Simultaneous morphological transformation of metal salt and conformations of DNA in a bio-based ionic liquid

Jitkumar Bhatt, M.M. Pereira, Kamalesh Prasad

PII: S0141-8130(19)31264-4
DOI: https://doi.org/10.1016/j.ijbiomac.2019.06.012
Reference: BIOMAC 12518

To appear in: *International Journal of Biological Macromolecules*

Received date: 18 February 2019
Revised date: 9 April 2019
Accepted date: 3 June 2019

Please cite this article as: J. Bhatt, M.M. Pereira and K. Prasad, Simultaneous morphological transformation of metal salt and conformations of DNA in a bio-based ionic liquid, International Journal of Biological Macromolecules, https://doi.org/10.1016/j.ijbiomac.2019.06.012

Simultaneous morphological transformation of metal salt and conformations of DNA in a bio-based ionic liquid

Jitkumar Bhatt [a,b] M. M. Pereira [c] and Kamalesh Prasad [a,b*]

[a] *Natural Products & Green Chemistry Division, CSIR-Central Salt & Marine Chemicals Research Institute, G. B Marg, Bhavnagar-364002 (Gujarat), India.*

[b] *AcSIR- Central Salt & Marine Chemicals Research Institute, G. B Marg, Bhavnagar-364002 (Gujarat), India.*
Corresponding Author : *E-mail: kamlesh@csmcri.res.in /drkamaleshp@gmail.com; Tel: +91-278-2567760 ; Fax : +91-278-2567562

[c] *CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal*

ABSTRACT: The extraordinary left handed conformation of DNA known as Z-DNA has attracted the attention of structural biologists due to its characteristic features such as its possible role in regulation of gene expression and genetic instability. There are number of physical parameters which can induce the conformational transformation of double helical B-DNA to Z-DNA. Among the various physical conditions, right-handed B-DNA can be transformed into left-handed Z-DNA *in vitro* at high salt concentrations or *in vivo* under physiological conditions. Herein DNA solubilized in a choline based ionic liquid namely choline formate was found to reduce Ag(I) salt into silver nanoparticles (AgNPs) with the size distribution of 10-20 nm. During the process, the interaction of DNA with the ionic liquid induces alteration in secondary structure of DNA (B-Z transition). The formation of the NPs was confirmed by UV-Vis spectrophotometer and Transmission Electron Microscopic (TEM) measurements, while the formation of Z-DNA was confirmed by circular dichroism (CD) spectroscopic measurements. Upon molecular docking studies, choline-formate was found to present different binding sites for its cation and anion and they promote torsions on DNA structure leading to possible changes in DNA three dimensional structures (B-Z transition).

Key words: DNA, Ionic liquid, Reduction, B-Z transition.

1. Introduction

DNA is one of the most explored biomolecules in various branches of chemistry and chemical engineering. The molecule is highly delicate in nature and sensitive to high temperature, organic solvents and pH. Under normal physiological conditions, DNA remains in its natural B-form with right handed double helical structure. [1] This B-form may adapt other secondary structures such as A or Z depending upon its interaction with other molecules or metal ions. The conformation transition from B (double helical) form to Z (left handed) form of DNA is one of the most complex and mysterious conformational changes occurring in biomolecules. Although Z form of DNA is quite unstable but several studies have been done to investigate the relative stability of this DNA form under environmental and physiological conditions and to find its possible role in biological processes. [2,3] The tremendous research efforts now established the occurrence of Z-DNA *in vivo* and the thermodynamic factors influencing the stability is well understood but still the pathway of inter-conversion of B to Z form DNA not clear. Apart from the other factors, proteins are also found to stabilize Z-DNA, which play important roles in several biological processes such as RNA editing, innate immune response, and viral infection. [4-6]

Since ionic liquids (ILs) are also a kind of salt and often termed as 'liquid salt', the conformation of DNA in such solvent must be influenced by the nature of the solvent via different types of interactions. Many ILs and their structure analogues known as deep eutectic solvents (DESs) are emerging as new and suitable solvent systems for DNA in recent times [7]. Apart from high concentration dissolution of DNA, which is important from processing point of view, the delicate molecule is found to be stable structurally for several months to years upon room temperature storage in certain such solvents. [8-10] Studies have established such solvents suitable for packaging of DNA. [11] Among the several ILs, we found DNA solubility up to 12% w/w in choline formate. [11] The DNA preserves its native structure for

six months in choline formate upon storage at room temperature. Apart from the dissolution, researchers have observed the folding and imaging of DNA nano structure in deep eutectic solvents both in anhydrous and hydrated form. [12] Interaction of several aqueous cholinium-based ILs such as cholinium chloride, cholinium dihydrogen citrate, cholinium bicarbonate and cholinium bromide with calf thymus DNA (***ct***-DNA) was studied. The researchers have found weak interaction between the cholinium-based ILs and double stranded ***ct***-DNA, mainly through minor grooves. [13]

In addition to the affects of these liquid salts on DNA conformation, DNA is found to adopt A-conformation at low salt concentration. [14, 15] However, at high salt concentration, almost complete inversion of the double-helical oligonucleotides was observed. [16] Rare earth metal binding also found to affect the DNA structure. In one of the studies, La^{3+} Y-shaped self-assembled B-DNA was found to be converted into Z-form. Herein, the La^{+3} ions binds with the major and minor grooves and stabilizes Z-DNA. [17]

Due to the above excellent characteristic properties of DNA, such as conformational transition from B to Z in high ionic strength and temperature, this has recently been exploited in the design of a nano thermometer and design of a nanomechanical motor device based on the B-to-Z transition has also been proposed. [18]

Further, due to the presence of multiple sites, DNA acts as a template for the synthesis of the nanoparticles. [19] In such morphological transformations, the metal ions get attached on the DNA and then with the help of reducing agents it converts into the nanoparticles. Using DNA as a template, a series of reactions have been done to reduce silver into silver nanoparticles (AgNP) and to make composites for various applications. [21-24] In addition to this, due to the excellent solvent properties of ILs, number of metals as well as their salts are

reported to be processed using various ILs. For example, silver nanoparticles are reported to be formed in [OMIm][PF_6] and water in the presence of a nonionic surfactant. [25]

Herein we have observed the reduction ability of choline formate and suitability of the DNA solution prepared in this IL for the formation of silver nano particles. DNA was further found to have Z-Conformation.

2. Experimental Section:

2.1. Materials

Deoxyribonucleic Acid extracted from Salmon testes in the sodium salt form (CAS No. 9007-49-2, ca. 20 kbp) was purchased from TCI Chemicals, Tokyo, Japan. The DNA was used as received, since the purity of DNA was sufficiently high as determined from optical measurements. The ratio of the absorbance of the DNA stock solution at 260 nm to that at 280 nm was found to be 1.92, which suggested the absence of proteins. [26] Choline hydrogen carbonate and tris (hydroxymethyl) aminomethane (Tris)-HCl was purchased from Sigma-Aldrich. Silver nitrate was purchased from S D Fine chemicals Ltd., Mumbai. All Chemicals were of analytical grade and were used as received.

2.2. Synthesis of choline formate

In the simple metathesis reaction to prepare choline formate, formic acid was added drop wise into choline hydrogen carbonate (1:1 molar ratio) with constant stirring at 150 rpm. The resulting solution was then heated at 80 ^{o}C for 1 h and then washed 3 times with ethyl acetate to remove excess of acid. Ethyl acetate was then removed by vacuum evaporator.

2.3. Synthesis of silver nano-particles

DNA (Salmon milt) was dissolved in choline formate (1% w/w) by constant stirring at room temperature for 6 h as reported earlier by us. [11] After complete dissolution of DNA,

$AgNO_3$ powder was added into it maintaining the ratio of $AgNO_3$ to DNA 1:0.5 and 1:1. These solutions were allowed for stirring for 1 h. The solutions turned reddish indicating the formation of silver nanoparticles.

2.4. Characterizations

For CD and UV spectroscopic measurements, 20 µL of samples containing DNA:AgNp (1:0.5 and 1:1 % w/w) in choline formate were added into 1 mL Milli-Q water. The CD spectra was acquired in a 1 mm path length quartz cell using the CD spectrometer (Jasco, J-815). The UV-Vis absorption spectra were recorded on a Varian CARY 500 UV-Vis-NIR spectrophotometer. For the preparation of TEM samples, DNA-AgNp hybrid was precipitated from the ionic liquid and washed several times to remove the IL completely. For TEM analysis of only AgNp, the above mentioned precipitated hybrid material was washed with water in order to remove DNA which left DNA free AgNps. The remaining solid materials were then dispersed in isopropyl alcohol and loaded on carbon coated copper grids (300 mesh size). As prepared samples were kept in desiccators for complete drying and analyzed on a JEOL TEM (Model-JEM 2100, Japan) instrument operated at accelerating voltage of 120 kV.

2.5. Melting point determination of DNA

To determine melting point of DNA, it was solubilized in saline-sodium citrate buffer to give a final concentration of approximately 1 mg DNA/mL. Then the cuvettes were placed into a water bath at 25 ° C and allowed to equilibrate the temperature. The absorbance were recorded at 260 nm using a UV-vis spectrophotometer at 25 °C using only buffer solution as blank. Then the temperature of the bath was raised steadily to 80° C and the absorbance was recorded. Above 80°C, the absorbance was recorded each after 2 ^{o}C intervals. After correction of the absorbance reading relative to that obtained at 25 °C, the absorbance values were plotted against the temperature. The midpoint of the graph indicating increase in the

absorbance values indicates the melting point T_m of the DNA samples. The GC% of standard DNA, regenerated DNA from choline formate was determined using the following equation and reported earlier by us. [11]

$$GC\% = (T_{m\text{-}DNA} - 69.3) \times 2.44 \quad \text{----------eq1}$$

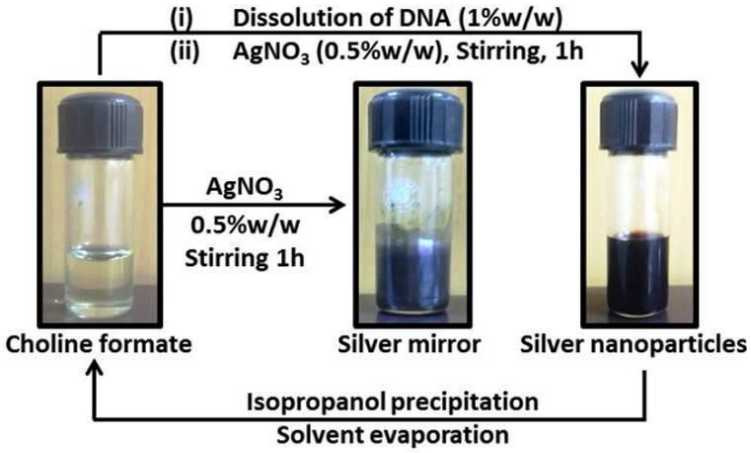
2.6. Molecular docking studies

The interaction sites of DNA with the IL ions were identified using the Auto-dock vina 1.1.2 program. [27] The crystal structure of DNA (PDB: 1bna was adapted in order to achieve AT:57% and GC:42% and used in the molecular docking and this is as per GC% calculated using eq 1 described above [Supporting Figure S1]. Auto Dock Tools (ADT) [28] was used to prepare the protein input files by merging non-polar hydrogen atoms, adding partial charges and atom types. Ligand (IL anion and cation) 3D atomic coordinates were computed by Gaussian 03w and ligand rigid root was generated using AutoDockTools (ADT), setting all possible rotatable bonds defined as active by torsions. The grid centre at the centre of mass (x-, y-, and z-axes, respectively) to cover the whole interaction surface of DNA was 48 Å×52 Å × 126 Å. The binding model that has the lowest binding free energy was searched out from 10 different conformers for choline formate.

3. Results & Discussion

In a typical reaction, upon addition of optimized amount of $AgNO_3$ in choline formate (0.5% w/w) under stirring for 1 h resulted formation of silver mirror (Scheme 1). Formic acid is a well known reducing agent for metal salts to metals and reduction of gold salts was observed using formic acid. [29] Further, DNA (Salmon milt) was dissolved in choline formate (1% w/w) by constant stirring at room temperature for 6 h. After complete dissolution of DNA, $AgNO_3$ powder was added into it maintaining the ratio of $AgNO_3$ to

DNA 1:0.5 and 1:1 followed by stirring for 1 h. Upon stirring, a wine red coloured solution formed instantly confirming formation of silver nanoparticles (Scheme 1).



Scheme 1 : Choline formate mediated reduction of silver nitrate and preparation of silver nano particles by DNA solution prepared in the IL.

The morphology of the NPs were studied under HR-TEM and shown in Fig 1. Size distribution of the NPs was found in the range of 10-20 nm.

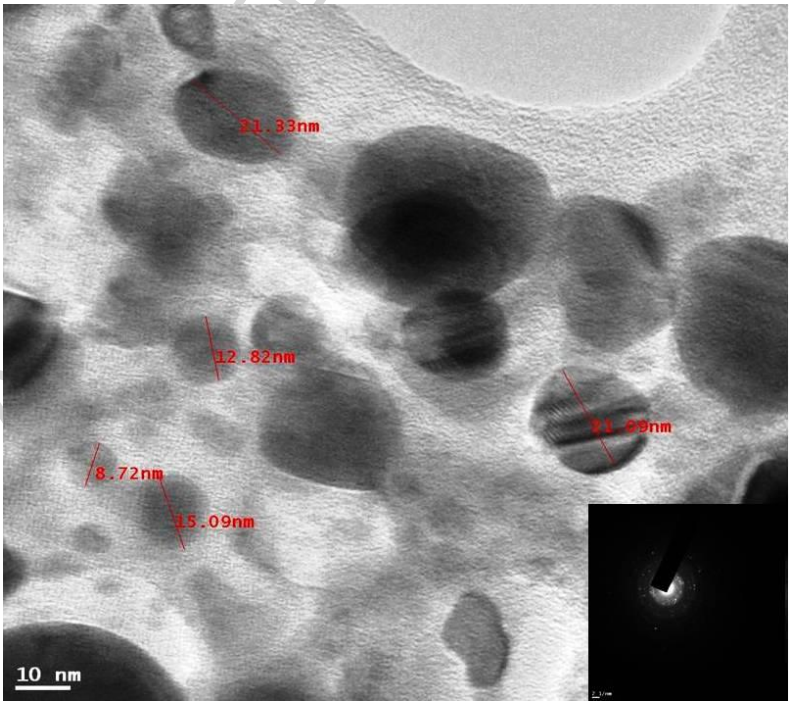


Fig 1. HR-TEM images of AgNPs formed in presence of DNA in choline formate.

As shown in Fig 2, the UV-Vis spectra of the nano particle solution were recorded for both DNA: AgNP in weight ratio 1:0.5 and 1:1 in IL and appearance of a peak at 435 nm indicates the formation of silver nanoparticles and also broadening of the peak suggested the polydispersity of the nanoparticles. [30] From the UV-Vis spectra, it is observed that, the characteristic peak for the nano particle is more pronounced when weight ratio of DNA and silver nitrate is 1:1 in comparison to 1:0.5 indicating the former ratio as optimized ratio condition for the formation of the NPs.

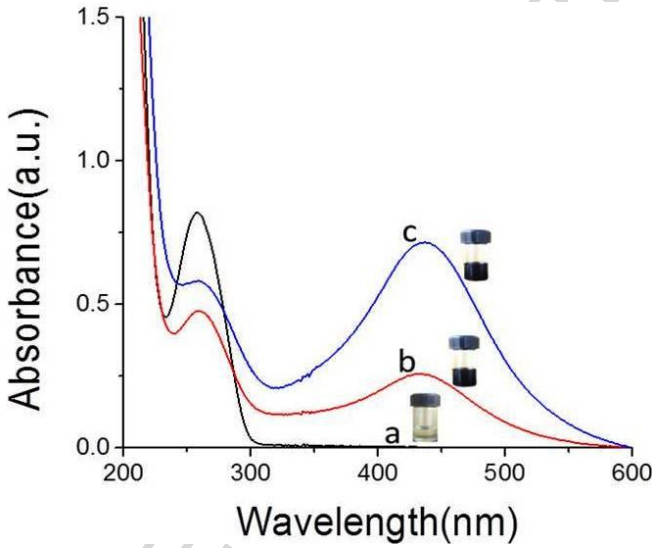


Fig 2 : UV Spectra of solutions of (a) DNA, (b) DNA:AgNP in weight ratio 1:0.5 in IL and (c) DNA:AgNP in weight ratio 1:1 in IL.

A circular dichroism (CD) spectroscopy is one of the vital tools to monitor conformational changes in DNA. The standard DNA solution in water or buffer shows a negative peak at 248 nm characteristic of helicity and another peak at 278 nm characteristic of the π-π stacking in the base pairs of the DNA. [31] The DNA in the choline formate shows the similar spectra characteristic to B-form of DNA (Fig 3a) indicating the preservation of the native B-form of the DNA in the IL. However, the spectrum of DNA in the presence of silver nanoparticles in IL shows the red shift of both 248 nm and 278 nm peaks. The red shift was observed was found to be dependent on the concentration of the AgNPs. When the ratio of

DNA:AgNP was 1:0.5 in IL, the negative peak was found to shift from 245 nm to 250 nm and the positive peak was found to shift from 278 to 285 nm (Fig 3b). By increasing concentration of silver nanoparticles the extent of increment of red shift is still more i.e. 248 shifted to 260 nm and 278 shifted to 295 nm (Fig 3c). This result clearly suggests the AgNP concentration dependent change in conformation of DNA in IL. Further, decrease in the DNA helicity was also observed with increased concentration of the AgNP. The red shift in CD peaks and fall in helicity is indicative of the B-Z transition of the DNA. [32] It was proposed that the B-Z transition in DNA occurs with unwinding and negative super coiling of DNA. [33] So, it can be concluded that, in the presence of AgNPs, DNA tends to move from its B-form to Z-form.

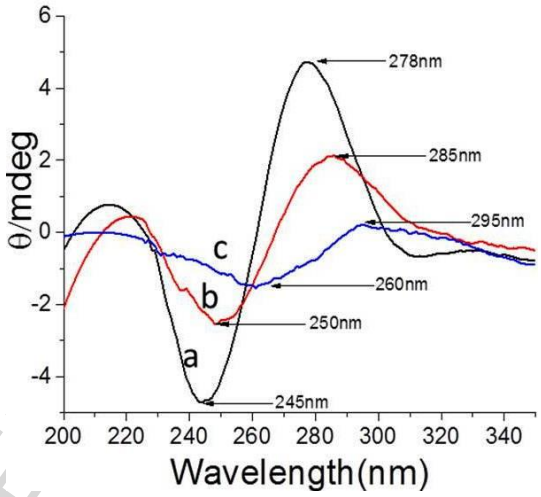
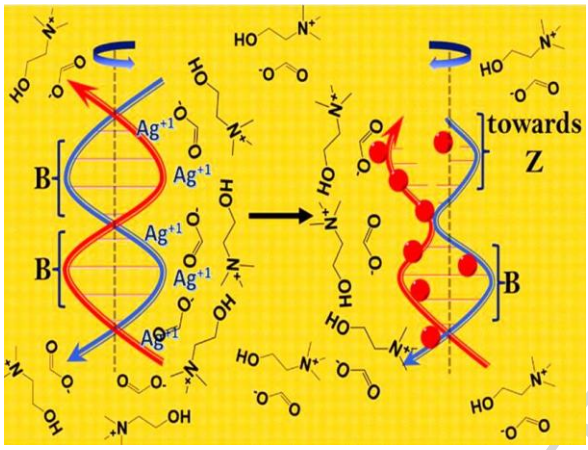


Fig 3. CD spectra of (a) DNA, (b) DNA:AgNP in weight ratio 1:0.5 in IL and (c) DNA:AgNP weight ratio 1:1 in IL.

From above studies it is confirmed that, Ag (I) salt is reduced by choline formate and DNA solution in the ionic liquid resulted formation of silver nano particles and alteration secondary structure of DNA took place (B-Z transition) during such morphological transformation (Scheme 2).



Scheme 2. Schematic representation of formation of silver mirror and silver nanoparticles and structural alteration of DNA in choline formate

In order to investigate the interaction of choline formate with DNA which perhaps contributes to the switching of conformation, simulation studies were performed. In order to simulate the most suitable DNA pose for the DNA-docking studies the GC% of the DNA sample was determined by measuring the melting temperature of the biopolymer. The GC% present in standard DNA as determined using Eq1 was found to be 41.77%.

Further, in order to understand the impact of IL structure on DNA stability and transformation in conformations of DNA in presence of a metal salt as observed at experimental level, the DNA docking binding site of IL cation and anion was analyzed. The pose with the lowest absolute value of affinity (kcal/mol) for DNA with [Choline cation and anion is displayed in Fig. 4].

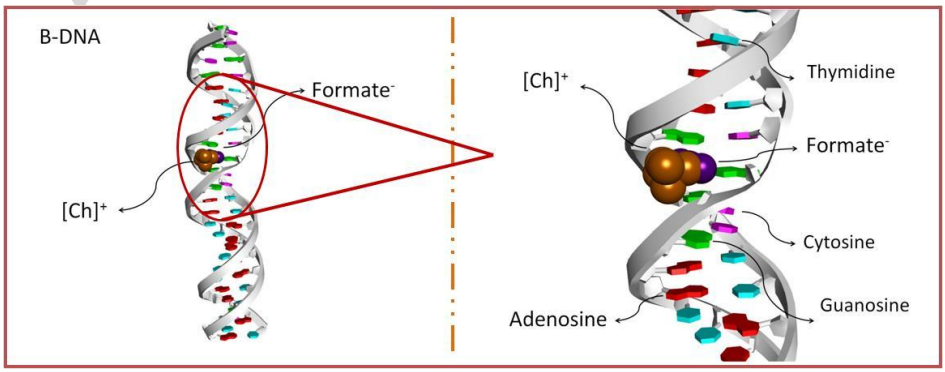


Fig. 4 : The DNA docking pose with the lowest absolute value of affinity (kcal/mol) for DNA with choline formate cation and anion.

The molecular interaction diagrams were displayed aiming to understand the interactions between the DNA and ILs at molecular level (Supporting Figures S 2 and S3). The best binding pose and docking affinities, interacting nucleic acids, type of interaction and geometry distance (Å) of IL anion and cation individually are presented in Table 1. The best B-form DNA pose for the DNA crystal was drawn based on the GC% obtained from the melting point studies (Supporting Figure S1).

Table 1. Docking affinity energy and interacting nucleic acids predicted by AutoDock vinna for DNA-IL [based on interaction shown in Figure S and S].

IL	Affinity (kcal/mol)	Interacting nucleic acids	Type of interaction	From	To	Distance (Å)
$[Ch]^+$	-3.1	Cytosine11	Electrostatic	$[Ch]^+$ - N	Cytosine - O	5.45
			Hydrogen Bond	$[Ch]^+$ - C	Cytosine - O	3.54
				$[Ch]^+$ - C	Cytosine - O	3.50
		Cytosine15		$[Ch]^+$ - H	Cytosine - C	2.33
		Guanosine16		Guanosine - H	$[Ch]^+$ - O	3.04
$[Formate]^-$	-2.3	Guanosine10	Hydrogen Bond	Guanosine -H	$[Formate]^-$ - O	2.05
				$[Formate]^-$ - O	Guanosine - N	3.21
		Cytosine11		$[Formate]^-$ - O	Cytosine - O	3.09
		Guanosine16		Guanosine -H	$[Formate]^-$ - O	2.30

According to the docking simulations of DNA and choline formate as shown in Fig. 4 and calculated based on parameters presented in Table 1, the IL present higher preference for

minor-groove over major-groove binding on DNA structure. This IL presents high ability to promote hydrogen bond with nucleic acids (Table 1, Supporting Fig. S2 and S3). It has been observed earlier that, during the interaction of ILs with DNA phosphate groups, the hydrogen bond and electrostatic interactions appears as major driving force for DNA stability in the ILs. [11] However, as can be seen from Fig. 4, choline formate displays lower ability to stabilize DNA structure. $[Ch]^+$ Interactions with phosphate backbone (N-O) appear to be responsible for this phenomenon. choline formate present different binding sites for IL cation and anion and are more willing to promote torsions on DNA structure leading to possible changes in DNA three dimensional structure inducing B-Z transition. Perhaps, the induced torsion is more prominent in presence of a metal salt and results conformational transformation (B-Z transition).

4. Conclusions

In conclusion, although significant research efforts are being made to understand biopolymer metal interactions but nanoparticle induced conformational change of oligonucleotide in any ionic liquid still not studied. To the best of our knowledge there is no any report available on reduction of silver salt using ionic liquid and DNA induced formation of silver nano particles in an ionic liquid with conformational transitions of the biomolecule. Herein, DNA solubilized in choline formate was found to reduce Ag(I) salt into silver nanoparticles and during the process, the interaction of DNA with the ionic liquid induces alteration in secondary structure of DNA (B-Z transition). Upon molecular docking studies, choline-formate was found to present different binding sites for its cation and anion and they promote torsions on DNA structure leading to possible changes in DNA three dimensional structures (B-Z transition).

Acknowledgement

KP thanks CSIR, New Delhi for overall financial support. JB thanks CSIR for Senior Research Fellowships and Analytical and Environmental Science Division and Centralized Instrument Facility of the Institute is acknowledged for providing instrumentation facilities.

Appendix A. Supplementary data

REFERENCES

[1] J.D. Watson and F.H.C. Crick. *Nature* 171 (1953) 737-738

[2] A. Rich and Z. Shuguang. 2003. Nature 4(2003) 566–573

[3] A. Herbert. *Communications Biology*. 2(2019) . doi : 10.1038/s42003-018-0237

[4] A.R. Lee, N.H. Kim, Y.J. Seo, S.R. Choi, J.H. Lee. Molecules. 23 (2018) doi: 10.3390/molecules23112748.

[5] T. Sunami, T. Chatake and H. Kono. *Acta Crystallographica Section D*. 73(2017) 600-608. https://doi.org/10.1107/S2059798317007707

[6] G.Wang, K.M.Vasquez. *Front Biosci*. 12(2007) 4424-4438

[7] H. Zhao. *J Chem Technol Biotechnol* 90(2015) 19–25.

[8] M. Sharma, D. Mondal, N. Singh, J. Bhatt and K. Prasad. *RSC Advances*, 5(2015) 40546 – 40551

[9] C. Mukesh, D. Mondal, M. Sharma, K. Prasad. *Chem. Commun.* 49(2013) 6849 -6851

[10] D. Mondal, M. Sharma, C. Mukesh, V. Gupta, K. Prasad. *Chem Commun* 49(2013) 9606-9608

[11] N. Singh, M. Sharma, D. Mondal, M.M. Pereira, K. Prasad. *ACS Sustainable Chem. Eng.* 2017, *5,* 1998-2005

[12] I. Gállego, M.A. Grover, N.V. Hud. *Angew Chem Int Ed Engl.* 54(2015)6765-9.

[13] A. Haque, I. Khan, S. I. Hassan, M.S.Khan. *Journal of Molecular Liquids*. 237 (2017) 201-207

[14] R. Wells and S. Harvey, editors. 1989. Unusual DNA Structures. Springer, New York.

[15] A. Ghosh and M. Bansal. *Acta Crystallogr. D*.59 (2003)620–626.

[16] F.M. Pohl, T. M. Jovin. 1972. *J. Mol. Biol*. 67(1972)375–396.

[17] A.K. Nayak, A. Mishra, B.S. Jena, B.K. Mishra, U. Subudhi. *Scientific Reports* 6(2016) 26855.

[18] M. A. Kastenholz, T. U. Schwartz, and P. H. Hünenberger. Biophys J. 91(2006) 2976–2990.

[19] Z. Liu, Y. Zu , Y. Fu, Y. Zhang, H. Liang. *Materials Letters* 62 (2008) 2315–2317

[20] S. Chen, K. Liu, Y. Luo, Y.Wei, F. Li, L. Liu. *Materials Letters* 147(2015)109-112.

[21] J. Liu, X. Zhang, M. Yu, S. Li, J. Zhang. *Small* 8(2012) 310-316.

[22] B. Nithyaja, H. Misha, V.P.N. Nampoori. *Nanoscience and Nanotechnology* 2(2012)99-103

[23] D. Majumdar, A. Singha, P.K. Mondal, S. Kundu. *ACS Appl. Mater. Interfaces* 5(2013) 7798–7807.

[24]T. Takeshima, L. Sun, Y. Wang, Y. Yamada, N. Nishi, T. Yonezawa, B. Fugetsu. *Polymer Journal* 46(2013) 36-41.

[25] M. Harada, M. Yamada, Y.Kimura, K. Saijo. *J Colloid Interface Sci* 406(2013) 94-104.

[26] W. Saenger, Principles of Nuclei Structure; *Springer-Verlag : New York*, 1984.

[27] O. Trott, A. Olson. *J. Comput. Chem*. 31(2010) 455−461.

[28] G. Morris, R. Huey. *J. Comput. Chem*. 30(2009) 2785−2791.

[29] A. Diamantatos. *Analyst*. 111(1986)213-215.

[30] V. S. A. Kiruba, A. Dakshinamurthy, P.S. Subramanian, P.M. Selvakumar. *J Exp Nanosci* 10(2015) 532–544.

[31] M. Cao, M. Deng, X.-L. Wang, Y. Wang. *J. Phys. Chem. B* 112(2008)13648-13654.

[32] J. Zhu, M. Lin, D. Fan, Z. Wu, Y. Chen, J. Zhang, Y. Lu, Z. Guo. *Dalton Trans.* 48(2009)10889-10895.

[33] R. Kelleher, M.J. Ellison, P.S. Ho, A. Rich. *Proc. Natl. Acad. Sci. USA* 83(1986) 6342-6346