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# Superparamagnetic nanoparticles stabilized by polymerized PEGylated coatings

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## Abstract

Novel superparamagnetic iron oxide nanoparticles coated with polymerized PEGylated bilayers were prepared. Bilayers composed of 10-undecenoic acid (UD) inner and UDPEG (PEG ester of UD) outer layers are resistant to aggregation after  $\gamma$ -irradiation. Various methods of coating were developed to prepare small (60–100 nm) and ultrasmall (20–35 nm) particles without size separation processes.

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*Keywords:* Superparamagnetism; Nanoparticles; Iron oxide; Bilayer; Poly(ethylene glycol); 10-Undecenoic acid; Synthesis; PEGylation

## 1. Introduction

Iron oxide nanoparticles offer valuable benefits in the *in vivo* biomedical applications due to their size-dependent superparamagnetism and non-toxic, metabolizable nature [1,2]. Superparamagnetic iron oxide nanoparticles are clinically used as contrast media in magnetic resonance imaging, and extensively evaluated for many applications such as magnetic drug delivery, cell tracking, hyperthermia [1,3–6]. These particles consist of

iron oxide magnetic cores coated with a protective layer providing stability and dispersability, and yet are often in agglomerated form in a wide size range (from nanometer to micrometer). Isolation of the size fraction for appropriate application requires excessive fractionation steps including successive centrifugation at various *g*-forces for different particle sizes, magnetic fractionation at high gradient field and column separation [7]. Therefore, elimination of aggregation is a major issue to control the particle size and simplify the process. Aggregation during the particle processing and/or use is another significant problem and a function of particle stability. Aggregation of cores can be reduced by *in situ* coating of the magnetic cores with surface binding molecules. However, stability

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of the coated particles depends on the strength of the bond between core and coating. Coating materials are mostly adsorbed on the surface of the magnetic core, and may desorb at low concentrations causing particles to aggregate. Aggregation is a significant problem in most applications of magnetic nanoparticles.

Pharmacokinetics of particles is largely determined by size and surface and major limitations for in vivo applications of magnetic nanoparticles are large particle size, quick blood clearance and non-specific uptake by macrophages. Small particles (<50 nm) and polyethylene glycol (PEG) surfaces have been shown to be effective in extending blood circulation time. Yet, there are no simple methods to prepare stable aqueous suspensions of PEG-coated superparamagnetic nanoparticles in small sizes that eliminate the need for excessive size separation processes.

Here, we demonstrate the preparation of novel superparamagnetic iron oxide nanoparticles that are coated with two layers of interdigitated and polymerizable surfactants. The inner layer is composed of a micelle forming ionic surfactant that covers the surface of the superparamagnetic core as a monolayer with the hydrocarbon tail extending out. The outer layer is composed of a non-ionic surfactant which is physically adsorbed on the inner layer and composed of a polymerizable hydrocarbon and a hydrophilic polymer, in particular PEG. Although few bilayer-coated particles such as gold and iron oxide have been reported recently, these systems are based on ionic surfactant bilayers that provide stabilization through electrostatic repulsion of the charged surfaces and do not contain PEG [8–10]. Tournier et al. [11] describe intertwined layers of glycerophospholipids and pluronics, however, with an overall

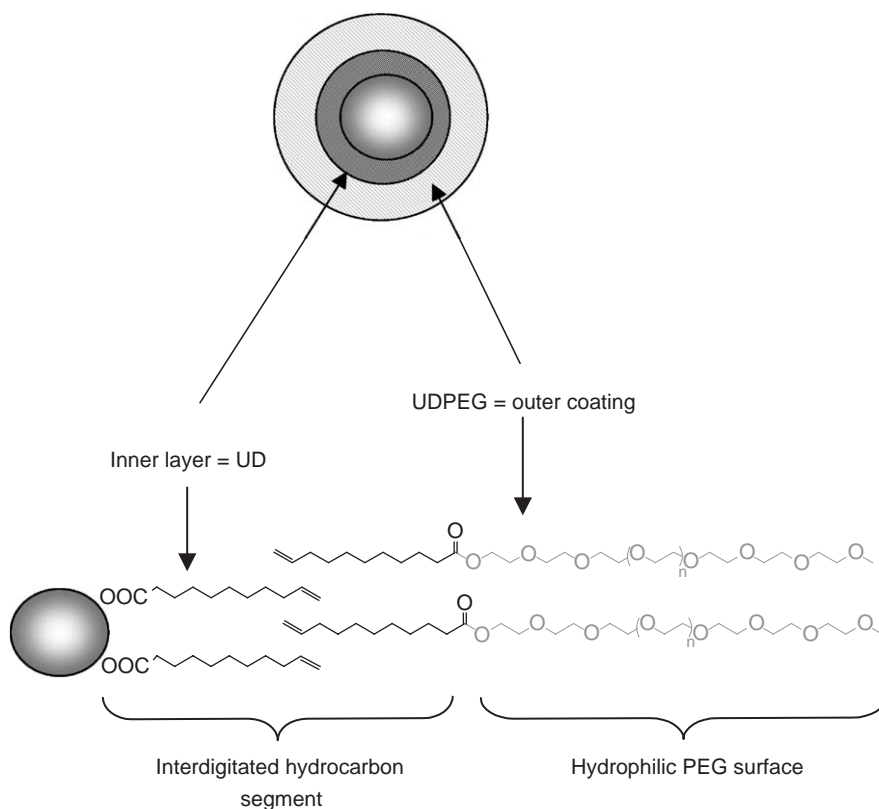


Fig. 1. Bilayer structure around magnetic core.

size of 120 nm and a broad size distribution. The stability of the bilayer structure under dilute conditions was not investigated [11]. Hatton et al. [10] demonstrated that if interdigitated bilayers of fatty acids are diluted, outer layer fatty acids desorb and hydrophobic particles agglomerate. Hatton and Brinker also demonstrated that stabilization of the bilayer structure with polymerization within or at the periphery is possible [10,12].

Preparation of particles with an inner layer of 10-undecenoic acid (UD) and an outer layer of PEG ester of UD (UDPEG) of different molecular weights of PEG will be discussed in the following sections (Fig. 1). Size distribution and aggregation of the magnetic cores are minimized by in situ coating of the magnetic cores with UD. Resistance to aggregation is induced by the crosslinking of two layers covering the magnetic cores through polymerization of the UD segments. To the best of our knowledge, this is the first report for the preparation of aqueous suspensions of small superparamagnetic iron oxide nanoparticles stabilized by polymerized PEGylated bilayers. We will discuss factors governing the aggregation and particle size and demonstrate methods for improved size control without multiple fractionation steps. Small (50–100 nm) and ultrasmall (20–35 nm) superparamagnetic nanoparticles with high saturation magnetization and stability have been achieved through these methods.

## 2. Experimental method

### 2.1. Characterization

The iron oxide core size was determined by transmission electron microscopy (TEM). TEM samples were prepared by placing a drop of the suspensions onto a 3 mm diameter, 300 mesh copper grid with a carbon membrane film. Bright-field images and an electron diffraction ring pattern from the particles were taken using a JEOL 2010 EM operated at 200 kV. The hydrodynamic size of the particles was measured by dynamic light scattering (DLS) at 25 °C using either a Brookhaven Instruments BI-200SM or

Malvern Particle Size Analyzer. Reported hydrodynamic sizes are based on the *z*-average of intensity-based distribution. The molecular weight of the polymerization product was measured by gel-permeation chromatography (GPC) using tetrahydrofuran (THF) eluent on a PLgel Mixed-E column and RI detector or using a HP Model 1050 LC system in-line to a Varex Evaporative Light Scattering Detector (ELSD). Iron oxide cores were etched by concentrated HCl and the organic material was analyzed by GPC. Magnetization was measured using a vibrating sample magnetometer with fields up to 2500 G at 25 °C. Iron concentration of the samples was measured by solution nebulization inductively coupled plasma emission spectrometry (ICP-AES, Varian Liberty II) after sulfuric acid digestion. All materials were purchased from Aldrich and used as received.

### 2.2. Synthesis of PEGylated surfactants

PEGylated non-ionic surfactants are named by the acronyms symbolizing the hydrocarbon portion and the molecular weight of PEG, such as UDPEG2000 indicating that the surfactant is composed of an undecene segment and PEG 2000 Da. For example, PEG (10.11 g, 2000 Da) was dissolved in 65 ml dry CH<sub>2</sub>Cl<sub>2</sub>. Then 1.1 ml triethyl amine (TEA) and 0.73 g dimethylamino pyridine (DMAP) were added to the solution and stirred under nitrogen in an ice bath. 10-Undecenoyl chloride (1.2 ml) in 1 ml dry CH<sub>2</sub>Cl<sub>2</sub> was added slowly to the chilled solution, stirred for a few hours in an ice bath and overnight at room temperature. The reaction mixture was filtered, diluted to 150 ml with CH<sub>2</sub>Cl<sub>2</sub>, washed three times with 0.1 N HCl, 0.1 N NaOH, brine solution and dried over anhydrous MgSO<sub>4</sub>. Product was obtained in quantitative yield after the solvent was removed in vacuum.

### 2.3. Synthesis of the magnetic nanoparticles

In a typical preparation, 0.36 g of NaNO<sub>3</sub>, 0.55 g of FeCl<sub>2</sub> (anhydrous beads) and 2.35 g of FeCl<sub>3</sub>·6H<sub>2</sub>O were dissolved under nitrogen in 45 ml of deoxygenated water with vigorous stirring. The solution was heated to 80–85 °C, and

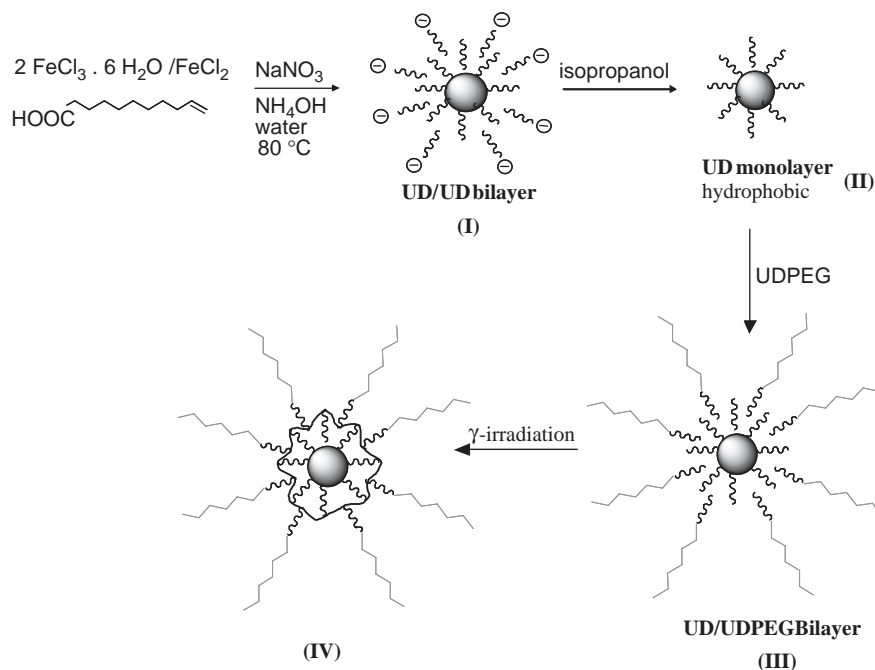


Fig. 2. Preparation of the coated particles.

then charged with rapid injection of 5 ml 28% (w/w)  $\text{NH}_4\text{OH}$  and 1.1 g of 10-undecenoic acid (UD). Crystal growth was allowed to proceed for about 30 min under vigorous stirring. The dark colloidal solution of nanoparticles (I in Fig. 2) was then cooled slowly to room temperature with stirring and was placed atop a handheld magnet overnight, and then decanted to remove any precipitate. Precipitates are assumed to be the aggregates of possible hydrophobic particles that were not dispersed in water. The dark-colored, stable suspension was added into acetone or alcohols, in particular in isopropanol, to isolate magnetic cores coated with a monolayer of UD (II in Fig. 2).

#### 2.4. Formation of PEGylated bilayers

**Method 1:** Isolated magnetic nanoparticles with a UD monolayer coating (II) were added in water at 5–30 mg/ml concentrations. A 5–40% aqueous solution of PEGylated ligand was added to the suspension slowly during continuous sonication at  $50$ – $60^\circ\text{C}$  until all or most of the monolayer-coated particles form a stable aqueous suspension when

placed atop a handheld magnet. For example, 101.5 mg of isolated UD monolayer-coated magnetic nanoparticles (II) (0.614 g Fe/g) was added to 8 ml water and sonicated for 20 min at  $60^\circ\text{C}$ . To this suspension, 4.9 ml 0.05 M aqueous solution of UDPEG5000 was added dropwise during continuous sonication. Suspension was allowed to sit atop a handheld magnet for 1 h. A small amount of non-dispersed particles was removed by magnetic decantation and the dark brown suspension (III) was isolated and filtered from a  $0.2\ \mu\text{m}$  syringe filter. No color change was observed after the filtration, indicating that a majority of the particles are 200 nm or less.

**Method 2:** Eight milliliters of an aqueous suspension of magnetic particles I (not precipitated in isopropanol) was shaken vigorously with 20 ml chloroform. A dark-colored organic phase was separated and chloroform was evaporated under reduced pressure and the residue was dried further under vacuum at  $60^\circ\text{C}$  for 2 h. Residue (0.242 g) was added in 10 ml water and 4 ml of 0.4 M aqueous solution of UDPEG750 was added in portions during continuous sonication at  $60^\circ\text{C}$

until a stable suspension was obtained. It was allowed to sit atop a handheld magnet for 1 h to remove any non-dispersed fraction from the dark suspension (III). The final colloidal suspension contained 22.6 mM Fe and 0.114 M UDPEG750.

*Method 3:* Six milliliters of an aqueous suspension of magnetic particles I (not precipitated in isopropanol) was sonicated at 60 °C with 12 ml toluene for 30 min. A dark-colored organic phase was separated and added to a 0.114 M aqueous solution of UDPEG750 in a centrifuge tube and then centrifuged at 15 000 rpm for 1.5 h. A dark-colored aqueous layer was separated and sonicated for about 20 min. It was allowed to sit atop a handheld magnet for a few hours; however no precipitation was observed. Stable aqueous suspension III (0.27 mM Fe) was easily filtered from a 20 nm filter with no color loss, suggesting that a majority of the particles are 20 nm or less.

### 2.5. Polymerization of the bilayers

Aqueous suspensions of PEGylated bilayers (III) were subjected to  $\gamma$ -irradiation before the removal of excess surfactants to keep the bilayer structure and hydrodynamic size intact during radiation. Irradiation was performed with a total dose of 3–5 MRad at MIT Nuclear Reaction Laboratory after sample deoxygenation. Irradiated particles (IV) were cleaned from the excess surfactant either by magnetic decantation after centrifuging at 17 000  $g$  for 1 h or through ultrafiltration through 50 000 Da cut-off Amicon ultra-centrifuge tubes. GPC analysis of the organic component of the particles after irradiation showed oligomers/polymers with 6–30 repeat units confirming polymerization.

## 3. Results and discussion

UD bilayer-coated superparamagnetic iron oxide nanoparticles were prepared from the aqueous solution of the iron salts ( $\text{Fe}^{2+}/\text{Fe}^{3+}$  molar ratio of 0.5) in the presence of excess UD, sodium nitrate with ammonium hydroxide (Fig. 2). Aqueous suspensions of monodisperse spinel-structured mixed iron oxide ( $(\gamma\text{-Fe}_2\text{O}_3)_{1-y}(\text{Fe}_3\text{O}_4)_y$

nanocrystals, each having a crystal size of about  $8.5 \pm 1.2$  nm, formed small clusters of 25–40 nm hydrodynamic size (Fig. 3a). The weakly adsorbed outer UD layer was initially removed by precipitation into polar solvents and aqueous suspensions of UD/UDPEG-coated nanoparticles (III) were obtained as PEGylated surfactants adsorbed on the inner hydrophobic layer (Fig. 2). In the PEGylated bilayer system, the hydrocarbons of both layers interdigitate through hydrophobic interactions and the outer PEG segment provides steric stabilization and water dispersability to the magnetic nanoparticles (Figs. 1 and 2). Magnetic nanoparticles coated with UD/UDPEG bilayers were obtained in clusters of 60–100 nm hydrodynamic size (Fig. 3b). Cluster size was insensitive to PEG molecular weight and concentration. Table 1 shows the sizes of a few of these particles measured by DLS after polymerization and before the removal of excess surfactants. Sizes do not change before and after polymerization. Precipitated UD monolayer-coated particles (II) were often insoluble in hydrocarbons unlike UD monolayer-coated particles prepared in organic solvents [13]. Aggregation might have occurred during the removal of the outer UD layer in the process of forming monolayer-coated particles (II). Desorption of some UD from the inner monolayer during the precipitation step would create partially uncoated particles that aggregate. We have observed that the precipitation solvent plays some role in this. Precipitation in methanol, for example, creates more aggregation than isopropanol. Previous reports on particles coated with ionic surfactant bilayers do not indicate such problems [10,12]. Ionic surfactants introduced as the outer layer can also adsorb on the surface of the magnetic core substituting the lost surfactants of the inner layer, and as the bilayer is formed, electrostatic repulsion prevents particle aggregation. However, non-ionic surfactants, such as UDPEG, lack the ability to do either and simply rely on the hydrophobic interactions and steric repulsion. Therefore, alternative methods are needed to prevent aggregation while forming bilayers with non-ionic surfactants.

In an alternative approach (Method 2), instead of precipitation into polar solvents, monolayer-

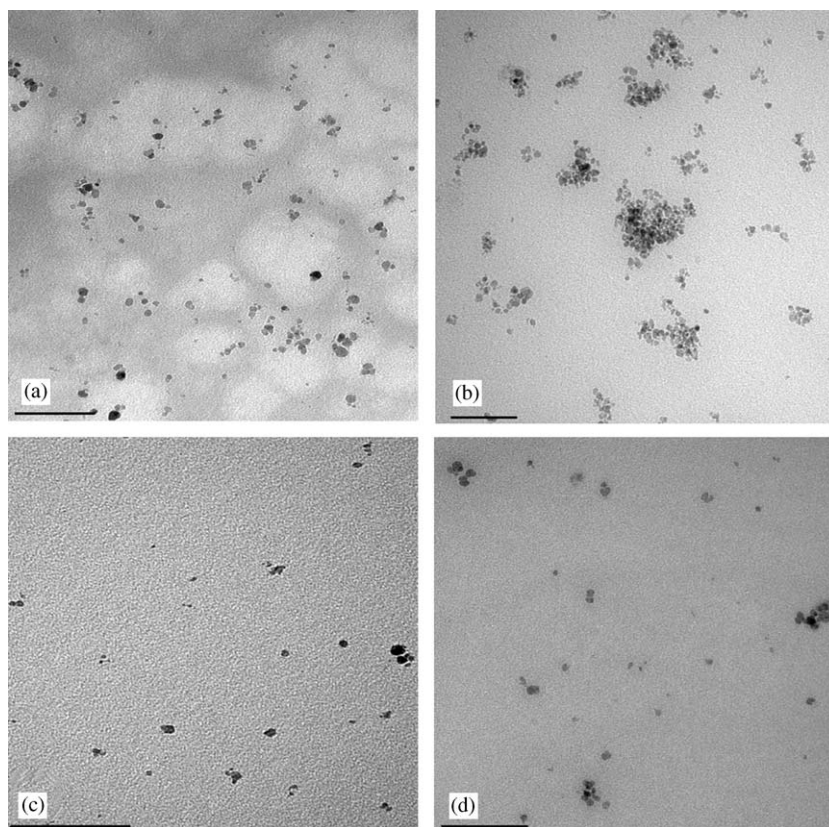


Fig. 3. TEM images of (a) UD/UD bilayer, (b) UD/UDPEG750 bilayer prepared by Method 1 (after  $\gamma$ -irradiation), (c) UD monolayer (in chloroform) and (d) UD/UDPEG750 bilayer prepared by Method 2 (after  $\gamma$ -irradiation). Scale bar represents 100 nm.

Table 1  
Hydrodynamic size of UD/UDPEG-coated magnetic nanoparticles

PEG molecular weight (g/mol)	Diameter (nm)
5000	100
2000	80
750	90

coated iron oxide nanoparticles (II) were extracted from the initial aqueous suspension (I) into organic solvents such as toluene or chloroform in which hydrophobic UD monolayer-coated magnetic particles are dispersible. Extraction into chloroform provided non-aggregated UD monolayer-coated nanoparticles with 20 nm diameter in chloroform (Fig. 3c). Once these monolayer-coated particles are dried and coated with UD-

PEG750 in water, 25–35 nm nanoparticles were obtained. A small amount of non-dispersed particles were removed by magnetic decantation. TEM clearly showed combination of individual particles and small clusters of few particles compared to those obtained after monolayer precipitation (Fig. 3d). Further improvement was achieved by the elimination of the dry state of the monolayer-coated particles after extraction of the monolayer-coated particles (Method 3). Centrifugal transfer of the hydrophobic particles from toluene into an aqueous solution of UDPEG750 generated aqueous suspension of UD/UDPEG750-coated magnetic nanoparticles with 20 nm hydrodynamic size. Elimination of dry state diminished monolayer aggregation dramatically. No non-dispersible fraction was observed through this method.

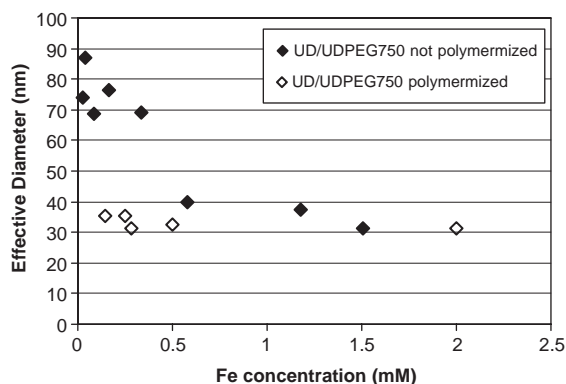


Fig. 4. Hydrodynamic size of the coated particles as a function of dilution: (◆) before and (■) after  $\gamma$ -irradiation measured by DLS.

Bilayer structure, however, is held together through weak interactions, and surfactants constituting the outer layer desorb easily upon dilution or removal of excess surfactants causing particles to agglomerate (Fig. 4) regardless of the PEG molecular weight (Fig. 5) within the studied molecular weight ranges. At further dilutions and at extended times, particles agglomerate and precipitate out of solution. However, hydrodynamic size of the irradiated particles was insensitive to dilution or removal of excess surfactant, indicating stability was gained by the polymerization (Fig. 4). Polymerization within the interdigitated hydrocarbon segments linked the surfactants of two layers together preventing desorption of the surfactants from the outer layer [10,12]. These superparamagnetic nanoparticles have a saturation magnetization of 85–100 emu/g Fe.

#### 4. Conclusions

Novel superparamagnetic iron oxide nanoparticles coated with interdigitated bilayers of 10-undecenoic acid (UD) and non-ionic surfactant UDPEG were prepared in small (60–100 nm) and ultrasmall (20–35 nm) sizes without excessive size separation processes. Stability was achieved by

linking the surfactants of the two layers together through polymerization of terminal olefins. Resistance to aggregation allowed removal of excess surfactant and stability at high dilutions, which is critical in many applications of magnetic nanoparticles. Especially ultrasmall sizes added to the PEG coating and stability make these particles suitable for in vivo applications. Particle sizes were affected by the method of preparation and dictated by the quality of the UD monolayer-coated particles due to the non-ionic nature and the lack of the electrostatic repulsion of PEG. These challenges of non-ionic systems require alternative preparative methods. Best practice is determined as the elimination of the precipitation and the dry state of the monolayer-coated particles. Nanoparticles with alternative water-soluble polymers, as well as other inorganic cores can be prepared with the same approach.

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