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Magnetic core–shell chitosan nanoparticles: Rheological characterization and hyperthermia application

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ABSTRACT

Stabilized magnetic nanoparticles are the subject of intense research for targeting applications and this work deals with the design, preparation and application of specific core–shell nanoparticles based on ionic crosslinked chitosan. The nanometric size of the materials was demonstrated by dynamic light scattering (DLS) and field emission scanning electron microscopy (FESEM) that also proved an increase of the size of chitosan nanoparticles (NPs) with the magnetite content. Steady oscillatory rheology measurements revealed a gel-like behavior of aqueous dispersions of chitosan NPs with concentrations ranging from 0.5% to 2.0% (w/v). The cytotoxicity of all the materials synthesized was analyzed in human fibroblasts cultures using the Alamar Blue and lactate dehydrogenase (LDH) assays. The measured specific power absorption under alternating magnetic fields (f = 580 kHz, H = 24 kA/m) indicated that magnetic core–shell chitosan NPs can be useful as remotely driven heaters for magnetic hyperthermia.

1. Introduction

Chitosan, a cationic polysaccharide obtained by the thermo-alkaline N-deacetylation of chitin, is the second-most abundant naturally occurring amino polysaccharide offering high biocompatibility (Muzzarelli, 2011; Muzzarelli et al., 2012). Chitosan has attracted intense attention as an important biopolymer to effectively stabilize colloidal dispersions of superparamagnetic iron oxide nanoparticles, conferring them an increased biocompatibility and chemical functionality (Dias, Hussain, Marcos, & Roque, 2011; Nicolás et al., 2013). These materials find applications in magnetic hyperthermia treatment of cancer, a promising approach to cancer therapy in which the temperatures of tumors are increased to 41–46 °C to induce apoptosis of the cells (Jordan, Scholz, Wust, Fähling, & Roland, 1999; Laurent, Dutz, Häfeli, & Mahmoudi, 2011). This therapy involves the introduction of ferromagnetic or superparamagnetic nanoparticles (mainly magnetite, Fe_3O_4) into the tumor tissue and then irradiation with an alternating magnetic field (AMF). The particles transform the energy of the AMF into heat by different physical mechanisms, and the transformation efficiency strongly depends on the frequency of the external field (Gellermann et al., 2006) as well as the nature of the particles such as particle size (Goya et al., 2008) or surface modification (Gupta & Gupta, 2005).

Magnetic hydrogels, a combination of hydrogels with micro- and/or nanomagnetic particles (e.g., γ-Fe_2O_3, Fe_3O_4, $CoFe_2O_4$) have been implemented as materials able to heat up target tumors remotely through an external magnetic field (Hernandez, Sacristán, Asi'n et al., 2010; Li et al., 2013). We reported on the employment of chitosan as a template for the oxidation of iron cations to yield simultaneously iron oxide nanoparticles and the formation of a chitosan gel (Hernández et al., 2009). These materials show a high quality thermal response in the presence of an AMF which makes them potential candidates for applications in magnetic hyperthermia (Hernandez, Sacristán, Asi'n et al., 2010).

In recent years, the preparation of magnetic chitosan NPs has attracted a great attention in order to develop thermoseeds for magnetic hyperthermia that can also be injected for localized therapy as in the case of ferrofluids (Jordan et al., 2001; Kim, Kim, Kim, & Lee, 2008; Zhao, Wang, Zeng, Xia, & Tang, 2009). Chitosan-Fe_3O_4 NPs can be prepared in situ with tiny pools of water-in-oil microemulsion containing chitosan and ferrous salt as micro-reactors by adding the basic precipitant, NaOH, into the micro-emulsion (Zhi, Wang, Lu, Ma, & Luo, 2006). The encapsulation of preformed iron oxide nanoparticles can be stabilized by crosslinking chitosan with glutaraldehyde (Jiang, Long, Huang, Xiao, & Zhou, 2005) or tripolyphosphate salts (TPP). Crosslinking of chitosan with TPP constitutes a mild and efficient method to

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achieve chitosan NPs (Calvo, Remuñán-López, Vila-Jato, & Alonso, 1997; Goycoolea, Lollo, Remuñán-López, Quaglia, & Alonso, 2009). Sodium tripolyphosphate (TPP) is a polyanion categorized as being GRAS (generally recognized as safe) by the FDA (Food and Drug Administration) (Ur-Rehman, Tavelin, & Gröbner, 2011). It is known that the chitosan particles are formed mainly through the electrostatic interaction between positively charged chitosan and negatively charged TPP molecules. The understanding of the macroscopic rheological properties of the resulting aqueous colloidal dispersions is of paramount importance for the design of biomedical applications (Guvendiren, Lu, & Burdick, 2012). In a recent report, the packing of chitosan NPs to form microgels from aqueous suspensions was ascertained through rheological measurements for samples with different particle sizes obtained by varying the chitosan to TPP ratio (Li & Huang, 2012).

In this paper, the encapsulation of Fe_3O_4 nanoparticles into chitosan NPs crosslinked with TPP is described and the rheological properties of the aqueous dispersions were investigated through dynamic rheological measurements. The rheological characterization will allow determining the structural organization of these materials by relating their linear viscoelastic properties to their properties in dispersion by means of scaling laws (Echeverria, Peppas, & Mijangos, 2012; Hernandez, Sacristan, Nogales et al., 2010). The work extends to a study on remote heating by a magnetic field and the analysis of cytotoxicity to evaluate the application of the materials obtained for magnetic hyperthermia.

2. Materials and methods

2.1. Materials

Chitosan with a deacetylation degree (DD) of 65% and molecular weight (M_w) of 362 kDa was supplied by Laboratorio de Polímeros, Universidad Nacional de Costa Rica. This chitosan was isolated from shrimp's shell (*Heterocarpus vicarious*).

Acetic acid (Aldrich) and sodium tripolyphosphate (Aldrich) were used as received. Oleic-acid-coated iron oxide nanoparticles dispersed in water as a ferrofluid (density = 1.08 g/mL), NGAP FeO-05#4, were provided by Nanogap subnmparticles, Spain. According to the manufacturer, the crystalline form is magnetite, Fe_3O_4 and the average size of nanoparticles is 18.55 ± 2 nm. Milli-Q (18.3 MΩ) water was used in all experiments.

2.2. Preparation of chitosan–sodium tripolyphosphate (CS + TPP) nanoparticles

CS + TPP nanoparticles were prepared as reported elsewhere (Calvo et al., 1997). Briefly, 0.5% (w/v) chitosan stock solutions were prepared by dissolving the appropriate chitosan weight into a 1% (v/v) acetic solution. Sodium tripolyphosphate (TPP) was dissolved in Milli-Q water to a final concentration of 0.5% (w/v). CS + TPP nanoparticles were formed by dropwise addition of TPP solution into a chitosan stock solution under severe magnetic stirring. A chitosan to TPP mass ratio of 5 was chosen based on previous studies (Li & Huang, 2012). Finally, aqueous dispersions of CS + TPP nanoparticles were centrifuged at 5000 rpm for 20 min and the supernatant was separated and subjected to freeze-drying.

The crosslinking degree of CS + TPP nanoparticles was 68% as determined by the ninhydrin test. Ninhydrin is extensively used in the analytical determination of amino acids and related structures because it can react with a variety of primary and secondary amines producing *Ruhemann* purple color. This product has a maximum absorbance at 570 nm (Wu, Hussain, & Fassihi, 2005).

2.3. Preparation of Fe_3O_4–chitosan nanoparticles (NP + Fe)

Fe_3O_4 chitosan nanoparticles (NP + Fe) with three different Fe_3O_4 contents were prepared based on the following steps: firstly, a determined amount of ferrofluid was dispersed in 5 mL of water to yield various concentrations of Fe_3O_4 nanoparticles (0.5%, 2.0% and 5.0%, w/v). The resulting ferrofluid was added under vigorous stirring to 30 mL of a chitosan solution in acetic acid (0.5%, w/v, pH = 3.5) in a N_2 atmosphere. Secondly, an aqueous TPP solution (0.5%, w/v, pH = 9.2) was dropped into the chitosan solution containing magnetite nanoparticles (final pH ~ 4, for all the samples). This step allowed encapsulating magnetite nanoparticles into CS + TPP nanoparticles. Finally, aqueous dispersions of NP + Fe nanoparticles were centrifuged at 5000 rpm for 20 min and the supernatant was separated and subjected to freeze-drying.

The magnetite concentration in the chitosan nanoparticles was determined through UV spectroscopy. Samples were digested in HNO_3 and HCl 6 M and iron concentration was measured spectrophotometrically at the λ_{max} of 478 nm.

2.4. Dynamic light scattering (DLS) and zeta potential

Dynamic light scattering (DLS, Malvern Nanosizer Nano S) was employed for the determination of electrophoretic mobility and hydrodynamic diameter of chitosan nanoparticles dispersed in Mili-Q water at 25 °C, the resulting pH of the dispersions was 4.5. For hydrodynamic diameter determinations, a backscattering detection angle of 173° was employed. The electrophoretic mobility was transformed into zeta potential using the Smoluchowski equation. All measurements were repeated three times and the average of three runs was taken as the result.

2.5. Morphology studies

Micrographs of the samples were taken using a FESEM Hitachi model SU8000 HRSEM used in the TE (electron transmission) detector bright field mode and SE operated at 0.5–30 kV. One drop corresponding to aqueous dispersions of each of the samples under study was deposited on the Formvar carbon-coated Cu grid.

2.6. Fourier-transform infrared spectroscopy (FT-IR)

Fourier-transform infrared spectroscopy was performed on a Perkin Elmer spectrometer. The pellets were prepared on a KBr press. The spectra were scanned over the wave number range of 4000–450 cm^{-1} at a resolution of 2 cm^{-1}.

2.7. Rheology

Dynamic oscillatory measurements were performed in a AR-G2 rheometer (TA Instruments, USA). The geometry used was 60 mm acrylic parallel plates. Samples under study were dispersed in ultrapure water at concentrations ranging from 0.5% to 2.0% (w/v). This range was chosen because concentrations higher than 2.0% w/v did not allow the homogeneous dispersion of the samples in water and lower than 0.5% w/v did not allow the rheological characterization due to the torque limit. Strain sweep tests at a constant, nondestructive 0.5 Hz frequency were carried out. All measurements were done at room temperature.

2.8. Cell culture conditions

The biological response to the materials was tested with fibroblasts of human embryonic skin (HFB, Innoprot). The culture medium was Dulbecco's modified Eagle's medium enriched with 4500 mg/mL of glucose (DMEM, Sigma) and supplemented with