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A novel polysaccharide gel bead enabled oral enzyme delivery with sustained release in small intestine

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ABSTRACT

We report a novel oral delivery system based on sodium alginate/κ-carrageenan binary polysaccharide gel bead. β-galactase was selected as a model molecule to evaluate the capacity of the beads as delivery system. After enzyme encapsulation, the hydrogel beads were subsequently coated with κ-carrageenan (κ-CG) and ε-polylysine (ε-PL). Scanning electron microscope (SEM) images revealed the microstructural differences between the beads synthesized with and without ε-PL coating. Fourier transform infrared spectroscopy (FTIR) spectra further proofed the successful coating of ε-PL and κ-CG. Releasing studies showed that the enzyme releasing time is significantly prolonged by ε-PL coating. In vitro experiments showed that, the hydrogel beads synthesized with 0.6% ε-PL were stable and compact in the simulated gastric fluid environment with negligible swelling, which can protect the loaded enzyme effectively. More than 94.6% lactase activity can be retained after treated with simulated gastric fluid (pH 4) for 2 h. After transferred into simulated small intestinal fluid for 14 h (pH 7.4), as much as 89.6% of the enzyme can be released from the beads. It is worthy to note that the activity of the released enzyme retained 76.0% of total enzyme activity. These unique properties of the hydrogel beads enabled the effectively maintenance of enzyme bioactivity and thus enhanced the enzyme delivery and therapeutic efficiency. The reported delivery system is an ideal oral delivery system for bioactive food compounds, especially for compounds that are unstable in gastric environment.

1. Introduction

β-galactase is an enzyme that hydrolyzes D-galactosyl residues from lactose and other substrates containing D-galactosyl. It plays an important role in human metabolism and growth process. Lactose intolerance or lactose malabsorption can lead to a lot of harmful effects (Misselwitz et al., 2013). When lactase deficiency occurred in the gut, the hydrolysis of lactose is incomplete and the undigested sugar will be decomposed by colonic microflora into lactic acid, hydrogen, methane and carbon dioxide, resulting in abdominal distension and diarrhea. Lactose intolerance occurs in children shortly after weaning due to the down regulation of lactase production in the gut. A good approach to reduce the harm from lack of lactase is to add lactase into lactose containing food, such as milk and whey (Nussinovitch, Chapnik, Gal, & Froy, 2012). However, lactase would lose its activity in the stomach due to the highly acidic condition in stomach for proteins. Similar problems are faced by some drugs. Compared with injection, the non-invasive oral administration route is considered a more convenient and acceptable way (Sonaje et al., 2010). The hydrochloric acid and pepsin in gastric fluid may reduce the stability and solubility of drugs and decrease drug bioavailability. A prolonged drug release at an appropriate concentration and location is also a challenge. There are some researches focus on the development of delivery systems for gastrointestinal tract (GI) drugs such as insulin (Fonte et al., 2015) and caffeine (Belščak-Cvitanović et al., 2015). It is of great important to develop a vehicle to protect the oral protein or peptide drugs from the harsh environment in the stomach, and more importantly to achieve a prolonged and targeted release in small intestine.

The altering pharmacokinetic and pharmacodynamics profiles of given drugs request for new carriers to improve efficacy and safety (Kim et al., 2014). A large number of carriers, including emulsions (Lawrence & Rees, 2000), nanoparticles (Karimi et al., 2016), microspheres (Ma, 2014) and liposomes (Li et al., 2015) have been developed for oral delivery (Muheem et al., 2016; Zhang, Chan, & Leong, 2013). Among these forms, the pH-sensitive hydrogel beads have attracted increasing attentions (Yang, Chen, Pan, Wan, & Wang, 2013). A variety of synthetic or natural polymers have been studied in fabricating pH-sensitive hydrogels (Caló & Khutoryanskiy, 2015; Singh & Lee, 2014). Alginate is

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a natural anionic polysaccharide extracted from brown algae, which can be used to prepare pH-sensitive hydrogels (Hong, Lee, & Kim, 2011). It is reported to be non-toxic and biodegradable when given orally, and thus broadly used in biomedical applications. Hydrogel beads can be formed under mild pH and temperature conditions when dropping alginate solution into a gelation medium containing hardening agent such as Ca^{2+} (Li, Hu, Du, Xiao, & McClements, 2011). Swelling of such hydrogel beads in the stomach is negligible and thus the drug can be protected from the gastric fluid. However, the major limitations of using alginate as the delivery system is its large pore size and thus poor drug loading efficiency (Belščak-Cvitanović et al., 2015; Yang et al., 2013). Moreover, these beads are difficult to implement controlled drug release. At pH 7.4, the swelling is still negligible due to the relatively strong ionic interaction between the carboxylic groups on alginate and Ca^{2+} (Lin, Liang, Chung, Chen, & Sung, 2005). To solve these problems, hydrogel beads of carrageenan-alginate were synthesized by Mohamadnia and coworker (Mohamadnia, Zohuriaan-Mehr, Kabiri, Jamshidi, & Mobedi, 2007). κ-carrageenan (κ-CG), a kind of polyanionic polysaccharide extracted from red seaweed, is widely used in the food industry as gelling agent. Owing to the good physical and chemical properties such as electronegativity, viscosity and gelling properties, the carrageenan carrier can improve the loading efficiency and reserve the active drugs for a long time in simulated gastric fluid (Keppeler, Ellis, & Jacquier, 2009; Leong et al., 2011; Li, Ni, Shao, & Mao, 2014). However, the swelling ratio increases dramatically due to the increase in pH as hydrogel beads go into the duodenum, and a burst release occurs at intestinal pH, which may cause side effects.

In this article, κ-CG and ε-polylysine (ε-PL) were used to coat the hydrogel beads of carrageenan-alginate to further improve the loading and protection efficiency and achieve a sustained release in the intestinal tract. ε-PL is a homopolymer of L-lysine. ε-PL has been approved as generally recognized as safe (GRAS) for food applications. Through the toxicology study of ε-PL, it was found that ε-PL did not cause any adverse effects on reproductive, neurological, immunological, or embryonic and fetal development and growth at dietary concentrations of up to 30000 ppm (FDA, 2010). The isoelectric point of ε-PL is about 9, so it is strongly cationic at pH < 9. Therefore ε-PL has strong electrostatic interact with polyanion such as κ-CG and alginate (Shukla, Singh, Pandey, & Mishra, 2012; Chang, McLandsborough, & McClements, 2014). Compared with cationic chitosan, ε-PL was easy to dissolve in water and had no viscosity, which could reduce the loss of active ingredients during preparation process and improve the encapsulation efficiency. The preparation process is very simple, and the raw materials are inexpensive and bio-safe natural products. β-galactase is selected as a model drug molecule to illustrate the potential application of our system with the aim of improving the protection effect and the releasing kinetics of the beads in gastrointestinal tract.

2. Materials and methods

2.1. Materials

β-galactase (food grade) was purchased from Pangbo Biological Engineering Co., Ltd. ε-PL (food grade) was purchased from Baina Biological Engineering Co., Ltd. κ-CG (≥99.5%), sodium alginate (≥99.5%), coomassie bright blue G-250 (ultrapure grade) and orthonitrophenyl-β-galactopyranoside (ONPG) were purchased from Beijing solarbio technology Co., Ltd. Citric acid, disodium hydrogen phosphate, sodium chloride, hydrochloric acid, acetic acid, sodium acetate, potassium dihydrogen phosphate, sodium dihydrogen phosphate, sodium hydroxide, phosphoric acid and ethanol (95%) were purchased from Sinopharm Chemical Reagent Co., Ltd. The reagents were of analytical grade and used as received without further purification.

2.2. Preparation of hydrogel beads with or without β-galactase loading

Firstly, 0.896 g sodium alginate (SA) and 0.224 g κ-CG were dissolved in 30 mL distilled water and stirred for 30 min in 80 °C water bather. After naturally cooled to room temperature, β-galactase (10 mL, 4%) was added and stirred at 1000 rpm for 30 min, then degassed with ultrasound for 15 min. For control experiment, 10 mL distilled water was added instead of β-galactase. The mixture was slowly extruded through a 5.0 mL syringe into the hardening solution containing ε-PL (0%, 0.2%, 0.6%) and 0.15 M calcium chloride. After gently stirred for 30 min, the beads were washed three times with distilled water to remove the hardener. The beads were further successively coated with 1.5% κ-CG and ε-PL for 15 min. The coating process was repeated one time. Finally, the beads were dried under 40 °C, then stored at 4 °C.

2.3. Scanning electron microscopy

The hydrogel beads were sputter coated with gold for 5 min. Further, the coated particles were examined under SEM (JEOL, JSM-6380LV, Kyoto, Japan) at room temperature. The acceleration voltage used was 15 kV.

2.4. FTIR spectroscopic analysis

The hydrogel beads were ground with mortar and pestle. Under a deuterium lamp, the crushed material was mixed with dry potassium bromide, grinded and compressed to pastilles. Infrared spectra over the wavelength range 4000–400 cm^{-1} were recorded as KBr pastilles with FTIR spectrophotometer (Bruker, VERTEX70, Karlsruhe, German). The resolution was 4 cm^{-1}.

2.5. Swelling studies of the hydrogel beads

The hydrogel beads were immersed into 50 mL release mediums with different pH respectively under constant shaking at 100 rpm for 4 h (37 °C), including NaCl-HCl buffer (pH 1.2), phosphate-citrate buffer (pH 3 and pH 4), phosphate buffer (pH 7.4) and glycine buffer (pH 8.4). After that, the beads were removed from the swelling medium and blotted with a piece of paper to absorb excess water on surface, and then weighed.

The swelling ratio (SR) was calculated by the dynamic weight change of the beads with respect to pH using the following formulae:

$$SR(\%) = \frac{W_s - W_d}{W_d} \times 100$$

Where W_s is the weight of the beads in the swollen state and W_d is the initial weight of the dry beads. Each experiment was repeated three times.

2.6. Enzyme release studies

In vitro release studies of the β-galactase-loaded beads (0.2 g each) were performed in 40 mL of different release mediums with pH 1.2, 3, 4 and 7.4, respectively. The samples were incubated at 37 °C under constant shaking at 100 rpm. At predetermined time intervals, 5 mL samples were collected from the release medium and were replaced by an equal amount of fresh medium. The collected samples were centrifuged at 3000 rpm for 20 min. The concentration of β-galactase in the solution was assayed by UV spectroscopy (Hitachi UV2450) at 595 nm by Coomassie Brilliant Blue G-250 dye-binding method. Bovine serum albumin was used as a standard protein. The beads prepared at the same condition but without β-galactase loading was used as a blank control. Cumulative enzyme release rate (Q) was calculated using the following expression:

$$Q(\%) = \frac{\sum_{n-1}^{i} C_i V_i + C_n V}{W}$$

Where n is the time of solution collection and $0 < i < n$. C_n is the β-galactase concentration of the nth time collected solution, C_i is the β-galactase concentration of the ith time collected solution, V_i is the volume of solution collected at the ith time and equal to 5 mL here, V is the volume of the immersing buffer and equal to 40 mL here. W is the weight of β-galactase in the beads. For the measurement of W, 0.2 g of beads was incubated in 40 mL buffer (pH 7.4) for 12 h, and grinded up to ensure fully swelling of the beads and release of the β-galactase.

2.7. Enzyme encapsulation rate

The hydrogel beads (0.1 g) were soaked in distilled water (20 mL) for 12 h at 37 °C. Then they were grinded and heated for 3 h at 40 °C to take out the lactase completely from polymeric network. After centrifuged at 10,000 rpm for 10 min, the enzyme in the supernatant was assayed by Coomassie Brilliant Blue G-250 dye-binding method. All experiments were repeated three times. The encapsulation efficiency (EE) was calculated according to the following expression:

$$EE(\%) = \frac{C_r}{C_0} \times 100\%$$

Where C_0 is the initial enzyme concentration of mixed solution, C_r is the practical encapsulated enzyme concentration of beads.

2.8. Enzyme protection effect against simulated gastric fluid

For enzyme protection effect assessment, the beads prepared with 0.6% ε-PL (0.1 g each) were firstly incubated in 20 mL simulated gastric fluid (pH 1.2, 2, 3 and 4) containing pepsin (100 mg/L) at 37 °C for a period of time. After washed three times, the beads were transferred into 20 mL distilled water and incubated at 37 °C for 12 h, grinded up in an agate mortar. In order to release the enzyme in the beads thoroughly, the solution was incubated in 40 °C for 3 h. The enzyme activity detection was according to a reported method (Talbert & Hotchkiss, 2012). Briefly, the supernatant (1 mL) was collected and added into a 2 mL of acetate buffer solution (0.1 M, pH 5.0) containing 12 mM of ONPG, and allowed to react at 50 °C under shaking for 15 min. The enzyme activity was detected by measuring the absorbance of the catalysate at 420 nm. The activity of enzyme released from beads without simulated gastric fluid treatment was considered as control (100%) for the calculation of remaining percent activity.

2.9. The simulated gastrointestinal tract enzyme release

The in vitro simulated enzyme release contained two steps. Firstly, the beads (0.6% ε-PL) were treated with various acidic mediums (pH 1.2, 2, 3 and 4) containing pepsin (100 mg/L) at 37 °C for 2 h to simulate stomach environment of different conditions. The corresponding beads are numbered as WQ1, WQ2, WQ3 and WQ4. Secondly, the beads were taken out and blotted with a piece of paper to absorb the

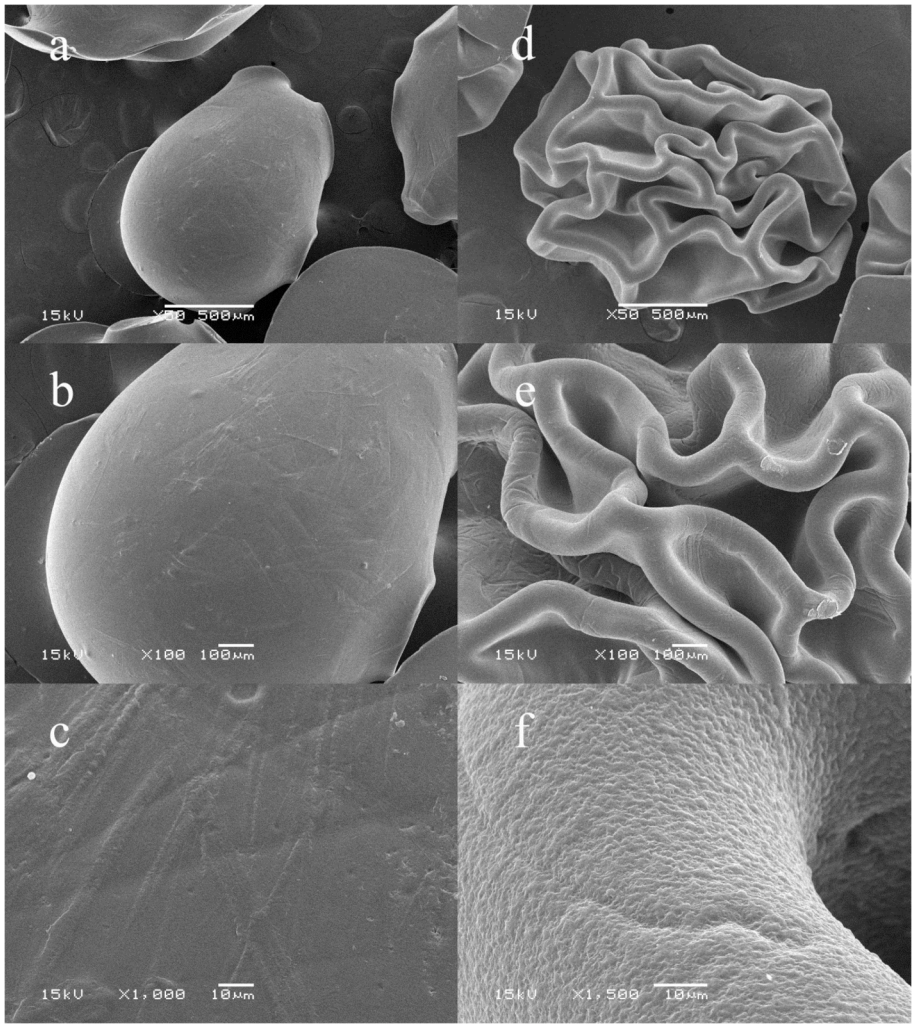

Fig. 1. SEM micrographs of hydrogel beads synthesized with different concentration of ε-PL. 0% ε-PL (a)–(c), 0.6% ε-PL (d)–(f).

medium, and then treated with buffer (pH 7.4) containing 100 mg/L of trypsin for 14 h to simulate the intestinal environment. The Q values were recorded. The activities of the released β-galactase in the second step were also measured.

3. Result and discussion

3.1. Characterization of the beads

In this article, we focus on the influence of ε-PL on the enzyme releasing behavior of the prepared hydrogel beads. The beads synthesized with and without ε-PL were characterized with scanning electron microscope (SEM). The average diameter of the synthesized beads was about 1.6 mm. From Fig. 1, it can be seen that, the surface of the bead synthesized without ε-PL is relatively smooth. In the presence of ε-PL, the surface became rough and wrinkled. It is due to the strong electrostatic interaction between ε-PL polycation and κ-CG/SA polyanion (Selvakumaran, Muhamad, & Razak, 2016).

The hydrogel beads were also characterized with Fourier transform infrared spectroscopy (FTIR), and the results are shown in Fig. 2. Curve a represents the blank beads without enzyme loading. Curve b, c and d represent the beads that loaded with β-galactase and encapsulated with 0.6%, 0.2% and 0% ε-PL, respectively. The encapsulation efficiency of beads was 89.58%, 85.22% and 9.67% in curve b, c and d, respectively. With the incorporation of β-galactase, the spectra were similar to that of the blank beads, which was in accordance with previous research (Sankalia, Mashru, Sankalia, & Sutariya, 2006). The main bands related to protein were not detected in Fig. 2, which could be due to the interaction between the protein and polysaccharide. Thus, the hydrogel beads would be ideal material for encapsulation of β-galactase. Compared with Curve a, the characteristic peaks of κ-CG at the 845 cm^{-1} wasn't appeared in the Curve b, c and d. The band at 850-840 cm^{-1} was caused by the stretching vibration of C4-O-S and represented the characteristic peaks of sulfuric acid ester group in C-4 position of the 1,3-β-D-galactose, which was the monomer of κ-CG (Sen & Erboz, 2010). It indicated that the β-galactase in the beads would interact with the sulfate group of the κ-CG. With the increase of the ε-PL content, the asymmetric stretching vibration peak of COO^- shifted from 1631 cm^{-1} to 1648 cm^{-1}, the symmetric stretching vibration peak of COO^- shifted from 1418 cm^{-1} to 1434 cm^{-1}, -OH stretching vibration band (3440 cm^{-1}) became smaller and narrower. These characteristic bands of SA were in accordance with previous research (Pasukamonset, Kwon, & Adisakwattana, 2016). With the increase of the ε-PL content, the peak represents S=O stretching vibration (1262 cm^{-1}) was disappeared. The band at 1270-1230 cm^{-1} was caused by the S=O symmetrical

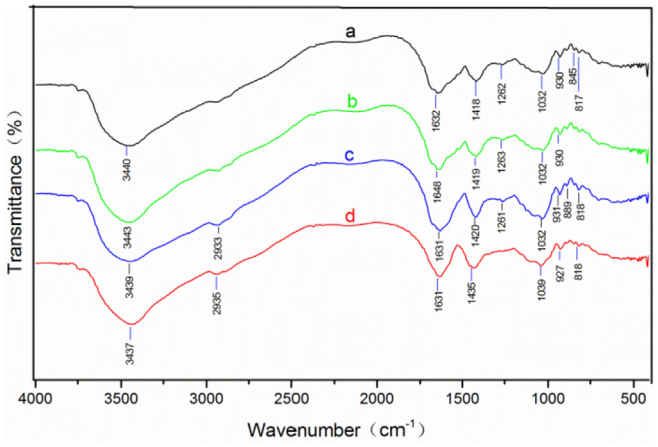

Fig. 2. FTIR spectra of gel beads. (a-Blank beads (0.6% ε-PL), b-lactase load beads (0.6% ε-PL), c-lactase load beads (0.2% ε-PL), D-lactase load beads (0% ε-PL)).

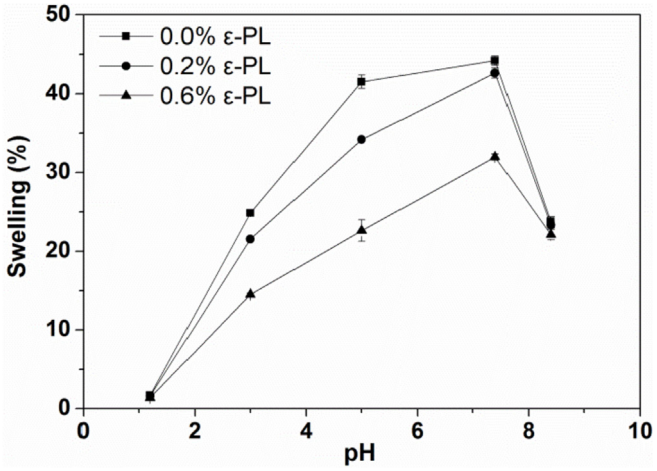

Fig. 3. Influence of pH on the swelling ratio of gel beads.

stretching vibration of sulfate ester of κ-CG (Sen & Erboz, 2010). These changes suggested the electrostatic interaction of ε-PL with sulfate ester of κ-CG and carboxyl group of SA.

3.2. Swelling behavior of the beads under various pH conditions

The pH changed a lot in the gastrointestinal tract (1.2–8.4) from mouth to stomach, small intestine and eventually colon (Y. Li et al., 2011). Therefore, studying the effect of pH on the swelling behavior of gel beads is extremely important. The swelling behavior of the gel beads in the medium with pH ranging from 1.2 to 8.4 was studied, which will provide a basis for subsequent lactase release in gastrointestinal tract. Various beads prepared with different concentration of ε-PL were used for the swelling studies. As shown in Fig. 3, the swelling ratio of the beads reached maximum at pH 7.4, and then decreased as the pH further increased. Such pH-responsive swelling property is attributed to the presence of negatively charged carboxyl groups in SA and the sulfate groups in κ-CG. The dissociation constant (pKa) of SA and κ-CG is reported to be 3.2–3.5 and 2.5, respectively (El-Ghaffar, Hashem, El-Awady, & Rabie, 2012; Y.; Li et al., 2011; Mahdavinia, Etemadi, & Soleymani, 2015). In strong acidic pH, carboxylic acid groups and the sulfate groups remain undissociated and therefore no net charge is developed in the polysaccharide network. Furthermore, strong hydrogen bond between the carboxylic groups, sulfate groups, and the hydroxyl groups on the polysaccharide ensure the compact structure and low swelling ratio of the beads, which can ensure a relatively low amount of enzyme release and guarantee the maintenance of its activity under harsh acidic conditions (Yang et al., 2013). Once exposed to neutral or alkaline medium with pH greater than the pKa of SA and κ-CG, the carboxylic acid groups and the sulfate groups dissociated. The electrostatic repulsion force among polymer chains broke the hydrogen bond, resulting in the expansion of the beads. The water was absorbed by the beads due to the presence of hydrophilic groups within its network, exhibiting swelling phenomenon (Selvakumaran et al., 2016). In the alkaline environment, the swelling ratio of the beads seemed to be decreased. This can be due to the degradation of the beads in alkaline condition, which result in the decrease of bead weight after alkaline treatment, and thus the decrease of calculated swelling ratio. The pH responsive swelling property that the beads remained compact under acidic condition and swelled under alkaline environment enables the protection of the enzyme in the stomach and targeted drug release in small intestine.

From Fig. 3 it can also be seen that, ε-PL had little impact on the swelling ratio at pH 1.2. However, the swelling ratio of gel beads decreased with the increase of ε-PL concentration under higher pH conditions (pH 3.0–7.4). This is due to the increased electrostatic

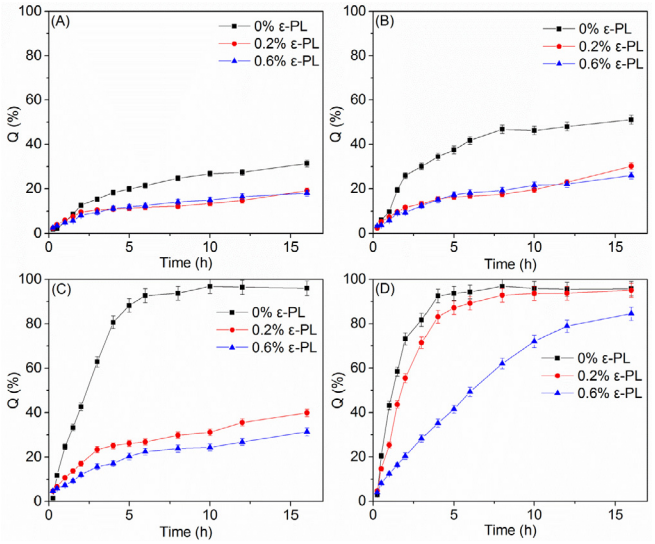

Fig. 4. Cumulative release enzyme rate of lactase loaded gel beads in (A) pH 1.2, (B) pH 3, (C) pH 4, (D) pH 7.4, respectively.

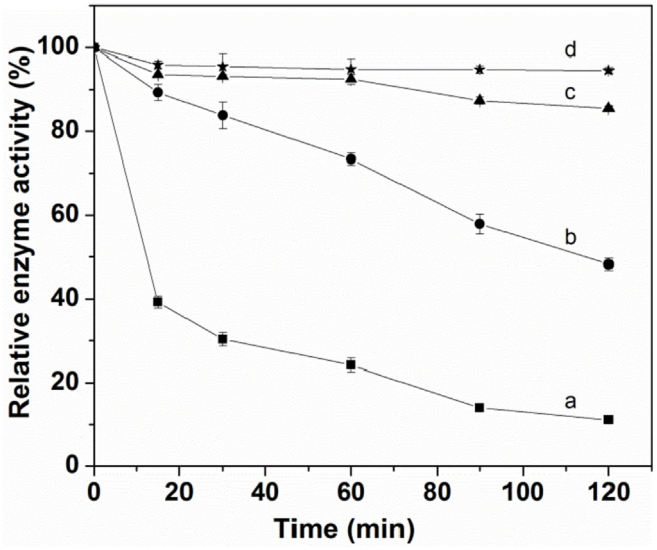

Fig. 5. Relative activity of enzyme in the beads treated with various simulated gastric fluid. (a) pH 1.2, (b) pH 2, (c) pH 3, (d) pH 4. (The concentration of ε-PL is 0.6%.)

interaction between ε-PL and SA or κ-CG, which formed a more stable network structure membrane to prevent the permeation of water, thus showed a slower swelling tendency (Chang et al., 2014; Hong et al., 2011). On the other hand, swelling ratio of the bead without ε-PL is much larger than that coated with 0.6% ε-PL. Therefore, it is potential to control the release rate of the cargo by adjusting the concentration of ε-PL to achieve sustained release.

3.3. Release behavior of the beads under various pH conditions

For the investigation of drug release behavior, beads were prepared using various concentration of ε-PL (0%, 0.2% and 0.6%). Buffer solutions (pH 1.2, 3, 4 and 7.4) were used as dissolving mediums. Release rates were calculated according to the method mentioned in section 2.5, and the releasing curves were drawn. According to Fig. 4A, in the dissolution medium of pH 1.2, the beads had low release rates. After released for 16 h, the Q values are 34.13%, 19.09% and 18.06% for beads prepared with 0%, 0.2% and 0.6% ε-PL, respectively. This can be due to part destruction of ε-PL and κ-CG membrane on the surface of the beads under long time acidic condition treatment, resulting in the release of lactase loaded in the outer layer (Hu, Huang, Situ, & Chen, 2011). Similar to the swelling behavior, with the increase of the ε-PL concentration, the release rate is decreased. This is due to the enhancement of the interaction between ε-PL and κ-CG in acidic condition, resulting in the formation of tighter layers to reduce the release rate (Muhamad, Fen, Hui, & Mustapha, 2011).

From Fig. 4 we can also see that, the enzyme release of beads prepared with 0.2% and 0.6% ε-PL was slow under the stomach condition (pH 1.2–4). On the contrary, under small intestine environment (pH 7.4, Fig. 4D), the beads made with 0.2% and 0.6% ε-PL exhibited obviously increased drug release rate. The swelling ratio of the gel beads in the medium with pH ranging from 1.2 to 7.4 increased with the increase of pH (see Fig. 3). The surface of beads incubated in a solution of pH 1.2 was non-porpus and in a solution of pH 7.4 was highly porpus (Kulkarni, Boppana, Krishna, Mutalik, & Kalyane, 2012). Because of the low swelling ratio under low pH, the high molecular weight enzymes were prevented by membranes which were formed by ε-PL and κ-CG on the surface of the beads, so the enzyme released less. Because the beads were obviously swelling under pH 7.4, the enzyme could release rapidly from the distensible pore of beads. It is worthy note that, the beads made with 0.6% ε-PL exhibited a relatively gentle and continues drug release, indicating the potential application for sustained drug release in the intestinal tract.

3.4. Enzyme activity assessment

The normal pH ranges of the human stomach vary from 1.2 to 4.0 (1.2–1.8 for empty stomach and 3.0–4.0 for after meal). In order to investigate the protection effect of the beads to the loaded enzyme when it goes through the stomach, the beads (prepared with 0.6% ε-PL) were treated with simulated gastric fluid of different pH for 2 h and during which the encapsulated enzyme activity was assessed. The results are shown in Fig. 5. It can be seen that, the activity decreased with the extension of treating time. The lower the pH of the medium, the lower the activity was. According to Fig. 4, the beads had low Q value under acidic condition. However, the strong acid (pH 1.2, 2) may destruct the membrane on the surface of the beads and then penetrate into the bead which resulted in the inactivation of the enzyme inside the beads (Hu et al., 2011). The gastric emptying time is usually less than 2 h. After 2 h treatment, the retained relative enzyme activities are 11.18%, 48.27%, 85.48% and 94.6% for pH 1.2, 2, 3 and 4, respectively. These results indicated that relative enzyme activity higher than 85% can be retained when the beads were taken after meal.

3.5. In vitro simulated release of enzyme loaded in hydrogel beads

In order to investigate the potential application of the hydrogel beads in practical oral delivery, in vitro release experiment was carried out. The enzyme loaded beads (0.6% ε-PL) were treated with simulated gastric fluid (pH 1.2, 2, 3 and 4) for 2 h, and then transferred into simulated small intestinal fluid. From Fig. 6, it can be seen that, the Q values are very low in simulated gastric fluid, indicating the protection of the loaded enzyme by the beads in the simulated stomach. When the beads were transferred into simulated intestinal fluid (pH 7.4), the Q value increased quickly in the initial ~8 h and increased slowly in the following time because of close to equilibrium state, indicating the sustained release in the simulated small intestine environment. These were related to the swelling property, which was consistent with the results of Section 3.2 and 3.3. The enzyme release trend is consistent with that of κ-CG/sodium carboxymethyl cellulose drug releasing system reported by Lohani's group (Lohani, Singh, Bhattacharya, Hegde, & Verma, 2016). In addition, the Q values were 54.1%, 66.9%, 80.3% and 89.6% for WQ1, WQ2, WQ3 and WQ4 at 16 h, respectively, indicating that the higher pH in the initial gastric acid environment may increase the final Q value in the intestinal environment. Although

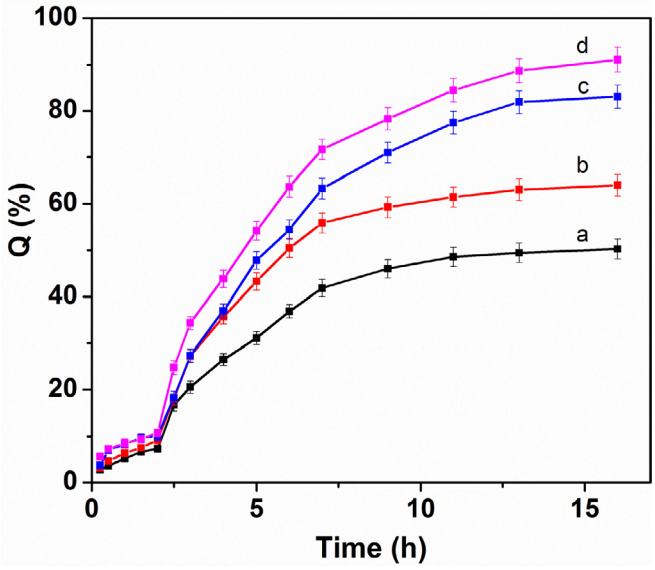

Fig. 6. Cumulative enzyme release rate of lactase loaded gel beads during in vitro simulated release. The pH of simulated gastric fluid for (a) WQ1, (b) WQ2, (c) WQ3, (d) WQ4 are 1.2, 2, 3 and 4, and the pH of simulated small intestinal fluid is 7.4.

the enzyme release rates are low in various simulated gastric solutions at 2 h. The smaller the pH, the smaller the swelling of hydrogel beads (see Fig. 3). Therefore, the swelling rate of the beads in subsequent simulated intestinal fluid was limited. Thus, the smaller the pH of the initial gastric fluid, the smaller the rate of Q value in the final environment.

The effect of gastric acidity on the relative activity of the released β-galactase is shown in Fig. 7. After treatment with highly acidic gastric fluid (pH 1.2 and 2) for 2 h, the relative activities of the released enzymes after 14 h releasing are 4.8% and 27.4% for WQ1 and WQ2, respectively. After treatment with low acidic gastric fluid (pH 3 and 4), the relative activities of enzymes released from WQ3 and WQ4 are 62.4% and 76.0%, respectively. These results indicated the effective protection of the beads to the enzyme and the targeted release of enzyme in small intestine when taken after meal.

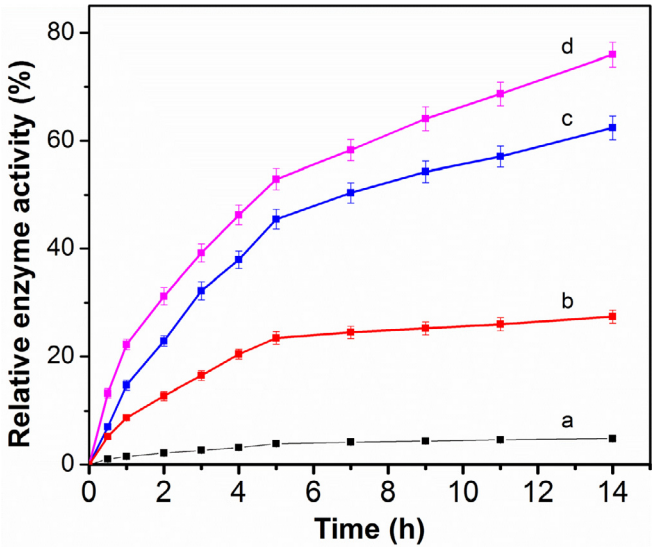

Fig. 7. Relative activity of the enzyme released from beads incubated in in vitro simulated small intestinal fluid after treated in various simulated gastric fluid for 2 h. (The pH of simulated gastric fluid for (a) WQ1, (b) WQ2, (c) WQ3, (d) WQ4 are 1.2, 2, 3 and 4.).

4. Conclusions

In summary, ε-PL coating was successfully applied on the surface of sodium alginate/κ-carrageenan hydrogel beads through electrostatic adsorption process. SEM was employed to characterize the ε-PL-coated hydrogel beads. FTIR showed the electrostatic interaction of ε-PL with κ-CG and SA. The pH responsive bead swelling and enzyme release were carefully studied before and after ε-PL coating. ε-PL coating greatly improved the function of the alginate/κ-carrageenan hydrogel beads. The higher the pH (pH 1.2–7.4), the higher the swelling of hydrogel beads, and the release rate increased accordingly. With the increase of the ε-PL concentration, the swelling and release rate was decreased. Enzyme activity assessment showed that, after coating with 0.6% ε-PL, the hydrogel beads exhibited satisfied protection effect on the loaded β-galactase activity under low acidic condition (pH 3–4) and 94.6% lactase activity could be retained after treated with simulated gastric fluid (pH 4) for 2 h. In vitro experiment showed that, after transferred to a simulated small intestinal fluid for 14 h (pH 7.4), as much as 89.6% of the enzyme can be released from the beads. It is worthy to note that the activity of the released enzyme retained 76.0% of total enzyme activity. Our work clearly demonstrated that the constructed system is promising for protein and enzyme store and handle during industrial operation and targeted delivery in GI tract.

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