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Preparation and characterization of chondroitin-sulfate-A-coated magnetite nanoparticles for biomedical applications

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ABSTRACT

Polysaccharides are promising candidates for manufacturing biocompatible core–shell nanoparticles with potential *in vivo* use. Superparamagnetic magnetite nanoparticles (MNPs) have prospective application in both diagnosis and therapy, and so developing a novel polysaccharide shell on MNP core is of great challenge. MNPs were prepared by co-precipitation, then the surface of purified MNPs was coated with chondroitin-sulfate-A (CSA) to obtain core–shell structured magnetite nanoparticles (CSA@MNP). The effect of the added amount of CSA on the surface charging and the aggregation state of MNPs at various pHs and 10 mM NaCl was measured by electrophoresis and dynamic light scattering. The amphoteric behavior of MNPs was fundamentally modified by adsorption of CSA polyanions. A very low CSA-loading induces the aggregation of MNPs, while four times more stabilizes the dispersions over the whole pH-range studied. The coagulation kinetics experiments measured at pH = 6.3 ± 0.3 showed that salt tolerance of CSA@MNPs rises up to ~150 mM NaCl.

1. Introduction

Superparamagnetic iron oxide (magnetite, Fe_3O_4 and maghemite, γ-Fe_2O_3) nanoparticles (SPIONs) are in the focus of scientific interest because of their potential biomedical applications such as MRI contrasting, targeted drug delivery and magnetic hyperthermia [1–6]. Most of these applications require the SPIONs to be non-toxic, chemically stable, sufficiently uniform in size, and well-dispersed in aqueous media. The colloidal stability of water-based magnetic fluids (MFs) prepared from SPIONs is of crucial importance under physiological conditions (e.g. in blood pH ~ 7.2–7.4 and salt concentration ~ 150 mM) because particle aggregation in blood vessels can be disastrous [1–7]. The SPIONs must be coated to prevent aggregation and dissolution of magnetite nanoparticles (MNPs) under physiological conditions [3]. Different organic compounds have been used to coat SPIONs, such as neutral polymers (e.g. natural dextran [8–11]) and polyelectrolytes (e.g. synthetic polyacrylic acid [8,12–14]). Innumerable SPION preparations have been synthesized for biomedical applications, but only a few of them were characterized systematically in respect of pH-dependent surface charging and aggregation state of coated nanoparticles. Moreover, their salt tolerance would be also important regarding the salty medium and different pH values in the human body.

Dextran- and modified dextran-coated iron oxide nanoparticles are very common magnetic products for biocompatible applications (e.g., Ferumoxides (Feridex or Endorem), Ferumoxtran-10 (Sinerem or Combidex), Ferucarbotran (Resovist) [14,15]). Other polysaccharides are frequently used as coating agents [16], too. One example is chondroitin-sulfate (CS), patented under US 5427767 A [17] and EP-1-433-482-B1 [18]. Chondroitin-sulfate is a natural polysaccharide, which contains a repeating unit of one glucuronic acid and one N-acetyl-galactoseamine, modified by sulfate groups replacing –OH groups. Depending on the positions and the quantities of the sulfate groups several types of CS can be distinguished, such as chondroitin-sulfate-A (CSA, chondroitin-4-sulfate) and chondroitin-sulfate-C (CSC, chondroitin-6-sulfate) [19] (see Fig. 1). The procedure of magnetic nanoparticle preparation in the presence of CS for potential MRI contrast agents has been patented [17]. Magnetic microspheres with CS-content are offered to use for magnetic targeting [20]. Furthermore, there is a potential for drug delivery, since some promising anticancer drugs, such as multivalent pseudopeptide, bind to chondroitin-sulfate with high affinity [21]. Based on all these, well-defined chondroitin-sulfate-coated core–shell magnetite nanoparticles can be promising candidate for theranostic application.

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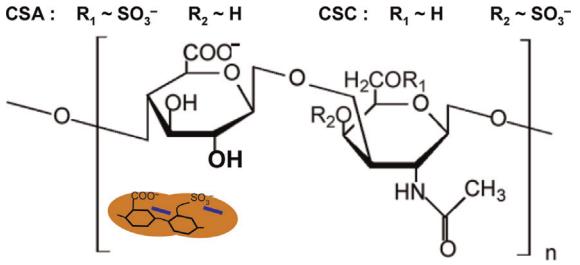

Fig. 1. The repeating unit of fully deprotonated CSA and CSC.

The fundamental aim of our research is the preparation of novel chondroitin-sulfate-coated core–shell magnetite nanoparticles (CSA@MNP) by post-coating method, which are presumably stable colloidally under physiological condition. We intend to synthesize magnetite nanoparticles by co-precipitation method and to coat them after their purification and characterization. We plan to describe the adsorption of CSA on magnetite and to study the pH-dependent surface charging and aggregation of the CSA@MNP particles. Finally we intend to test the feasibility of the prepared CSA@MNPs in biorelevant media by coagulation kinetics studies.

2. Experimental section

2.1. Materials

The $FeCl_2$, $FeCl_3$ salts and NaOH for magnetite synthesis by co-precipitation [22–26] were analytical grade reagents obtained from Molar, Hungary. The prepared material was purified carefully through washing and dialysis, and it was stored as stable sol at pH ~ 3 and 4 °C.

The chondroitin-sulfate-A (CSA) was purchased from Sigma-Aldrich as sodium-salt (Na_2CSA), which could contain a small amount of chondroitin-sulfate-C (CSC), too. One repeating unit of CSA (Fig. 1) contains one –COOH and one $–SO_3H$ group. The strongly acidic sulfate groups ($–SO_3^-$) in CSA are fully deprotonated at a wide pH-range [27,28]. However, the –COOH groups in CSA have a pH-dependent dissociation ($pK_{\beta\text{-glucuronic acid}} \sim 2.9$) [29] given by Eq. (1).

$$-\mathrm{COOH} \leftrightarrow -\mathrm{COO}^- + \mathrm{H}^+ \quad (1)$$

The notation "CSA" is used in this article for sodium-salt regardless of the actual degree of dissociation of the carboxylic groups $\alpha = [-\mathrm{COO}^-]/([-\mathrm{COO}^-]+[-\mathrm{COOH}])$. The amount of CSA is expressed through the mole of repeating units, which equals to the number of dissociable –COOH groups.

NaCl, HCl and NaOH, analytical grade products of Molar (Hungary) were used to adjust the pH and salt concentration in all experiments. Ultra pure water from a Milli-Q RG water purification system (Millipore) was used. All measurements were performed at 25 ± 1 °C.

2.2. X-ray diffraction (XRD)

A Bruker D8 Advance X-ray diffractometer operating in the reflection mode with Cu-Kα radiation was used to take the XRD patterns of synthesized iron oxides. The scanning range of 2Θ was between 20° and 80°. The samples of magnetite sol were dried on a glass holder before the measurements. The identification of magnetite was based on the characteristic peaks in the diffractograms using JCPDS database. The Scherrer equation (see Eq. (2)) was used to calculate the primary particle size:

$$d = (K\lambda)/(B\cos\Theta) \quad (2)$$

where d is the average particle size, K is the Scherrer constant (its value is 0.9 for magnetite), λ is the X-ray wavelength (0.154 nm), B is the peak broadening and Θ is the position of the peak maximum.

2.3. Magnetic measurement

A vibrating sample magnetometer VSM 880 (DMS/ADE Technologies-USA) was used to measure the magnetization curves at the NCESCF-UP Timisoara. The analysis was performed at room temperature on stable aqueous MNP sol at ~10% by weight; the maximum of the applied field was ~840 kA/m. The value of specific magnetization was related to the actual amount of MNP.

2.4. Transmission electron microscopy (TEM)

A Philips CM-10 transmission electron microscope supplied with a Megaview-II camera was used to take the TEM micrographs of iron oxide nanoparticles. The accelerating voltage of 100 kV was applied; the maximum resolution of the instrument is 0.2 nm. One drop of highly diluted magnetite sol was dried on to Formwar-coated copper under infrared lamp. The average size distribution was determined by evaluating 100 particles using the JMicroVision 1.2.7 software.

2.5. Surface modification of MNPs

The surface of the purified, bare magnetite was modified by chondroitin-sulfate-A to prepare core-shell nanoparticles. The effect of the CSA-adsorption on the particle interaction was determined first in concentrated systems at pH = 6.3 ± 0.3 and 10 mM NaCl. The MNPs were equilibrated for 24 h with CSA solutions of concentration between 0 and 10 mM at a solid/liquid ratio of 20 g/L. The pH was adjusted at the beginning of adsorption. The adsorption series was evaluated after a day.

2.6. Particle size determination

For characterization of the aggregation state of nanoparticles, the average hydrodynamic diameter (Z-Ave) of bare magnetite particles and of CSA-coated nanoparticles were determined at 25 ± 0.1 °C using dynamic light scattering (DLS) method, an apparatus Nano ZS (Malvern) with a 4 mW He–Ne laser source (λ = 633 nm) operating in backscattering mode at an angle of 173°. The dispersions were diluted to get an optimal intensity of ~10^5 counts per s, thus the samples contained 100 mg/L of magnetite. Prior to the measurements, the samples were homogenized in an ultrasonic bath for 10 s, after which 2 min relaxation was allowed. Any changes in the aggregation state of the bare or the CSA-coated nanoparticles in aqueous dispersions was characterized by the hydrodynamic diameter (Z-Ave). The influence of the added CSA amount was determined at pH = 6.3 ± 0.3 and 10 mM NaCl. The effect of pH variation (between 3 and 10) at different CSA-loadings (0.0, 0.05, 0.1, 0.2, and 0.4 mmol/g) was studied at 10 mM NaCl. For evaluation, we used the second- or third-order cumulant fit of the autocorrelation functions, depending on the degree of polydispersity.