Controlled Release of Riboflavin and Insulin through Crosslinked Poly(vinyl Alcohol)/Chitosan Blend Membrane

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SYNOPSIS

The permeation of riboflavin and insulin through poly(vinyl alcohol) (PVA) and chitosan blend membrane was conducted. The permeability coefficients of both solutes through the crosslinked PVA and chitosan blend membrane were in the order of 10^{-6}–10^{-7} cm^3 cm/cm^2 s and showed a pH dependence. The pH-dependent permeation behavior was discussed in terms of water content and water structure inside of the swollen membrane. Riboflavin and insulin were presumed to permeate through the free water region in the swollen blend membrane. The DSC thermograms of these membranes indicated that the content of free water and the amount of freezing bound water increased with the water content in the membrane. The greater permeation rate of solutes in acidic solution rather than in neutral solution was due to an increase in both water content and the amount of free water and freezing bound water.

INTRODUCTION

Controlled release of insulin is useful for the treatment of diabetics because the conventional insulin administration by injection causes a wide fluctuation in blood glucose level. Many studies on the controlled release of insulin using polymeric materials have been reported.[1-5] For an ideal insulin delivery system, insulin release should be controlled directly by the amount of blood glucose present at any particular time. This requires a continuous feedback between the glucose level in the blood and insulin release rate.

Chemical systems for insulin delivery have been reviewed recently by Heller, who identified two classes of mechanism.[1] The first mechanism is termed *competitive desorption* and have been pioneered by Brownlee and Cerami[2] and Kim and coworkers.[3] The second class of chemical mechanisms identified by Heller involves the conversion of glucose to gluconic acid by enzyme glucose oxidase. This mechanism has been studied by Horbett and coworkers[4] and Ishihara et al.[5]

We reported in our previous paper that PVA/chitosan blend membrane changes their ability to absorb water when the environmental pH is altered.[6] We also showed that the amount of crosslinking agent in the blend membrane played an important role in the degree of swelling in the membrane. The present article discusses the control of riboflavin and insulin permeation through PVA/chitosan blend membrane in response to pH and glucose concentration. In addition, differential scanning calorimeter (DSC) studies for the membrane have been performed to understand the state of water in the water-swollen polymer membrane.

EXPERIMENTAL

Materials

Riboflavin was purchased from Junsei Chemical Co., Ltd. Insulin from bovin pancreas (24.1 IU/mg) and glucose oxidase type VII from *Aspergillus niger* (125,000 U/g) were obtained from the Sigma Chemical Co.

Preparation of the pH sensitive membrane used in this paper (Table I), characterization, and the degree of swelling of the membrane were discussed in detail in our previous paper.[6]

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Table I Preparation of PVA/Chitosan Blendmer

Blendmer	PVA (g)	Chitosan (g)	Glutaldehyde (mol/g Polymer)
P-1	2.0	1	6.0×10^{-6}
P-2	1.5	1	6.0×10^{-6}
P-3	1.0	1	6.0×10^{-6}
G-1	1.5	1	1.2×10^{-5}
G-2	1.5	1	6.0×10^{-6}
G-3	1.5	1	3.0×10^{-6}

Permeation Experiments

A permeation cell used in the present study is shown in Figure 1. It has two compartments of equal volume (100 mL). Each chamber was mechanically stirred at 750 rpm to eliminate the boundary layer resistance. All measurements were made at 37°C in this study. One compartment of the cell was filled with a different pH phosphate buffer or with glucose solution in phosphate buffer at pH 7.4, and the other side with a solution of riboflavin or insulin in phosphate buffer at pH 7.4. Aliquots of the buffer solution were taken out after a given period of time. The UV absorbance of solution was measured with a spectrophotometer (Spectronic 21, Milton Roy Co.) at 444 cm^{-1} in wavelength to determine the concentration of riboflavin in the feed and in the permeate. The concentration of insulin in the feed and in the permeate was measured from the UV absorbance appeared at 274 cm^{-1}. The solute permeability coefficient P was calculated from the following equation which was obtained from mass balance equation,[7] i.e.,

$$P = \frac{-d}{A(1/V_1 + 1/V_2)t} \ln\left[\left(1 + \frac{V_1}{V_2}\right)\frac{C_t}{C_0} - \frac{V_1}{V_2}\right] \quad (1)$$

where V_1, V_2, A, d, C_0, and C_t were the volumes of the concentration and the dilute compartment, membrane area (7.96 cm^2), thickness, and concentrations of the concentrated compartment at times 0 and t, respectively.

Differential Scanning Calorimetry (DSC)

A DuPont 910 thermal analyzer and the cell base was used for all the melting measurements. The DSC was calibrated by using indium and distilled water as standards. About 6–15 mg membrane sample equilibrated at 37°C was sealed in an aluminum pan and cooled down with liquid nitrogen to −70°C in DSC cell. The cell was slowly heated in a stream of nitrogen gas at a program rate of 5°C/min up to 20°C.

RESULTS AND DISCUSSION

Effects of pH and Glucose Concentration

In our previous paper, we reported the preparation of PVA/chitosan blend membrane and studied the mechanical and thermal properties and swelling characteristics of the membrane.[6] The important fact drawn from the previous study was that the degree of swelling changed markedly with pH ranging from 4 to 7 and was also affected by the amount of crosslinking agent applied. From this knowledge the pulsatile release of riboflavin and insulin in response to a change in pH and glucose concentration was investigated using the membrane equilibrated in a phosphate buffer at pH 7 and 37°C for 3 days.

Swelling measurements were first performed on the membrane prepared as discussed previously.[6] It was observed that as pH in an equilibrated solution was lowered, water content in the membrane increased. The magnitude of the change in water content from pH 7 to 4 rose as the amount of crosslinking agent decreased.

Second swelling measurement was performed in a different concentration of glucose solution containing glucose oxidase (GOD) (Fig. 2). To explain this phenomenon in detail, a schematic representation of glucose-induced permeation of drug using a blend membrane is proposed and shown in Figure 3. It was noted that the higher glucose concentration and thus a lower pH led to a more swollen membrane. A decrease in pH is attributed to the enzymatic reaction between GOD and glucose to form gluconic acid (steps 1 through 4 in Fig. 3). The increase in water content in the PVA/chitosan blend membrane is due to the protonation of amino groups

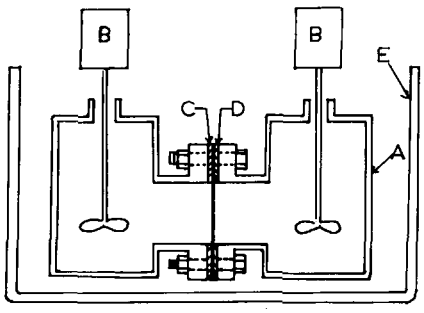

Figure 1 Apparatus for drug permeability measurement: (A) glass compartment; (B) mechanical stirrer; (C) sealing rubber; (D) membrane; (E) water bath.

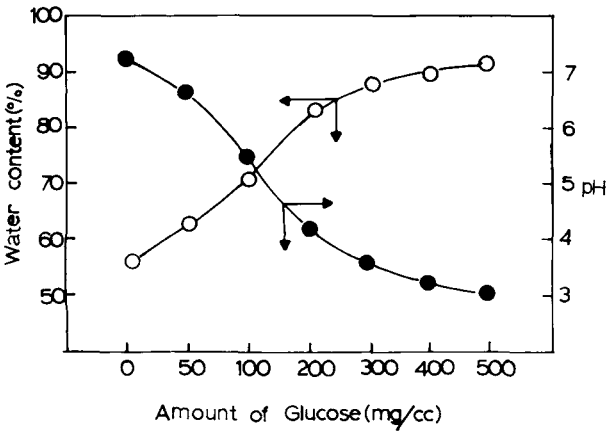

Figure 2 Change of pH (●) and water content (○) in G-2 membrane from reaction between glucose oxidase and glucose.

in the membrane in response to a decrease in pH (step 5). Therefore, the water content and thus the insulin permeation of PVA/chitosan blend membrane can be changed by the enzymatic reaction between GOD and glucose (step 6).

Riboflavin Permeation

Figure 4 illustrated results of the permeation of riboflavin through the blend membrane at a different pH buffer solution. It can be seen that the permeability of riboflavin through this membrane was changed with pH. The permeation rate of riboflavin in pH 4 buffer solution was greater than that in pH 7. The permeability data also indicated that G-3 membrane, having higher water content and lower crosslinking degree, exhibited the faster permeation of riboflavin than that G-1 membrane did. The permeability decreased monotonously with increasing the concentration of crosslinking agents.

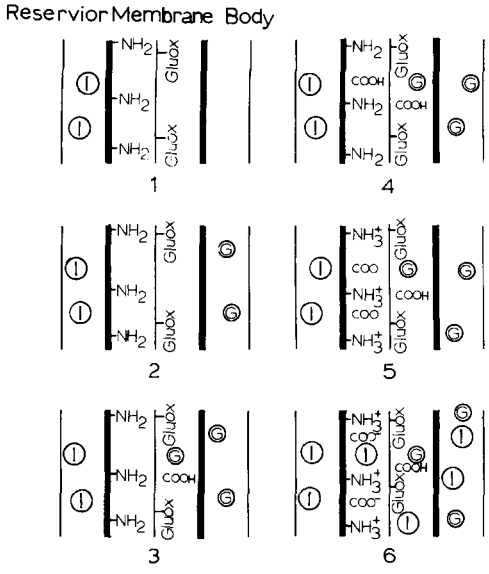

Figure 3 The proposed insulin delivery system: (①) insulin; (◎) glucose; Gluox = glucose oxidase; COOH = gluconic acid; COO— = ionized gluconic acid; NH_2 = PVA/chitosan membrane.

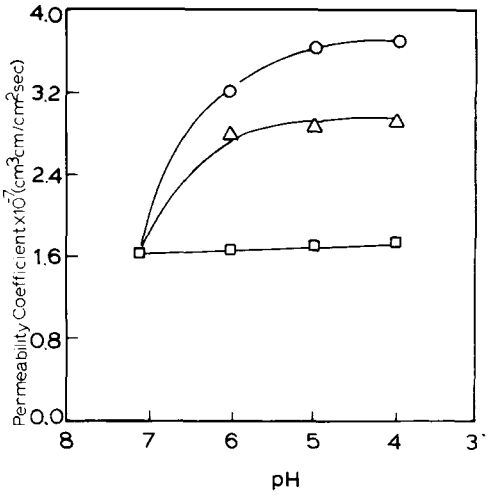

Figure 4 Effect of pH on the permeation of riboflavin through crosslinked PVA/chitosan blend membrane: (□) G-1; (△) G-2; (○) G-3.

Figure 5 showed riboflavin permeation through the blend membrane at a different glucose concentration. The change in permeability induced by an environmental change in pH is very similar to a change induced by glucose. This trend indicates that

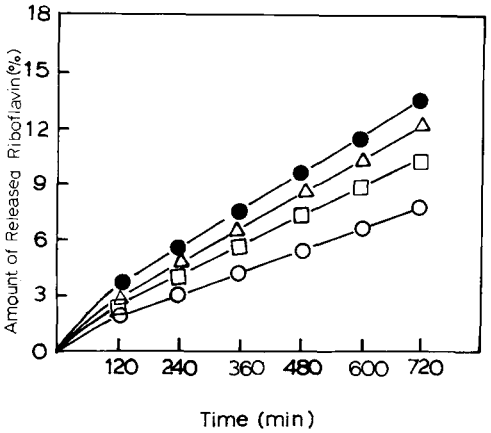

Figure 5 Release profiles of riboflavin through G-2 membrane at glucose concentration (mg/cc) of: (○) 50; (□) 100; (△) 200; (●) 400.

the production of gluconic acid catalyzed by the glucose oxidase in glucose solution might alter the pH of the solution, cause the swelling in blend membrane, and consequently change the permeability.[8]

The equal and parallel change in riboflavin permeability caused by either pH or glucose is a significant observation because it means that a maximum change in the permeability of membrane, which can occur as a result of drop in pH in the external environment, can also be induced by the change in glucose concentration in a physiological range.

Insulin Permeation

The permeability of insulin through blend membrane (G-2) was studied as a function of pH in the solution (Fig. 6). It could be seen that the permeation rate of insulin in a buffer solution at pH 4 was greater than that in a buffer solution at pH 7. The permeability of insulin through this membrane increased as the external solution became acidic due to a swelling. In other words, the permeability of insulin through the membrane was greatly influenced by the water content of the membrane induced by a change in pH in buffer solution.

According to Horbett et al.'s relationship between the diameter of a hydrogel and permeability, the rate of 4 mg insulin transport per day is required to obtain a normal blood glucose concentration.[8] This rate is sufficient to meet the normal daily requirement of insulin for a non-insulin-resistant diabetic. They also proposed that an effective implantable device

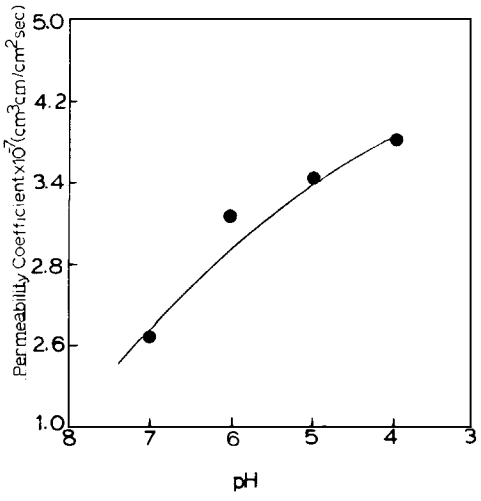

Figure 6 Effect of pH on the permeation of insulin through G-2 membrane.

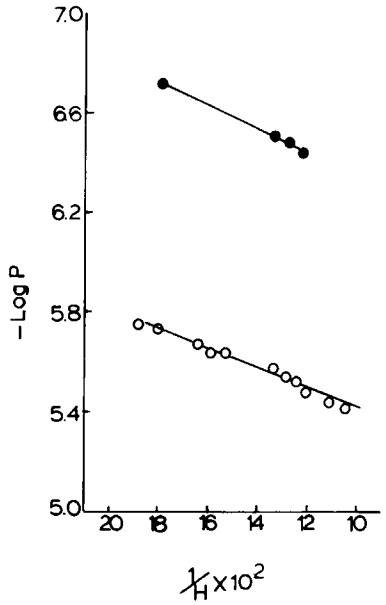

Figure 7 Relationship between log P and $1/H$ in PVA/chitosan membrane system for riboflavin (○) and insulin (●).

could be obtained if the permeability was in the order of about 10^{-7} cm^3 cm/cm^2 s for a membrane having thickness of 0.02–1 mm and a diameter of a hydrogel of 0.5–4.3 cm. In our case, the thickness of the present membrane was 0.1 mm and a diameter was 1.565 cm. Therefore, our data on the blend membrane indicated that it met the requirement for such a device on the sole ground that the insulin permeability was in the order of 10^{-7} cm^3 cm/cm^2 s.

Figure 7 shows the relationship between the logarithm of permeability and the reciprocal of the water content ($1/H$) in the blend membrane. From this result and according to the theory proposed by Yasuda et al.,[9] it is considered that riboflavin and insulin permeate through the region of free water in the swollen membrane. They also showed that a linear relationship between log P and $1/H$ indicated that solute might permeate through the water region contained in hydrophilic polymer membrane where no interaction between the polymer chains took place. On this basis, this result leads to a conclusion that the permeability of insulin through the hydrophilic membrane can be controlled by a change in the water content of the membrane. Therefore, in controlled release of insulin using this membrane, the desired release rate can be obtained by means of incorporation of crosslinking technique in the preparation of polymer membrane by which the swelling of the membrane is controlled.

Effect of Water Structure

Figure 8 shows DSC heating curves of water-swollen PVA/chitosan membrane. With the increase in swelling ratio of membrane, it was found that an endothermic peak appearing at around 0°C due to the presence of free water was separated into two peaks, one remaining at close to 0°C and the other shifting toward −8°C. These endothermic peaks confirm the existence of two types of water in the membrane,[7,10] i.e., a free water and a freezing bound water. The former has a melting temperature comparable with that of bulk water, whereas the latter shows a lower temperature peak presumably by a weak interaction between water and the polymer chain.

There are some controversies about what causes the deficit in the heat effect of water in macromolecular gels. Pouchly et al. suggested that the deficit might be due to the heat of mixing or heat of dilution,[11] whereas some authors believed that this might be due to the structure of water in the gel network which was indirectly verified by the studies such as dilatometry, specific conductivity, dielectric measurement, and pulse NMR.

Since solutes are permeated only into the water region in hydrophilic polymer, the structure of water in the swollen polymer matrix may play a significant role in the permeation of solutes. The amounts of

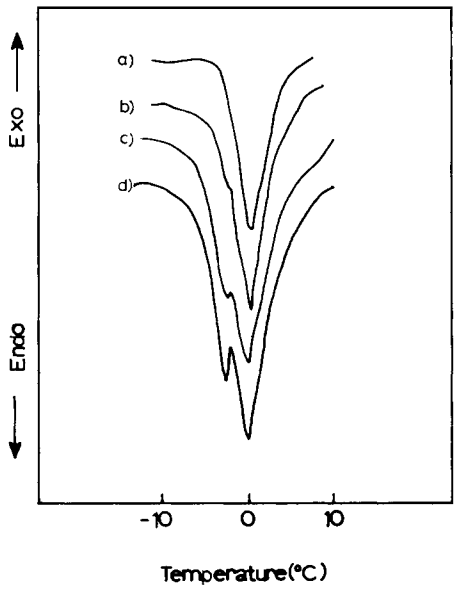

Figure 8 DSC thermograms of PVA/chitosan membrane measured in nitrogen atmosphere when water contents in the membrane are: (a) 54%; (b) 75%; (c) 82%; (d) 92%.

Table II Calculated Bound Water, Freezing Bound Water, and Free Water in PVA/Chitosan Membrane from DSC Results[a]

Polymer Sample	pH	Total Water (W_t)	Bound Water (W_b)	Free Water ($W_f + W_{fb}$)
G-3	7	0.54	0.49	0.057
G-2	6	0.75	0.46	0.29
G-1	6	0.82	0.46	0.36
G-1	4	0.92	0.46	0.46

[a] Unit: g water/g polymer.

free, freezing bound, and nonfreezing water in the membrane are estimated and listed in Table II for different polymer membranes. The values were calculated from enthalpies given by the area of peaks appearing at −8 and 0°C. The amount of bound water in the membrane is approximately estimated from the following equation[7]:

$$W_b = W_t - (W_f + W_{fb}) \quad (2)$$

$$= W_t - Q_{endo}/Q_f \quad (3)$$

where W_b and W_t are the weight of bound and total water in the membrane, respectively. W_f and W_{fb} are the weight fraction of free water and freezing bound water, respectively. The sum of W_f and W_{fb} is the ratio of Q_{endo}/Q_f, where Q_{endo} is the observed endothermic heat (cal/g) originating from bound and freezing bound water for water-swollen membrane and Q_f is the heat of fusion of ice (79.7 cal/g). We used heat of fusion of ice in place of heat of fusion for free water in PVA/chitosan membrane since it was recently reported that the heat of fusion for free water in PVA hydrogel was almost identical to that of pure ice.[12] It can be seen that free and freezing bound water begin to appear at a degree of total water content of over 0.75. Unfortunately, the respective amount of free and freezing bound water could not be estimated because of the uncertainty in deriving three peak areas exactly. The amounts of nonfreezing bound water remains constant at around 0.46 for all the sample whereas the amount of free and freezing bound water increase from 0.057 to 0.46. The more the increase in total water content, the greater the permeability of solute through the membrane. Free and freezing bound water may form water cluster by hydrogen bonding between themselves. The strength in hydrogen bonding between free and freezing bound water is obviously weaker

than that between bound water. Bound water is difficult to move because it strongly combines with polymer chains. Therefore, solutes find difficulty in diffusing through this region and may rather transport through the region of free and freezing bound water. Note that the permeation rate increased with the amount of free and freezing bound water.

The permeation rate of solute in acidic buffer solution was greater than that in neutral buffer solution. This is mainly due to an increase in the amount of free and freezing bound water. Considering all the facts for PVA/chitosan blend membrane, riboflavin and insulin permeated only through the region of free water. In the controlled release of insulin using this membrane, the desired release rate can be controlled by means of the amount of free water in the membrane.

CONCLUSION

The PVA/chitosan membrane was prepared and the permeation of riboflavin and insulin through this membrane was conducted. The water content in the membrane showed a pH dependence. As the glucose concentration in the phosphate buffer solution increased, the pH in the solution decreased from 7 to 4 and water content in the membrane increased due to an enzymatic reaction between glucose oxidase and glucose to form a gluconic acid. An increase in water content in PVA/chitosan membrane was attributed to the protonation of the amino group of the chitosan in response to a decrease in pH.

The results on the permeation of riboflavin and insulin through the blend membrane indicated that the permeation coefficients were in the order of 10^{-6}–10^{-7} cm^3 cm/cm^2 s at pH ranging from 7 to 4 and were dependent on both pH and glucose concentration. The pH dependence of riboflavin and insulin permeation was interpreted in terms of water content and water structure inside of the membrane. Riboflavin and insulin permeated through free water region in the swollen membrane. The DSC thermograms of crosslinked PVA and chitosan blend membrane indicated that there appeared two endothermic peaks, one around 0°C due to the presence of bulk water, and the other around −8°C caused by a weak interaction between polymer chains and water. The bound water content in the membranes from G-1 to G-3 stayed constant while the amount of free water and freezing bound water increased with the water content in the membrane. The greater permeation rate of solutes in an acidic solution rather than in a neutral solution was attributed to both an increase in water content and the amount of free and freezing bound water inside of the membrane. These results led to a conclusion that the permeability of riboflavin and insulin through the hydrophilic membrane could be controlled by a change in the water content in the membrane through crosslinking technique.

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