Electronic supplemental material

Rapid dissolution of DNA in a novel bio-based ionic liquid with long-term structural and chemical stability: successful recycling of the ionic liquid for reuse in the process

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Experimental Section

Deoxyribonucleic Acid extracted from Salmon testes in the sodium salt form (CAS No. 9007-49-2, ca. 20kbp) 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. [1] Choline hydrogen carbonate and tris (hydroxymethyl) aminomethtane (Tris) was purchased from Sigma-Aldrich. Other chemicals employed were of analytical grade and were used without further purification. Deionized water (conductivity lower than 12.95 $\mu s.cm^{-1}$) was used throughout the experiments. A stock solution of DNA was prepared by dissolving an appropriate amount of the solid DNA in tris-HCl buffer solution and stored at 4 ^{o}C for more than 24 h with occasional gentle shaking to get homogeneity. DNA concentration were determined by using an extinction coefficient of 13200 $M^{-1}.cm^{-1}$ at 260 nm and expressed in terms of base molarity. [2]

The ionic liquids containing indole 3-acetic acid and indole 3-butyric acid and choline (Chol-IAA & Chol-IBA) were synthesized following method reported by Petkovic et al., (2010). [3] In a typical reaction, indole 3-acetic acid or indole 3-butyric acid was dissolved in methanol / water mixture (1:1) and aqueous choline hydrogencarbonate (molar ratio 1:1) was added into the solution under stirring at ambient temperature and pressure. Water was then removed under reduced pressure using a rotary evaporator (*e.g.* 80°C, 2h) (supporting Scheme S1). The ionic liquids were finally washed with ethyl acetate. Structure of the synthesised ILs was confirmed by ^{1}H NMR and electro spray-mass spectrometry (ESI-MS) (supporting Figures S9 & S10).

Agarose gel electrophoresis was carried out with salmon testes DNA (10 µl, 200 µg/ml) and a DNA marker (10 kb) (10 µl). Presence of insoluble DNA particles in the solutions was monitored using ordinary light microscope with 100 X magnification (Fine Vision Microscope, India). Circular dichroism (CD) spectra were recorded on a Jasco model J-815 CD Spectrometer, using measurement range at 230–330 nm and sample concentration of 2 x 10^{-5} M in tris-HCl Buffer (pH = 7.2) at a scanning speed of 10 nm/min and band width of 1 nm. The spectra were acquired in a 1.0 cm path-length quartz cuvette at 25 ^{o}C. The ratio of peak height to trough depth was calculated using Eq. (1), described by Morris, Rees, and Thom (1980). [4]

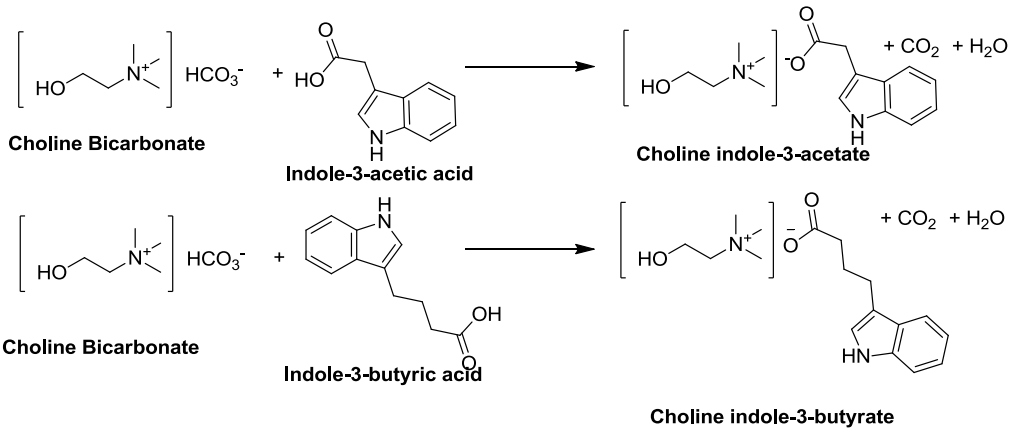
$$\text{Peak / trough ratio} = (\theta_{trough} - \theta_{peak} / \theta_{trough}) \qquad [1]$$

The UV–Vis absorption spectra of standard DNA at concentration of 7.5 x 10^{-5} M and regenerated DNA from chol-IAA (1.02 x 10^{-4} M) and from chol-IBA (1.56 x 10^{-4} M) in tris-HCl buffer (pH = 7.2) were recorded on a Varian CARY 500 UV–Vis-NIR spectrophotometer. Thermo gravimetric (TGA) measurements were carried out on a Mettler Toledo STAR^{e} TGA, Switzerland machine with ionic liquids (10 mg) using a temperature programme 30–600 °C at a heating rate 10 °C min^{-1} in a nitrogen atmosphere. The shear viscosity of DNA solutions was measured on an Anton Paar, Physica MCR 301 rheometer USA, using parallel plate PP50/P-PTD200 geometry (50 mm diameter; 0.1 mm gap). Temperature was maintained at 25 ^{o}C ± 1 ^{o}C by Viscotherm VT2 circulating water bath. The ^{31}P NMR spectra of standard DNA and recovered DNA solution in tris-HCl buffer were recorded at 25 ^{o}C on a Bruker Avance 200 MHz spectrometer. The measurements were carried out with a DDO probe at a resonance frequency of 80.96 MHz. The phosphorous chemical shifts of DNA were externally referenced to 85% o-phosphoric acid.

Dissolution of sodium salt of DNA in ionic liquid

As a typical procedure for the dissolution experiment, consecutive additions of 10 to 45 mg of DNA powders into the vials containing 1 g of solvent (Chol-IAA & Chol-IBA) were done under gentle stirring (1h – 6h) at room temperature (25 ^{o}C) under nitrogen gas atmosphere to ensure good contact between the phases. Once the turbidity or the presence of particles was noticeable by the cloud point method, the samples were equilibrated for at least 24 h to check the disappearance of the cloudiness. Additionally the solubility was further confirmed by monitoring the aliquots of the solutions under ordinary light microscope with 100 X magnification. Zero solubility was considered when the addition of 0.1 wt% of solute showed turbidity and appearance of particles under microscope. DNA was recovered from the above solutions by adding ice cold IPA (IPA to IL = 2:1) followed by addition of ethyl acetate to the rest of the solution to recover the IL as shown in Fig. 1 in the text. DNA was solubilised in the recycled ILs by the same method described above.

1. W. Saenger, Principles of Nuclei Structure; *Springer-Verlag : New York*, 1984.
2. M. E. Reichmann, S. A. Rice, C. A. Thomas and P. Doty, *J. Am. Chem. Soc*., **1954**, *76*, 3047-3053.
3. M. Petkovic, J. L. Ferguson, H. Q. N. Gunaratne, R. Ferreira, M. C. Leit˜ao, K. R. Seddon, L. P. Rebelo and C. S. Pereira, *Green Chem.*, **2010**, *12*, 643–649.
4. E. R. Morris, D. A. Rees and D. Thom, *Carbohydr Res*, **1980**, *81*, 305–314.



Scheme S1 : Synthesis of choline-Indole-3-acetate and choline-Indole 3-butyrate

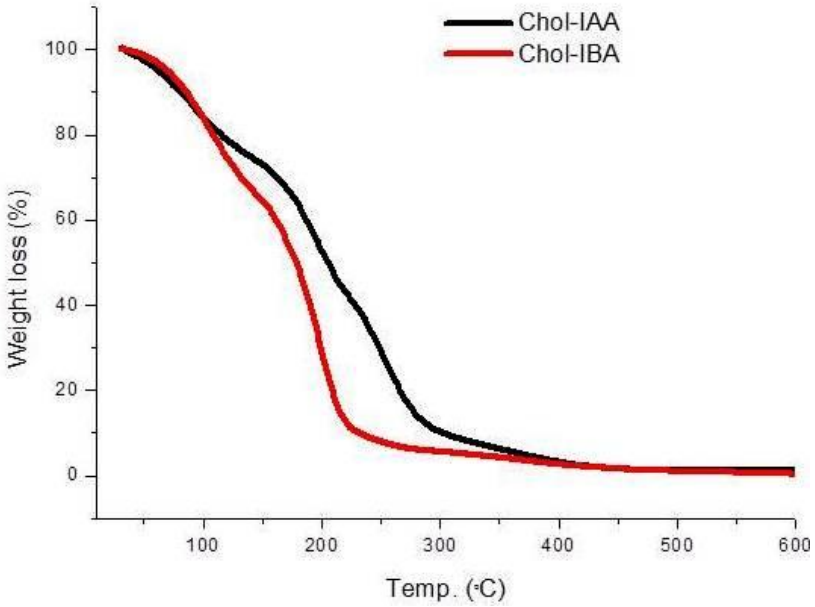


Fig. S1**:** TGA thermogram of chol-IAA and chol-IBA ionic liquids.

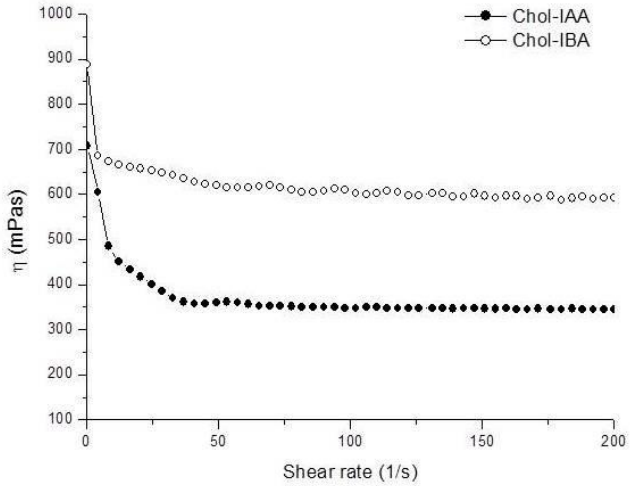


Figure S2 *:* Steady shear viscosity of chol-IAA and chol-IBA ionic liquids.

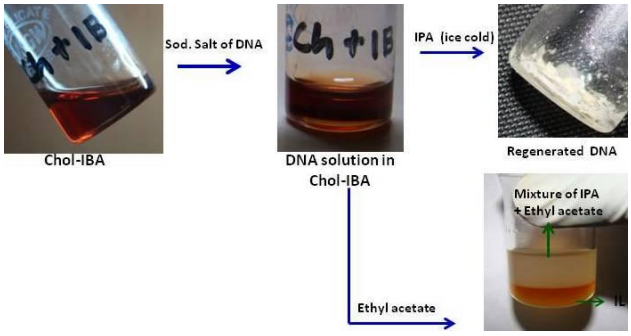


Fig. S3: Dissolution of sodium salt of DNA in chol-IBA. Pictorial demonstration of the recycling of the IL

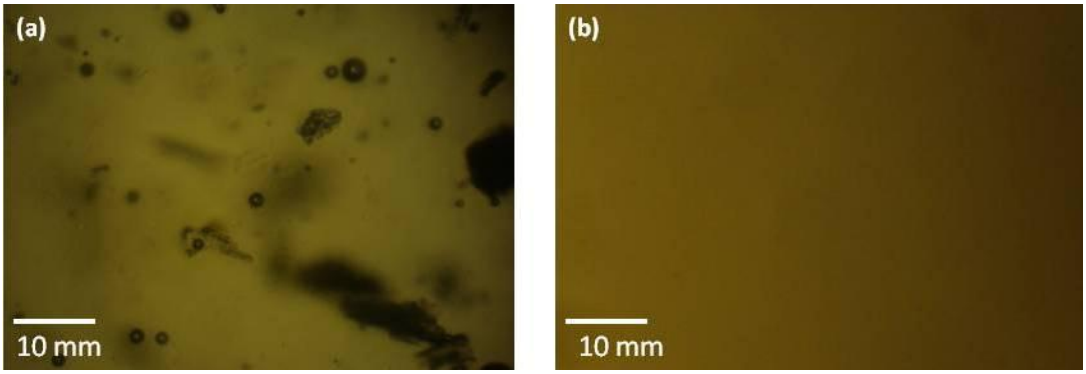


Fig.S4 : Phase contrast microscopic image of DNA in chol-IAA (a) before dissolution and (b) after dissolution

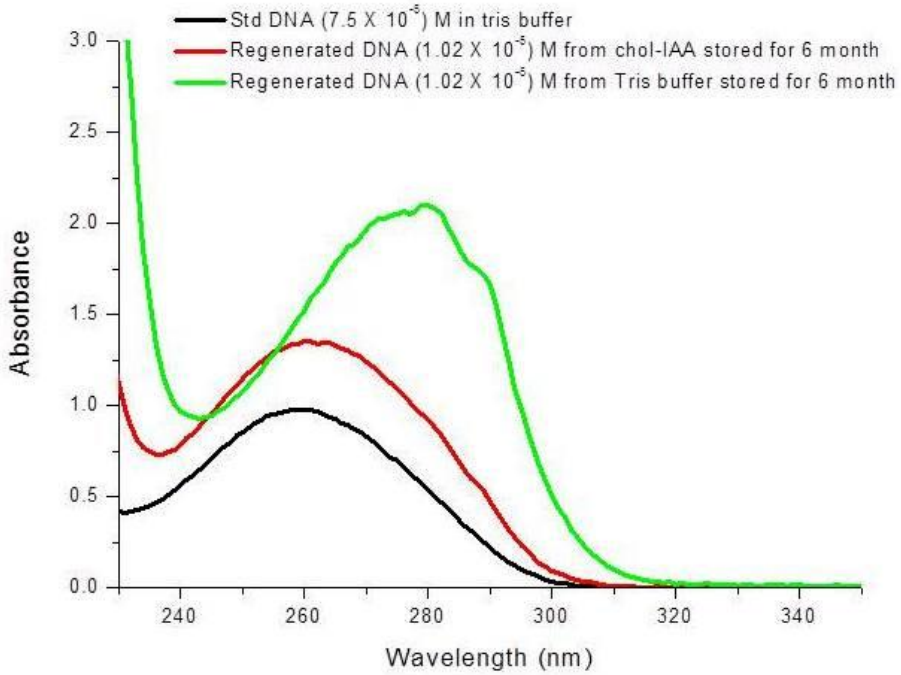


Fig. S5. UV-Vis spectra of regenerated DNA in chol-IAA stored for six months and DNA stored in Tris-HCl buffer at room temperature for six months along with standard DNA in Tris-HCl buffer.

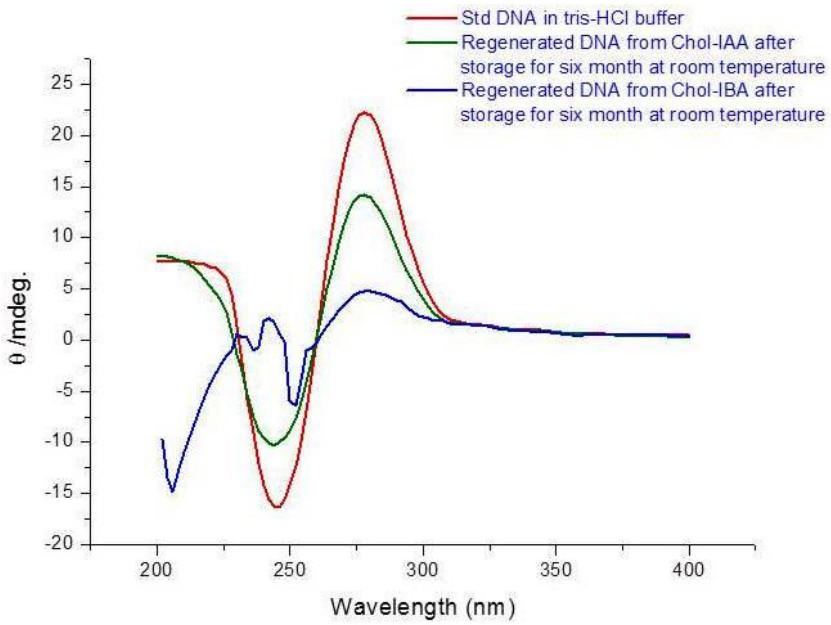


Fig. S6. CD spectra of regenerated DNA in chol-IAA and chol-IBA stored for six months along with standard DNA in Tris-HCl buffer.

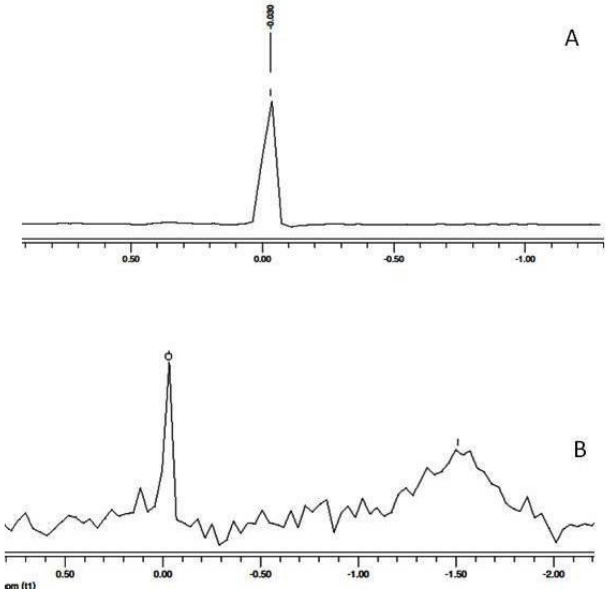


***Fig. S7* :** ^{31}P NMR of (A) ortho phosphoric acid and (B) regenerated DNA from chol-IBA in presence of ortho phosphoric acid.

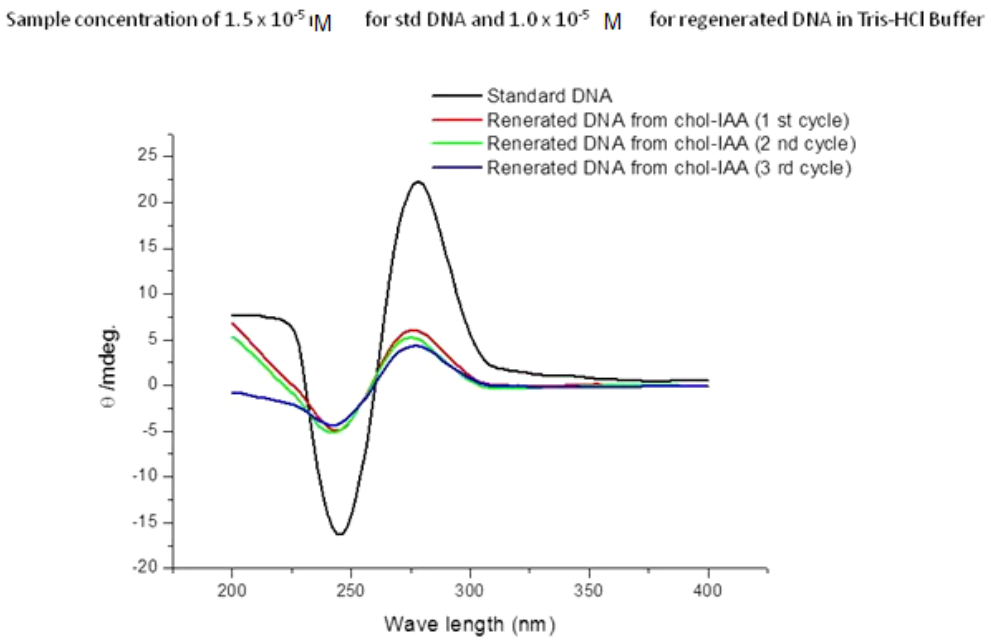


Fig. S8 : Circular dichroism spectra of standard DNA (1.5 X 10^{-5} M) and regenerated DNA (1 X 10^{-5} M) from chol-IAA at 25 ^{o}C after three consecutive dissolution in recycled chol-IAA.

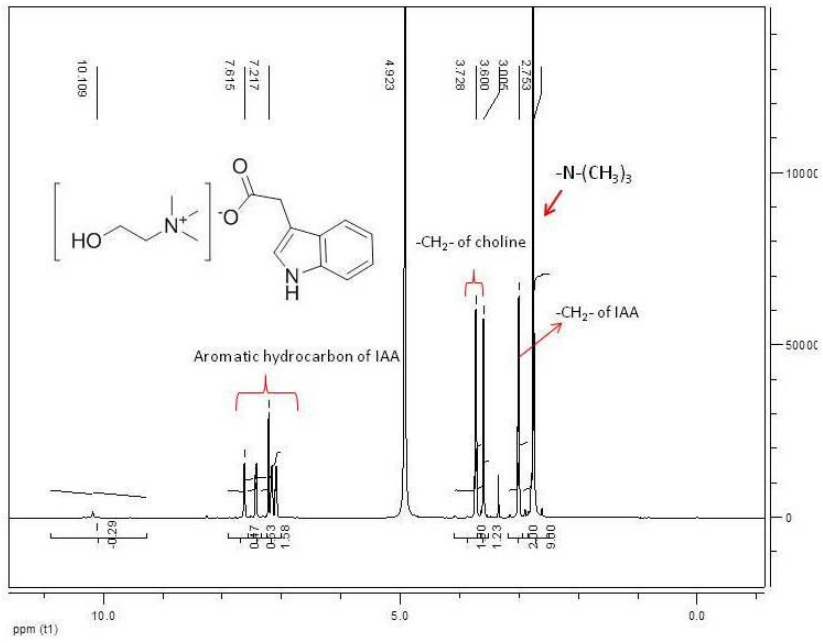


Fig. S9: ^{1}H NMR of choline-IAA

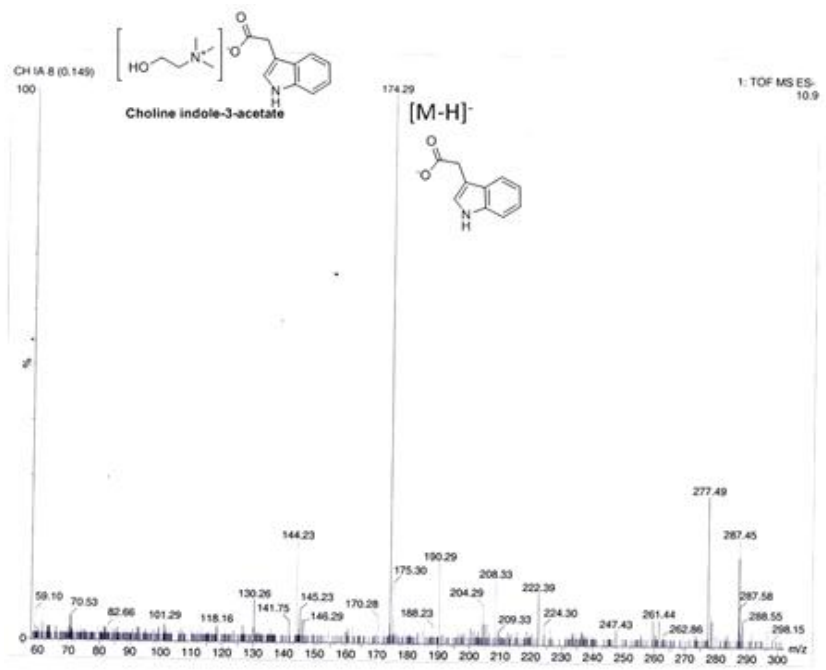


Fig. S10: ESI-MS spectra of chol-IAA.