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RESEARCH ARTICLE

EFFECT OF IONIC LIQUIDS ON DNA-LIGANDS INTERACTION: STUDIED BY FLUORESCENCE

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ABSTRACT

Ionic Liquids having great green properties such as low vapour pressure, tunable viscosity, tunable electrochemical window and wide liquid range which attract the attention for their study. In this course of project the introduction of Ionic Liquids with DNA-Ligand complex was applied, and the effect of ionic liquids on DNA-Ligands interaction were studied by fluorescence spectrophotometry titration. For this purpose Calf-thymus (Ct-DNA) and EtBr was taken under study, a UV-Vis Abs spectroscopic titration at different salt condition were studied and then two different ILs [bmim]Cl and [bpip]NtF_2 were introduced with EtBr to study their effect on Ct-DNA-EtBr interaction. It was observed that there was a gradual decrease in binding affinity of EtBr with Ct-DNA as concentration of ILs was being increased. This result coincides with the result as observed with the event when binding affinity was measured at different salt concentration of phosphate Buffer in traditional methods.

INTRODUCTION

Ethidium Bromide (3, 8-diamino-6-phenyl-5-ethylphenanthridinium Bromide) first synthesized by Watkins in 1952, interacts strongly between the Adenine and Thymine base pairs in DNA. The interaction of EB with Ct-DNA was studied by UV-Vis Absorbance, fluorescence spectrophotometry. In UV-Vis Abs spectroscopy, on binding to DNA the absorption spectrum of EtBr underwent Bathochromic (Red Shift) and Hypochromic shifts. EtBr is a fluorescent dye with appearance of purple red colour most commonly used for DNA detection in gel. The experiment described in this dissertation was undertaken with the objective of characterizing the DNA-ligand complex and obtaining quantitative data on its formation. *EtBr*forms complexes with DNA and in which the Ethidium is strongly bound to the hydrophobic region of Ct-DNA. The DNA-bound EtBr cannot be removed by dialysis but may be dissociated from DNA at high sodium chloride concentrations or by chromatography on cation exchange resins. Ionic liquids are green solvent having many useful properties are being used by chemists widely. In this dissertation effect of ionic liquids on DNA-Ligand complexes is taken under interest of study.

MATERIALS AND METHODS

Ct-DNA Solution-Deoxyribonucleic Acid, Sodium Salt from Calf Thymus (Type-1) was obtained from Sigma-Aldrich Co. LLC. The Stock Solution was made in 10mM, pH 7.0 Sodium Phosphate buffer. Some threads of DNA with Buffer were taken in a falcon tube and put it on a rotator for dissolving overnight. With the help of Beer-Lambert's law using the extinction coefficient of DNA at 260nm ($13200M^{-1}Cm^{-1}$Base-Pair) the concentration of Ct-DNA was measured. The concentration of stock Ct-DNA Solution was found to be 2.64mM. (Appendix-I)

EtBr Solution: MB071 levelledEthidium Bromide, supplied by HIMEDIA Laboratories Vadhani Industrial Estate, Mumbai, India, and was used during the whole work of this project. Solution was prepared in milli Q water and stored in $3\text{-}4^0$c in dark. Again the concentration of EtBr Stock Solution was determined by same preceding method, the $\lambda_{max}$was found to be at 479nm and extinction coefficient was $5600M^{-1}Cm^{-1}$. The concentration of stock EtBr Solution was found to be 13.3 mM.(Appendix-II).

Buffer: All solutions used in this course of work were prepared in milli Q water, unless otherwise noted. Experiments was performed in the presence of 0.01M Sodium Phosphate Buffer (pH 7.0) containing primary standard grade Sodium Phosphate dibasic. (Sigma-Aldrich Co. LLC).

Ionic Liquids: The supply of following ILs was done by Sigma- Aldrich Co. LLC.

- 1-Butyl-3-methylimidazolium Chloride – [bmim] Cl

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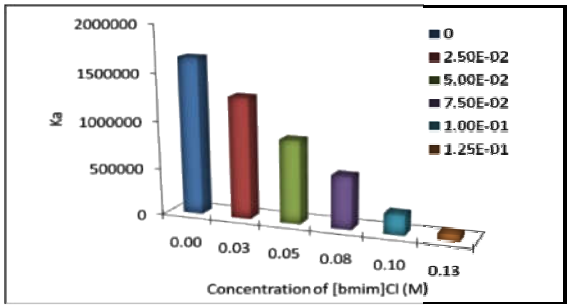
- 2, 5 bis (1-phenyliminoethyl) pyrazinebis (trifluoromethylsulfonyl) imide – [bpip] NtF2

Spectroscopic Measurements-The spectral absorption of Ct-DNA and EtBr were measured using *Agilent Technology Cary series UV-Vis Cary 100* Spectrophotometer and fluorescence spectra were measured using *Horiba Scientific Fluoromax 4 Spectrofluorometer* at *CSIR-IGIB, New Delhi-India.* In UV-Vis Abs spectrum a red shift and hypochromic effect was observed as EB interact to Ct-DNA (Figure-I). On subsequent addition of 2.5×10^{-4} ml of 2.64×10^{-3} M Ct-DNA, a hypochromic effect and red shift to 520 nm was observed with an isobestic point at 510 nm. Free EtBr had a maximum Absorbance at 479 nm. Here hypochromic effect can be studied by fluorescence spectrophotometry. While we observe a decreasing order in Absorbance on addition of Ct-DNA to EtBr, there was an increase in fluorescence intensity on such addition (Figure-II). When ionic liquids were introduced in these set-ups of experiments we observed a regular decrease in the fluorescence of EtBr with increasing concentration of ionic liquids (Figure-III).This result led us to perform a fluorescence titration experiment using different concentration of ionic liquids (Figure-IV to VII) and calculate binding constant of DNA-EtBr Interaction at varying concentration of ILs using scatchared analysis. (Appendix-III)

RESULTS

In order to illustrate the binding strength of the EB with Ct-DNA, the intrinsic binding constant was determined using spectral titration data with the help of the scatchared plot, A graph between r and r/Cf gives a straight line with a negative slope with the help of this we can extract binding constant. The binding constant of EtBr to Ct-DNA in absence of IL were determined by using UV-Vis Abs spectroscopy and Fluorescence spectroscopy, however the binding constant in presence of IL were obtained by using fluorescence spectroscopic titration data only .On account of Fluorescence spectrophotometry titration data it was observed that there was a linear decrease in binding affinity as concentration of IL was being increased. [bmim]Cl at different conditions were taken under study, and it was observed that there is a linear decreasing trend in binding affinity of ligand as concentration of [bmim]Cl was being increased.(Appendix-IV).as shown in attached graph.



A calibration between binding affinity of ligand versus concentration of ionic liquid [bmim]Cl.

Conclusion

EtBr interact with Ct-DNA and binds between base pair, a hydrophobicregion, removing water molecule from ethidiumcation, this phenomenon result in decrease in Absorbance , a hypochromic shift, a shift in wavelength maxima, a red shift and an increase in fluorescence intensity. These events can be understood by energy level profile, i.e. change in electronic and vibrational energy level. As EtBr binds to Ct-DNA it requires less energy to absorb electromagnetic radiations due to change in transition level which result a shift in λ_{max}, resulting a red shift, similarlya decrease in absorbance can be observed because when an excited electron come back to ground state in transfer its energy through singlet state to triplet state and then ground state, this singlet to triplet state transition cannot be detectable by UV-Vis bsorbance spectroscopic tools, hence there detector detect only absorbance of triplet to ground state transition and hence a decrease in absorbance, hypochromic shift.

When ILs are introduced in the system, the hydrophobic region of ILs takes place between base pair of DNA removing bounded EtBr and hence decreasing the fluorescence of EtBr. This hydrophobic interaction increase with increasing concentration of ILs, and cause a gradual decrease in binding affinity of Ct-DNA-EtBr interaction. The screening effects of ionic liquids is again an another reason, The screening effect of ILs are much larger than screening effect of sodium ion and hence, there is much influence of ILs on binding affinity of Ct-DNA EtBr complex than sodium salt.

The counter ions of ILs in the solution shield the phosphate groups of Ct-DNA, reducing the electrostatic repulsion force between Ct-DNA and EtBr, hence decrease the binding affinity of DNA-EtBr complex. With increasing concentration of ILs there is induced shielding (screening) in phosphate group of Ct-DNA to EtBr and hence due to much reduction in electrostatic repulsion there is a linear decreasing trend in binding affinity.

Acknowledgment

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Figure and Appendixes

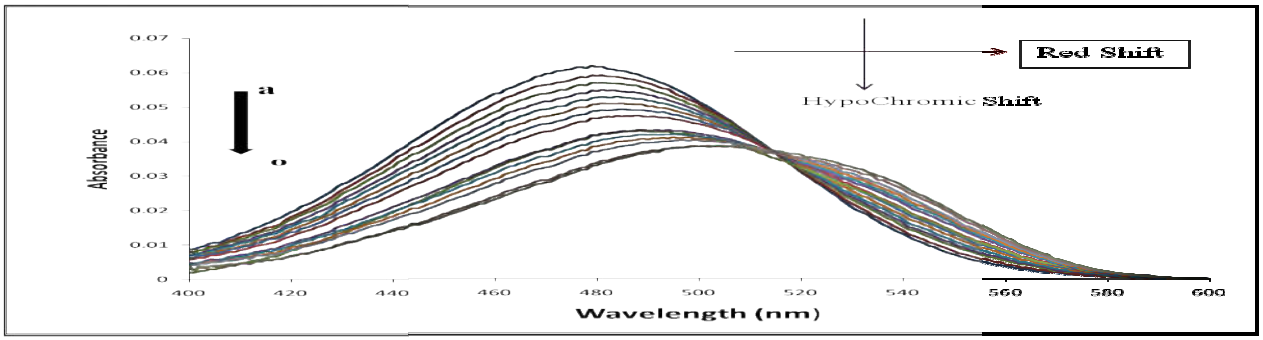


Figure 1. UV-Vis Abs spectrum of interaction of 1×10^{-5} M EtBr with Ct-DNA in Phospate buffer, Arrow indicates Red Shift and Hypochromic shift on subsequent addition of Ct-DNA solution to EtBr. Bold Arrow indicate increasing concentration of Ct-DNA, a) 0M, b) 1.3×10^{-6} M ,c) 2.6×10^{-6} M, d) 3.9×10^{-6} M, e) 5.2×10^{-6} M f) 6.5×10^{-6} M, g) 7.8×10^{-6} M h) 9×10^{-6}M, i) 1.05×10^{-5} M, j)1.18×10^{-5}M, k) 1.31×10^{-5} M, l) 1.45×10^{-5} M, m) 1.57×10^{-5} M, n) 1.84×10^{-5} M, o) 2.0×10^{-5} M

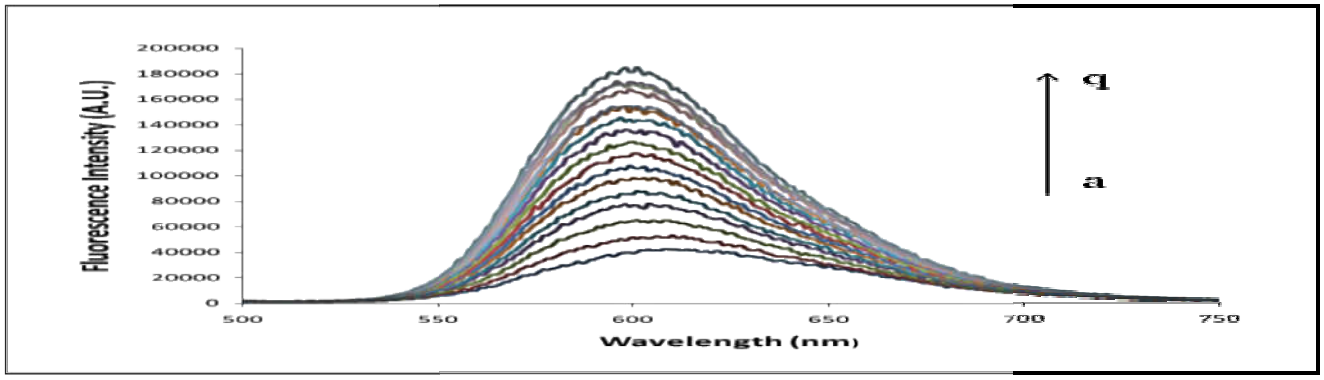


Figure 2. Fluoroscence spectrum of interaction of 1×10^{-6} M EtBr with Ct-DNA, Arrow indicates the increasing Concentration of Ct-DNA. a) 0M b) 1.248×10^{-7} M c) 2.493×10^{-7} M, d) 3.736×10^{-7} M, e) 4.975×10^{-7} M, f) 6.211×10^{-7} M, g) 7.444×10^{-7} M, h) 8.674×10^{-7} M, i) 9.9×10^{-7} M, k) 1.112×10^{-6} M, l) 1.234×10^{-6} M, m) 1.356×10^{-6} M, n) 1.477×10^{-6} M, o) 1.599×10^{-6} M, p) 1.720×10^{-6} M, q) 1.85×10^{-6} M

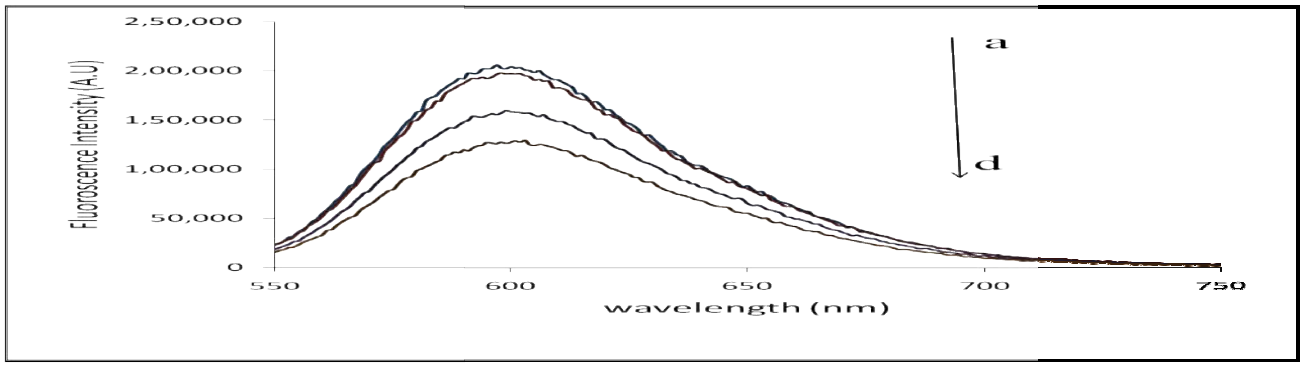


Figure 3. Fluorescence spectrum of Ct-DNA and EtBr Compex with IL, [bmim]Cl, arrow indicates increasing concentration of

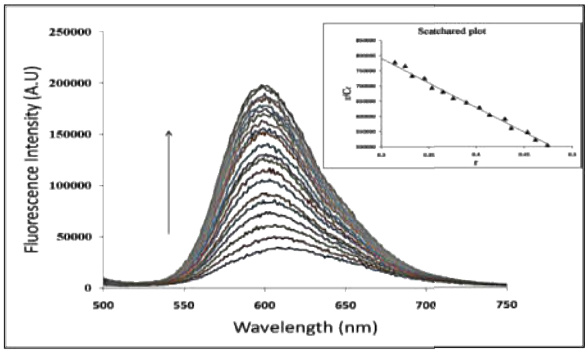


Figure 4. Fluoroscence spectrum of interaction of 1×10^{-6} M EtBr in presence of 2.5×10^{-2} M [bmim]Cl with Ct-DNA, Arrow indicates the increasing Concentration of Ct-DNA a) 0M b) 1.248×10^{-7} M c) 2.493×10^{-7} M, d) 3.736×10^{-7} M, e) 4.975×10^{-7} M, f) 6.211×10^{-7} M, g) 7.44×10^{-7} M, h) 8.67×10^{-7} M, i) 9.9×10^{-7} M, k) 1.112×10^{-6} M, l) 1.234×10^{-6} M, m) 1.356×10^{-6} M n) 1.477×10^{-6} M, o) 1.599×10^{-6} M, p) 1.720×10^{-6} M, q) 1.85×10^{-6} M, r)1.96×10^{-6} M, s) 2.08×10^{-6} M, t) 2.20×10^{-6} M, u) 2.31×10^{-6} M, v)2.44×10^{-6} M. In checkbox Scatchard plot

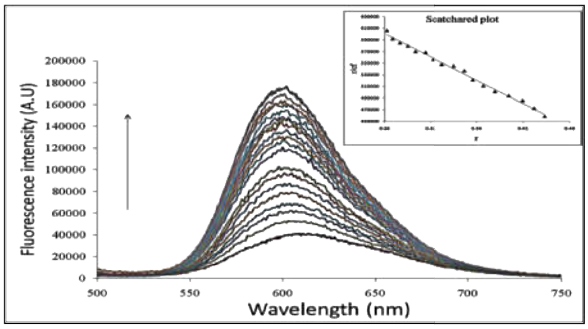


Figure 5. Fluoroscence spectrum of interaction of 1×10^{-6} M EtBr in the presence of 5.0×10^{-2} M [bmim]Cl with Ct-DNA, Arrow indicates the increasing Concentration of Ct-DNA. a) 0M b) 1.248×10^{-7} M c) 2.493×10^{-7} M, d) 3.736×10^{-7} M, e) 4.975×10^{-7} M, f) 6.211×10^{-7} M, g) 7.44×10^{-7} M, h) 8.67×10^{-7} M, i) 9.9×10^{-7} M, k) 1.112×10^{-6} M, l) 1.234×10^{-6} M, m) 1.356×10^{-6} M n) 1.477×10^{-6} M, o) 1.599×10^{-6} M, p) 1.720×10^{-6} M, q) 1.85×10^{-6} M, r)1.96×10^{-6} M, s) 2.08×10^{-6} M, t) 2.20×10^{-6} M, u) 2.31×10^{-6} M, v)2.44×10^{-6} M. In checkbox Scatchard plot.

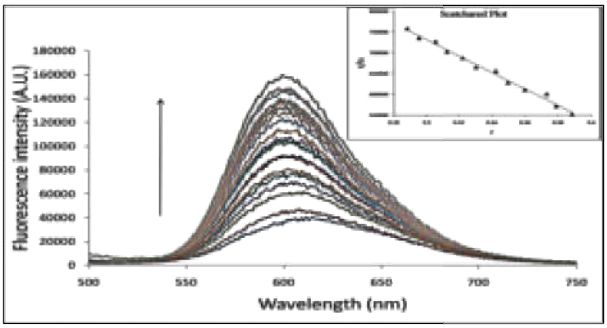


Figure 6. Fluoroscence spectrum of interaction of 1×10^{-6} M EtBr in the presence of 7.5×10^{-2} M [bmim]Cl with Ct-DNA, Arrow indicates the increasing Concentration of Ct-DNA. a) 0M b) 1.248×10^{-7} M c) 2.493×10^{-7} M, d) 3.736×10^{-7} M, e) 4.975×10^{-7} M, f) 6.211×10^{-7} M, g) 7.44×10^{-7} M, h) 8.67×10^{-7} M, i) 9.9×10^{-7} M, k) 1.112×10^{-6} M, l) 1.234×10^{-6} M, m) 1.356×10^{-6} M n) 1.477×10^{-6} M, o) 1.599×10^{-6} M, p) 1.720×10^{-6} M, q) 1.85×10^{-6} M, r)1.96×10^{-6} M, s) 2.08×10^{-6} M, t) 2.20×10^{-6} M, u) 2.31×10^{-6} M, v)2.44×10^{-6} M. In checkbox Scatchard plot.

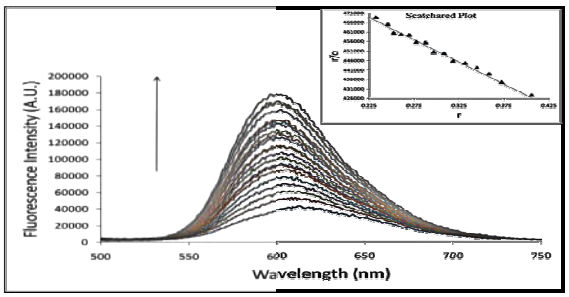


Figure 7. Fluoroscence spectrum of interaction of 1×10^{-6} M EtBr in the presence of 1.0×10^{-1} M [bmim]Cl with Ct-DNA, Arrow indicates the increasing Concentration of Ct-DNA a) 0M b) 1.248×10^{-7} M c) 2.493×10^{-7} M, d) 3.736×10^{-7} M, e) 4.975×10^{-7} M, f) 6.211×10^{-7} M, g) 7.44×10^{-7} M, h) 8.67×10^{-7} M, i) 9.9×10^{-7} M, k) 1.112×10^{-6} M, l) 1.234×10^{-6} M, m) 1.356×10^{-6} M n) 1.477×10^{-6} M, o) 1.599×10^{-6} M, p) 1.720×10^{-6} M, q) 1.85×10^{-6} M, r)1.96×10^{-6} M, s) 2.08×10^{-6} M, t) 2.20×10^{-6} M, u) 2.31×10^{-6} M, v)2.44×10^{-6} M. In checkbox Scatchard plot.

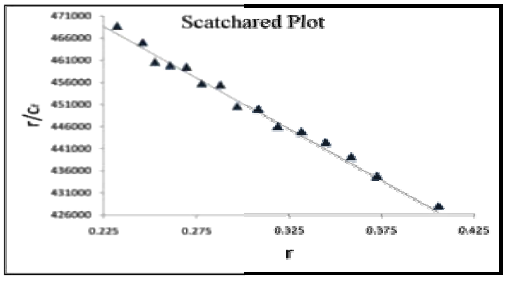
Appendix-I: Using Beer-Lambert's law and following observations the concentration of stock DNA Solution was calculated.

Volume(µL)		Abs	Concentration
Buffer	Stock DNA		
700	5	0.253	0.002708 M
700	10	0.471	0.002537 M
700	15	0.741	0.002676 M
Average Concentration			0.002640 M (2.64mM)

Appendix-II: Using Beer-Lambert's law and following observations the concentration of stock EtBr Solution was calculated

Volume(µL)		Abs	Concentration
Solvent	Stock EB		
700	5	0.353706	0.014801 M
700	10	0.616474	0.012953 M
700	15	0.863526	0.012148 M
Average Concentration			0.013301 M (13.3mM)

Appendix-III: The value of Binding coefficient was calculated by Scathared Analysis. This is ploting between r/C_f vs r, with a slope - K_a and a Y-interceypt of nK_a . Where r is number of ligand molecule bound, and C_f is concentration of free ligands at that instant. Following is the scatchared plot for Figure VI.



Appendix-IV: Trend in binding affinity of ligand as concentration of [bmim]Cl at 298K temp and 10 mM Buffer concentration.

Concentration of [bmim]Cl	Binding Affinity
0 mM	1.8×10^{6} M
25mM	1.4×10^{6} M
50mM	8.9×10^{5} M
75mM	4.5×10^{5} M
100mM	2.1×10^{5} M
125mM	5.5×10^{4} M

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