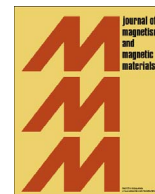




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Clustering of carboxylated magnetite nanoparticles through polyethylenimine: Covalent versus electrostatic approach

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ABSTRACT

Carboxylated magnetite nanoparticles (MNPs) are frequently used to develop materials with enhanced properties for MRI and hyperthermia. The controlled clustering of MNPs via covalent or electrostatic approaches provides opportunity to prepare high quality materials. MNPs were prepared by co-precipitation and coated by poly(acrylic acid-co-maleic acid) (PAM@MNP). The clusters were synthesized from purified PAM@MNPs and polyethylenimine (PEI) solution via electrostatic interaction and covalent bond formation (ES-cluster and CB-cluster, respectively). The electrostatic adhesion ($-\text{NH}_3^+$ and $-\text{COO}^-$) and the formed amide bond were confirmed by ATR-FTIR. The averaged area of CB-clusters was about twice as large as that of ES-cluster, based on TEM. The SAXS results showed that the surface of MNPs was smooth and the nanoparticles were close packed in both clusters. The pH-dependent aggregation state and zeta potential of clusters were characterized by DLS and electrophoresis measurements, the clusters were colloidally stable at $\text{pH} > 5$. In hyperthermia experiments, the values of SAR were about two times larger for the chemically bonded cluster. The MRI studies showed exceptionally high transversion relaxivities, the r_2 values are $457 \text{ mM}^{-1} \text{ s}^{-1}$ and $691 \text{ mM}^{-1} \text{ s}^{-1}$ for ES-cluster and CB-cluster, respectively. Based on these results, the chemically clustered product shows greater potential for feasible biomedical applications.

1. Introduction

The biomedical application of magnetic nanomaterials has been in the focus of scientific interest over the past few decades. Magnetite (and partially maghemite) nanoparticles (MNPs) are one of the most promising candidates for this purpose. These superparamagnetic iron oxide nanoparticles (SPIONs) are planned to be used in diagnostics (e.g., contrast agent for magnetic resonance imaging (MRI), magnetic particle imaging) and in different therapies (e.g., targeted drug delivery and magnetic hyperthermia) as well [1–11]. Nevertheless, the most progressive trend is the so-called theranostic approach, i.e., the combination of diagnostics and therapy to get more efficient clinical applications [11–14].

The SPIONs used for these purposes must fulfill a number of criteria, including the MNPs have to be non-toxic, chemically stable, uniform in size and should be well-dispersed in the relevant media. This latter means that the colloidal stability of magnetite nanoparticles is of crucial importance under physiological conditions, for example in blood, in which the pH is ~ 7.3 and the salt concentration is $\sim 150 \text{ mM}$

[1,3,6,7,11,12,15,16]. The naked particles do not meet this criterion, so the MNPs must be coated to prevent their aggregation [16]. There is a wide variety of options for the surface modification of nanoparticles. For example covering the surface of the magnetite NPs by carboxylated polyelectrolytes [17–20] is a very favorable choice (e.g., polyacrylic acid [21], chondroitin-sulfate-A [22], poly(acrylic acid-co-maleic acid) [23], etc.), because this not only provides the necessary colloidal stability of coated nanoparticles, but the free (not bonded) $-\text{COOH}/-\text{COO}^-$ groups are perfect even for binding different drugs (e.g., tissue plasminogen activator [24]).

Many promising SPION products can be found in the literature [11] with the potential of being used in future biomedical applications, but many questions still need to be clarified and for a greater potential in theranostics further enhancement of MNPs is required. One possibility is the preparation of particle clusters. The particle-particle interactions in clusters can modify the magnetic behavior of nanoparticles [25–27], which basically influence their contrast-enhancing effects during MRI diagnostics and heating efficiency during magnetic hyperthermia treatment.

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In the past decade the significant advances made in controlled clustering of magnetic nanoparticles have enabled the preparation of high quality nanoparticles with tailored properties (e.g., size, composition) enabling biomedical use [28]. Recently Illés et al. published that clustered oleate double layer (OA) coated MNPs covered by poly(ethylene glycol) showed enhanced MRI contrast compared to the non-clustered nanoparticles [18]. Similar enhancement was realized by Berret et al. [29] in the r_2 relaxivities of MNPs clustered electrostatically via oppositely charged polyions. Nesztor et al. synthesized poly(acrylic acid-co-maleic acid) coated magnetite nanoparticles (PAM@MNP) clustered by branched polyethylenimine (PEI-cluster). These magnetic clusters were prepared via electrostatic adhesion of oppositely charged PEI and PAM@MNP. Two different mixing methods, as well as the effect of PEI's relative amount and electrolyte (NaCl) concentration on the cluster formation were tested to choose the optimal preparation of electrostatically bonded clusters [30]. Friedrich et al. attached tissue plasminogen activator (tPA) proteins to PAM@MNP nanoparticles via electrostatic adsorption and covalent binding strategy using an amino-reactive activated ester reaction. The behavior of these products was particularly different [24]. The covalent binding strategy could also be used in case of the PEI and PAM@MNP particles and the clusters prepared by this reaction should be slightly different from the electrostatically bonded clusters.

The fundamental aim of this work is the preparation and comprehensive characterization of PEI-clusters obtained by electrostatic interaction or chemical bonding of the components. First, we synthesized the magnetite by co-precipitation and after the purification the nanoparticles were coated by PAM. The clusters were prepared from purified PAM@MNP nanoparticles and PEI solutions via electrostatic interaction and chemical bond formation. Then we characterized the clusters by TEM and SAXS, and we studied their pH-dependent surface charging and aggregation properties. Next, we identified the bonds between the PAM@MNP and PEI by FTIR-ATR. Finally, we tested the products in magnetic hyperthermia and in MRI diagnostics.

2. Materials and methods

2.1. Synthesis and materials

2.1.1. Synthesis of MNPs

Magnetic nanoparticles were prepared by co-precipitation method published in our earlier paper [17,31]. $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and NaOH were analytical grade and purchased from Molar, Hungary. The purified sol was stored at pH~3 and it was identified as magnetite by X-ray diffraction. The magnetite nanoparticles (MNP) were spherical and their average diameter was ~10 nm based on TEM [22,32].

2.1.2. Synthesis of PAM-coated MNPs

Carboxylated magnetite nanoparticles were prepared by surface modification. Poly(acrylic acid-co-maleic acid) with molecular weight ~1800 Da (PAM, purchased from Sigma-Aldrich) was mixed with MNP sol in a ratio of 1.1 mmol PAM (total amount of carboxylate and carboxylic groups) to 1 g MNP (i.e., mmol/g). The pH was set to ~6.5, and one day was ensured for the adsorption process [23]. The amount of PAM which was not adsorbed on MNPs was completely removed from the sol by an excessive washing process. During this, the pH was set to ~3 which resulted in the aggregation of PAM-coated magnetite nanoparticles (PAM@MNP). The PAM@MNP were settled by a strong magnet and the supernatant was removed, after which the particles were re-dispersed in ultra-pure water. This process was repeated three times. Finally, the pH was set to ~6.5, and the stable sol of PAM@MNP contained approximately 0.9 mmol adsorbed PAM/1 g MNP [23,30]. Due to the presence of $-\text{COOH}$ and $-\text{COO}^-$ functional groups on the surface of PAM@MNP particles, at pH~6.5, the amount of charge on the surface is approximately -0.175 mmol/g based on the potentiometric acid-base titration as published earlier [30].

2.1.3. Preparation of PEI-solution

For the preparation of clusters, branched polyethylenimine with the molecular weight of ~25000 Da (PEI, purchased from Sigma-Aldrich) was used. PEI has three different, N-containing moieties: primary, secondary and tertiary amino groups, which can be protonated at different pH-ranges. At pH~6.5 the positive charge on PEI is derived from the protonated amino groups, and the amount of charge is +10.30 mmol/g based on potentiometric acid base titration [30]. As a result of this opposite charge formation the interaction between deprotonated carboxylates (PAM@MNP) and protonated amino groups (PEI) was controlled during the preparation of clusters. The synthesis of clusters was performed in two different ways, i.e., the electrostatic structural self-assembly and the chemical bond formation.

2.1.4. Synthesis of electrostatically bonded PEI-cluster

The "electrostatic way" relies on the extensive charge screening and concomitant loss of repulsive double layer forces upon adsorption of PEI [33,34]. This method is a type of self-assembly. The clusters were prepared by mixing the PEI-solution (5 mL, 56.9 mg/L) and the PAM@MNP sol (5 mL, 20 g/L) adjusted to the same pH and ionic strength (pH~6.5 and 10 mM NaCl). The synthesis was performed by synchronous flow method, i.e., the two precursors were simultaneously and promptly injected into a vial and sonicated for 1 min immediately after mixing [30]. In the prepared clusters (ES-cluster) the PEI to PAM@MNP charge ratio is approximately 0.17. The exact iron content of the sample was determined by inductively coupled plasma (ICP) method using an Optima 7000 DV ICP-OES instrument (Perkin-Elmer, Shelton, CT, USA), thus the magnetite content of the ES-cluster's sol is 9.1 g/L.

2.1.5. Synthesis of chemically bonded PEI-cluster

In case of the novel "chemical way", 1.8 mL MES buffer (192.1 mg MES- H_2O , pH ~6.2, purchased from Sigma-Aldrich) was added to 22 mL PAM@MNP sol (1.75 g/L), the mixing was provided by ultrasonication (US) treatment (impulse mode). During further sonication, 0.35 mL PEI solution (0.1 mg/mL) was dosed into the mixture, then the vessel was put into cold water (~0 °C). Finally, 31.5 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide monohydrochloride (Sigma-Aldrich) was added to the mixture under US treatment. The vessel was stored in refrigerator for 2 days, occasionally shaking and ultrasonating (5 impulses) the sample. The purification was accomplished by dialysis (MWCO=6–8 kDa) against ultra-pure water, the end-point of dialysis was ~5 $\mu\text{S}/\text{cm}$. The sample was let to settle on a magnet for two days, and the supernatant was removed. The prepared clusters (CB-cluster) were dispersed in 5 mL ultra-pure water. The PEI to PAM@MNP charge ratio is approximately 0.06. The exact iron content was determined by ICP as mentioned above, thus the magnetite content of the CB-cluster's sample is 5.3 g/L. It is important to note that according to our previous experience, the use of MES as a buffer agent is strongly recommended for obtaining a suitable degree of functionalization [35].

2.1.6. Materials

NaCl, HCl and NaOH, used to set the pH and ionic strength, were analytical grade products of Molar (Hungary). The majority of experiments were performed at $\text{pH}=6.5 \pm 0.3$ so we simplify the notion of this pH value as ~6.5 and omit to note pH unless it has special significance or the values are different. Ultra-pure water was provided by a Zeener water purification system (HumanCorp, Korea). The experiments were performed at 25 ± 1 °C.

2.2. Transmission electron microscopy (TEM)

The TEM micrographs of clusters were taken by a JEOL JEM 1400+ transmission electron microscope. The accelerating voltage of 80 kV was applied; the maximum resolution of the instrument is

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