Available online at www.sciencedirect.com
ScienceDirect
Catalysis Today 118 (2006) 158–165
CATALYSIS TODAY
www.elsevier.com/locate/cattod

Preparation of urease-immobilized polymeric membranes and their function

T. Uragami *, K. Ueguchi, M. Watanabe, T. Miyata

Unite of Chemistry, Faculty of Engineering and High Technology Research Center, Kansai University, Suita, Osaka 564-8680, Japan

Available online 11 July 2006

Abstract

Two kinds of urease-immobilized polymer membranes were prepared. One was prepared by bulk-copolymerizing a mixture consisting of vinylized urease (VU), acrylamide (AAm), 2-hydroxylethylmethacrylate (HEMA) and a cross-linker (urease-immobilized poly(VU-AAm-HEMA) membrane) and another was prepared by ultrafiltrating a mixture composed of urease, quaternized chitosan and sodium carboxymethylcellulose in an aqueous sodium bromide solution (urease-immobilized polyion complex membrane). The permeation and hydrolytic characteristics of aqueous urea solutions were kinetically investigated under various conditions through urease-immobilized membranes. The hydrolysis of urea through urease-immobilized membranes followed Michaelis–Menten kinetics and is discussed herein.

Keywords: Enzyme; Immobilization; Copolymerization of vinylized urease; Polyion complex; Hydrolysis of urea; Ultrafiltration; Michaelis–Menten kinetics

1. Introduction

The relationship between humans and enzymes has evolved over time. Even during historical times where there was no concept of enzymes, ancient people produced beer and wine by enzymatic fermentation and had a taste for drinking them. After several thousand years, enzymatic studies have significantly progressed. In particular, the world market for the industrial application of enzymes has spread, and could be attributed to the fact that enzymes have high catalytic activities and substrate specificities under moderate conditions at standard temperatures and pressures. The utilization of these enzymatic characteristics can eventually resolve environmental problems associated with natural resources, energy, etc. However, enzymatic activities are remarkably lowered under the presence of heat, strong bases, strong acids and organic solvents. Additionally, the recovery of enzymes from reaction mixtures is very difficult because of their water-solubility. Thus, for the purpose of the continuous regeneration of enzymes, the latter have been immobilized by various methods such as covalent bonding and adsorption onto carriers, cross-linking, gel entrapment and microcapsulation [1–4]. In particular, the immobilization of enzymes in polymeric membranes separates the dual natures associated with immobilization–enzymatic catalysis and spatial separation from the membrane.

Martino et al. immobilized urease on nylon membranes grafted with cyclohexyl-methacrylate. When the urease-immobilized membranes were used as a contactor, the optimum pH, optimum temperature and K_m value of urease-immobilized membrane shifted toward more acidic values, higher temperatures and higher values, respectively [5]. Godjevargova and Gabrovska immobilized urease by covalently bonding onto poly(acrylonitorile-methylmethacrylate-sodium) vinylsulfonate membranes with different chemical modifications [6]. They did not carry out the experiments of the membrane permeation but reported characteristics of these membranes. For positively or negatively charged membranes with immobilized urease, optimum pH was shifted to higher or lower pH, respectively, and the charge of the matrix also affected the rate of the enzymatic reaction. Chen and Chiu prepared the urease-immobilized membrane by forming covalent bonds on a composite membrane made of cross-linked poly(*N*-isopropylacrylamide-*co*-*N*-acryloxysuccinimide-*co*-2-hydroxylethyl methacrylate) hydrogel on polyester nonwoven support [7]. They reported that the

* Corresponding author. Fax: +81 6 6330 3770.
E-mail address: uragami@ipcku.kansai-u.ac.jp (T. Uragami).

0920-5861/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.cattod.2005.12.007

thermal stability of the immobilizing urease was enhanced and the hydrolysis rates of urea in a diffusion experiment with temperature swing were increased as compared with isothermal operation. Lin and Yang immobilized urease on teh surface of hydrolyzed polyacrylonitrile hollow fiber dialyzer membranes [8]. The activity of urease-immobilized membranes was higher than that of native urease at various pHs and the stabilities of the former to pH were higher than those of the latter. The removal of urea using urease-immobilized dialyzer in vitoro dialysis was faster than a regular dialyzer.

In this study, urease-immobilized polymer membranes were prepared by covalent binding and gel entrapment, i.e., the copolymerization of a mixture consisting of vinylized urease (VU), acrylamide (AAm), 2-hydroxylethylmethacrylate (HEMA) and *N*,*N*′-methylenebisacrylamide and the entrapment of enzymes in polyion complexes formed from quaternized chitosan (q-Chito) and sodium carboxymethylcellulose (CMCNa). Permeation and hydrolytic characteristics were studied under various conditions through urease-immobilized poly(VU-AAm-HEMA) membranes and urease-immobilized polyion complex membranes obtained from ultrafiltration.

2. Experimental

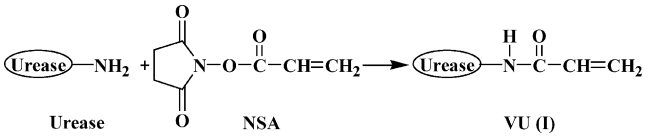
2.1. Materials

Urease (4.5 units/mg) was purchased from Funakoshi Reagent Co. Ltd., Japan. *N*-Succinimidylacrylate (NSA) as a vinylization reagent of urease, acrylamide (AAm) and 2-hydroxyethylmethacrylate (HEMA) as comonomers, *N*,*N*′-methylenebisacrylamide (MBAA) as a cross-linker, ammonium persulphate (APS) and *N*,*N*,*N*′,*N*′-tetramethylenediamine (TEMED) as redox initiators were purchased from Wako Pure Chemical Industry, Japan. A BCA protein assay reagent kit was also purchased from Wako Pure Chemical Industry, Japan, for the determination of immobilized enzymes.

Deacetylized chitosan (100%) with an average molecular weight of 3–4 $\times$ 10^5, was supplied by Koyo Chemical Co. Ltd., Japan. Sodium carboxymethylcellulose (CMCNa) (7.5% carboxmethylation) was supplied by Wako Pure Chemical Industry, Japan, and was used as a polyanion. The anion exchange capacity of CMCNa was 1.8 meq/g, and was determined by the colloid titration method [9]. All other reagents and solvents used in this study were supplied by commercial sources.

2.2. Synthesis of vinylized urease

Urease was chemically modified by coupling with NSA in a phosphate buffer solution using the method reported by Hoffman and co-workers [10]. NSA (10 mg) was added to a phosphate buffer solution 20 mM (pH 7.4) containing urease (120 mg), and the reaction was performed at 36 °C for 1 h to introduce vinyl groups into the urease molecule. After the resulting vinylized urease (VU) (**I**) was purified by gel filtration. The degree of vinyl group introduction was 15.9% which was determined by the TNBS method [11].



2.3. Synthesis of quaternized chitosan

The synthesis of quaternized chitosan (q-Chito) was carried out according to a previously described procedure [12,13]. Purified chitosan powder (6.0 g) was dispersed into an aqueous solution of 42 wt% methanol (760 ml) at room temperature, and a desired amount of methyl iodide was added to this heterogeneous mixture and stirred at 50 °C. Quaternization of chitosan was performed for 6 h at 50 °C. Excess sodium chloride was added to the reaction mixture to convert iodide ammonium salts to chloride ammonium salts. The solution was poured into excess acetone to precipitate the q-Chito. The crude q-Chito was dissolved in pure water and purified by repeated re-precipitation in acetone. The degree of quaternization of purified q-Chito was determined by 270 MHz ^{1}H nuclear magnetic resonance (^{1}H NMR) (JEOL; EX-270) spectra. In this study, q-Chito with a degree of quaternization of 18% was synthesized. The cation exchange capacity of q-Chito as a polycation was 5.1 meq/g, and was determined by the colloid titration method [9].

2.4. Preparation of urease-immobilized membranes

In this study, we prepared two kinds of urease-immobilized membranes. One was prepared by copolymerizing VU with comonomers. Urease-immobilized poly(VU-AAm-HEMA) membranes were prepared by the copolymerization of VU with AAm and HEMA using MBAA as a cross-linker, and aqueous solutions of APS and TEMED as redox initiators, in a molded plate between two glass plates at 25 °C for 3 h, as shown in Fig. 1.

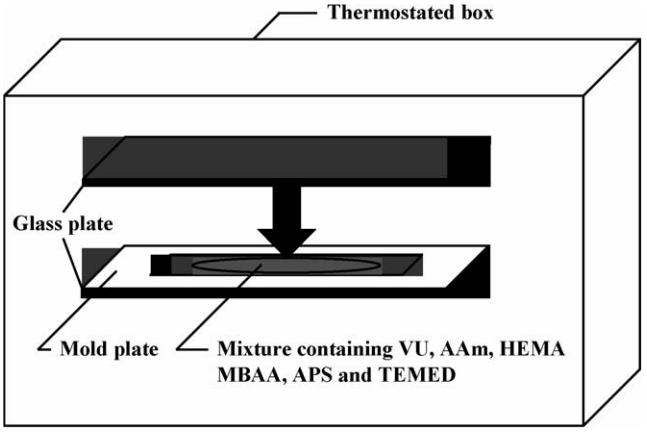
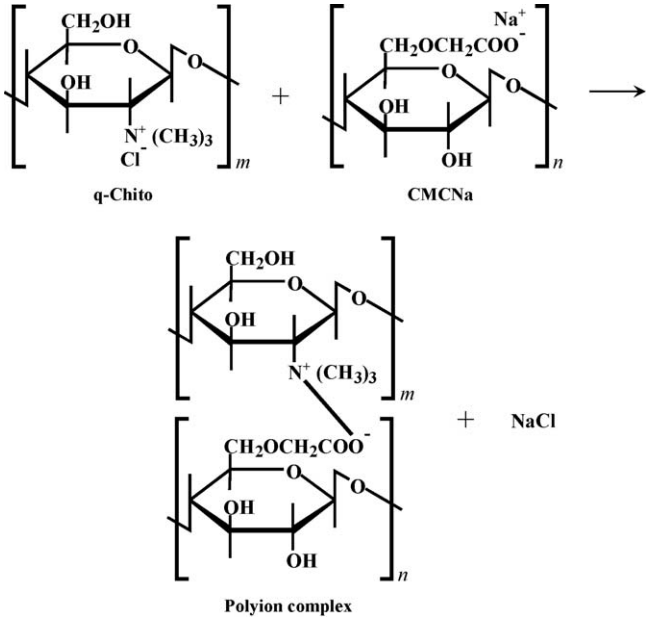


Fig. 1. Schema for the preparation of urease-immobilized poly(VU-AAm-HEMA) membranes.



Scheme 1. Polyion complexes between q-Chito and CMCNa.

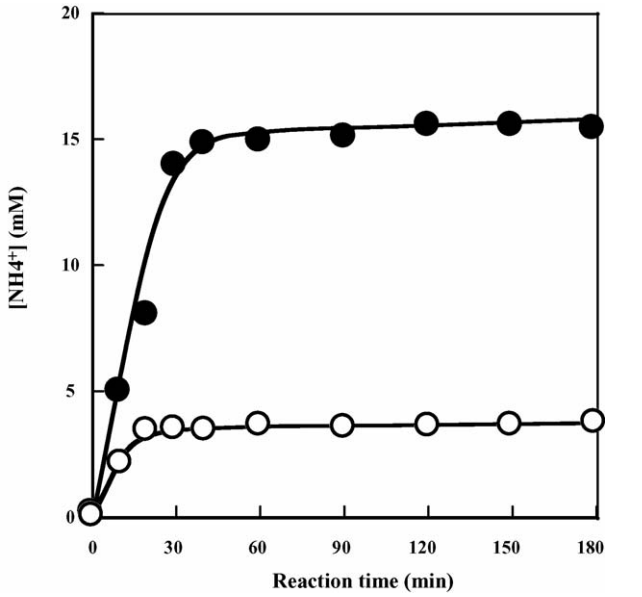


Fig. 2. Enzymatic activities of vinylized urease (○) and native urease (●) for aqueous solutions of urea (100 mg/ml) as a function of time. Operating conditions: 37 °C, pH 7.4.

Another urease-immobilized membrane was prepared by entrapping urease in a polyion complex between a polycation and a polyanion (Scheme 1). Q-Chito and CMCNa dissolved in an aqueous solution of NaBr (20 wt%) were mixed at a weight ratio of 1 part of q-Chito with 2.5 parts of CMCNa. The total polymer concentration of this mixed solution was 1 wt%. Urease was added to the mixture and a solution containing urease was poured onto the microporous cellulose acetate membrane mounted on the porous support in the ultrafiltration cell and ultrafiltered at 25 °C and 1 kg/cm^2. During the ultrafiltration process, NaBr and water were removed from the mixed solution and consequently urease was immobilized in a polyion complex membrane from q-Chito and CMCNa.

2.5. Apparatus and measurements

The ultrafiltration cell used in this study was presented in previous papers [14–16]. The effective membrane area was 13.8 cm^2. Urease-immobilized poly(VU-AAm-HEMA) membranes and urease-immobilized polyion complex membranes were exposed to urea for permeation and hydrolysis in a buffered solution (pH 7.4) at 37 °C and 1 kg/cm^2 using the ultrafiltration cell. The permeation rate was determined by weighing the permeate through the urease-immobilized membrane. Concentrations of urea in the feed solution were measured by the *p*-dimethylaminobenzaldehyde method [17]. Concentrations in the permeate were determined by the indophenol method [8,18].

3. Results and discussion

3.1. Enzymatic activity of vinylized urease

Fig. 2 shows the enzymatic activity of VU for an aqueous solution of urea as a function of reaction time in comparison to native urease. In an initial reaction time using VU, the concentration of ammonium ion formed by hydrolysis of the urea molecule, increased remarkably until about 20 min and reached a plateau. That of the native urease increased significantly until about 40 min and then reached a plateau. As can be seen from these results, the enzymatic activity of VU was lower than the native urease. The decrease in the enzymatic activity of the former could be attributed to protein denaturation and a concomitant change in the higher-structure of the urease molecule.

3.2. Amounts of enzyme-immobilized in polymer gels

The HEMA content effect in HEMA to AAm ratios in terpolymers consisting of VU, AAm and HEAMA to urease-immobilization was investigated. Immobilized urease was determined by the BCA protein method [8]. All the urease-immobilized poly(VU-AAm-HEAMA) membranes were about 190 mg. This result suggests that the amount of immobilized urease in terpolymer gels was not significantly influenced by the composition of components consisting of AAm and HEAMA. If all the urease used in preparing the urease-immobilized poly(VU-AAm-HEAMA) membranes had been immobilized, the theoretical amount of urease would have been 300 mg. Approximately, 65% of the total urease was immobilized by the poly(VU-AAm-HEAMA) membranes.

3.3. Permeation and hydrolysis characteristics of urease-immobilized poly(VU-AAm-HEAMA) membranes

Fig. 3 shows the permeation rate and the ammonium ion concentration in the permeate through the urease-immobilized poly(VU-AAm-HEAMA) membranes during ultrafiltration as a function of HEMA to AAm contents. The permeation rate, PR

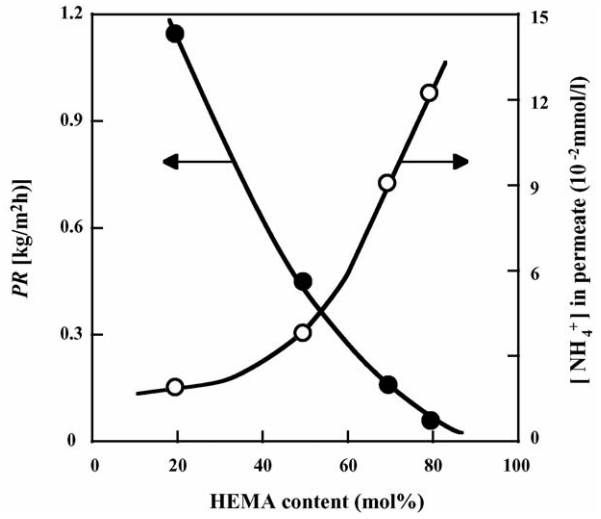


Fig. 3. HEMA concentration effects to AAm on permeation rates (●) and ammonium ion concentrations in permeates (○) through urease-immobilized poly(VU-AAm-HEMA) membranes in ultrafiltrations. Operating conditions: 25 mm mol/l urea solution, pH 7.4, 37 °C, 1 kg/cm^2 under N_2 gas.

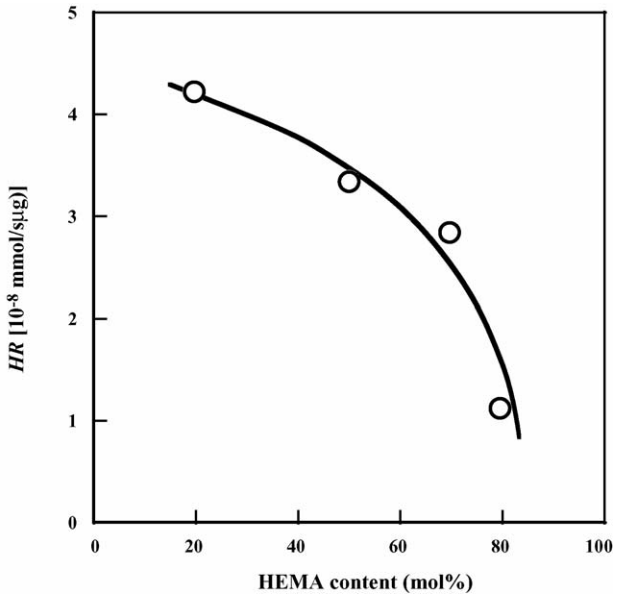


Fig. 4. HEMA concentration effects to AAm on permeation hydrolysis rates of urea through urease-immobilized poly(VU-AAm-HEMA) membranes in ultrafiltrations. Operating conditions: 25 mm mol/l urea solution, pH 7.4, 37 °C, 1 kg/cm^2 under N_2 gas.

(kg/(m^2 h)) was determined by Eq. (1):

$$\mathrm{PR} = \frac{W}{At} \tag{1}$$

where W (kg), A (m^2) and t (h) are the weight of permeate, membrane area and permeation time, respectively.

As can be seen in this figure, the permeation rate decreased and the ammonium ion concentration in the permeate increased with increasing HEMA. The decrease in the permeation rate is due to the fact that the degree of swelling of the urease-immobilized poly(VU-AAm-HEMA) membrane decreased with an increase in the content of HEMA which has a lower hydrophilicity than AAm. On the other hand, an increase in the ammonium ion concentration could be attributed to the fact that the contacting probability between the urease molecule immobilized in the urease-immobilized poly(VU-AAm-HEMA) membrane and the urea molecule as a substrate increased with a decrease in the permeation rate.

In order to estimate the permeation hydrolysis reaction of urea molecules through the urease-immobilized poly(VU-AAm-HEMA) membranes synthetically, the permeation hydrolysis rate, HR (mol/(h μg)) of the urea molecule was defined as expressed by Eq. (2):

$$\mathrm{HR} = C_2 \frac{\mathrm{PR}'}{I_\mathrm{U}} \tag{2}$$

where C_2 (mol/l), PR′ (ml/h) and I_U (μg) are the ammonium concentration in the permeate, the amount of the permeate per the permeation time and the amount of urease-immobilized in the membrane, respectively.

In Fig. 4, the relationship between the permeation hydrolysis rate of urea through the urease-immobilized poly(VU-AAm-HEMA) membranes during ultrafiltration and HEMA contents to AAm in urease-immobilized poly(VU-AAm-HEMA) membranes is shown. With increasing HEMA, the permeation hydrolysis rate of urea decreased. This result suggests that although the absolute number of hydrolyzed urea molecules increased with increasing HEMA as shown in Fig. 3, the number of synthetically hydrolyzed urea molecules decreased.

3.4. Temperature and pH effects on permeation and hydrolytic characteristics

It is well known that the activity of enzymes is significantly affected by reaction conditions such as temperature, pH and so on. Thus, temperature and pH effects on permeation rates and hydrolysis rates of urea molecules through urea-immobilized poly(VU-AAm-HEMA) membranes and urea-immobilized polyion complex membranes obtained from ultrafiltration were investigated. Fig. 5 shows temperature effects on permeation rates and hydrolysis rates of urea molecules through these membranes. As can be seen from Fig. 5, an optimum temperature for urea-immobilized poly(VU-AAm-HEMA) membranes was about 45 °C and that of the urea-immobilized polyion complex membranes was about 37 °C. The optimum temperature of the latter membrane is closer to that of native urease. The difference in optimal temperatures between the urea-immobilized membranes is due to a difference in the immobilized state of urease molecules within these membranes, i.e., urease molecules in urease-immobilized poly(VU-AAm-HEMA) membranes are immobilized by covalent bonds based on copolymerization, but those in urease-immobilized polyion complex membranes are immobilized by entrapment within polymer gels. This discussion may be further supported by the behavior of permeation hydrolysis rates at higher temperatures. Permeation hydrolysis rates in urease-immobilized polyion complex membranes with increasing temperatures decreased remarkably but those in urease-immobilized poly(VU-AAm-HEMA) membranes

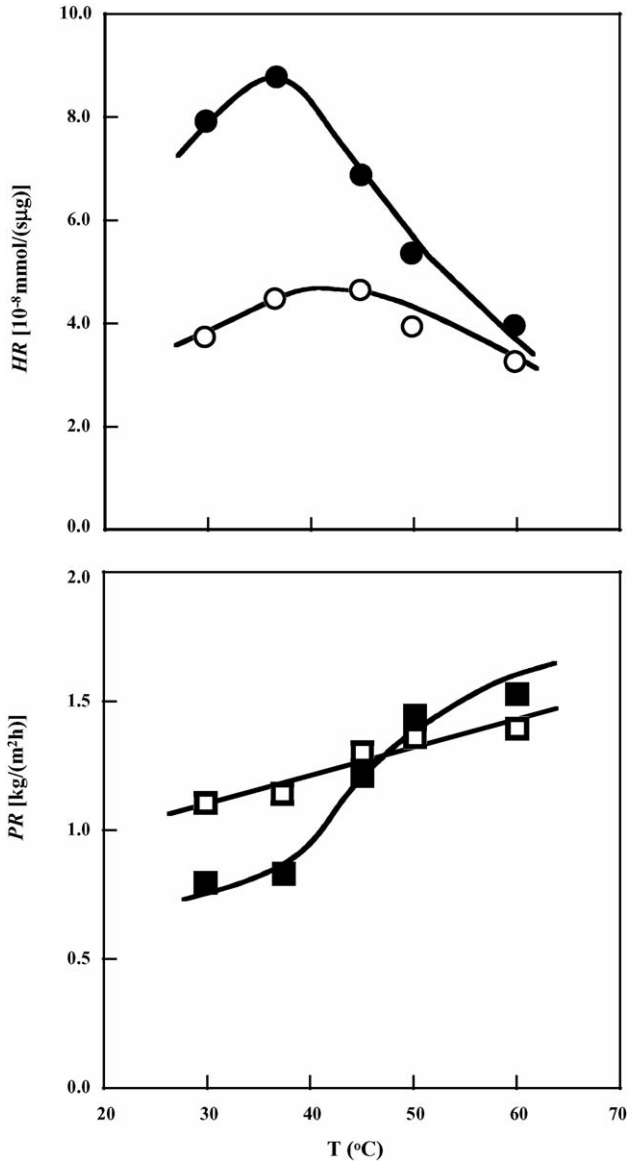


Fig. 5. Temperature effects on permeation rates and hydrolysis rates of urea through urease-immobilized poly(VU-AAm-HEMA) membranes containing 20 mol% HEMA to AAm and urease-immobilized polyion complex membranes in ultrafiltrations: (□) permeation rate for urease-immobilized poly(VU-AAm-HEMA) membrane; (○) hydrolysis rate for urease-immobilized poly(VU-AAm-HEMA) membrane; (■) permeation rate for urease-immobilized polyion complex membrane; (●) hydrolysis rate for urease-immobilized polyion complex membrane. Operating conditions: 25 mm mol/l urea solution, pH 7.4, 1 kg/cm^2 under N_2 gas.

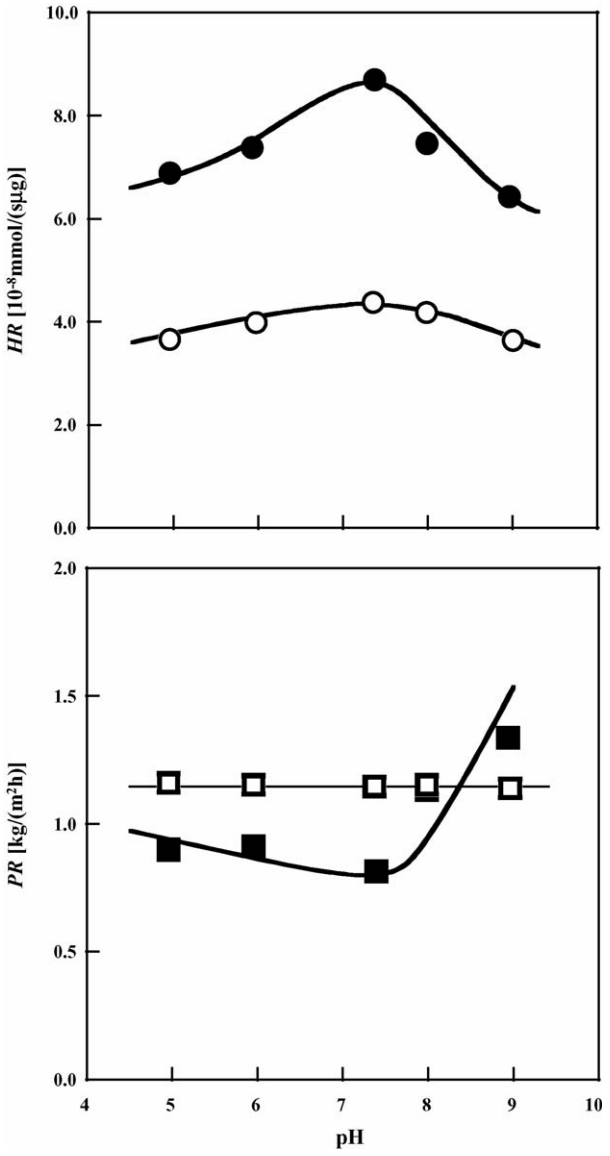


Fig. 6. Effects of pH on permeation rates and hydrolysis rates of urea through urease-immobilized poly(VU-AAm-HEMA) membranes containing 20 mol% HEMA to AAm and urease-immobilized polyion complex membranes in ultrafiltrations: (□) permeation rate for urease-immobilized poly(VU-AAm-HEMA) membrane; (○) hydrolysis rate for urease-immobilized poly(VU-AAm-HEMA) membrane; (■) permeation rate for urease-immobilized polyion complex membrane; (●) hydrolysis rate for urease-immobilized polyion complex membrane. Operating conditions: 25 mm mol/l urea solution, 37 °C, 1 kg/cm^2 under N_2 gas.

decreased slightly; it is difficult to maintain native conformations in higher-structure for urease molecules immobilized by covalent bonds when changes in temperature are involved.

On the other hand, the permeation rates increased with increasing permeation temperature. The increase in the permeation rate is due to the increase in the motion of the permeating molecules and the polymer chains composing the membrane matrix.

In Fig. 6, effects of pH on permeation rates and hydrolysis rates of urea molecules through both urease-immobilized poly(VU-AAm-HEMA) membranes and urease-immobilized polyion complex membranes from ultrafiltrations are shown. Optimum pHs for both urease-immobilized membranes were about 7.5. In this case, the pH dependence in the latter membrane was larger than that in the former. This result can be understood by the above discussion, i.e., the urease molecule immobilized in the former membrane is stable for external environmental conditions. In Figs. 5 and 6, permeation hydrolysis rates with changes in temperature and pH through the latter urease-immobilized membranes were greater than those through the former membrane. As mentioned above, this difference could be attributed to the fact that the urease molecule in the latter can

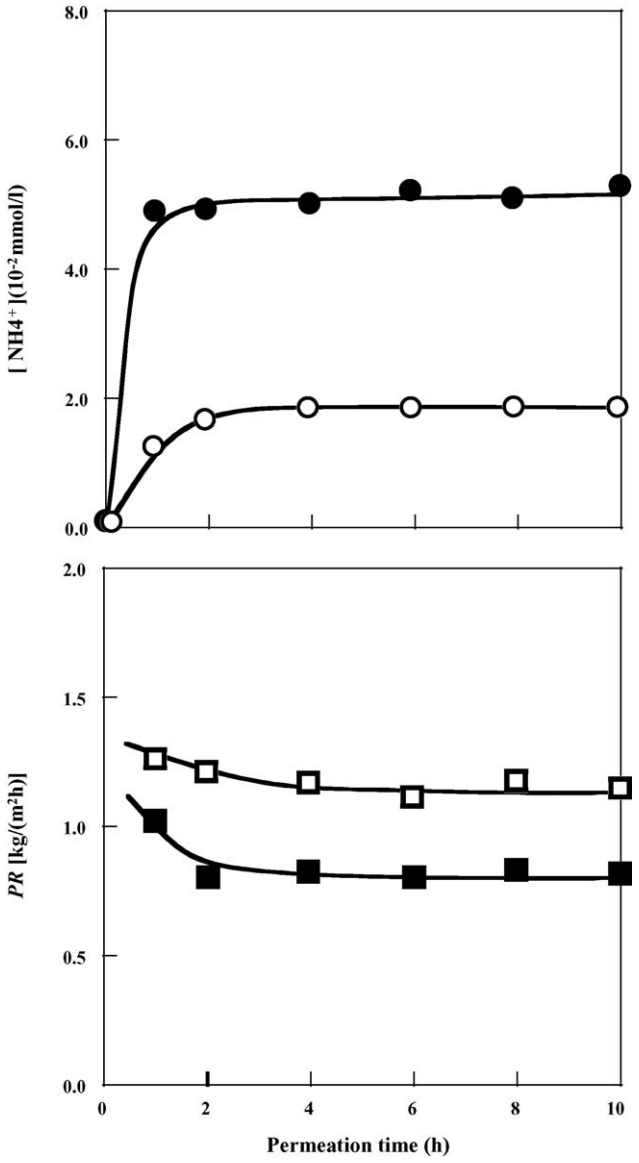


Fig. 7. Effects of permeation time on permeation and hydrolysis characteristics of an aqueous urea solution through urease-immobilized poly(VU-AAm-HEMA) membranes containing 20 mol% HEMA to AAm and urease-immobilized polyion complex membranes in ultrafiltrations: (□) permeation rate for urease-immobilized poly(VU-AAm-HEMA) membrane; (○) ammonium ion concentration in permeate for urease-immobilized poly(VU-AAm-HEMA) membrane; (■) permeation rate for urease-immobilized polyion complex membrane; (●) ammonium ion concentration in permeate for urease-immobilized polyion complex membrane. Operating conditions: 25 mm mol/l urea solution, 37 °C, pH 7.4, 1 kg/cm^2 under N_2 gas.

behave relatively freely because of entrapment immobilization but the behavior of the urease molecule in the former is limited because of covalent immobilization.

The permeation rate of urease-immobilized poly(VU-AAm-HEMA) membrane was constant regardless of pH change, but that of urease-immobilized polyion complex membrane was dependent on pH. Such a difference of the permeation rate with pH change can be attributed to a difference of membrane structure based on a change of dissociation state of polyion complex membrane.

3.5. Effect of permeation time

In Figs. 5 and 6, optimum temperatures and pHs for both urease-immobilized membranes were determined 37 °C and 7.5, respectively. Thus, effects of permeation time on permeation rate and hydrolysis characteristics of an aqueous urea solution through both urease-immobilized membranes during ultrafiltrations under the above optimum conditions are shown in Fig. 7 at initial permeation time, a little decrease in the permeation rate of both urease-immobilized membranes was observed. This decrease is due to the compaction of polymer gel membranes swollen with water under the permeation pressure. After about 2–4 h, the permeation rates of both urease-immobilized membranes were constant. The permeation rate of urease-immobilized poly(VU-AAm-HEMA) membrane was higher than that of urease-immobilized polyion complex membrane. On the other hand, the hydrolysis rate approximately corresponded to the behavior of permeation rate. This result suggests that the hydrolysis of urea molecules in the urease-immobilized membranes is significantly related to the residence time of urea molecules in the urease-immobilized membranes. The hydrolysis rates of the urease-immobilized polyion complex membrane was greater than that of the urease-immobilized poly(VU-AAm-HEMA). As can be seen from Fig. 7, it is found that the permeation and hydrolysis characteristics of an aqueous urea solution through both urease-immobilized membranes during ultrafiltration reach after a few hours in the permeation time.

3.6. Analysis of permeation and hydrolysis characteristics

When the rate of hydrolysis for a substrate through an enzyme-immobilized membrane is determined, the residence time of the substrate molecule in the enzyme-immobilized membrane corresponds to the reaction time of the batch reaction. The hydrolysis rate, V (mol/(l s)) of the substrate through the enzyme-immobilized membrane is defined as Eq. (3):

$$V = \frac{\mathrm{HA}}{T_\mathrm{r}} \tag{3}$$

where HA (mol/l) is the hydrolyzed amount of the substrate and T_r (s) is the residence time of the substrate in the enzyme-immobilized membrane as expressed by Eq. (4):

$$T_\mathrm{r} = \frac{Al}{\mathrm{PR}'} \tag{4}$$

where A (cm^2), l (cm) and PR′ (cm^3/s) are the membrane area, membrane thickness and amount of permeate per the permeation time, respectively.

Fig. 8 shows the effect of the initial urea concentration on the hydrolysis rate through both the urease-immobilized poly(VU-AAm-HEMA) membrane and the urease-immobilized polyion complex membrane in the ultrafiltration. As can be seen from Fig. 8, in low concentrations of urea in the feed solution hydrolysis was dependent on the urea concentration but high feed urea concentrations did not affect the rate of hydrolysis. These hydrolytic profiles

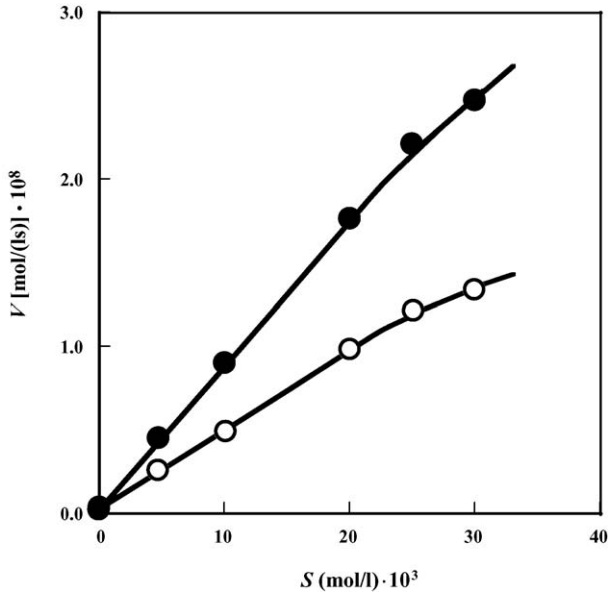


Fig. 8. Relationships between initial urea concentrations and hydrolysis rates through urea-immobilized poly(VU-AAm-HEMA) membranes containing 20 mol% HEMA to AAm (○) and urease-immobilized polyion complex membranes (●) in ultrafiltrations. Operating conditions: pH 7.4, 37 °C, 1 kg/cm^2 under N_2 gas.

through the above two urease-immobilized membranes in the ultrafiltration suggest a first order rate equation or Michaelis–Menten kinetics. In order to confirm whether hydrolysis of the urea molecule through both membranes can be explained by conventional enzyme kinetics, results from Fig. 8 were transformed to Lineweaver–Burk plots.

Fig. 9 shows Lineweaver–Burk plots for the hydrolysis of urea through urease-immobilized poly(VU-AAm-HEMA)

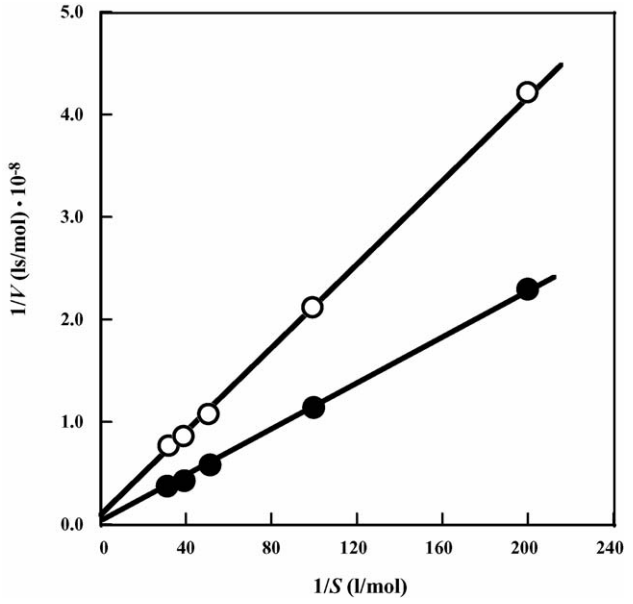


Fig. 9. Lineweaver–Burk plots for the hydrolysis of urea through urea-immobilized poly(VU-AAm-HEMA) membranes containing 20 mol% HEMA to AAm (○) and urease-immobilized polyion complex membranes (●) in ultrafiltrations.

Table 1
Kinetic data for the hydrolysis of urea through urea-immobilized poly(VU-AAm-HEMA) membranes containing 20 mol% HEMA to AAm and urease-immobilized polyion complex membranes in ultrafiltrations

Membrane	K_m (mmol/l)	$1/K_m$ (l/mmol)	V_{max} (mol/(l s))
Urease-immobilized polyion complex membrane	1.27	0.78	11.10×10^{-7}
Urease-immobilized poly(VU-AAm-HEMA) membrane HEMA: 20 mol%	0.92	1.08	4.43×10^{-7}

membranes and the urease-immobilized polyion complex membranes. As can be seen from this figure, Lineweaver–Burk plots for the urease-immobilized membranes result in good linear relationships. These results support that hydrolysis of urea through urease-immobilized membranes obtained by ultrafiltration obey Michaelis–Menten kinetics.

Kinetic data for hydrolysis reactions of urea molecules through the above two urease-immobilized membranes in the ultrafiltration were determined from intercepts of axes in Fig. 9 and are listed in Table 1.

In general, the enzymatic reaction is represented by Eq. (5):

$$\mathrm{E} + \mathrm{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_3} \mathrm{E} + \mathrm{P} \tag{5}$$

where E, S and P are the enzyme, substrate and product, respectively. k_1, k_2 and k_3 are the rate constants for each reaction. The maximum rate, V_{max}, is given by Eq. (6):

$$V_{max} = k_3[\mathrm{E}] \tag{6}$$

Eq. (6) means that the V_{max} value is significantly dependent on the enzyme concentration. In Table 1, the V_{max} value for the hydrolysis reaction of the urea molecule through the urease-immobilized polyion complex membrane was about 2.5 times of that through the urease-immobilized poly(VU-AAm-HEMA) membrane.

On the other hand, a Michaelis constant, K_m, is expressed by Eq. (7):

$$K_m = \frac{k_2 + k_3}{k_1} \tag{7}$$

The reciprocal of the Michealis constant, $1/K_m$, is evaluated as an affinity constant between the substrate molecule and the enzyme molecule. The $1/K_m$ value in the urease-immobilized poly(VU-AAm-HEMA) membrane was greater than that in the urease-immobilized polyion complex membrane. This result suggests that the former membrane has a stronger affinity for the urea molecule. On the basis of the above kinetic data such as V_{max} and $1/K_m$ values for the urease-immobilized membranes, it seems difficult to analyse static systems such as batch reactions versus kinetic systems such as enzymatic reactions through enzyme-immobilized membranes.

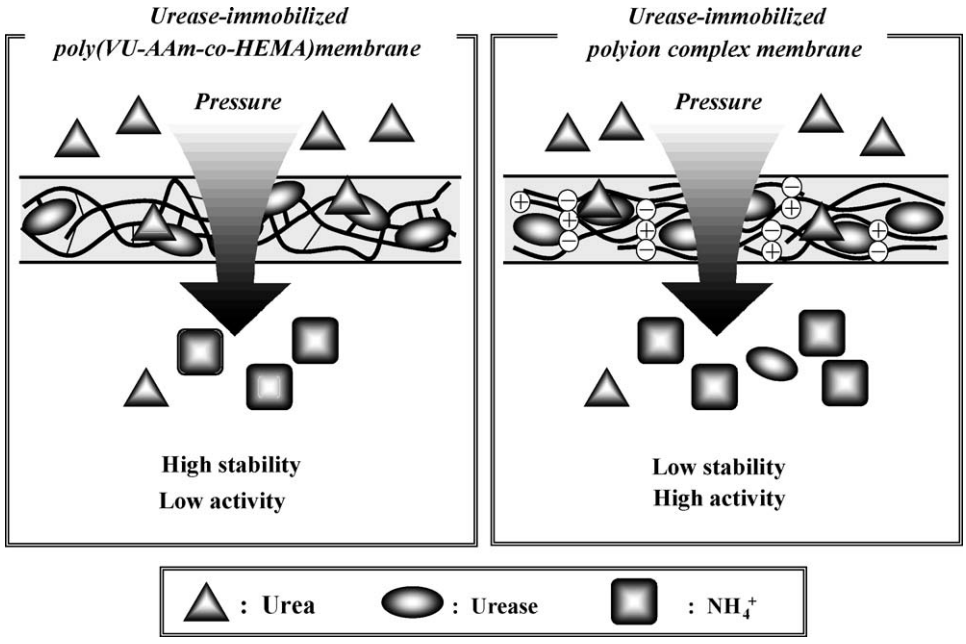


Fig. 10. Illustration for performance of the urea-immobilized poly(VU-AAm-HEMA) membranes and urease-immobilized polyion complex membranes.

4. Conclusions

In this study, two urease-immobilized polymer membranes were prepared. They were a immobilized poly(VU-AAM-HEMA) membrane and a urease-immobilized polyion complex membrane. Permeation and hydrolysis characteristics of aqueous urea solutions through the urease-immobilized membranes were kinetically investigated under various conditions. The hydrolysis rate of urea through urease-immobilized membranes followed Michaelis–Menten kinetics. When comparing kinetic data for the hydrolysis of urea through these membranes, enzymatic activities of urease-immobilized poly(VU-AAm-HEMA) membranes were lower than those of urease-immobilized polyion complex membranes, but stabilities of the former were higher than those of the latter. Illustrations of these membrane performances are shown in Fig. 10. These differences could be significantly attributed to differences in the higher-structure of urease molecules obtained during immobilization. Although the enzyme activity in conventional and membrane reactors cannot be discussed in a similar way, the membrane reactor has advantages such as the keeping of the stability of enzyme, and the ease of separation of the enzyme and product and the recovery of the enzyme. Therefore, in the near future, if the immobilized amount of enzyme in polymer membranes can be increased, the performance of the enzyme immobilized membrane reactor will be improved.

References

[1] I. Chibata (Ed.), Immobilized Enzyme, Kodansha, 1975.
[2] Immobilized enzyme, K. Mosbach (Ed.), Methods in Enzymology, vol. 44, Academic press, 1976.
[3] S. Fukui, I. Chibata, S. Suzuki (Eds.), Enzyme Technology, Kagaku Dojin, Tokyo, 1981.
[4] T. Uragami, Bio Ind. 3 (22) (1986) 33.
[5] S. Di Martino, H. El-Sheriff, N. Diane, A. De Maio, V. Grano, S. Rossi, U. Bencivenga, A. Mattei, D.G. Mita, Appl. Catal. B: Environ. 46 (2003) 613.
[6] T. Godjevargova, K. Gabrovska, J. Biotech. 103 (2003) 107.
[7] J.-P. Chen, S.-H. Chiu, Enzym. Microb. Technol. 26 (2000) 359.
[8] C.-C. Lin, M.-C. Yang, Biomaterials 24 (2003) 1989.
[9] K. Senjyu, Colloid Titration Method, Nankoudou, 1969.
[10] S.G. Shoemaker, A.S. Hoffman, J.H. Priest, Appl. Biochem. Biotechnol. 15 (1987) 11.
[11] A.K. Hazra, S.P. Chock, R.W. Albers, Anal. Biochem. 137 (1984) 437.
[12] T. Uragami, T. Aketa, S. Gobodami, T. Sugihara, Polym. Bull. 15 (1986) 101.
[13] T. Uragami, S. Yamamoto, T. Miyata, Biomacromolecules 4 (2003) 137.
[14] T. Uragami, K. Fijino, M. Sigihara, Angew. Makromol. Chem. 55 (1976) 29.
[15] T. Uragami, M. Tamura, M. Sugihara, Angew. Makromol. Chem. 55 (1976) 59.
[16] T. Uragami, M. Tamura, M. Sugihara, J. Membr. Sci. 4 (1979) 305.
[17] G.W. Watt, Anal. Chem. 26 (1954) 456.
[18] C.-C. Lin, Anal. Chem. 39 (1967) 971.