International Journal of Pharmaceutics 438 (2012) 258–265

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Quaternized chitosan–organic rectorite intercalated composites based nanoparticles for protein controlled release

Ruifen Xu[a,b,1], Shangjing Xin[c,1], Xue Zhou[d,1], Wei Li[c], Feng Cao[e], Xuyang Feng[e,*], Hongbing Deng[b,c,**]

[a] Department of Anesthesiology, School of Stomatology, Fourth Military Medical University, Xi'an 710032, China
[b] Department of Environmental Science, College of Resource and Environmental Science, Wuhan University, Wuhan 430079, China
[c] College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
[d] State Key Laboratory of Environment Health (Incubation); Key Laboratory of Environment and Health, Ministry of Education; Key Laboratory of Environment and Health (Wuhan), Ministry of Environmental Protection; Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
[e] Department of Cardiology, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, China

ARTICLE INFO

Article history:
Received 25 July 2012
Received in revised form 27 August 2012
Accepted 6 September 2012
Available online 12 September 2012

Keywords:
Quaternized chitosan
Organic rectorite
Intercalation
Nanoparticles
Drug delivery

ABSTRACT

Organic rectorite (OREC) was added in the quaternized chitosan (QC)/alginate (ALG) nanoparticles using an ionic gelation method to fabricate a controllable release system for proteins for the first time. The morphology of nanoparticles, the intercalated structure of OREC, bovine serum albumin encapsulation efficiency and *in vitro* release properties were investigated. Fourier transform infrared spectra, energy dispersive X-ray, X-ray photoelectron spectroscopy, small angle X-ray diffraction and size distribution analysis were performed to characterize the composite nanoparticles. With the addition of OREC, the encapsulation efficiency and the loading capacity of nanoparticles had increased from 21.2% to 44.9% and from 13.7% to 25.0%, respectively. In addition, the rapid initial release was inhibited successfully from 20.15% to 11.07% in stimulated gastric fluid and from 14.69% to 4.52% in stimulated intestinal fluid. The results verified that the addition of OREC could make these nanoparticles effective carriers to encapsulate drug and slow the drug controlled release of nanoparticles.

1. Introduction

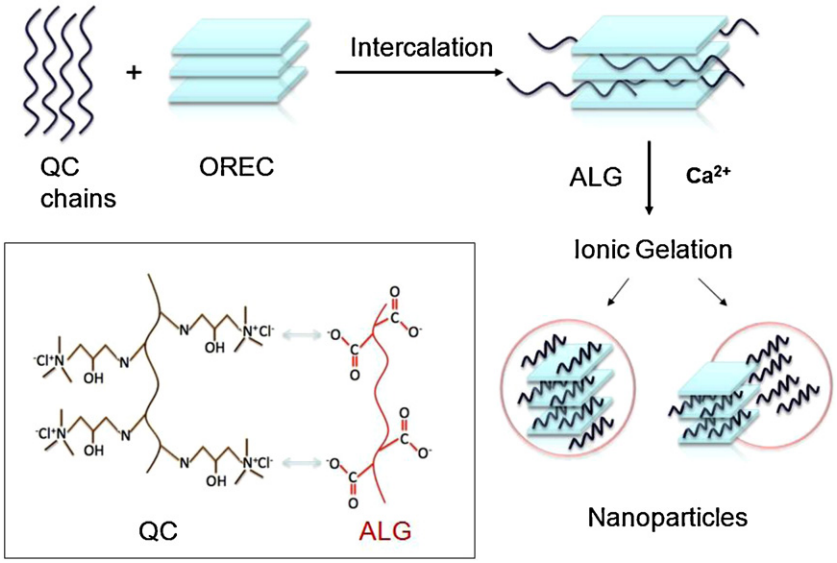
Nowadays, proteins and peptides have drawn many attentions in biotechnology because of their effective properties for disease treatment (Castro et al., 2005). However, they are hard to be absorbed in stomach and intestinal tracts as their hydrophilic property. In order to overcome this disadvantage, colloidal materials such as nanoparticles (NPs) have been used to protect proteins and peptides from quick dissolving and fabricate controlled release systems for the enduring utilization of drugs (Khalil and Mainardes, 2009; Mora-Huertas et al., 2010).

Chitosan (CS) is not only naturally abundant, but also nontoxic, biodegradable, and regenerable. Because of these properties, CS has been used in a variety of application areas such as bacterial inhibition (Deng et al., 2011a), cell culture (Deng et al., 2010), cosmetic skin masks (Huang et al., 2003), tissue engineering (Thein-Han et al., 2008) and drug delivery (Yuan et al., 2008). Some researchers have reported that CS NPs was applied as drug controlled release systems for hydrophilic drugs delivery by utilizing the hydrophobic property of CS (Wu et al., 2005; Xu and Du, 2003). Quaternized chitosan (QC), a water soluble derivative of CS, has been found that it is with better properties of permeability and mucoadhesion than CS (Kotze et al., 1999; Thanou et al., 2000), when used as an absorption enhancer transporting across the intestinal epithelia. In addition, QC NPs were with smaller particle size and remarkable advantages such as weakly alkaline values in drug delivery system (Bayat et al., 2008). Moreover, some reports have covered that QC NPs were used as a protein carrier (Xu et al., 2003) and that NPs cross-linking with alginate (ALG) were with better controlled release properties (Wang et al., 2011). However, the encapsulation efficiency of drug in the NPs is too low because the superabundant negative charged ALG could compete for the positive charged QC with drugs (Li et al., 2007).

In order to solve this problem, inorganic materials will be added for preparing QC/ALG organic–inorganic hybrid NPs. So far, CS based organic–inorganic hybrid has been reported as a nontoxic and biodegradable scaffold for tissue engineering and drug delivery response (Depan et al., 2011a; Thein-Han and

* Corresponding author. Tel.: +86 29 84775183; fax: +86 29 84775183.
** Corresponding author at: Department of Environmental Science, College of Resource and Environmental Science, Wuhan University, Wuhan 430079, China. Tel.: +86 27 68778501; fax: +86 27 68778501.
E-mail addresses: fengxuyang@gmail.com (X. Feng), alphabeita3000@yahoo.com.cn (H. Deng).
[1] Co-first authors with the same contribution to this work.

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2012.09.010



Scheme 1. Schematic diagram illustrating the fabrication process of QC–OREC nanoparticles.

Misra, 2009; Thein-Han et al., 2009; Yuan et al., 2010a) and the structure–process–property relationship has been studied to show the effect of adding inorganic component (Depan et al., 2011b). Based on these previous researches, the organic–inorganic composites might form a novel potential system with both of high encapsulation efficiency and loading capacity of drugs. Layered silicate, such as montmorillonite (MMT) and rectorite (REC), can be easily intercalated into layered structure by nature polymer chains and has been used in drug delivery recently (Burgentzle et al., 2004). CS-layered silicate nanocomposite carriers have been determined to be effective in cancer therapy. In the carriers, the positively charged chemotherapeutic drug was strongly bounded to the negatively charged layered silicate and release of the drug was slow (Yuan et al., 2010b). REC, especially organic rectorite (OREC) organic modified from REC, has larger interlayer distance, better separable layer thickness and larger aspect ratio than MMT (Wang et al., 2006). REC has been intercalated by QC chains and fabricated to QC–REC nanocomposites for antibacterial application (Wang et al., 2009) and gene delivery (Wang et al., 2008). In addition, layered silicate has been verified safe enough as food additives by European Food Safety Authority (ESFA) last year so that it is good for drug delivery (European Food Safety Authority, 2011).

While CS-layered silicate composites are biocompatible and become a potential candidate to drug controlled release, the mechanical properties and biological response of these composites are inadequate to qualify them for drug delivery or facilitate transfer of the applied load at the implant site. Furthermore, when considered intercalation between polymer chains and layered silicate, larger interlayer distance and better electrostatic force still need to be achieved. In the present study, novel QC–OREC/ALG composite NPs with intercalated structure and controllable OREC interlayer distance were fabricated and used as drug carrier for the first time. Bovine Serum Albumin (BSA) was selected as hydrophilic drug model for investigating the drug delivery properties of NPs. Transmission scanning electron microscope (TEM), field emission scanning electron microscopy (FE-SEM), energy-dispersive X-ray (EDX), X-ray elemental spectroscopy (XPS), X-ray diffraction (XRD), Fourier transform infrared (FT-IR) and NPs size distribution analysis are performed to characterize the properties of NPs. Encapsulation efficiency, loading capacity and *in vitro* release properties of BSA will be evaluated.

2. Materials and methods

2.1. Materials

Chitosan ($M_w = 2.1 \times 10^5$ kDa) was provided by Yuhuan Ocean Biochemical Co. (Taizhou, China) and the degree of deacetylation was 92%. Alginate (ALG) was purchased from Aladdin reagent Inc. (Shanghai, China). BSA ($M_w = 6.8 \times 10^4$ kDa) was supplied by Amresco Inc., USA. Calcium rectorite (Ca^{2+}–REC) was provided by Hubei Mingliu Inc. Co. (Wuhan, China). QC and organic rectorite (OREC) were prepared in our lab as previously reports (Wang et al., 2006; Xu et al., 2003). The degree of substitution of QC was 71%. The average diameter of OREC particles was about 100 nm. All other chemicals were of analytical grade.

2.2. Preparation of QC/ALG NPs

QC–ALG NPs were labeled with NP0. QC–OREC/ALG NPs were prepared in mass ratios of QC:OREC were at 12:1, 6:1 and 3:1, which were labeled with NP121, NP61 and NP31, respectively. NPs were prepared as shown in Scheme 1. QC–OREC nanocomposites were moderately stirred for 24 h in 60 °C water bath. Aqueous calcium chloride (0.5 mg/mL) was dispersed by ultrasonic for 5 min and then added into aqueous sodium alginate (1.0 mg/mL) to form the pre-gel. The QC solution or the QC–OREC composites was distilled into the pre-gel with stirring and continued stirring for another 30 min. The solution volume ratio of $CaCl_2$, ALG and QC or QC–OREC composites was at 2:6:1. The obtained opalescent emulsion was stored overnight and centrifuged at 15,000 × *g* for 30 min at 4 °C. The sediment lyophilized to produce solid NPs aggregates. The BSA-loaded QC/ALG NPs were prepared by using the same way as above by adding BSA (1.0 mg/mL) into ALG solutions, and the pH value of BSA/ALG mixture solution was determined at 6.78.

2.3. Morphology and characterization of QC/ALG NPs

The morphology of the NPs was observed by transmission electron microscope (TEM, JEM-2100, JEOL, Japan). QC/ALG NPs were stained with phosphotungstic acid solution (2%, w/v) and other samples were observed on a micro grid mesh scaffold. All samples were dried on copper grill at room temperature. The