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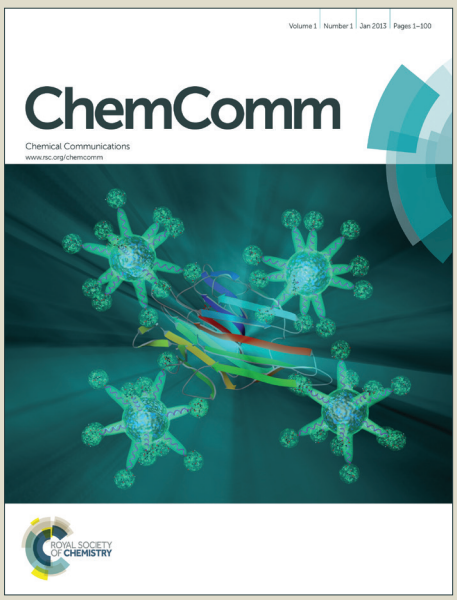

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Communication

A facile approach to prepare dual functionalized DNA based material in a bio-deep eutectic solvent

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DNA (Salmon testes) was functionalized by Fe_3O_4 nanoparticles and protonated layered dititanate sheets ($H_2.Ti_2O_5.H_2O$) in the mixture of choline chloride and ethylene glycol (a deep eutectic solvent) to yield a hybrid material having magnetic and anti bacterial properties. Ti sheets were found to interact with the phosphate moieties, while Fe interacted with the base pair of DNA in the hybrid material.

Unique properties such as nanoscale structural distribution, molecular recognition properties etc., make DNA an interesting candidate for designing novel nano and advanced materials. [1,2] DNA and its hybrid materials are being designed for possible applications in molecular sensing, intelligent drug delivery and programmable chemical syntheses, bioanalysis and biomedicine. [3,4] Research endeavours are being made to prepare composite materials having different inorganic species or molecules associated to DNA. Gold nanoparticles were the first thought to induce nano structural geometry utilizing the molecular recognition properties of DNA.[5,6] The combination of DNA with carbon-based nanomaterials such as carbon nanotubes (CNTs) for the development of novel bio-materials and devices has attracted great attention in the bio-medical field such as gene therapy. [7]

Magnetic particles, magnetic microspheres/nanospheres and ferrofluids are being investigated for their applications in various fields in biology and medicine such as magnetic targeting drugs, genes, radiopharmaceuticals, magnetic resonance imaging (MRI) diagnostics etc..[8] In most of the magnetic nano-composites reported so far, the main component is mostly Fe_3O_4, which is widely used to prepare number of nanocomposite materials. Titanium dioxide (TiO_2) is extensively used in medicine as bio compatible material. TiO_2 nanoparticles of size about 5 nm are known to penetrate through cell membranes. [9,10] Levina *et al*., (2013) discovered a new unique method to show specific effect on nucleic acids in cells using TiO_2-DNA nanocomposite materials.[11]

We have recently demonstrated solubilization of DNA in bio-ionic liquids and deep eutectic solvents (DESs) with long term chemical and structural stability of the macromolecule. [12,13] The DES consisting of the mixture of choline chloride and ethylene glycol (choCl-EG 1:2) was able to solubilize DNA upto 5.5% *w/w*. [13] Considering the ability of this DES for DNA solubilization, herein we have studied suitability of the solvent for dual functionalization of DNA with Fe_3O_4 and $H_2.Ti_2O_5.H_2O$ (protonated layered dititanate sheets). The dititanate sheets were synthesized following literature procedure (ESI†).[14] The DES thus used was recycled and reused in the process.

2.5% *w/w* of DNA (salmon testes) was solubilized in choCl-EG 1:2 as shown in Fig.1 followed by the addition of Fe_3O_4 and $H_2.Ti_2O_5.H_2O$. The DNA hybrid material thus formed was isolated by precipitating in isopropyl alcohol (IPA). Total time required for obtaining the hybrid material was 6 h. IPA was evaporated from the mixture of DES and IPA and the DES was recovered. Absence of impurities in the DES was confirmed by ^{1}H NMR measurements. 2.5% *w/w* DNA could be resolubilized in the recycled DES and the hybrid material could again be prepared following the same methodology (Fig.1). The DNA solution obtained upon addition of Fe_3O_4 was magnetic in nature and ordered arrangement of the particles can be seen under optical microscope (ESI †, Fig. S1). The solution upon addition of $H_2.Ti_2O_5.H_2O$ partially lost the magnetic characteristics, however the hybrid material obtained after IPA precipitation exhibited magnetic property. The interaction of DNA with these two different types of metal ions was further studied.

Fig. 1: Pictorial demonstration for the preparation of DNA based hybrid material in chol.Cl-EG 1:2 as well as recyclability and reusability of the deep eutectic solvent.

The FT-IR bands at 1073 and 1223 cm^{-1} are assigned to the symmetric and anti symmetric stretching vibrations of the PO_2^- groups of DNA, and that at 1713 cm^{-1} is due to the in plane C=O and C=N stretching vibrations of the bases. [15] The spectral region 1750-1550 cm^{-1} is considered for base pairing and base stacking. [16] The anti symmetric band due to PO_2^- groups of the DNA was found to be affected in the case of the mixture of DNA and $H_2.Ti_2O_5.H_2O$ (Fig. 2c) indicating interaction of the dititanate sheets with the phosphate side chains of the DNA. Similar trend was observed in the case of the hybrid material (Fig. 2d). However in the case of the mixture of DNA and Fe_3O_4 and the hybrid, the band due to phosphate was not affected but the bands due to base pairs were found to be partially affected (Fig. 2b) indicating interaction of the base pair of DNA with Fe. Moreover, the intact bands at 1713 cm^{-1} is due to the in plane C=O and C=N stretching vibrations of the bases of DNA in the hybrid material indicated chemical stability of the bio macromolecule in the hybrid material. Powder XRD of Fe_3O_4 recovered from choCl-

EG 1:2 showed crystalline peaks at 2θ of 36°, 57 ° and 63 °, while $H_2.Ti_2O_5.H_2O$ showed a crystalline peak at 26 ° (ESI †, Fig. S2). [14] Standard DNA showed an amorphous XRD profile and DNA + Fe_3O_4 mixture recovered from the DES showed predominantly amorphous characteristics with little crystallinity due to Fe (ESI †, Fig. S3). The hybrid material showed the similar trend indicating attachment of both the metals in the DNA backbone.

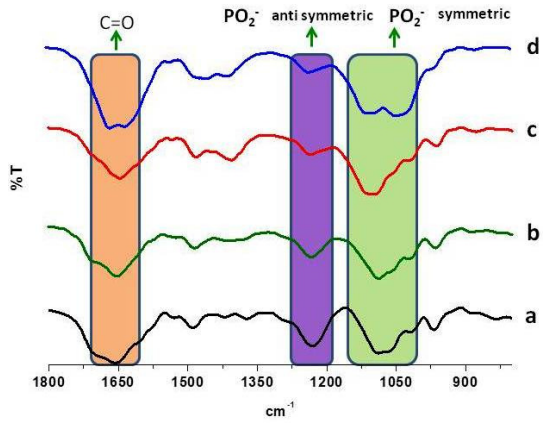


Fig. 2: FT-IR spectra of (a) Standard DNA (b) recovered DNA + Fe_3O_4 mixture (c) recovered DNA + $H_2.Ti_2O_5.H_2O$ mixture and (d) DNA based hybrid material.

The SEM image of the hybrid material showed morphology that resembles the $H_2.Ti_2O_5.H_2O$ sheets indicating attachment of this metal on the periphery of DNA i.e., phosphate groups (Fig. 3a). SEM-EDX measurements indicated presence of Ti, Fe in the composite along with phosphorous, carbon and oxygen confirmed formation of the hybrid by the interaction of DNA with Ti and Fe (ESI †, Fig. S4). Further to establish the interaction sites, HR-TEM images were recorded for the hybrid material. Ti particles could be seen attached to the phosphate imprints of the DNA molecule (Fig. 3b), while Fe particles were found to be distributed towards the centre of the DNA molecule (Fig. 3b) indicating binding of Fe particles to the DNA base pair. The morphology of the metal ions were compared those with pristine metal ions (ESI †, Fig. S5).

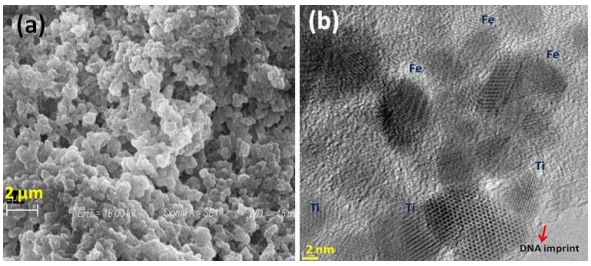


Fig. 3: (a) SEM and (b) HR-TEM image of dual functionalized DNA hybrid material.

The three dimensional surface morphology of the dual functionalized hybrid material was further investigated by AFM (Fig. 4). The DNA regenerated from the deep eutectic solvent was found to have double stranded structure with inter helical distance of ca. 2 µm (Fig. 4a). The Fe_3O_4 particles of height 25 nm were found to have orderly arranged in the hybrid (Fig. 4e). The periphery of the DNA consisting of phosphate showed presence of metal ions, which may be the Ti sheets (Fig. 4c & 4d).

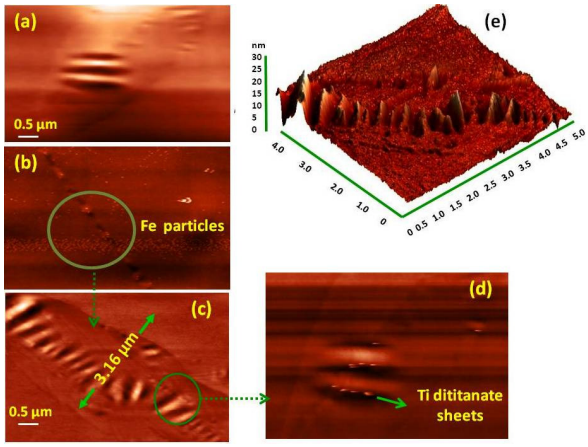


Fig.4 : 2D and 3D AFM image of dual functionalized DNA hybrid material.

Chemical and structural stability of DNA is very important for its molecular recognition. The standard DNA in Tris-HCl buffer showed UV-Vis absorption patterns with ratio of absorbance at 260 to 280 nm being 1.89. The UV-Vis spectra of the regenerated mixture of DNA and Fe_3O_4 (A_{260}/A_{280} = 1.86), regenerated mixture of DNA and $H_2.Ti_2O_5.H_2O$ (A_{260}/A_{280} = 1.90) as well the regenerated hybrid material (A_{260}/A_{280} = 1.86) in tris-HCl buffer showed similar UV spectral pattern in comparison to standard DNA indicating preservation of the double helical structure of the macromolecule in the hybrid material (ESI †, Fig. S6). The circular dichroism (CD) spectrum of standard DNA at 25 °C showed a long wave positive band at 278 nm corresponding to π-π base packing and a short wave negative band at 243 nm corresponding to helicity (Fig. 5) and characteristics of B-form of DNA. [17] The regenerated mixture of DNA and Fe_3O_4, DNA and Ti complex and the hybrid material showed similar CD spectra (Fig. 5) indicating preservation of structural and chemical stability of DNA in the hybrid material.

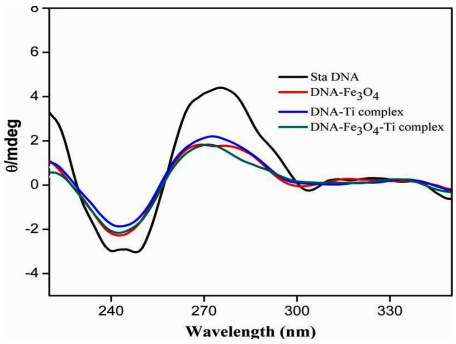


Fig. 5: CD spectra of dual functionalized DNA hybrid material along with regenerated mixture of DNA (1.46 x 10^{-5} M) with Fe_3O_4 and $H_2.Ti_2O_5.H_2O$.

^{31}P NMR has been applied to characterize normal phosphates fragments of the DNA. The chemical shift of the normal phosphate group of standard DNA was observed at -1.230 ppm (Fig. 6a) and the phosphate group showed the similar chemical shift in the mixture of DNA and Fe_3O_4 (ESI†, Fig.S7) indicated that, phosphate groups of DNA did not interact with Fe. On the other hand the phosphate groups showed chemical shift of -1.123 ppm in the mixture of DNA and $H_2.Ti_2O_5.H_2O$ indicated interaction of phosphate with Ti sheets (ESI†, Fig.S8). The

phosphate showed chemical shift value of -1.167 ppm in the hybrid material (Fig. 6b).

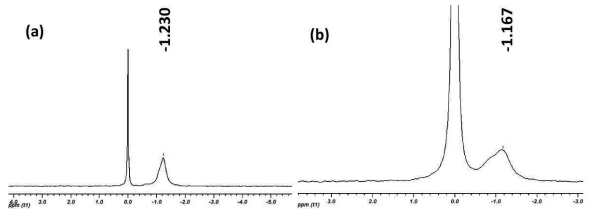


Fig. 6: ^{31}P NMR spectra of (a) standard DNA and (b) dual functionalized DNA hybrid material

The bacterial growth inhibition with dual fuctionalized DNA hybrid material was studied in gram positive and gram negative bacteria. As shown in ESI †, Table S1, when 5 mg/mL of the hybrid material was applied to each bacterial culture, after 4 h of incubation it showed growth inhibition in all the four bacteria in comparison to the control sample (without hybrid material) (Fig. 7). Colony formation unit (CFU) was calculated after overnight incubation at 37 °C and result in CFU/mL (mean± SD) was calculated. In LB agar plate, difference of one logarithm was observed in CFU of *P. fluorescens*, *S. fexineri* and *B. subtilis* containing 5 mg/mL of hybrid material in comparison to the control sample. No bacterial colony was observed after overnight incubation in comparison to the control sample in *E.coli* (ESI †, Table S1). Further, the LB containing 4 mg/mL DNA did not inhibit growth of the bacterial colonies, which confirms that antibacterial activity was the signature of the hybrid material formed by the dual functionalization of the DNA. Microscopic images of all the bacteria culture showed distinct differences in the morphology of the colonies after treatment with the hybrid material (ESI †, Fig. S9). Visible cell rupture was observed in the gram stained bacterial colonies of all the bacteria.

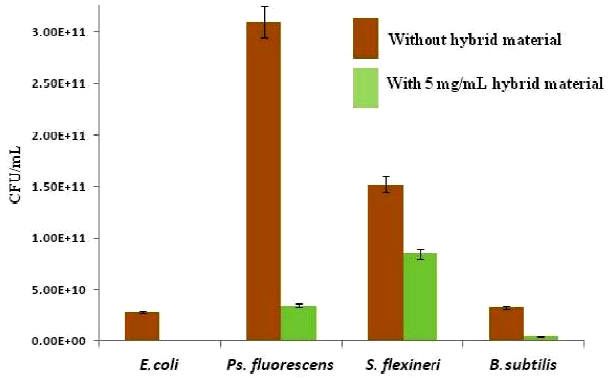
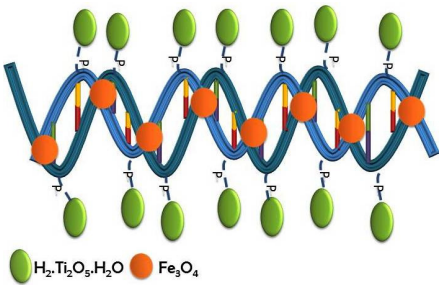


Fig.7- Colony Formation Unit (CFU) per mL of *Escherichia coli, Pseudomonas fluorescens, Shigella flexineri* and *Bacillus subtilis* in the LB agar.



Scheme 1: Schematic representation of dual functionalized DNA hybrid material.

In summary dual functionalization of DNA (salmon testes) by Fe_3O_4 nanoparticles and protonated layered dititanate sheets ($H_2.Ti_2O_5.H_2O$) in choline chloride-ethylene glycol (a deep eutectic solvent) resulted formation of a hybrid material having magnetic and antibacterial property. The studies done employing various analytical tools confirmed interaction of dititanate sheets with the phosphate moieties and interaction of Fe with the base pair of the DNA as shown in Scheme 1. The structural and chemical stability of DNA in the hybrid material was also established. This type of material would be used in biomedical for diagnostics applications and as biosensors.

Notes & References

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1. T. H. LaBean and H. Li., *Nano today*, **2007**, *2*, 26.
2. W. Saenger, *Principles of Nucleic acid structure*, Springer-Verlag, *New York*, 1984.
3. J. Bath and A. J. Turberfield, *Nature Nanotechnol,* **2007**, *2*, 275
4. L Peng , C. S. Wu , M. You , D. Han , Y. Chen , T. Fu , M. Ye and W. Tan, *Chem. Sci*., **2013**,*4*, 1928
5.C.M. Nimeyer, *Angew. Chem., Int. Ed*. **1997**, *36*, 585-587
6. C.A. Mirkin, R.L. Letsinger, R.C. Mucic, J.J. Storhoff, *Nature*, **1996**, *382*, 607
7. B. S. Harrison, A. Atala, *Biomaterials* **2007**, *28*, 344 – 353.
8. U. Häfeli, W. Schütt, J. Teller, (1997). Scientific and clinical applications of magnetic carriers, 1st edn. Plenum, New York
9. H. Suzuki, T. Toyooka, Y. Ibuki, *Environ. Sci.Technol.,* **2007**, 41, 3018–3024.
10. V.F. Zarytova, V.V. Zinov'ev, Z.R. Ismagilov, A.S. Levina, M.N. Repkova, N.V. Shikina, A.A. Evdokimov, E.F. Belanov, S.M. Balakhnin, O.A. Serova, S.I. Baiborodin, E.G. Malygin, S.N. Zagrebel'nyi, *Ross.Nanotekhnol.*, **2009**, *4*, 115–118.
11. A. S. Levina, Z. R. Ismagilov, M. N. Repkova*a,* N.V. Shikina, S. I. Baiborodin*d*,N. V. Shatskaya*a,* S. N. Zagrebelnyi*b*, and V. F. Zarytova, Bioorg. Khim, 2013, 39, 78-86
12. C. Mukesh, D. Mondal, M. Sharma, K. Prasad, K. C*hem. Commun.***2013**, *49*, 6849-6851.
13. D. Mondal, M. Sharma, C. Mukesh, V. Gupta, K. Prasad. *Chem. Commun.*, **2013**, 49, 9606-9608
14. N. Sutradhar, A. Sinhamahapatra, S. K. Pahari, H.C. Bajaj, A. B. Panda, *Chem.Commun.* **2011**, *47*, 7731-7733
15. S. L. Lee, P. G. Debenedetti, J. R. Errington, B. A. Pethica, D. J. Moore, *J. Phys. Chem. B,* **2004**, *108*, 3098.
16. J. A. Taboury, J. Liquier, E. Taillandier, *Can. J. Chem.*, 1985, **63**, 1904.
17. M. Cao, M. Deng, X. L. Wang and Y. Wang, *J. Phys. Chem. B*, **2008**, *112*, 13648