Magnetite ferrofluids stabilized by sulfonato-calixarenes†

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Magnetite (Fe_3O_4) nanoparticles stabilised by sulfonato-calixarene macrocycles are readily accessible by a rapid *in situ* co-precipitation, and exhibit ferro-fluidic and superparamagnetic behaviour.

Superparamagnetic magnetite (Fe_3O_4) nanoparticles have been widely studied for various scientific and technological applications such as magnetic storage media,[1] contrast agents for magnetic resonance imaging (MRI),[2] biolabelling and separation of biomolecules,[3] and magnetic targeted drug delivery.[4] All these applications require the magnetic nanoparticles to be chemically stable, have particle size <20 nm with a narrow size distribution, and to be well dispersed in aqueous medium.[5] Preparation of stable magnetic nanoparticles is a challenge as the particles have large surface area to volume ratios and thus they tend to aggregate to reduce their surface energy. In addition, there are strong magnetic dipole–dipole attractions between the particles that also cause the particles to aggregate.

Tetramethylammonium hydroxide (TMAOH) is well known as a surfactant to stabilize such magnetite nanoparticles.[6] However, TMAOH is highly basic and is not biocompatible. Stabilization of magnetic nanoparticles can also be achieved by coating the particle surfaces with organic surfactants or polymers. Oleic acid[7] and lauric acid[8] are commonly used for this purpose. Khalafalla and Reimers[9] and Wooding *et al.*[10] have stabilised suspensions of magnetite using various saturated and unsaturated fatty acids as primary and secondary surfactants. Synthetic polymers and biopolymers have also been used to coat and stabilize magnetic nanoparticles. Examples of the former include poly(vinyl alcohol),[11] poly(acrylic acid),[12] and triblock co-polymers (PEO–COOH–PEO).[13] Among the biopolymers used to stabilize magnetite nanoparticles are dextran[14] and alginic acid.[15] Most of this work involves adding the surfactant to the preformed magnetite nanoparticles.

One of the drawbacks of the above approach is that the particle size distribution is difficult to control, and the process is time-consuming requiring further work up. On the other hand, the synthesis of magnetite nanoparticles in the presence of a surfactant can circumvent aggregation and control the size of the nanoparticles. Yaacob *et al.* have prepared magnetite nanoparticles at room temperature <15 nm in diameter with a narrow size distribution of the particles which are inside vesicles based on a variety surfactants, namely cetyltrimethylammonium bromide (CTAB) and dedecylbenzenesulfonic acid (DBSA).[16]

p-Sulfonato-calix[*n*]arenes are cyclic phenolic oligomers with a hydrophobic cavity, which can form host–guest inclusion complexes in a similar way to cyclodextrins. Such water soluble calixarenes display interesting biological properties such as anti-viral and anti-bacterial activity,[17] and form inclusion complexes with a variety of small molecules.[18] Complexes with hydrophobic drugs impart increased solubility of the drug molecules in aqueous medium.[19] Complexation of Bovine Serum Albumin (BSA), an arginine- and lysine-rich protein, with sulfonato calixarenes has been demonstrated by Memmi *et al.*[20] Furthermore, both *in vitro* and *in vivo* toxicity studies show that sulfonato-calixarenes have low toxicity.[21] Overall, *p*-sulfonato-calixarenes have potential for biomedical applications, and in this context we note that sulfonato-calix[4,5,6,8]arenes act as surfactants in stabilizing *trans*-β-carotene nanoparticles.[22]

In this study we report the stabilization of superparamagnetic magnetite nanoparticles by coating them with *p*-sulfonato-calix[6 and 8]arenes, **1**, $n = 6,8$, and sulfonated *p*-benzylcalix[4,5,6 and 8]arenes, **2**, $n = 4,5,6,8$, Fig. 1.‡ Remarkably, stable ferrofluids are formed by a rapid and simple *in situ* co-precipitation from a solution of Fe(II) and Fe(III) chloride in the appropriate ratio, with aqueous ammonia, in the presence of the *p*-sulfonato-calixarenes and sulfonated *p*-benzylcalixarenes. The calixarenes not only serve as surfactants to stabilize the magnetite nanoparticles, they also functionalize the magnetite nanoparticles for potential biomedical applications, Fig. 1. To the best of our knowledge, this is the first report in the literature of coating magnetite nanoparticles with calix[*n*]arenes.

The formation of superparamagnetic magnetite nanoparticles was confirmed by SQUID measurements and TEM, while the

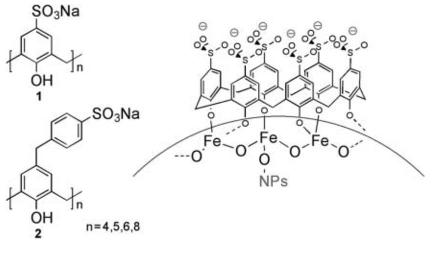

Fig. 1 *p*-Sulfonato-calix[*n*]arenes and sulfonato *p*-benzylcalix[*n*]arenes showing a possible mode of interaction of **1**, $n = 6$, at the surface of the nanoparticles (NPs).

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interaction of the magnetite with the calixarenes surfactants was investigated using FTIR, and DLS (dynamic light scattering) studies in solution. TEM images indicate that the samples prepared in the presence of *p*-sulfonato-calix[6]arene and sulfonated *p*-benzylcalix[4,5,6 and 8]arene have a narrow particle size distribution with diameters ranging from 5 to 10 nm. Whereas, the magnetite nanoparticles synthesized in the presence of *p*-sulfonato-calix[8]arene have a broader size distribution with the particle size ranging from 5 to 20 nm, Fig. 2. The *d* spacing values calculated from selected area diffraction patterns obtained from each of the samples are in good agreement with those for bulk magnetite (Joint Committee on Powder Diffraction Standards, JCPDS, card 19-0629, see supporting information†).

The particles are roughly spherical in shape and high resolution TEM imaging of the sample prepared in the presence of *p*-sulfonato-calix[6]arene show an amorphous material surrounding the iron oxide nanocrystals, Fig. 3. DLS measurements show the particles are approximately 30% larger, which is consistent with the assembly of calixarenes on the surface, and associated aquated environment.[23]

Elemental maps obtained by energy-filtered TEM showed that the iron oxide nanoparticles are surrounded by a carbon-rich shell, and thus the calixarenes are coating the surface of the nanoparticles. The thickness of the coating is around 1.2 to 1.6 nm, as measured from high resolution TEM images and elemental maps, which is consistent with the thickness of a monolayer of calix[*n*]arene sulfonates and associated aquated sodium ions, Fig. 4.

Calixarenes form complexes with Fe(II and III), with the iron centres bound to deprotonated phenolic OH groups.[24] This is also likely in the present study for iron centres on the surface of the nanoparticles, noting that the phenolic groups are deprotonated under basic conditions and unless the generated phenolate groups form one calixarene are associated with the same nanoparticle, there would be spontaneous aggregation. Thus complexation necessitates the $-SO_3^-$ groups of the calixarenes to be facing outward form the surface of the magentite nanoparticles, thereby electrostatically repelling other nanoparticles, Fig. 1. At the same time, the presence of calix[*n*]arenes during the formation of the nanoparticles may also limit the rapid growth of the crystals and control the particle size.

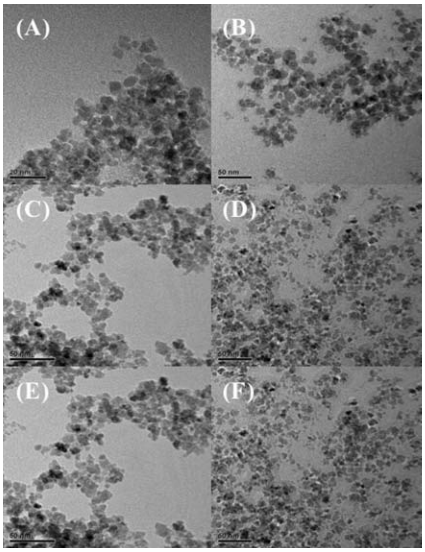

Fig. 2 TEM micrographs of *p*-sulfonato-calix[*n*]arene and sulfonated *p*-benzylcalix[*n*]arene coated magnetite: (A) **1**, *n* = 6; (B) **1**, *n* = 8; (C) **2**, *n* = 4; (D) **2**, *n* = 5; (E) **2**, *n* = 6; (F) **2**, *n* = 8.

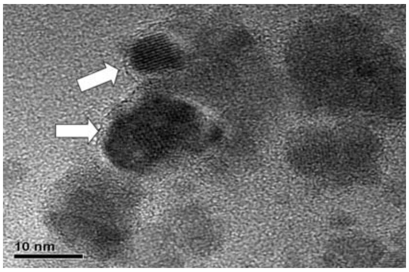

Fig. 3 TEM micrograph show the coating of *p*-sulfonato-calix[6]arene on the surface of the magnetite nanoparticles.

Magnetite prepared in the presence of *p*-sulfonato-calix[4 and 5]arenes did not form stable suspensions, in contrast to stable dispersed nanoparticles using *p*-sulfonato-calix[6 and 8]arenes. This could be due to the cone shaped *p*-sulfonato-calix[4 and 5]arenes tending to form the well known bilayer arrangement of these calixarenes with their cavities alternating up and down, which is associated through hydrophobic interplay.[25] Such an arrangement would effectively have adjacent nanoparticles locked together through sharing a common bilayer. *p*-Sulfonato-calix[6 and 8]arene are more fluxional and binding of metal centres to the calixarenes is less likely to result in bilayer formation between surface bound calixarenes from adjacant nanoparticle, and thus favour dispersion of the nanoparticles. Sulfonated *p*-benzylcalix[4, 5, 6 and 8]arene do not from crystalline bilayer arrangements, and the dangling benzyl moieties are more likely to act as surfactants for the attached nanoparticle rather than intertwine in the form of a bilayer or some other arrangement between adjacent particles, thereby stabilizing the nanoparticles.

FTIR spectra of the nano-particles are dominated by water absorption bands which is expected from the aquated sodium ions. This aside, there is a broad absorption band at *ca* 580 cm^{-1} (see supporting information†) which corresponds to ν_{Fe-O} in the crystal lattice of Fe_3O_4.[26] Peaks in the region 1036 to 1164 cm^{-1} correspond to S–O–C stretching.[27] Importantly, the usual broad peak v_{C-O} at 1450–1460 cm^{-1} for free calixarene[27] is shifted to 1400 cm^{-1}, with the intensity increased. This is consistent with the *p*-sulfonato-calix[6 and 8]arenes and sulfonated *p*-benzylcalix[4,5,6 and 8]arenes on the surface of the magnetite essentially in the deprotonated form, with the iron centres attached to the phenolic O-centres, Fig. 1.

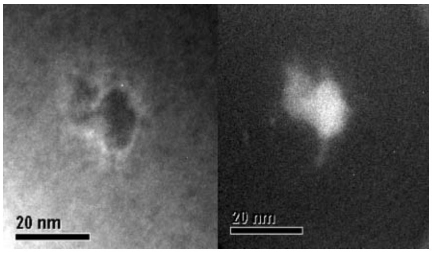

Fig. 4 Carbon and iron elemental maps (left and right respectively) of magnetite nanoparticles coated with *p*-sulfonato-calix[6]arene.

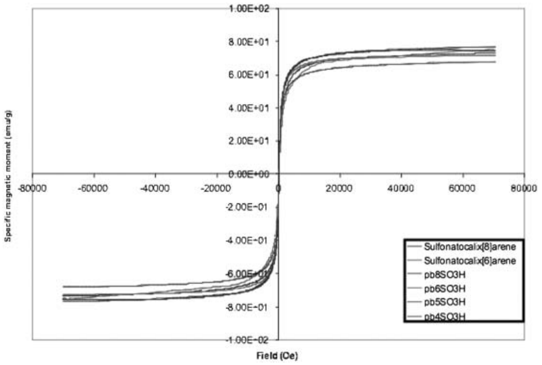

Fig. 5 Hysteresis loops for calix[*n*]arenes **1** and **2** coated magnetite nanoparticles at 300 K.

SQUID measurements all show superparamagnetism. The hysteresis loops of all the samples measured at room temperature are presented in Fig. 5. The superparamagnetic behaviour is evidenced by zero coercivity, zero remnance and the absence of hysteresis loops. The specific saturation magnetization of the samples ranged from 68–76 emu g^{-1}. The theoretical specific saturation magnetization of bulk magnetite is reported to be 92 emu g^{-1}.[28] Some studies suggested that the lower specific saturation magnetization of nanoparticles as compared to the bulk materials are due to the reduction of crystalline magnetic anisotropy constant K for the material.[29] Nevertheless, some studies also suggest that the effects of particle size in the nanolength scales are complex and alter relaxation processes and inter-particle interactions.[30] Therefore, values of 60–70 emu g^{-1} of Fe_3O_4 may be approaching the limit in the specific magnetization for magnetite nanoparticles with diameters less than 20 nm.

In summary, we have simultaneously stabilized and modified the surface of magnetite nanoparticles using *p*-sulfonato-calix[6 and 8]arene and sulfonated *p*-benzylcalix[4, 5, 6 and 8]arenes, in a convenient *in situ* process. The nanoparticles have good colloidal stability at physiological pH and exhibited superparamagnetic behaviour with high saturation magnetic moment at room temperature. These findings along with the ferrofluidic behaviour have implications in materials, contrast agents and drug delivery, and more.

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Notes and references

‡ Sodium *p*-sulfonato-calix[n]arenes and sulfonated *p*-benzylcalix[4, 5, 6 and 8]arenes were prepared using literature methods[31] while iron chlorides were purchased from Fluka. Stable suspension ferrofluid of calix[n]arene sulfonates coated magnetite were prepared by coprecipitation of Fe(II) and Fe(III) chloride (1:2 molar ratio) with aqueous ammonia in the presence the calix[n]arene. The solutions were stirred vigorously at room temperature under N_2 gas until stable black magnetite suspensions are formed. The magnetite suspensions were then centrifuged and the supernatant decanted. The collected solids were re-dispersed in deoxygenated ultra pure Mili-Q water. This process was repeated a few times to remove the excess of surfactants and ammonia.

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