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Preparation and release behavior of pH and ionic sensitive chitosan/poly(vinylpyrrolidone) semi-IPN beads for coenzyme A

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Abstract: A series of pH- and ionic sensitive semi-interpenetrating polymer network (semi-IPN) beads of chitosan (CS) and poly(vinyl pyrrolidone) (PVP) were prepared using glutaraldehyde as crosslinking agent and characterized for the release of coenzyme A (Co A). The swelling behavior of the semi-IPN beads and the influential factors, such as the component ratio of CS and PVP, the loaded amount of Co A, the pH and ionic strength of the release medium on Co A release were studied. The results showed that within 48 h the cumulative release rate of Co A decreased with Co A loading content, ionic strength increase or crosslinking time increase; when the weight ratios of CS to PVP in the drug-loaded beads were 100:0, 80:20, 60:40, 50:50, 40:60 and 20:80, the cumulative release rates of Co A at pH 2.1 solution were 95.2, 72.1, 73.2, 74.2, 86.5 and 80.1%, respectively, and they were 22.7, 30.1, 28.0, 33.1, 38.4 and 35.2% at pH 7.4 solution, respectively. All the results indicated that the CS/PVP semi-IPN beads were suitable for drug delivery systems.

Key words: pH and ionic sensitivity, biomaterials, semi-IPN beads, drug delivery systems

Introduction

New controlled drug delivery systems response to changes in environmental conditions, e.g. temperature [1-2], pH [3-4], exposure to ultraviolet [5] and visible radiation [6], electric field [7], and in the presence of certain chemicals [8] are being explored. Biodegradable polymers have been used extensively in biomedical areas in the form of sutures, wound covering materials and artificial skin, and for the controlled release of drugs [9-10]. Natural polymers have become more and more important for their rich resources and low costs, especially for their unique properties, such as nontoxicity, degradability, and good biological compatibility [11]. Chitosan is one of the most important natural polymers. It contains a large number of hydroxyl and amino groups, and it can thus be modified by various chemical reactions to prepare series of chitosan derivative as non-toxic biocompatible biomaterials [12-15], such as wound healing, anti microbial agent, drug delivery carrier, blood vessel, metal chelating agent, immobilization of enzymes and food processing technology, etc.

Poly (vinyl pyrrolidone) (PVP) is a typical neutral water-soluble polymer. It has good biocompatibility and has been applied as a biomaterial or additive to drug compositions, e.g. as a blood plasma expander [16] and vitreous humor substitute [17]. Several reports have discussed the interaction of PVP with other polymers, surfactants, and adsorbents [18-19].

A large number of pH-sensitive polymer network were made of homopolymers or copolymers containing acrylic acid, methacrylic acid, and *N*-isopropyl acrylamide groups [20-22]. In recent years, it was found that some of the polymer complexes could also show pH sensitivity and ion sensitivity due to the dissociation of two components in the complexes, which was different from the mechanism of those materials containing acrylic acid, methacrylic acid, and *N*-isopropyl acryl amide groups [23-24]. In this article, a series of CS/PVP semi-IPN beads was synthesized. Their swelling behaviors under different pH and ionic strength and controlled Co A release were investigated.

Results and discussion

FTIR spectra of semi-IPN beads

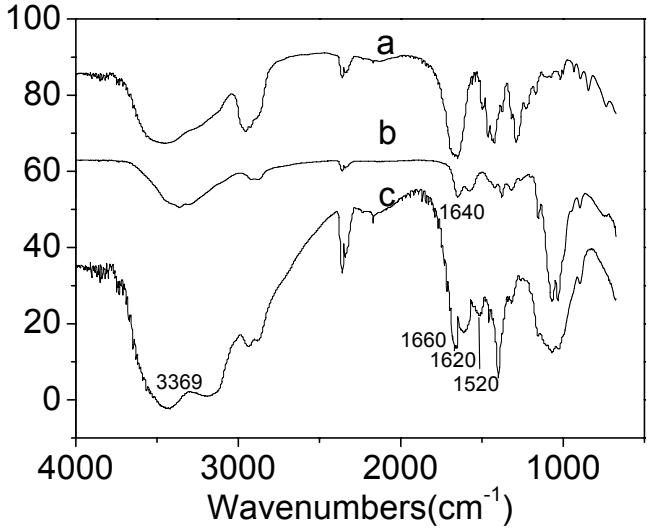

Fig. 1. FTIR spectra of PVP (a), CS (b) and semi-IPN beads B_4 at pH 2.1(c).

Fig. 1 shows the IR spectra of PVP (a), CS (b) and sample B_4 at pH 2.1. The non-modified chitosan (b) showed signals at 1647 and 1590 cm^{-1} for the C-O stretching (amide) and N-H bending (amine), respectively. In contrast with the spectra PVP (a) and CS (b), a significant new peak at 1621 cm^{-1} in the spectra of B_4 is attributed to the formation of the C=N groups by imine reaction between amino groups of CS with aldehydic group of glutaraldehyde, which demonstrated that the crosslinking reaction occurred in the semi-IPN beads. Sample B_4 (c) has a new peak appearing around 1520 cm^{-1} corresponding to the protated NH_3^+ in the acid condition (pH 2.1). In addition curve c having all the characteristic peaks of curves a and b, the peak corresponding to –OH and $–NH_2$ groups at around 3419 cm^{-1} become broader, indicating strong hydrogen bond formation in this polymer system.

Scanning electron microscope (SEM) of the beads

SEM micrograph of dried semi-IPN beads B_4 is shown in Fig. 2. Their shape is like round ball. The size of the beads are about 300-500μm with a narrow size distribution.

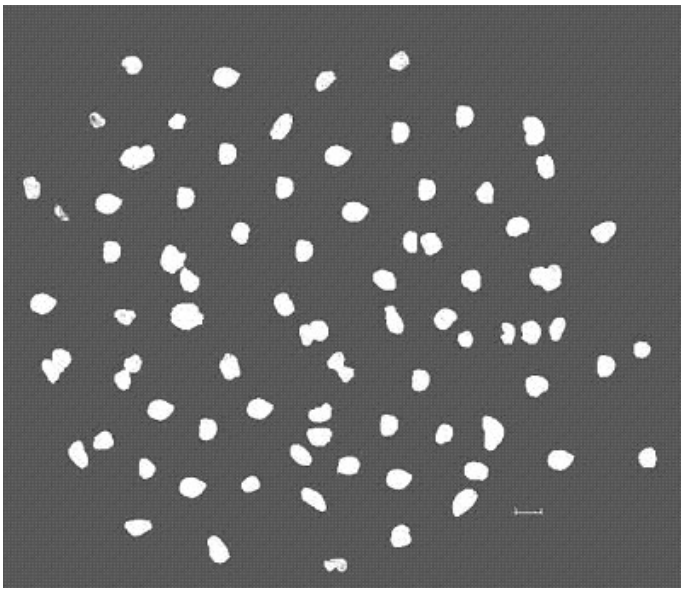

Fig. 2. SEM micrograph of the semi-IPN beads B_4 (bar indicates 500μm).

Effect of crosslinking time on the swelling ratio

It is known that crosslinking agent has great effect on the swelling ratio of the beads. We use crosslinking time in 1 wt% glutaraldehyde solution to control the crosslinking density. SR values for sample A_1, A_2, A_3, A_4 and A_5 prepared at different crosslinking time are shown in Tab.1. With crosslinking time in 1 wt% glutaraldehyde solution increasing, SR decreased accordingly. This can be explained by the fact that the crosslinking density increased with the crosslinking time increase. The hydrophilicity of the polymer network decreases, and it is difficult for water to invade into the polymer matrix. For 5 min crosslinking time, the crosslinking density is too low, and for 20 min or longer time, the SR was small. So in the subsequent experiment, crosslinking time is 10 min in 1 wt% glutaraldehyde solution for all the samples.

Tab. 1. Influence of crosslinking time on swelling ratio and percent weight loss of the beads at pH 2.1 and 7.4 values solution.

pH	Swelling Ratio					Weight loss (%)[a]				
	Crosslinking time in 1wt% Glutaraldehyde (min)					Crosslinking time in 1wt% Glutaraldehyde (min)				
	5	10	20	40	80	5	10	20	40	80
2.1		7.8	5.4	3.2	2.9	2.8	45.7	30.4	22.4	18.6	16.9
7.4		3.4	2.3	1.4	1.3	1.2	27.6	21.4	17.2	14.8	12.4

a) The average value from three experiments.

Effect of pH on the swelling ratio of different blend composition samples

Generally, the swelling process of the beads at pH<6 involves protonation of amino/imine groups in the beads, and mechanical relaxation of coiled polymeric chains. Although protonation takes place in a very short time, the overall process is much more complex [25] and mainly completed in two stages. In the first stage, amino/imine groups protonizes at the sample surface, which lead to breaking of the

hydrogen bonding between amino/imine and other groups, which results in penetrants invading the polymer from the surface and forming a sharp boundary or moving front separating the unsolvated polymer region ahead of the front from the swollen bead phase behind it. In the second stage, proton and counterions diffuse into the bead, inward amino/imine groups are protonated, and the hydrogen bonding further dissociates causing the complex structure of the beads to collapse with further inward moving of the front. The process repeats itself and drives the front to move forward until the beads are completely solvated. Finally, mechanical relaxation of the strained macromolecular chains of the beads takes place, and redistribution of mobile ions occurs in the interior and the external regions of the beads.

We investigate the effect of pH on SR at 37 ^{0}C for sample B_1-B_5 and A_3 in this experiment, and the results are shown in Fig. 3. It appears that the highest values of SR are obtained at pH 2.1, and the SR of the semi-IPN beads tend to decrease as the pH of the swelling solution increase. This can be explained by the fact that in an acidic medium the amino groups of chitosan are protonated, resulting in the hydrogen bonds between CS and PVP being broken and the network dissociating [26]. The semi-IPN beads exhibits a lower degree of swelling in basic medium (pH=7.4). This may correspond to the decrease in the number of protonated amino groups of chitosan at this pH. The pKa of chitosan is 6.3–6.5, indicating that chitosan tends to protonate in acidic solution. Therefore, the SR of the beads at pH 7.4 is lower than those of the beads in acidic solution. In Fig.3, it is also found that 40:60 samples have a relative high SR than other composition samples. This may also relate to the hydrogen bond and electrostatic repulsion in the polymer network.

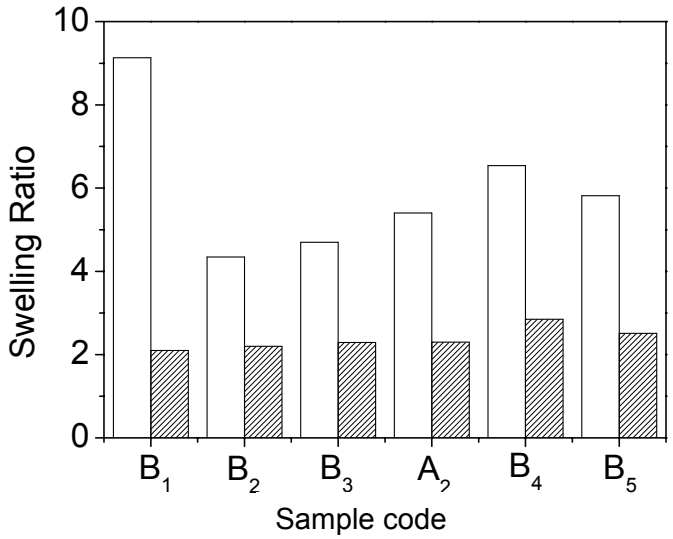

Fig. 3. Influence of pH on swelling ratio of different blend composition beads: (blank) pH 2.1, (hatched) pH 7.4.

Effect of ionic strength on SR at different pH values solution

Fig. 4 shows swelling ratio in various aqueous NaCl concentrations for sample B4 at 37 ºC. These beads were prepared with constant crosslinking time (10 min in 1 wt% glutaraldehyde solution). In Fig. 4, as ionic strength increases, the gels shrink s accordingly. The driving force for the swelling and shrinking of beads is the difference between the concentration of free ions inside and outside the beads according to the

Donnan equilibrium [27]. When the concentration of mobile ions inside the beads is lower than that in the surrounding condition, the osmotic pressure in the surrounding causes the beads to shrink; otherwise, a large osmotic pressure inside the beads causes the beads to swell.

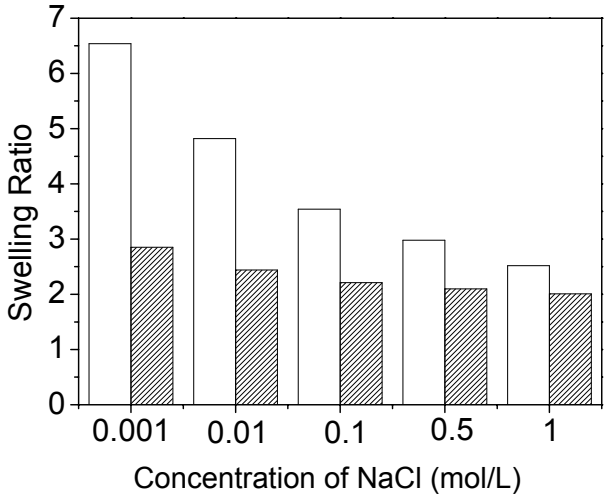

Fig. 4. Influence of ionic strength on swelling ratio of the beads at different pH values solution: (blank) pH 2.1, (hatched) pH 7.4.

As the concentration of NaCl increases, the exterior osmotic pressure increase, and interior osmotic pressure of the semi-IPN beads remains the same. The exterior osmotic pressure is larger than that in the semi-IPN, so SR of the semi-IPN beads decreases with the increase of concentration of NaCl.

Drug release study

-Effect of drug loading content on Co A release

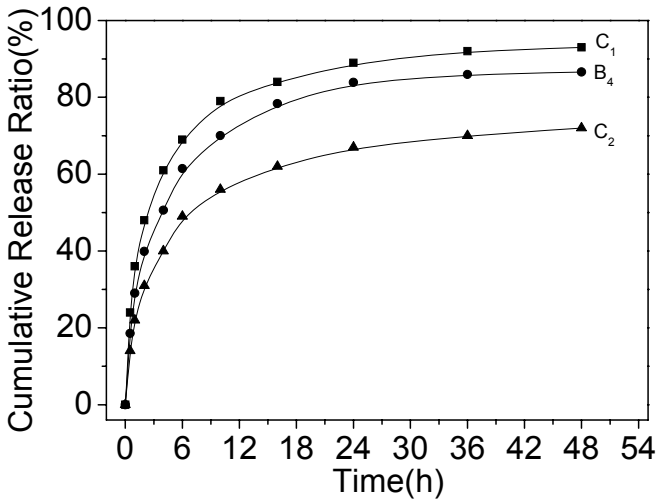

Fig. 5. Influence of drug loading content on Co A release at pH 2.1 values solution.

In order to study the effect of drug loading content on release rates, semi-IPN beads B_4, C_1 and C_2 with different drug loading content are studied. The pH 2.1 values solution with the same ionic strength is used as the release medium. From Fig. 5, we can conclude that the release pattern of the higher drug loading content beads is found to be similar to that of the beads with a lower loading of Co A. The percentage of released Co A from the beads is decreased with increase in Co A loading content. However, the total amount of Co A released is found to be more from higher loading beads in comparison to the beads with lower loading content. This result is in accordance with the literature [28].

-Effect of composition ratio and time on Co A release at pH 2.1 and 7.4 solution

The effect of blend composition on Co A release is shown in Fig. 6 and 7 at pH 2.1 and 7.4 values solution, respectively. Samples B_1, B_2, B_3, A_3, B_4 and B_5 are used in this study. It is found that the maximum release of Co A is observed for sample A_1, and the second largest release amount of Co A in sample B_4. This can be explained by the swelling behavior of the semi-IPN beads. As shown in Fig.3, It is found that the chitosan beads shows the maximum swelling ratio. It is known that for beads delivery systems, the release of the drug is controlled by the swelling behavior of the beads. The swelling of the carrier increases the aqueous solvent content within the polymer matrix, enabling the drug to diffuse through the swollen network into the external environment.

Tab. 2. Percent weight loss of semi-IPN Chitosan/PVP beads.

Weight ratio of CS to PVP	Weight loss [a)]	
	pH 2.1	pH 7.4
100:0	40.2	12.3
80:20	22.5	13.2
60:40	25.2	18.1
50:50	29.1	21.4
40:60	33.2	22.6
20:80	30.4	21.5

(a) The average value from three experiments.

The pH of the release medium also has significant effect on Co A release. As shown in Fig. 6 and 7, there is a burst release initially for the first hour in both acidic and basic medium, followed by an almost constant release of Co A from the matrix for the studied period of 48 h. The amount and percentage of Co A release is much higher in acidic than in basic solution. This can be explained considering the rate of diffusion from the swollen beads in acidic and basic solution. In basic medium (pH 7.4), there is a limited swelling of the beads (see Fig. 3), which inhibits the diffusion of drug at a faster rate as it occurs in acidic medium (pH 2.1). Initially, the magnitude of swelling of beads in acidic medium is very high and gives rise to a significant burst effect through uncontrolled diffusion, but becomes almost constant due to controlled diffusion at beads equilibrium swelling.

Besides the release of drug being controlled by the swelling behavior of the carrier, drug release may also be concerned with an erosion process. This process is associated with macroscopic changes in the appearance of the device, including changes in the physicomechanical properties of the polymeric material, deformation

or structural disintegration, weight loss, and the eventual loss of functions. Tab. 2 shows the weight losses of the semi-IPN beads under the studied conditions. It was found that the weight losses of the beads at pH 2.1 is higher than the value at 7.4. This indicates that drugs released by an erosion process can also be occurring in this system.

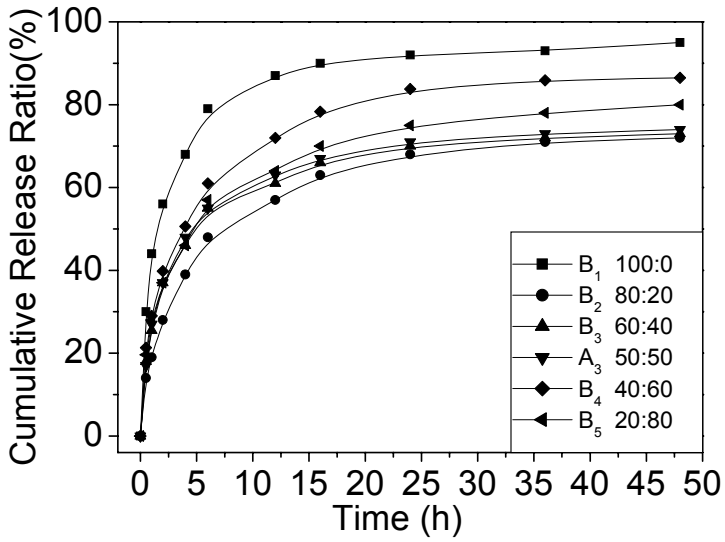

Fig. 6. Effect of composition ratio of CS and PVP in beads and time on Co A release as a function of time at pH 2.1 solution.

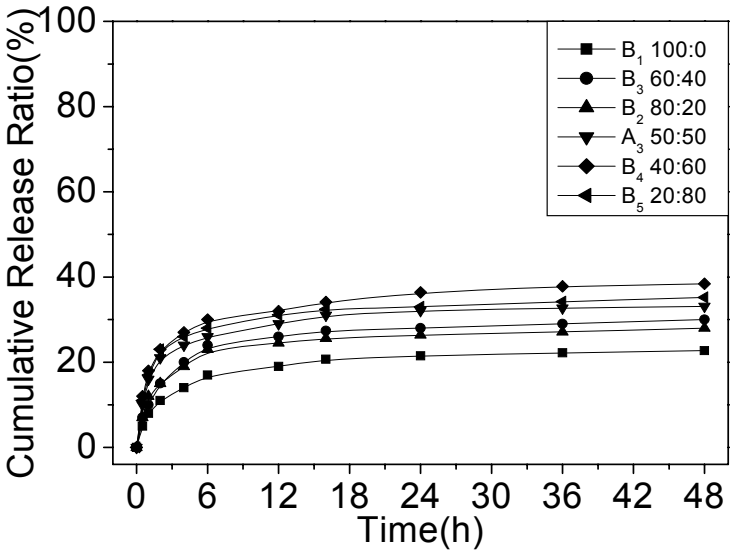

Fig. 7. Effect of composition ratio of CS and PVP in beads and time on Co A release as a function of time at pH 7.4 solution.

In Fig. 6, it is also found that the release of Co A from the chitosan beads is very fast and takes the shortest time to reach equilibrium as compared with the other samples. It may also relate to the fastest swelling and the biggest SR in all the samples. In Fig.

7, the cumulative release rate of Co A from all the samples is very low, except the limited SR and weight lose in the pH 7.4 solution, drug–polymer interaction between Co A and matrix may also play an important role. Since Co A has several polar groups, including an hydroxyl group, amino group, and phosphoric group, which can interact with the polymer matrix, an interaction between Co A and the polymer matrix may occur. As a result, the amount of Co A release from the semi-IPN beads is only about 20–30%.

-Effect of ionic strength on Co A release at different pH values solution

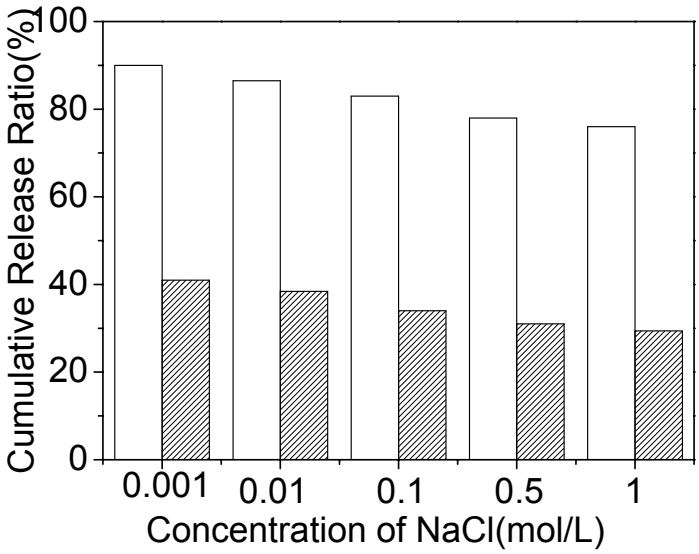

Fig. 8. Influence of ionic strength of the solution on Co A release: (blank) pH 2.1, (hatched) pH 7.4.

The effect of ionic strength on Co A release is shown in Fig. 8. Drug-loaded semi-IPN beads B_4 are used in this experiment as the release matrix. Adding appropriate amounts of NaCl to the buffer solution with pH 2.1 and 7.4 produces five different release medium. It is found that the highest amounts of Co A release from the systems are observed at pH 2.1 and the cumulative release rate of Co A decreases with the concentration of NaCl increasing both at pH 2.1 and 7.4 values solution. This is in good agreement with the results of swelling of the semi-IPN beads shown in Fig. 4.

-Effect of crosslinking time on Co A release at different pH values solution

The effect of crosslinking time on Co A release from the beads is shown in Fig. 8. To study the effect of the crosslinking time on Co A release, sample A_1-A_5 are used in this study. It is found that the amount of Co A release from the beads decreases with an increase of crosslinking time both at pH 2.1 and 7.4 values medium. It can possibly be explained by the term of degree of swelling (Tab.1). The results reveals that the SR of the Co A loaded beads decreases with increasing crosslinking time. This is attributed to the swelling behavior of the crosslinked network. For short crosslinking time samples, the density of crosslinking is low, which make the beads swell extensively.

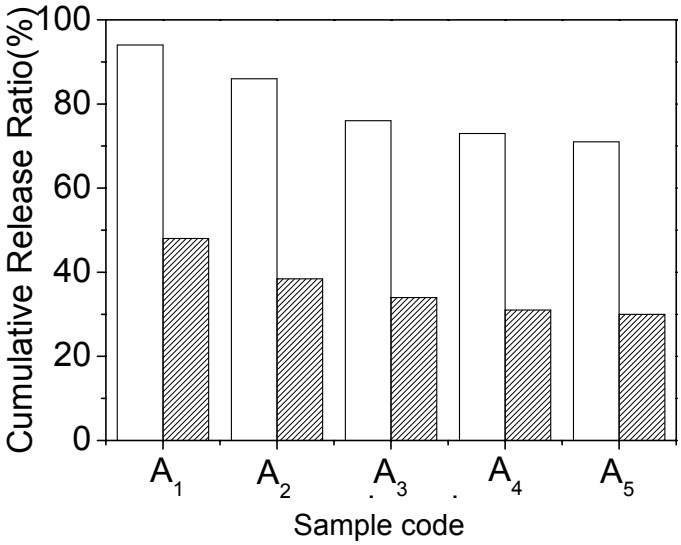

Fig. 9. Influence of crosslinking time on Co A release at pH 2.1 and 7.4 values solution: (blank) pH 2.1, (hatched) pH 7.4.

While the mesh size of the network became bigger, more Co A molecules are able to penetrate to the external environment. On the other hand, for long crosslinking time samples, the swelling ratio is limited. Therefore, the mesh size of the network is closer to the size of Co A molecule, and Co A has difficulty penetrating to the external environment. In Tab.1, it was found that the degradation of the beads also plays an important role in the release process. The rule is that the shorter the crosslinking time, the bigger the weight lose rate, and the degradation of the beads leads to Co A release from the polymer network.

Conclusions

A series of semi-IPN beads composed of CS and PVP were synthesized by using glutaraldehyde as crosslinking agent. The semi-IPN beads structure, swelling behaviour at different experimental conditions, especially its potential use in drug delivery systems were studied. The results suggested that the semi-IPN beads were sensitive to pH and ionic strength of the release medium. Furthermore, the beads' composition, drug loading content, and crosslinking time all had relevant influence on the release property of the beads. Thus we could control the drug release rate through changing some influential factors of the drug-loaded beads. The differences in the rates and the amounts of drug released from the semi-IPN beads could be attributed to the swelling capacity of the beads, the degradation of the baeds, and interaction between the drug molecule and the polymer matrix. These results suggested crosslinked chitosan/PVP semi-IPN beads cloud be used as a drug delivery carrier.

Experimental

Materials

Chitosan (CS) was obtained from Tokyo Kasei Kogyo Co., Ltd. The degree of deacetylation was 0.85 as measured by elemental analysis [29]. Coenzyme A (Co A)

was purchased from Aldrich. PVP (Mw: 80,000) was supplied by Kisida Chemical Co., Ltd, Japan. Glutaraldehyde was obtained from ICN Biomedicals, Ohio, USA. All other chemicals used were of analytical grade, without further purification.

Preparation of drug loaded semi-IPN beads

Chitosan was dissolved in 2 wt% acetic acid under stirring for 3 h at room temperature. PVP was added into this solution, and again stirred for 1h. Then calculated amount of Co A as model drug was added to the viscous solution of chitosan/PVP, and stirred for 2 h. The homogeneous mixture was extruded in the form of droplets using a syringe into NaOH-methanol solution (1:20 w/w) under stirring conditions. The resultant beads were placed in 1 wt% glutaraldehyde aqueous solution and maintained at 50 ^{0}C. Finally, the crosslinked beads were washed with hot and cold water successively and vacuum dried at 30 ºC to a constant weight. All the preparation parameters of the samples are described in Tab.3.

Tab. 3. Composition of CS/PVP semi-IPN beads.

Code	CS (g)	PVP (g)	Crosslinking time in 1% glutaraldehyde (min)	2wt% Acetic Acid (mL)	Coenzyme A (g)	Loading Content (%)	Loading Efficiency (%)
A_1	0.5	0.5	5	50	0.2	15.2	74
A_2	0.5	0.5	10	50	0.2	15.4	72
A_3	0.5	0.5	20	50	0.2	14.9	75
A_4	0.5	0.5	40	50	0.2	14.8	76
A_5	0.5	0.5	80	50	0.2	15.0	73
B_1	1.0	0	10	50	0.2	15.1	74
B_2	0.8	0.2	10	50	0.2	15.5	73
B_3	0.6	0.4	10	50	0.2	15.2	74
B_4	0.4	0.6	10	50	0.2	15.2	73
B_5	0.2	0.8	10	50	0.2	15.2	72
C_1	0.4	0.6	10	50	0.1	7.5	76
C_2	0.4	0.6	10	50	0.4	30.1	71

By the above method, different crosslinking agent beads were prepared by immersing the beads in 1 wt% glutaraldehyde solution for different times coded as A_1, A_2, A_3, A_4 and A_5 in Tab.3.
For different drug-loading content beads, 0.1 or 0.4 g Co A was dissolved in the mixed solution of CS and PVP with a mass ratio of CS to PVP of 40: 60, thus producing different drug loading content semi-IPN beads C_1 and C_2, respectively.

Characterization

IR spectra of the vacuum dried semi-IPN beads were recorded using KBr pellets on AVATAR-360FT-IR at a resolution of 4 cm^{-1}. The shape of the beads was examined using a scanning electron microscope (S-4300, Japan). All the UV spectrum of the release medium were recorded with a UV-visible spectrophotometer (UV-540, US).

Swelling and degradation studies

The swelling ratio (SR) was determined by immersing the dry semi-IPN beads with a water jacket in aqueous solutions of the desired pH or ionic strength in sealed containers. After regular periods of time, they were removed from the aqueous solution. After the removal of excess surface water with filter paper, they were weighed and returned to the same container. SR was calculated from the equation $SR = (Ws–W_d)/W_d$, where Ws and W_d represent the weights of the swollen and dry-state samples, respectively.

The crosslinked CS/PVP semi-IPN beads were expected to undergo degradation by the hydrolysis of the amino/ imine bonds. This process was associated with macroscopic changes in the appearance of the device, including changes in the physical mechanical properties of the polymeric material, deformation or structural disintegration, weight loss, and the eventual loss of functions. Hence, hydrolytic degradation [30] of the beads (A_1-A_5 and B_1–B_5) were studied under physiological conditions. Semi-IPN beads were placed in 100 mL solutions of pH 2.1 and pH 7.4 at 37 °C under unstirred conditions and the hydrolytic degradation of the beads were determined by the following equation: Weight lose (%) = (Wo- Wt)/Wo× 100, where Wo is the initial weight of the beads and Wt is a weight of the vacuum-dried beads after immersion for 48 h.

Determination of drug loading content and loading efficiency of the beads

The semi-IPN beads (10 mg) were kept in 100 mL water with pH 2.1 values at 37 ^{0}C under stirring for 72 h. After centrifugation, the amount of free Co A was determined in the clear supernatant by UV spectrophotometer (UV-540, US) at 260 nm using a calibration curve constructed from a series of Co A solutions with standard concentrations. Such experiments allow the calculation of the drug loading content (%) and the loading efficiency (%). The loading content (%) of drug and the loading efficiency (%) were calculated as following equation:

$$\text{Drug loading content \%)} = \frac{\text{amount of drug in the semi-IPN beads}}{\text{mass of semi-IPN beads}} \times 100 \quad (1)$$

$$\text{Loading efficiency (\%)} = \frac{\text{amount drug loading}}{\text{theoretical loading}} \times 100 \quad (2)$$

In vitro drug release

The release rate experiments were performed in a glass apparatus at 37 ^{0}C under unstirred conditions, in acidic (pH 2.1) and basic (pH 7.4) solutions or under different ionic strength solution. At a time interval, 1mL sample were withdrawn and assayed for the amount of released Co A as a function of time. The amount of released Co A was analyzed with a spectrophotometer as described previously. The results were expressed as cumulative release ratio (amount of released Co A/ all amount of loaded Co A). The experiments were done in triplicate.

Acknowledgments

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