Porous Polydimethylsiloxane Membranes for Enzyme Immobilization

HONG YING WANG, TAKAOMI KOBAYASHI,* HITOSHI SAITOH, and NOBUYUKI FUJII

Nagaoka University of Technology, Department of Chemistry, Kamitomioka, Nagaoka, 940-21 Japan

SYNOPSIS

Porous polydimethylsiloxane (PDMS) membranes with α-amylase or glucose oxidase activity were prepared by catalytic hydrosilylation cure of PDMS in the presence of the enzyme–water solution. The pores in the membrane are the result of hydrogen foams, which are generated during the curing reaction. The enzyme reactions were examined in batch and permeation experiments by using glucose and starch solutions as substrates. For the permeation set-up, the reaction yields of the immobilized α-amylase increased as the permeation rate of the starch solution decreased. The Michaelis–Menten type of reaction kinetics for the immobilized enzyme indicated that the permeation system is effective for the diffusion through the solute of the matrix, as compared with the batch system. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Porous membranes have an advantage of low resistance to mass transfer of solutes in solution, because the presence of the pores in the membrane result in an increase of the permeation rate.[1] If there are catalyst sites such as an enzyme in the porous matrix, the catalytic reaction is effectively performed by an extensive internal surface area for the reaction of the immobilized enzyme. So far, porous membranes have been employed with immobilized enzyme systems by a number of authors.[2–8] The importance of solute transport through membranes with covalently bound enzyme was studied by Staude and co-workers.[9–11] They indicated that synthetic porous membranes with enzyme activity offer some advantages compared to particulates or gels in packed columns. In these reports, polysulfone and chemically modified polysulfone were used as the membrane materials.

On the other hand, little is known concerning the development of porous membranes made of polydimethylsiloxane (PDMS). Further, PDMS has not been applied to the enzyme immobilization except for the work by Kawakami et al.[17] They reported the use of an alginate–PDMS composite for enzyme immobilization. The interest in developing PDMS with enzyme activity, derives from the unique characteristics of PDMS.[12,13] The vulcanizates have been applied to numerous industrial uses related to membrane technology[14–16] in the form of nonporous membranes.

Recently, we have developed porous PDMS membranes by hydrosilylation curing of PDMS having a vinyl group and an SiH group, in the presence of water or other alcohol derivatives.[18] During the hydrosilylation cure, many pores are formed in the PDMS membrane as result of hydrogen foams produced by the reaction between the SiH and OH groups. In the present work, a similar technique as used in the preparation of PDMS membranes is applied to enzyme immobilization. The properties of the immobilized enzyme in the porous PDMS membrane were examined and kinetic data of the catalytic behavior were determined for batch and permeation reaction modes.

EXPERIMENTAL

Materials

Glucose oxidase and α-amylase were from Toyobo and Sigma, respectively. Other reagents were com-

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 60, 2339–2346 (1996)
 CCC 0021-8995/96/132339-08

mercial types of special grade. Water was purified by distillation and then passed through an ion-exchange resin.

PDMS membranes were formed by hydrosilylation of PDMS compounds consisting of the vinyl type PDMS (PDMS-V) and the SiH type PDMS (PDMS-H) in the presence of 20 ppm of platinum catalyst (Pt cat.). To obtain high mechanical strength of the cured PDMS membrane, PDMS-V contains a *Q* resin structure, i.e., SiO_2. Details of the preparation of PDMS and properties of the cured PDMS were reported previously.[18]

Immobilization of Enzyme

In the presence of enzyme aqueous solution, porous PDMS membrane was prepared via hydrosilylation and hydrogen generation during the cure as schematically illustrated in Figure 1; PDMS-V containing Pt cat. was well mixed with aqueous enzyme solution and then PDMS-H was added. After mixing, degassing of the compound was carried out *in vacuo* and the PDMS mixture was cast on a teflon sheet of about 200-μm thickness. The hydrosilylation cure was carried out at 30°C for 1 h. The resulting porous membrane was well rinsed with a large excess of water and used for experiments.

Measurements of Enzyme Activity

The catalytic activity of the enzyme immobilized by the porous membrane was carried out by means of batch and permeation experiments. For measuring the enzyme activity in a batch reaction, a vessel (200 mL) containing 100 mL substrate solution and a piece of the immobilized enzyme membrane was incubated at 30°C with shaking in a swinging shaker at 60 rpm. The effective surface area of the membrane used is 30 cm^2. Activity of α-amylase and glucose oxidase was measured by following the precedence the substrate of starch and β-D-glucose (glucose), respectively. The phenol sulfonic acid method[19] was used for the measurement of the glucose concentration by detection of the absorbance at 490 nm. The starch concentration was determined from the absorption of a starch–iodine mixture at 600 nm. The measurements of the absorbance were carried out by using a Shimadzu UV-190 double beam spectrophotometer. The molecular weight of starch in the permeated solution was measured using GPC (Tosoh; pump, CCPD; Column, TSK_{gel} $5000PW_{xl}$, 30 cm). Dextran samples (Pharmacia) were used as standards to calculate the molecular weight of the starch.

The pH was measured with a Horiba F-11 pH meter equipped with a Horiba combination electrode no. 6366.

The morphology of porous PDMS membranes was observed by scanning electron microscopy (SEM), JXA-733 (JEOL). The cross section of the membrane was obtained by fracturing the membrane at liquid nitrogen temperature; and the membrane was coated with gold by use of spatter SPM-112 (Anelva).

Permeation Experiments

Permeation experiments were carried out with an apparatus (Milipore Minitan-S), which is a tangential flow device. A peristaltic tubing pump draws the fluid sample from the unpressurized reservoir and pumps it into the membrane holder. The membrane size used was 180 × 130 mm^2. In the membrane holder, the effective area of the membrane for permeation is 30 cm^2. Permeation of 0.1 wt % starch solution was carried out with the porous membrane having α-amylase activity under 3 kPa applied pressure. The flow rate of the solute streams was determined by means of a flow meter (Model RK400, Kofloc).

RESULTS AND DISCUSSION

Preparation and Characteristics of Porous PDMS Membranes

In our previous work,[18] we described the preparation of porous membranes, using the hydrosilylated vulcanizate of PDMSs having a vinyl group and a SiH group in the presence of Pt catalyst. As shown in Figure 1, the porous vulcanizate of PDMS was used as a matrix for the entrapment of the enzyme. Formulation of PDMS-V, PDMS-H, and enzyme aqueous solution (10 mg/L) in PDMS compound was fixed at 10 : 1 : 0.2 (w/w/w). The molecular weights of PDMS-V and PDMS-H containing 0.22 mmol/g of vinyl and 16 mmol/g of SiH group are 1 × 10^5 and 2 × 10^2, respectively. In the enzyme-free compound of PDMS-V/PDMS-H = 10/1, the cure of the PDMS was completed in 8 min, as reported previously.[18] It was found that the addition of water to the compound slows the curing time to about 15 min, because the curing site of the SiH group is consumed by hydrogen generation as follows.

$$-SiH + H_2O \rightarrow -SiOH + H_2 \qquad (1)$$

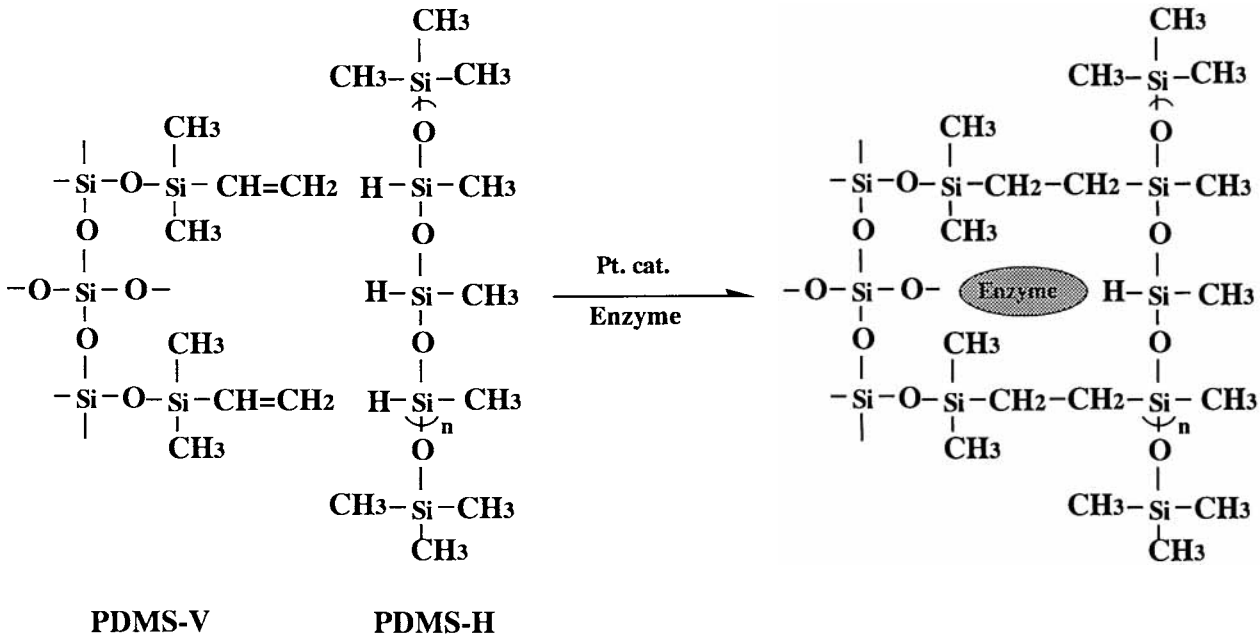

Figure 1 Schematic representation of hydrosilylation of PDMS-V and PDMS-H for enzyme immobilization.

The apparent curing time of PDMS containing enzyme aqueous solution is about 18 min for both glucose oxidase and α-amylase. In the presence of the enzyme in the water additive, the curing time of the PDMS compound becomes longer than that of the enzyme-free compound. The delay of the cure may be due to the inhibition of hydrosilylation curing of PDMS by the added enzyme. In practice, the addition of enzyme powder to the PDMS compound causes a marked inhibition of the cure around the enzyme powder. Therefore, as mentioned in the experiment section, a dilute enzyme solution was used in the present work.

During the curing reaction, the hydrogen generation occurs according to reaction (1) and the hydrogen-filled foam remains in the cured PDMS membrane. As previously reported,[18] we examined the amounts of the hydrogen generation during the cure after the mixing of the PDMS compound and the water additive. The hydrogen generation rate for PDMS–α-amylase and PDMS–glucose oxidase is 1.1 and 0.9 (mL/min g-PDMS), respectively. The value of the PDMS compound containing water without the enzyme is 1.5 mL/min g-polymer. This comparison indicates that enzyme addition to the PDMS results in a lower rate of the hydrogen generation. We observed that the hydrogen generation continues during the curing process. As a result, hydrogen foam remains in the PDMS membrane.

In order to check the hydrogen consumption of the SiH group by the hydrosilylation cure, FT-IR spectra of the PDMS membrane were measured by ATR-IR method. As reported,[18] the spectra of the membranes without the water additive have a peak at 2200 cm^{-1}, assigned to the SiH group. We confirmed the consumption of the SiH group of PDMS-H on the cured membrane, because the IR peak intensity of the SiH group in the cured membrane decreases when the water additive is present in the compound. In the presence of the enzyme aqueous solution, the peak intensity in the cured membrane did not change. This result suggests that the consumption of an SiH group only occurs by the reaction (1) for the hydrogen generation. However, the curing reaction becomes slow when the enzyme solution is added to the PDMS. Hence, the enzyme may inhibit the catalytic reaction by the Pt catalyst.

Figure 2 shows an SEM photograph of the porous membrane made of the PDMS compound with the α-amylase solution. The photograph of the membrane cross section indicates that the PDMS membranes have a heterogeneous, porous cross section consisting of many large voids of about 200 μm diameter. The diameter of the pores is almost equal to the cross section thickness. As reported,[18] the resulting enzyme-free PDMS membrane has a similar structure to that of the membrane containing α-amylase or glucose oxidase. This result suggests that the enzyme addition does not influence the formation of the porous membrane.

As illustrated by Figure 1, we assume that the enzyme is entrapped in the network of cured PDMS.

However, since the silicone compound is immiscible with the aqueous enzyme solution,[20] the enzyme uptake of the PDMS is very low. The porous membrane obtained seems to have a non-uniform enzyme distribution in the matrix. Immobilization may take place only on the interface between the enzyme aqueous solution and the PDMS, because the penetration of the aqueous enzyme solution to the interior of the PDMS networks is restricted.

In order to examine the non-uniform distribution of enzyme in the PDMS membrane, the X-ray microanalysis (XMA) for the immobilized α-amylase membrane was carried out: since α-amylase contains Ca in the molecular units combined with COO^- groups[21] the Ca content becomes a probe for the location of the immobilized enzyme in the membrane. Figure 3 shows the XMA data obtained at up to 2600× magnification for (a) the pore surface and (b) the inner part of the porous membrane, as presented in Figure 2. The obtained XMA spectra in Figure 3 show strong peaks at 1.8 and 2.2 keV due to Si of PDMS and Au scatter, respectively. In addition, the peak of Ca at about 3.7 keV for spectrum (a) is much stronger than for (b). This fact results from the concentration of the immobilized α-amylase in the surface network of the PDMS. This demonstrates that immobilization by entrapping in the PDMS network occurs only near the PDMS surface.

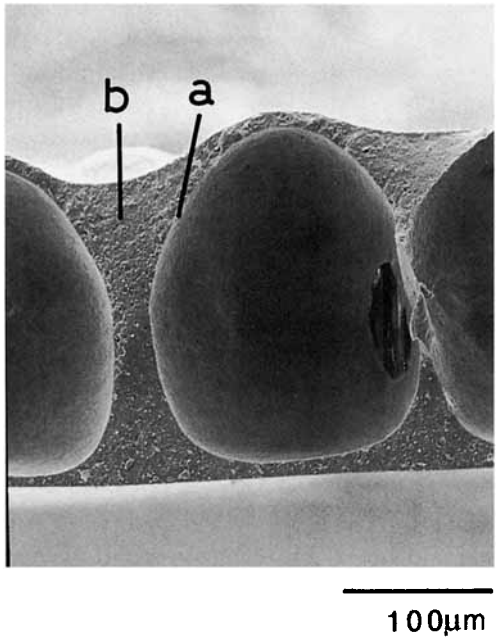

Figure 2 SEM photograph of the cross section for the PDMS–α-amylase immobilizate, ×500 magnification.

Enzyme Activity for the Immobilized Porous Membranes

It is well known that glucose oxidase and α-amylase are highly efficient enzymes for the oxidation of glucose to gluconic acid and the hydrolysis of starch to sugars,[22] respectively. At first, the catalytic reaction of the enzyme immobilized in the membrane was performed with a batch system at 30°C. The enzyme reaction was followed by measuring the substrate concentration remaining after the batch solution in various reaction times. The substrate was exchanged with fresh solution every 24 h and 9 h for the immobilized glucose oxidase and α-amylase, respectively.

Figure 4 shows the change of glucose concentration for the immobilized glucose oxidase. With increasing reaction time, the glucose concentration decreased due to the catalytic reaction of immobilized glucose oxidase. Similar plots for α-amylase are shown in Figure 5. Three different experiments are shown for each case, always with a concentration of 36 mg/L (glucose) or 100 mg/L (α-amylase). The time profile of the substrate concentration with immobilized α-amylase is similar to that of glucose oxidase. However, between the second and third experiments, the immobilized enzyme concentration gradually decreases. The resulting decrease in activity may be due to enzyme elution from the porous PDMS matrix, because the enzyme was entrapped only on the PDMS matrix surface.

As mentioned in our previous report,[18] this type of porous membrane can be applied to ultrafiltration experiments. We examined the enzyme activity by using a filtration process in a cross-flow permeation system.[23] The schematic diagram of the cross-flow system is shown in Figure 6. The flow rate of 0.1 wt % starch solution from the feed tank (300 mL) to the membrane holder was controlled in the range between 0.083 and 7.8 mL/s. The liquid flow is divided into two streams in a membrane process, i.e., the retentate and permeate streams. The divided streams were back to the feed tank. Consequently, the substrate solution is continuously circulated during the permeation experiment. Before the experiments, the membrane was washed by water permeation during 90 min in order to prevent the influence of any free enzyme on the catalytic reaction.

Figure 7 shows trans-membrane volume flux of 0.1 wt % starch solution for the α-amylase membrane at various permeation times. Here, the permeation was operated at various flow rates at the inlet of the membrane holder at 2.6, 3.8, and 7.8 mL/s. The permeation rate is defined as the volume flowing through membrane per area and time; ex-

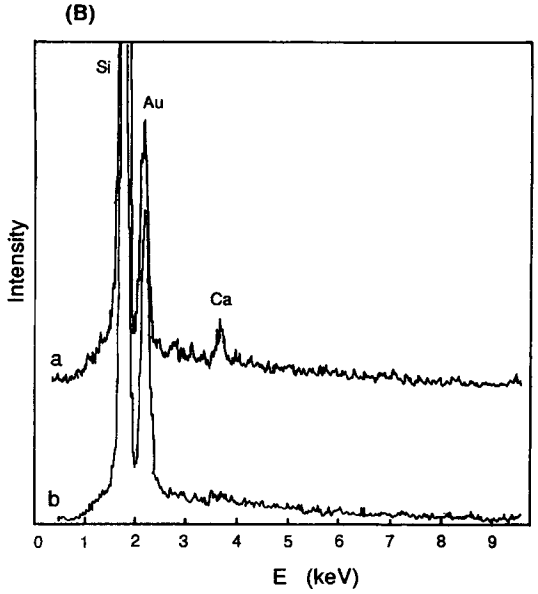

Figure 3 XMA spectra for (a) the surface of macrovoid and (b) the inner membrane corresponding to the location indicated in Figure 2, ×2600 magnification.

pressed in m^3/m^2 s. The volume flux obtained for the permeate stream is almost constant during the circulation of the starch solution.

Figure 8 shows GPC profiles of the starch solution at various circulation times. Before permeation, the starch molecule has a broad molecular weight distribution over the range 1×10^4–2×10^6 Dalton and exhibits three peaks in the GPC trace. As shown in the figure, the observed peaks appear in the GPC trace at 6–10 min elution time disappear with increasing the circulation time. Also, the observed area of the curve is reduced with the increase of the circulation time. This shows that the immobilized α-amylase membrane decomposes the starch molecule to smaller units during circulation of the substrate solution.

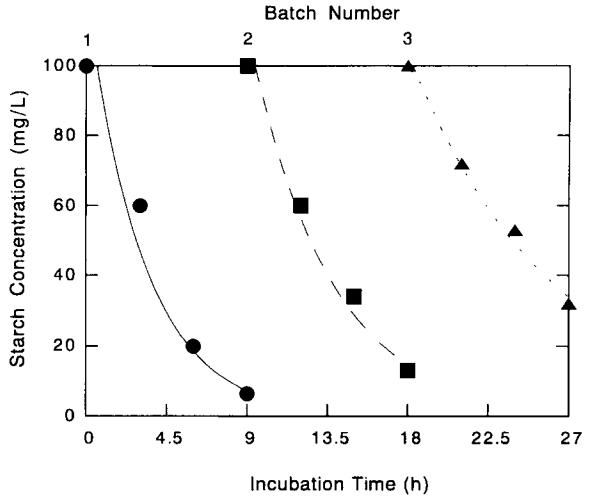

Figure 5 Plots of starch concentration vs. incubation time for batch system of the immobilized α-amylase membrane.

Using the starch–iodine method, we measured the total starch concentration as a function of the circulation time. Figure 9 shows the starch concentration in the permeate for circulation rates between 2.6 and 7.8 mL/s. The data show that the starch concentration in the feed tank decreases with circulation time. It is noted that the starch decompo-

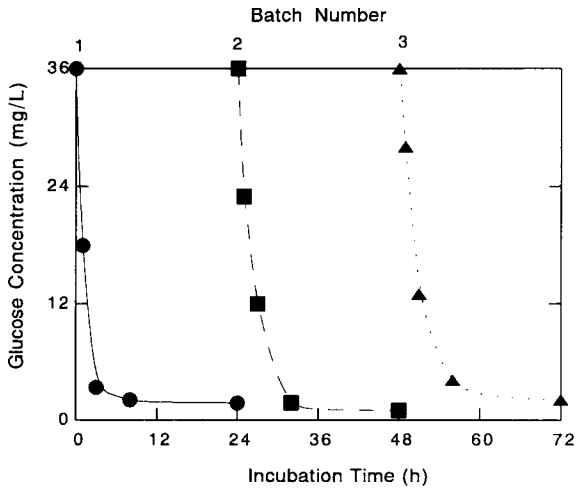

Figure 4 Glucose concentration at various incubation times for batch reaction of the immobilized glucose oxidase system.

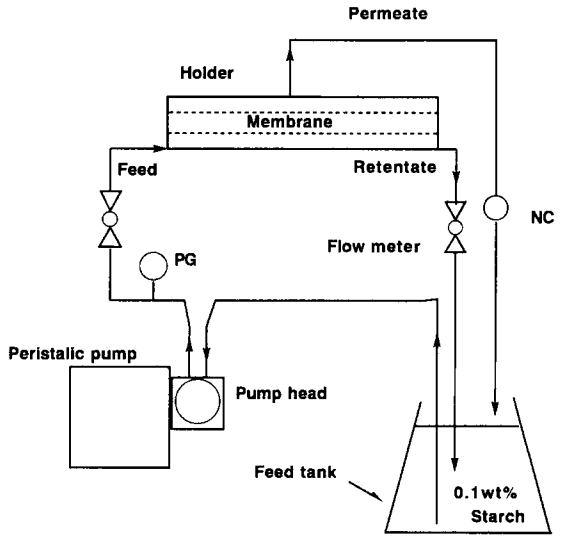

Figure 6 Schematic diagram of the set-up of the permeation experiment: NC, needle valve; PG, pressure gauge.

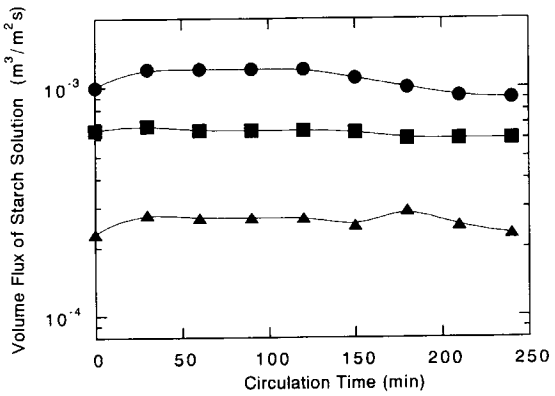

Figure 7 Volume flux of 0.1 wt % of starch solution at various feed circulation rates of 2.6 (▲), 3.8 (■), and 7.8 mL/s (●) under 3 kPa applied pressure. The effective area of porous membranes for immobilized α-amylase is 30 cm^2.

sition depends on the circulation rate. The decomposition becomes slower as the feed rate to the membrane holder increases. Perhaps the contact ability of the solute with the immobilized enzyme on the surface depends on the circulation rate of the solute solution.

Kinetic Parameters of the Immobilized α-Amylase in the Membrane

In the following experiments for kinetic analysis, the outlet for retentate flow (without permeation) was closed, i.e., all of the solute solution was permeated through the porous membrane without circulation. According to the investigations by Staude and co-workers,[2,9] the immobilized enzyme reactivity in the membrane was estimated by considering the residence time of the substrate solution in the activated zone of the immobilizate. The mean residence time, t_r, of solute in the porous membrane can be calculated as

$$t_r = L/J \tag{2}$$

where L is membrane thickness and J is volume flux of solution. For the calculation of t_r, a membrane thickness of 200 μm is used in the present work.

According to Michaelis-Menten kinetics,[2,24]

$$v = V_{max}s/(K_m + s) \tag{3}$$

where v, V_{max}, s, and K_m denote reaction rate, maximum reaction rate, substrate concentration, and Michaelis constant, respectively. In the permeation experiments for the porous PDMS membrane having α-amylase activity, the starch concentration in the permeate was sampled downstream of the membrane, and the starch–iodine method was applied to determine the starch concentration. The experiments were performed at various permeation rates in the range 0.083–2.5 mL/s. The reaction rate v of starch decomposition is calculated as

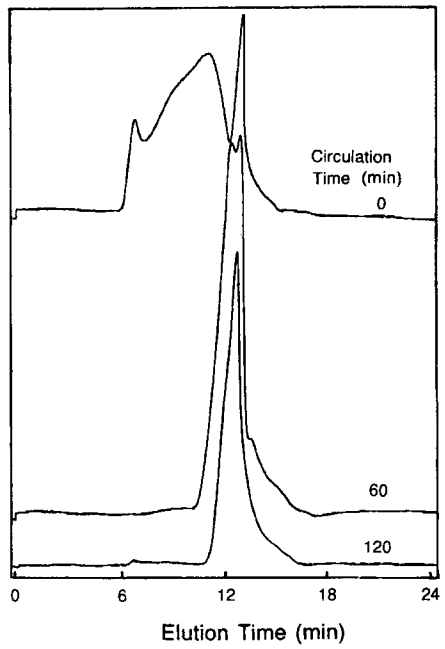

Figure 8 GPC traces of 0.1 wt % starch solution at various circulation times for 7.8 mL/s feed rate.

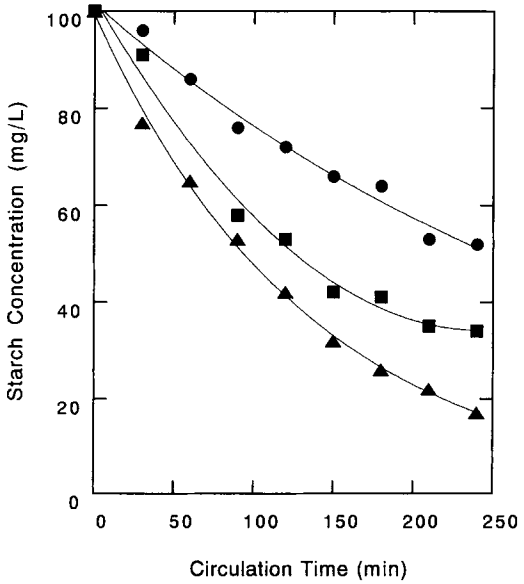

Figure 9 Starch concentration in feed tank vs. circulation time for various feed rates of 2.6 (▲), 3.8 (■), and 7.8 (●) mL/s.

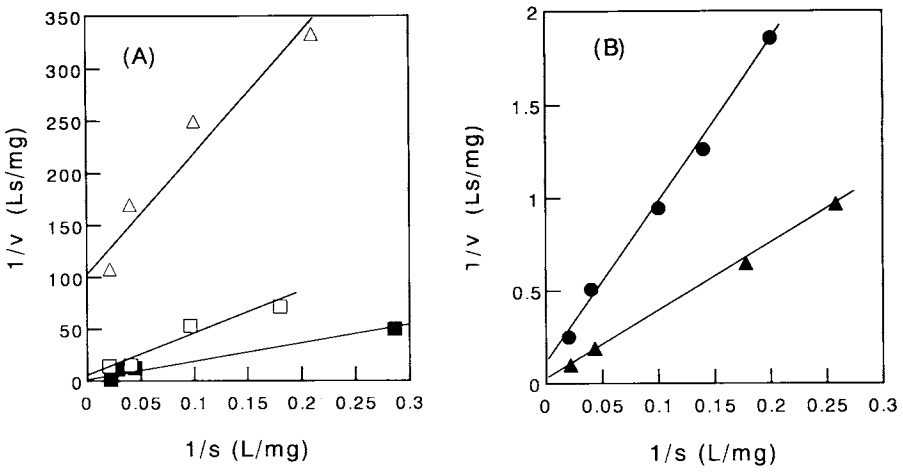

Figure 10 Lineweaver–Burk plots for the immobilized α-amylase and native α-amylase in permeation and batch systems. (A) native α-amylase (□) and immobilized α-amylase in the batch system (△); the permeation system with feed rate of 0.083 (■); (B) Permeation with feed rates of 1.3 (●) and 2.6 (▲) mL/s.

$$v = \Delta C/t_r \tag{4}$$

where ΔC is the decrease of starch concentration after permeation. The reaction rates were determined at different starch concentrations ranging from 3.5 to 49 mg/L.

Figure 10 shows Lineweaver-Burk plots for the immobilized α-amylase membrane, plotting $1/v$ vs. $1/s$. From the obtained slope (K_m/V_{max}) and intercept ($1/V_{max}$) for the linear relationship, both K_m and V_{max} are estimated as listed in Table I. A large value of K_m means that a high substrate concentration is required for saturation of the catalytic site of the enzyme. Here, the enzyme E and substrate S combine to form an intermediate complex ES and then dissociation to product P and E with rate of k_2 as follows:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \tag{5}$$

Namely, K_m is expressed as eq. (6):

$$K_m = (k_2 + k_{-1})/k_1 \tag{6}$$

when k_2 is small and can be neglected compared with k_{-1}, $k_2 \ll k_{-1}$, K_m can approximate to $k_{-1}/k_1 = [E][S]/[ES]$. So, K_m is an expression of the activity of the enzyme and related to dissociation constant for species $[ES]$.

The apparent K_m values for the batch system with the immobilized enzyme are almost the same as that of the native enzyme. This suggests that there is very little deactivation of the enzyme activity by the immobilization. However, the value of the apparent

Table I Kinetic Parameters, Apparent K_m and V_{max}, of PDMS-Immobilized α-Amylase Membranes in Various Reaction Systems

System	Feed rate (mL/s)	t_r (s)	K_m (mg)	V_{max} (mg/Ls)
Permeation[a]	0.083	6.8	54	0.33
	1.3	0.47	81	9.4
	2.5	0.24	153	42
Batch[b]	—	—	47	0.008
Native[c]	—	—	58	0.15

[a] Temperature of feed tank is kept at 30°C during experiments.

[b] The reaction vessel containing the immobilized α-amylase membrane (effective area: 30 cm^2) is shaken with 60 rpm at 30°C. The amount of native α-amylase is 0.1 mg in the batch reaction vessel.

[c] The reaction of native α-amylase was carried out by similar batch system of the immobilized enzyme. Amount of used enzyme is 1 mg/L.

V_{max} for the immobilized enzyme is extremely small compared to that for the native enzyme. The data comparison indicates that the diffusion of the starch to the enzyme sites in the porous matrix is very low in the batch system.

On the other hand, for the permeation experiments, the value of the apparent V_{max} becomes similar to that of the native enzyme. With increasing permeation rate of the solution, the apparent V_{max} value increases. This means that the diffusion of the substrate molecules through the porous membrane is enhanced by the permeation set-up. The value of K_m for the permeation system increases when the permeation rate of the solute solution increases. This is due to the low concentration of the ES complex at a high permeation rate; the "innate" affinity between the enzyme and the substrate cannot change in the various circulating conditions. So it must be due to a residence time effect.

CONCLUSIONS

Novel porous PDMS membranes having enzyme activity were prepared by hydrosilylation curing. Properties of the immobilized enzyme were examined in batch and permeation experiments. By Michaelis–Menten analysis, the apparent K_m and V_{max} of the immobilized α-amylase were estimated. The effectiveness of the porous PDMS matrix for enzyme immobilization was demonstrated in the permeation system.

The authors wish to express their thanks to Yoshimasa Hoshino, Masanori Takanashi and Bunjiro Murai of Toshiba Silicone Co. for supplying various reagents. They are also grateful for a grant from the Nakano Foundation Fund of 1993.

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Received May 31, 1995
Accepted October 7, 1995