it can be said that the molecules of these compounds repulse each other when mixed. The concept of excess thermodynamic functions can be used for the improvement of new theoretical models, for the development of many empirical correlations, to carry out engineering applications in the process industries, e.g., for designing industrial separation processes properly.

Commonst among molecular interactions are (i) Van der Waals interactions, (ii) Short range repulsions, (iii) Electrostatic interactions, (iv) Hydrogen Bonding interactions, (v) Interactions among Dipolar substances. Dipolar interactions are also of various kind such as: (a) Dipole-dipole interactions (Keesom Interactions), (b) Dipole-induced dipole interactions, (c) Ion-Dipole Interactions, (d) Variable Dipoles interaction, which include Dispersive interactions and London Forces.

Incorporation of partial charges in molecules result in the dipole-dipole forces, dipole-induced dipole forces, hydrogen bonding interactions etc. These are collectively called as intermolecular forces of interactions. Intermolecular forces control the thermodynamic properties of a solution. The understanding of solvation thermodynamics is very essential for the categorical elucidation of a process accompanying in the liquid phase. These thermodynamic properties are either a feature of the whole system or the functions of such a state that do not vary much in excess of nano distances, apart from the positions where, there are abrupt changes at borders between varied phases of the system. A decent idea, regarding the nature of the interactions that exist within the constituents of a solution, is obtained from the thermodynamic investigations along with the transportation behaviour of the solution. [14-17]

By the determination of precise and accurate thermophysical properties of the components in a solution, quantitative elucidation of the solvent consequences and evaluation of the nature of various interactions are possible. By studying partial molar volumes, limiting ionic conductivity as well as viscosity B-coefficient, ion-solvent interactions can be estimated. Approximate single-ion values are used to purify the models of ion-solvent interactions. Evaluation of ion-solvent interactions allow chemists choosing solvents that will progress the solubility of minerals in discharging operations, the rates of chemical processes, or the reversal of the route of equilibrium reactions. Nowadays, the value and importance of Chemistry of electrolytes in mixed solvents, has paved researcher's attraction. [18,19].

Although, an extensive collection of data on different solution of electrolyte and non-electrolyte in water are obtainable, the structure of water and different types of interactions that water goes throughout with electrolytes are so far properly understood. The study of physicochemical properties of solutions provide sufficient knowledge on various thermodynamic properties of electrolytes and non-electrolytes, the effects of the variation in ionic constructions, mobility of ions along with their common ions. [20]

The idea of whatever happening in aqueous medium, enhances interest to study the behaviour of electrolytes in aqueous and mixed solvents by investigating solute-solute and solute-solvent interactions at different circumstances. However, the behaviour of solutes in these solvents are quite different than in aqueous medium. The difference in sequence of solubility, dissimilarity in solvating power and possibilities of chemical reactions unfamiliar with aqueous chemistry opens a new gateway for physical chemists, and, the intense use of these organic solvents has somehow overshadowed the conventional boundaries of organic, inorganic, physical, analytical and electrochemistry. [21] These facts exhort us to extend the study of binary or ternary solvent systems by choosing some industrially important polar, weakly polar or nonpolar solvents along with some important electrolytes (solutes).

The consequences on non-aqueous electrolytic solutions has outstretched their extensive usage in diverse fields. The high flexibility of non-aqueous electrolytic solutions, by making choice of numerous solvents, additives and electrolytes with widely varying properties render them to compete with ionic conductors, particularly at ambient and at low temperatures. Non-aqueous electrolyte solutions are extensively used in high energy primary and secondary batteries, in double layer capacitors, electroplating and electro deposition devices, photo-electrochemical cells, electro-chromic displays and smart windows, polishing and electro-synthesis. [22,23]

Beside the wide technical applications, quantitative understanding of these systems are still not very clear. This may be attributed to the lack of detailed information regarding the nature and strength of ion-molecular interactions and their effect on structural as wee as dynamic properties of non-aqueous electrolytic solutions. [24]

Ionic liquids (ILs) recently have been attracted as ground-breaking compound. For their unique intrinsic properties[25], the application is also increasing exponentially in many academic, industrial, and research field. [26,27] The applications of individual ionic liquid may be understood clearly by investigation of their nature and mode of interaction with the solvent molecules.

The electrolytic behaviour of the ionic liquid, tetrabutylphosphonium methanesulfonate in solvents of high and intermediate or modarate dielectric constant ($\varepsilon_r > 12$) have been studied extensively but the behaviour of this ionic liquid in solvents of very low dielectric constant ($\varepsilon_r < 12$ or 10) have not been examined before. Therefore, an attempt has been made to ascertain the nature of ion-association of tetrabutylphosphonium methanesulfonate [$Bu_4PCH_3SO_3$] in methylamine solution.

Dynamic of molecular interactions occurring between ionic liquid namely 1-ethyl-3-methylimidazolium chloride-aluminum chloride and alcohols (viz., methanol, ethanol, 1-propanol, and 1-butanol) have been studied. Limiting apparent molar volume, molar refraction and limiting apparent molar isentropic compressibility have been calculated using thermodynamic properties like density, refractive index, and, speed of sound.

The properties of material like densities and viscosities of some lithium salts, namely, lithium nitrate, lithium iodide and lithium acetate have been measured in acetonitrile-water binary mixed solvents at the temperature 298.15K. Studies on thermophysical properties namely, viscosities, densities, and refractive index, conductivity of ionic solutions assist in characterizing structures and properties of binary solutions.

Supramolecular chemistry concern chemical systems composed of a discrete number of molecules i.e., it is the study of systems containing more than one molecular assembly. Lehn truely defined Supramolecular chemistry as “chemistry beyond molecule”. It deals with understanding the structure, function and properties of these assemblies. The basic aim of this domain of chemistry is to design new functional systems of interest by combining multiple chemical entities through various non-covalent interactions. Supramolecular chemistry features phenomena like molecular self-assembly, molecular recognition, host-guest chemistry, mechanically-interlocked molecular architectures, folding of protein etc.

The stature of supramolecular chemistry came into focus, when the Nobel Prize for Chemistry in the year 1987, was awarded to Donald J. Cram, Charles J. Pedersen and Jean-Marie Lehn in recognition of their work on "host-guest" assemblies. Since then, the field is growing, by the effort of researchers from all over the world. It is highly interdisciplinary in nature and attracts biologists, biochemists, physicists, environmental scientists, crystallographers, theoreticians, in addition to the chemists.

The thermodynamics associated with supramolecular chemistry can be studied quite precisely utilising binding constants of the inclusion complexes. The binding affinities of such host-guest complexes can be governed by external stimuli, for example, temperature, ion, pH, redox, enzyme and light.[28-32] Alongside calculating binding energies, designing of the supramolecular systems, their functionings are also of primary interest. In a typical supramolecular complex, a number of interactions contribute, and, nevertheless, the loss in entropy of translation due to intermolecular association is paid by a sole association step. A set of phenomena, crucial in biological systems, for example, enzyme action, genetic information and processing, molecular transport, protein assembly, and many, has been monitored by studying specific non-covalent interactions between molecules. These non-covalent interactions are no doubt much weaker while being compared with covalent interactions (see, **Table I.1**). Modern synthetic tools help to develop a suitable host structure, so that there can be an extra potential for a particular guest molecule.

Type of Interactions	Strength (in KJ/mole)
Covalent Bond	200–400
Ion-Ion	100–360
Ion-Dipole	50–200
Dipole-Dipole	5–50
Hydrogen Bonding	4–120
Cation-π	5–80
π–π	0–50
van der Waals	< 5 (varies with surface area)
Hydrophobic	Related to solvent-solvent interaction

Table I.1 Different type of Interactions with relevant energies

So far as the remarkable developments in supramolecular chemistry is concerned, macrocycle based Host-Guest interactions have played the most significant role. The cyclized and constrained conformations of macrocyclic hosts offer the benefit of molecular selectivity. In this regard, cyclodextrins (CDs) are immensely interesting owing to their amphiphilic nature, that can be applied in different fields ranging from nanodevices to cell imaging and drug delivery. [33-35] Supramolecular chemistry, over the past decade, has a significantl contribution towards the advancement of nanotechnology. Cyclodextrin-modified nanoparticles have been in the centre of attraction for long for their ability to improve the characteristics of the resulting assemblies, such as the electronic, conductance, thermal, fluorescence, and catalytic properties thereby enhancing the potential applications of such assemblies as nanosensors, and drug delivery vehicles.

The cyclodextrin (CD) molecules have a distinct structure with a hydrophobic cavity and a hydrophilic surface which can form inclusion compounds with a wide range of guest molecules. Cyclodextrins CDs) based host-guest inclusion complexation has earned a significant value in consumer goods, food industries, pharmaceuticals , cosmetics, and bio-medical devices. [36-38] The encapsulation of different bioactive compounds, drugs, enzymes, vitamins [39] etc., using cyclodextrins and their derivatives, can protect the compounds from environmental conditions, and, often associated with an improvement of aqueous solubility, bioavailability and shielding side effects.

The problem of poor solubility of many drugs in water that restrict their applications, can be surpassed by forming CD-drug inclusion complexes. Compared with other macrocycles, CDs are the most significant additives in pharmaceutical products for many reasons: (i) CDs are seminatural products, and can be produced in thousands of tons in a year from starch with very low cost, (ii) for being highly biocompatible in nature, CDs can directly be used as ingredients of foods, drugs, and cosmetics, (iii) their strong binding affinity for a specific guest, stabilizes the complex in physiological environment, (iv) on arriving at the destination, the loaded cargoes can be easily released from the cavity, (v) the cavity provides an hydrophobic environment to shield the drug molecules from enzymatic hydrolysis during the circulation and delivery processes. In modern pharmacology, the stabilisation as well as controlled release of drug molecules have inclined a significant attraction. It is quite common now that the encapsulation of many biologically active molecules into cyclodextrin cavities can shield those molecules from environmental effects. The controlled release of drug molecules, so as to minimise their inherent side effects, have been achieved by complexing them with CDs. So, to get the benefit, primitive requisite is to verify whether the drug can form inclusion complex with CDs. Following this, the inclusion complex formation of drug molecules like 1-hydrazinophthalazine Hydrochloride with β-Cyclodextrin, have been studied in detail utilising different physicochemical measurements alongwith some spectroscopic techniques. [40-42]

N,N-Dimethyl-p-phenylenediamine dihydrochloride, is a stain used in microscopy, encapsulated into the cavity of β-CD in aqueous medium was explored and characterized by employing several physicochemical and spectroscopic methods.

Host–guest inclusion complexes formed from 4-chloro-1-napthol with α- and β-cyclodextrin in a 50% aqueous ethanol solvent have also been investigated by various methodologies.

1.2. METHODS OF INVESTIGATION

In order to get a better understanding into the phenomena of diverse interactions upon solvation and inclusion complexation, various experimental methods in solution and in solid phase have been employed. A number of noteworthy methodologies like densitometric, conductometric, viscometric, refractometric techniques have been utilised to inquire the solvation and inclusion phenomena.

Study of thermodynamic and transport properties play key role in characterizing the structural aspects and properties of solutions. The partial molar volumes obtained from density measurements are suitable parameters to interpret solute-solute, solute-solvent interactions taking place in solution. The sign and magnitude of another thermodynamic quantity, partial molar volume (ϕ_V^0), offers information regarding the nature and extent of ion-solvent interaction. Beside, the experimental slope (S_V^*) gives information about ion-ion interactions . [43] The change in viscosity due to the addition of electrolyte solutions has been attributed to inter-ionic and ion-solvent effects. Conductance data obtained as a function of concentration have been used to evaluate the ion-association by employing appropriate equations.

These interactions, in a broad sense, can be highly defined by applying suitable spectroscopic investigations. The exact characteristic properties of different molecules are seen in various spectroscopic studies in aqueous and mixed solvents. The spectroscopic studies like UV-Vis, Proton-NMR, 2D-ROESY, FT-IR spectroscopic and Mass spectrometric studies have been made to elucidate a variety of interactions.

Following are the various physicochemical and spectroscopic methods used during the research work:

- UV-VIS Spectroscopy
- Fluorescence Spectroscopy

- Scanning Electron Microscopy (SEM)
- FTIR Spectroscopy
- ^{1}H NMR Spectroscopy
- Mass Spectroscopy
- 2D ROESY
- Surface tension study
- Conductivity study
- Density study
- Viscosity study
- Refractive Index study

1.3. CHOICE OF BIOLOGICALLY ACTIVE MOLECULES, HOST MOLECULES, IONIC LIQUIDS, SALTS AND SOLVENTS USED IN THE RESEARCH WORK

Names of the Biologically Active Molecule, Host Molecules, Ionic Liquids and Solvent molecules are listed below:

Biologically Active Molecule:

- 1-Hydrazinophthalazine Hydrochloride
- N,N-Dimethyl-p-Phenylenediamine
- 4-Chloro-1-Napthol

Host Molecules:

- α-Cyclodextrin
- β-Cyclodextrin

Ionic Liquids and salts:

- Tetrabutylphosphonium Methanesulfonate
- 1-Ethyl-3-Methylimidazolium Chloride-Aluminum Chloride
- Lithium Iodide

- Lithium Nitrate
- Lithium Acetate

Solvents:

- Water
- Acetonitrile
- Methanol
- Ethanol
- 1-Propanol
- 1-Butanol
- Dimethyl sulfoxide
- Methyl amine