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Alginate–konjac glucomannan–chitosan beads as controlled release matrix

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Abstract

Controlled release beads were prepared by using alginate (ALG), konjac glucomannan (KGM) and chitosan (CHI). Bovine serum albumin and insulin were used as model proteins for in vitro assessments. It was observed that KGM could be contained within beads, and faintness hydrogen binding and electrostatic interaction exist between ALG and KGM by infrared spectra. Clear dents were found on the surface of beads using KGM by scanning electron microscopy. Use of KGM could help increase the payload of drug. After beads were treated by 0.1 N HCl for 4 h and put into pH 7.4 buffers, protein was released from ALG–CHI beads within 1 h, while it was lost from ALG–KGM–CHI beads for 3 h. However, the leaking of protein from ALG–KGM–CHI beads was also increased in 0.1 N HCl solution. Concentration of gelling ion had great effect on release rate and gel structure. Studies of water of hydration had shown that swelling of ALG–KGM–CHI beads was higher than that of ALG–CHI beads in acidic solution, but the opposite result was obtained in alkali solution. The result indicated that the diffusion of protein was related to the viscosity and swelling properties of KGM.

Keywords: Alginate; Konjac glucomannan; Chitosan; Insulin; BSA; Controlled release

1. Introduction

There are a variety of both natural and synthetic polymeric systems that have been investigated for the controlled release of drug. Hydrophilic polyionic carbohydrates such as alginate (ALG) (Wayne and Siow, 1998) and chitosan (CHI) (Thanou et al., 2001) have been paid much attention in recent years. Since the preparation of beads by these materials involves the use of aqueous solvents, environmental problems associated with organic solvents would be minimized. These beads would also have nonimmunogenicity and bioadhesive properties (Park and Robinson, 1984), which could serve as a potential advantage in mucosal drug delivery.

In this work, we used ALG and konjac glucomannan (KGM) as the core material for preparing drug-loaded beads. ALG, a naturally occurring copolymer of guluronic and manuronic acids, can be ionically cross-linked by the addition of divalent cations in aqueous liquid. The relatively mild gelation process has enabled not only proteins

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(Hari et al., 1996) but also cells (Machluf et al., 2000) and DNA (Quong et al., 1998) to be incorporated into ALG matrices with retention of full biological activity. ALG is chemically very stable between pH 5 and 10. High acid concentrations cause decarboxylation of ALG. In order to improve the stability of ALG capsules in acidic gastric juice, complex coacervation of oppositely charged polyelectrolytes such as CHI (Gaserod et al., 1999) and polylysine (PLL) (Thu et al., 1996) has been commonly used. But these just had a certain degree of success.

It has been recently reported that the drug delivery particulates were prepared using ALG, PLL and pectin to release theophylline, chlorothiazide and indomethacin (Liu and Krishnan, 1999). Pectin is more resistant to breakage in the gut than ALG gel, breaking down in the presence of micro-flora in the colon, and has been used for colonic drug. Use of pectin especially helped in forming a more robust particulate that was more resistant in acidic pH and modulated the release profiles of the encapsulated model drugs in the alkaline pH. This particulate system may have potential use as a carrier for drugs that are poorly absorbed after oral administration.

KGM is a high-molecular weight water-soluble non-ionic glucomannan extracted from tubers of the *Amorphophallus konjac* plant. It is a linear random copolymer of β (1→4) that linked D-mannose and D-glucose, and approximately one in 15 of the sugar units are acetylated. It is similar to pectin which is not hydrolyzed by digestive enzyme in human being and is considered as an indigestible dietary fiber that has received recognition for reducing the risk of developing diabetes and heart disease. A diet rich in high-viscosity KGM is generally recommended for improved glucose and lipid profile control, suggesting a therapeutic potential in the treatment of the insulin-resistance syndrome (Vuksan et al., 2000). KGM could be hydrolyzed by β-mannanase to manufacture manno-oligosaccharides which play important roles in biological systems (He et al., 2001). In addition, KGM could form strong, elastic, heat-stable gels when heated with mild alkali (Dave et al., 1998), and has also been used as drug carrier (Nakano et al., 1979), food preservative (Xiao et al., 2000a,b) and enzyme entrapment (Pérols et al., 1997). However, the encapsulation yield of protease remained only about 50%, and KGM loses the above physiological action after alkali treatment and heating (Pérols et al., 1997).

A more stable complex gel of ALG and KGM may be formed in the presence of calcium ion. Without heating and alkali treatment, activity of KGM can be retained completely. The preparation procedure was simple and would be easily scalable. The model drugs chosen for the in vitro experiments were insulin and bovine serum albumin (BSA). Recently, oral peptide drug delivery has shown considerable promise.

It is our aim in this study to utilize ALG–KGM as a matrix to deliver insulin and BSA orally in the gastrointestinal tract. A CHI coating is employed to increase the capsule strength and flexibility. We used infrared (IR) spectra and scanning electron microscopy (SEM) to discuss the interaction between ALG and KGM and the bead structure. Through release experiments, the effect of KGM and concentration of $CaCl_2$ on the protein delivery were investigated. It seems that ALG–KGM–CHI beads have potential use as a carrier for oral delivery system.

2. Materials and methods

2.1. Materials

Sodium ALG (low viscosity, 87 cps for a 2% solution at 25 °C) was obtained from Beijing Xudong Co. (Beijing, China). CHI, whose viscosity-average molecular weight was 8.55×10^6, was procured from Qingdao Hengsheng Co. (Qingdao, China). Konjac powder was purchased from Dazhou Wufeng Co. (Sichuan, China). Insulin (bovine) 28.5 IU mg^{-1} was imported. BSA, fraction V, was a product of Blood Research Center of Chinese Academy of Medical Sciences (Tianjin, China). All other chemicals are of AR grade. The estimation of insulin and BSA was done as per Bradfold's method of estimation of protein. Absorbance study was done with the help of a spectrophotometer (Hitachi U-2001, Tokyo, Japan) at 595 nm.

2.2. KGM preparation

A 0.5% (w/v) solution of KGM was prepared in water, and the solution was centrifuged to remove the insoluble material. The supernatant liquid was poured into an equal volume of methanol to precipitate KGM. The solid was then filtered, redispersed in water and freeze-dried. This yielded a fluffy white material, approximately 92% of original powder, which was used for this study.

2.3. Bead formation

Sodium ALG (2.0 or 2.5% w/v) and the mixture of sodium ALG (2% w/v) and KGM (0.5% w/v) were allowed to dissolve in deionized water containing BSA (3 mg ml^{-1}) or insulin (3 mg ml^{-1}). Approximately 1 ml of this solution is dropped through a needle, from a plastic syringe into a beaker containing 20 ml of $CaCl_2$ solution (0.2 M in pH 5 HAc–NaAc buffer). ALG and ALG–KGM capsule were hardened in $CaCl_2$ solution for 30 min, and then was filtered and rinsed with distilled water. The wet bead diameter is 2.8 ± 0.1 mm that was determined microscopically with a vernier scale micrometer. In another method, a CHI solution (20 ml) containing 0.2 M, 0.3 M or 0.4 M $CaCl_2$ was used for gellation of ALG, and then ALG–CHI or ALG–KGM–CHI beads were prepared. Study of bead formation processes and varying concentration of KMG and $CaCl_2$ was discussed. The rinsed capsules were allowed to dry in air at room temperature until constant weight could be achieved.

2.4. IR spectra

IR spectra of beads without protein were recorded with a Fourier transform infrared (FTIR) spectrometer (Hitachi 270–30, Tianjin, China).

2.5. SEM

Blank beads were put into deionized water and lyophilized. For SEM (PHILIPS XL 30 ESEM, Amsterdam, The Netherlands), cross-sections of the freeze-dried blank beads were obtained using a razor blade. The sections were then coated with gold–palladium for 70 s in an argon atmosphere, before observing them under the microscope. The mean bead diameter and standard deviation are calculated from a sample population of at least 20 lyophilized beads randomly selected from the population. Partial beads become ellipses due to shrink. The average over the short diameter and the long diameter is the size of the bead.

2.6. In vitro BSA/insulin release

The release of encapsulated proteins was carried into Tris–HCl pH 7.4 after 4 h of acid treatment (in 0.1 M HCl) in agitator. The stirrer speed was set to 90 rev min^{-1} and thermostat-controlled bath was set to 37 °C. Samples at appropriate intervals were withdrawn and assayed using Bradfold's method for protein estimation. An equal volume of same dissolution medium was added to maintain a constant volume. The amount loaded is estimated by dissolving a known amount of beads in PBS, pH 7.4. The percentage of loading efficiency is determined from the following equation:

$$\text{Loading efficiency } (\%) = \frac{L}{L_0} \times 100,$$

where L and L_0 are the amount of protein loaded within a known amount of beads according to experimental data and calculation of concentration, respectively.

2.7. Effect of KGM concentration on water of hydration of beads

ALG beads of various KGM concentrations are placed into 20 ml Tris–HCl, pH 7.4, or 0.1 M HCl for 4 h. Then beads were filtered. The excess water was blotted with a filter paper, and their wet weight was determined. The percentage water of hydration is determined from the following equation:

$$\%\ \text{Water of hydration} = \frac{w_1 - w_2}{w_1} \times 100,$$

where w_1 is the wet weight of beads and w_2 the dry weight of beads before hydration.

3. Results and discussion

3.1. IR spectra

KGM was a non-ionic carbohydrate and impossible to form a gel in the presence of multivalent ions. Then perhaps KGM could be leaked out from gel. We use IR spectra to discuss whether KGM exits within beads after gelling and the interaction between carbohydrates.

Fig. 1 gives the IR spectra of the beads at the range of 4000–900 cm^{-1}. IR spectra of ALG–Ca beads in Fig. 1 showed that the absorption band around 2950, 1620, 1438 and 1040 cm^{-1} corresponds to the stretching of –CH, COO–, –CH and C–O–C, respectively. Furthermore, from IR spectra of KGM, we can know that the stretching peaks of –CH were presented at 2920, 2885, 1415 and 1380 cm^{-1} and that of the carbonyl of aceto groups at 1730 cm^{-1}. The absorption band at 1640 cm^{-1} was the intra-molecular hydrogen bonds. The peaks at 1031 and 1069 cm^{-1} were assigned to the stretching of C–O–C.

For ALG–KGM beads, some peaks disappeared or became weak due to interaction or superposition between groups of ALG and KGM. The stretching of –CH at 2950 cm^{-1} in ALG and that of at 2920 cm^{-1} and 2885 cm^{-1} in KGM disappeared. The carbonyl of aceto groups at 1730 cm^{-1} of KGM also cannot be seen. The absorption band at 1620 cm^{-1} of COO– in ALG may be coupled with the absorption band at 1640 cm^{-1} of intra-molecular hydrogen bonds in KGM and forms a new little peak at 1630 cm^{-1}. At the same time, peak of 1620 cm^{-1} was relatively weak perhaps because the concentration of COO– was decreased in the mixture. It is similar for peak at 1438 cm^{-1} which has almost disappeared. Moreover, the absorption band at 1040 cm^{-1} in ALG interacts with KGM and forms a broad peak at about 1070 cm^{-1}. The above phenomenon indicated that KGM was contained within beads and faintness hydrogen binding and electrostatics interaction exists between ALG and KGM.

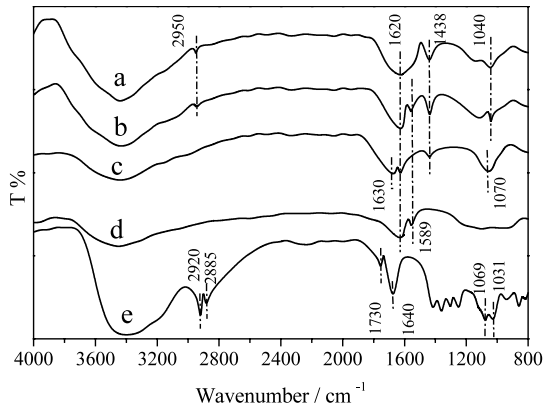

Fig. 1. IR spectra of the beads: (a) ALG (2%) beads; (b) ALG (2%)–CHI beads; (c) ALG (2%)–KGM (0.5%) beads; (d) ALG (2%)–KGM (0.5%)–CHI beads; (e) KGM.

The solution of ALG and CHI can form polyelectrolyte complex (PEC). The peak at 1589 cm^{-1} is assigned to the characteristic absorption band of amino group in CHI. It can be seen from IR spectra of beads coating CHI that the peak at 1589 cm^{-1} emerged due to the complex coacervation formed on the surface of beads. We also observed that the peaks of ALG–KGM–CHI were much more weakened. One reason is that more KGM was contained within beads because the leaking of KGM was retarded by the compact polyelectrolyte skin; other one may be multi-interaction among ALG, KGM and CHI.

3.2. SEM

The SEM micrograph of the beads of ALG and ALG–KGM is shown in Fig. 2. There are evident differences of morphology between bead without KGM and with KGM. The surface of ALG bead was smooth (Fig. 2a), while that of ALG–KGM bead had clear cylindroid dents (Fig. 2b). The above phenomenon indicated that the phase separation happened when mixture of sodium ALG and KGM was dropped into a cross-linking solution containing Ca^{2+}. After gelling for a while, beads were rinsed with distilled water. At this time, KGM was lost from the surface of beads and dents were created. At the inner of the beads, homogenous and honeycomb-like open cavities were formed in ALG bead (Fig. 2a), while a

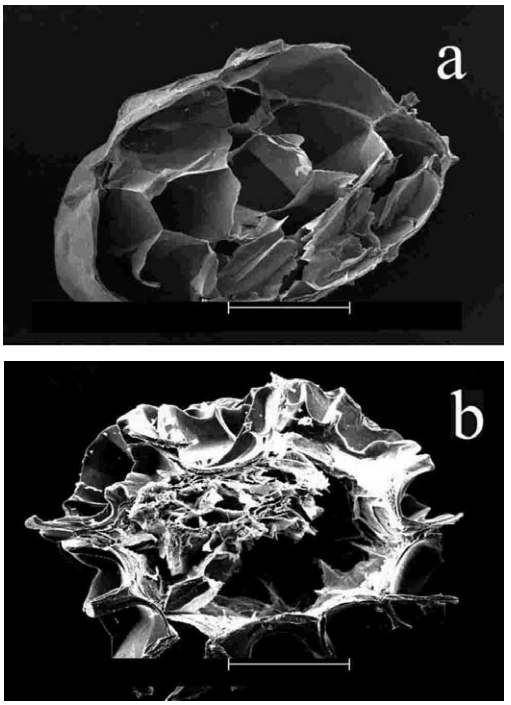

Fig. 2. Scanning electron micrographs of the cross-section of beads: (a) ALG beads; (b) ALG–KGM beads. Bar = 500 μm.

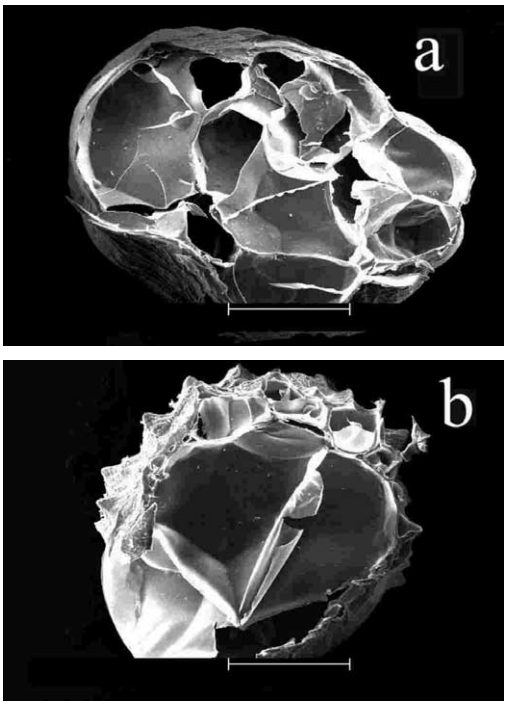

Fig. 3. Scanning electron micrographs of the cross-section of ALG–CHI beads: (a) gelling solution containing 0.2 M $CaCl_2$; (b) gelling solution containing 0.4 M $CaCl_2$. Bar = 500 μm.

compact core was found in ALG–KGM bead and separated partly from the surface due to the inner phase separation (Fig. 2b).

Fig. 3 gives the SEM micrograph of the beads of ALG coating CHI. The skin of beads obviously becomes thicker. With increased concentration of calcium ions, fine goffer (Fig. 3a) changed into ridgy (Fig. 3b) on the surface of beads. It seems that the calcium ions increase the binding of CHI by a more specific mechanism (Gaserod et al., 1998). Furthermore, inner pores become bigger within ALG–CHI beads using 0.4 M $CaCl_2$ due to entirely complexation reaction (Fig. 3b). It can be obviously seen from Fig. 4 that the outer layer of ALG–KGM–CHI beads also becomes thicker wall, compared with that of ALG–KGM. Moreover, since the mixture of ALG and KGM had high viscosity, the solidified beads exhibit tails (Fig. 4), indicative of high deformation upon landing in the bath and gelation prior to regaining spherical shape. The head and the tail of beads were found, which means that the first and the last part were touching the gelling solution, respectively. When 0.4 M $CaCl_2$ was used as gelling solution, the inner part was separated mostly from outer layer (Fig. 4b). While in lower concentration of $CaCl_2$, the relatively evident core was not found (Fig. 4a).

The effect of gelling processes on morphology of beads is summarized in Table 1. Size of beads without KGM was smaller when increasing the concentration of $CaCl_2$, but opposite result was obtained from beads containing KGM. It is indicated that the shrink of ALG–KGM–CHI is small due to the high viscosity of KGM contained within the bead. It was also observed that the range of diameter of exterior dents in ALG–KGM–CHI beads (0.4 M $CaCl_2$) was bigger than that of ALG–KGM–CHI beads (0.2 M $CaCl_2$). The reason was perhaps the faster gelling rate that led to the head of bead forming big dents and the tailing of bead forming small dents (Fig. 4b).

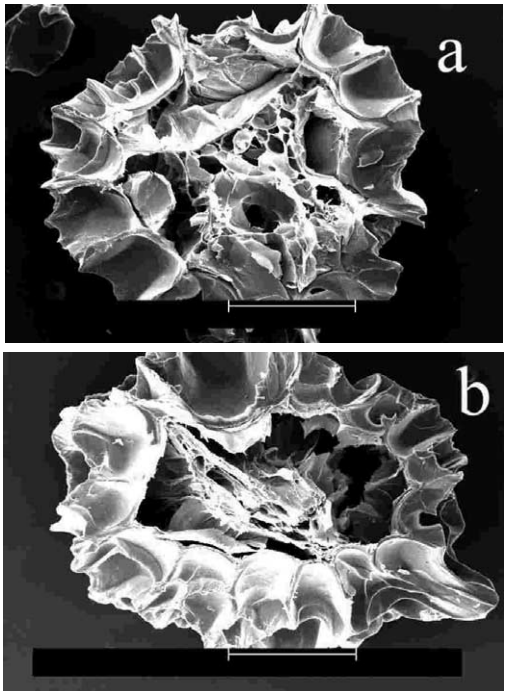

Fig. 4. Scanning electron micrographs of the cross-section of ALG–KGM–CHI beads: (a) gelling solution containing 0.2 M $CaCl_2$; (b) gelling solution containing 0.4 M $CaCl_2$. Bar = 500 μm.

In summary, the presence of KGM obviously affects the gel structure. The clear dents emerge on the surface of beads containing KGM, and this could increase in surface area after bead swelling in release solution. On the other hand, with increase in concentration of gelling ions, the more inhomogeneous structure was found both in inner and outer beads using KGM.

3.3. Effect of bead formation processes on protein load and release

The effect of bead formation processes on BSA-loading efficiency and -release efficiency in 0.1 N HCl is showed in Table 2. It was observed that the payload was higher by providing a skin coating of CHI in situ. This was in agreement with the results reported by Hari et al. (1996); CHI was bound instantaneously to ALG, preventing the protein from leaking out. The use of KGM system for BSA encapsulation appears to increase the protein loading within the matrix compared to the classical system of protein entrapment by calcium ALG gellation, and the highest loading efficiency was obtained from ALG–KGM–CHI system. It is also observed that the leaking of protein from ALG beads was more than ALG–KGM beads, and opposite results were got from beads coated with CHI.

From Fig. 5 it was found for equal concentration of polysaccharide that the use of KGM obviously delays the release of a protein drug in the alkaline pH after acid treatment. Time of 100% BSA released from ALG, ALG–KGM, ALG–CHI and ALG–KGM–CHI were 0.67, 1.0, 1.5 and 3.0 h, respectively. When the release time was 0.5 h, the cumulative release of ALG, ALG–KGM, ALG–CHI and ALG–KGM–CHI reached to 95, 60, 50 and 27%, respectively. When the release time was 1 h, the protein loss from ALG–KGM–CHI was just 62.5%, and the others released almost all. The burst effect at the beginning release disappeared when the beads use both KGM and CHI.

This could be attributed to the presence of KGM gel that was stronger and more stable

Table 1
Effect of gelling processes on morphology of beads

Bead	ALG (2%)	ALG (2%)–CHI		ALG (2%)–KGM	ALH (2%)–KGM (0.5%)–CHI	
Concentration of $CaCl_2$ (M)	0.2	0.2	0.4	0.2	0.2	0.4
Morphology of surface	Smooth	Goffer	Fine ridgy		Clear cylindroid dent	
Skin and inner		Linking		Separation partly	Separation partly	Separation mostly
Beads size (mm)	1.2±0.09	1.2±0.1	1.2±0.12	1.24±0.09[a]	1.26±0.1[a]a	1.4±0.1[a]
Diameter of exterior sink (μm)	None	None	None	210–330	210–320	70–370

[a] Outer diameter including sink.

Table 2
Effects of bead formation processes on loading efficiency and release of BSA

Sol solution (w/v)	2.5% ALG		2% ALG and 0.5% KGM	
Beads	ALG[a]	ALG–CHI[b]	ALG–KGM[a]	ALG–KGM–CHI[b]
Loading efficiency (%)	40	93	55.4	96
Release in 0.1 N HCl solution for 4 h[c] (%)	21.7	1.8	9.6	3.06

[a] Gelation solution containing 0.2 M $CaCl_2$ and 0.2 M pH 5 HAc–NaAc buffer.
[b] Gelation solution containing 0.2 M $CaCl_2$ and 0.5% (w/v) CHI (wt = 8.55×10^6).
[c] Generally, foods stay in stomach for about 4 h, then we also treat the beads by 0.1 N HCl for 4 h.

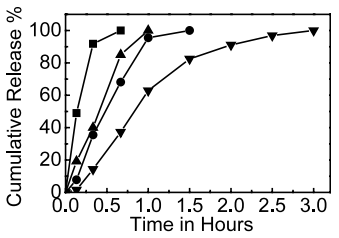

Fig. 5. Effect of bead formation processes on cumulative release of BSA in Tris–HCl, pH 7.4, after acid treatment (0.1 NHCl) for 4 h. (■) ALG (2.5%), (●) ALG (2.5%)–CHI, (▲) ALG (2.0%)–KGM (0.5%), (▼) ALG (2.0%)–KGM (0.5%)–CHI.

than ALG gel in gastrointestinal solution. It is worth noting that ALG–KGM–CHI beads kept sphere and integrity at the end of release, while others were hydrolyzed and broken. An ion cross-linked ALG delivery system when exposed to low pH can undergo a reduction in ALG molecular weight, which results in faster degrading and release of a molecule when the gel is reequilibrated in a neutral pH solution (Mumper et al., 1994). KGM was not hydrolyzed in acid solution and it stabilizes the gel.

3.4. Effect of concentration of KGM on protein load and release

Table 3 gives the effect of KGM concentration on insulin-loading efficiency and release efficiency in 0.1 N HCl. The payload is enhanced when increasing the concentration of KGM within gel. It is also indicated that the losing of insulin is higher than that of BSA in 0.1 N HCl solution (Tables 2 and 3). Insulin and BSA had isoelectric point (PI) of 5.3 and 4.9, respectively, and hence, at pH 7.4, they were all negatively charged molecules. The molecular weight of BSA (67 000) was about tenfold larger than that of insulin (6000), which led to the lower release of BSA in 0.1 N HCl.

From Table 3, one knows that more KGM within ALG–KGM–CHI beads result in faster release of protein. This phenomenon is similar to results reported by Coa and colleagues (Cao et al., 1999). They used gelatin and BSA as additives, and the release time increased to 140 and 80 min, respectively, in mimic intestinal juice, while the leaking of protein also got to 27.8 and 37.49%, respectively, in mimic gastric solution. Protein drugs that come into stomach will lose activity via the hydrolysis by the enzyme. The leaking of insulin from ALG (2%)–KGM (0.5%)–CHI was 14.6% in 0.1 N HCl and release time reached 3.0 h in mimic intestinal solution, which is a better result.

The effect of KGM concentration on release of insulin in Tris–HCl, pH 7.4, after acid treatment is depicted in Fig. 6. Time of 100% protein release from beads containing 0, 0.25, 0.375, and 0.5% (w/v) KGM were 1.0, 2.0, 2.5 and 3.0 h, respectively, which showed that a high-KGM gel had low diffusion coefficients of protein. One reason could be that high viscosity of KGM solution leads to a reduction in insulin diffusion coefficient within the complex gel of ALG and KGM. Viscosity of 2.5% ALG was 122 cps, while that of the mixture of 2.0% ALG and 0.5% KGM reached 550 cps. Other one may be, as shown in Section 3.1, the multi-interaction among ALG, KGM and CHI via supermolecular interaction, hydrogen binding and electrostatic interaction.

Liu and Krishnan (1999) had reported that release rates of drug from ALG–pectin–PLL

Table 3
Effect of KGM concentration on loading efficiency and release of insulin

Concentration of ALG (w/v)	2%			
Concentration of KGM (%w/v)	0	0.25	0.375	0.5
Loading efficiency (%)	92.8	92.1	94.8	98
Release efficiency in 0.1 NHCl for 4 h (%)	6.2	7.06	13.1	14.6

Gelation solution containing 0.2 M $CaCl_2$ and 0.5% CHI (wt = 8.55×10^6) solution.

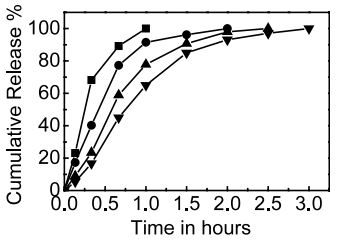

Fig. 6. Effect of KGM concentration on release of insulin in Tris–HCl, pH 7.4, after acid treatment (0.1 NHCl) for 4 h. (■) ALG–CHI, (●) ALG–KGM (0.25%)–CHI, (▲) ALG–KGM (0.375%)–CHI, (▼) ALG–KGM (0.5%)–CHI.

particulate were slower than capsule without pectin, both in acid and in alkaline solutions (Liu and Krishnan, 1999). This could be attributed to the same and the different characteristic between KGM and pectin system. KGM and pectin were more resistant to breakage in the gut than ALG, just breaking down in the presence of micro-flora in the colon, which stabilizes the complex gel of ALG with them and prolong the release of drug. On the other hand, pectin was similar to ALG, which is a polyanion polysaccharide, and can cross-link with calcium ions, but KGM was non-ionic glucomannan and impossible to form gel in the presence of multivalent ions. However, more detailed studies are needed to confirm these observations.

3.5. Effect of concentration of gelling ion on protein load and release

The effect of concentration of calcium ions on insulin-loading efficiency and release efficiency in 0.1 N HCl is showed in Table 4. It was observed that the loading efficiency of beads without KGM was not obviously varying, while that of beads using KGM was decreased from 98 to 90.5% with increase in concentration of calcium. Furthermore, using higher concentration of gelling ion leads to more losing insulin in 0.1 N HCl.

Fig. 7 gives the influence of concentration on release of insulin in Tris–HCl, pH 7.4, after acid treatment. May be because of faster release rate, no evident difference was found between the diffusion of protein from beads without KGM. On the contrary, it was for beads containing KGM that the obvious varying release rate was obtained when the concentration of gelling ion was changed. When the release time was 0.5 h, the cumulative release of ALG–KGM–CHI gelling in 0.2 M, 0.3 M and 0.4 M calcium chloride solutions reached about 60, 80 and 95%, respectively. The release was almost identical between ALG–CHI and ALG–KGM–CHI using 0.4 M gelling ion. Above phenomenon indicated that the concentration of gelling ion was a very important parameter that had distinct effect on the gel structure of beads with KGM. This result was similar to the SEM observation that comes from the result of Section 3.2. The beads containing KGM would become unstable due to the more inhomogeneous structure formed in the case of higher concentration of gelling ions.

3.6. Effect of KGM concentration on water of hydration of beads

In pH 1 and 7.4 solutions, the influence of concentration KGM on water of hydration of beads was studied. Percentage water of hydration for 4 h exposed beads in buffer as a function of concentration of KGM and pH is presented in Fig. 8. Significant pH effect was noted for the water

Table 4
Effect of calcium ion concentration on loading efficiency and release of insulin

Sol solution (w/v)	2.0% ALG			2% ALG and 0.5% KGM		
Calcium ion concentration (M)	0.2	0.3	0.4	0.2	0.3	0.4
Loading efficiency (%)	92.6	93	91	98	93.4	90.5
Release efficiency in 0.1 NHCl for 4 h (%)	7.4	10.3	12.4	12.7	15.4	16.9

Gelation solution containing 0.5% CHI (wt = 8.55×10^6).

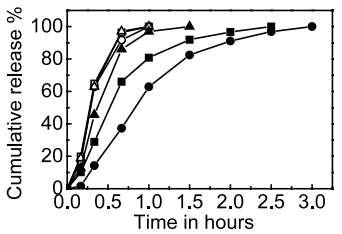

Fig. 7. Effect of concentration of gelling ion on release of insulin in Tris–HCl, pH 7.4, after acid treatment (0.1 NHCl) for 4 h. ALG–CHI: (○) 0.2 M $CaCl_2$, (□) 0.3 M $CaCl_2$, (△) 0.4 M $CaCl_2$; ALG–KGM–CHI: (●) 0.2 M $CaCl_2$, (■) 0.3 M $CaCl_2$, (▲) 0.4 M $CaCl_2$.

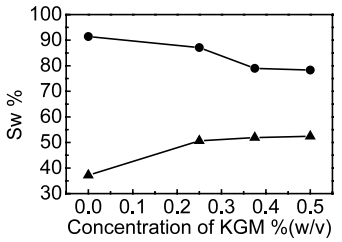

Fig. 8. Effect of KGM concentration on water of hydration of beads (2% ALG). (▲) 0.1 N HCl, (●) Tris–HCl, pH 7.4.

content within the beads. Water of hydration is lower in acidic pH than that in pH 7.4.

In a 0.1 N HCl solution ALG beads undergo a decrease in diameter (Mumper et al., 1994). Release of macromolecules from ALG beads in low pH solution is also significantly reduced, which could be advantageous in the development of oral delivery system. Fig. 8 reveals that the water of hydration of ALG bead (37.2%) is the lowest in 0.1 N HCl solution. However, it is obvious that the water of hydration increase to 50.6% within the beads containing KGM (0.25%), and 52.4% with higher concentration of KGM (0.05%). When gel with higher concentration of KGM (0.5%), the water of hydration rises slightly to 52.4%. This is the reason why beads with KGM lost more insulin in 0.1 N HCl solution (Table 3).

In Tris–HCl pH 7.4, the water of hydration of ALG–CHI beads was 91.4%, while that of beads with KGM (0.25%) reduced to 87.1% (Fig. 8). With an increase in the concentration of KGM, water of hydration declined. There is no evident difference between beads containing KGM of 0.375 and 0.5%. The above phenomenon indicated that greater the content of KGM in the complex gel, the stronger and more stable is the gel, which makes the gel less swelled in pH 7.4 solution.

As a result, beads with KGM could reduce the swelling differences of gel between acidic pH and alkaline pH, and cause the gel to swell less and shrink in different conditions. This could be attributed to the stability and high viscosity of KGM in different pH solutions. Furthermore, enhancement or drop in water of hydration was limited by the content of KGM.

Gursoy and Cevik had reported tabletting of ALG microspheres using carrageenan (carr), ALG, pectin, NaCMC, tragacanth (trgh) and HPMC as additives (Gursoy and Cevik, 2000). This process produced tablets with good physical properties and also better controlled release of diclofenac sodium (Dna). The rank order of Dna release from tablets was carr < ALG < pectin < NaCMC < trgh < HPMC which relates to the viscosity and swelling properties of polymers. Thus, the use of KGM and other polysaccharides within ALG–CHI capsules as oral drug delivery should be further studied. It is necessary to

compare their properties which influence the release of drug in acidic or alkaline solution.

In addition, more in vivo studies were needed to confirm these observations and the bioactivity of the encapsulated insulin.

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