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ORIGINAL PAPER

Adsorption of serum protein in chitosan-coated and polyethyleneimine-coated magnetite nanoparticles

Christian Chapa-González[1] · Karla Valeria Sosa[1] · Jorge Alberto Roacho-Pérez[2] · Perla Elvia García-Casillas[1]

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Abstract

Magnetite (Fe_3O_4) nanoparticles provide several possibilities for a compelling platform for medical applications due to their magnetic properties. In the same way, functionalization with polymers provides several properties seeking to achieve colloidal stabilization in physiological fluids. Nonetheless, the use of magnetite nanoparticles as a medical agent is still in its early stages and is faced with many doubts and challenges as the study of coatings interactions with biological molecules. In this work, Fe_3O_4 nanoparticles were synthetized by co-precipitation method and further coated with chitosan, as well as coated with polyethyleneimine. The nanoscale size of magnetite nanoparticles was confirmed using scanning electron microscopy. Fourier-transform infrared spectroscopy corroborated that characteristic functional groups of chitosan and polyethylenimine were present in the surface modified magnetite samples. The evaluation of protein immobilization was carried out by incubating bovine serum albumin at different concentrations followed by magnetic decantation using a permanent magnet. Coated magnetite nanoparticles have a protein absorption greater than bare MNP.

Introduction

Nanomedicine is an area of great interest due to the wide variety of applications that can be conferred by modifying or re-engineer properties such as size, shape, or composition of nanostructures. Controlling these properties, especially in magnetic nanoparticles, has interesting applications in the treatment of diseases or leverage the new knowledge to focus work directly on translational studies to treat a disease. Magnetite (Fe_3O_4) nanoparticles have been developed for a variety of biomedical applications including hyperthermia [1], magnetic separation [2], imaging [3], biosensors [4], gene therapy [5], and drug delivery [6].

The biomedical performance of the magnetic nanostructures in vivo or in vitro is not determined solely by their physicochemical or magnetic properties. In addition, it depends on the interaction between the nanoparticles and the immediate biological environment. Due to their higher percentage of surface atoms and their colloidal nature, once they encounter a physiological or simulated physiological medium, nanoparticles undergo processes that transform them toward more stable thermodynamic states including aggregation, and interaction with biomolecules such proteins in the media.

Several cationic polymers are used to improve the colloidal stability or biocompatibility of magnetite nanoparticles, including chitosan [7, 8] and polyethyleneimine (PEI) [9, 10]. Furthermore, it was shown that the properties of polymer-coated magnetite nanoparticles depend on the nature of the stabilizing agent [11, 12]. Increasing evidence has shown that positively charged-coated nanoparticles are capable of favor the interaction with proteins [13]. However, limited research has been conducted to compare the effect of protein immobilization in relation to the availability of amino functional groups. In chitosan the apparent range of pKa is from 6.2 to 7.0 [14]. The amount of primary amino groups on the units of D-glucosamine of its backbone gives chitosan the positive charge, at neutral pH [15]. While PEI protonation has been shown to be influenced by intra-chain repulsion forces, the net protonation of PEI at neutral pH gives it a highly charged character [14, 16–18]. Branched PEI demonstrated buffering capacity over almost the entire pH range [19], this attributed to the fact that the polymer

✉ Christian Chapa-González
christian.chapa@uacj.mx

[1] Grupo de Nanomedicina, Instituto de Ingeniería y Tecnología, Universidad Autónoma de Ciudad Juárez, Ciudad Juárez, Mexico

[2] Departamento de Bioquímica y Medicina Molecular, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Mexico

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contains primary, secondary (pKa 8–9) and tertiary amine groups (pKa 6–7) [20]. For the above, we hypothesize that, for a region of neutral-physiological pH, the arrangement of amino groups in positively charged polymers, and, consequently, physicochemical properties such as pKa, determine the amount of protein that adheres to the surface of the complexes (cationic polymers/magnetite nanoparticles). These multifunctional nanoparticles are intended for medical practices in oncology such hyperthermia clinical protocols, and drug or gene delivery. For this reason, the objective of this work is to study the interaction of these nanostructures with serum proteins in a phosphate buffer solution (PBS, pH 7.4).

Materials and methods

Preparation of Fe_3O_4 nanoparticles and coating with chitosan and polyethyleneimine

The Fe_3O_4 nanoparticles were obtained by co-precipitation reaction where a stoichiometric ratio of 1–2 with respect to the Fe^{2+} and Fe^{3+} ions is maintained. First, 100 ml of 0.05 M of $FeSO_4$ and 100 ml of 0.1 M $FeCl_3$ were prepared separately. Then, with vigorous shaking for 15 min both solutions were mixed and immediately a strong base was added which caused the formation of a black precipitate. The pH was adjusted to 7.0 and the precipitate was recovered by magnetic decantation using a permanent magnet. Following this, the coating with the polymers was carried out separately via in situ surface modification method.

Chitosan-coated magnetite was obtained as described in [15] with modifications. Briefly, 90 mg of chitosan of medium molecular weight, were weighed, dissolving them in 45 ml of 2% acetic acid. This solution was added drop by drop to a suspension of magnetite nanoparticles, and the pH was adjusted to 7.0. The precipitate was recovered for characterization and for experiments with the protein. Similarly, branched polyethylenimine-coated magnetite samples were obtained following the protocol described in [9] with modifications. In brief, 375 µl of a 50 µg/µl polyethylenimine solution were added to a mixture of the precursor salts with ferrous and ferric ions, then, this mixture was added to a NH_4OH solution heated up to 80 °C, the pH was adjusted to 7 and the precipitate formed was recovered by magnetic decantation.

Characterization

Using the field-emission scanning electron microscope (FE-SEM) (JEOL), the morphology, and size of samples were characterized, operating at a 15 kV. Each sample was mounted with double side carbon tape. In addition, the nanostructures were washed three times with distilled water, dried, and then was analyzed by the Fourier-transform infrared FTIR technique at room temperature. The spectra were recorded using the Nicolet 6700/Thermo Electron spectrometer to identify functional groups present in the samples. Subsequently, the polymer-coated magnetite samples were analyzed by DLS in distilled water a Nanotrac Wave Particle Size Analyzer (Microtrac) to determine the hydrodynamic size, polydispersity index (PDI), and zeta potential.

Immobilization of serum protein

The protein solution (bovine serum albumin, BSA) was prepared diluting 4.4 g in 100 ml of phosphate buffer solution (PBS, 1X, pH 7.4) previously prepared and sterilized. A calibration curve was performed measuring the absorbance of serial dilutions of the solution at 280 nm in a Nanodrop; this was done in triplicate. A suspension of 312.5 µg/ml of nanoparticles was incubated for 24 h with various concentrations of BSA, from 0.172 to 22 µg/µl, keeping the temperature at 37 °C. A permanent magnet was used to carry out magnetic decantation and immediately the amount of adsorbed BSA was determined by calculating the final concentration with the absorbance of the supernatant. Measurements were made in triplicate.

Results and discussion

The morphology of the samples is observed in Fig. 1. Agglomerates are present in the magnetite sample (Fig. 1a) and that the processing with chitosan and polyethylenimine (Fig. 1b, c, respectively) led to the formation of larger nanoparticles and agglomerates persist which was corroborated with the results of the hydrodynamic size by DLS (Fig. 2). It should be noted that the measurements were made using distilled water without surfactants, which could influence the result since when surfactants are used, the degree of aggregation is minimized. However, measurements were made to show whether the polymer itself improved the degree of aggregation. Still, PDI for chitosan-coated magnetite, and polyethylenimine-coated magnetite were 0.383 and 0.225 respectively. In addition, DLS showed the net charge of the samples where it is shown the successful surface modification by changes on the net charge. The zeta potential of the bare magnetite was − 6.5 mV and changed to − 2.7 mV, and + 7.5 mV when a layer of chitosan and polyethylenimine were added respectively.

Likewise, it was possible to corroborate the modification of the surface of the magnetite nanoparticles with the Fourier-transform infrared spectroscopy (FTIR). FTIR spectra (Fig. 3) corroborated bands near to 500 cm^{-1} due to Fe–O bond, while the bands at 1250 cm^{-1} in chitosan-coated magnetite were attributed to the vibratory stretching mode of

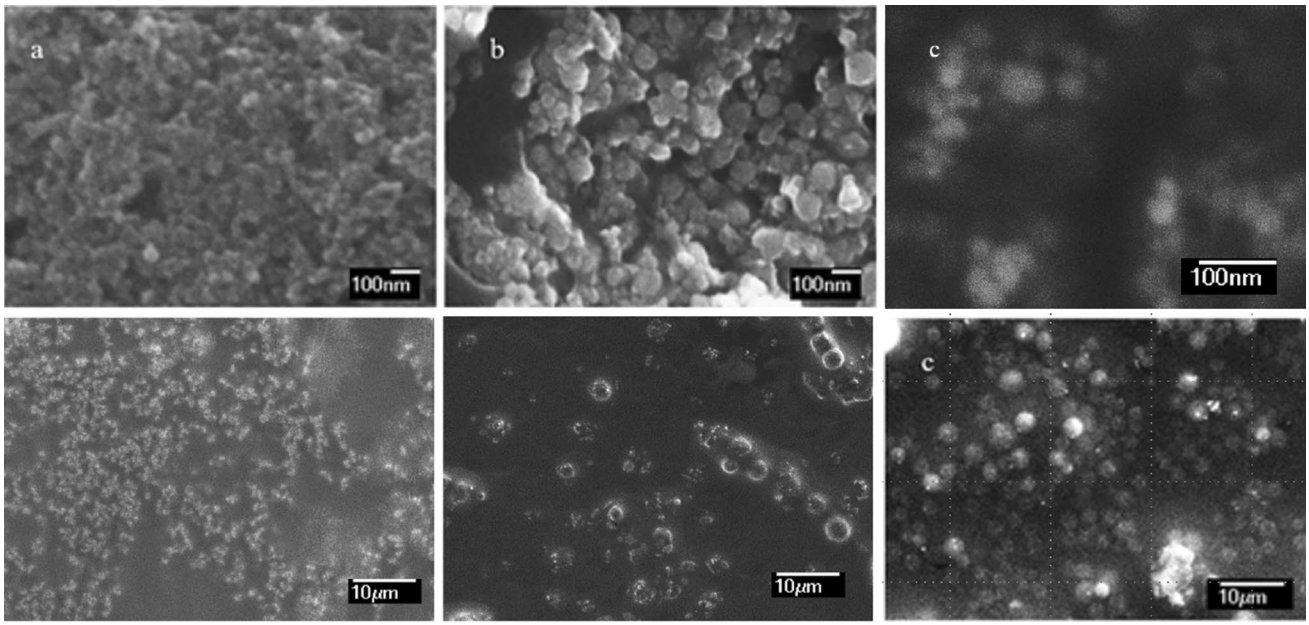

Fig. 1 Micrographs of column (**a**) magnetite, column (**b**) chitosan-coated magnetite, and column (**c**) polyethylenimine-coated magnetite yielded at different magnifications

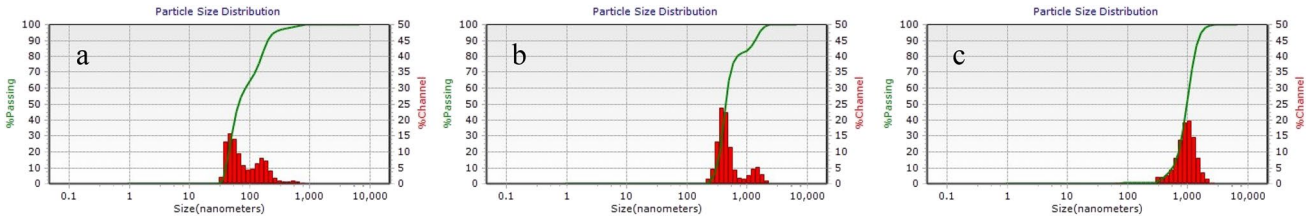

Fig. 2 Particle size distribution of (**a**) magnetite, (**b**) chitosan-coated magnetite, and (**c**) polyethylenimine-coated magnetite

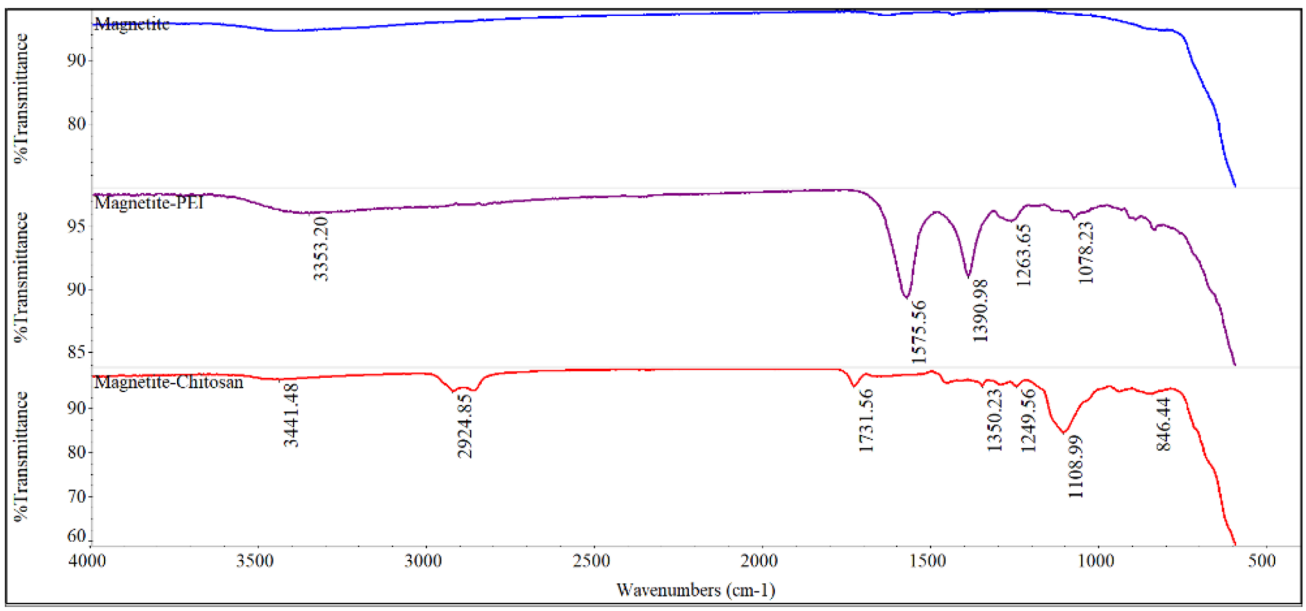

Fig. 3 FTIR spectra of bare magnetite, chitosan-coated magnetite, and polyethylenimine-coated magnetite

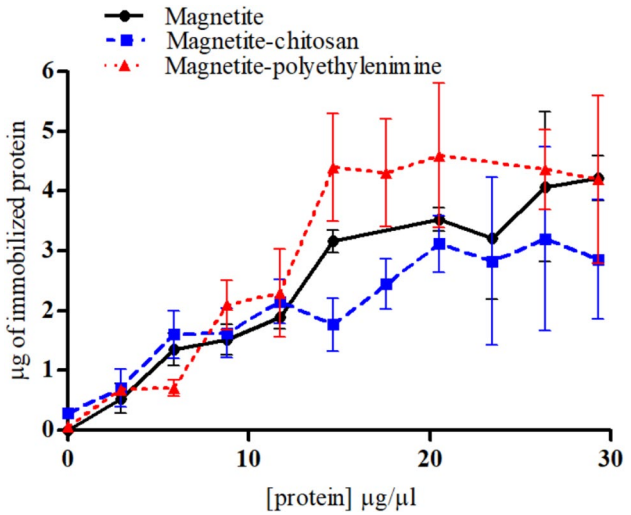

Fig. 4 Effect of different concentrations of protein after 24 h of incubation with the bare and coated magnetite samples

C–N; likewise 3441 cm^{-1} band and 1575 cm^{-1} were attributed to N–H stretching and N–H bending of aliphatic amine in polyethylenimine-coated magnetite.

The elaborated calibration curve of protein made it possible to relate the concentration of BSA in the supernatant after carrying out the incubation of the protein in the suspension of nanoparticles. In Fig. 4, the effect of the different concentrations of BSA with the amount of protein immobilized by the samples is shown. All the samples show a similar behavior at low protein concentrations, there are no differences between the amount of protein adsorbed between the uncoated and coated magnetite nanoparticles. The difference in the amount of immobilized protein is remarkable using a protein concentration of approximately 15 µg/µl. Chitosan-coated magnetite has lower protein adhesion compared to poly-coated magnetite, even the adhesion is less than bare magnetite. This can be attributed to the fact that polyethyleneimine has a higher number of amino groups, compared to chitosan, which can form interactions with the negative side chains of amino acids in the protein.

Conclusion

The polymeric-coated magnetite nanoparticles have been prepared via in situ surface modification method. The characteristic functional groups of the polymers used to modify the surface of the magnetite nanoparticles were identified in the FTIR spectrum of the samples. Which indicates that the in situ coating procedure was carried out successfully. In this way, the addition of polymeric coating on nanoparticles induced an increase in size according to the SEM images. After evaluation of protein immobilization, it was clearly concluded that at concentrations greater than 15 µg/µl the nature of the polymeric coating played an important role in protein separation. Therefore, polyethylenimine-coated nanoparticles will have a better performance in applications where it is required to adhere proteins to the surface of the magnetic nanoparticles, while those coated with chitosan are recommended to avoid biochemical phenomena such as formation of protein corona.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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