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Novel carboxylated PEG-coating on magnetite nanoparticles designed for biomedical applications

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ABSTRACT

Fabrication of PEG coating on magnetite nanoparticles (MNPs) is one of the most favoured ways to ensure biocompatibility. Surface modification of magnetite by an own-prepared comb-like PEG-copolymer (PEGA-AA) was compared with two commercially available ones (carboxy-PEG (PEG-C) and phosphate-PEG (PEG-P)). ATR FT-IR data revealed that all polymers form complexes on the surface of MNPs. Electrophoresis and dynamic light scattering (DLS) experiments showed that both the type and quantity of the polymers' anchoring groups influence the aggregation of coated nanomagnets. PEG-C shell does not provide excess negative charges, so magnetite particles became aggregated. However PEG-P and PEGA-AA gradually modify the surface: neutralizing the originally positively charged MNPs below loading 0.5 mmol/g, while above it a polyanionic layer forms on nanomagnets dispersing them in salty media at pH ~6.5. The PEGA-AA comb-like copolymer is more efficient for MNPs PEGylation due to the uniform distribution of carboxylates and PEG chains along the carbon skeleton.

1. Introduction

Designing hydrophilic magnetic nanoparticles (MNPs) for diagnostic and/or therapeutic applications, such as MRI contrast enhancement, magnetic hypertermia, drug-delivery, etc. has been in the focus of scientific interest for the last decades [1–7]. Aqueous magnetic fluids (MF) designed for biomedical applications should be non-toxic, biocompatible, chemically stable, and remain uniformly sized even under physiological conditions [8,9]. Bare MNPs (nanomagnets) inherently aggregate in biorelevant media (neutral pH, high salt and protein content) so they cannot be applied in living systems. A protective layer on the particle's surface is necessary to prevent the aggregation, to stabilize the dispersion and to hinder their chemical and biological degradation.

Wide varieties of coating compounds have been published in the literature, among them are frequently used small molecules (e.g. citrate [9–12], surfactants [13–15]) and larger ones (e.g. dextrane [16,17], polyelectrolytes [9,10,18], PEO–PPO–PEO triblock copolymers [2,19]) as well. Beside the chemical resistance of the shell, very hydrophilic surfaces are required to avoid the non-specific protein adsorption (opsonisation) in the blood. Recently the PEGylation, i.e. creating protective polyethylene glycol (PEG) (also called as polyethylene oxide (PEO)) layer on MNPs is the most widely applied method to ensure the biocompatibility of nanomagnets [2,20]. The superhydrophilic PEG coating can prevent opsonisation in blood [2,19] and hence it presumably increases the life-time of the PEGylated MNPs in the circulation system. The prolonged circulation of PEG coated MNPs in living systems raises their theranostic potential.

Numerous methods related to the binding of PEG to MNP surface have been described elsewhere [1,2,20–27]. Among several approaches including in situ and post-synthesis coatings, one of the popular ways is to prepare PEG coating on surfacted (mainly oleate double layer covered) nanoparticles [28–31]. Although these PEGylated MNPs exhibit remarkable relaxivity (r_2) values promising for MRI contrast enhancement, the colloidal stability is not satisfactory due the weakly bound top shell of the triple layer coated MNPs [32]. Currently published results proved that the protective layer can be fastened properly on the surface of nanomagnets via functional (e.g. carboxyl, phosphate) groups of organic molecules [9,24,25,27,31,33]. Therefore polymers harnessing the joint combination of high hydrophilicity of the PEG chains and anchoring sites that form chemical bonds through carboxylic/phosphate groups open opportunities to construct a new generation of biocompatible core–shell MNPs.

The colloid stability is essential for biomedical applications, since the aggregation of MNPs can lead to embolism or thrombosis

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in the blood vessels. An optimization procedure of carboxylate@MNP core–shell nanoparticle preparation was established in our group [9], which guides us through a sequence of the physicochemical tests (e.g. adsorption, electrophoresis and dynamic light scattering measurements) to select the potential candidates for biomedical applications. Briefly only the samples stable under the physiological conditions (pH ~7, 150 mM NaCl) can be chosen for in vitro tests. The steps of this optimization procedure can be used as a guideline to compare the efficiency of PEG-polymers in MNP stabilization.

The main goal of this paper is to prepare water-based, biocompatible magnetic fluids consisting of core–shell PEGylated MNPs stable under physiological conditions. We compared the adsorption and colloidal stabilizing efficiency of three different PEG-polymers with various structures and anchoring (carboxyl and phosphate) groups on magnetite in order to choose the most efficient PEG-coating.

2. Materials and methods

2.1. Materials

Magnetite nanoparticles (MNPs) were synthesized by co-precipitation method starting from analytical grade $FeCl_2$ and $FeCl_3$ salts (Molar, Hungary) as described in detail in our previous and recent papers [8,9,18,32]. The size of naked nanomagnets is ~10 nm determined by TEM (picture is not shown here) [9], the material is of black colour and exhibits strong magnetism. The crystalline structure was identified by X-Ray diffraction using the six characteristic peaks of magnetite according to the JCPDS database [34].

Two commercially available linear PEG-polymers with different end groups and an own-synthesized one were chosen for coating the MNPs. The α-hydroxy-ω-carboxy PEG (Iris Biotech, Germany) is abbreviated as PEG-C, and the α-methoxy-ω-phosphate PEG (Chemicell, Germany) is marked as PEG-P. The PEG-acrylate-acrylic acid comb-like copolymer (PEGA-AA) is synthesized by quasiliving atomic transfer radical polymerisation (ATRP) using a complex of Cu(I)-chloride and hexamethyl-triethylene-tetramine as catalyst. PEGA-AA was purified by passing it through a neutral Al_2O_3 column; PEG-C and PEG-P were used as received. The chemical structures of the studied polymers are shown in Fig. 1 and their main features are summarized in Table 1.

All experiments were performed at room temperature (25 ± 1 °C) and at atmospheric pressure. Considering future biomedical application the coated nanomagnets were studied at near physiological pH. The pH was adjusted to 6.5 ± 0.2 (denoted as ~6.5) to avoid the remarkable carbon dioxide absorption at higher pHs and the use of various buffers during experiments, which can influence the adsorption equilibrium. This pH approaches the conditions in cancer cells (pH=6.3–6.8) [35].

2.2. Preparation of PEG-coated core–shell nanomagnets

The nanomagnets were coated by the adsorption method, equilibrating a given mass of MNP with various amounts (0–1.5 mmol/g MNP) of PEG-polymers (PEG-C, PEG-P and PEGA-AA respectively) in the volume of 10 mls. The adsorbed amount of PEG polymers is expressed in mmol/g, where the molar amount of their functional groups is related to the unit mass of magnetite. The pH was set to pH ~6.5 with a 0.1 M NaOH solution. The samples containing MNPs and polymer were left to stand for an hour (adsorption time). The MFs consisting of core–shell nanoparticles designated as PEG-C@MNP, PEG-P@MNP and PEGA-AA@MNP, were further characterized without any additional purification process and stored in fridge prior to use.

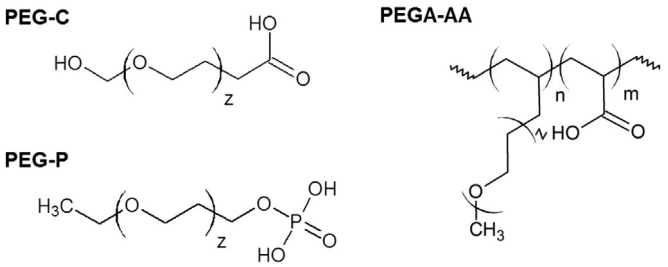

Fig. 1. Chemical structure of the studied PEG-polymers.

Table 1
Some characteristics of compounds used for coating MNPs.

Coating materials	M_n (g/mol)	Specific amount of functional groups[a] (mmol/g)	Type of compound
PEG-C	3000	0.3	Linear polymer
PEG-P	5000	0.2	Linear polymer
PEGA-AA	4500	5.0	Comb-like polymer

[a] Characteristic functional group is COOH for PEG-C and PEGA-AA, and phosphate for PEG-P expressed in millimoles per mass of polymer.

2.3. Infrared spectroscopy (ATR FT-IR)

Bio-Rad Digilab Division FTS-65A/896 spectrometer (with DTGS detector) equipped with a Harrick's Meridian Split Pea Diamond ATR accessory was applied to record the ATR FT-IR spectra. The absorbance of the samples was measured in single reflection mode over the 400–4000 cm^{-1} range (with resolution of 2 cm^{-1}), accumulating 256 scans. One drop of the nanomagnets' dispersions (MNP, PEG-C@MNP, PEG-P@MNP and PEGA-AA@MNP) or of the pure polymer solutions (PEG-C, PEG-P and PEGA-AA) was dried onto the surface of the diamond crystal. The polymer loading was 1 mmol/g MNP, the pH was set to ~6.5 and the NaCl concentration to 10 mM. The background spectra were measured on a clean and dry diamond crystal.

2.4. Electrophoresis measurements

Zeta potentials of the bare and coated MNPs (MNP, PEG-C@MNP, PEG-P@MNP and PEGA-AA@MNP) were determined using NanoZS (Malvern, UK) apparatus with a 4 mW He–Ne laser source (λ=633 nm). Electrophoretic mobilities of dilute dispersions (0.1 g MNP/L) were measured in DTS 1061 cells at 25 ± 0.1 °C and the Smoluchowski equation was used to convert them to zeta potentials. The surface modification effect of the PEG-polymers was studied at various loadings (0–1.5 mmol/g MNP) at pH ~6.5 and 10 mM NaCl. The pH-dependent surface charging of the nanomagnets was examined between pH ~3 and ~10 set by using 0.1 M HCl and NaOH solutions.

2.5. Particle size determination

The mean diameter of the bare and coated nanomagnets (MNP, PEG-C@MNP, PEG-P@MNP and PEGA-AA@MNP) was measured by NanoZS (Malvern, UK) dynamic light scattering (DLS) instrument operating at 173° (backscattering mode) at 25 ± 0.1 °C. Same solution conditions were ensured as for electrophoresis experiments (0.1 g MNP/L, pH=3–10, 10 mM NaCl), the amount of the added PEG-polymer was varied between 0 and 1.5 mmol/g MNP. Aggregation state of the core-shell MNPs was characterized by the change of the average hydrodynamic diameter (Z-Ave). We used