Materials Science and Engineering C 80 (2017) 243–251

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Synthesis and characterization of pectin-6-aminohexanoic acid-magnetite nanoparticles for drug delivery

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ARTICLE INFO

Article history:
Received 28 November 2016
Accepted 14 May 2017
Available online 15 May 2017

Keywords:
Magnetite nanoparticles
Curcumin
Pectin
Serum proteins
Superparamagnetism

ABSTRACT

In this study, we have synthesized magnetic nanocomposites of magnetite nanoparticles coated with 6-aminohexanoic acid and pectin (MAP). The size of the aqueous dispersion of the nanocomposites was 147 nm with a Polydispersity index (PDI) of 0.32, and the nanocomposites were stable in NaCl up to a concentration of 0.45% (w/v) after which they aggregated. The dispersion of the nanocomposites was stable in Dulbecco's Modified Eagle's medium (DMEM) in the presence of 5 and 10% fetal bovine serum (FBS). Curcumin was used as a model drug to evaluate the potential of the nanocomposites for drug delivery applications. The release behavior of curcumin from the nanocomposites showed a biphasic pattern with initial burst release followed by a slow release, and the size of the aqueous dispersion of curcumin loaded nanocomposites was 159 nm with a PDI of 0.34.

1. Introduction

Magnetic nanoparticles (Fe_3O_4), due to their bio-compatible and superparamagnetic nature, are emerging as a potential candidate for biomedical applications such as magnetic targeting, diagnostic and real-time monitoring of disease using Magnetic Resonance Imaging (MRI[1]) and for hyperthermia treatment of cancer [1]. Superparamagnetic materials are characterized by a large magnetic moment and negligible remanence and coercivity [2]. Magnetic nanoparticles are prone to aggregation due to magnetic dipole-dipole interactions or Van der Waals forces which lead to their detection and clearance from the body by the mononuclear phagocytic system (MPS[2]) before they can reach their target site thus limiting their biomedical applications [3,4]. Magnetite nanoparticles are coated with various polymers to prevent their oxidation and aggregation by introducing steric or electrostatic repulsion between them [5]. When coated with polymers, magnetite nanoparticles can also be used as a carrier for delivery of drug molecules. Drug release can be modulated by using polymer coatings that respond to stimuli such as temperature [6], pH [7] or presence of enzymes [8].

Curcumin, also known as diferuloylmethane, is an orange-yellow colored polyphenolic compound derived from the rhizomes of the *Curcuma longa* and is known for its tumor suppression and chemopreventive effects [9]. Curcumin downregulates the expression of various cell cycle modulators such as cyclin D1, Bcl-2, Bcl-XL and also activates caspase-8 which is involved in the sequential release of enzymes leading to apoptosis [10]. Curcumin has also been demonstrated to increase the efficacy of anticancer drugs like cisplatin and radiation therapy [11]. Despite its therapeutic potential, use of curcumin is limited due to its low aqueous solubility and limited bioavailability. Upon intravenous administration, it quickly disappears from the blood and is found along with its metabolites in bile [12]. Various attempts have been made to increase bio-availability of curcumin by encapsulation in polymer nanoparticles [13], polymer coated nanoparticles [14] and micelles [15]. Curcumin has also been conjugated with polyethylene glycol, amino acids, fatty acids to increase its water solubility and antiproliferative activity [16,17].

In this study, we have optimized the synthesis of pectin and 6-aminohexanoic acid coated magnetite nanoparticles to yield a stable aqueous dispersion. The nanoparticles were characterized for their size, purity, coating and magnetic properties using Transmission electron microscopy (TEM[3]), X-ray diffraction (XRD[4]), Fourier transform infrared spectroscopy (FT-IR[5]), Thermogravimetric analysis (TGA[6]) and Vibrating sample magnetometer (VSM[7]) respectively.

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[1] Magnetic resonance imaging.
[2] Mononuclear phagocytic system.
[3] Transmission electron microscopy.
[4] X-ray diffraction.
[5] Fourier transform infrared spectroscopy.
[6] Thermogravimetric analysis.
[7] Vibrating sample magnetometer.

http://dx.doi.org/10.1016/j.msec.2017.05.097
0928-4931/

We have also measured the size of the nanoparticles in different physiological media using Dynamic light scattering (DLS[8]) to make sure that the nanoparticles are ready for *in vitro* and *in vivo* applications. Curcumin was used as a model drug for encapsulation studies in the nanoparticles.

2. Materials and methods

2.1. Materials

Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, 99%) was purchased from Thomas Baker (Chemicals). Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, 98%), Sodium Hydroxide (NaOH, 97%), and Hydrochloric acid (HCl, 37%) were purchased from Merck Chemicals, India. Pectin (Poly-D-galacturonic acid methyl ester, degree of esterification 65–70%), Curcumin ($C_{21}H_{20}O_6$, 98%), Dulbecco's Modified Eagle Medium (DMEM[9]); High glucose, Fetal bovine serum (FBS[10]) and Tween-80® were purchased from Himedia Laboratories. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 99%) and 6-Aminohexanoic acid (6-Aha, 99%) were purchased from Spectrochem, *N*-Hydroxysuccinimide (NHS, 98%) was purchased from Alfa Aesar. Deionized water was used for all experiments, and all chemicals were used as received without any further purification.

2.2. Synthesis of magnetite nanoparticles (Fe_3O_4, MNP)

Magnetite nanoparticles (MNP[11]) were synthesized by coprecipitation of Ferrous sulfate heptahydrate and Ferric chloride hexahydrate using NaOH. Briefly, $FeCl_3 \cdot 6H_2O$ (10 mmol) and $FeSO_4 \cdot 7H_2O$ (5 mmol) were dissolved in 20 ml deionized water containing HCl (5 mmol) to form a homogenous solution. The solution was dropwise added to a stirring solution of 125 ml of 1.5 N NaOH under a nitrogen gas atmosphere. The dispersion was stirred for an hour and the black precipitate formed was purified by using an alloy magnet (FeNdB, 2500 gauss). The precipitate was redispersed in deionized water and separated again using the magnet; this procedure was repeated three times. The purified black precipitate was dispersed in nitrogen purged deionized water and stored at room temperature (RT[12]) for further use.

2.3. Synthesis of 6-Aminohexanoic acid coated magnetite nanoparticles (MA)

Magnetite nanoparticles were dispersed in an aqueous solution of 6-aminohexanoic acid (6-Aha, 40% weight of magnetite nanoparticles) and sonicated in a water bath sonicator (50 W) for 20 min. The dispersion was stirred at 80–85 °C for 30 min under nitrogen gas atmosphere and allowed to cool to room temperature. Excess 6-Aha was removed by magnetically separating the particles from the dispersion followed by washing with water. 6-Aha coated magnetite nanoparticles (MA[13]) were dispersed in deionized water for coating with pectin.

2.4. Synthesis of pectin-6-Aha-magnetite nanoparticles (MAP)

The carboxyl groups in pectin were first activated using EDC-NHS and then reacted with free amine groups present in MA. Briefly, pectin (1 g) was dissolved in 100 ml water; EDC (20 mg, 0.104 mmol) and NHS (12 mg, 0.104 mmol) were added to it. The solution was stirred for 20 min at room temperature (RT) followed by addition of 200 mg of MA. The pH of the dispersion was adjusted to 8 using 0.01 M NaOH followed by sonication in a water bath sonicator for 20 min and overnight stirring at RT. MAP[14] nanoparticles were separated from the excess pectin and other reactants using the magnet and washing with water.

3. Characterization

The X-ray diffraction patterns of MNP, MA, and MAP were taken on a PANalytical X-Ray diffractometer (X'Pert Pro) over a 2θ range of 20° to 60° using Cu-Kα radiation (λ = 1.54 Å) operating at 30 mA and 40 kV. The magnetic properties of MNP and MAP were measured on a Lakeshore Vibrating sample magnetometer (VSM) at room temperature. The size and morphology of MNP, MA, and MAP were studied using a Transmission electron microscope (TEM), a drop of an aqueous dispersion of nanoparticles (0.01 mg/ml) was placed on a carbon coated copper grid and the grid was dried at RT. TEM images were obtained using a G2 20 twin, Tecnai 200 kV twin microscope at an accelerating voltage of 200 kV. The mean particle size was measured using Image J software (www.imagej.nih.gov/ij/) by calculating the area of the individual nanoparticles assuming spherical morphology and the average of the area was used to calculate the size of the nanoparticles. The TGA thermograms of MNP, MA, and MAP were taken on a TG Analyzer Model Q50, TA Instruments. The analysis was carried out from 40 °C to 800 °C at the heating rate of 20 °C/min under air atmosphere. The TGA thermogram of pectin was taken from 40 °C to 800 °C in both air and nitrogen atmosphere. Fourier Transform-Infrared spectroscopy (FT-IR) spectra of MNP, MA, MAP, Pectin, Curcumin and Curcumin loaded MAP were taken from 4000 cm^{-1} to 400 cm^{-1} on a Jasco 4600 FT-IR, Japan.

The size distribution and zeta potential of various MAP formulations were measured using Malvern Zetasizer Nano S for size measurement and Malvern Zetasizer Nano ZS for zeta potential at a concentration of (0.01 mg/ml). All formulations were sonicated in the water bath sonicator for 10 min before measurements. In an another experiment, MAP nanoparticles incubated in DMEM containing 5% FBS by volume (D5S) and 10% (D10S) FBS for 2 h at 37 °C were centrifuged at 15,000 rpm for 20 min, and the pellets were resuspended in water and centrifuged again. The pellets were then resuspended in deionized water and 0.9% NaCl for measuring the size and zeta potential.

3.1. Drug loading and release study

For encapsulation of curcumin in MAP; 10 mg of MAP were dispersed in 5 ml deionized water using the water bath sonicator for 20 min and 1 mg of curcumin in 300 μl ethanol was added to it. The dispersion was purged with nitrogen to allow ethanol to evaporate and stirred overnight at RT. Curcumin loaded nanoparticles were separated from free curcumin using the alloy magnet and were washed with deionized water; this process was done twice. Free curcumin was solubilized with 0.2% (w/v) tween-80® and its concentration was determined by measuring absorbance at 450 nm using UV–Visible spectrophotometer [18]. A standard curve of curcumin (1 to 10 μg) in Phosphate buffer saline (PBS[15]) containing 0.2% (w/v) tween-80 (PBST-80) was prepared, and the encapsulation efficiency was calculated as follows:

$$\text{Encapsulation efficiency } (\%) = \frac{(\text{amount of drug added} - \text{amount of free drug})}{\text{amount of drug added}} \times 100$$

[8] Dynamic light scattering.
[9] Dulbecco's modified eagle medium.
[10] Fetal bovine serum.
[11] Magnetite nanoparticles.
[12] Room temperature.
[13] 6-Aminohexanoic acid coated magnetic nanoparticles.
[14] Pectin-6-Aha-magnetic nanoparticles.
[15] Phosphate buffer saline.