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Fabrication of rectorite-contained nanoparticles for drug delivery with a green and one-step synthesis method

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ABSTRACT

The composite nanoparticles (NPs) consisted of quaternized chitosan (QC)/bovine serum albumin (BSA)/rectorite (REC) were prepared successfully just by adding BSA solution into QC-REC nanocomposites solution via electrostatic interactions. The average diameter of NPs increased with the accretion of REC, which was demonstrated with dynamic laser scattering (DLS) and transmission electron microscopy (TEM). The results of small angle X-ray diffraction (SAXRD) and selected area electron diffraction (SAED) demonstrated that the intercalated structure of REC was enlarged with the addition of REC. Besides, it can was proved that the interaction had occurred between QC and REC in NPs with fourier transform infrared (FT-IR) and X-ray photoelectron spectroscopy (XPS). In addition, doxorubicin (DOX) was used to investigate the entrapment efficiency and release pattern in NPs. It turned out to be that the addition of REC could increase the encapsulation efficiency (EE) and loading capacity (LC). The results also exhibited that the drug release in simulated gastric fluid reduced apparently with the addition of REC, which could ensure more DOX released in intestines.

1. Introduction

Nanoparticles (NPs) as the carriers for drug delivery have gained increasing attention in the last few decades. NPs become an important carrier in the field of drug delivery because they possess the ability to deliver drugs to varied areas of the body for sustained periods of time (Liu et al., 2008). However, the synthetic processes of NPs are usually complex, such as introduction of crosslinking agent, dispersing agent and other toxic chemicals. Furthermore, the stability of NPs is easily affected by experimental conditions such as temperature and pH value. Due to those restrictions chemical-sensitive, pH-sensitive and temperature-sensitive drugs cannot be entrapped efficiently. Therefore, it is important to seek for a green and efficient method for the preparation of NPs.

Based on the consideration above, some natural polymers with several unique advantages such as nontoxicity, good biological compatibility and high hydrophilicity, comparing with synthetic organic materials, are preferable candidates for fabricating drug carriers (Lu et al., 2006; Sayin et al., 2008).

In our previous research, Xu et al. have prepared NPs by adding extra cross-linking agent (Xu et al., 2012); Zhu et al. have reported that NPs could be synthesized with the process of heating and pH adjustment (Zhu et al., 2013). All these methods are not simple or efficient. Recently, we found that NPs can be synthesized rapidly by mixing negatively charged bovine serum albumin (BSA) and positively charged quaternized chitosan (QC) via electrostatic interactions. BSA is a natural globular protein with a molecular weight of 68 kDa, which contains three domains specified for metal-ion binding, lipid binding, and nucleotide binding (Boye et al., 1996). Because of its extensive medical applications, unusual ligand-binding properties and wide acceptance in the pharmaceutical industry, BSA can be widely used for drug delivery (Hu et al., 2006; Tantra et al., 2010). Besides, BSA can also be applied in fabrication of NPs due to its static electricity, coacervation and emulsification (Galisteo-González and Molina-Bolívar, 2014; Meziani and Sun, 2003; Rajith and Ravindran, 2014); QC, a water soluble derivative of chitosan, has been found that it has better properties of permeability and mucoadhesion than chitosan when it used as an absorption enhancer transporting across the intestinal

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epithelia (Kotzé et al., 1999; Thanou et al., 2000). Besides, QC-based NPs have smaller particle size and more advantages than those of chitosan-based NPs, such as weak alkaline values in drug delivery system (Bayat et al., 2008). Therefore, BSA and QC are ideal candidates to form the carrier for drug delivery (Chen et al., 2013; Kotzé et al., 1999).

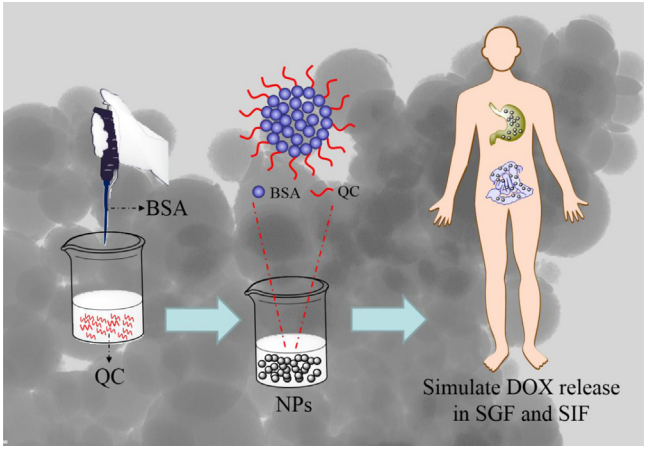
It is well-known that the encapsulation efficiency, loading capacity and controlled release of drugs are critical issues for NPs drug delivery systems. Interestingly, the efficiency of drug delivery of NPs carriers could be enhanced with the addition of rectorite (REC) in previous report, because REC-based composites have higher surface area and adjustable interlayer distance which can cause efficient contact and interaction with drugs (Wang and Du, 2008). REC is a kind of layered silicate, which has larger interlayer distance, better separable layer thickness and layer aspect ratio than montmorillonite (Farmer and Russell, 1964). REC constitutes with alternate pairs of dioctahedral mica-like layers and montmorillonite-like layers at the ratio of 1:1. Besides, it contains exchangeable cations, which can enlarge its interlayer distance by intercalating cations or polar molecules. The tunable interlayer distance of REC can obviously affect the efficiency of drug loading and delivery (Su et al., 2014). In addition, layered silicates have been verified to be safe enough as food additives by European Food Safety Authority (Xu et al., 2012).

In presented study, QC/BSA or QC-REC/BSA NPs were fabricated with one-step synthesis in a simple and green method. The NPs were synthesized without further process, such as pH adjustment or gelation. Besides, doxorubicin (DOX) was selected as hydrophilic drug model for investigating the drug delivery properties of NPs. Dynamic laser scattering (DLS), transmission electron microscope (TEM), field emission scanning electron microscope (FE-SEM), energy-dispersive X-ray (EDX), selected area electron diffraction (SAED), fourier transform infrared (FT-IR), X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) were performed to characterize the properties of NPs. Encapsulation efficiency (EE), loading capacity (LC) and *in-vitro* release properties of DOX in NPs had also been evaluated.

2. Materials and methods

2.1. Materials

Chitosan (CS, $M_w = 2.0 \times 10^5$ Da) from shrimp shell with 92% deacetylation was supplied by Zhejiang Yuhuan Ocean Biochemical Co., China. Calcium rectorite (Ca^{2+}-REC) was provided by Hubei Mingliu Inc., Co., China. BSA ($M_w = 6.8 \times 10^4$ Da) was purchased from Amresco Inc., USA. DOX ($M_w = 580$ Da) was received from Aladdin Co., China. All other chemicals were of analytical grade and were used without any further purification. All aqueous solutions were prepared by using purified water with a resistance of 18.2 MΩ· cm as solvents.



Scheme 1. Schematic diagram illustrating the fabrication process of QC/BSA NPs.

2.2. Preparation of QC and QC-REC composites

Quaternized chitosan (QC) was prepared according to our previous report (Deng et al., 2012). Briefly, CS (5 g) was dissolved into 2% w/v acetic acid (250 mL) and then adjusted the pH to 9.0 by adding sodium hydroxide solution to precipitate CS. After being soaked for 8 h, the solution was filtered to obtain purified CS, and then dried in oven at 40 °C.

The purified CS powder was transferred into a boiling flask, followed by addition of 2,3-epoxypropyl trimethylammonium chloride and adjustment of pH to 9. The mixture was kept under gentle agitation in 80 °C bath for 6 h. Afterwards, the reaction mixture was dialyzed against deionized water for 3 days and then precipitated by acetone. At last, the product was dried by lyophilization.

QC-REC composites were synthesized via solution intercalation method (Wang et al., 2009). Briefly, REC suspension was left still standing for 24 h after vigorous stirring for 30 min. QC aqueous solution was dissolved in water to obtain the 2% (w/v) solution. Then the resulting solution was added slowly into the pretreated REC suspension under stirring at 60 °C to obtain the nanocomposites with QC/REC weight ratios of 12:1, 6:1 and 3:1, respectively. The resulting mixture was stirred moderately for 2 days. The nanocomposites with initial QC/REC weight ratios of 3:1, 6:1 and 12:1 were designated as QR31, QR61 and QR121.

2.3. Preparation of the NPs

The concept for fabrication of NPs is shown in Scheme 1. QC and BSA powder were dissolved in aqueous solution with the concentration of 1.0 mg/mL, respectively. The obtained BSA solution was added dropwise into the QC solution or the QC-REC composites at the weight ratios of QC to BSA of 1:2, 1:3, 1:4 and 1:5, respectively. The obtained opalescent emulsions were stored overnight. Here, QC-BSA NPs were labeled with NP0. QC-REC/BSA NPs with the weight ratios of QC: REC were at 12:1, 6:1 and 3:1, which were designated as NP121, NP61 and NP31, respectively. The DOX-loaded NPs were prepared by applying the same method as above by adding BSA/DOX solutions into QC solution or QC-REC composites.

2.4. Characterization and morphology of QC/BSA or QC-REC/BSA NPs

The size distribution of the NPs were determined with Nano 3690 (Malvern, UK). The wavelength for the size measurement was 532.0 nm and the angle detection was 90° at 25 °C. The morphology of the NPs was observed with transmission electron microscope (TEM, JEM-2100, JEOL, Japan). Only NP0 were stained with phosphotungstic acid solution (2%, w/v), and then observed on a micro grid mesh scaffold. The intercalated structure of REC in NPs was investigated with selected area electron diffraction (SAED, JEM-2100, JEOL, Japan). Energy-dispersive X-ray (EDX) analysis of NP61 was conducted with Sirion 200 (FEI, Netherlands). Fourier transform infrared (FT-IR) spectra were recorded using a Nicolet 170-SX (Thermo Nicolet Ltd., USA). The composition of the NPs was examined with X-ray elemental spectroscopy (XPS) using an axis ultra DLD apparatus (Kratos, UK). The small angle X-ray diffraction