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Investigation of tannic acid cross-linked onto magnetite nanoparticles for applying in drug delivery systems

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ABSTRACT

With the purpose of developing a drug delivery nanocarrier with good stability in the body and enhanced efficacy, we synthesized a novel nanoparticles (NPs) composed of tannic acid, chitosan and magnetite NPs. Magnetite NPs were synthesized by the sonication of iron salts in alkaline condition and then coated with chitosan (Ch-Fe_3O_4). Tannic acid coating of these NPs was carried out by cross-linking with glutaraldehyde (T-Fe_3O_4). All results from experiments (FT-IR, DLS, zeta potential, AAS and TEM) showed that products have been successfully prepared and were in nanoscale. Investigation of NPs degradation in phosphate buffered saline (PBS) solution showed that these NPs have lost their morphology in acidic medium. This suggested that T-Fe_3O_4 NPs can be a good candidate for pH sensitive nanocarrier by decomposing in acidic environment and releasing their contents in target tissue. In order to understand the unspecific adsorption and interaction of NPs (as a nanocarrier for drug delivery) with plasma protein, the interaction between NPs and bovine serum albumin (BSA) were investigated by spectroscopy methods. NPs coated with tannic acid showed low BSA adsorption, which candidate them as a nanocarrier for clinical applications with long circulation in the body by preventing the protein adsorption.

1. Introduction

Using nanocarriers in therapeutics, nanotechnology has changed the area of drug delivery. These nanocarriers have great potentials that improve some restrictions of conventional medicine, such as poor drug solubility, inadequate pharmacokinetics and diverse side effects [1]. Nanocarriers that have been involved drugs and diagnostic imaging agents, and decorated with targeting agents are considered for effective guided therapy [2]. Among all nanoparticles (NPs) being applied for biomedicine and drug delivery, magnetite NPs are very attractive nanocarriers for diagnostic, therapeutic and targeting applications. These NPs present some unique properties, such as well-documented biosafety, ease of preparation and handling, the possibility of controlling the characteristics of the nanocarriers, availability, affordability of the materials needed for this procedure. These NPs have been considered excellent contrast agents for magnetic resonance imaging (MRI), and also, can be targeted locally by using an external magnetic field, and released drugs in the required tissue (magnetic drug targeting) [3–5]. However, these NPs tend to form agglomerates and will be cleared from blood circulation by the reticular endothelial system (RES) after administration. This causes NPs not to be able to reach the intended target site. Therefore, NPs should be coated by materials such as polymers that must be hydrophilic, biocompatible and biodegradable. These coatings enhance circulation time in the blood stream, and also, can be used for encapsulation and/or conjugation of drugs and targeting agents [4,6–8].

One of the materials is used for stabling magnetite in colloidal NPs is chitosan. The stabilization of NPs by chitosan is dominated by electrostatic repulsion force and steric effects. But, high charged polymers have colloidal instability in biological media, such as plasma, through opsonization. Various strategy such as blending, grafting or cross-linking of synthetic polymers have applied to enhance biocompatibility and stability of NPs coated with chitosan [5].

Herein, we present a facile and effective strategy for improving chitosan coated NPs by tannic acid. Tannic acid is a plant polyphenolic compound that achieves through decomposing organic material in the environment. Tannic acid is good soluble in water

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and can be hydrolyzed in acidic or alkaline surroundings resulting glucose and phenolic acids such as gallic acid. Also, hot water and enzymes can be caused tannic acid to decompose [9–11]. One of the challenges that has been encountered with nanocarriers is to control the release of drug from them. It depends on the biodegradation of the nanoparticles in vivo. Also, developing stimuli-responsive drug delivery systems is another strategy, which can release drugs at the diseased site through sensitivity to stimuli such as temperature, pH and etc [1].

In this work, tannic acid was used to form a structure involved NPs coated with chitosan. These NPs can be destructed in acidic medium and can release their drug content in targeted tissue because of hydrolysis of tannic acid. In other hand, protein adsorption can be reduced by reducing surface charge and changing functional groups on the surface of NPs. The present study showed that use of tannic acid onto chitosan-coated magnetite NPs is a good strategy for obtaining magnetic nanocarrier with low protein adsorption and can also be used in vivo applications.

2. Experimental

2.1. Materials

$FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, tannic acid, glutaraldehyde, aqueous ammonia (NH_4OH, 25 wt %) and bovine serum albumin (BSA) all were purchased from Merck. Chitosan with medium molecular weight and deacetylation degree of $\geq$75% was purchased from Sigma-Aldrich.

2.2. Equipment

The size and morphology of the NPs were obtained by transmission electron microscopy (TEM) on a Philips EM208S operating at accelerating voltage of 150 kV. Degrading and changing process of NPs morphology were studied by scanning electron microscopy (SEM) with Hitachi S-4160 instrument. Dynamic light scattering (DLS) and zeta potential measurements were assessed on dispersed NPs by sonication in water using a Malvern- DTS Ver 4.20 at room temperature. The magnetic properties of NPs were measured by using a vibrating sample magnetometer (VSM) (Meghnatis Daghigh Kavir Co, Iran) at room temperature to confirm superparamagnetic properties of the NPs. Fourier transform infrared (FTIR) spectra were taken on a magna-IR spectrometer 550 Nicolet, by using the conventional KBr disk method. Atomic absorption spectrometry was performed on Perkin-Elmer 2380 by flame (air/C_2H_2). UV–Vis absorption spectra were recorded with a TU-1901 dual beam UV–Vis spectrophotometer (Purkinje General Instrument, Beijing, China).

2.3. Synthesis of magnetic Fe_3O_4 nanoparticles and coating with chitosan

Fe_3O_4 and Ch-Fe_3O_4 NPs were obtained according to our previous reported procedure with some modifications [5]. Briefly, Fe_3O_4 NPs were synthesized using $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 4H_2O$ (2:1 M ratio), into an ultrasonic water bath, in alkali condition and in the presence of N_2. After beginning the sonication, 20 mL of ammonia was added to solution. Converting of color to black showed forming of Fe_3O_4 NPs. The mixture was left under sonication for 15 min in room temperature, and then nanoparticles were collected by an external magnet. NPs were washed with ethanol and deionized water for several times and dried in oven in 45 °C for 30 h. For coating of NPs with chitosan, Fe_3O_4 NPs were dispersed in deionized water by sonication, and chitosan were dissolved in mixture of water/acetic acid at 4:1 ratio in 50 °C, separately. Solutions were mixed together and stirred at a speed of 700 rpm at room temperature for 12 h. Ch-Fe_3O_4 NPs were separated by centrifugation at a speed of 12000 rpm, and were dried at room temperature.

2.4. Modification of Ch-Fe_3O_4 NPs with tannic acid (T-Fe_3O_4)

0.4 g of tannic acid was dissolved in 10 ml deionized water. Then, 0.06 g of Ch-Fe_3O_4 NPs dispersed homogeneously in 15 ml of deionized water by sonication. Two prepared solutions were mixed together and stirred at a speed of 400 rpm at room temperature. After 15 min, 1 ml of glutaraldehyde (GA) was added to the mixture in the presence of 1 ml of HCl (1 M). The product was separated after 15 min by magnet and was dried at room temperature.

2.5. Iron content

The iron content of NPs was assayed by AAS with the procedure which we had reported [5] with some changes. Specific amount of each NP was dissolved in 5 mL of HCl, 12 M and the process was maintained for overnight. Then, the volume of each solution was reached to 50 ml by adding deionized water. In last step, the amount of iron in NPs was determined by AAS and the results were used to calculate the amount of coating in NPs. A calibration curve was used with standard solutions to determine iron concentration.

2.6. Protein adsorption studies

1 mg of each synthesized NPs was incubated in 10 ml of BSA solution (1 mg/mL) and after dispersing by ultrasonic bath, they were kept for 24 h at 37 °C. NPs were separated by centrifuge. Then, UV–Vis spectrophotometer was used to measure BSA concentration in the supernatant. The absorbance of all solutions were recorded around 278 nm.

2.7. Degradation study of T-Fe_3O_4 NPs

T-Fe_3O_4 NPs were immersed in phosphate buffered saline (PBS) solutions at 37 °C with different pHs (7.4 and 5.8) and times (3 and 8 days). These NPs were separated from solution at definite time by magnet. NPs were dried at room temperature and then, SEM was used to study the changes in the morphology of T-Fe_3O_4 NPs.

2.8. Drug loading and release study

In this work, ascorbic acid was used as a model drug to evaluate the loading and release behavior of T-Fe_3O_4 NPs. 4 mg of ascorbic acid was dissolved in 10 ml of deionized water and then 7 mg of NPs was added to the solution. The mixture was sonicated for 2 min and remained in room temperature for 24 h. During this time, ascorbic acid can diffuse and can be trapped in branch-like structure of tannic acid in T-Fe_3O_4 NPs. Then, NPs were separated from solution by a magnet and washed with water for removing the unloaded ascorbic acid. The separated solution was used to estimate the ascorbic acid loading in T-Fe_3O_4 NPs by a UV–Vis spectrophotometer and measure the absorbance at 254 nm. Then, by using a calibration curve, drug loading contents (Eq. (1)) and drug entrapment efficiency (Eq. (2)) of NPs were calculated.

$$\text{drug loading contents } (\%) = \frac{\text{weight of drug in NPs}}{\text{weight of prepared NPs}} \times 100 \quad (1)$$