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Preparation and Properties of Alginate/Polyaspartate Composite Hydrogels

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Abstract: This study examined the swelling behavior and *in vitro* release of a model drug, tetracycline-HCl, from alginate and alginate-polyaspartate (Alg-PASP) composite gel beads. The alginate and Alg-PASP composite beads were prepared using an ionic crosslinking method with aqueous Ca^{2+}. Their microporous morphology was observed by scanning electron microscopy. The swelling ratio of the beads in different media varied according to their composition, cross-linking density (Ca^{2+} concentration), and pH of the aqueous medium. The *in vitro* release experiment of the tetracycline-HCl encapsulated beads in different media suggests that the release of the drug is governed mainly by the swelling properties of the polymer network. The presence of PASP was found to significantly influence the swelling properties and drug release profile.

Keywords: alginate, polyaspartate, ionic crosslinking, swelling behavior, drug release.

Introduction

Hydrogels based on both natural and synthetic polymers are of considerable interest for the encapsulation of drugs and cells. Most recently, such hydrogels have become particularly attractive to the new field of "tissue engineering" as matrices for repairing and regenerating a wide variety of tissues and organs.[1-4]

Poly(aspartic acid), PASP, is a synthetic water-soluble and biodegradable polymer that can be produced from the hydrolysis of polysuccinimide (PSI), which is the thermal condensation polymer of an aspartic acid monomer.[5-7] PASP has been commercialized as a biodegradable polymeric dispersant, and has also been widely investigated as a biodegradable polymeric drug carrier.[8] In previous studies, a PASP-based hydrogel was prepared by chemical crosslinking, and the swelling property was investigated.[9,10] Alginate (Alg) hydrogels are proving to have a wide range of uses as biomaterials, such as a scaffold for tissue engineering, delivery vehicles for drugs, and model extracellular matrices for basic biological studies.[11-13] Alginate, an anionic polysaccharide composed of (1-4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G), has found a wide range of applications in the bioencapsulation of drugs, proteins and cells. The gelling properties of its guluronic residues with divalent ions, such as calcium, permit the formation of alginate matrices for gels, films, beads, and pellets.[14,15]

In this study, composite hydrogels from a natural alginate and a synthetic PASP were used to modify the gel properties of the component polymers. The homo and composite hydrogels based on PASP and alginate were prepared by ionic crosslinking in an aqueous medium. The characterization of these composite gels and their swelling properties are discussed. The incorporation and release behavior of a model drug, tetracycline hydrochloride, were also examined using different aqueous media.

Experimental

Materials and Measurements. Sodium alginate (20-40 cP, 1% in H_2O (lit.)) L-aspartic acid (98+%), *o*-phosphoric acid (98%), and tetracycline-HCl were used as purchased from the Aldrich Chemical Co. Phosphate buffered saline (pH=7.4) powder was purchased from the Sigma-Aldrich Co. The buffer pH solutions for swelling measurement were obtained from the Samchun Chemical Co. (Korea). The water was purified using a reverse osmosis system (Sartorius, arium 61315).

The IR spectra were obtained on a FT-IR spectrometer (JASCO 660 plus, Japan). The morphology of the gel beads was observed by scanning electron microscopy (FESEM

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Table I. The Composition of Hydrogel Preparation

Hydrogel	wt. ratio	PAA-Na (g)	Alginate (g)
a	1:0	0.40	-
b	7:3	0.56	0.24
c	5:5	0.40	0.40
d	3:7	0.24	0.56
e	0:1	-	0.40

Model JSM6700F, JEOL Inc. Japan).

Synthesis of Polysuccinimide (PSI) and Sodium Polyaspartate (PAA). L-Aspartic acid (20 g) and *o*-phosphoric acid (20 g) were charged into a round-bottom flask, and the mixture were stirred at 200 °C under reduced pressure for 5 h. The reaction mixture was then cooled and *N,N*-dimethylformamide (DMF) was added to dissolve the product. The resulting solution was precipitated into an excess of water, and the precipitate was washed several times with fresh water to remove the residual phosphoric acid. The filtered polymer, PSI, was then dried at 80 °C under vacuum. The molecular weight of PSI was estimated to be approximately 120,000 Da, as calculated from an empirical equation that relates the solution viscosity to the molecular weight.[7] The corresponding sodium polyaspartate (PAA) was obtained by the hydrolysis of PSI using NaOH as follows: 1 M NaOH solution was added dropwise into a fine dispersion of 6.7%(w/v) PSI in water, and the reaction mixture was stirred for 7 h in an ice bath at a pH < 10.8.

Preparation of Alg-PAA Composite Gel Beads. Mixed PAA and 4%(w/v) alginate solutions with different compositions were prepared under magnetic stirring for 2 h to use in the next steps. Table I gives a summary of the gel composition of the samples. The Alg-PAA composite gel beads were prepared by the dropwise addition of 20 mL of an Alg-PAA mixed solution into 20 mL of a calcium chloride solution (2%, 4%, 6%, and 10%) through a stainless steel needle (gauge #22). The in-situ formed gel beads were left to stand in the gelling medium for 15 min, and then separated from the solution using a stainless steel grid. The beads were washed repeatedly to remove the excess $CaCl_2$ solution, and then freeze-dried to obtain the final dry beads. An appropriate amount of tetracycline-HCl was added to the calcium chloride solution to prepare the drug loaded beads. The mixture was dissolved with magnetic stirring and the beads were formed by ionic gelation as previously described.

Measurement of Swelling. The swelling behavior of the composite gel beads were examined in three different aqueous media, i.e. deionized water, phosphate buffer saline (PBS, pH=7.4), and buffer pH solution (pH=3). An accurately weighed amount of beads (ranging from 45 to 50 mg) was immersed in aqueous media and removed from the medium in a predetermined time interval. Immediately, the beads were wiped gently with paper and weighed. The degree of swelling (swelling ratio) was calculated using the formula:

$$\text{Swelling ratio} = W_s / W_d$$

Where, W_s is the weight of the swollen beads and W_d is the weight of dried beads.

Drug Release Behavior. The *in vitro* release studies were performed in three different aqueous media, i.e. deionized water, PBS (pH=7.4), and buffer pH solution (pH=3), respectively. Tetracycline hydrochloride, which is a non-steroidal anti-inflammatory drug, was used as a model drug. An accurately weighed amount of the beads (wet or dry) was placed in a beaker containing 200 mL of the release medium. The samples were then incubated at 37 ± 0.1 °C with magnetic stirring. At predetermined time, a 3 mL aliquot was sampled from the release medium and replaced with the same volume of fresh medium. The concentration of tetracycline-HCl in solution was assayed by UV spectroscopy (Biochrom, Biowave II, Germany) at 359 nm using a standard curve of known concentrations ranging from 10 to 50 μg/mL with a correlation coefficient of $R = 0.9998$.

The drug encapsulation efficiency (EE) was estimated using the formula:

$$EE(\%) = (M_i - M_d) / M_i \times 100$$

Where, M_i is the initial weight of the drug dissolved in the $CaCl_2$ solution and M_d is the weight of the drug in the gelling media measured immediately after preparing the drug-loaded beads.

Results and Discussion

Preparation and Morphology of the Hydrogel Beads. The alginate and IPN type composite hydrogels with PAA were prepared by coagulating the mixed polymer solution in a Ca^{+2} aqueous medium. The gel product was obtained in

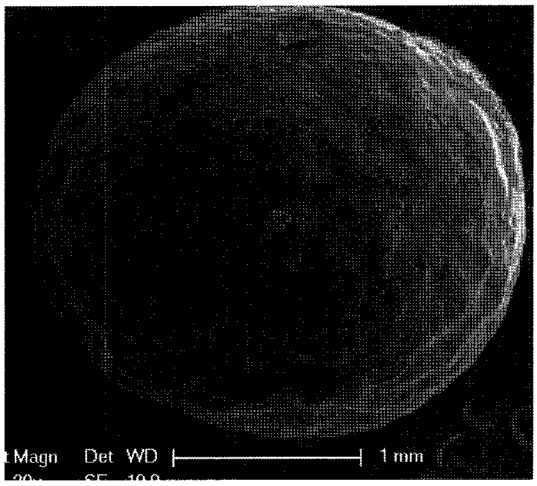


Figure 1. SEM image of the surface of the calcium alginate bead.

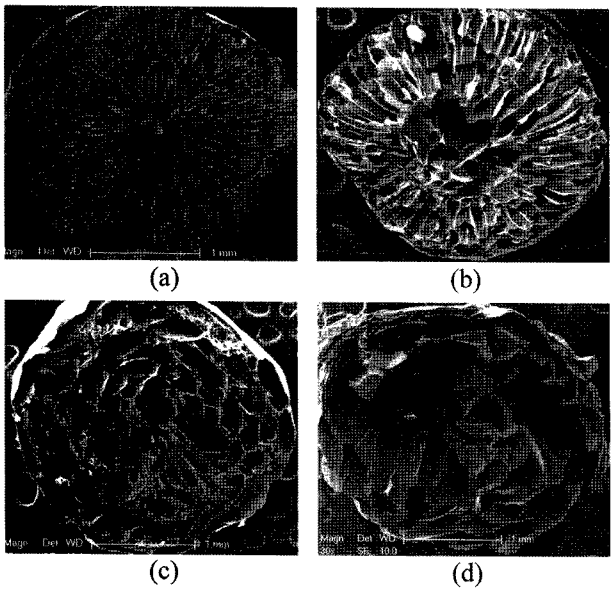


Figure 2. SEM images of the composite hydrogels made with (a) PAA:Alg=5:5 in 10% $CaCl_2$, (b) PAA:Alg=5:5 in 6% $CaCl_2$, (c) PAA:Alg=3:7 in 10% $CaCl_2$, and (d) homo Alg in 10% $CaCl_2$.

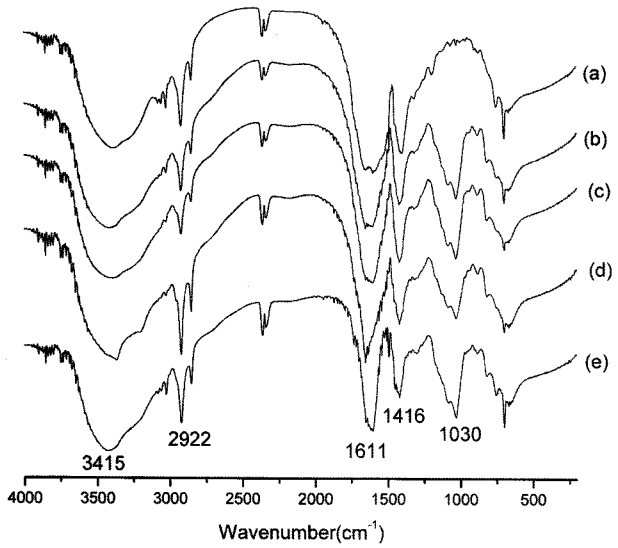


Figure 3. FT-IR spectra of (a) homo PAA, (b) PAA:Alg=7:3, (c) PAA:Alg=5:5, (d) PAA:Alg=3:7, and (e) homo Alg.

bead form. Figure 1 shows a typical scanning electron micrograph of the dry bead. The gel beads had a smooth surface with a spherical shape. The freeze-drying method could reduce the roughness and eliminate cracks on the surface. The mean diameter of the dried beads was measured to be approximately 2.0 mm with a relatively uniform size distribution. Figure 2 shows a series of SEM images of the cross-section of composite hydrogels indicating that the prepared composite gels are highly porous. Moreover, their porosity was changed according to the hydrogel composition. The higher Ca^{2+} ion content in the gelling medium results in

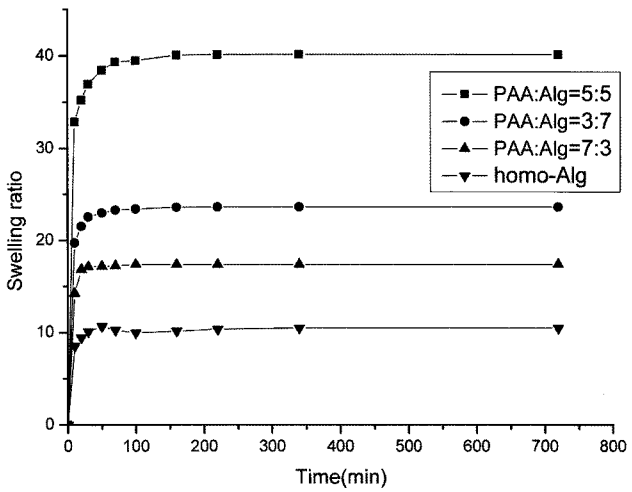


Figure 4. Typical swelling curves of the gels in water at RT.

a more densely cross-linked hydrogel network (a *vs.* b). Also the pore size of hydrogel tended to increase with increasing alginate content (a→c→d). Figure 3 shows the FT-IR spectra of alginate, PAA, and a series of composite gels. The gel products show the characteristic absorption bands corresponding to carboxylate groups near 1611 cm^{-1} (symmetric COO^- stretching vibration) and 1416 cm^{-1} (asymmetric COO^- stretching vibration). In addition, the alginate containing gels showed an absorption band at 1033 cm^{-1} corresponding to the C-O-C vibration of the carbohydrate ring with a broad and strong band at approximately 3450 cm^{-1} due to the hydroxyl moiety.

Swelling Properties of Composite Gels. The swelling of the dry gel beads can be attributed mainly to the hydration of the hydrophilic groups of PAA and alginate. In this case, free water penetrates the beads in order to fill the inert pores that developed within the gel matrices, contributing to a greater degree of swelling. The swelling properties of the prepared gels were tested in aqueous solutions. Figure 4 shows the typical water swelling curves of the different gel samples (prepared using 2% $CaCl_2$ solution) in distilled water. The initial fast swelling appeared to reach equilibrium within 1-2 h, and the swelling degree remained almost constant thereafter. The degree of swelling was much higher at the intermediate composition compared with the homo alginate gel. A lower effective crosslinking density obtainable from composite gels along with more hydrophilic nature of PAA might be responsible for this result. On the other hand, the neat sodium polyaspartate solution did not form a bead at the given condition. Instead, a gummy precipitate was formed on the bottom of the reaction flask. This will probably due to a less efficient or weak binding by Ca^{+2} in case of PAA in the given reaction condition. Figure 5 shows the degree of swelling in deionized water and the PBS (pH 7.4) solution using the gel samples prepared at different calcium chloride concentrations. As the Ca^{+2} concentration in gelling medium increased, the swelling degree decreased. The

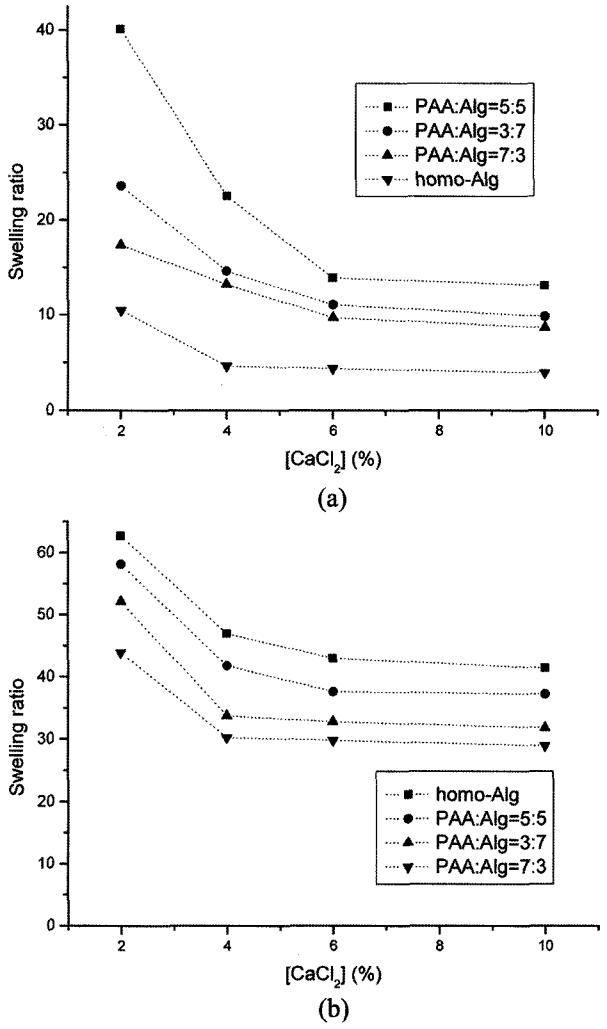


Figure 5. Swelling ratio of the hydrogel in different $CaCl_2$ concentration in (a) water and (b) PBS (pH 7.4).

crosslinking density is expected to increase linearly with increasing $CaCl_2$ concentration in the medium. Hence, the resulting hydrogels should have a denser network structure. Therefore, the degree of swelling of the hydrogels decreases according to the trends shown in Figures 5(a) and (b). The overall degree of swelling in the PBS solutions was high compared with those in water, probably due to the ionic nature of the hydrogel materials. When gel beads are placed in the PBS solution, the Na^{+1} ions present in the external solution undergo ion-exchange process with Ca^{+2} ions which are binding with COO^- groups mainly in the polymannuronate sequences. As a result the electrostatic repulsion among negatively charged COO^- groups increases which ultimately causes the chain relaxation and enhances the gel swelling.[15] However, the degree of swelling in the PBS solution tended to decrease with extended time, indicating disintegration and final dissolution of the network structure caused by the continued ion-exchange between Ca^{+2} and Na^+ to break the ionic crosslinking between polymer chains. A similar result

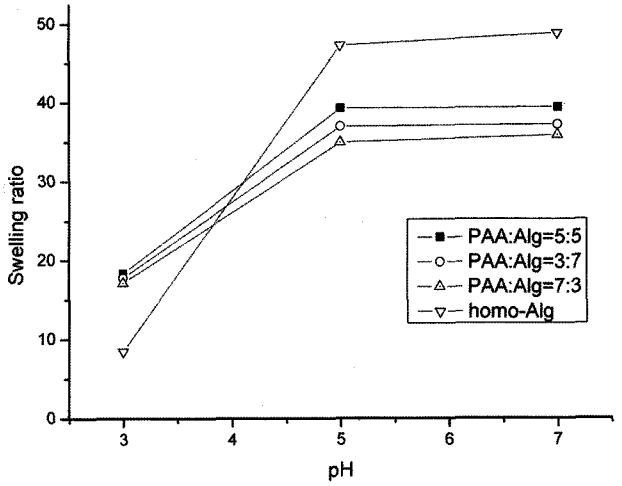


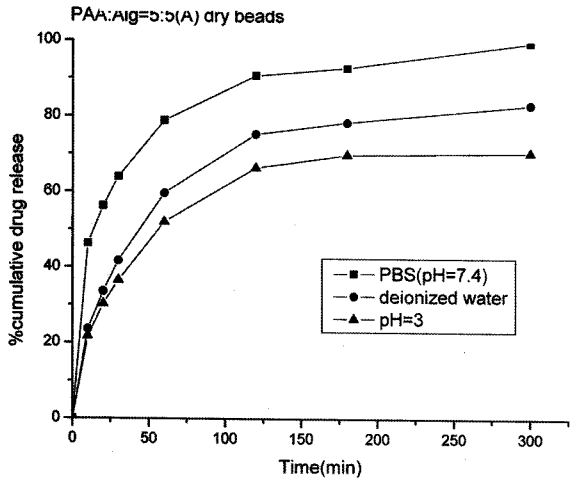
Figure 6. Swelling dependence on the pH of buffer solutions at RT.

Table II. Drug Loading Efficiency

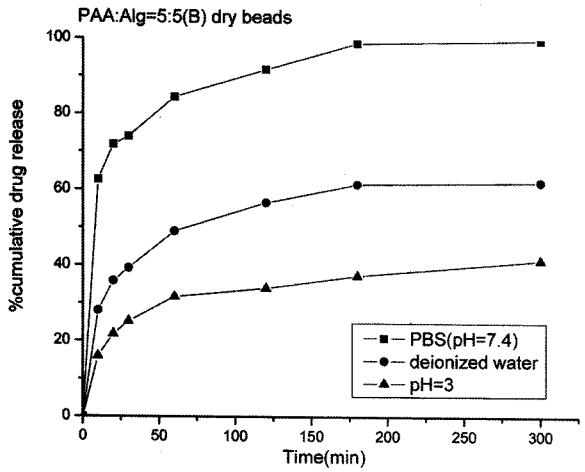
Sample	PAA: Alg (wt. ratio)	[tetracycline-HCl]	EE(%)	Amount of drug (mg/g gel)
A	5:5	0.250%	73.3	28.4
B	5:5	0.125%	45.4	17.6
C	0:1	0.125%	35.6	14.8

and discussion has been reported earlier by Bajpai and Sharma.[15] In PBS solution, the alginate bead showed the highest swelling degree among the others. The reason might be resulted from a more pronounced effect of ion-exchange as discussed above in case of homo alginate gel. Figure 6 shows the pH-sensitive swelling characteristics of the hydrogels, which were investigated using a swelling test at pH 3, 5, and 7. As shown in the figure, the degree of swelling was much lower at pH 3 compared to those at pH 5 or 7. The same beads tended to shrink when exposed to an acidic environment. For example, at low pH (< 4), the carboxylate groups on the gel polymer will be protonized and the electrostatic repulsion between these groups will be reduced to favor shrinkage of gel. On the other hand, at a pH above the pKa value of the carboxylic acid group, the groups will mostly remain ionized to absorb more water.

***In-vitro* Drug Release Behavior.** Table II shows the encapsulation efficiency of tetracycline-HCl for the composite (5:5) and neat Alg hydrogels. Tetracycline hydrochloride is a non-steroidal anti-inflammatory drug used as a model compound for this releasing test. The encapsulation efficiency increased with increasing drug concentration (sample A *vs* B). This result might be due to the additional adsorption of drug onto the hydrogel bead when higher drug concentration was used for the gelation. On the other hand, the increased encapsulation efficiency was obtained from the composite gel containing PAA (sample B *vs* C). This can be ascribable to the higher swelling of composite gel compared to neat



(a)



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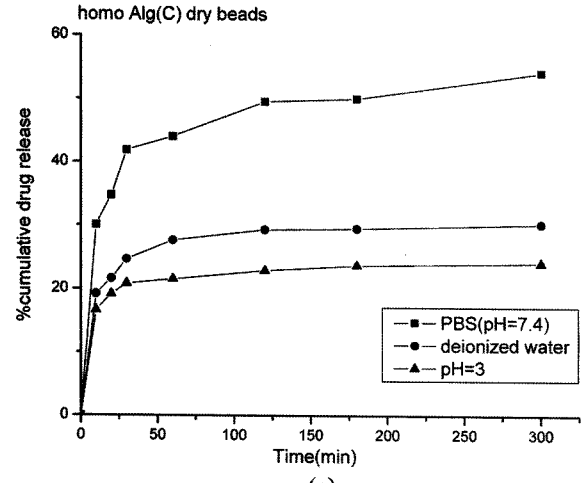
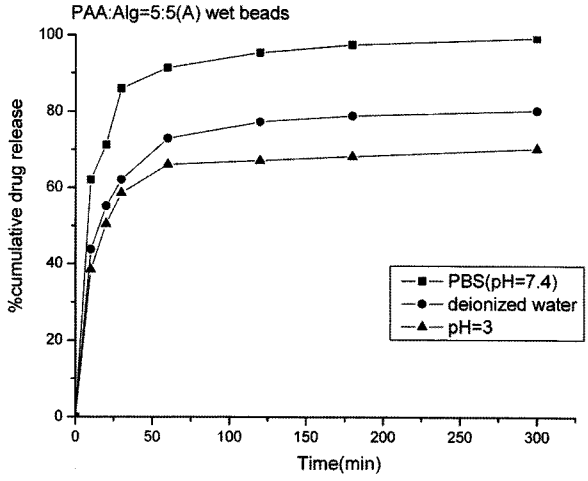
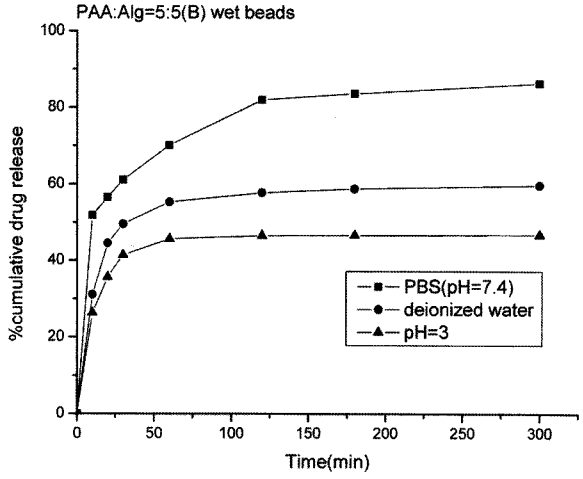


(c)

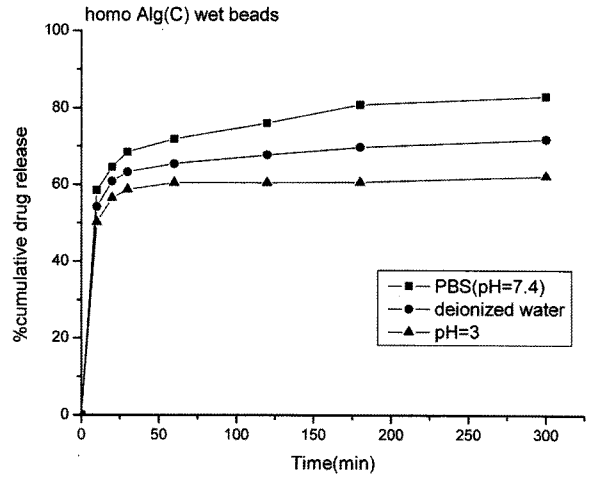
Figure 7. Cumulative release of tetracycline-HCl from the dry beads; (a) PAA:Alg=5:5(A), (b) PAA:Alg=5:5(B), and (c) homo Alg(C).



(a)



(b)



(c)

Figure 8. Cumulative release of tetracycline-HCl from the wet beads. (a) PAA:Alg=5:5(A), (b) PAA:Alg=5:5(B), and (c) homo Alg(C).

Alg gel. Figures 7 and 8 show the cumulative drug release profiles of the dry and wet gel beads, respectively. Overall, the wet and dry beads had similar release profiles. However, the initial release rate was much slower in the case of the dry gel (Figure 7) compared with the wet gel (Figure 8). In the case of dry gel beads, the release profiles might be char-

acterized by a biphasic behavior. For example, in the first phase, from zero to about 60 min, two apparent mechanisms, swelling and diffusion, appear to govern the overall drug release from the beads. During the second phase, from 60 min, the swelling of the beads is constant. Therefore, only diffusion will affect the release of drug molecules. In both systems, the initial release rate and the total amount of drugs at a given time were in the following order: PBS > deionized water > pH 3 buffer solution. This suggests that the drug release of the beads is governed mainly by the swelling of the polymer network. In the same release medium, the amount of drug released from the dry alginate beads was much small compared to that in composite gel, probably due to the more condensed network structure with lower swelling. In addition, there is a possibility that the drug molecules are more tightly bound within the gel matrix during the freeze-drying process. A high drug loading (A) showed a higher level of cumulative drug release in a given time than a low loading (B) in both composite gel systems.

Conclusions

A series of IPN gel beads composed of alginate and PAA with various compositions were prepared by ionic crosslinking in aqueous Ca^{+2} solutions. The freeze-dried gel beads showed a microporous structure, as evidenced by SEM. The swelling of the composite gel beads varied according to composition, pH of the aqueous media, and the $CaCl_2$ concentration. Tetracycline-HCl, as a model drug, was encapsulated in the alginate/PAA composite gels. The *in vitro* release of the drug showed a modified release profile depending on the gel composition and related swelling behavior.

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