Journal of Colloid and Interface Science 437 (2015) 147–155

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Effects of phase transfer ligands on monodisperse iron oxide magnetic nanoparticles

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ARTICLE INFO

Article history:
Received 6 June 2014
Accepted 10 September 2014
Available online 18 September 2014

Keywords:
Superparamagnetic iron oxide nanoparticles
Ligand-exchange
Citric acid
DMSA
Magnetization
Colloidal stability
Gum Arabic

ABSTRACT

Oleic acid coated iron oxide nanoparticles synthesized by thermal decomposition in organic medium are highly monodisperse but at the same time are unsuitable for biological applications. Ligand-exchange reactions are useful to make their surface hydrophilic. However, these could alter some structural and magnetic properties of the modified particles. Here we present a comprehensive study and comparison of the effects of employing either citric acid (CA) or meso-2,3-dimercaptosuccinic acid (DMSA) ligand-exchange protocols for phase transfer of monodisperse hydrophobic iron oxide nanoparticles produced by thermal decomposition of $Fe(acac)_3$ in benzyl ether. We show the excellent hydrodynamic size distribution and colloidal stability of the hydrophilic particles obtained by the two protocols and confirm that there is a certain degree of oxidation caused by the ligand-exchange. CA revealed to be more aggressive towards the iron oxide surface than DMSA and greatly reduced the saturation magnetization values and initial susceptibility of the resulting particles compared to the native ones. Besides being milder and more straightforward to perform, the DMSA ligand exchange protocol produces MNP chemically more versatile for further functionalization possibilities. This versatility is shown through the covalent linkage of gum Arabic onto MNP-DMSA using carboxyl and thiol based chemical routes and yielding particles with comparable properties.

1. Introduction

Superparamagnetic iron oxide nanoparticles (SPION) have been gaining increased attention in the last decades due to their applications in environmental sciences, memory storage and, mainly, in biomedical and pharmaceutical sciences [1,2]. Superparamagnetism, large surface to volume ratio and biocompatibility are unique physical properties of iron oxide at the nanoscale that make it one of the most studied nanomaterials nowadays. There are several forms of iron oxide but the more explored ones are magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) due to their improved magnetic properties and biocompatibility [3]. The different crystal structure of these two iron oxides influences the net spontaneous magnetization of the particles: at 300 K, 92–98 emu/g for magnetite, and 76–84 emu/g for maghemite [4–6].

There are several methods to synthesize SPION, ranging between physical, chemical and biological methods. The most common are chemical methods and among those, co-precipitation is widely used to obtain large amounts of hydrophilic nanoparticles. However, SPION produced by this method have a broad size distribution and tend to easily aggregate and become colloidally unstable [7]. This behavior is not desirable for most applications, as it is associated with wide size distributions, increased average hydrodynamic diameters and decreased surface to volume ratio. For example, regarding biomedical applications, the increase in size of the aggregates reduces the area available for grafting of bioactive molecules and compromises the biocompatibility and biodistribution of the administered particles [8]. Similarly, in the case of environmental remediation applications (e.g. removal of heavy metals from water), the formation of large agglomerates reduces the adsorbing area and particle transport becomes hampered, limiting the system efficiency [9].

Up to now, the synthesis method that allows better control of sizes and produces the narrowest size distributions is the thermal decomposition of organometallic precursors of iron in organic sol-

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http://dx.doi.org/10.1016/j.jcis.2014.09.019
0021-9797/

vents. The magnetic properties of SPION obtained by this route are enhanced in comparison with other methods due to their more crystalline structure. However, there is a limitation: the precursors and solvents are not biocompatible, and the resulting ferrofluids are only stable in hydrophobic media. Therefore, an intermediate step of phase transfer to aqueous media is required before any biological application [3,7]. There are two approaches for the phase transfer: adding an amphiphilic molecule that binds through hydrophobic interactions to the original surfactant layer and forms a micellar structure that encapsulates the magnetic cores [10], or replacing the native hydrophobic surfactants by hydrophilic molecules that have higher affinity for iron [11,12]. This last process is called ligand-exchange and has been widely used [11,13–18] because it is a simple and effective method. The used ligands consist of an anchoring chemical group that binds to the surface of the SPION (e.g. carboxylic acid, phosphonic acid, dopamine) and a hydrophilic region that becomes exposed to the surrounding water molecules, affording physiological stability and conjugation capability [19]. The anchoring group of the ligand is important as it is responsible for the stability of the new coating and also because it can influence the magnetic properties of the hydrophilic MNP [20]. Due to their small size and presence of multiple carboxylic groups that readily coordinate with iron, citric acid (CA) [11,14,17] and *meso*-2,3-dimercaptosuccinic acid (DMSA) [12,16,21–23] are two frequently used molecules in ligand-exchange procedures. Good colloidal stability is generally obtained in both cases but the overall effects of the process on structural and magnetic properties of the resulting particles, to our knowledge, have never been directly compared. Some studies claim no alteration of the saturation magnetization value (M_S) compared to the precursor hydrophobic particles [24] and others show consequences on the magnetization characteristics [12,25] but this comparison is often neglected [11,14,17,23,26]. Therefore, the benefits of using one ligand instead of the other are not clear. In this work, we present a comprehensive comparison between two ligand-exchange protocols that employ either citric acid or DMSA, discussing the effects of these two ligands on the colloidal and magnetic properties of SPION intended for biological and biomedical applications. We analyze hydrophobic SPION synthesized by the thermal decomposition method and the hydrophilic SPION resulting from the ligand exchange regarding their core size, hydrodynamic diameter and colloidal stability along with surface characterization by FTIR and evaluation of the magnetic properties by VSM. DMSA-modified MNP were finally coated with gum Arabic to show their chemical versatility, taking advantage of the carboxyl and thiol groups available at the particles surface.

2. Experimental section

2.1. Materials

All chemicals were purchased from Sigma–Aldrich and used without ulterior purification.

2.2. Synthesis of hydrophobic magnetic nanoparticles (MNP-OA)

Hydrophobic magnetic nanoparticles were synthesized following Sun's method [10] with slight modifications. Briefly, iron tri(acetylacetonate) (2 mmol), 1,2-tetradecanediol (10 mmol), oleic acid (6 mmol), oleylamine (6 mmol), and benzyl ether (10 ml) were mixed and stirred magnetically under a constant flow of nitrogen. The mixture was heated to 200 °C at a rate of 3 °C/min and kept at 200 °C for 2 h. Then, under a blanket of nitrogen, the mixture was heated to reflux (300 °C) at a rate of 4.5 °C/min and kept in reflux for 1 h. The resultant black-colored mixture was cooled to room temperature by removing the heat source. Ethanol (about 20 ml) was added to the mixture, to precipitate the particles after which they were separated via centrifugation (9000 rcf, 15 min). The supernatant was discarded and the pellet was re-dispersed in hexane (10 ml) in the presence of oleic acid (50 μl) and oleylamine (50 μl) with the aid of vortexing and sonication. Centrifugation (7000 rcf, 10 min) was then applied to remove any undispersed material. The pellet was discarded and the supernatant was precipitated with ethanol (about 20 ml), centrifuged (9000 rcf, 10 min) to remove the solvent and redispersed into hexane.

2.3. Phase transfer by ligand-exchange with citric acid (MNP-CA)

Ligand-exchange using citric acid was performed following the protocol described by Lattuada et al. [11] MNP-OA (120 mg) were previously dried, and dispersed in a 50/50 mixture of 1,2-dichlorobenzene and N,N′-dimethylformamide (15 ml of total volume), to which 0.1 g of citric acid was added. The mixture was incubated in a rotating agitator at 100 °C for approximately 18 h. MNP-CA were precipitated by the addition of diethyl ether (about 40 ml) and recovered by magnetic separation. The particles were redispersed in acetone and reprecipitated by means of a permanent magnet three times to remove all traces of free citric acid. After the final magnetic precipitation, traces of acetone were removed with a nitrogen flow and the particles were re-dispersed in milliQ water and filtered through a 0.1 μm pore membrane.

2.4. Phase transfer by ligand-exchange with meso-2,3-dimercaptosuccinic acid (MNP-DMSA)

A standard ligand-exchange protocol [16] was used to replace oleic moieties by DMSA. MNP-OA (50 mg Fe_3O_4) were precipitated from the hexane suspension by adding ethanol and centrifuging (9000 rcf, 20 min) several times. The supernatant was discarded. After the final precipitation, the MNP-OA were re-dispersed in toluene (20 ml), added to a solution of DMSA (90 mg) in DMSO (5 ml) and mixed with sonication. The mixture was incubated at room temperature for 48 h in a rotating agitator. After the reaction, the translucent solvent containing the oleic acid and oleylamine was discarded and the black particles (MNP-DMSA) attached to the walls of the flasks were re-dispersed in ethanol with sonication and vortexing. This mixture was centrifuged and re-dispersed in ethanol several times to clean the particles. Finally, the MNP–DMSA were re-dispersed in milliQ water, basified to pH 10 with sodium hydroxide and dialyzed against milliQ water for about 48 h. Filtration through a 0.1 μm pore membrane and adjustment of the pH to 7 was carried out as a final step.

2.5. Preparation of MNP-DMSA-Cyst-GA

MNP-DMSA were first aminated with cysteamine hydrochloride (Cyst) and then covalently bond to carboxylic groups of GA. For that, MNP-DMSA thiol groups were activated with 2,2′-Dithiodipyridine (DTDP) as follows. MNP-DMSA (15 mg) were precipitated from the stock solution by centrifugation and re-dispersed in a previously filtered saturated solution of DTDP (15 ml) in phosphate buffer (10 mM, pH 8). The activation reaction continued overnight in a rotating agitator at room temperature. The suspension was then centrifuged (9000 rcf, 20 min) and the unreacted DTDP in the supernatant removed. The precipitated particles were redispersed in a solution of cysteamine (15 ml, 120 mM) in phosphate buffer (10 mM, pH 8) with sonication and vortex and incubated for approximately 5 h in a rotating agitator at room temperature. To remove unreacted cysteamine, dialysis was performed (48 h). Finally, the MNP-DMSA-Cyst were precipitated by centrifugation