IMPACT OF IONIC LIQUIDS ON CONFORMATIONAL CHANGES OF BOVINE SERUM ALBUMIN - A FOURIER TRANSFORM INFRARED SPECTROSCOPIC STUDY

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By

GURUDAS CHAKRABORTY

ROLL NO.- 412CY2007

UNDER THE SUPERVISION OF

DR. HAREKRUSHNA SAHOO

DEPARTMENT OF CHEMISTRY

NATIONAL INSTITUTE OF TECHNOLOGY

ROURKELA-769008, ODISHA

CERTIFICATE

This is to certify that the dissertation entitled ***"Impact of Ionic Liquids on Conformational Changes of Bovine Serum Albumin - A Fourier Transform Infrared Spectroscopic Study"*** being submitted by Mr. Gurudas Chakraborty to the Department of Chemistry, National Institute of Technology, Rourkela, Odisha, for the award of the degree in Master of Science is a record of bonafide research carried out by him under my supervision and guidance. To the best of my knowledge, the matter embodied in the dissertation has not been submitted to any other University/ Institute for the award of any Degree or Diploma.

Dr. Harekrushna Sahoo

Assistant Professor

Department of Chemistry

National Institute of Technology

Rourkela, Odisha

Rourkela

Date:

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Last but not the least; I would like to express my deep sense of gratitude to my parents

and almighty for bestowing their blessings on me.

DECLARATION

I, Gurudas Chakraborty hereby declare that the dissertation entitled **"Impact of Ionic Liquids on Conformational Changes of Bovine Serum Albumin-A Fourier Transform Infrared Spectroscopic Study"** is the original work carried out by me under the supervision of Dr. Harekrushna Sahoo, Department of Chemistry, National Institute of Technology, Rourkela and the present work or any part of this work has not been presented in any other University or Institute for the award of any other degree to the best to my belief.

Gurudas Chakraborty

ABSTRACT

Ionic liquids (ILs) are low melting salts composed of an organic cation and an inorganic or organic anion. Ionic liquids are of interest for their wide range of applications and unique properties, such as the negligible vapor pressure of some types of ionic liquids, and the ability to modify ionic liquid properties by selection of the cation or anion. It has been hypothesized that over one million binary ionic liquids (meaning a single cation/anion pair) are possible. Due to the vast number of potential combinations, it should be possible to design ionic liquids specifically for an application of interest. Ionic liquids not only provide a novel and highly competent reaction medium, that is the solvent, but they also serve as efficient participants in a variety of chemical and biological reaction processes. However, there is a little understanding on how ionic liquids affect proteins. Here, we investigated the impact of different ionic liquids as a function of their alkyl chain length of the cationic moiety and concentrations on the conformational changes of the Bovine Serum Albumin (BSA) protein. In this work, we focused on the alternation in the secondary structures of the model protein. Fourier Transform Infrared (FTIR) spectroscopy is used to examine the changes in the secondary structure of BSA on varying concentration of a particular ionic liquid from 0 M to 1.0 M. It is observed that on increasing the concentrations of particular ionic liquid, structured regions are transformed to unstructured regions and thereby, assisting protein unfolding. Similarly, a gradual disruption of the native structure of BSA is observed with increase in the alkyl chain length of ionic liquids.

CONTENTS

CHAPTER-1

INTRODUCTION

1.1 Introduction to Protein:

The word protein is derived from a Greek word *proteios* meaning primary. Proteins are biomolecules and are composed of one or more chains of amino acids. They can also be defined as natural polymers or biopolymers in which the amino acids serve as the monomer units and are bonded to each other by a peptide bond. A peptide bond is formed between the carboxyl and amino groups of adjacent amino acid residues. Hence a protein is also known as a polypeptide which is responsible for a specific function in a living system. Proteins play a very vital role in all living organisms by performing a spectrum of functions *viz.* governing the biochemical reactions *i.e.* catalyzing the metabolic reactions, transporting molecules from one location to another, responding to stimuli, replicating DNA etc. For example, actin and myosin in muscle and the proteins in the cytoskeleton form a scaffold that maintains the shape of cell. Fibrin is responsible for blood clotting and so on. Proteins differ from one another primarily in their sequence of amino acids. Which is dictated by the nucleotide sequence of their genes, and which usually results in folding of the protein into a specific three dimensional structure that determines its activity. In general, the genetic code specifies 20 standard amino acids, however, in certain organisms the genetic code can include selenocysteine and in certain archae- pyrrolysine. Sometimes proteins have non-peptide groups attached, which can be called prosthetic groups or cofactors[1]. It is the combined effect of all the amino acid side chains in a protein that ultimately determines its three dimensional structure and its chemical reactivity. The amino acids in a polypeptide chain are linked by peptide bonds. Once linked in the protein chain, an individual amino acid is called a residue, and the linked series of carbon, nitrogen, and oxygen atoms are known as the main chain or protein backbone. Protein is generally used to refer to the complete biological molecule in a stable conformation, whereas peptide is generally reserved for a short amino acid oligomers often lacking a stable three dimensional structure. Polypeptide can refer to any single linear chain of amino acids, usually regardless of length, but often implies an absence of a defined conformation[2].

Most proteins fold into unique 3-dimensional structures. The shape into which a protein naturally folds is known as its native conformation[3]. Although many proteins can fold unassisted, simply through the chemical properties of their amino acids, others require the aid of

molecular chaperones to fold into their native states. There are four distinct aspects of a protein's structure:

- *Primary structure*: the amino acid sequence. A protein is a polyamide.
- *Secondary structure*: regularly repeating local structures stabilized by hydrogen bonds. The most common examples are the alpha helix, beta sheet and turns. Because secondary structures are local, many regions of different secondary structure can be present in the same protein molecule.
- *Tertiary structure*: the overall shape of a single protein molecule; the spatial relationship of the secondary structures to one another. Tertiary structure is generally stabilized by nonlocal interactions, most commonly the formation of a hydrophobic core, but also through salt bridges, hydrogen bonds, disulfide bonds, and even posttranslational modifications. The term "tertiary structure" is often used as synonymous with the term *fold*. The tertiary structure is what controls the basic function of the protein.
- *Quaternary structure*: the structure formed by several protein molecules (polypeptide chains), usually called *protein subunits* in this context, which function as a single protein complex.

Proteins are not entirely rigid molecules. In addition to these levels of structure, proteins may shift between several related structures while they perform their functions. In the context of these functional rearrangements, these tertiary or quaternary structures are usually referred to as "conformations", and transitions between them are called *conformational changes.* Such changes are often induced by the binding of a substrate molecule to an enzyme's active site, or the physical region of the protein that participates in chemical catalysis. In solution proteins also undergo variation in structure through thermal vibration and the collision with other molecules. Proteins can be informally divided into three main classes, which correlate with typical tertiary structures: globular proteins, fibrous proteins, and membrane proteins. Almost all globular proteins are soluble and many are enzymes. Fibrous proteins are often structural, such as collagen, the major component of connective tissue, or keratin, the protein component of hair and nails. Membrane proteins often serve as receptors or provide channels for polar or charged molecules to pass through the cell membrane[4].

1.2 Serum Albumin:

Serum Albumin, a very stable, highly soluble multi domain protein, is commercially available at high purity and low cost. It does not contain any prosthetic group or bulky appending carbohydrates. It is of elliptical shape with low intrinsic velocity. Albumin is a very stable protein although more than 50 human albumins exists which are slight variants of the 585 amino acid sequence. The structure of Human Serum Albumin (HSA) has been revealed by the high resolution X-ray image of the protein [5]. Since, serum albumin molecule can change its structure and conformation with variation in environmental conditions and with binding of ligands, therefore, this particular protein can be described as a very flexible protein. Besides this, albumin has a resilient structure and regains its shape easily owing to the disulphide bridges, which provides strength in physiological conditions[6].

Albumin is the most abundant extracellular protein. It contains total 60% of the total serum content in human. It is a single polypeptide with 585 amino acids having molecular weight of 66.2kD and is expressed in the liver. It differs from the extracellular proteins by its primary structure. Structurally, it has multiple cystein (Cys-34) and has two tryptophan. X-ray crystallography studies reveal that the secondary structure of serum albumin consists of approximately 67% of α-helix as well as there are 9 loops and 17 disulphide bridges giving a heart shaped 3D structure [5, 7]. The tertiary structure of serum albumin is composed of three domains I, II, and III, and each domain is constituted of two sub-domains A and B. Some of the most important and commonly studied proteins are Human Serum Albumin (HSA), Bovine Serum Albumin (BSA), Equin Serum Albumin (ESA) and Rat Serum Albumin (RSA). Albumin is responsible for the 75-80% of the osmotic pressure. It constitutes the main protein in the blood plasma. Therefore it can be considered as the COP gradient rather than the absolute plasma value. It determines the flow of fluid in and out of the capillaries [6]. It is responsible for the transportation of drugs and reduces the serum concentration of the compounds. Albumin has four different binding sites with varying specificity for different substances. It can be considered as the carrier of numerous exogenous and endogenous compounds in the blood [5, 7]. Albumin is a negatively charged protein present in high concentration in the plasma. It contributes heavily towards the “anion gap”. The concentration of anions and cations in plasma should be equal so that the remaining anions come predominantly from albumin, inorganic phosphate and

hemoglobin. Thus, when the concentration of albumin is high in blood plasma, the anion gap should be narrow[6].

1.2.1 Bovine Serum Albumin (BSA):

BSA is derived from cows and it is used as a standard for protein concentration. “Fraction V” is another name of BSA which refers to the fifth fraction of the original Edwin cohn purification methodology. This particular method is first used with human albumin for medical use and later it was used for the production of BSA.

Structure of BSA:

The primary structure of BSA is composed of 583 amino acid residues, the sequence has 17 disulphide bonds resulting in 9 loops (figure 1). It contains multiple cysteine and 8 pairs of disulphide bonds similar to HSA [5].

According to the amino acid sequence, the structure of BSA shows that it is composed of three homologous domains[10, 11]. The circular dichroism measurement shows that the secondary structure of BSA contains α-helix, β-sheet, turn and random coil with 48.7 %, 0%, 10.9% and 30.7% contribution respectively [8, 12].It has been suggested that the α-helices are uniformly placed in the sub-domains and in the connections between the domains. Most of the residues in the long loops (except at the end) and the regions linking the domains possibly form α-helices, whereas the intra-domain hinge regions are mainly non-helical structure. The three long helices in the sub-domain are considered as principle elements of the structure. These run parallel with each other, and a trough is formed owing to the middle helix being slightly lower in position. The helices are mainly linked together by disulphide bridges.

Properties of BSA:

The full length BSA protein has 583 amino acid residues[13].The N-terminal having 18 residual single peptide is cut off from the precursor protein on secretion, hence the initial protein product contains 589 amino acid residues. For the efficient expression and purification of a mature BSA protein (containing 585 amino acids), additional 4 amino acid is cleaved[14, 15].

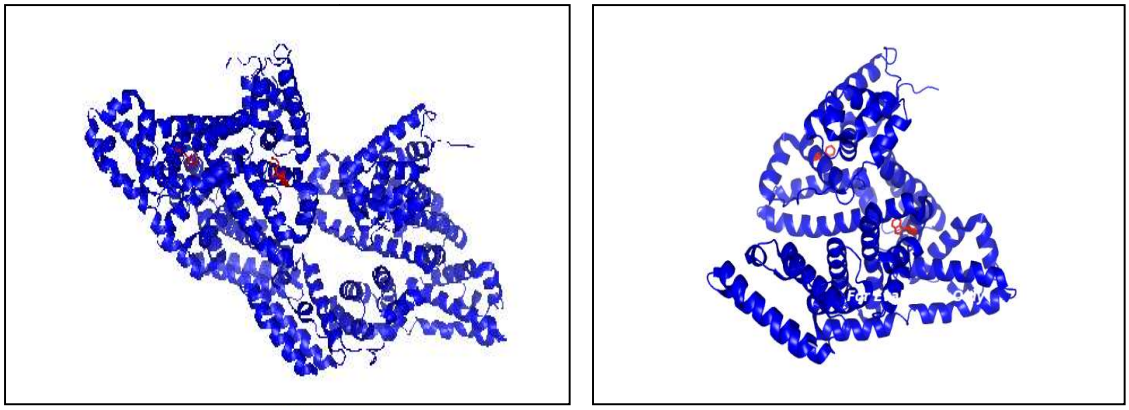


Figure 1: Crystal structure of BSA (PDB ID: 4F5S). Left panel represents the dimeric form where as right panel shows the monomeric form with the highlighted tryptophan (red in color).

Application of BSA:

- It has numerous biochemical application including ELISA (Enzyme Linked Immunosorbent Assay), immunoblots and immunohistochemistry. It is also used as a nutrient in cell and microbial structure.
- It can be used for the stabilizing of some enzymes during digestion of DNA and to prevent adhesion of the enzyme to the reaction tubes, pipet tips and to the other vessels.
- It is commonly used as a standard protein marker to determine the unknown quantity of other proteins by comparing with the known quantity of BSA. Because of its stability, it can also be used to increase the signal in assay. The other properties of BSA that made it a widely used protein are, it is less effective towards many biochemical reactions, cheaper in cost and large quantities of it can be readily purified from bovine blood[13].

1.2.2 Comparison of HSA and BSA:

HSA and BSA are the most commonly studied serum albumin proteins. Almost 76% homology and a repeating pattern of disulphide occur between HSA and BSA. The major difference between these two proteins is the number and position of tryptophan residues present. HSA has only one tryptophan, located at position 214 and it is equivalent to the tryptophan present in BSA at position 212 which is buried in a hydrophobic pocket at sub domain IIA[9]. BSA has two

tryptophan located at position 212 and 134, the later one is more exposed to solvent than that of trp-212 and is found at sub domain IB [6]. Because of the medical importance, low cost, ready availability, and unusual ligand binding properties, BSA is used as the model protein [5, 8].

1.3 Ionic Liquids:

Ionic liquids can be defined as ionic compounds composed of an organic cation and an anion that can be organic or inorganic [16]. Till date it is known that ionic liquids have a melting point less than 100°C. Many ionic liquids have a melting point lower than room temperature (so-called room temperature ionic liquids – RTILs) making them useful as solvents [19, 20]. Ethylammonium nitrate is the first credited ionic liquid discovered by Gabriel and Weiner's in 1888 [17, 18]. It has a melting point of 52°C to 55°C, ethylammonium nitrate was the first recorded organic salt with a melting point less than 100°C [17, 18]. Ethylammonium nitrate, the first RTIL was prepared by Walden and reported in 1914. In 1997 Seddon reported that ionic liquids can be used as promising tools for clean technology[21]. The reason behind the subsequent upsurge in ionic liquid research is The *Chemical & Engineering News* article in 1998, "Designer solvents-Ionic liquids may boost clean technology development", which highlighted the environmentally friendly chemical processes that can be achieved using ionic liquids [22].

As ionic liquids are composed of cations and anions, ionic liquids have dual functionality and the ability, through careful selection and design of each ion, to dissolve disparate materials in a single solvent [18]. It has been estimated that over a million different binary ionic liquids, each with a particular pairing of cation and anion, are possible. The variety of ionic liquids has led to a wide range of applications taking advantage of their useful properties. One exclusive and significant property is that aprotic ionic liquids have negligible vapor pressure until decomposition, which facilities their handling and means that aprotic ionic liquids cannot evaporate [18, 23]. Ionic liquids have also received attention as "green" solvents as they are usually non-flammable and it is also possible to recycle those [18, 24, 25]. However, ionic liquids are frequently made from toxic compounds, so their "greenness" is always discussed with respect to their function and what solvent they may replace [25, 26]. Much work has been performed to prepare ionic liquids from environmentally benevolent compounds such as choline and amino acids, which opens the door to non-toxic and potentially truly green ionic liquids [27-31].

1.3.1 Applications of Ionic Liquids:

Ionic liquids have been used in a number of research fields. The different research areas in which ionic liquids have been used include carbon dioxide capture,[32] organometallic catalysis,[33] electrochemistry,[34] nanoparticle synthesis,[35-36] gas chromatography,[37, 38] and even as solutions for enzyme catalysis[39]. The solution thermodynamics of ionic liquids with water and alcohols,[40-42] and gases [43, 44] have also been examined. Ionic liquids have also been used as solvents for aluminophosphate molecular sieve synthesis [45-52]. Researchers have effectively prepared both already known and novel crystal structures in ionic liquids [45-56].

During the ionothermal molecular sieve research it was documented that water plays less of a role in the synthesis of molecular sieves than expected by water's behavior in other solvents [49, 57]. The "water deactivating" effect is due to the lack of chemical activity of water to the degree expected by its concentration[57]. The "water deactivating" effect has been described as the molecular dispersion of the water because of water's strong nucleophilic attraction to the anion of the ionic liquid [49]. This dispersion reduces the hydrolysis activity of the water, effectively deactivating its ability to participate in reactions [49]. This phenomenon has been used to prepare compounds in ionic liquids that would otherwise be unstable in the presence of catalytic amounts of water, such as phosphorus trichloride [58, 59]. The flexibility of ionic liquids because of their wide range of cations and anions is important to their versatility.

1.3.2 Ionic Liquids in Protein Assay:

The increase in use of ionic liquids to perform protein assays is due to the fact that ionic liquids not only provide a novel and highly efficient reaction medium but they also serve as efficient participants in the various chemical / biological reaction processes [60].

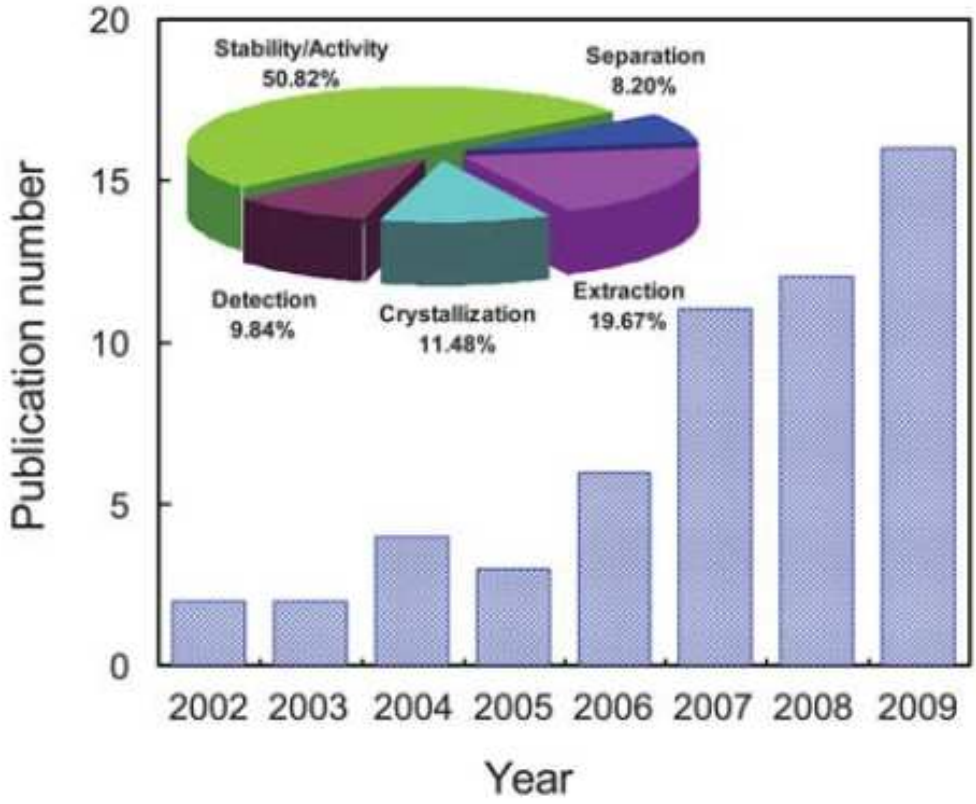


Figure 2: The number of publications per year concerning the applications of ionic liquids related to protein assays in the last decade.(Source of information: ISI Web of Knowledge).

1.4 Aim of the Present Work:

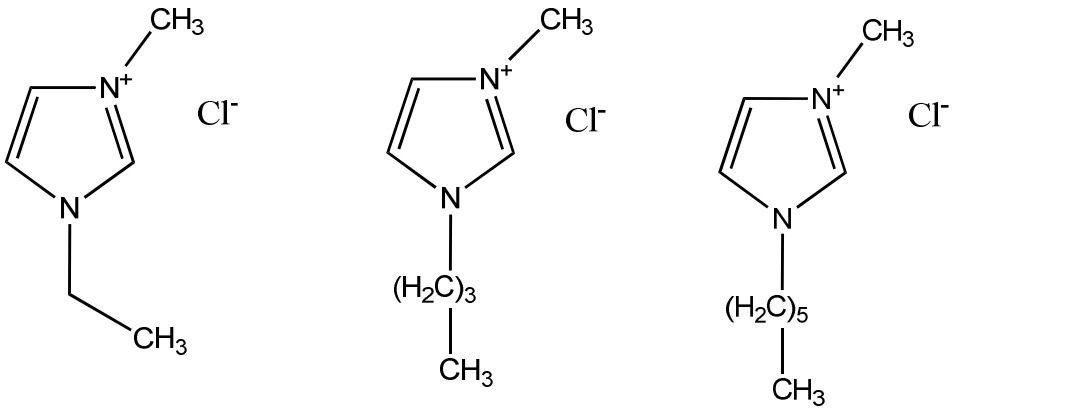
The main objective of this research work is to investigate the impact of different ionic liquids as a function of their alkyl chain length of the cationic moiety and concentrations on the conformational changes of the Bovine Serum Albumin (BSA) protein. Fourier Transform Infrared Spectroscopic technique is used to monitor the changes in the secondary structures of the model protein in presence of the proposed ionic liquids.

CHAPTER-2

MATERIALS AND METHODS

2.1 Materials:

Here in the proposed work Albumin from bovine serum (BSA) (Pcode-1001324686, Sigma-Aldrich) is used. The commercially available protein was used without further purification. 1-Ethyl-3-methyl-imidazolium chloride (EMImCl)(Pcode-101087489, Sigma- Aldrich), 1-Butyl-3-methylimidazolium chloride (BMImCl)(Pcode-101203951, Sigma- Aldrich) and 1-Hexyl-3-methylimidazolium chloride (HMImCl)(Pcode-101306345, Fluka) are used to study the conformational changes in BSA protein.



1-Ethyl-3-methylimidazolium chloride 1-Butyl-3-methylimidazolium chloride 1-Hexyl-3-methylimidazolium chloride

Figure 3: Structures of the Ionic Liquids Used in This Project.

PREPARATION OF 0.1 M SODIUM PHOSPHATE BUFFER SOLUTION

In order to prepare 100 ml of 0.1 M sodium phosphate buffer solution, 10 ml of buffer solution was prepared by mixing 7.7 ml of 1M disodium hydrogen phosphate (Na_2HPO_4) with 2.3 ml of 1 M sodium dihydrogen phosphate (NaH_2PO_4). Then 90 ml of distilled water was added to it in order to maintain the volume in the volumetric flask. A pH of 7.4 was maintained.

PREPARATION OF BSA STOCK SOLUTION

0.0005 M of BSA stock solution was prepared by dissolving 0.0825 gm of BSA in 2.5 ml of freshly prepared buffer solution. The protein solution was allowed to mix homogeneously. The protein solution which was prepared was allowed for degassing in a sonicator in order to remove the undesirable gases from the solution which may interfere during spectral analysis.

PREPARATION OF IONIC LIQUID (IL) STOCK SOLUTION

4 ml of 1 M stock solutions of the three ILs were prepared by measuring 0.58648 g, 0.69868 g and 0.81088 g of EMImCl, BMImCl and HMImCl followed by the addition of required amount of buffer solution as solvent medium. Then further dilution was done to get two more concentrations (i.e. 0.2 M, 0.6M).

PREPATION OF SAMPLE SOLUTIONS (PROTEIN WITH IONIC LIQUIDS)

The sample solutions (3 ml) i.e. the protein in different concentrations of ionic liquids were prepared by dissolving of 0.5 ml freshly prepared protein in 2.5 ml of each IL.

2.2 Methods:

Fourier Transform Infrared Spectroscopy is used for the analysis of the protein secondary structures.

Working procedure:

For 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.

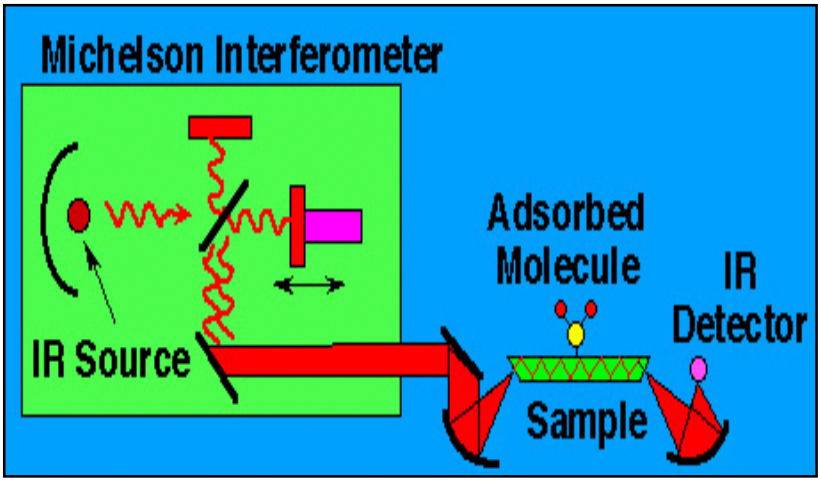


Figure 4: Schematic representation of Fourier Transform Infrared Spectroscopy.

Infrared spectroscopy is the study and characterization of modes of vibration of bonds between two atoms in a molecule using electromagnetic radiation between 10 and 14000 cm^{-1}. The infrared region is broadly divided into three regions:

- The near IR region (14000 to 4000 cm^{-1})
- The mid IR region (4000 to 400 cm^{-1})
- The far IR region (400 to 10 cm^{-1})

FTIR spectroscopy is a measurement of wavelength and intensity of the absorption of IR radiation by a sample. 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}.

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 hydrogen. 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. Each secondary structure has a different and unique, peak location.

Table 1 List of Amide bands in mid-infrared region

Designation	Approximate frequency (cm-1)	Description
Amide A	3300	N-H stretching
Amide B	3100	N-H stretching
Amide I	1690 – 1600	C=O stretching
Amide II	1575 – 1480	C-N stretching, N-H bending
Amide III	1300 – 1230	C-N stretching, N-H bending
Amide IV	770 – 625	O-C-N bending
Amide V	800 – 400	Out-of-plane N-H bending
Amide VI	610 – 530	Out-of-plane C=O bending
Amide VII	200	Skeletal torsion

Table 2 Infrared spectroscopy Amide I locations of secondary structure of proteins

Secondary structure element	Band position (cm-1)
β-turn	1690-1665
α-helix	1665-1655
Random coil	1655-1645
β-sheet	1645-1610

The advantage of FTIR over other techniques is that spectra can be obtained for proteins in a wide range of environments, requiring less time and sample and direct correlations between the IR amide I Band frequencies and the secondary structure components can be found.

CHAPTER-3

RESULTS AND DISCUSSION

The relative contributions of different types of secondary structures in proteins are known to be estimated quantitatively from their amide I spectra in solution by several methods. The different methods include FSD- curve fitting, second derivative analysis, partial least- squares analysis and data basis analysis. The FSD- curve fitting and second derivative analysis are the two most extensively used methods. Here, we have used the second derivative analysis for investigating the relative contributions of different types of secondary structures in BSA protein in presence and absence of different aprotic ionic liquids. The experimental observations are analyzed in two different ways, i.e., effect of (i) concentration of different ILs and (ii) alkyl chain lengths.

3.1 Effect of Concentration:

In order to study the effect of concentration, we employed three different ILs with variations in concentrations from 0 to 1 M (0.2, 0.6 and 1.0 M). Figure 5, 6 and 7 show the concentration-dependency for different ILs.

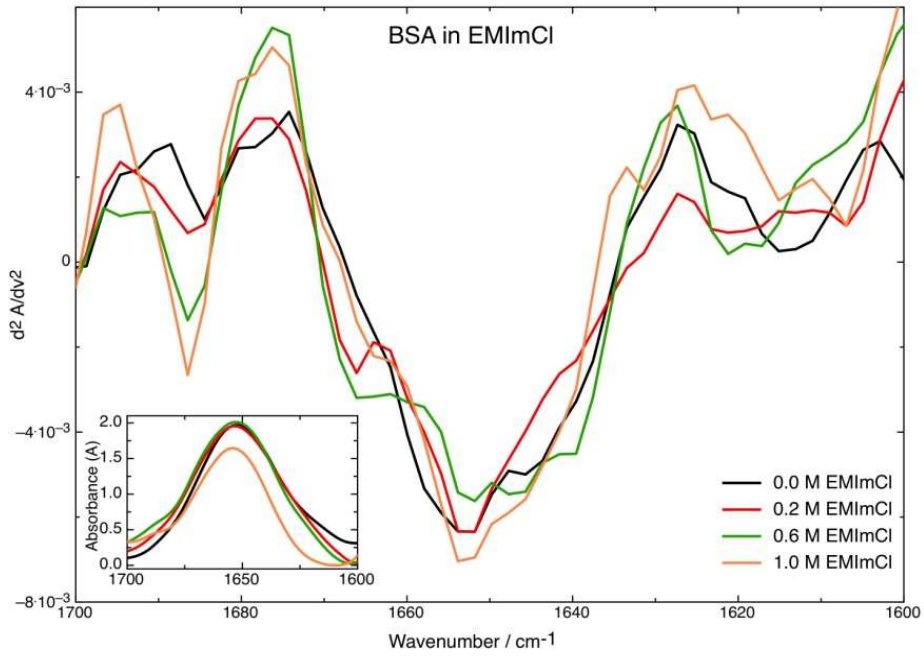


Figure 5: Effect of EMImCl concentrations on the secondary structure of BSA. Inset: FTIR (absorbance vs wavenumber) spectra showing the variation of concentration.

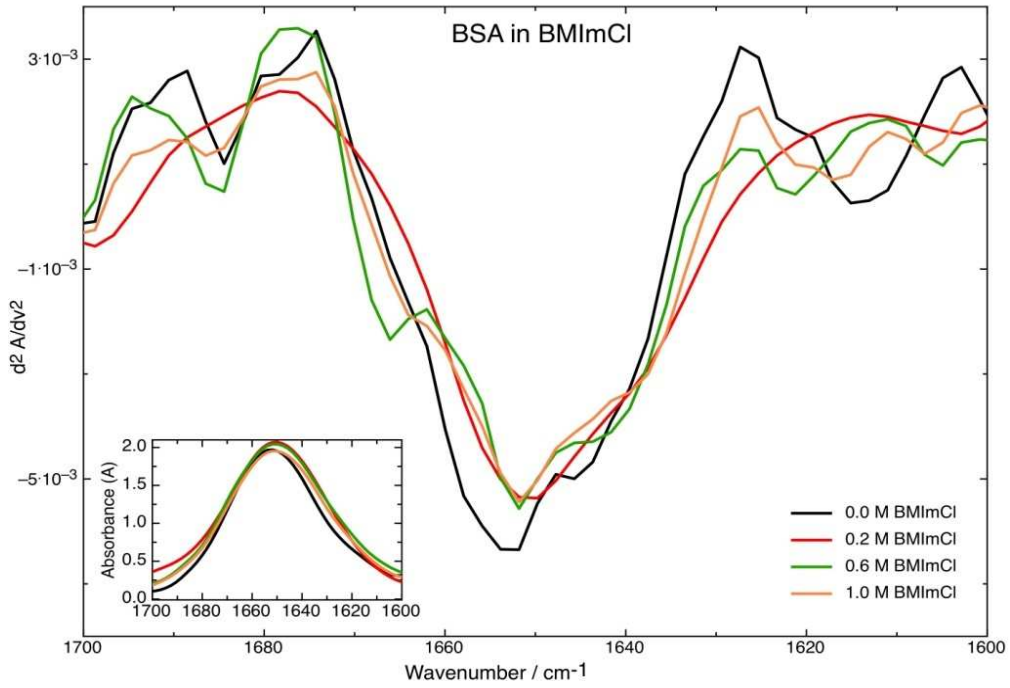


Figure 6: Effect of BMImCl concentrations on the secondary structure of BSA. Inset: FTIR (absorbance vs wavenumber) spectra showing the variation of concentration.

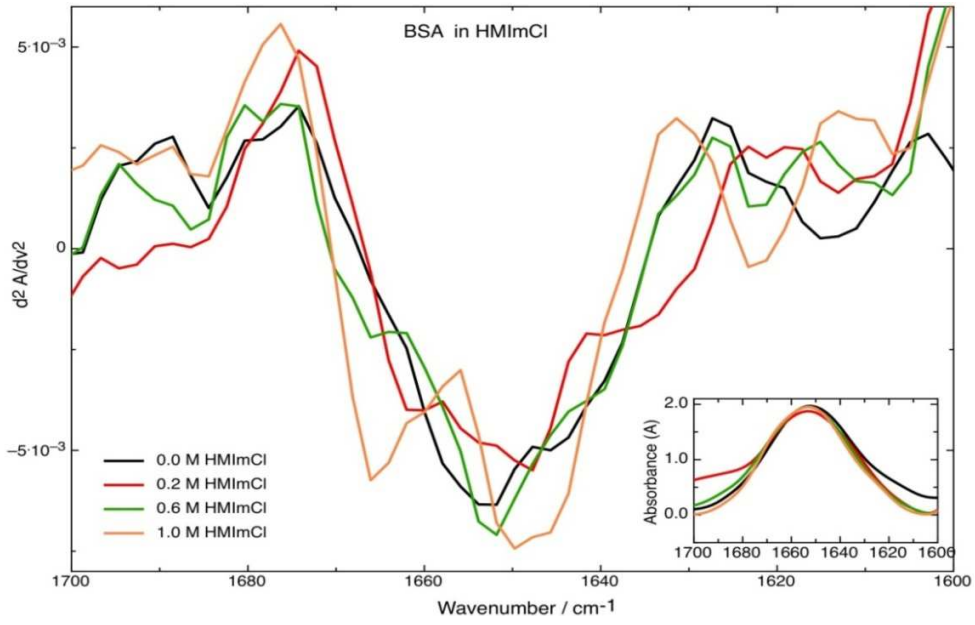


Figure 7: Effect of HMImCl concentrations of on the secondary structure of BSA. Inset: FTIR (absorbance vs wavenumber) spectra showing the variation of concentration.

The processed FTIR spectra (second derivative analysis) open up the relation between secondary structure conformational changes in BSA and ionic liquid properties, i.e., on increasing the concentrations of ionic liquids the corresponding vibration bands for α-helix, β-sheet, random coil and β-turn undergo an alternation (either increases or decreases or shows a shifting towards either of the directions). In all of the above cases, there are three distinct peaks (α-helix at 1660-1650 cm^{-1}, β-turn at 1690-1665 cm^{-1} and β-sheet at 1635-1610 cm^{-1}) observed with two more additional peaks (random coil at 1650 – 1640 cm^{-1} and 3_{10} helix at 1665 cm^{-1}) in the form of shoulders to the main peak. In case of EMImCl (figure 5), at higher concentration (1.0 M), percentage of β-turn is more compared to that of in the absence. Similarly, a peak at 1665 cm^{-1} is observed showing the appearance of 3_{10} helix in the presence of EMImCl, which increases as a function of concentration. The existence of 3_{10} helix leads to the distortion of α-helix. This shows that on increasing the concentration of EMImCl from 0 to 1.0 M, contribution of unstructured regions overshadows the structured conformations leading towards the destabilization of BSA. Similar types of observations are found in case of BMImCl (figure 6) as a function of increasing concentrations. On the other hand, the observations that are recorded in the case of HMImCl (figure 7) is slightly different compared to EMImCl and BMImCl. In case of HMImCl, the existence of 3_{10} helix peak is much more intense than the other two employed ILs at higher concentrations. At 1.0 M HMImCl, besides of having high population from the 3_{10} helix 'so called distorted α-helix', there is a huge shifting of the α-helix towards random coil region. This is the direct evidence in favor of destabilization of the native structure of BSA in presence of ILs.

Hence, on increasing the concentrations of particular ionic liquid, structured regions are transformed to unstructured regions and thereby, assisting protein unfolding. This can be attributed to the fact that with increase in concentrations more number of molecules are available for interaction with the protein resulting into the disruption of the native state. Therefore, increase in ILs concentrations leads to the breaking of several bonds, i.e., hydrogen bonding among the side chains of amino acids, salt-bridges, van der Waals interactions, hydrophobic interactions, that play an important role in forming the tertiary or native structure of protein.

3.2 Effect of Alkyl Chain Length:

In order to study the effect of alkyl chain length attached to the cationic moiety of the ionic liquids on the conformational changes of BSA, we used three different ILs i.e. 1-Ethyl-3-methylimidazolium chloride, 1-Butyl-3-methylimidazolium chloride and 1-Hexyl-3-methylimidazolium chloride. The following figure highlights the changes in the secondary structure of BSA due to increase in the hydrophobic nature in terms of the alkyl chain length of the ionic liquids.

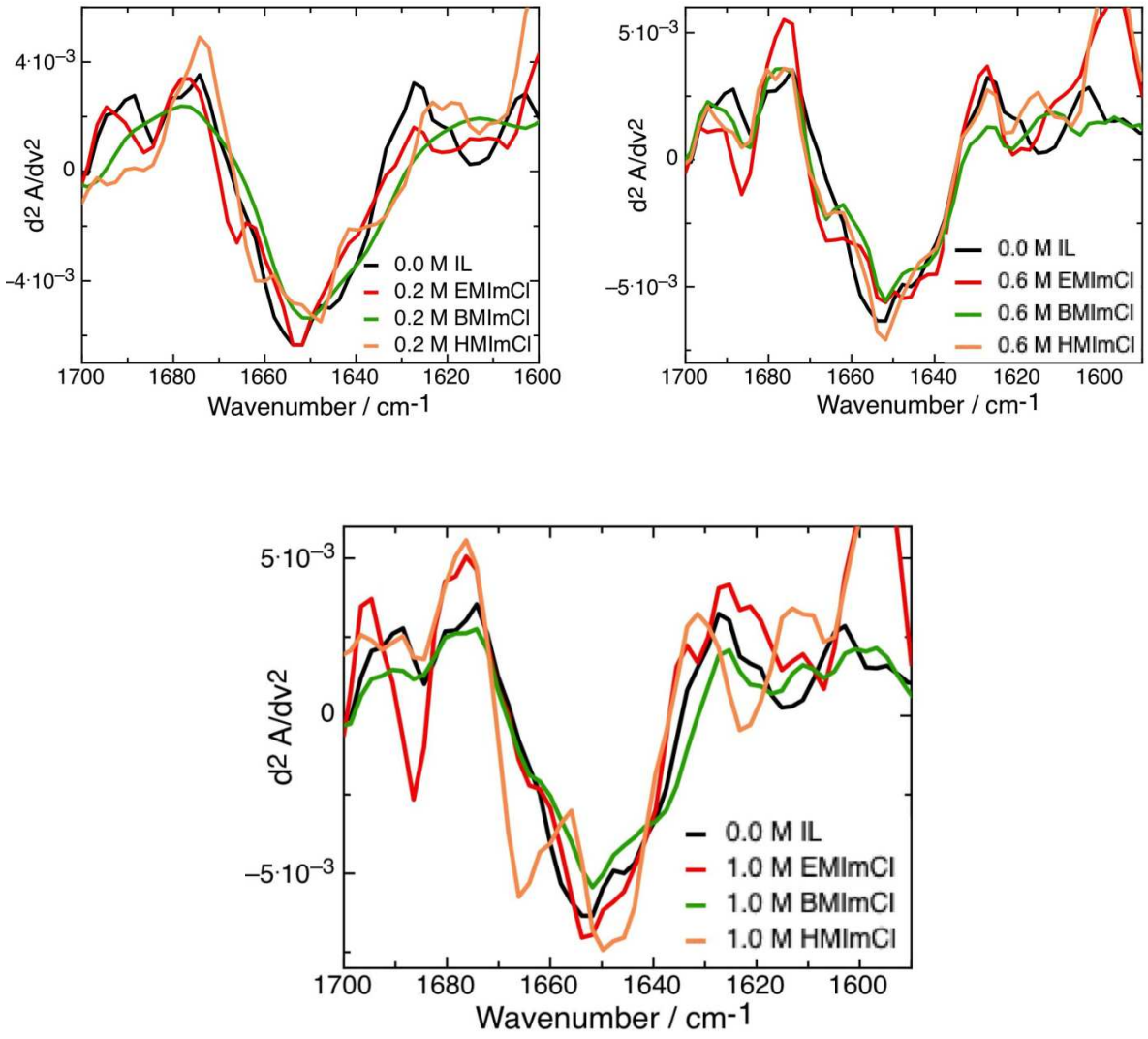


Figure 8: Effect of alkyl chain lengths (EMImCl, BMImCl and HMImCl) at different concentrations on the secondary structures of BSA.

In order to unleash the effect of hydrophobic groups on the protein secondary structures, cationic moiety was modified with different hydrophobic groups (ethyl, butyl and hexyl) on keeping the anionic group constant to nullify the effect from the anion. At 0.2 M ILs (figure 8), it is observed that there is a shifting in α-helical band towards random coil for BMImCl and HMImCl. As well, existence of 3_{10} helical structure is prominent in the presence of HMImCl. On increasing the concentration to 0.6 M from 0.2 M does not bring any significant change. Whereas, at 1.0 M ILs, HMImCl displays the strongest effect among other ILs employed. There is an appearance of 3_{10} helix, which is very much intense compared to others. Along with, band shifting is observed for α-helical conformation from 1655 to 1650 cm^{-1} towards random coil region. The changes in the secondary structures of BSA are attributed to non-polar interactions between the alkyl chain of the ionic liquids and the protein backbone as well as side chains. The changes in the secondary structures seemed to be more significant in case of ionic liquids with higher chain lengths i.e. HMImCl and BMImCl in comparison to EMImCl, because of increase in the hydrophobic nature of the alkyl chains.

CONCLUSION

The present work based on FTIR study revealed the impact of ionic liquids on BSA secondary structures. From the experimental observations, it is concluded that BSA secondary structures depend on the concentration as well the nature of the substituent attached to the cationic moiety, which differs among themselves with their hydrophobicity. The results suggest that different forces such as hydrophobic, electrostatic and hydrogen bonding interactions are governing the tertiary structure of BSA and gets disrupted in the presence of ILs, where the intensity of disruption depends on the nature (hydrophobicity) and physical properties (concentration of IL). Also, at lower concentrations and alkyl chains with lower hydrophobicity, the specific interactions partially get affected compared to that of higher concentrations as well as higher alkyl chain lengths with higher hydrophobic nature.

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