Linking Hydrophilic Macromolecules to Monodisperse Magnetite (Fe_3O_4) Nanoparticles via Trichloro-*s*-triazine

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Linking hydrophilic macromolecules, especially biomolecules, to magnetic nanoparticles is a vital step for producing water-based ferrofluids for biomedical applications. Magnetic nanoparticles present in these fluids can be used as highly sensitive labels as they exhibit a magnetic signal that far exceeds that from any of the biomolecules. This, plus their capability of being manipulated under a magnetic field, provides a controllable means of magnetically tagging biomolecules or cells, leading to highly efficient bioseparation, drug delivery, bio-sensing, magnetic fluid hyperthermia, and magnetic resonance imaging contrast enhancement.[1] Functionalization of magnetic nanoparticles with biomolecules can be achieved through an organic linker that is designed to couple two different kinds of molecules. Such organic linkers have been well developed to couple macromolecules/biomolecules with organic dyes so that the molecules can become optically active and be detected.[2] However, using the similar coupling chemistry to link macromolelcules/biomolecules to monodisperse magnetic nanoparticles has been limited to date,[3] and the linkers used for this purpose, such as succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) and sulfo-SMCC, are often not economically available.

Here we report a simple approach to conjugate monodisperse Fe_3O_4 nanoparticles with various poly(ethylene glycol) (PEG)-based hydrophilic macromolecules via a readily available linker, trichloro-*s*-triazine (TsT). TsT is a symmetrical heterocyclic compound containing three acyl-like chlorines, as shown in Scheme 1. In aqueous solution, these three chlorines show different reactivities toward nucleophiles. For example, at pH = 9, the first chlorine is reactive toward hydroxyls as well as alkylamine groups at 4 °C. After this first chlorine is coupled to the nucleophile, the second one requires at least room-temperature conditions to do so. Once two chlorines are conjugated to nucleophilic groups, the third one is even more difficult to react, requiring at least 80 °C.[2] Such sequential activation capability of TsT in different temperatures has been used extensively to label proteins with different dyes or molecular carriers for sensing and separation applications.[2] As a first test of TsT's applications in magnetic nanoparticle functionalization, we chose to link monomethoxy-poly(ethylene glycol) (mPEG) with monodisperse Fe_3O_4 nanoparticles. PEG, along with several other types of polymeric molecules of dextran, poly(ethylene oxide), poloxamers, and polyoxamines, can form a robust coating layer around nanoparticles to prevent them from aggregation in physiological conditions or absorption by the reticulo-endothelial system (RES).[4] Fe_3O_4 nanoparticles have been tested vigorously for various biomedical applications because of their chemical stability and low toxicity.[1] Our experiments indicate that TsT can indeed link hydrophilic mPEG to monodisperse Fe_3O_4 nanoparticles, forming stable nanoparticle dispersions in phosphate buffered saline (PBS) or borate buffered saline (BBS) at pH = 7 or above, and the coupling can be readily extended to link avidin or other amine-terminated nucleotides and peptides.

The monodisperse Fe_3O_4 nanoparticles (9 nm in diameter) were prepared by one-pot high temperature (300 °C) reductive decomposition of $Fe(acac)_3$ (2 mmol) in oleylamine (10 mL) and benzyl ether (10 mL). The procedure was adopted from recent publications on the synthesis of MFe_2O_4 nanoparticles[5] and was modified slightly to make Fe_3O_4 nanoparticles here.[6] Note that for the particles prepared without

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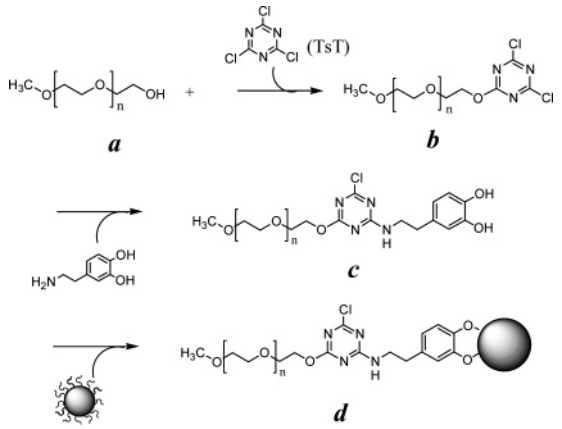
Scheme 1



the presence of oleic acid, their dispersion stability can be improved significantly by adding 0.2 mL of oleic acid into the product dispersion after the reaction. The monodisperse Fe_3O_4 nanoparticles are coated with a layer of oleate and oleylamine and are only soluble in hexane and other nonpolar or weakly polar organic solvents.

To functionalize the Fe_3O_4 nanoparticles with mPEG, TsT (22 mg) was first used to react with mPEG 2000 (**a**; 200 mg) at room temperature in anhydrous benzene (20 mL) to give **b**,[2] as shown in Scheme 1. **b** (20 mg) further reacts with dopamine, or 4-(2-aminoethyl)benzene-1,2-diol (adduct with hydrogen chloride) (1.7 mg) in 1,4-dioxane solvent, forming compound **c**. The molecular structures of both **b** and **c** are confirmed by ^{1}NMR spectroscopy of the compounds in $CDCl_3$ solvent.[6] The catecol unit in dopamine-based molecule **c** is used to replace oleate/oleylamine around the iron oxide nanoparticles as reported,[7] but in $CHCl_3$ solvent.[6] Such replacement gives m-PEG coated Fe_3O_4 nanoparticles (**d**). It can be seen from **d** that TsT acts as a bridge to link mPEG and dopamine-Fe_3O_4 nanoparticles. The PEG-dopamine-coated nanoparticles (**d**) are readily dispersed in water, PBS (137 mM NaCl, 2.7 mM KCl, and 10 mM Na_2HPO_4/KH_2PO_4, pH = 7.4) or BBS (H_3BO_3/Na_3BO_3, 10 mM H_3BO_3 with pH adjusted by sodium hydroxide).

Dynamic light scattering (DLS) measurements (Figure 1) on the dispersed 9 nm Fe_3O_4 nanoparticles show that before surface modification, the nanoparticles have an average hydrodynamic diameter of 11.9 nm (Figure 1A). This is close to a simple addition of the particle core diameter (9 nm) and the shell coating (~4 nm). After ligand exchange, the organic shell coating in **d** is increased to ~30 nm (2 × 15 nm). This, plus a 9 nm core, gives an overall diameter of 39 nm, while the average hydrodynamic diameter of the structure from DLS measurement is at 40.3 nm (Figure 1B), a size that is close to the addition of PEG + dopamine + nanoparticle diameter. A transmission electron microscopy (TEM) image of the nanoparticles from hexane is shown in Figure 2A, and that from PBS is given in Figure 2B.

(6) Supporting Information.
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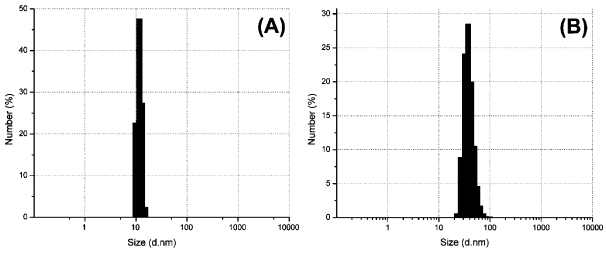

Figure 1. Hydrodynamic diameter distribution of (A) 9 nm Fe_3O_4 nanoparticle dispersion in hexane and (B) nanoparticles **d** (Scheme 1) in PBS measured by DLS.

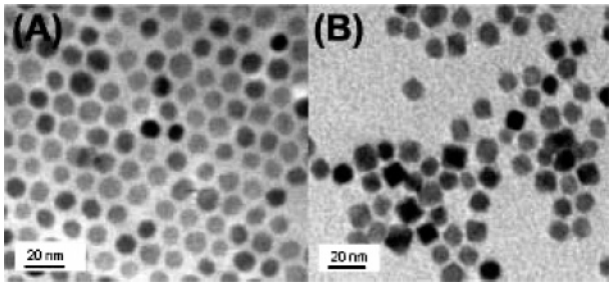

Figure 2. TEM images of (A) 9 nm Fe_3O_4 nanoparticles coated with oleate/oleylamine from their hexane dispersion and (B) nanoparticles **d** in Scheme 1 from PBS dispersion.

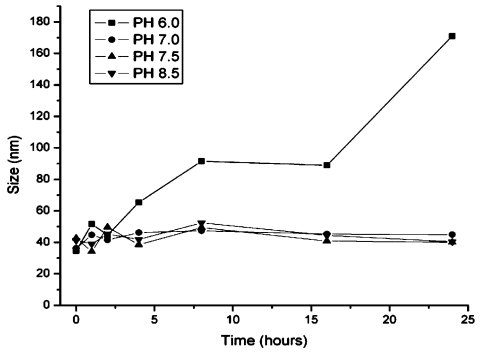

Figure 3. DLS measured average hydrodynamic diameters of the nanoparticles **d** (Scheme 1) in BBS at different pH values after incubation at 70 °C.

Comparing the two TEM images, one can see that the nanoparticles are well dispersed in both hexane and PBS. However, the morphology of the nanoparticles after dopamine replacement does change from sphere/polyhedron-like to cube-like, indicating slight Fe_3O_4 surface corrosion during the exchange.

Stability of the mPEG-dopamine coated Fe_3O_4 nanoparticles was tested in BBS (10 mM) at various pH values with an incubation temperature of 70 °C. DLS was used to track the size change of the nanoparticles in these pH conditions during the incubation. Figure 3 shows the variation of hydrodynamic diameters of the nanoparticles in BBS incubated at 70 °C. It can be seen that the initial average size of the nanoparticles is at ~40 nm. In the solutions with pH = 7 or above, there is no increase in size of the particles in the tested incubation time (24 h), indicating no particle aggregation/sintering. The TEM image of the nanoparticles after incubation is similar to what is shown in Figure 1B. On the other hand, at lower pH = 6, the particles can be stabilized for only 2 h before serious aggregation occurs. After 15 h, the sizes of the clustered nanoparticles reach 100 nm. This sintering behavior of the dopamine coated particles may

result from the chemical bond cleavage between iron oxide and the catecol unit under low pH and incubation conditions, destabilizing the nanoparticle dispersion. In neutral or basic conditions, the nanoparticles are well stabilized. Note that the stability test in PBS showed similar results as in BBS.

This report demonstrates that TsT can be used as an easy linker to couple magnetic Fe_3O_4 nanoparticles with hydrophilic mPEG. At the temperatures tested in this work, 25−70 °C, the mPEG-dopamine coated nanoparticles are stable in buffer at various pH values greater than 7. At pH values less than 7, the nanoparticles start to aggregate shortly after 2 h of incubation at 70 °C. Our further experiments indicate that the TsT-based linking strategy can be extended to the coupling of various hydrophilic macromolecules, including nucleotides and peptides, to iron oxide nanoparticles. Work on linking NH_2-terminated oligonucleotide and avidin molecules using TsT for highly sensitive DNA sequence detection is underway.

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Note Added after Print Publication: In the version of this paper posted on the Web October 13, 2006, and published in the November 14, 2006, issue, Nader Pourmand's name was misspelled. The electronic version was corrected and re-posted to the Web issue on February 13, 2007, and an Addition and Correction appears in the March 6, 2007, issue.

Supporting Information Available: Experimental procedures and characterization data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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