COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Magnetic nanoparticle assemblies on denatured DNA show unusual magnetic relaxivity and potential applications for MRI†

Stephen J. Byrne,[a] Serena A. Corr,[a] Yurii K. Gun'ko,*[a] John M. Kelly,*[a] Dermot F. Brougham*[b] and Swapankumar Ghosh[b]

[a] *The Department of Chemistry, Trinity College, University of Dublin, Dublin 2, Ireland. E-mail: igounko@tcd.ie*

[b] *National Institute for Cellular Biotechnology, School of Chemical Sciences, Dublin City University, Dublin 9, Ireland*

Received (in Cambridge, UK) 25th June 2004, Accepted 18th August 2004
First published as an Advance Article on the web 27th September 2004

Denatured (substantially single-stranded) herring sperm DNA acts as a template for the preparation of magnetic nanowires, forming stable aqueous suspensions, which exhibit unprecedentedly high relaxivity at low field, suggesting that the material may be a potentially useful reagent for MRI.

The preparation of uniformly dispersed colloidal ferrofluids of particle sizes in the range of 10 nm continues to be of interest for many areas of technology including the biosciences.[1] Particles of this size are referred to as "superparamagnetic", as each particle possesses a constant magnetic dipole moment proportional to its size, is attracted to a magnetic field, but retains no residual magnetism after the field is removed.[2] This aspect of the particles' magnetism is important for their use as MRI contrast agents, where signal intensity relies on the effect of the magnetic particles on the NMR signal of water in the tissues being imaged.[3] While there are some obstacles to the clinical application of colloids, materials with high relaxivity at low field will become important with the advent of low-field MRI.[4] One approach to obtaining such materials is to prepare conjugates with biomolecules. As a consequence of its nanometre-sized cross-section, functional groups, and regular structure, DNA can be used to construct assemblies of nanoparticles.[5–8] Indeed previously Mornet *et al.* have reported the association of magnetite nanoparticles with double stranded DNA,[9] while the use of DNA-modified magnetic nanoparticles as recognition devices has been studied.[10] In this paper, we report that the use of denatured DNA as both a surfactant and a template allows the preparation and organisation of magnetite nanoparticles, yielding materials with a remarkably high relaxivity at low field.

Magnetite nanoparticles were prepared by reacting a mixture of ferrous and ferric chlorides (2 : 1 molar ratio; 2×10^{-2} mol dm^{-3} and 1×10^{-2} mol dm^{-3} respectively) with ammonia in a degassed aqueous solution at room temperature[11] containing herring sperm-DNA (hs-DNA), either natural (double-stranded) or after heat-induced denaturation (substantially single-stranded) [1.7×10^{-3} mol dm^{-3} nucleotide].‡ This controlled precipitation method leads to the formation of magnetite nanoparticles (as shown by XRD, IR and Raman spectroscopy†). Transmission electron microscopy (TEM) revealed that in each case the Fe_3O_4 nanoparticles have a size of 9.0 $\pm$ 2 nm (estimated for a sample of 100 particles). TEM also demonstrated that denatured hs-DNA–magnetite nanocomposites (Fig. 1(a)) were comprised of randomly distributed chains whereas by contrast, in the double-stranded DNA–magnetite samples the chains are much more entangled (Fig. 1(c)).

To test whether these materials could be further aligned by exposure to a magnetic field, 20 μL samples of the denatured or double-stranded DNA nanocomposites dispersed in deoxygenated water were placed on copper TEM grids and then introduced perpendicular to a magnetic field of 7 T for 30 minutes. These were then removed and allowed to dry in air at room temperature (~18 hours). TEM showed that this treatment caused the single-stranded DNA sample to align in an end-to-end fashion, forming ordered ropes of many microns long (Fig. 1(b)) (each rope appears to be composed of bundles of smaller chains of magnetite arranged on the DNA template). By contrast, TEM shows that the magnetic field has very little effect for either the duplex-DNA nanocomposites (Fig. 1(d)) or the nanoparticles prepared by controlled precipitation in the absence of DNA.† This markedly differing behaviour of the duplex and denatured DNA may be attributed to a more efficient binding of the magnetic nanoparticles to the phosphate backbone of the single-stranded DNA. This was confirmed by IR studies as a band attributable to a Fe–O–P stretch is observed at 1157 cm^{-1}.[12]

The effect of these nanoparticle composites on the water proton spin–lattice relaxation time T_1 has been measured by NMR dispersion (NMRD), allowing the determination of the frequency dependence of the relaxivity r_1 *via* eqn. (1) {where $T_{1(water)}$ is the native relaxation time of the supporting fluid (water) and r_1 is independent of the concentration of the magnetic fluid}.[3a]

$$R_{1(obs)} = \frac{1}{T_{1(obs)}} = \frac{1}{T_{1(water)}} + \frac{1}{T_{1(para)}} = \frac{1}{T_{1(diam)}} + r_1[\mathrm{Fe}] \qquad (1)$$

For complex particulate systems, it has been established that the contributions to the overall relaxivity from magnetic material in different components of the suspension is often additive.[13] In such

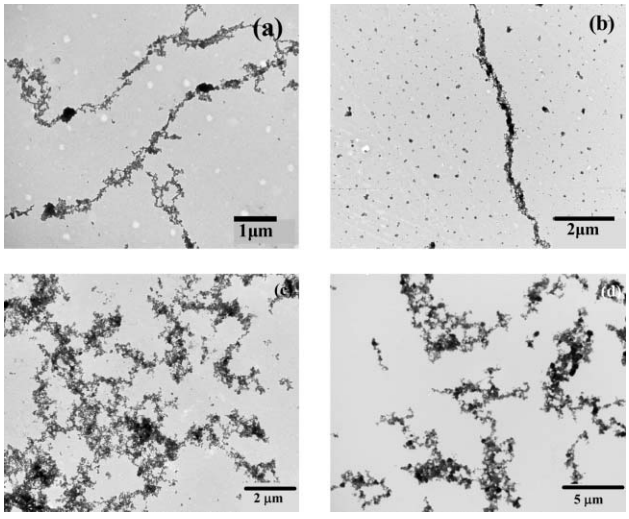

Fig. 1 Representative TEM images of denatured hs-DNA–magnetite nanocomposites (a) without and (b) with subjection to a magnetic field of 7 T; (c) double-stranded hs-DNA–magnetite nanocomposites without and (d) with subjection to a magnetic field of 7 T (see ESI for further TEM images†).

† Electronic supplementary information (ESI) available: NMRD experimental details, TEM images, XRD data, IR and Raman spectra. See http://www.rsc.org/suppdata/cc/b4/b409603g/

DOI: 10.1039/b409603g

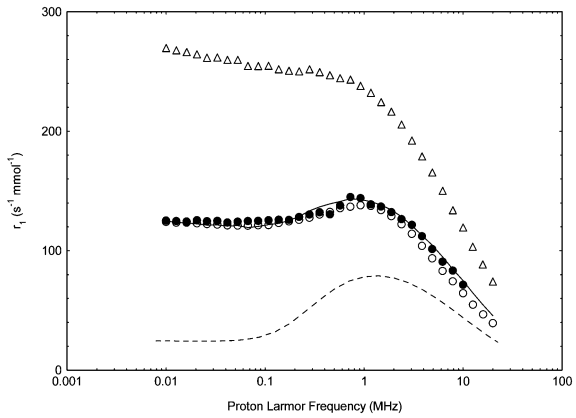

Fig. 2 NMRD relaxation curve recorded at a measuring frequency of 9.25 MHz for (a) (Δ) denatured hs-DNA–magnetite nanocomposites; (b) (●) double-stranded hs-DNA–magnetite nanocomposites; (c) (——) simulated curve for a two component system (see text below); (d) (– – –) simulated curve for a distribution of superparamagnetic particles of magnetite of diameter 9 ± 2 nm and (e) (○) double-stranded salmon sperm-DNA–magnetite nanocomposites. All measurements were carried out at 25 ± 1 °C. All measured NMRD curves were stable; errors in r_1 are 1%, with repeat preparations giving the same r_1 to 3%.

cases the paramagnetic contribution to the relaxivity is given by eqn. (2):

$$r_1 = \sum_i x_i r_{1i} \quad (2)$$

(x_i is the mole fraction of Fe in component i, with relaxivity r_{1i}.)

The NMRD curves obtained for both DNA composites differ dramatically from that expected for a purely superparamagnetic magnetite sample, which can be successfully predicted by the theory[3a,b] developed previously for dispersed magnetite suspensions (Fig. 2(d)). In particular the relaxivity at low field in both cases, but especially for the single-stranded sample, is extraordinarily high. The denatured DNA nanocomposites (Fig. 2(a)) show an unusual dual power law dependence of T_1 on frequency, which is not consistent with the predictions of outer sphere theory.[14] We propose that the behaviour of the two different DNA-stabilised systems (Figs. 2(a) and 2(b)) can be explained on the basis that the denatured DNA material is composed of 'magnetically aggregated' particles of relaxivity r_{1agg} while the double-stranded DNA composite also contains 'magnetically dispersed' superparamagnetic particles. The solid curve (Fig. 2(c)) is a fit to the data for the double-stranded hs-DNA sample (Fig. 2(b)) based on eqn. (2). We assume a two-phase system, using r_{1spm} taken from the simulated NMRD response (Fig. 2(d)) and r_{1agg} from the denatured DNA nanocomposites and further postulating that the relaxation due to the dispersed and aggregated populations is additive ($x_{spm} = 0.59$ and $x_{agg} = 0.41$). The materials therefore have a larger local magnetic moment, consistent with improved magnetic ordering and higher relaxivity. The differing behaviour of the two types of DNA samples may be attributed to the greater binding efficiency of the single-stranded segments of the denatured DNA which results in higher concentration of the magnetite particles. Also included (Fig. 2(e)) are data for an analogously prepared double-stranded salmon-sperm-DNA–magnetite nanocomposite, which exhibits very similar two-phase behaviour. For this sample the r_1 maximum due to the superparamagnetic fraction is more apparent.

In summary, we have successfully prepared long-range ordered chains of denatured hs-DNA–magnetite nanocomposites. TEM analysis and spectroscopy results are consistent with magnetite nanoparticles bound to the DNA phosphate groups, acting as a cross linker between strands, thereby increasing the chain length to micron size. It is found that, in contrast to the behaviour with duplex DNA, the denatured hs-DNA–magnetite nanocomposites can be aligned in a magnetic field. The denatured DNA composites also exhibit an unprecedented dual power law dependence of the water T_1 on frequency and a remarkably high relaxivity at low field. This suggests that such materials might be potentially useful for low field MRI.[4] Future work will include a study of these particles bound to synthetic DNA of defined sequence and varying length and a detailed examination of the causes of the very high relaxivity.

We thank Dr John O'Brien for assistance with magnetic field trials, Sean Quilty of Particular Sciences Ltd. for performing PCS measurements, Dr Alain Roch and Prof. Robert Muller for helpful discussions. This work is supported by the Enterprise Ireland Research Innovation Fund (IF-2001-364) and HEA PRTLI programme.

Notes and references

‡ *Experimental procedure:* the sodium salt of herring sperm (hs) DNA (0.06 g in 100 mL autoclaved water) purchased from Aldrich was denatured by boiling to 100 °C. The extent of denaturation was monitored *via* the UV absorbance of the DNA solutions. Both double-stranded and denatured DNA solutions were added directly to a solution of ferric and ferrous salts in autoclaved water [$FeCl_3$, 2×10^{-2} mol dm^{-3}; $FeCl_2$, 1×10^{-2} mol dm^{-3}]. The magnetite nanoparticles are formed *via* addition of ammonia solution until a pH of 9 is reached. The resultant magnetic precipitate was then washed several times with autoclaved, double distilled water and dried once a neutral pH was achieved.

1 (*a*) B. M. Berkovsky, V. F. Medvedev and M. S. Krokov, *Magnetic fluids: Engineering Applications*, Oxford University Press, Oxford, 1993; (*b*) B. Bonnemain, *J. Drug Targeting*, 1998, **6**, 167; (*c*) A. Wooding, M. Kilner and D. B. Lambrick, *J. Colloid Interface Sci.*, 1991, **144**, 236; (*d*) K. Butter, P. H. H. Bomans, P. M. Frederik, G. J. Vroege and A. Philipse, *Nat. Mater.*, 2003, **2**, 88.
2 H. E. Horng, C.-Y. Hong, S. Y. Yang and H. C. Yang, *J. Phys. Chem. Solids*, 2001, **62**, 1749.
3 (*a*) R. N. Muller, A. Roch, J.-M. Colet, A. Ouakssim and P. Gillis, *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, ed. A. E. Merbach and E. Toth, John Wiley and Sons Publishers, New York, 2001, p. 417; (*b*) A. Roch, R. Muller and P. Gillis, *J. Chem. Phys.*, 1999, **110**, 5403; (*c*) D. K. Kim, Y. Zhang, J. Kehr, T. Klason, B. Bjelke and M. Muhammed, *J. Magn. Magn. Mater.*, 2001, **225**, 256.
4 (*a*) G. Navon, Y. Q. Song, T. Room, S. Appelt, R. E. Taylor and A. Pines, *Science*, 1996, **271**, 1848; (*b*) C. H. Tseng, G. P. Wong, V. R. Pomerpy, R. W. Mair, D. P. Hinton, D. Hoffmann, R. E. Stoner, F. W. Hersman, D. G. Cory and R. L. Walsworth, *Phys. Rev. Lett.*, 1998, **81**, 3785.
5 N. C. Seeman, *Nature*, 2003, **421**, 427.
6 H. Yan, T. H. LaBean, L. Feng and J. H. Reif, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 8103.
7 M. G. Warner and J. E. Hutchison, *Nat. Mater.*, 2003, **2**, 272.
8 (*a*) J.-M. Nam, C. S. Thaxton and C. A. Mirkin, *Science*, 2003, **301**, 1884; (*b*) J.-M. Nam, S.-J. Park and C. A. Mirkin, *J. Am. Chem. Soc.*, 2002, **124**, 3820.
9 S. Mornet, A. Vekris, J. Bonnet, E. Duguet, F. Grasset, J.-H. Choy and J. Portier, *Mater. Lett.*, 2000, **42**, 183.
10 L. Josephson, J. M. Perez and R. Weissleder, *Angew. Chem., Int. Ed.*, 2001, **40**, 3204.
11 (*a*) R. Massart, *IEEE Trans. Magn.*, 1981, **17**, 1247; (*b*) X.-P. Qiu, *Chin. J. Chem.*, 2000, **18**, 834.
12 K. V. P. M. Shafi, A. Ulman, X. Yan, N.-L. Yang, C. Estournès, H. White and M. Rafailovich, *Langmuir*, 2001, **17**, 5093.
13 A. Ouakssim, A. Roch, C. Pierart and R. N. Muller, *J. Magn. Magn. Mater.*, 2002, **252**, 49.
14 J. H. Freed, *J. Chem. Phys.*, 1978, **68**, 4034.