INTERACTION BETWEEN IONIC LIQUID AND LYSOZYME: A CONFORMATIONAL AND STABILITY ASPECT

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CERTIFICATE

This is to certify that the dissertation entitled **"Interaction between ionic liquid and Lysozyme: a conformational and stability aspect"** submitted to the Department of Chemistry, National Institute of Technology, Rourkela-769008 in partial fulfilment of the requirement for the award of M.Sc. degree in Chemistry is a record of original work done by **Ms. Litun Swain** under my supervision and guidance.

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Litun Swain

DECLARATION

I, **Ms**. **Litun Swain**, hereby declare that all my research works are original and no part of this report had been submitted for any other degree or diploma. All the given information and works done are true to my sense and knowledge.

Place: Rourkela **Litun Swain**

Date: **(412CY2029)**

ABSTRACT

In this project the recent developments in Fourier transform infrared (FTIR) spectroscopy technique and its applications to protein structural studies were introduced. The experimental skills, data analysis and correlations between the FTIR spectroscopic bands and secondary structure components of lysozyme are discussed here. Here, we are studying the conformational changes of lysozyme protein in the presence of different concentrations of various ionic liquids, like 1-ethyl-3-methylimidazolium chloride (EMImCl), 1-butyl-3-methylimidazolium chloride (BMImCl) and 1-hexyl-3-methylimidazolium chloride (HMImCl) in FTIR spectroscopy, and also, the thermal stability of lysozyme in different concentrations of BMImCl have been investigated by temperature dependant absorbance study. The applications of FTIR to the secondary structure analysis, conformational changes and structural dynamics and stability studies of lysozyme are also discussed. Moreover, the overall objective of our research is to qualitatively investigate the effect of ILs on protein conformation and stability.

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CHAPTER- 1

INTRODUCTION

1.1 OVERVIEW OF PROTEIN:

Proteins are the most abundant bioorganic molecules in the living system. They occur in every part of the cell and constitute about 50% of the cellular dry weight. Proteins form the fundamental basis of structure and function [1]. Basically the term protein is derived from a Greek word ***proteios***, meaning holding the first place. Mulder (Dutch chemist) in 1838 used the term proteins for the high molecular weight nitrogen-rich and most abundant substances present in animals and plants. Proteins perform essential functions in the living cells along with great variety and specialization [2]. These functions may be broadly grouped as static (i.e. responsible for the structure and strength of body) and dynamic (i.e. acting as enzymes, hormones, blood clotting factors, immunoglobulin's, membrane receptors, storage proteins etc.). Proteins are the large biological molecules or macromolecules consisting of one or more number of amino acid residues. These are predominantly constituted by five major elements such as; Carbon (50-55 %), Hydrogen (6-7.3 %), Oxygen (19-24 %), Nitrogen (13-19 %), Sulfur (0-4 %). Besides the above, proteins may also contain other elements such as P, Fe, Cu, I, Mg, Mn, Zn etc [2]. Usually many proteins for example enzymes *ribonuclease A* and *chymotripsin*, contain only amino acid residues and no other chemical constituents; these are considered as simple proteins. However, some proteins contain permanently associated chemical components in addition to amino acids, these are called conjugated proteins. The non-amino acid part of conjugated protein is usually called as prosthetic group [1, 2].

As far as the applications are considered proteins are the biomolecules which are of greater importance in the biochemical processes such as the medical, pharmaceutical, and food fields, since they exhibit their excellent biological activities under mild condition. However, most of proteins dissolved in an aqueous solution are immediately denatured and inactivated at high temperatures due to the breakdown of weak interactions like ionic or electrostatic interactions, hydrogen bonds and hydrophobic interactions, which are prime determinants of protein tertiary structures [3]. In particular, protein aggregation easily occurs upon the exposure of the hydrophobic parts of proteins, which are usually located inside of native proteins and this phenomenon becomes the major problem due to its the fast irreversible inactivation. Thermal denaturation of proteins is a serious problem not only in the separation and storage of proteins

but also in the processes including biotransformation, biosensing, drug production and food manufacturing. Several strategies such as chemical modification, immobilization, genetic modification, and addition of stabilizing agents have so far been proposed in order to prevent thermal denaturation of proteins. The addition of stabilizing agents is the most convenient method for minimizing this thermal denaturation, in comparison to other methods. It has been reported that inorganic salts, polyols, sugars, amino acids, amino acid derivatives, chaotropic reagents and water-miscible organic solvents are used for improving protein stability. However, these additives do not sufficiently prevent irreversible protein aggregation or some of the additives are no longer stable at high temperatures [4].

1.2 LYSOZYME AND ITS STRUCTURE:

Lysozyme also known as muramiase or; N-acetylmuramide is a small protein (Molecular Weight.- 14.3 kDa) which, catalyses the denaturation of bacterial cell wall or; the hydrolysis of 1, 4-$\boldsymbol{\beta}$ linkages between the N-acetyl muramic acid and N-acetyl-D-glucosamine. Lysozyme is nothing but a natural antibacterial agent found in tears and egg whites. The hen egg white lysozyme is a monomer with 129 amino acid residues, in which lysine is the N-terminal amino acid and leucine is the C-terminal one [5]. The active site of lysozyme is formed by the contribution of amino acid residues numbered as 35, 52, 62, 63 and 101 [2].

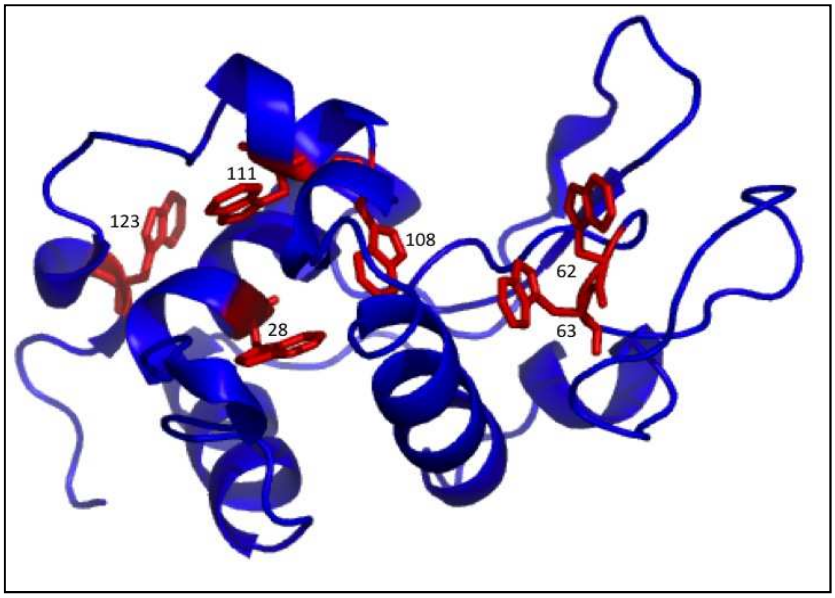


Figure 1: Crystal structure of Hen egg white lysozyme (PDB ID 1AZF). The intrinsic Trps are numbered as per their occurrence in the native protein structure. Trp62, Trp63, Trp111, Trp123 are fully or partially exposed to solvent, whereas Tro28 and Trp108 are in the hydrophobic core.

This was the first enzyme to have its three dimensional structure determined by David Phillips and colleagues in 1965. It is an enzyme notably found in tears, nasal secretions and the white of avian eggs which hydrolyzes the polysaccharides found in many bacterial cell walls. Lysozyme has four disulfide bonds (S-S) that cause high thermal stability of the enzyme and also it contains six alpha-helical regions and five beta-sheets, which is shown in Fig.1. The four disulfide bridges cause thermal stability of the enzyme. Along with this a number of $\boldsymbol{\beta}$-turns and a large number of random coils are present in order to make the remainder of the polypeptide backbone. A rich and easily available source of lysozyme is the egg white of birds. In the hen egg white, lysozyme accounts for 3.5% of the total egg white proteins. /in the hen egg white, lysozyme occupies only 3.5% of the total egg white proteins.

1.3 APPLICATION OF LYSOZYME:

Lysozyme is extensively used in the pharmaceutical field for destroying gram positive bacteria and can be used to support already existing immune defenses to fight against bacterial infection.

However, bacteriostatic and bactericidal properties of lysozyme have also been used against a certain number of food spoilage bacteria and pathogens. It is now been evident that, aside from the lysozyme bacteriolytic-action, a dimeric form of lysozyme exhibits therapeutic, antiviral and anti inflammatory properties. The studies conducted so far show that it induces the activity of phagocytizingcells, influences the immunological processes by the stimulation of immunoglobin synthesis, stimulates alpha-interferon synthesis, and most importantly – modulates TNF (Tumor Necrosis Factor) generation [Kiczka, 1994]. The enzyme lysozyme kills bacteria by hydrolyzing the β1-4 glycosidic linkage between N-acetyl glucosamine and N-acetylmuramic acid (Fig. 2) [1, 5].

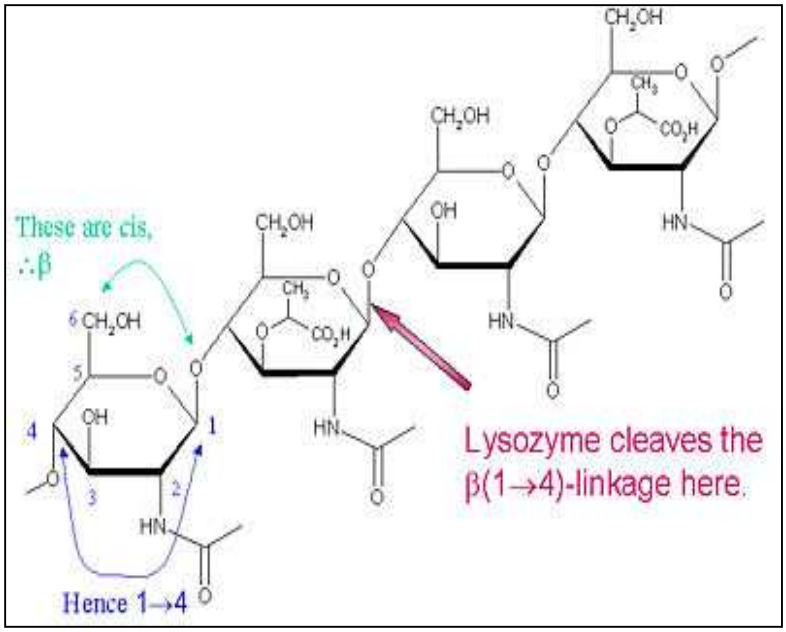


Figure 2: Glycosidic Cleavage site in bacterial cell wall.

Hen egg white lysozyme has been studied in great detail, because the enzyme is a very effective catalyst and as well as it is used as a model protein. Since it is well investigated in the research field due to its structure, properties, functions, and thermo stability.

1.4 IONIC LIQUIDS AND THEIR SIGNIFICANCE:

As per the Fluka product bulletin ionic liquids (ILs) are the organic salts with melting points under 100^{o}C, often below room temperature (RT) [6, 7]. Ionic liquids are simply the mixture of cations and anions which, do not pack well among them and therefore they remain as liquid at low to moderate temperatures [8]. The low melting point is often achieved by incorporating bulky asymmetric cations into the structure, together with weakly coordinating anions. Within the last decade, ionic liquids have come to the fore as environmentally-responsible designer solvents which are claimed to be as *green solvents* [9-12]. As a non-conventional class of novel solvents, ionic liquids are becoming increasingly important and of particular interest [9, 13-16]. This is because they have a number of characteristics including negligible volatility, non-flammability, high thermal stability, low melting point, broad liquid range, eco-toxicity, biodegradability and controlled miscibility with organic compounds, especially some heterocyclic compounds [17-20] .Due to this significant properties, ionic liquid is widely used in many field including biotechnology, chemistry, chemical engineering, pharmaceutical, coating etc which is shown in the figure 3.

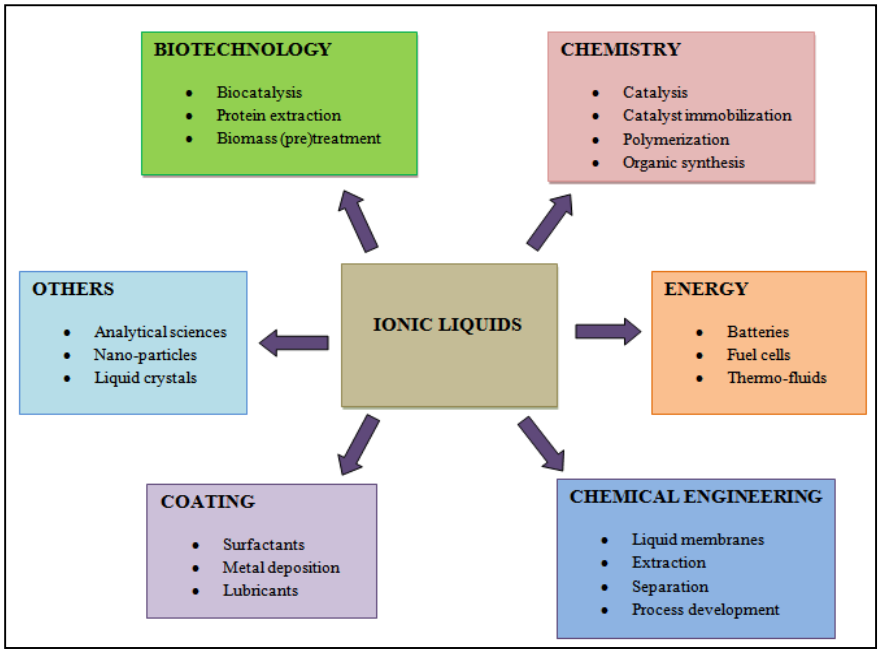


Figure 3: Overview of different possible applications of Ionic Liqiuds.

Ionic solvent that is ionic liquid at room temperature has attracted increasing attention as a green solvent for the chemical processes because of the lack of vapor pressure, the thermal stability, and the high polarity. Chemical and physical properties of ionic liquids can be changed by the appropriate modification of organic cations and anions, which are the constituents of ionic liquids [21]. It has recently been reported that protic ionic liquids such as alkylammonium salts keep the stability of proteins in an aqueous solution at high temperatures, and amyloid fibrils of proteins are dissolved in protic ionic liquids and are refolded by dilution with an aqueous solution [22-24]. On the other hand, biotransformation in ionic liquids has increasingly been studied. Aprotic ionic liquids such as immidazolium salts have mainly been employed as reaction media.

1.5 PROPOSSED IONIC LIQUIDS IN THE EXPERIMENT:

Among a plethora of biochemical applications of ILs enhancement of thermal and functional stability of proteins are one among them [12-15,17,18]. Because natural media are usually crowded by ions, the role of inorganic salts in biomolecular processes has been studied

for a long time. At low concentrations salt effects on proteins are dominated by electrostatic forces between ions and the charged protein. At concentrations above 0.05 M ion-specific effects become detectable [25], which largely increase with increasing salt concentration.35, 36. Here in the experiment the ILs is basically taken above 0.5M concentration (i.e. 0.2, 0.6 and 1M).

Here, we are studying the conformational change of lysozyme protein in the presence different concentrations of various ionic liquids like 1-ethyl-3-methylimidazolium chloride (EMImCl), 1-butyl-3-methylimidazolium chloride (BMImCl) and 1-hexyl-3-methylimidazolium chloride (HMImCl) in FTIR spectroscopy. Then after the melting temperature (T_m) variation protein in different ionic liquid medium was studied using UV- visible spectrophotometer. Among so many ionic liquids we are mainly concentrated with *imidazolium* based ionic liquids. Such as, EMImCl, BMImCl and HMImCl. Because early developed ILs are corrosive and toxic. Whereas the ILs possessing imidazolium cations exhibits higher ionic conductivities and lower viscosities [26]. There are also a lots of investigations are going on for capacitors, solar cells, fuel cells and batteries. Moreover, the overall objective of our research is to qualitatively investigate the effect of ILs on protein conformation and stability.

CHAPTER- 2

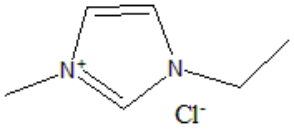
MATERIAL & METHODS

2.1 MATERIALS:

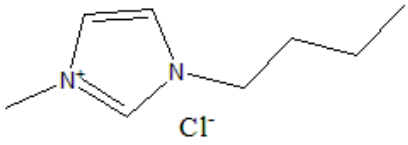
Here in the proposed work, hen egg white lysozyme (product number-L6876) was purchased from Sigma. The FTIR study of lysozyme was done at three different concentrations (0.2M, 0.6M, and 1.0M) of three different ionic liquids (i.e. 1-Ethyl-3-methyl-imidazolium chloride, 1-Butyl-3-methylimidazolium chloride, 1-Hexyl-3-methylimidazolium chloride) varying in their alkyl chain attached at cationic moiety, in order to study the conformational and their respective stability. The commercially available lysozyme was used without further purification. Temperature-dependent absorption study was also performed to monitor the melting temperature (T_m) as a function of concentrations (i.e. 0.2 M, 0.6 M and 1.0 M) of 1-Butyl-3-methyl imidazolium chloride (BMImCl).

Ionic liquids used in the experiment:

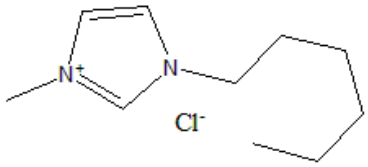
1. 1-Ethyl-3-methyl immidazolium chloride



2. 1-Butyl-3-methyl immidazolium chloride



3. 1-Hexyl-3-methyl immidazolium chloride



Sodium phosphate buffer is used as solvent and maintain at pH 7.4 throughout the experiment.

2.2 METHODS:

Preparation of 0.1 M Sodium Phosphate Buffer (SPB):

100ml sodium phosphate buffer was prepared by mixing 7.74 ml of 1 M disodium hydrogen phosphate (Na_2HPO_4) with 1 M monosodium hydrogen phosphate (NaH_2PO_4) and 90ml of distilled water was added to it in order to maintain the volume in the volumetric flask.

Preparation of Lysozyme Stock Solution:

500 μM of lysozyme stock solution was prepared by dissolving 0.029 gm of lysozyme (solid powder) in 4 ml of freshly prepared buffer solution. The protein solution was allowed to incubate homogeneously. The protein solution which was prepared was allowed for degassing (20-30 minutes) in a sonicator in order to remove the undesirable gaseous impurities from the solution which may interfere during spectral analysis.

Preparation of Ionic Liquid (IL) Stock Solution:

The 1 M stock solution of three different kind of ILs (EMImCl, BMImCl, HMImCl) were prepared by measuring required amount of solute followed by the addition of required amount of buffer solution as solvent medium. Then further dilution was done to get four more concentrations (i.e. 0.6, 0.2 M respectively).

Preparation of Sample Mixture (Protein with Ionic Liquids):

The sample mixtures protein in different concentration of different ionic liquid (3 ml each) were prepared by dissolving required volume of freshly prepared protein (0.5 ml) with required volume of different ILs (2.5 ml each).

2.3 TECHNIQUES USED:

A) Fourier Transform Infrared Spectroscopy (FTIR):

Infrared spectroscopy is nothing but the study and characterization of modes of vibration of bonds between two atoms in a molecule using electromagnetic radiation between wavenumber 10 to 14000 cm^{-1}. The infrared region is broadly divided into three regions. Such as;

- Near (14000 to 4000 cm^{-1})
- Mid (4000 to 400 cm^{-1})
- Far (400 to 10 cm^{-1})

FTIR analyzes the absorption of infrared radiation over a range of wavelengths around 700 nm to 1 nm [27,28]. FTIR is the most widely used experimental technique because it has the ability to examine the entire range of radiations of our interest simultaneously. During the last few years the use of FTIR to determine the structure of biological macromolecules has dramatically being increased. A new technique of FTIR spectroscopy is arises which, requires only small amount of samples (1 mM) for characterization. Thus we can get high quality of spectra without any problems of background fluorescence, light scattering and problem related to the size of the proteins. Infrared spectroscopy is able to view nine different characteristic "Amide" bands of the protein, which includes the important "Amide I" band referring to C=O stretching vibration, occurring between 1690 to 1600 cm^{-1}[29, 31-33].

The *Amide I* band is of particular importance because of its sensitivity towards the changes in the secondary structure of proteins. The oxygen of the Amide I band is hydrogen bonded to nearby amide hydrogens. Changes in the secondary structure may alter the length of the hydrogen bond as a result of which the frequency of absorption can also be changed.[27, 31]

Each secondary structure has a different and unique, peak location [32]. For example, α-helix bonds are present in the range of 1665 to 1655 cm^{-1}, while β-sheet bonds are also found in this range, 1645 to 1610 cm^{-1}[27, 34]. The β-sheet region is interesting from a quantitative perspective because often more than one peak is found from 1645 to 1610 cm^{-1}. Along with $\boldsymbol{\alpha}$-

Helix and $\boldsymbol{\beta}$- Sheet a number of other peaks are there in Amide I band, which are summarized in Table 1.

TABLE 1: Assignment of Amide I band to protein secondary structures.

Secondary structure element	Band position (in cm^{-1})
$\boldsymbol{\beta}$-turn	1690-1665
$\boldsymbol{\alpha}$-helix	1665-1655
Random coil	1655-1645
$\boldsymbol{\beta}$-sheet	1645-1610

Again the half width of the alpha-helix band depends on the stability of the helix. For the most stable helices, the half-width is of about 15 cm^{-1}. Whereas, the other α-helices display half-widths of 38 cm^{-1}. Amide I mode of vibration is directly related to the backbone of protein. Changes in the secondary structure due to solvent, chemical, or thermal effects can also be studied under FTIR [27, 28, 31, 32]. As an example Lau et al. (2004) showed the loss of protein's enzymatic activity which was dissolved in ionic liquids through changes in the enzyme's secondary structure using infrared spectroscopy. Similar manner here we have studied the how the conformation of lysozyme is affected by dissolving it in a range of concentration of different ILs.

Working procedure:

In case of FTIR, a continuum source of light is required (such as Nernst Globar) which, produces wide range of infrared wavelengths. Then, the light coming from the source is splitted into two paths using a half-silvered mirror. The splitted out light is then reflected from two mirrors back onto the beam splitter, where these are recombined. In between the above two mirrors one is fixed and the other one is movable. The distance between the beam splitter to the fixed mirror should be exactly same as the distance from the beam splitter to the second mirror. In FTIR spectroscopy, the light is directed onto the sample and the intensity is measured by using an infrared detector as shown in fig. 4. The intensity of light striking the detector is

measured as a function of the mirror position, and this is then Fourier-transformed to produce a plot of intensity vs. wavenumber.

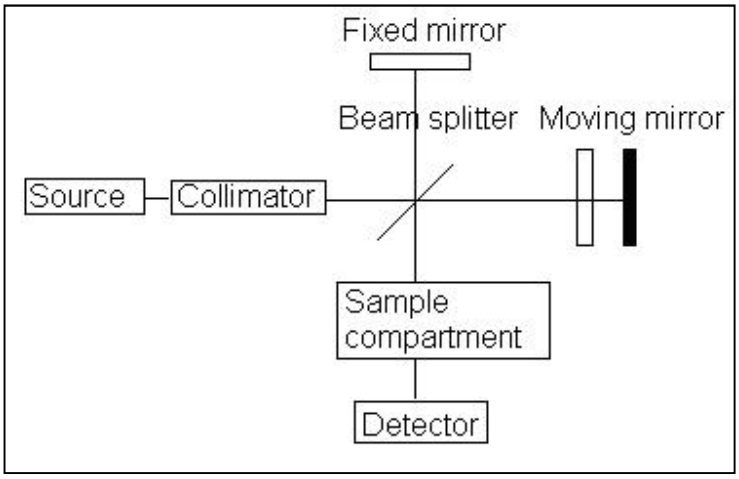


Figure 4: Schematic representation of FTIR.

B) Ultraviolet-Visible Spectroscopy:

The ultraviolet-visible (UV-Vis) spectrophotometer is an instrument which is commonly used in the laboratories and analyzes compounds in the ultraviolet (UV) and visible (Vis) regions of the electromagnetic spectrum. Unlike infrared spectroscopy, which looks at vibrational motions; ultraviolet-visible spectroscopy looks at electronic transitions. It allows one to determine the wavelength and maximum absorbance of compounds. From Beer's Law;

$$A = \varepsilon bc$$

Where A = absorbance, ε = molar extinction coefficient, b = path length, and c = concentration,

From the above equation one can be able to determine either the concentration of a sample if the molar extinction coefficient is known or the molar absorptivity, if the concentration is known. Molar extinction coefficients are specific to particular compounds; therefore UV-Vis spectroscopy helps us to determine the identity of the unknown compounds. Furthermore, the energy of a compound can be determined from this technology by using the equation;

$$E = hc/\lambda$$

Where; E = energy, h = Planck's constant, c = speed of light, and λ = wavelength.

In the present work we have done the temperature dependant absorption experiment by using Cary 300 UV- Vis spectro photometer. The temperature was changed in the range of 25 to 95 ^{o}C. Usually the thermal stability of lysozyme in aqueous buffer solutions was studied at selected temperatures (73–100 °C) and pH values (4.2–9.0).

CHAPTER- 3

RESULTS & DISCUSSIONS

3.1 FTIR STUDY:

Secondary structures of the protein are very much important to study the conformational changes in the native or non-native structures. Here, we analyzed the secondary structures of lysozyme as a function of different ionic liquids and their concentrations ranging from 0 to 1 M. The FTIR measurements were done in Bruker FTIR-STIR (Germany). For the analysis purpose of the FTIR results, the amide I band is specifically adopted because of its usefulness in terms of sensitivity [4,11]. In amide I, due to unique molecular geometry and H-Bonding pattern each type of secondary structure gives rise to a different C=O stretching frequency. In most of the cases, the second derivative of the FTIR spectra is followed to analyze the secondary structures of protein because of the high resolution and well separated bands. In our case, we avoided the second derivative transformation because the original FTIR spectra show well-separated bands for each secondary structure.

3.1.1 Effect of Ionic Liquid:

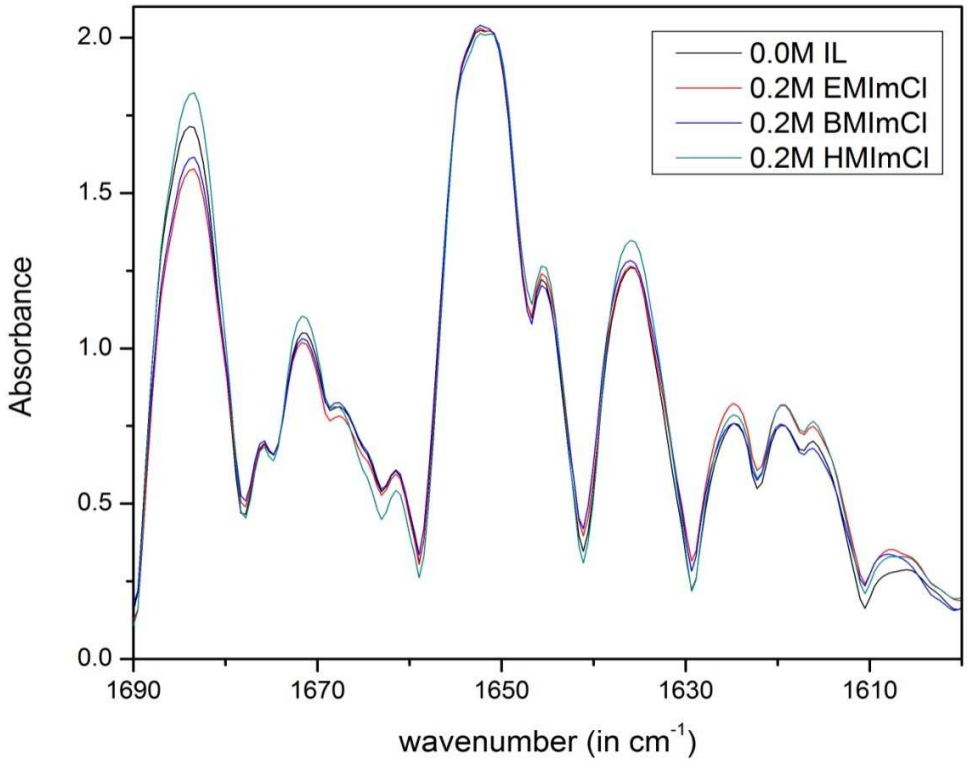


Figure 5: Comparison of Amide I band for lysozyme at 0.2 M of different ionic liquids.

In fig. 5, there are no significant changes observed in the absorbance intensity and the peak width against the ***α***-helical region (1665-1655 cm^{-1}) in different ionic liquids. Its FWHM (full width half maximum) is about 15 cm^{-1}, thus ***α***-helical region is found to be stable at 0.2 M concentration of different ILs. Whereas the ***β***-turn arising in between the range 1690-1665 cm^{-1} displays a slight change in the absorbance intensity confirming a minor conformational change. Furthermore, the peak around 1640-1630cm^{-1} suggests the existence of random coil. The significant increase in absorbance peaks of secondary structures suggests a change in composition from structural to unstructured region. And, consequently destabilizes the lysozyme.

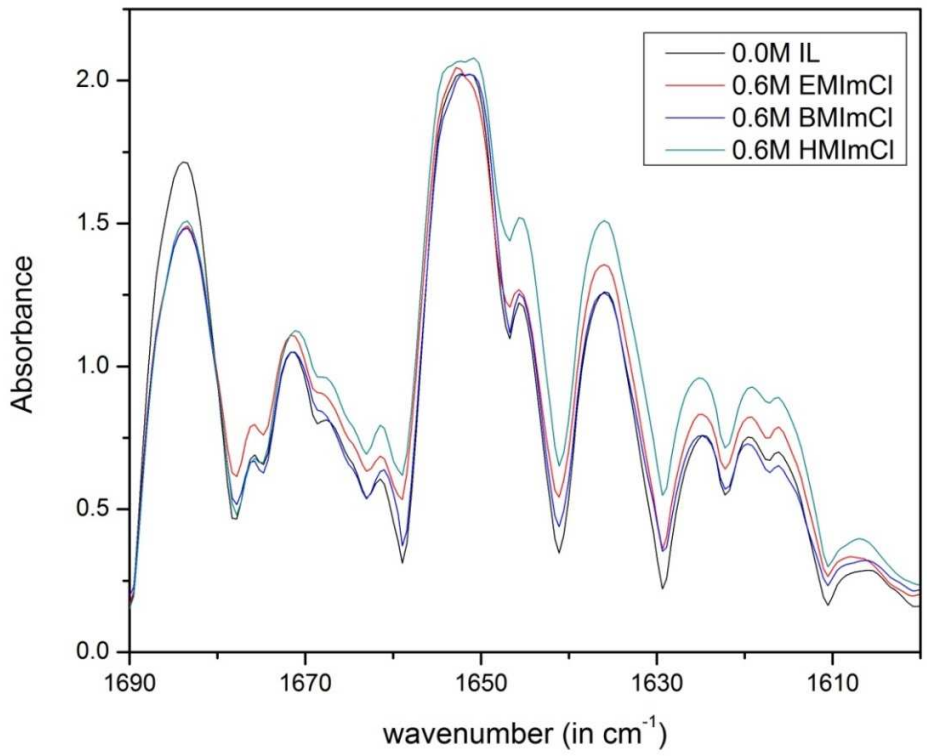


Figure 6: Comparison of Amide I band for lysozyme at 0.6 M of different ionic liquids.

In fig. 6, the spectrum corresponding to 0.6 M HMImCl is shifted a little towards right (1650 cm^{-1} compared to the 1652 cm^{-1} in the absence of IL). There is a significant increase in the intensity of the random coil at 1635 cm^{-1} for 0.6 M HMImCl. This suggests the transformation towards unstructured regions and hence, the protein gets destabilized or proceeds towards unfolded state. But in the $\boldsymbol{\alpha}$-helical region lysozyme at 0.6M HMImCl shows high absorbance and increment in the peak width, thus stability of lysozyme in 0.6 M HMImCl is reduced. On the other hand, the stability and structure of $\boldsymbol{\alpha}$-helix remain constant in 0.6M EMImCl and 0.6M BMImCl. The peaks around 1640-1633cm^{-1} leads to random coil followed by the $\boldsymbol{\beta}$-sheet in the wave numbers around 1630 and 1610cm^{-1}.

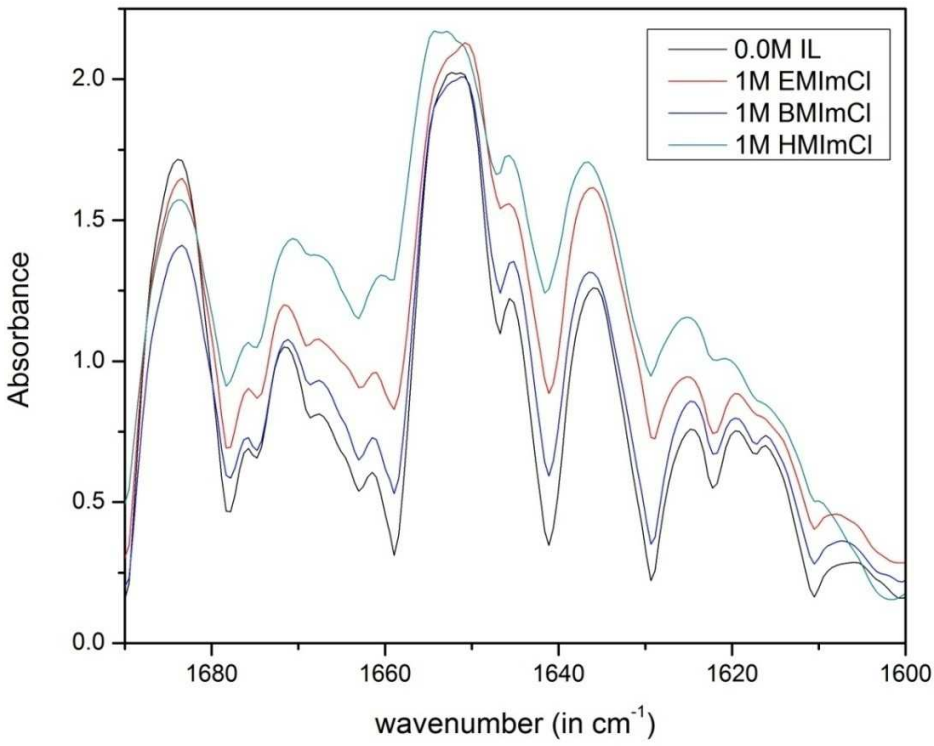


Figure 7: Comparison of Amide I band for lysozyme at 1.0 M of different ionic liquids.

In fig. 7 the peaks of 1.0 M HMImCl are shifted towards left. The ***α***-helix peak of 1 M HMImCl is shifted to 1654 cm^{-1} compared to 1651 cm^{-1} in the absence of IL and also the random coil peak of 1 M HMImCl is shifted to 1637 cm^{-1}compared to 1636 cm^{-1} in the native lysozyme. Again the peak corresponding to 1 M EMImCl is also shifted towards right. The ***α***-helix peak is shifted from 1651 to 1650 cm^{-1} as compared to lysozyme in the absence of IL and the random coil peak is also shifted a little from 1636 to 1637 cm^{-1} as compared native protein. The above datas indicates the transformations towards unstructured regions. Due to this reason lysozyme gets destabilized and still proceeds towards the unfolding state. Further the increment in peak width occurs in 1 M concentration at all the three different ILs, which also contribute towards the destabilization of lysozyme. On the other hand the stability and structure of ***α***-helix remain same in 1 M BMImCl as in lysozyme. Again the peaks around 1688 to 1659 cm^{-1} leads to ***β***-turn

showing the absorbance decrement as compared native lysozyme. There is also random coil and $\boldsymbol{\beta}$-sheet arises at 1636 and 1625 cm^{-1} respectively.

Hence, in conclusion, the changes in the amide I band could be attributed to the interaction between ionic liquids and the protein, which is obvious. In detail, the changes in the secondary structures of lysozyme are because of the increase in the alkyl chain lengths at the cationic moiety of different ILs. In our case, the ILs that is employed has different hydrophobicity which is as a function of alkyl chain length. Thus, the increase in hydrophobic behavior of the cationic group in ILs decreases stability of lysozyme.

3.1.2 Effect of Concentration:

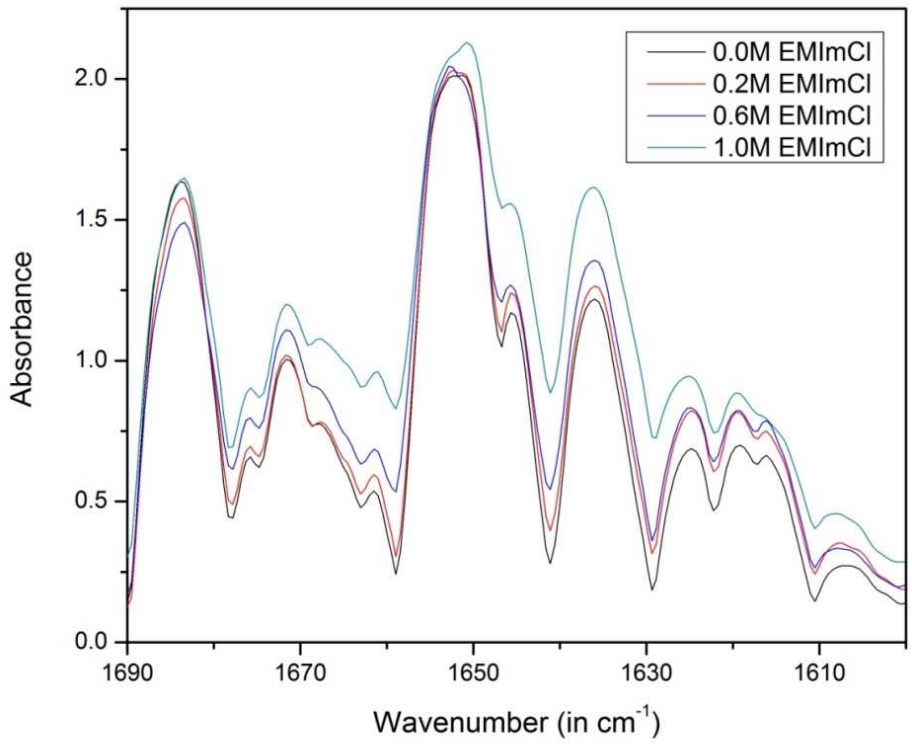


Figure 8: Comparison of Amide I band for lysozyme in EMImCl at different concentration.

At 1.0 M of EMImCl, the FWHM (full width half maximum) is more than 15 cm^{-1} for α-helical amide I band (1665 to 1655 cm^{-1}). This suggests the transformation of structured $\boldsymbol{\alpha}$-helix

region to unstructured random coil region or 3_{10}-helix structure; hence the lysozyme gets destabilized in 1 M EMImCl. Furthermore, the random coil appears in between 1641-1629 cm^{-1} with increase in absorbance intensity from 0.0 M EMImCl to 1 M EMImCl. But the FTIR band (1689-1658 cm^{-1}) for ***β***-turn shows a reverse trend (decreasing) in absorbance intensity with increase in concentration from 0.0 M EMImCl to 0.6 M EMImCl. Whereas, there is an increasing trend observed for ***β***-sheets between1628-1611 cm^{-1} region with increase in concentration from 0.0 to 1.0 M.

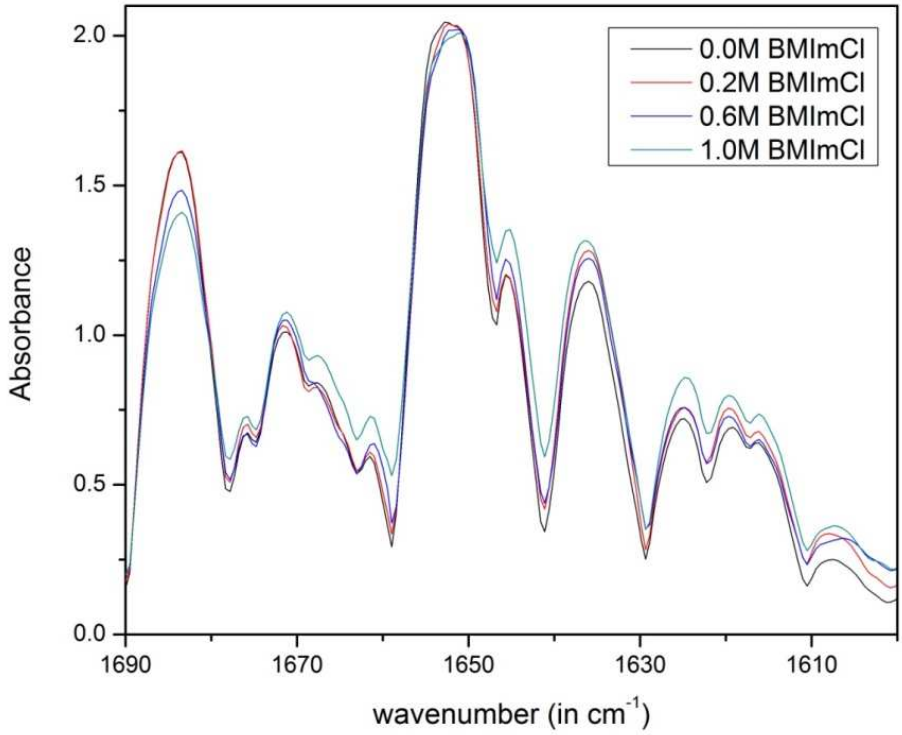


Figure 9: Comparison of Amide I band for lysozyme in BMImCl at different concentration.

In the case of BMImCl, there is a significant change observed for random coil and β-sheet absorbance intensities. In addition, there is a minor shifting of peak for α-helix towards random coil suggesting the transformation towards unstructured region.

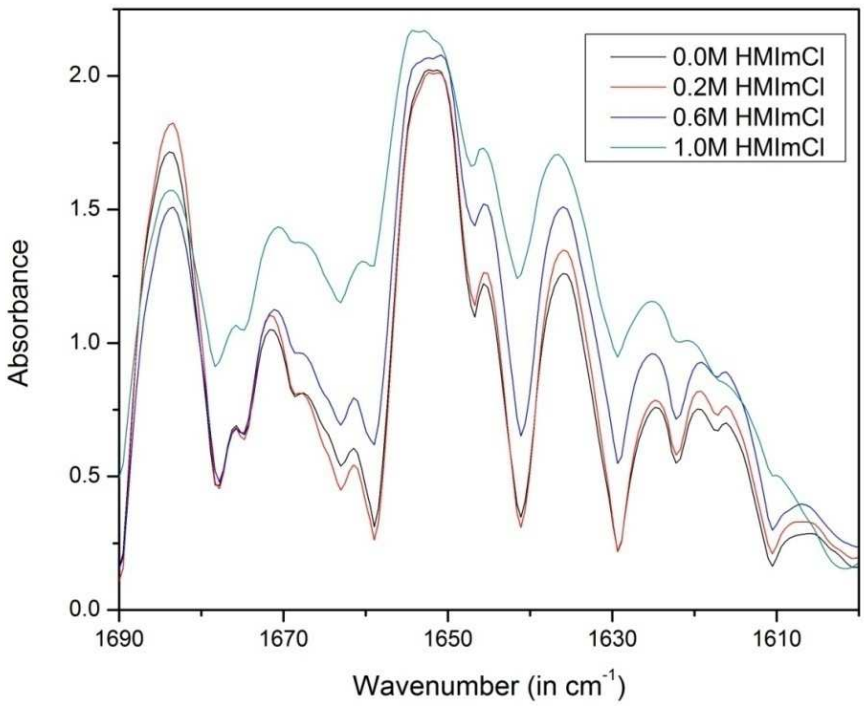


Figure 10: Comparison of Amide I band of lysozyme in HMImCl at different concentration.

There is an eye-catching observation observed in the case of 0.6 and 1.0 M HMImCl. At these concentrations, there is a shifting of α-helical band towards β-turn region as well increase in intensities suggesting the distortion of the helical moiety in to β-turn and gradually, towards destabilization and unfolding. As far as FWHM is considered, at all most all concentrations of HMImCl, there is an increasing trend observed as a function of concentration.

In a nutshell, irrespective of the types of ILs, the results reflect that the secondary structure of lysozyme gets distort gradually with increase in concentration. It is presumed that the destabilization or transformation towards more unstructured regions could be as a result of the breakdown of the hydrogen bonds involved with the backbone amide group and hydrophobic interactions that are responsible for the tertiary structure.

3.2 TEMPERATURE DEPENDANT ABSORPTION STUDY:

Melting temperature (T_m) of protein is actually the temperature at which there is existence of equilibrium between unfolded and folded structure. Usually, the aprotic ionic liquids consisting 1-Butyl-3-methylimidazolium cations and several kinds of anions affect the thermal stability of lysozyme. As a model protein, chicken egg-white lysozyme has been employed, since it is well investigated with respect to its structure, properties, functions, and thermostability [24, 25]. Melting temperature is a popular laboratory measurement to check the protein stability. T_m is directly related to protein stability. i.e., if T_m is more, then the protein is more stable and vice-versa.

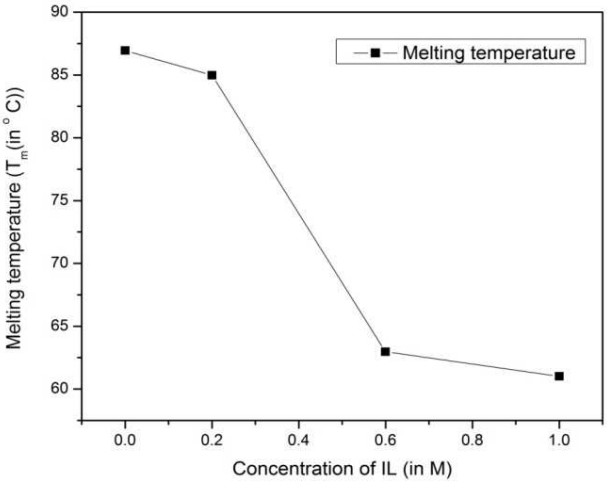


Figure 11: Melting temperature plot of lysozyme in different concentration of BMImCl**.**

As per the above figure (Figure 11), the melting temperature (T_m) of lysozyme is determined in different concentration of BMImCl. From the experimental observations and earlier reported values, T_m of lysozyme is 86.95^{o}C at room temperature [35]. Whereas, the observed Tm at 0.2 M, 0.6 M, and 1 M BMImCl is around 84.97, 62.97 and 61.02 °C, respectively. From the above trend, it shows that the Tm value decreases with increase in the concentration of BMImCl. This is because of the enhancement of bond breaking processes such as salt-bridges, hydrophobic interactions, hydrogen bond among the side chains of the amino acids in the presence of BMImCl.

4. CONCLUSIONS:

In this report the effect of aprotic ionic liquids on lysozyme structural stability as well as dynamics and the effect of water miscible ionic liquids on thermal stability lysozyme have been described. FTIR spectroscopy is basically a well established experimental technique for studying the secondary structural composition of structural dynamics of proteins. The only advantage of FTIR over other technique is convenience. Further the IR spectra can be obtained for protein in wide range of environments with a small amount of sample. It not only provides the knowledge about the content of the protein but also provides the information on protein structural stability and dynamics.

Again during the analysis thermal stability of lysozyme, different ionic liquids with which differ in their alkyl chain length present in the cationic part is responsible for lysozyme destabilization by lowering the melting temperature (T_m). Especially BMImCl exhibited the thermal stabilization effect on lysozyme at low concentrations. But mainly worked as a denaturant at high concentrations.

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