



Synthesis and characterization of stable dicarboxylic pegylated magnetite nanoparticles

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ABSTRACT

The coating of implantable nano- or micro-objects with polyethylene glycol (PEG) enhances its biocompatibility and biodistribution. Herein, we describe a new protocol that enhances and maintains MNPs stability in biological media, simulating multiple conditions to which they would be subjected in the human body. Magnetite nanoparticles (MNPs) prepared via a facile way at room temperature by co-precipitation reaction, were coated with dicarboxylic polyethylene glycol (DCPEG) via covalent bonds. The surface of the nanoparticles was first coated with 3-aminopropyl trimethoxysilane by a silanization reaction and then linked with DCPEG of different molecular weight ($M_w=5000$, $10,000$ and $20,000 \text{ g mol}^{-1}$). The uncoated magnetite nanoparticles, with an average size of 20 nm , exhibited superparamagnetism, high saturation magnetization and a negative surface charge (with a zeta potential value of -40 mV). Increase of M_w enhances the colloidal stability of MNPs and makes them more suitable to tolerate high salt concentrations (1 M NaCl) and wide pH (from 5.5 to 12) and temperature ranges ($24 \text{ }^\circ\text{C}$ to $46 \text{ }^\circ\text{C}$). The results indicate that magnetite nanoparticles coated with DCPEG with $M_w=20,000$ have improved properties over their counterparts, making them our best choice for biomedical studies.

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1. Introduction

The stability of magnetic nanoparticles (MNPs) in aqueous media results from the equilibrium established between attractive and repulsive forces. Attractive forces come from van der Waals and magnetic dipolar attractions, while repulsive forces mainly originate from electrostatic and steric repulsions [1]. In aqueous solutions, the Fe atoms coordinate with water, which dissociates readily to leave the iron oxide surface hydroxyl functionalized. These hydroxyl groups are amphoteric and may react with acids and bases [2]. Depending upon the pH of the solution, the surface of the magnetite will be positively or negatively charged. For superparamagnetic iron oxide nanoparticles (SPIONs) to be stable in aqueous media at physiological pH ($7.35\text{--}7.45$ for human blood), it is necessary to bring in additional charges to the nanoparticle surface. This is very important for electrostatically stabilizing the colloids due to the fact that the isoelectric point (IEP) of “naked” SPIONs is observed at pH 6.8 [3]. Around this point of zero charge

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(PZC), the surface charge density is too small and the particles are no longer stable in water and flocculate. Matching both electrostatic and steric stabilization, allows to obtain stable SPIONs [4–6].

Nevertheless, satisfying colloidal stability around neutral pH is not enough for magnetic nanoparticles to be useful as magnetic-field-directed drug targeting and as contrast agents for magnetic resonance imaging administered intravenously; they should also have good enough colloidal stability at physiological ionic strength around 0.17 M . Phosphate buffered saline (PBS) is often used to mimic the pH and ionic strength of physiological conditions as the osmolarity and ion concentrations of the PBS buffer match those of the human body [1]. However, in biological media, electrostatically stable MNPs are prone to aggregation due to neutralization of the surface charge [7]. Therefore, polymers are often used as stabilizing agents as they provide steric repulsion to the MNPs in addition to electrostatic repulsion, thus being able to reduce the influence of ionic strength on the colloidal stability.

Under physiological conditions, the effective minimization of SPIONs aggregation, caused by protein adsorption, must also be taken into account. Thus, anti-biofouling polymers are preferred to modify the MNPs for producing reticuloendothelial system evading nanoparticles with long blood half-lives [8]. Among anti-biofouling polymers, polyethylene glycol (PEG) is one of the good

choices. It has been demonstrated that PEG coated Fe_3O_4 nanoparticles present long blood circulation time [9].

Dicarboxyl-terminated PEG (HOOC-PEG-COOH), DCPEG, was adopted as the nanoparticle surface capping agent for providing the magnetic nanoparticle biocompatibility, meanwhile offering free surface carboxylic acid groups for further covalently conjugating the Fe_3O_4 nanoparticles to bioligands bearing amine groups, via the EDC/NHS mediated amidation reaction [10–14]. It has been demonstrated that Fe_3O_4 nanoparticles coated by dicarboxyl-terminated poly(ethylene glycol) (HOOC-PEG-COOH) can be used for constructing MRI dual modality molecular probes for *in vivo* colorectal carcinoma and gastric carcinoma detection [10,15,16]. Herein, we study the important effect of the molecular weight of DCPEG on the colloidal stability of magnetite nanoparticles, and present a new surface engineering approach that produces a highly stable polymeric coating and MNPs.

2. Experimental section

Preparation of DCPEG- Fe_3O_4 MNPs: All the reagents used in this work were analytical grade without further purification. Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\geq 99\%$), iron (II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 99%), ammonium hydroxide (NH_4OH , 33% NH_3 in H_2O) were obtained from Sigma. DCPEG₅₀₀₀ ($M_w \approx 4900 \text{ g mol}^{-1}$ —Figure S1 of Supporting Information), DCPEG₁₀₀₀₀ ($M_w \approx 11,400 \text{ g mol}^{-1}$ —Figure S2 of Supporting Information), DCPEG₂₀₀₀₀ ($M_w \approx 20,000 \text{ g mol}^{-1}$) were all purchased from Nano OcS. (3-aminopropyl) triethoxysilane (APTES) was a coupling agent from Sigma. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, $\geq 98\%$) and N-hydroxysuccinimide (NHS)

were bought from Sigma. In all experiments, deionized water was used.

The procedure used for the preparation of MNPs and coating by silanization reaction was based on a previously described protocol [17]. The magnetite (Fe_3O_4) nanoparticles were synthesized by the co-precipitation reaction of ferrous and ferric salts with NH_4OH acting as a reducing agent. Then the MNPs were coated with APTES and finally linked with dicarboxyl-terminated DCPEG via the EDC/NHS reaction between $-\text{NH}_2$ and $-\text{COOH}$ to form well-dispersed surface functionalized biocompatible MNPs.

Characterization: The morphology of Fe_3O_4 and DCPEG- Fe_3O_4 was observed using a Philips CM-12 transmission electron microscope (TEM) operating at an acceleration voltage of 100 kV. Samples for TEM were obtained by the evaporation of a drop of the solution sample ($C = 1 \text{ mg mL}^{-1}$) on a carbon-coated copper grid (400 mesh), under ambient conditions. Later the images were analyzed by *Image J* software (NIH). The dynamic light scattering (DLS) and zeta (ζ) potential measurements were performed with a Nano-ZS, Malvern Zetasizer. Ultrasonic dispersion was performed for 15 min for each sample before DLS and ζ potential measurements. All measurements were repeated three times. Magnetic properties were measured on a vibrating sample magnetometer (VSM) (PPMS, Quantum Design) at 25 °C in a field up to 100 kOe. Mass spectroscopy measurements (MALDI-TOF) were carried out using a ULTRAFLEX III TOF/TOF (BRUKER).

3. Results and discussion

Fig. 1A presents the average size of MNPs coupled with DCPEG with M_w of 5000, 10,000 and 20,000 g mol^{-1} , in aqueous medium obtained by DLS. Before modification the MNPs exhibit an average

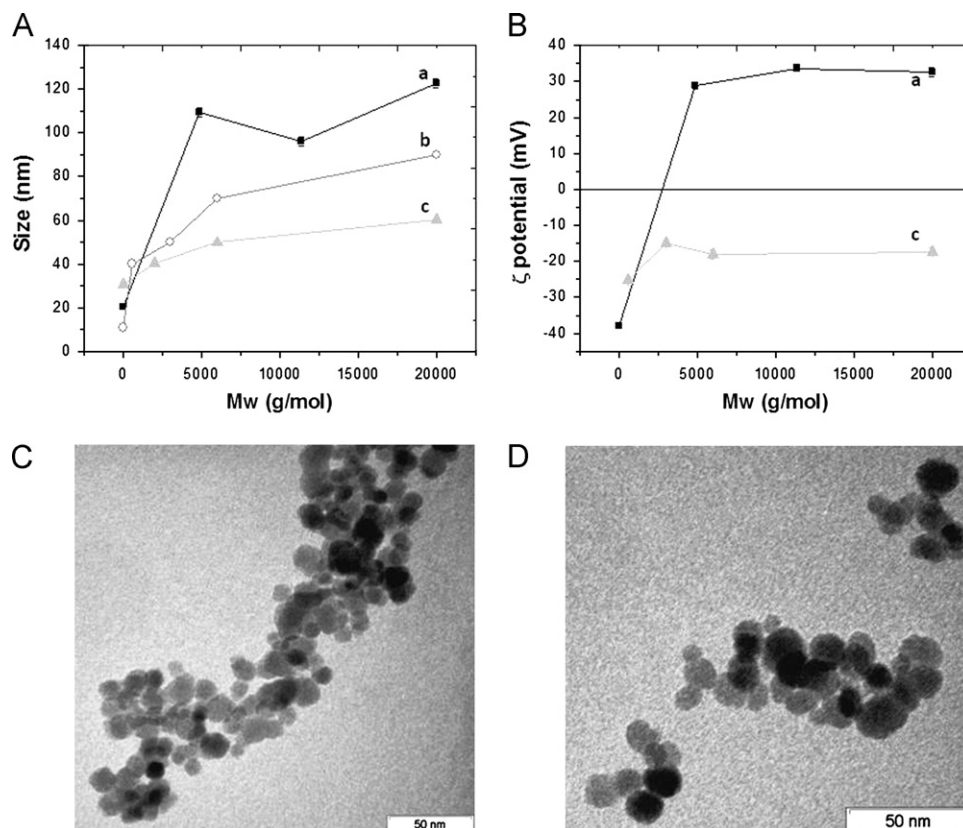


Fig. 1. (A) Evolution of hydrodynamic size with the increasing M_w of DCPEG (a: results from the work, b: results from [18], c: results from [17]). (B) Evolution of ζ potential with the increasing M_w of DCPEG (a: results from the work, c: results from [18]). TEM images of (C) Fe_3O_4 and (D) DCPEG- Fe_3O_4 .

hydrodynamic diameter of 20 nm. After modification the MNPs' size increases with the increasing length of DCPEG chains from 110 nm up to around 120 nm. Both Xie et al. [18] and Feng et al. [17] also found a similar behavior—see comparison in Fig. 1A. ζ potential measurements show that before the modification the MNPs are stable ($|\zeta| \geq 25$ mV) and negatively charged. However, when coated with DCPEG, the MNPs exhibit a positively charged ζ potential that remains positive regardless of DCPEG Mw (Fig. 1B). The positive ζ potential can be explained by the hydration of polymer coating surrounding the magnetite core, which increases steric repulsion. Another factor is that not all the $-\text{NH}_2$ groups in APTES react with DCPEG and, in culture medium, these groups can exist in a cationic form.

TEM images show that both Fe_3O_4 (Fig. 1C) and DCPEG- Fe_3O_4 (Fig. 1D) MNPs present spherical shape and a core size of 14.8 ± 2.9 nm and 13.8 ± 3.4 nm, respectively. The low contrast of the organic coating explains the similarity of these values.

For clinical purposes it is important that DCPEG- Fe_3O_4 MNPs maintain the same magnetic properties as before the modification. Both Fe_3O_4 and DCPEG- Fe_3O_4 MNPs show a superparamagnetic behavior at room temperature, with negligible hysteresis and remanence ($M_R(\text{Fe}_3\text{O}_4) = 1.19 \text{ emu g}^{-1}$ and $M_R(\text{DCPEG}_{10000}\text{-Fe}_3\text{O}_4) = 0.93 \text{ emu g}^{-1}$)—Fig. 2. The saturation magnetization of the samples is 70.0 emu g^{-1} (iron oxide) for the Fe_3O_4 and 67.9 emu g^{-1} for the DCPEG- Fe_3O_4 . Thereby it becomes possible to conclude that the magnetite core remains intact and the magnetic properties were kept.

Stability of the PEG coated nanoparticles was tested by measuring the ζ potential through a range of temperatures. Fig. 3A shows that uncoated MNPs exhibit a negative potential whereas, when coated with DCPEG, the MNPs' potential increases with increasing Mw. In all cases no significant changes of ζ potential were noticed with temperature indicating that DCPEG offers a stable coating to the MNPs.

To study the effects of pH and ionic strength on colloidal stability, MNPs were exposed to a range of pHs from 2 to 12 and to different NaCl concentrations (from 0 to 1 M). Fig. 3B shows that the MNPs coated with DCPEG₂₀₀₀₀ are more stable in pHs ranging from 9–12 and 2–4, and the ones coated with DCPEG₅₀₀₀ reveal more stability for basic pHs. The size distribution shown in Fig. 4 demonstrates that the MNPs size remains almost unchanged at NaCl concentrations as high as 1 M proving that the influence of ionic strength on the colloidal stability can effectively be reduced

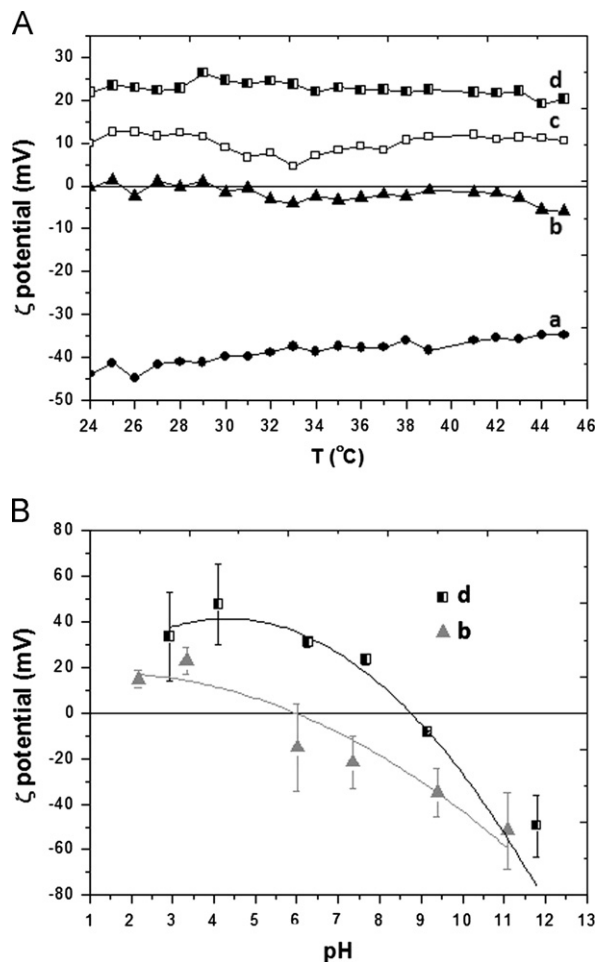


Fig. 3. Evolution of ζ potential measurements with (A) temperature and (B) pH (a: Fe_3O_4 , b: DCPEG₅₀₀₀- Fe_3O_4 , c: DCPEG_{10,000}- Fe_3O_4 , d: DCPEG_{20,000}- Fe_3O_4).

with the DCPEG₂₀₀₀₀ coating. In the literature there are calculations that estimate the maximum chain length of DCPEG₂₀₀₀₀ as being 200 nm [19]. Thereby, in Fig. 4 it is observed that the distribution peak is always below 200 nm, except for graphs B and C, in which the distribution is not as narrower as for the other concentrations, and a second peak appears indicating that for 0.001 M and 0.1 M concentrations, some aggregation must be present. For the DCPEG₅₀₀₀ (see supporting information) it was found that the MNPs were not stable for concentrations below 0.1 M.

4. Conclusions

In summary, biocompatible, water-soluble DCPEG- Fe_3O_4 MNPs of varying size were synthesized by surface modification of the Fe_3O_4 core by silanization reaction. MNPs colloidal stability was evaluated based on DLS and ζ potential measurements performed at room temperature. We concluded that DCPEG has an important effect on size, and charge properties of the MNPs. Among the different formulations tested, DCPEG₂₀₀₀₀- Fe_3O_4 MNPs kept the morphology of Fe_3O_4 core and showed colloidal stability, with non-detectable aggregation, when exposed to different salt concentration, pH and temperature. This, plus the multiple free carboxylic groups present on the nanoparticles surface makes DCPEG- Fe_3O_4 MNPs suitable for enhancing contrast in MRI and for drug delivery purposes.

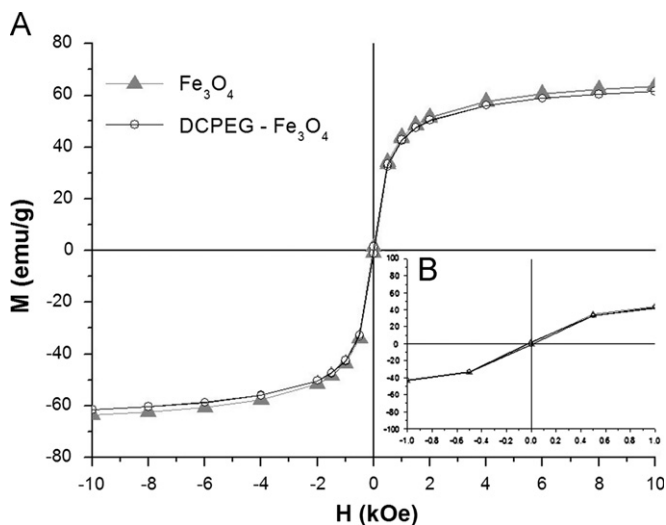


Fig. 2. Magnetization curves obtained by VSM at room temperature of Fe_3O_4 and DCPEG_{10,000}- Fe_3O_4 MNPs. (A) Scale from -10 kOe to 10 kOe and (B) Scale from -1 kOe to 1 kOe.

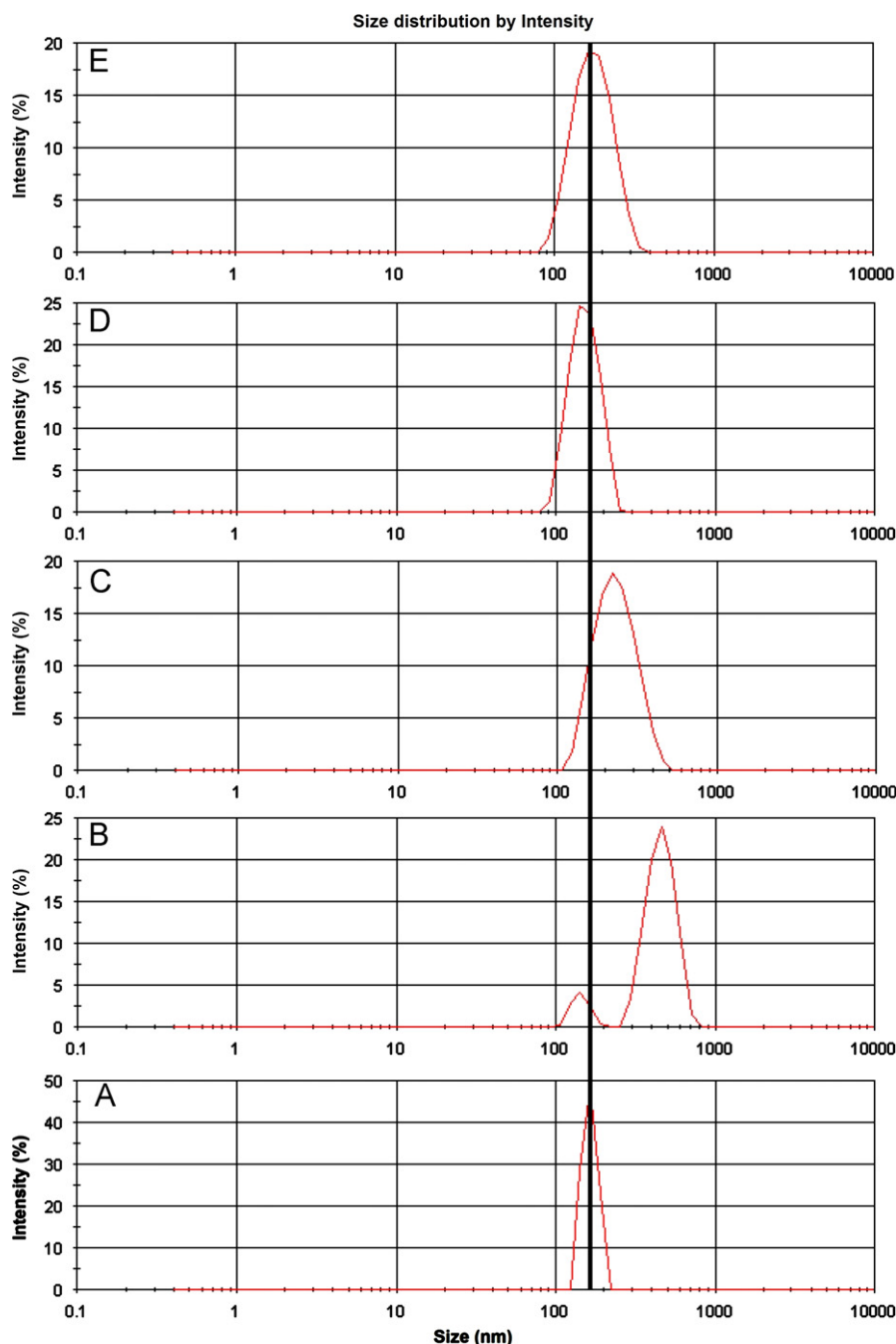


Fig. 4. Distribution of hydrodynamic sizes of DCPEG₂₀₀₀₀-Fe₃O₄ MNPs incubated in NaCl solution with different concentrations (A: 5×10^{-4} M, B: 0.001 M, C: 0.1 M, D: 0.5 M, E: 1 M).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.matlet.2013.03.058>.

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