Journal of Industrial and Engineering Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

Controlled pH- and glucose-responsive drug release behavior of cationic chitosan based nano-composite hydrogels by using graphene oxide as drug nanocarrier

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ARTICLE INFO

Article history:
Received 26 October 2016
Received in revised form 13 December 2016
Accepted 25 December 2016
Available online xxx

Keywords:
Cationic chitosan
Graphene oxide (GO)
Drug nano-carrier
pH-/glucose-sensitivity
Initial burst release

ABSTRACT

To realize tight control of hyperglycemia for diabetic patients, cationic chitosan (HTCC) based nano-composite hydrogels were prepared by using graphene oxide (GO) as nano-carrier for the model drug (bovine serum albumin, BSA). BSA intercalated into the layers of GO and the intercalation process was mainly driven by the mutual electrostatic interaction. By introducing GO-BSA, a more compact GO-centered network structure formed for the hydrogel. Compared with HTCC/BSA, HTCC/2.0wt%GO-BSA hydrogels exhibited a more distinct pH-/glucose-sensitivity and a much lower initial burst release, which was attributed to the compact structure and strong interactions among HTCC, GO and BSA in the hydrogel system.

Introduction

Diabetes mellitus, a disorder of glucose regulation, is a global burden affecting 366 million people across the world [1]. For tight control of hyperglycemia and prevention of the resulting complications in diabetic patients, it is highly desirable to develop simple, effective, and continuously self-regulated drug delivery systems [2]. Glucose-responsive hydrogels, known as stimuli-responsive or "intelligent" systems, can adapt the rate of drug release in response to changes in glucose concentration in order to maintain blood glucose levels within the normal range. Enzyme glucose oxidase (GOD), a widely used natural receptor, can be entrapped or immobilized within a pH-sensitive matrix, which results in enzyme-catalyzed conversion of glucose to gluconic acid, thereby lowering the pH in the microenvironment of the hydrogel and causing drug release [3–5].

Chitosan (CS), a copolymer of D-glucosamine and *N*-acetylglucosamine derived from chitin, is a potentially useful pharmaceutical material owing to its good biocompatibility and low toxicity [6,7]. In our previous study, it was demonstrated that chitosan microspheres based on the amino group were pH-sensitive in a wide range of pH 1.0–9.0, which was not suitable in the field of glucose responsive drug release systems with a narrow physiological pH-sensitive variation range from 7.4 to 6.8 for the conversion of glucose to gluconic acid through GOD [8]. Meanwhile the cationic chitosan (HTCC) synthesized by grafting with glycidyltrimethylammonium chloride (GTMAC) showed more distinct pH sensitivity as compared with chitosan and satisfied the above mentioned narrow physiological pH variation for glucose responsive drug release systems [9]. However, the initial burst effect was the main problems for the application of such HTCC hydrogels systems. The burst release of drugs is usually due to the weak interaction between polymer matrix and drugs, and the quick swollen rate of the polymer gel during the initial release process.

Recently, the preparation and application of novel biopolymer/nanomaterial composites [10–12] as controlled drug delivery vehicles have attracted much attention owing to their unique structure and properties. Graphene oxide (GO), an oxidative derivative of graphene, has attracted extensive interests in drug delivery. GO sheets are enriched with oxygen-containing functional groups such as hydroxyl and epoxy group on the basal planes and carbonyl and carboxylic groups at the sheet edges. Thus, the GO sheets with one-atom thickness and two-dimensional plane structure can provide large specific surface area to carry drugs via surface adsorption, hydrogen bonding, and other types of interactions [13], which makes it a promising material for drug carrier [14–21].

Very limited works have been published regarding the use of CS/GO nano-composites for drug delivery. Justin and Ben [22]

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http://dx.doi.org/10.1016/j.jiec.2016.12.023
1226-086X/

studied the drug release behavior of CS/GO nano-composite film, which offered a faster and a more substantial release of drug than CS, and the pH-sensitive release functionality of the nano-composite was also demonstrated, releasing 48% less drug in an acidic condition than that in a neutral environment. Chen et al. [23] prepared a series of porous CS/GO composite xerogels, which can absorb and slowly release an anticancer drug, doxorubicin hydrochloride (DOX). The cumulative release percentage of DOX from the xerogels at pH 4 was much higher than that at pH 7.4. In summary, for the current reported intelligent CS/GO drug release systems, the pH-sensitive variation range was too wide to satisfy the glucose-responsive drug release systems. And up to now, no literature can be available for the effect of GO on the glucose-responsive drug release behavior of CS.

In this study, GO was used as nanocarrier for bovine serum albumin (BSA) as the model drug. A series of GO-BSA intercalation complexes were prepared at first, and then, by using the cationic chitosan (HTCC) prepared in our previous study as the pH-sensitive polymer matrix and enzyme glucose oxidase (GOD) as the glucose-sensitive receptor, GO-BSA loaded intelligent hydrogels (HTCC/GO-BSA) were prepared. The intercalation behavior of GO-BSA was studied, and the network structures as well as pH- and glucose-responsive drug release behavior of HTCC/GO-BSA hydrogels were investigated.

Experimental

Materials

Chitosan (molecular weight 1×10^6 Da, degree of deacetylation 85%) was purchased from Zhejiang Jinke Biochemical Co. Ltd. (Zhejiang, China). Epichlorohydrin and sodium tripolyphosphate (TPP) were purchased from Tianjin Tianda Chemical Reagent Co. (Tianjin, China). Glycidyltrimethylammonium chloride (GTMAC) was obtained from Dongyingguofeng Fine Chemical Co. Ltd. (Shandong, China). Bovine serum albumin (BSA) was provided by Huayi Bioengineering Co. Ltd. (Hubei, China). Glucose, enzyme glucose oxidase (GOD) and insulin were purchased from Baoxin Biotechnology Co. Ltd. (Chengdu, China). All other reagents were of analytic reagent grade. Double distilled water was used throughout.

Preparation of GO-BSA intercalation complexes

The preparation procedure for the GO-BSA intercalation complexes was carried out as follows: 0.5 g of GO was dispersed in 200 ml of deionized water under ultrasound for 30 min at room temperature, and in this process the stable GO/H_2O dispersion solution formed. Then BSA was added and the mixture solution was stirred for another 4 h. Afterwards, GO-BSA dispersion solution was centrifugated and washed with distilled water to remove the free BSA. The resulting solids were freeze-dried for 12 h, finally yielding GO-BSA intercalation complexes.

Preparation of HTCC/GO nano-composite hydrogels

Chitosan was dispersed in water/isopropanol media at 37 °C and stirred for 30 min prior to dropwise addition of GTMAC. Then the reaction mixture was stirred at 60 °C for another 6 h. After being precipitated and washed with cold acetone, cationic chitosan (HTCC) was obtained by filtration.

Afterwards, HTCC with GO-BSA and GOD was dissolved in distilled water at room temperature and crosslinking agent (TPP) was added. After incubating at 37 °C for 2 h, HTCC/GO-BSA hydrogels formed. The samples were freeze-dried for 24 h and stored at 4 °C before use.

Measurements

X-ray diffraction analysis (XRD)

The interlayer spacing of the samples of GO and GO-BSA was measured at room temperature over the scanning range of $2\theta = 3–20°$ with Rigaku D/max III B x-ray diffraction equipment (Japan). Copper (Cu) K_α radiation ($\lambda = 0.154$ nm) was used at a generator voltage of 40 kV, current of 35 mA, and the scanning speed was 2.4 deg min^{-1}. The d-spacing of the GO-BSA layers was calculated with the Bragg equation:

$$2d\sin\theta = n\lambda \quad (1)$$

where θ is the diffraction angle; n is the order of diffraction, and λ is the incident wavelength.

Thermo-gravimetric analysis (TGA)

The thermo-gravimetric analysis (TGA) was used to characterize the thermal stability and intercalation ratio of GO-BSA. TGA was performed with a TA2950 thermo-balance from TA Co. (USA) under nitrogen atmosphere with the flow rate of 50 ml/min. The granulated samples of about 10 mg were heated from ambient temperature to approximately 800 °C at a heating rate of 10 °C/min.

Atomic force microscopy (AFM)

The surface morphologies and thickness of GO and GO-BSA were examined by AFM. The AFM measurements were performed with a SPM-9700 Scanning Probe Microscope (Japan) in a tapping mode from Digital Instruments with a Nanoscope IV controller. Samples for AFM imaging were prepared by drop-casting the GO and GO-BSA dispersions onto freshly cleaved mica substrates, which were then allowed to dry in air at ambient temperature and pressure.

Scanning electron microscopy analysis (SEM)

The morphology of the fractured surface of HTCC/GO-BSA hydrogels was observed with a JEOL JSM-5900LV scanning electron microscopy (SEM) (Japan). The operating voltage was 5 kV. The samples were ion beam sputter-coated with gold and the thin layer thickness was about 1–20 nm.

Transmission electron microscopy analysis (TEM)

The morphologies of GO-BSA intercalation complexes were observed with a Titan G2 60-300 transmission electron microscopy (TEM) (U.S.A.) at an accelerating voltage of 300 kV. Samples were dispersed in distilled water and dropped onto 200-mesh copper grids for TEM observation.

Rheological behavior

The visco-elasticity behaviors of HTCC/GO-BSA hydrogels were analyzed with Rheometer System Gemini 200 of Malvern Instrument Co. (UK), using parallel plates with 25 mm diameter and 1–2 mm plate-to-plate distance. For the strain scan experiment, the shear elastic modulus (G′) and viscous modulus (G″) were measured at 0.01–100% strain and 1 Hz frequency. For the frequency scan experiment, G′ and G″ were measured in the linear visco-elastic region, at 0.01–100 Hz frequency and 0.1% maximum strain.

Swelling ratio

The swelling properties of the HTCC/GO-BSA hydrogels were investigated in phosphate buffered saline (PBS) with pH 6.8. Hydrogels of a known weight (W_0) were immersed in PBS buffer solutions at 37 °C. Then, the hydrogels were taken out at predetermined intervals and weighed after removing excess solution on the surface. The swelling ratio can be determined

Please cite this article in press as: X. Zhao, et al., Controlled pH- and glucose-responsive drug release behavior of cationic chitosan based nano-composite hydrogels by using graphene oxide as drug nanocarrier, J. Ind. Eng. Chem. (2017), http://dx.doi.org/10.1016/j.jiec.2016.12.023