Journal of Colloid and Interface Science 424 (2014) 141–151

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

The formation of linear aggregates in magnetic hyperthermia: Implications on specific absorption rate and magnetic anisotropy

Steven L. Saville [a], Bin Qi [a], Jonathon Baker [a], Roland Stone [a], Robert E. Camley [b], Karen L. Livesey [b], Longfei Ye [c], Thomas M. Crawford [c], O. Thompson Mefford [a,*]

[a] Clemson University, Department of Materials Science and Engineering, Center for Optical Materials Science and Engineering Technologies, 91 Technology Dr., Anderson, SC 29625, USA
[b] UCCS Biofrontiers Center and Department of Physics, University of Colorado at Colorado Springs, 1420 Austin Bluffs Parkway, Colorado Springs, CO 80918, USA
[c] Department of Physics and Astronomy and Smart State Center for Experimental Nanoscale Physics, University of South Carolina, 712 Main St., Columbia, SC 29208, USA

ARTICLE INFO

Article history:
Received 2 October 2013
Accepted 1 March 2014
Available online 16 March 2014

Keywords:
Magnetic
Nanoparticle
Self assembly
Chaining
Magnetite
Polymer
Colloidal arrangement

ABSTRACT

The design and application of magnetic nanoparticles for use as magnetic hyperthermia agents has garnered increasing interest over the past several years. When designing these systems, the fundamentals of particle design play a key role in the observed specific absorption rate (SAR). This includes the particle's core size, polymer brush length, and colloidal arrangement. While the role of particle core size on the observed SAR has been significantly reported, the role of the polymer brush length has not attracted as much attention. It has recently been reported that for some suspensions linear aggregates form in the presence of an applied external magnetic field, i.e. chains of magnetic particles. The formation of these chains may have the potential for a dramatic impact on the biomedical application of these materials, specifically the efficiency of the particles to transfer magnetic energy to the surrounding cells. In this study we demonstrate the dependence of SAR on magnetite nanoparticle core size and brush length as well as observe the formation of magnetically induced colloidal arrangements. Colloidally stable magnetic nanoparticles were demonstrated to form linear aggregates in an alternating magnetic field. The length and distribution of the aggregates were dependent upon the stabilizing polymer molecular weight. As the molecular weight of the stabilizing layer increased, the magnetic interparticle interactions decreased therefore limiting chain formation. In addition, theoretical calculations demonstrated that interparticle spacing has a significant impact on the magnetic behavior of these materials. This work has several implications for the design of nanoparticle and magnetic hyperthermia systems, while improving understanding of how colloidal arrangement affects SAR.

Introduction

One of the more promising biomedical applications of magnetic nanoparticles is cancer therapy via magnetic hyperthermia [1–5]. In the past few years there have been significant advances in the treatment of cancerous tissue using magnetic hyperthermia as part of an ongoing effort to reduce invasiveness of current medical treatments [6–8]. Magnetic hyperthermia is a general term that refers to the introduction of biocompatible magnetic nanomaterials into cancerous tissue by either specific targeting or passive intake, then heating the tissue with an alternating magnetic field in order to induce cell necrosis [9]. Cancerous tissue has been shown to have a higher sensitivity to heat due to decreased blood flow and therefore higher acidity, thus allowing most cancerous cells to be destroyed in the 42–45 °C range while healthy cells remain relatively unchanged [1,10]. In the case of magnetic hyperthermia, heating to this temperature is achieved by the interaction of magnetic nanoparticles with an external alternating magnetic field [11–13]. In order to bypass the reticular endothelial (REU) system and other biological responses, most particle systems used for this treatment are smaller than 25 nm [3,14–16] and therefore exhibit superparamagnetic behavior under certain conditions [17]. Heating attributed to this system in an alternating magnetic field, according to the Linear Response Theory (LRT) [18,19], is from hysteretic losses from several relaxation mechanisms [18,20]. The LRT is capable of calculating the hysteretic losses (defined as the area of hysteresis) of magnetic nanoparticles in the superparamagnetic regime at low magnetic field strengths [21]. The first relaxation mechanism to consider is Brownian relaxation, which is caused

* Corresponding author.
E-mail address: mefford@clemson.edu (O. Thompson Mefford).

http://dx.doi.org/10.1016/j.jcis.2014.03.007
0021-9797/

by the particle both moving randomly throughout the media and physically rotating to align under an alternating induced field. The other main relaxation process is Néel relaxation, which is defined as the spontaneous flip of spin state direction given enough thermal energy. There is no external rotation of the particle in this mechanism, rather an internal rotation of the magnetic moment. There are many factors that affect both Brownian motion and Néel relaxation, including concentration, particle type, nanoparticle core size, stabilizing ligand brush length, frequency, and magnetic field. The particle size contribution to heating has been fairly well established, and has been analyzed elsewhere [22–25]. One of the more interesting factors in a nanoparticle system is its stabilizing layer length, whose contribution to heating has yet to be analyzed and is not understood. Theoretically, increasing the hydrodynamic diameter should decrease the overall specific absorption rate according to LRT [4]. Furthermore, there may be other effects related to the length of the polymer brush, such as controlling the van der Waals and magnetic attractive forces. The ligand length may control the structure of any magnetically induced colloidal arrangement, including clustering and chaining [26]. However, this relationship has yet to be fully validated.

One of the more common ways to identify the amount of thermal energy produced per mass of particle is by calculating the specific absorption rate (SAR), which relates the amount of energy converted to mass and time. This value does have some limitations, as it does not account for the magnetic field strength or frequency. For this reason, if accurate relationships between core and ligand size and SAR are to be determined, all measurements must be made on the same instrument under the same conditions.

Here we investigate the effect of particle size and stabilizing ligand molecular weight on the heating rate and SAR of magnetite nanoparticles, as well as how ligand length affects interparticle interactions in an alternating magnetic field. A matrix of varying molecular weight poly(ethylene glycol) (PEG) ligands (1 k, 2 k, 5 k, 10 k, 20 k, and 40 k g/mol) were attached to 6.5, 17, 20, and 22 nm magnetite nanoparticles, and these nanoparticle/brush combinations were systematically studied.

Experimental section

Materials

Linear monofunctional hydroxyl terminated poly(ethylene glycol) monomethyl ether (PEG-OH) of molecular weights of 1000, 2000, and 5000 g/mol were purchased from Sigma Aldrich and were dried at 80 °C in a vacuum oven overnight prior to use to remove any residual water. For the 10,000, 20,000 and 40,000 MW polymer, *N*-hydroxysuccinimide terminated PEG was purchased from JenKem and used without further purification. Iron(III) chloride hexahydrate, sodium oleate, dimethyl aminopyridine (DMAP), N,N′-dicyclohexycarbodiimide (DCC), *N*-hydroxysuccinimide (NHS), succinic anhydride, 3,4 dihydroxyphenylalanine (L-DOPA), chloroform, ethylene glycol methyl methacrylate, azobisisobutyronitrile (AIBN) and ethyl ether were purchased from Sigma Aldrich and used without further purification. Iron(III) acetylacetonate was purchased from Fluka and used without further purification. Oleylamine and oleic acid (from Sigma Aldrich, 99% purity) was fractionally distilled before use. Tetrahydrofuran (THF, from Sigma Aldrich) was distilled over calcium hydride before use to remove water. Dimethyl foramide (DMF, from Sigma Aldrich) was dried over molecular sieves (4A) before use to remove water.

Magnetite nanoparticle synthesis

Magnetite particles of 6.5 nm diameter were prepared via a modified method first presented by Sun et al. [27]. Briefly, magnetite particles were produced by adding iron (III) acetylacetonate (Fe(acac)3, 0.35 g, 1 mmol) and oleylamine (OAm, 2.5 mL, 17.09 mmol) in 17.5 mL of benzyl ether to a round bottom flask under a nitrogen blanket. The solution was then heated at 3 °C per minute to 300 °C and held isothermally for 1 h. The particles were purified by precipitating particles from solution using ethanol along with centrifugation and then redispersed in hexane.

A separate synthesis method was needed for larger particles, and nanoparticles of 17, 20, and 22 nm were produced using a method first developed by Park et al. [28]. This method involves the thermal decomposition of iron oleate, which first requires the synthesis of the precursor. First, iron oleate was prepared by the reaction of 20 mmol iron chloride hexahydrate with 60 mmol of sodium oleate in a mixture of 40 mL of ethanol, 30 mL of deionized water, and 70 mL of hexane in a three neck round bottom flask. The solution was heated up to 70 °C while stirring and kept at this temperature for 4 h. The solution was then cooled to room temperature and the upper organic layer was separated and washed with water resulting in the iron–oleate product.

For particle synthesis, 20 mmol of iron–oleate, which was the product from the previous reaction, was mixed with 60 mmol oleic acid and 100 g of 1-octadecene. The solution was heated to 105 °C under nitrogen, and was held isothermally for 30 min. The reaction was then further heated to 320 °C with a heating rate of 3 °C/min and kept at reflux for 1 h. The reaction solution was then cooled to room temperature. Particles were size fractionated into three separate sizes by selective precipitation using acetone and resuspension into toluene.

Synthesis of nitroDOPA

In order to provide a robust anchor to magnetite, 3,4 dihydroxyphenylanaline (L-DOPA) was nitrated using a method described by Yang et al. [29]. To nitrate the catecholamine, 1.97 g of L-DOPA (10 mmol) was dissolved in 25 mL of deionized water along with 1.52 g (22 mmol) of sodium nitrite. The water solution was then placed in an ice bath and allowed to cool down to approximately 4 °C. A 17.4 mM solution of sulfuric acid (0.927 mL concentrated sulfuric acid in 10 mL of deionized water) was then slowly dripped into the system at 0.2 mL/min addition rate. The formation of a yellow/brown precipitate indicated the presence nitro-3,4 dihydroxyphenylanaline (nitroDOPA). The reaction mixture was then filtered to remove the nitroDOPA and the resultant precipitant was washed three times with methanol. Formation of nitroDOPA was confirmed by nuclear magnetic resonance spectroscopy (NMR) by peaks at 6.1 ppm (CH, ring, nitroDOPA), 6.8 ppm (CH, ring, nitroDOPA), 1.8 ppm and 2.4 ppm (ring—CH_2—CH_2—C).

Synthesis of PEG-nitroDOPA of varying molecular weights

The synthesis of varying molecular weights of PEG polymers with a nitroDOPA terminal group was performed by reacting NHS-terminated PEG with nitroDOPA. For the 1000, 2000 and 5000 g/mol molecular weight polymers a hydroxyl terminated monofunctional PEG (2000 g/mol MW, 5 g, 2.5 mmol) was reacted with succinic anhydride (0.5 g, 2.5 mmol) and DMAP (0.12 g, 1 mmol) in anhydrous THF (20 mL) at room temperature for 8 h, yielding a carboxylic acid terminated monofunctional PEG (4.12 g, 78.5% yield). The polymer was then purified by dissolving it in water and extracting with chloroform. In the next reaction step, the carboxylic acid terminated PEG (4.12 g, 1.96 mmol) was then reacted with DCC (0.53 g, 2.58 mmol) and NHS (0.23 g, 1.96 mmol) in THF (20 mL) at room temperature for 4 h, which resulted in an NHS terminated PEG. Impurities were removed from the system using vacuum filtration and precipitation into ethyl ether (2.98 g, 68.6% yield). Finally, PEG-NHS (2.98 g, 1.34 mmol)