Molecular interaction on imidazolium based ionic liquids and serum albumin: A spectroscopy approach

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Abstract

Interaction between globular proteins with imidazolium based ionic liquids (ILs) is extremely significant considering that of the vast use of ILs, since protein stabilizer in the current years. The present work, the interaction of human serum albumin (HSA) and bovine serum albumin (BSA) with 1-butyl-3-methylimidazolium octylsulphate (BmimOS) and 1-decyl-3-methylimidazolium tetrafluoroborate (DmimBF$_4$) have been examine the use of fluorescence and FTIR. Fluorescence spectra of HSA/BSA are extinguished by BmimOS /DmimBF$_4$ ILs by way of the dynamic method. The various thermodynamic parameters were revealing that very susceptible interactions exist between HSA/BSA and BmimOS /DmimBF$_4$. The conformational adjustments of HSA/BSA were observed by means of FTIR analysis. Fluorescence methods were completed to find out about the thermal balance of HSA/BSA at different temperature. The thermal balance of BSA in the presence of ILs follows the style BmimOS/DmimBF$_4$ and presence of extra hydrophobic IL, decay increases swiftly as a characteristic of concentration.

Keywords: *FTIR; Fluorescence; Ionic Liquids; Serum Albumin; Complexation.*

Graphical abstract

Interaction of human serum albumin (HSA) and bovine serum albumin (BSA) with 1-butyl-3-methylimidazolium octylsulphate (BmimOS) and 1-decyl-3-methylimidazolium tetrafluoroborate (DmimBF$_4$) have been study by fluorescence and FTIR. Fluorescence of HSA/BSA is quenched by (BmimOS)/ (DmimBF$_4$) ILs with the steady state fluorescence method.
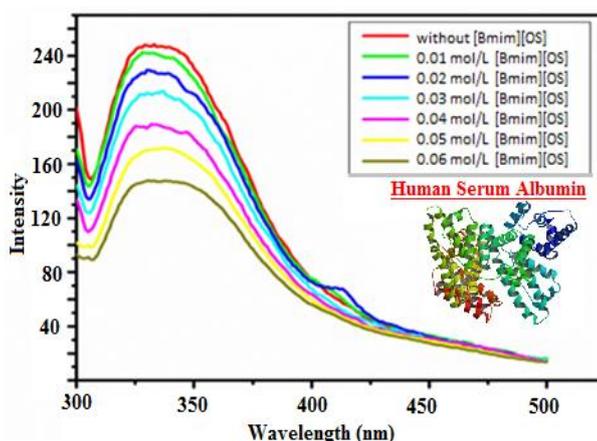

1. Introduction

Effect of proteins on the ionic liquids (ILs) provides important information about interactions between them which can change the secondary or tertiary structure of protein or denature them, thereby modifying the function of the molecule (Venkatesu et al. 2012, Venkatesu et al. 2014, Weingartner et al. 2012, Zhao et al. 2005). When a protein is denatured, the characteristic 3D arrangement is disorder (the protein unfolds) and as a result, its biological function is lost. Describing is an abrupt method that receive place within a partial variety of environment (Steinhauser et al. 2011). The abruptness of the unfolding transition indicates a cooperative process (Garab et al.

2001). Protein-ILs systems have manifold applications in food industry, pharmaceutical industry and analytical biochemistry (Crevenna et al. 2012, Coutinho et al. 2012, Figueiredo et al. 2013, Shao et al. 2013, Perssion et al. 2012).

ILs are the combination of two cation and anion, have emerged as the capable solvents that have captured special attention from biotechnology and biophysical chemistry (Pal et al. 2015, Chai et al. 2015, Cummings et al. 2015). ILs represents an exciting novel class of co-solvents for biochemical process that include protein stability. The present study is contributed to improve considerate of function of the molecular structure of ILs in protein-ILs interactions and as a result, in the behaviour of ionic ILs since denaturants (Schroder et al. 2016, Sairi et al. 2014, Roy et al. 2016).

Kumar et al. 2012, studied the structural modify of bovine serum albumin (BSA) and 3-methyl-1-octylimidazolium chloride and 1-butyl-3-methylimidazolium octylsulfate have been studied by spectroscopic methods. The interactional behaviour of ILs toward BSA in diverse ILs concentration systems at the air/solution interface and the bulk is study. CD mutual with the fluorescence provided expensive nearby into the unfolding of BSA as a result of IL binding. Patel et al. 2016, studied the interaction among N, N-dimethyl-2-oxopyrrolidinium iodide and BSA by fluorescence, UV-vis and FTIR in mixture among molecular docking method. The FTIR results confirm the conformational modify of BSA on binding with N, N-dimethyl-2-oxopyrrolidinium. Patel et al. 2017, studied the interaction between N-butyl-N-methyl-morpholinium bromide, [Mor1,4] [Br] IL with human serum album (HSA). The result was analyzed by CD spectroscopic, UV-vis, fluorescence, and molecular docking method. The results show that [Mor1,4] [Br] has a binding site on HSA and bind in the hydrophobic pocket of sub domain IIA of HSA. It was observed that [Mor1,4] [Br]IL maintain native conformation of HSA positive to definite concentration variety.

In our earlier work (Ghosh et al. 2018, Ghosh et al. 2016), we successfully deal with the interaction between globular protein with carbon quantum dot and surfactants. Herein, the present investigated the molecular interaction between ionic liquids i.e., 1-butyl-3-methyl - imidazolium octyl sulfate (BmimOS) and 1-decyl-3-methylimidazolium tetrafluoroborate (DmimBF$_4$) and serum albumins i.e., human serum albumin (HSA), bovine serum albumin (BSA) by fluorescence and FTIR spectroscopy. Gibbs free energy changes of ILs and serum albumin has also been calculated. The present works give precious information concerning the mechanism of interaction of ILs and serum albumins. Furthermore, the current research supply as a important for improved considerate of the vital behaviour of ILs in industrial application. Molecular structures of ionic liquids, human serum albumin and bovine serum albumin are shown in Scheme1.

1-Butyl-3-methylimidazolium octylsulphate   1-Decyl-3-methylimidazolium tetrafluoroborate
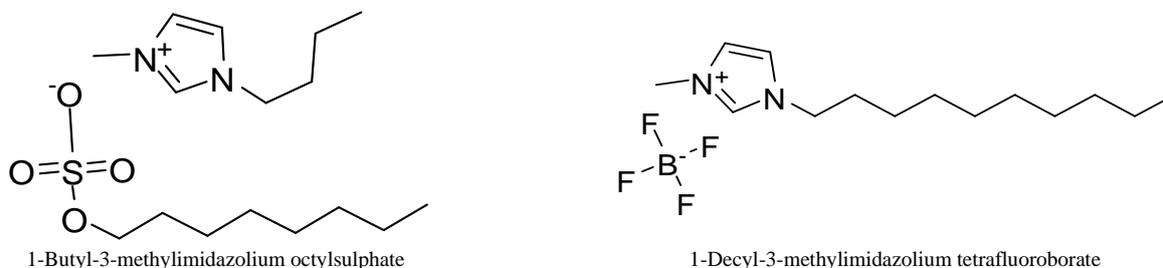
Scheme 1: Molecular Structure of Ionic Liquid 1-Butyl-3-Methylimidazolium Octylsulphate and 1-Decyl-3methylimidazolium Tetrafluoroborate.

2. Experimental and methods

2.1. Materials

1-Butyl-3-methylimidazolium octylsulphate, 1-decyl-3methylimidazolium tetrafluoroborate, human serum albumin and bovine serum albumin, potassium bromide was obtained sigma Aldrich Pvt. Ltd.

2.2. Fluorescence spectrometry

Fluorescence spectra of two BSA/HSA with changed concentration of ILs i.e., 1-Butyl-3-Methylimidazolium Octylsulphate (BmimOS) and 1-Decyl-3methylimidazolium Tetrafluoroborate (DmimBF$_4$) were recorded at 295K, 298K and 305K temperature in the wavelength range between 200-500 nm.

2.3. FT-IR spectrometry

FT-IR spectra were performed on a Nicolet iS10 FTIR (Thermo Fisher) by KBr pellets. The FTIR data of HSA and BSA during the existence and deficiency of ILs at 298K were study in the range of 4000-400 cm^{-1}. The FT-IR spectra of serum albumins and HSA-ILs, BSA-ILs structure are evidence during the range of 4000-400 cm^{-1}.

2.4. pH meter

pH learn was prepared with a crucial pH meter (pH 700 EUTECH instrument).

3. Results and discussion

3.1. Impact of ionic liquids on BSA/HSA

Basic fluorescence of globular albumins is mostly endorsed in single-handed tryptophan residue[23] and fluorescence quenching efficiency of albumins by other molecules was broadly studied by fluorescence quenching method (Xiao et al. 2016, Kanvah et al. 2019). The present investigation in molecular interaction between ILs [BmimOS]/ [DmimBF$_4$] and HSA and BSA, the influence of ILs at different concentration by using steady-state fluorescence and observed result are shown in Fig. 1. Serum albumin was showed the tough fluorescence emission under excitation wavelength of λ_{max} = 340 nm (Ghosh et al. 2018, Ghosh et al. 2016). Furthermore, [BmimOS]/ [DmimBF$_4$]

emitted nearly no fluorescence spectra below the same surroundings, which suggested that ILs might not affect the fluorescence belongings of serum albumins on all. Provisional, the basic fluorescence intensity of serum albumins was quenched regularly through the raise of the attentiveness of ILs which shows that ILs quenched the intrinsic fluorescence of albumins mainly during concentration dependent relative behaviour. The current result approved with the Stern-Volmer early linearity at higher concentrations of ILs.
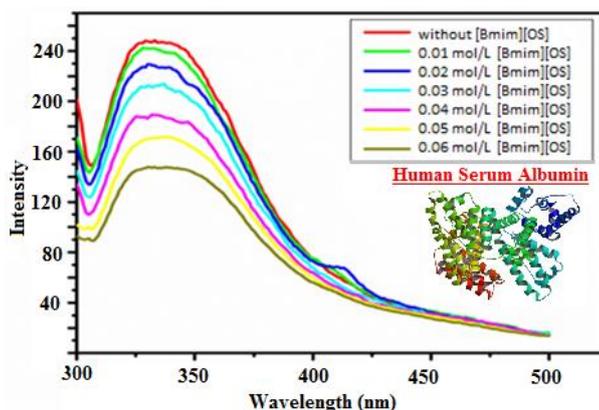
Fig. 1: Fluorescence Spectra of Human Serum Albumin into the Incidence of Different Concentration of 1-Butyl-3-Methylimidazolium Octylsulphate [Bmimos].

Steady static quenching mechanism is generally order like two types; (i) quenching and (ii) dynamic (Arik et al. 2011, Turner et al. 2019). Steady static fluorescence method reduce by the raise of temperature used for static quenching, since rising the temperature resulted rise in the flow coefficient. As a result, the persuade of different concentration of ILs on the fluorescence intensity of serum albumins on 298 K are shown in Figure.1. Fluorescence static quenching constants of BSA-BmimOS, HSA-BmimOS and BSA-DmimBF$_4$, HSA-DmimBF$_4$ system at 3 different temperatures can be considered using Stern-Volmer equation (1) (Ghosh et al. 2018, Yi and Liu et al. 2019).

$$\ln\frac{I_0}{I} = 1 + K_{sv}[Q] \tag{1}$$

Herein I_0 and I are the intensities of fluorescence within the occurrence and deficiency of ILs, correspondingly. Hence, the K_{SV} is considered by using the linear decay of the plot of I_0/I vs $[Q]$ according to the equation (1). The graph plots on Stern-Volmer plots of BSA-BmimOS, HSA-BmimOS and BSA-DmimBF$_4$ system at two changed temperatures are revealed in Fig 2 and the values were scheduled here Table 1. As a results the Stern-Volmer quenching constant K_{SV} are decreasing according with increased temperature. This is confirm that the steady static quenching mechanism of BSA-BmimOS, HSA-BmimOS and BSA-DmimBF$_4$, HSA-DmimBF$_4$.

3.2. Calculate the binding constant and binding number

The fluorescence static quenching method (Behere et al. 2019) to calculate the binding constant (K_b) as well as binding amount (n) was calculated using subsequent equation (2).

$$\log\frac{(I_0 - I)}{I} = \log K_b + n\log[Q] \tag{2}$$

Graph plot between $\log(I_0-I)/I$ vs $\log[Q]$, as a results a straight line observed and the number of binding sites (n) and binding constant (K_b) was achieved is shown in figure 3-4. In Table 1, shows that the value of binding constant (K_b) increase significantly with the increase of temperatures, representing that the binding ability of ILs-BSA/HSA systems would be greatly increased when the temperature increased. These phenomenons have shown that ILs bound stoutly with BSA/HSA according to mole ratio of 1:1 and self-sufficient of the binding of ILs to BSA/HSA. Tayyab et al.[30] studied the interaction of bovine serum albumin and bilirum (BR) by absorption spectroscopy. Analysis the association constant (K) of BSA and BR is 6.9 x 104 M^{-1}. As a result suggest that ErB binds to a site in the vicinity of BR binding site on BSA (Seetharamappa et al. 2004).
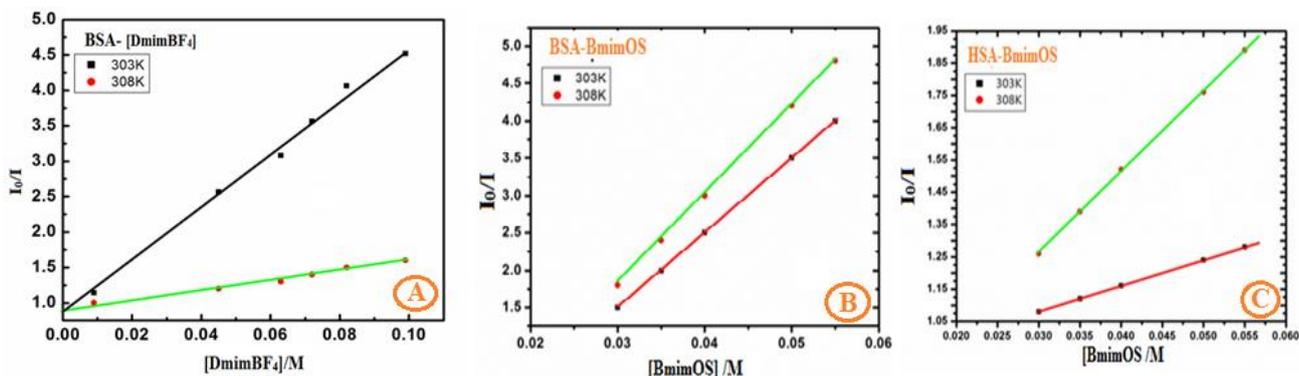
Fig. 2: [A] Graph Plot Between I_0/I Vs Concentration of [Dmimbf$_4$] (M). [B] Graph Plot Between I_0/I Against Concentration of [Bmimos] (M). [C] Graph Plot Between I_0/I vs. Concentration of [Bmimos] (M).
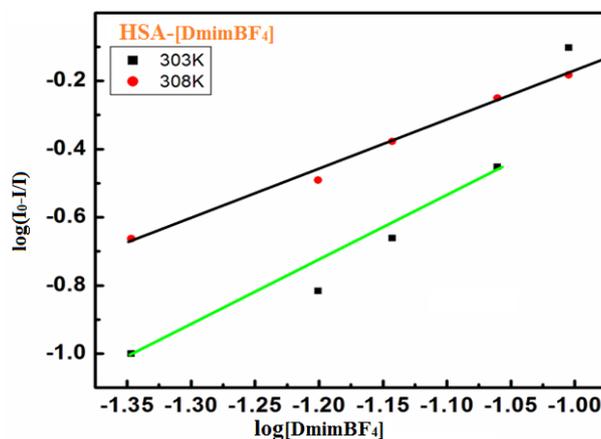
Fig. 3: Graph Plot between Log[(I$_0$-I)/I] Against Log [Dmimbf$_4$] for Binding Constant of HSA

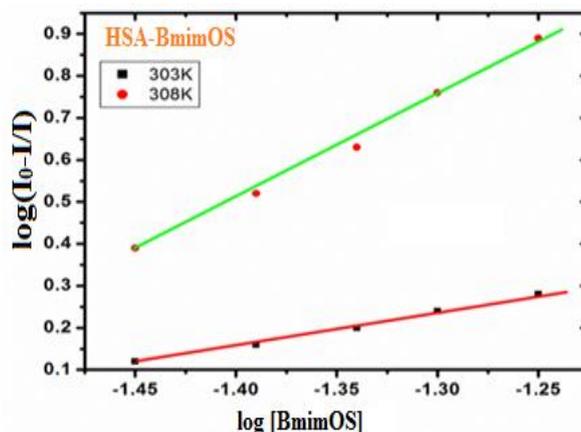**Fig. 4:** Graph Plot between Log[(I$_0$-I)/I] Against Log [Bmimos] for Binding Constant Of HSA.

The Gibbs free energy change (ΔG) for the complexation can be considered from the binding constant value (Ka) using the following equation (3).

$$\Delta G = -2.303 RT \log Ka \tag{3}$$

Where, ΔG is the standard Gibbs free energy, R is gas constants (8.314 J/mol K), T is temperature (in Kalvin), Ka is binding constants. The calculated values of ΔG, the complexation process are found to be presented here Table 2. The negative values of ΔG indicate the spontaneity of complexation method at 298/303/308K temperature. In general, the binding constants/ interaction between BSA/HSA and ILs may electrostatic interaction, H-bonding, hydrophobic force and van der Waal interaction. Binding military can be determined by variant some thermodynamic parameters i.e., enthalpy change (ΔH), entropy change (ΔS) and Gibbs free energy change (ΔG) (Ghosh et al. 2018).

Table 1: Binding Constant (K$_a$) (Mol/L) of [Bmim] [OS] /[Dmim] [BF$_4$] with Serum Albumins

Temperature (Kelvin)	[BmimOS]				[DmimBF$_4$]			
	HSA	R	BSA	R	HSA	R	BSA	R
298	20.50	0.996	20.02	0.979	23.65	0.980	14.32	0.966
303	28.43	0.991	36.98	0.993	36.0	0.957	28.0	0.992
308	49.02	0.998	61.36	0.995	39.34	0.993	16.79	0.991

3.3. Binding interaction between ionic liquids and serum albumins

The Gibbs free energy change (ΔG) for the complexation can be considered from the binding constant value (Ka) using the following equation (3).

$$\Delta G = -2.303 RT \log Ka \tag{3}$$

Where, ΔG is the standard Gibbs free energy, R is gas constants (8.314 J/mol K), T is temperature (in Kalvin), Ka is binding constants. The calculated values of ΔG, the complexation process are found to be presented here Table 2. The negative values of ΔG indicate the spontaneity of complexation method at 298/303/308K temperature. In general, the binding constants/ interaction between BSA/HSA and ILs may electrostatic interaction, H-bonding, hydrophobic force and van der Waal interaction. Binding military can be determined by variant some thermodynamic parameters i.e., enthalpy change (ΔH), entropy change (ΔS) and Gibbs free energy change (ΔG) (Ghosh et al. 2018).
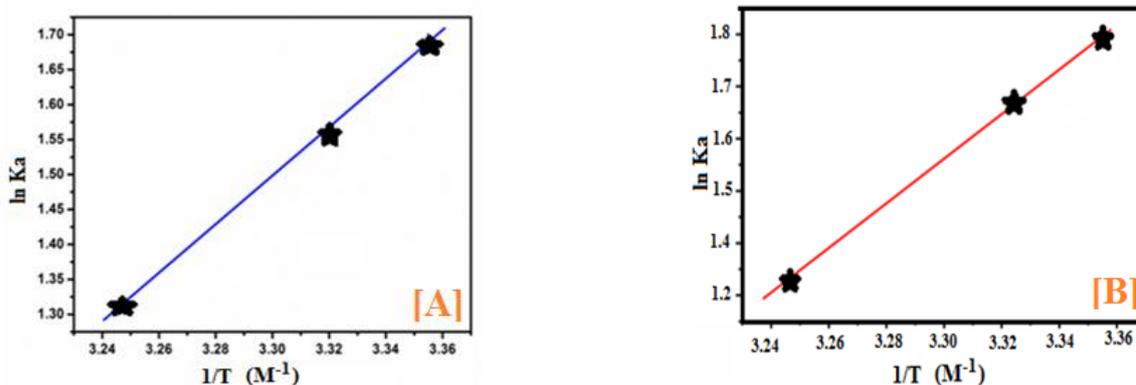
$$\ln K_a = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \tag{4}$$

Fig. 5: Van't Hoff plot of lnKa Against 1/T (M⁻¹) i.e., (A) BSA-BmimOS and (B) HSA-BmimOS.

Where, K_a is comparable to the binding constant at the resultant T, and R is the gas constant (8.314 J/molK), correspondingly. Fig. 5, viewing a good linear correlation between $\ln K_a$ vs $1/T$. As a result, ΔH, ΔS and ΔG were calculate according to the linear regression equation include in Fig.7. The calculate valuesΔH, ΔS, and ΔG were all shown Table 2. The calculated negative value of ΔG shows the binding process of ILs and HSA/BSA were spontaneous and negative values of ΔH indicated that the binding process of ILs. HSA/BSA with IL complex was exothermic in nature. As a results suggest that higher the temperature is show the weaker interaction between [BmimOS]/ [DmimBF₄] with HSA/BSA.

Table 2: Thermodynamic Parameter of Complexation between BSA/HSA with [BmimOS]/ [DmimBF₄]

Thermodynamic parameters	Temperature (K)	[BmimOS]		[DmimBF₄]	
		HSA	BSA	HSA	BSA
ΔG (kJmol⁻¹)	298	-2.8	-1.8	-7.8	-6.6
	303	-2.5	-1.0	-8.8	-8.2
	308	-2.2	-0.7	-9.0	-6.9
ΔH (kJmol⁻¹)	298	-6.31	-6.46	-5.47	-9.02
	303	-4.62	-3.53	-3.65	-4.69
	308	-2.72	-2.17	-3.39	-7.96
ΔS (Jmol⁻¹K⁻¹)	298	24.59	24.39	25.82	21.47
	303	27.43	29.68	29.43	27.28
	308	32.08	33.98	30.22	22.86

3.4. FT-IR spectrometry

IR spectra of [BmimOS] (Fig. 6 A)/ [DmimBF₄] (Fig. 7A) dispersed with HSA/BSA (Fig.6-8 B and 5B) were evidence using potassium bromide pellet in the scan range among 4000-400 cm⁻¹. As a results obtained between the interaction of the HSA/BSA with [BmimOS]/ [DmimBF₄] by FTIR is exposed in Figure 6-8. Globular proteins i.e., BSA/HSA shown two absorbing bands i.e., (i) amide first band C=O str. at 1644 cm⁻¹ and (ii)) amide second band C-N str. and N-H bending mode at 1540 cm⁻¹. IR spectra of amide first band is shifted from 1644 cm⁻¹ to 1659 cm⁻¹. Furthermore, amide II band shifted faintly from 1537 cm⁻¹ to 1542 cm⁻¹. These results convey that [BmimOS] also change the stretching vibration of C=O groups in the protein polypeptides beside by the C-N stretching and N-H bending vibration modes. Usually, the aggregation of [BmimOS] with BSA/HSA resulted in the delay of the serum albumins 2⁰ structure and [BmimOS] on larger concentration explain greater persuade on the structure change of the serum albumins BSA/HSA which confirm well with the results experimental from fluorescence experiments.
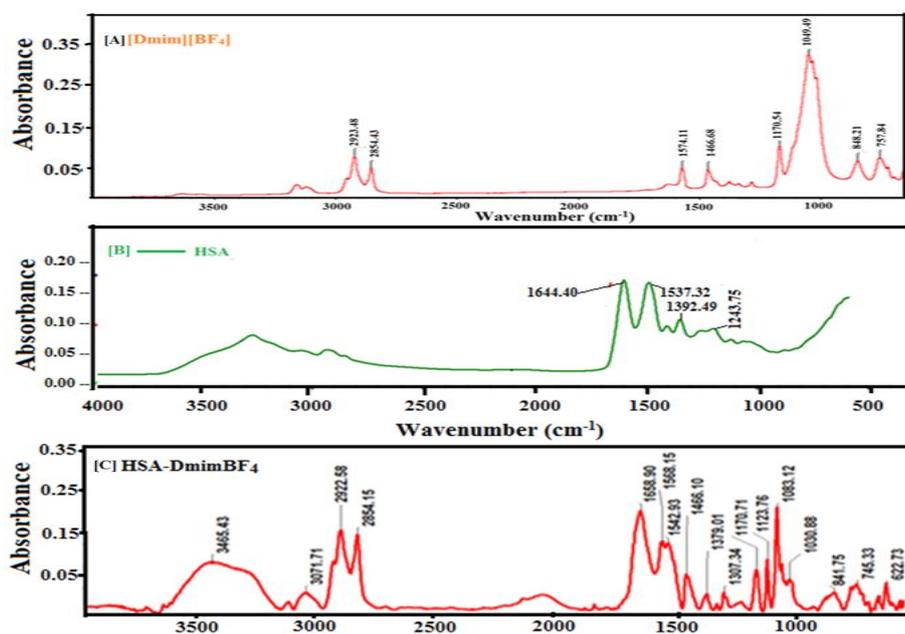

Fig. 6: FTIR Spectra of (a) [DmimBF₄] (b) HSA and (c) HSA with [DmimBF₄].
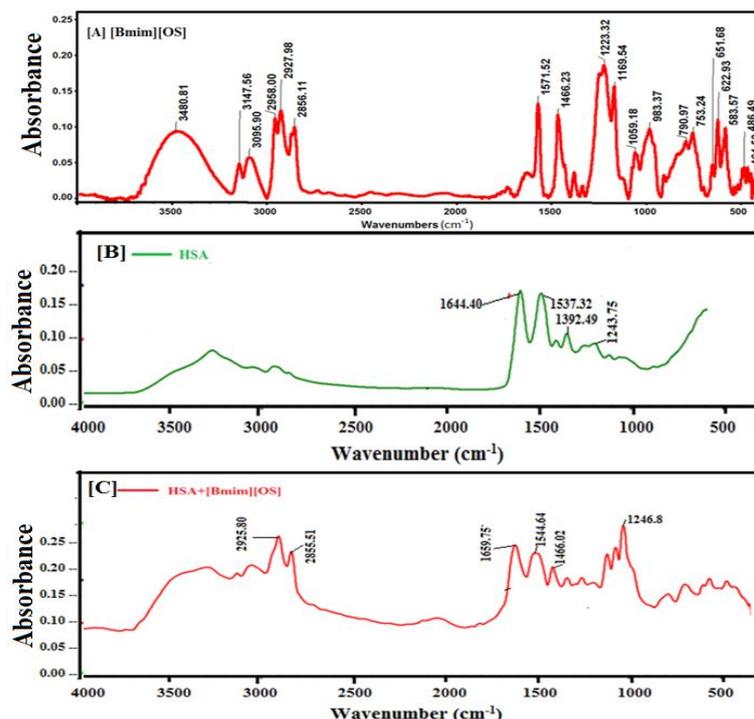
Fig. 7: FTIR Spectra of (a) [BmimOS] (b) HSA and (c) HSA with [BmimOS].

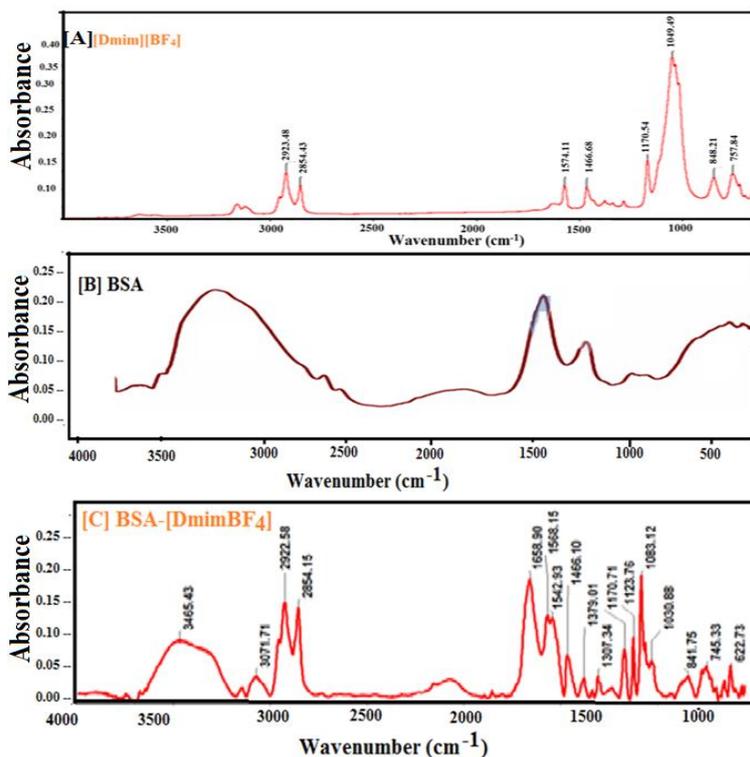**Fig. 8:** FTIR Spectra Of (A) [Dmimbf₄] (B) BSA and (C) BSA With [Dmimbf₄].

Zhao et al. 2011, has been investigated the interaction between reserpine and bovine serum albumin (BSA) were studied using UV-vis absorption, fluorescence, and FTIR spectroscopy. The calculated binding constant K_A of reserpine with BSA at three different temperatures 293, 301 and 309 K was 1.63×10^5, 1.78×10^5 and 2.35×10^5 moL^{-1} L respectively. As results of fluorescence and FT-IR data show that the conformation of BSA was changed by the binding of reserpine.

4. Conclusion

Spectroscopic revealed that 1-butyl-3-methylimidazolium octylsulphate (BmimOS) and 1-decyl-3-methylimidazolium tetrafluoroborate (DmimBF₄) interact with HSA/BSA in the bulk earlier to interaction. The results explain that the fluorescence quenching of ILs with BSA/HSA go to static quenching. The fluorescence quenching decreased among the temperature rising. Thermodynamic parameters are responsible to the joining force between ILs and BSA/HSA was mostly hydrophobic interaction. Fluorescence expose the verify of HSA/BSA altered in the existence of (DmimBF₄)/(BmimOS). We have previously reported in our paper (Ghosh et al. 2018, Ghosh et al.

2016) the interaction between serum albumin with nonmaterial's and surfactants from the description of conductivity, surface tension, fluorescence, UV-Vis, FT-IR and this study is an peace by our results.

Acknowledgement

Conflicts of interest

Authors do not have any conflicts for this manuscript.

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