Journal of Magnetism and Magnetic Materials 323 (2011) 7–13

Contents lists available at ScienceDirect

Journal of Magnetism and Magnetic Materials

journal homepage: www.elsevier.com/locate/jmmm

Preparation and characterization of polyvinyl alcohol—chitosan biocompatible magnetic microparticles

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ARTICLE INFO

Article history:
Received 15 December 2009
Received in revised form
2 June 2010
Available online 25 June 2010

Keywords:
Magnetic particle
Biocompatible
Polyvinyl alcohol
Chitosan

ABSTRACT

This work addresses the obtaining of biocompatible magnetic polyvinyl alcohol—chitosan microspheres, specifically tailored/functionalised to bind directly blood toxins using an emulsion crosslinking preparation method. The following synthesis parameters were studied: water to oil phase ratio, polyvinyl alcohol molecular weight, chitosan to polyvinyl alcohol weight ratio, surfactant composition and concentration of the crosslinking agent. These parameters were optimized for producing a high yield of colloidally stable and uniformly sized particles with significant magnetization of saturation, bearing surface amino groups that can be further used to bind blood toxins directly. The particles were characterized regarding their size distribution and surface charge (laser diffraction analysis), morphology (transmission electron microscopy), magnetic properties, chemical composition (Fourier transform infrared spectroscopy) and concentration of the surface amino groups (conductometric titration).

1. Introduction

In the last decade, magnetic nano- and micro-particles received a lot of attention related to their use in biomedical applications, such as diagnosis, separation and purification of biomolecules, carriers for drug delivery and as magnetic resonance imaging contrast enhancement agents [1]. Among other applications, the possibility of using magnetic particles to detoxify blood in patients suffering from hepatic/renal deficiencies was studied [2,3].

The most common magnetic material used in biomedical applications is iron oxide Fe_3O_4 (magnetite) or its oxidized version, γ-Fe_2O_3 (maghemite). Both are used either as a core covered with a matrix or uniformly dispersed within a matrix containing a natural or a synthetic polymer [4]. The biocompatibility of the magnetite is already proved by the fact that it is used as a magnetic resonance imaging contrast enhancement agent. Chitosan (CS), a product obtained by partial deacetylation of chitin in alkaline conditions, is a natural cationic polyaminosaccharide polymer. Due to its biocompatibility and advantageous functional groups (amino and hydroxyl), CS is widely used in medicine (wound dressing material, drug and gene delivery vehicle, candidate for tissue engineering), agriculture, food, biotechnology and water treatment [5,6]. Polyvinyl alcohol (PVA), a synthetic biocompatible polymer that is also attractive due to its high functionality and hydrogel-like properties, is widely used as a dispersing agent for preventing particle coagulation and contributing to their stability and monodispersity [7,8].

Experimental studies using CS–PVA mixtures (formulated as microgels or microspheres) indicated favorable drug controlled release properties [9,10], improved comfort, reduced irritation, ease of processing, improved flexibility and enhanced dissolution [11]. Although magnetic chitosan [12–14] and magnetic polyvinyl alcohol [7] particles with a wide size distribution were obtained and studied, to the authors' knowledge there is no example in literature of magnetic particles containing PVA/CS blends as polymeric matrix.

In many cases the preparation of biocompatible particles for biomedical purpose relies on the initial production of the particles followed by their subsequent surface modification, and finally by the attachment of the desired biological substance on the modified surface of the host carriers [7,15,16]. However, in this way the particles may not be sufficiently biocompatible due to the successive chemical treatments they were subjected to.

In this context, the main goal of this work was to achieve a high concentration of surface functionality directly from synthesis, the chemical treatment steps for functionalization of the particles surface being kept to a minimum.

For an efficient magnetic capture in separation systems with magnetic gradient and flow-through [17], the dimension of particles is an important parameter. Previous studies on high gradient magnetic capture of nanoparticles demonstrated the fact that the capture of small particles (<40 nm) is affected by thermal fluctuations and the parameters of the capturing process must be carefully chosen to avoid this drawback [18]. Nanometer

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0304-8853/$ - see front matter © 2010 Published by Elsevier B.V.
doi:10.1016/j.jmmm.2010.06.025

size particles [19] do not ensure a good magnetic recovery, while, by contrast, micrometer size particles [7] often have an insufficient surface area, although they are suitable for magnetic separation purposes. Therefore, intermediate size particles (400–600 nm) have to be considered. Moreover, monodisperse particles are easier to manipulate in magnetic fields.

In this context, monodispersed biocompatible composite magnetic particles (BCMP) bearing high functionality obtained directly from synthesis of intermediate size were prepared in this work using CS–PVA mixtures by an emulsion crosslinking method. The parameters used in the synthesis process were optimized for obtaining uniformly distributed BCMPs with a high surface area and a high concentration of surface amino groups. The products were characterized in terms of the following parameters: size distribution, particle morphology, composition, magnetic properties, and the presence and concentration of surface functional groups.

Due to their surface functionality, the particles may be used in direct binding of specific toxins that are present in the blood of patients suffering from hepatic/renal deficiencies (bilirubin, 5-hydroxyindole acetic acid, homocysteine).

2. Materials and methods

2.1. Materials

Iron(III) chloride hexahydrate p.a., iron(II) chloride tetrahydrate p.a., PVA for synthesis with varying molecular weights (MWs) and hydrolysis degrees (67,000/86–89%, 72,000/$>$99% and 205,000/$\sim$90%), oleic acid for synthesis, Tween 20, Span 80 and Span 83 for synthesis, 25% glutaraldehyde aqueous solution were all purchased from Merck. The sodium hydroxide p.a. grade, chitosan with a low molecular weight (deacetylation degree minimum 75%, viscosity of 1% solution in 1% acetic acid: 20–300 CPS) and Pluronic F127 for synthesis were purchased from Sigma-Aldrich. 1 N HCl aqueous solution and glacial acetic acid p.a. grade were received from Chemical Company, Romania. All solutions were prepared with double distilled water. The other chemicals used in this work were of analytical grade purity.

2.2. Preparation and characterization of stabilized magnetite

The magnetite nanoparticles were prepared by co-precipitation from an aqueous solution containing Fe^{3+}/Fe^{2+} ions with a molar ratio of 2:1, on addition of 2.66 M aqueous sodium hydroxide solution, at the temperature of 70 °C [20]. The obtained magnetite suspension was cooled to room temperature and washed repeatedly with distilled water until neutral pH was reached. The particles were then stabilized in suspension by adding a 2% aqueous solution of a non-ionic biocompatible surfactant (Pluronic F127). A stable suspension of magnetite, with 1.45% w/v solid content, was obtained. This stable suspension of magnetite (SMAG) was later utilized as the magnetic material for obtaining the composite magnetic particles.

The size distribution of SMAG particles was measured by laser diffraction analysis using a Shimadzu SALDI-7001 particle size analyzer (semiconductor laser, 405 nm) after sonicating the samples for 15 min. The magnetization of SMAG was measured using a Lakeshore VSM 7400 magnetometer.

2.3. Preparation and characterization of BCMP

The BCMP were produced by an emulsion crosslinking method. The aqueous phase of the emulsion was prepared by mixing polymers (consisting of a 5% PVA aqueous solution and 1% CS solution in 0.5% acetic acid) and the previously prepared magnetic material (SMAG suspension). Ratio of the magnetic material to polymers mixture was 1/10 w/w in all cases. The aqueous phase was dispersed in an oil phase consisting of vegetable oil containing 2.23×10^{-3} mole of oil soluble surfactants (Tween 20 and oleic acid/Span 80/Span 85) using a high speed homogenizer (Ultraturrax; speed: 10^4 rpm). After homogenizing for 1 min, a glutaraldehyde aqueous solution with variable concentrations was added as a crosslinker in the presence of 1 N HCl aqueous solution. The mixture was homogenized further for 2 min and then aged for 20 min. The particles were cleaned successively in a separatory funnel with n-heptane, acetone and distilled water and then dried in air.

The following synthesis parameters were studied: CS–PVA w/w ratio (in 1/0, 0/1, 1/1, 2/1 and 1/2 w/w ratio), type of PVA with different molecular weights, crosslinker concentration (6.25, 12.5 and 25%) and the type of oil soluble surfactant.

Particle size distribution and magnetic properties were measured in the same conditions as described above for SMAG. BCMP morphology was evaluated by transmission electron microscopy (Philips CM 20 apparatus operating at 100 keV) performed on a sample of BCMP dispersed in ethylic alcohol–water media, which was deposited as wet dispersion on the grid and then air dried before being loaded into the microscope. The formation of composite particles was confirmed by FTIR (FTIR Bomem MB 104 spectrometer). Particle ζ-potential was measured in water on a Malvern Zetasizer ZS90 system (with a HE–Ne Laser, 4 mW, 633 nm).

For explaining the size distribution for BCMPs, the PVA samples were analyzed using a Varian gel permeation chromatography (GPC) PL 120 system, with three columns ($3\times$ PL aquagel-OH 300×7.5 mm^2). Analysis parameters were as follows: flow rate$=$1 ml/min, $t=25$ °C, sample injection volume$=$100 μl and run time analysis$=$40 min. For calibration, polyethylene glycol standards having MWs between 895,500 and 106 were used. The eluent was aqueous 0.2 M $NaNO_3$ and 0.01 M NaH_2PO_4, pH$=$7.

The concentration of the surface amino groups for the BCMP was estimated by conductometric titration. A concentrated BCMP suspension (*batch 6*) (0.6002 g particles in 20 mL double distilled water), with pH adjusted to 6.7 (using a 0.01 N NaOH solution), was used for titration. The suspension was titrated with 0.05 N HCl while monitoring the conductivity with a Consort C831 analyzer.

Information regarding the experimental batches and some characterization data are presented in Table 1.

3. Results and discussions

3.1. Size of BCMPs

Average diameters of the SMAG particles were 24 nm, and the average diameters of the BCMPs are presented in Table 1.

The size analysis of the BCMP synthesized with PVA having different MWs (67,000, 72,000 and 205,000) showed that the molecular weight of the polymer influences the diameter and the size distribution of the resulting particles. The particles obtained with PVA 72,000 were more uniformly sized (Figs. 1 and 2).

The gel permeation chromatography results regarding the MW and polydispersity index for the various PVA with different MWs are presented in Table 2.

The polydispersity index of a polymer is defined as the ratio of the weight-average molecular weight (MW) to the number-average molecular weight (Mn) and it is a measure of the width of