Journal of Colloid and Interface Science 336 (2009) 510–518

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Cell toxicity of superparamagnetic iron oxide nanoparticles

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ARTICLE INFO

Article history:
Received 19 February 2009
Accepted 8 April 2009
Available online 22 April 2009

Keywords:
Superparamagnetic nanoparticles
Magnetite
Polyvinyl alcohol
Polymeric nanocomposite
MTT assay
Cell toxicity

ABSTRACT

The performance of nanoparticles for biomedical applications is often assessed by their narrow size distribution, suitable magnetic saturation and low toxicity effects. In this work, superparamagnetic iron oxide nanoparticles (SPIONs) with different size, shape and saturation magnetization levels were synthesized via a co-precipitation technique using ferrous salts with a Fe^{3+}/Fe^{2+} mole ratio equal to 2. A parametric study is conducted, based on a uniform design-of-experiments methodology and a critical polymer/iron mass ratio (*r*-ratio) for obtaining SPION with narrow size distribution, suitable magnetic saturation, and optimum biocompatibility is identified. Polyvinyl alcohol (PVA) has been used as the nanoparticle coating material, owing to its low toxicity. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is used to investigate the cell biocompatibility/toxicity effects of the samples. From the MTT assay results, it is observed that the biocompatibility of the nanoparticles, based on cell viabilities, can be enhanced by increasing the *r*-ratio, regardless of the stirring rate. This effect is mainly due to the growth of the particle hydrodynamic size, causing lower cell toxicity effects.

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1. Introduction

Superparamagnetic iron oxide nanoparticles (SPIONs) have potential biomedical applications such as magnetic drug targeting, enhanced resolution magnetic resonance imaging, tissue repair, cell and tissue targeting and transfection [1–5]. Superparamagnetism in many biomedical applications such as drug delivery is useful because the SPION can be transported by electrical field effects to the desired site and once the external magnetic field is removed, magnetization disappears and the SPION can remains at the target site for a certain period.

Several methods such as arc discharge, mechanical grinding, laser ablation, microemulsions and high temperature decomposition of organic precursors have been reported for the synthesis of Fe_3O_4 nanoparticles [6]. The chemical coprecipitation method is a common technique used to produce dispersed (water-based) Fe_3O_4 nanoparticles at low temperatures. If a suitable surfactant is employed and the processing parameters (pH, reaction temperature, stirring rate, solute concentration, etc.) are controlled, the size, distribution and shape of the particles can be tailored [7–10]. Individual nanoparticles and nanoparticle agglomerates can be characterized by the magnetic core size and the hydrodynamic diameter. The parameters are important for targeting purposes. The first one is responsible for the magnetic response in applied inhomogeneous magnetic fields and the second parameter is important for targeting and cell interactions [11]. A coating layer can prevent the agglomeration of the particles, increase the circulation time, and provide biocompatibility. Monodispersed particles with a high saturation magnetization and functionalized with suitable coatings are required for targeting and imaging in hyperthermia, transfection and MRI (magnetic resonance imaging) applications.

Despite the pros and cons of using nanoscale iron oxides for *in vivo* applications, superparamagnetic iron oxide nanoparticles (SPIONs) remain the only magnetic nanoparticles that have been approved for clinical use to date [12]. Investigators seeking fast-track developments of magnetic-guided therapy often prefer this tried-and-tested option. One solution to the nanoparticles' weak magnetic responsiveness is to maximize the magnetic field at the target sites [12]. It has been recognized that the core size of iron oxide nanoparticles determines the magnetic properties; however, less work has been conducted to investigate the effect of hydrodynamic size on magnetic properties [13]. Recently, we have reported the effect of both stirring rate and base molarities variations on the characteristics of superparamagnetic nanoparticles with the reaction temperature fixed at 35 °C [14].

The aim of the present work is to study the effect of other important synthesis parameters including polymer/iron mass ration and homogenization rate while the reaction temperature is not fixed. The temperature changes via the heat produced by the

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doi:10.1016/j.jcis.2009.04.046

chemical reaction of iron salts and base in the water medium. It is shown that temperature increase due to chemical reaction can have significant effects on optimum hydrodynamic diameter size, shape and maximum magnetic saturation of SPION. Synthesis is conducted and compared for different polymer/iron mass ratio and homogenization rates in the presence of polyvinyl alcohol (PVA). The nanoparticles are coated by PVA to prevent coagulation of particles during bio-applications. PVA is selected due to its thermo-mechanical stretching and crystallinity properties [15].

The application of design of experiments (DOE) methods in optimization of nanomaterials processing is beneficial, particularly given the high cost of experimentation and complex random error structures [14,16–18]. In the present study, a uniform design (UD) of experiments method, with two variables and six levels each, was applied to study the effects of stirring rate and PVA/iron mass ratio parameters on the magnetic properties and the size of synthesized nanoparticles.

2. Experimental procedure

2.1. Preparation of samples

Iron chloride salts with analytical grades and PVA were supplied by Merck Inc. (Germany) and Fluka, respectively, and used without further purification. Deionized water was utilized for preparation of the solutions after deoxygenating with argon gas bubbles for 30 min. The mole fraction of Fe^{2+} to Fe^{3+} was 1:2 (0.368 g $FeCl_2$ and 1 g $FeCl_3$). Precipitation was performed by the addition of iron salts (with 1 M HCl) to a solution of sodium hydroxide and PVA (MW = 30,000–40,000) under an argon atmosphere at pH of 13. The amount of PVA is defined via different polymer/iron mass ratio, *r*, ranging from 0 (i.e., no polymer) to 5. Homogenization at different stirring rates in the range of 720–4320 rpm was applied according to the DOE layout. The temperature of the reaction is increases due to the induced heat by chemical reaction. After the reaction, the samples are cooled to 4 °C; this temperature is below the glass transition temperature of PVA [19,20]. A stable polymer complex is formed at low temperature and as a result the obtained polymer configuration should remain stable. The particles were collected by centrifugation at 10,000*g* and redispersed in DI water, several times. The final ferrofluid was kept at 4 °C for future usage. In order to refer to the synthesis parameters of a sample during the analysis, the samples are labeled by *S*(*x*) *R*(*y*), where *S* is the stirring and *x* is the stirring rate, *R* is the polymer/iron mass ratio and *y* is the amount. For example, *S*(3600) *R*(2) refers to a sample prepared with a stirring rate of 3600 rpm and a *r*-ratio of 2.

To perform the necessary experiments based on a uniform DOE approach, levels of the *r*-ratio and stirring rate parameters within the aforementioned ranges were selected according to the layout shown in Fig. (A) of the Supporting Information. A conventional $U_{12}(6^2)$ design is employed which refers to a layout with 12 experiments, 2 factors and 6 levels for each.

2.2. Characterization of samples and the MTT method

The synthesized nanoparticles were characterized by various analytical techniques. TEM (ZEISS Model EM-10C) operating at 100 kV, SEM (Philips-XL30) and HRSEM (FEG LECO) are used for size and morphology characterization similar to our previous studies [14]. XRD (Siemens D5000) with Cu Kα radiation was used for the phase characterization and particle size determination using Scherrer method. Thermo-gravimetric analysis (TGA) measurements were performed with a DSC/TGA thermal analysis system (SDT Q600, USA). A mass loss from 9 mg of dried sample was monitored under the argon gas (inert atmosphere) at temperatures ranging from 30 to 800 °C at a rate of 5 °C min^{-1}. Size distribution of nanoparticles was determined by a zeta series Malvern instrument (Zetasizer model ZEN 1600, nano laser 633 nm). The magnetization of the samples in a variable magnetic field was measured using a vibrating sample magnetometry (VSM) with a sensitivity of 10^{-3} emu and magnetic field up to 8 kOe. The magnetic field was changed uniformly with a time rate of 66 Oe/s.

To quantitatively measure cell cytotoxicity, proliferation or viability of the particles, a simple, non-radioactive and colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used. The assay was a yellow and water soluble tetrazolium salt which is converted to a water insoluble and dark blue Formazan derivative by reductive cleavage of the tetrazolium ring with metabolically active cells [21]. Formazan crystals can be dissolved in an organic solvent such as dimethyl sulphur oxide (DMSO) or isopropanol and quantified by measuring the absorbance of the solution at 545 nm. For the MTT assay viability studies, the primary mouse connective tissue cells (L929 fibroblast) from the National Cell Bank of Iran (NCBI) Pasteur Institute were seeded onto a glass cover-slips in a 96 well-plate at a density of 10,000 cells per well in 150 μl of medium for 24 h, after which the medium containing SPION in amount of 20 mM (iron concentration confirmed with atomic absorption) was added in a dilution series (cell medium contained 0.2, 1, 5, and 20 mM magnetite). The control well was a culture medium with no particles. All specimens, as well as the control, were placed in five wells to provide statistically reliable results. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C in a 5% CO_2 incubator. After 3, 24 and 48 h of incubation of cells with SPIONs, 100 μl of MTT were added to wells. After incubation for 2 h the medium was removed and formazan crystals were solubilized by a 20 min-treatment with 150 μl isopropanol in the incubator. The absorbance of each well was read on a microplate reader (stat fax-2100, AWARENESS, Palm City, USA) at 545 nm. The relative cell viability (%) related to control wells containing cell culture medium without nanoparticles was calculated by $([A]_{test}/[A]_{control}) \times 100$ (*A* refers to the area).

3. Results and discussion

Several samples (out of the 12 prepared SPION samples according to the layout in Fig. (A) of the Supporting Information) are used as illustrative examples to visualize the effects of the polymer/iron ratio and the stirring rate parameters on the size and shape of SPI-

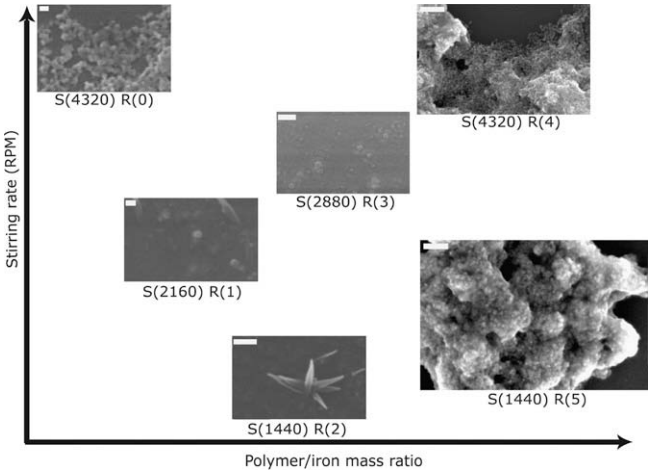

Fig. 1. Variation of SEM and HRSEM results for different stirring rates and polymer/iron mass ratio; bar scale is 100 nm.

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