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Sulfasalazine release from alginate-N,O-carboxymethyl chitosan gel beads coated by chitosan

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ABSTRACT

In the present study, spherical beads were prepared from a water-soluble chitosan (N,O-carboxymethyl chitosan, NOCC) and alginate with ionic gelation method. Then, swollen calcium–alginate–NOCC beads were coated with chitosan. To prepare drug loaded beads, sulfasalazine (SA) was added to the initial aqueous polymer solution. The effect of coating, as well as drying procedure, on the swelling behavior of unloaded beads and SA release of drug loaded ones were evaluated in simulated gastrointestinal tract fluid. The rate of swelling and drug release were decreased for air-dried and coated beads in comparison with freeze-dried and uncoated ones, respectively. No burst release of drug was observed from whole tested beads. Chitosan coated beads released approximately 40% of encapsulated drug in simulated gastric and small intestine tract fluid. Based on these results, the chitosan coated alginate–NOCC hydrogel may be used as potential polymeric carrier for colon-specific delivery of sulfasalazine.

1. Introduction

Interest in polymeric matrices for pharmaceutical formulation continues to grow, especially those that react to external stimuli, allow targeting the release of drugs in specific conditions or sites. Chitosan is a natural cationic polysaccharide obtained via deacetylation of chitin that is found particularly in crustacean shells. Chitosan is biocompatible, non-toxic, biodegradable and mucoadhesive polymer, with a gel-forming ability at low pH (Agnihorti, Mallikarjuna, & Aminabhavi, 2004; Berger, Reist, Mayer, Felt, & Gurny, 2004; Hamidi, Azadi, & Rafiei, 2008; Peniche, Arguelles-Monal, Peniche, & Acosta, 2003; Prabaharan, Reis, & Mano, 2007; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Also the degradation of chitosan occurs by the available microflora in the colon. These properties could provide a basis for preparation of controlled release formulations, particularly for colon-specific drug delivery (Hejazi & Amiji, 2003).

N,O-carboxymethyl chitosan (NOCC) is a chitosan derivative having carboxymethyl substituents on some of both the amino and primary hydroxyl sites of the glucosamine units of the chitosan structure (Lin, Linang, Chung, Chen, & Sung, 2005; Ravi Kumar et al., 2004). The biodegradability of NOCC films was significantly greater than its chitosan counterpart (Chen et al., 2004). NOCC is non-toxic, either *in vitro* or *in vivo* and improve retention time and bioavailability of drugs in ophthalmic preparations (George & Abraham, 2006). Carboxymethylated chitosan has already been used extensively in a wide range of biomedical applications due to its unique properties especially its good solubility in water and excellent biocompatibility (Aiping, Jianhong, & Wenhui, 2006; Chen & Park, 2003; Fan et al., 2006; Ramesh, Viswanatha, & Tharanathan, 2004; Zhang, Guo, Peng, & Jin, 2004).

Alginate is a water-soluble linear polysaccharide extracted from brown seaweed. Alginate beads can be prepared by extruding a solution of sodium alginate, as droplets, in to a divalent cross-linking aqueous solution such as calcium ions. Although calcium–alginate beads can be prepared by this simple and mild procedure, this method has major limitations such as drug loss during bead preparation, by leaching through the pores in the beads, and rapid drug release caused by physical instability and high solubility of alginate in neutral and weak alkali media (George & Abraham, 2006). To overcome these limitations, Lin et al. (2005) prepared a complex of alginate blended with NOCC by dropping into a Ca^{2+} solution. These microencapsulated beads demonstrated excellent pH-sensitivity and could be a suitable polymeric carrier for site-specific bioactive protein drug delivery in the intestine.

The carboxylate moieties on alginate can ionically interact with the protonated amines on chitosan, forming physical crosslinked hydrogels known as polyelectrolyte complex. This process has been widely used in the preparation of alginate-chitosan membrane with a solid calcium-alginate gel core. There are many advantages of the chitosan coating, such as the improvement of drug payload and bioadhesive property, as well as the prolonged drug release properties (Abreu, Carla, Maria, & Tarso, 2008; Douglas & Tabrizian, 2005;

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Gåser?d, Sannes, & Skjåk-bræk, 1999; Liu et al., 2004; Park, Kang, Haam, Park, & Kim, 2004; Shi et al., 2008; Shu & Zhu, 2002; Sæther, Holme, Maurstad, Smidsrød, & Stokke, 2008).

A great deal of research work has been devoted to specific drug delivery to the colon. Indeed, colon-specific delivery with a controlled release pattern provides more effective therapy for such chronic diseases as irritable bowel syndrome and inflammatory bowel disease, including Crohn's disease and ulcerative colitis. Sulfasalazine is a compound composed of 5-aminosalicylic acid and sulfapyridine and used as a prodrug for the treatment of ulcerative colitis (Lamprecht, Torres, Schafer, & Lehr, 2000; Mladenovska et al., 2007; Zambito & Colo, 2003).

At present study, to enhance the properties of calcium-alginate beads, an aqueous solution composed of alginate and NOCC was added dropwise into a Ca^{2+} solution and then coated by chitosan. Uncoated and chitosan coated calcium–alginate–NOCC beads loaded with SA were prepared. Then, the morphology of the beads, the swelling characteristics and release profile of the model drug (SA) from these carriers in simulated gastric and intestinal media were investigated.

2. Experimental

2.1. Materials

Chitosan ($M_W \sim 2 \times 10^5$) with an 85% degree of deacetylation was provided from Sigma (USA). Sodium alginate was obtained from BDH laboratory (England). Calcium chloride, monochloroacetic acid and isopropyl alcohol were purchased from Merck. Sulfasalazine was obtained from Zhejlang Jiuzhou pharmaceutical Co. Ltd (Zhejlang, China). N,O-carboxymethyl chitosan (NOCC) was synthesized according to the literature (Chen et al., 2004) and characterized by the method described by Sugimoto, Morimoto, Sashiwa, Saimoto, and Shigemasa (1998). All the other used chemicals and reagents were of analytical grade.

2.2. Preparation of beads

2.2.1. Calcium–alginate–NOCC beads

Firstly, aqueous alginate and NOCC solutions, with a distinct composition (3% (w/v)), were prepared. Then equal volumes of these solutions were mixed to form a homogenous blend solution and maintained 5 h for complete removal of bubbles. The final pH of solution was found to be ~7.5 ± 0.1. Five milliliters of this solution was dropped into a 30-ml gently stirred 2.5% (w/v) $CaCl_2$ solution through a syringe needle (0.4 mm in diameter) at a dropping rate of 1.0 ml/min. The distance of the needle tip from the gelling solution surface was 10 cm. The prepared beads were allowed to harden in the calcium chloride solution for 30 min. These beads were filtered, washed with distilled water three times and dried at 40 °C for 24 h or freeze-dried. The freeze-dried beads were obtained through rapid freezing at −80 °C, followed by drying in a freeze-drier (Zirbus, Denmark).

2.2.2. Calcium–alginate–NOCC–chitosan beads

The calcium–alginate–NOCC beads, prepared at first step (immediately after washing), were transferred to a solution of 1.5% (w/v) chitosan in a 1.5% (w/v) acetic acid and 0.2 M sodium acetate buffer at pH 5 and kept for 1 h. The beads were filtered and rinsed three times with distilled water, and subsequently dried at 40 °C for 24 h or freeze-dried as described above.

2.2.3. Drug loaded beads

To prepare drug loaded beads, SA with a final concentration of 1% (w/v) was added to the initial aqueous alginate solution with continuous stirring and the pH of the solution was adjusted to 7.5 by adding 2 M NaOH. This solution was used for preparation of SA-loaded beads by the same procedure described in Section 2.2.1.

2.3. Characterization of beads

2.3.1. Scanning electron microscopy (SEM)

The shape and surface characteristics of air and freeze-dried alginate–NOCC–chitosan beads were determined by SEM. The beads were sputter-coated with Au using a vacuum evaporator and examined using a scanning electron microscope (Philips, Netherlands).

2.3.2. Bead diameter analysis

The diameter of beads was determined using an optical microscope and digital micrometer, and the average values were taken for at least 25 beads.

2.3.3. Drug content and encapsulation efficiency determination

The encapsulation efficiency (wt.%) was calculated from the difference between the amount of SA dissolved in aqueous polymer solution and that of SA released in gelation medium divided by the amount of SA dissolved in aqueous polymer solution. For this purpose, the concentration of SA in gelation and washing solution was determined spectrophotometerically at 359 nm. The drug content (wt.%) was determined as the ratio of encapsulated SA weight to the total weight of the dried beads. This was accomplished by immersion of drug loaded beads in sodium phosphate buffer at pH 7.4. The total released drug after 24 h was determined spectrophotometerically and was considered as encapsulated SA.

2.3.4. Swelling studies

The swelling characteristics of beads were determined by immersing them at dry state into conical flask containing 40 ml of release medium that incubated at 37 °C under shaking at 150 rpm. At first, dry beads were swollen in 0.1 M HCl solution at pH 1.2 (simulated gastric fluid) for 2 h. Afterwards, the beads were transferred to sodium phosphate buffer solution at pH 6.8 (simulated small intestinal fluid) and kept for 3 h. Subsequently, they were transferred to sodium phosphate buffer solution at pH 7.4 (simulated colonic fluid) until complete dissolution was obtained. At specific time intervals, samples were taken out from the swelling medium and blotted with a piece of paper towel to absorb excess water on surface. The degree of swelling, $S(t)$, at each time was calculated using the following expression, where W_t and W_d are the sample weights at time t and in the dry state, respectively. Each experiment was repeated three times.

$$S(t) = \frac{W_t - W_d}{W_d} \quad (1)$$

2.3.5. Drug release studies

The SA release from drug loaded beads was studied at conditions described in swelling studies. In some cases after release at pH 6.8, the beads were transferred to the suspension of fresh rat cecal content (20%, (w/v)) in sodium phosphate buffer at pH 7.4 until complete release of the drug was obtained. At predetermined time intervals, 2 ml of samples were withdrawn from the dissolution medium and immediately replaced by the same volume of fresh medium. The amount of SA released from beads was determined spectrophotometerically (UV/Vis Varian Cary 50, USA) at 359 nm using previously calibrated standard curves at different pHs. To determine the total release in 0.1 M HCl solution (after 2 h), the pH of the release medium was adjusted to 7.4, by adding

NaOH, and the concentration of SA was determined from calibration curve at this pH. Each experiment was repeated three times.

3. Results and discussion

3.1. Characterization of beads

It is well known that dropwise addition of aqueous alginate solution into $CaCl_2$ solution immediately induces ionic cross-linking of alginate chains, thus forming Ca-alginate beads (Liu et al., 2004). Gel formation was observed upon the addition of aqueous NOCC into a $CaCl_2$ solution. This indicated that ionic crosslinks between the carboxylate ions ($-COO^-$) on NOCC and alginate can be established by Ca^{2+}. Thus, in the preparation of calcium–alginate–NOCC beads, alginate entangled through the NOCC network, resulting in the formation of interpenetrating polymeric network (IPN).

Then, calcium–alginate–NOCC beads were coated by chitosan as a result of ionic interaction between $-NH_3^+$ and $-COO^-$ groups. Liu, Jiao, and Zhang (2007) prepared chitosan coated NOCC beads by extruding carboxymethyl chitosan solution into a $CaCl_2$/chitosan gelation medium at pH 6, as a result of polyelectrolyte complex membrane forming through interpolymeric ionic interactions between carboxymethyl chitosan and chitosan. Lin et al. (2005) showed the FTIR spectra of the calcium–alginate–NOCC hydrogel at pH 1.2 and 7.4, which demonstrated the presence of carboxylate ions (COO^-) in hydrogel at both pHs (Lin et al., 2005). This is clearly an indication for the presence of carboxylate ions at pH 5. Lee, Park, and Ha (1997) mentioned that the chitosan coated alginate microcapsules prepared at pH 4.8 showed a minimum release because both amine and carboxylic groups in both polyelectrolytes have about 70–80% degree of dissociation near pH 5. The binding of chitosan with alginate was also found at pH between 4 and 6 which increased with increasing pH in this range (George & Abraham, 2006).

The wet beads prepared at present study were initially spherical in shape with a smooth surface and 1.1–1.3 mm diameter. The mean diameter of uncoated and chitosan coated calcium–alginate–NOCC beads loaded with SA in dry state was 0.5–0.7 and 0.65–0.85 mm, respectively. Chitosan-coating resulted in increased size of beads, as expected.

Scanning electron micrographs of SA-loaded alginate–NOCC beads and their surface morphologies are shown in Fig. 1. Air-dried beads with a rather rough surface have lost their spherical shape, but freeze-dried beads with a sponge-like structure remained almost spherical. This structural difference causes different swelling and drug release behavior, as described in the following sections.

3.2. Swelling studies

At pH 1.2, the swelling degree of the beads was limited due to the formation of strong hydrogen bonds between (–COOH and –OH) groups of both alginate and NOCC polar chains. At this pH, the beads reached to their maximum swelling degree within 30 min and then shrunk gradually towards their equilibrium state. For alginate–NOCC–chitosan beads, this phenomenon may be attributed to the reduced chemical potential of the network resulted from protonation of carboxylic acid groups. The initial increase of swelling degree is mostly driven by counterions which neutralize $-NH_3^+$ groups of polymer chains in the network (Dolatabadi-Farahani, Vasheghani-Farahani, & Mirzadeh, 2006).

At pH 6.8 the swelling of beads increased considerably due to the swelling force resulted from the presence of counterions which neutralized created carboxylic groups on alginate and NOCC at neutral media. The swelling degree of beads reached to a maximum and then decreased due to disintegration and dissolution of hydrogel network.

Fig. 2 shows the swelling behavior of beads, as a function of pH. The swelling degree of freeze-dried beads at whole pHs was higher than that of air-dried ones due to their sponge-like structure

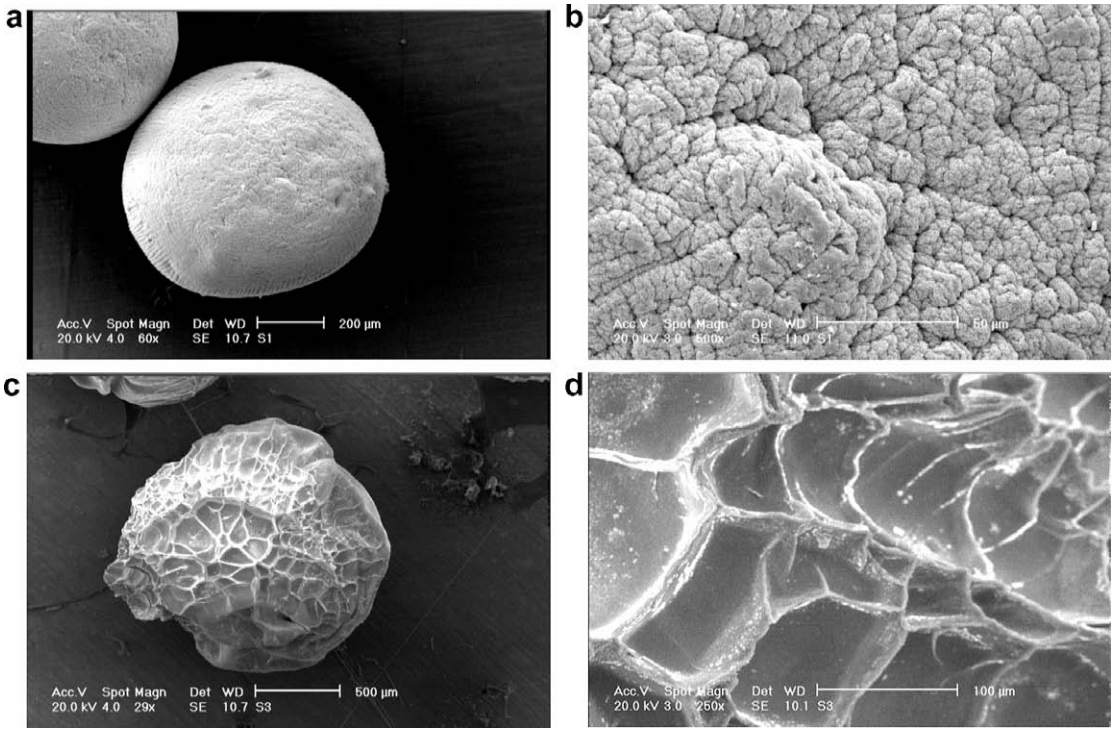

Fig. 1. SEM micrographs of (a) air-dried SA-loaded alginate–NOCC beads, (b) surface morphology of air-dried SA-loaded alginate–NOCC beads, (c) freeze-dried SA-loaded alginate–NOCC beads, and (d) surface morphology of freeze-dried SA-loaded alginate–NOCC beads.

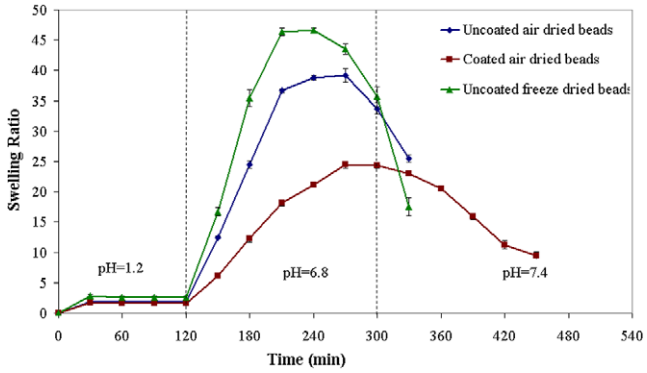

Fig. 2. Swelling characteristics of the beads in simulated gastrointestinal tract fluid.

($p < .05$). At pH 1.2, the maximum swelling degree of air and freeze-dried calcium–alginate–NOCC beads were 1.8 and 2.8. The corresponding values at pH 6.8, were 38 and 46, respectively.

The swelling degree and swelling rate of uncoated alginate–NOCC beads were higher than those of chitosan coated beads at pH 6.8 ($p < .05$). Swelling of the coated beads was limited mainly due to the presence of outer membrane resulted from ionic complexation of chitosan with alginate–NOCC blend as well as hydrogen bounding of amine groups of chitosan and hydroxyl and carboxyl groups of alginate and NOCC. Also, the swelling of the coated beads is limited due to deprotonation of $-NH_3$ groups, particularly in the outer shell which results in decreased chemical potential of the network as a whole. The rate of disintegration and consequent weigh loss of the samples were limited in the presence of coating layer.

3.3. Drug release behavior

The encapsulation efficiency of SA in both uncoated and chitosan coated calcium–alginate–NOCC beads were found to be 65% and 60%, respectively. The release profiles of SA from the beads are shown in Fig. 3. As shown, the cumulative SA release at acidic condition (pH 1.2) from freeze-dried, uncoated and coated beads was 6.8%, 4.7%, and 1%, respectively. The relatively slow SA release from these carriers at pH 1.2 is due to limited swelling degree of the beads and solubility of SA at this pH.

The SA release rate at pH 6.8 increased significantly ($p < .05$) in accordance with increased swelling degree of the hydrogel network and solubility of SA. At neutral and alkali medium, the release

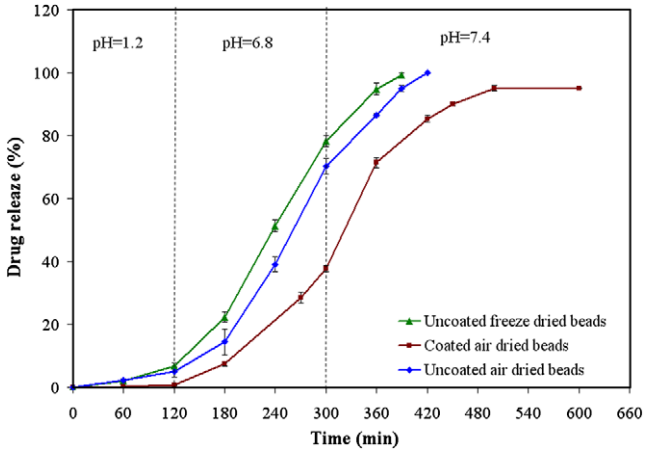

Fig. 3. The release profiles of SA from the beads in simulated gastrointestinal tract fluid.

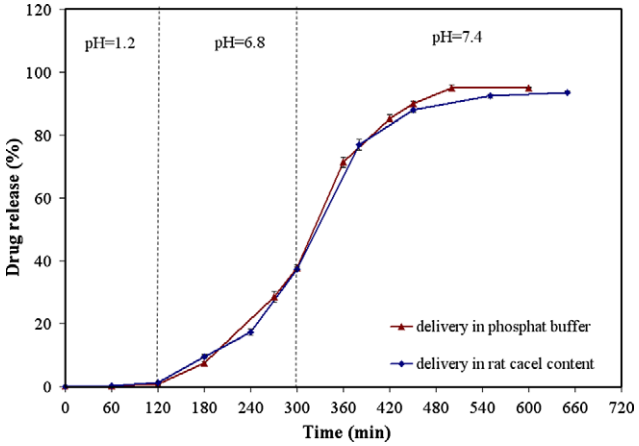

Fig. 4. The release profiles of SA from coated air-dried beads.

rate of encapsulated drug from coated alginate–NOCC beads was slower than that of uncoated beads ($p < .05$) due to the lower degree of swelling, lower rate of disintegration and resistance of the polyelectrolyte complex membrane against drug diffusion and release. Tapia et al. (2004) showed that, chitosan-alginate complex erodes slowly in phosphate buffer at pH values higher than 6.5 and this behavior leads to suppression of the initial drug release in the upper segments of GIT occurring for uncoated microparticles and controls release in the colon.

Coating with chitosan decreased significantly the rate of release and approximately 40% of encapsulated drug was released in simulated gastric and small intestine tract fluid during 5 h of dissolution. Incomplete SA release was also observed for chitosan coated alginate–NOCC beads. This result indicated that some drug molecules were entrapped within the polyelectrolyte complex membrane and cannot be released unless the polymer matrix is degraded. It is worth to mention that no burst release was observed from these carriers which are a desirable property for drug delivery systems. This observation and high solubility of SA in initial polymer solution are indirect evidence of homogeneous distribution of drug throughout the polymeric matrix.

Fig. 4. shows the release profiles of SA from coated air-dried beads when the third step of drug release was performed in the suspension of fresh rat cecal content (20%, (w/v)) and sodium phosphate buffer at pH 7.4. As shown, the release profile in the suspension of fresh rat cecal content was very similar to that in phosphate buffer. But it is found that the coated beads in this suspension collapsed slower than in sodium phosphate buffer solution. This phenomena result in slower rate of release. It is seemed that the release in pH 7.4 was controlled by disintegration of the hydrogel network.

4. Conclusion

Drug delivery systems of calcium–alginate–NOCC and calcium–alginate–NOCC–chitosan beads, prepared at present study, demonstrated distinct pH-sensitivity. The beads were sphere-like and had dense structure. At pH 1.2, the swelling degree of the beads was limited. At pH 6.8, the swelling degree of beads reached to a maximum and then decreased due to disintegration and dissolution of hydrogel network. The method of drying had a profound effect on the swelling and drug release behavior of these carriers. No burst release of drug was observed from both uncoated and coated alginate–NOCC beads. Coating with chitosan decreased significantly the rate of release and approximately 40% of encapsulated drug was released in simulated gastric and small intestine tract fluid

during 5 h of dissolution. Based on these results, chitosan coated calcium–alginate–NOCC beads may be used as potential polymeric carrier for colon-specific drug delivery of sulfasalazine. This can be further supported by performing *in vivo* trials.

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