# Alternative Molecularly Imprinted Membranes from a Derivative of Natural Polymer, Cellulose Acetate

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**ABSTRACT:** Molecularly imprinted polymeric membranes were prepared from cellulose acetate (CA), of which acetyl content was 40%, by applying the alternative molecular imprinting technique. The *Z*-D-Glu imprinted polymeric membranes thus obtained recognized D-Glu in preference to L-Glu from racemic Glu mixtures and vice versa. The affinity constants between Glu and the chiral recognition site for two kinds of membranes were determined to be $3.1 \times 10^3$ $mol^{-1}$ $dm^3$ from the adsorption isotherm of D-Glu or L-Glu in the molecularly imprinted CA membranes. Enantioselective electrodialysis was attained with the present membranes reflecting their adsorption selectivity. D-Glu was preferentially permeated through the *Z*-D-Glu imprinted CA membrane, whereas L-Glu was permeated through the *Z*-L-Glu imprinted CA membrane. The present study suggests that the molecularly imprinted CA membranes are applicable to the optical resolution of racemic amino acids. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 72: 493–499, 1999

**Key words:** molecular imprinting; chiral recognition; optical resolution; electrodialysis; enentioselective permeation; membrane; amino acids

## INTRODUCTION

Development of novel membrane materials for optical resolution has attracted much attention in connection with the pharmaceutical industry, food preparation, agricultural chemicals, and so forth. The optical resolution was investigated by both liquid[1–4] and polymeric membranes.[5–36] It is necessary to introduce physical stereoselectivity into synthetic membranes so that they can separate mixtures of optically active compounds. The authors adopted oligopeptides,[23,25,27,30,33,34,36] a derivative of natural polymer,[31] and nonchiral synthetic polymer[35] as candidate materials to form chiral recognition sites; these materials were converted into chiral recognition sites toward amino acids or amino acid derivatives by applying an alternative molecular imprinting technique. That is, *molecular memory* of the print molecule, which is separated or recognized, is introduced in the membrane simultaneously while the polymeric membrane is prepared from its polymer solution (i.e., membrane preparation with simultaneous molecular imprinting).

It was reported preliminarily that chiral recognition and enantioselective permeation of racemic amino acid were attained by cellulose acetate (CA) membranes imprinted by Boc-L-Glu. It is interesting to study whether the CA membrane having D-isomer recognition site can be obtained by using D-isomer as a print molecule and vice versa like membranes with oligopeptides[33] or nonchiral synthetic polymer.[35] To this end, molecularly imprinted CA membranes were prepared using *N*-α-*Z*-D- or *N*-α-*Z*-L-glutamic acid as a print molecule, and their abilities of chiral recognition and enantioselective separation of racemic glutamic acids were investigated.

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## EXPERIMENTAL

### Materials

The CA, of which acetyl content was 40%, was purchased from Wako Pure Chemical Industries, Ltd., and used without purification. *N*-α-*Z*-D-glutamic acid [*Z*-D-Glu] or *N*-α-*Z*-L-glutamic acid [*Z*-L-Glu], purchased from Calbiochem–Novabiochem AG, was used as a print molecule in this study. D-Glu, L-Glu, sodium azide, copper sulfate, methanol, ethanol, and tetrahydrofuran (THF) were used without purification. Distilled water was employed.

### Preparation of Molecularly Imprinted Polymeric Membranes

The polymeric membranes studied in this article were prepared from THF solution. The mol ratios of print molecule to unit mol of cellulose in membrane preparation process were fixed to be 0.5, 1.0, and 3.0. As for the unit mol of cellulose, a single repeating unit of D-glucose ring was adopted in the present study. Two hundred milligrams of CA and a prescribed amount of print molecule, *Z*-D-Glu or *Z*-L-Glu, were dissolved in 2 $cm^3$ of THF. The amounts of the print molecule were 132, 264, and 792 mg for the ratios of 0.5, 1.0, and 3.0, respectively. The THF solution thus prepared was poured into a flat laboratory dish, of which the diameter was 8.9 cm, and the solvent was allowed to evaporate at ambient temperature for 24 h. The obtained membrane was further dried at 50°C for 2 h. After drying, the print molecule was extracted from the resultant membrane by a known large volume of methanol until the print molecule could be hardly detected in methanol by ultraviolet (UV) analysis. In the present study, 89.3% of added *Z*-L-Glu was recovered from the membrane, of which the imprinting ratio was 0.5; 99.6% of added *Z*-D-Glu for the ratio of 3.0, and 96.0% of added *Z*-L-Glu for that of 3.0. As for the other three membranes, the print molecule was nearly quantitatively recovered from the membranes. Thickness of the membrane thus obtained was 102–114 μm.

### Adsorption of Racemic Mixtures to Membranes

The molecularly imprinted polymeric membranes were immersed in the racemic Glu solution, which was the same mixture studied in the electrodialysis (i.e., a 50 vol % aqueous ethanol solution of racemic Glu, with concentrations of 1.0 mmol $dm^{-3}$) and the mixture was allowed to be equilibrated at 40°C during 216 h. Aliquots of the solution at the initial stage and after 216 h were used for quantitative estimation by high-performance liquid chromatography (HPLC) (JASCO PU 980) equipped with a UV detector (JASCO UV 970) by using a TSKgel Enantio L1 column {150 × 4.6 [inside diameter (i.d.)] mm} (Tosoh Corp.) and aqueous copper sulfate solution as an eluent.

The amount of Glu in the supernatant subtracted from the amount initially in the solution gave the amount of Glu adsorbed in the membrane.

Adsorption selectivity $S_{A(i/j)}$ is defined as

$$S_{A(i/j)} = ((i\text{-Glu})/(j\text{-Glu}))/(C_{i\text{-Glu}}/C_{j-\text{Glu}})$$

where ($i$-Glu) and ($j$-Glu) are the amounts of Glu in the membrane. The $C_{i\text{-Glu}}$ and $C_{j\text{-Glu}}$ denote the concentration in the solution after equilibrium was reached.

### Adsorption Isotherms of D-Glu and L-Glu

The membrane was immersed in various concentrations of pure D-Glu or L-Glu solution and allowed to equilibrate at 40°C for 216 h. The 0.02 wt % of sodium azide was added as a fungicide. The quantitative analyses were done as described above.

### Enantioselective Electrodialysis

A 50 vol % aqueous ethanol solution of racemic amino acids was placed in both chambers of the permeation cell. Each concentration of racemic amino acid was fixed to be 1.0 mmol $dm^{-3}$. The electrodialysis was carried out with a prescribed applied voltage between platinum black electrodes (10-mm square; distance between the electrodes, 65 mm for single direction electrodialysis and 90 mm for dual direction electrodialysis) at 40°C with stirring. Aliquot was drawn from the permeate side at each sampling time. The amounts of D- and L-Glu that permeated through the membrane were estimated on an HPLC instrument described above.

The separation factor $\alpha_{i/j}$ is defined as the flux ratio $J_i/J_j$ divided by the concentration ratio $C_i/C_j$.

$$\alpha_{i/j} = (J_i/J_j)/(C_i/C_j)$$

## RESULTS AND DISCUSSION

### Adsorption Selectivity of Racemic Glu

The dependence of adsorption of racemic Glu on imprinting conditions is summarized in Figure 1.

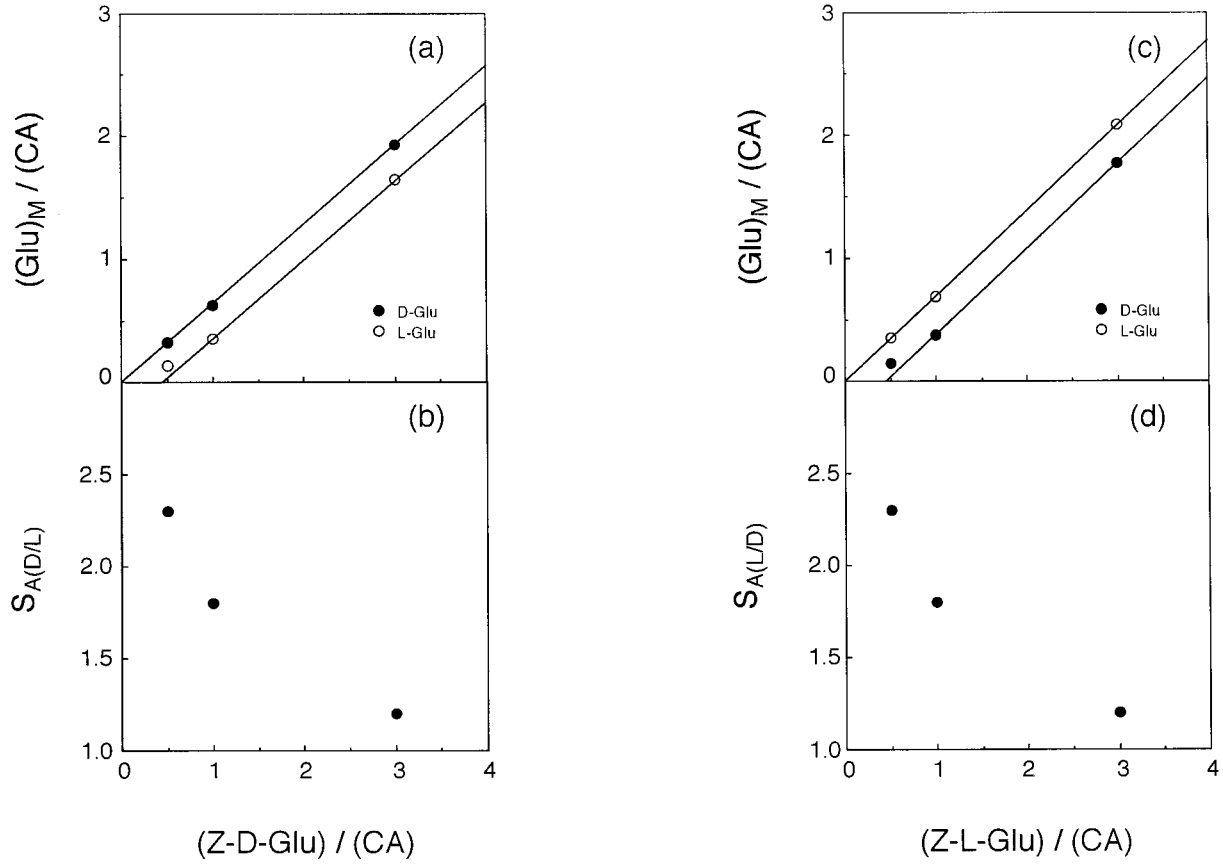


**Figure 1** Effect of the membrane preparation condition on Glu adsorption (a), on adsorption selectivity toward D-Glu (b) of the membrane imprinted by *Z*-D-Glu and that on Glu adsorption (c), and on adsorption selectivity toward L-Glu (d) of the membrane imprinted by *Z*-L-Glu.

The results for *Z*-D-Glu imprinted CA membranes are shown in Figure 1(a) and those for *Z*-L-Glu imprinted CA membranes in Figure 1(c), respectively. In Figure 1, the amounts of adsorbed Glu by the membrane are given in relative ones, which were converted to those of a single repeating unit of D-glucose ring basis. All plots give straight lines; that is, the adsorbed amounts increase linearly with the increase in the molecular imprinting ratio. These two membranes show adsorption selectivity (i.e., give chiral recognition). The membrane imprinted by (*Z*)-D-Glu recognizes the D-isomer in preference to the corresponding D-isomer, and that imprinted by (*Z*)-L-Glu recognizes the L-isomer in preference to the D-isomer. As observed in nonchiral carboxylated polysulfone membranes,[35] the membrane imprinted by the D-isomer recognizes the D-isomer and vice versa. The adsorption selectivities for both membranes ($S_{A(i/j)}$) increase from 1.2 to 2.3 with the decrease in the molecular imprinting conditions from 3.0 to 0.5. In the *Z*-D-Glu imprinted membranes, the excess amount of amino acid preferentially adsorbed by the membrane was 0.29 times that of the D-glucose ring in the membrane. In the L-isomer imprinted membrane, the excess amount was 0.31 times of the D-glucose ring in the membrane.

### Adsorption Isotherms of D-Glu and L-Glu

From the results shown in Figure 1, it can be said that the chiral recognition site was formed by the presence of the print molecule in the membrane preparation process. The tentative schemes for formation of molecularly imprinted polymeric membranes and the chiral recognition are shown in Figure 2. The hydrogen bonding between the carboxyl group in Glu and the hydroxyl and/or carbonyl group in CA and the absolute configuration of side chain of Glu might be the dominant factor to the chiral recognition of racemic Glu. It

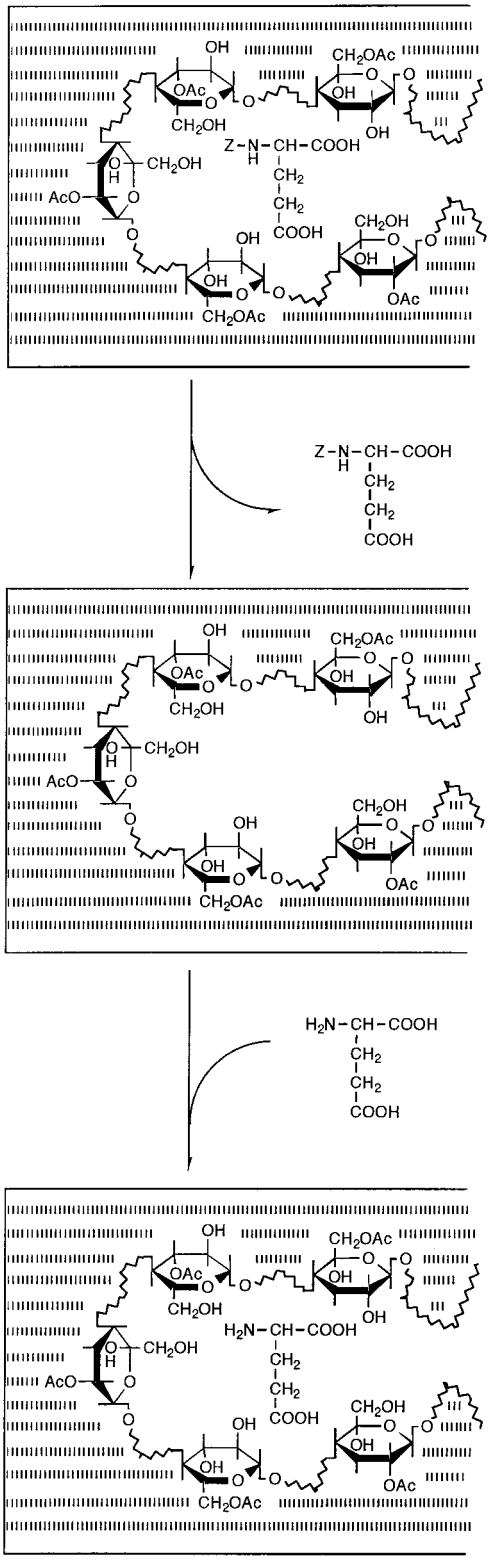


**Figure 2** Tentative scheme of the formation of molecularly imprinted CA membrane and its chiral recognition.

is interesting to study the substrate specificity of the recognition site. To this end, adsorption isotherms of D-Glu and L-Glu in *Z*-D-Glu or *Z*-L-Glu imprinted CA membrane, of which molecular imprinting condition (print molecule)/(CA) was fixed to be 0.5, were investigated.

The adsorption isotherms for the D-isomer imprinted membrane are shown in Figure 3 and those for the L-isomer imprinted membrane in Figure 4, respectively. The adsorption isotherm of each Glu in the membranes imprinted with the opposing optical isomer are straight lines passing through the origin. This implies that L-Glu in Figure 3 and D-Glu in Figure 4 were adsorbed in the membrane without any specific interaction with the imprint site. On the other hand, the adsorption isotherms of D-Glu in Figure 3 and that of L-Glu in Figure 4 in the like optical isomer-imprinted membrane show a complicated profile. This implies that the adsorption consists of nonspecific adsorption combined with adsorption on specific recognition sites toward the same print molecule family, which has the same absolute configuration as the print molecule. The linear isotherm for L-Glu in Figure 3 and D-Glu in Figure 4 can be represented by the following equation:

$$[\text{Glu}]_{M,i} = k_D[i\text{-Glu}]$$

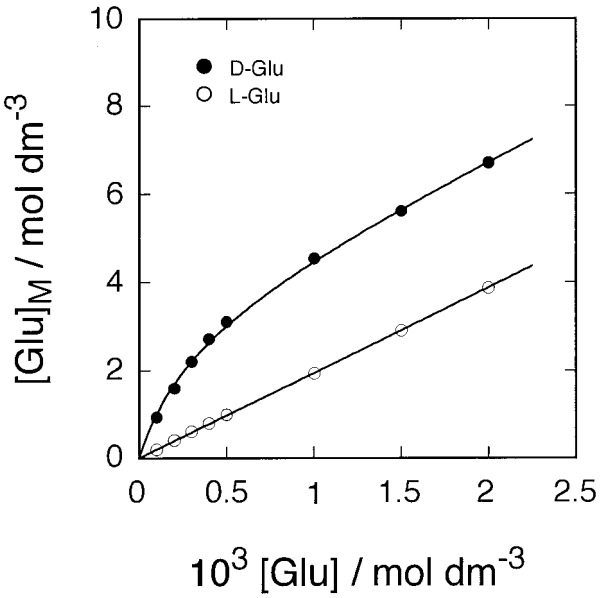


**Figure 3** Adsorption isotherms of Glu's on the CA membrane imprinted by *Z*-D-Glu. [(*Z*-D-Glu)/(CA) = 0.5; $k_D = 1.9 \times 10^3$; $n = 0.26$; $K_C = 3.1 \times 10^3$ $\text{mol}^{-1}$ $\text{dm}^3$; [CA] = $1.3 \times 10$ mol $\text{dm}^{-3}$.]

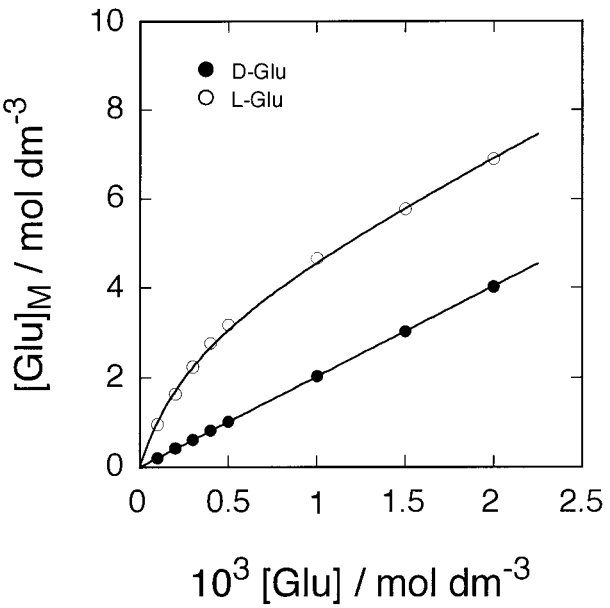


**Figure 4** Adsorption isotherms of Glu's on the CA membrane imprinted by *Z*-L-Glu. [(*Z*-L-Glu)/(CA) = 0.5; $k_D = 2.0 \times 10^3$; $n = 0.26$; $K_C = 3.1 \times 10^3$ $\text{mol}^{-1}$ $\text{dm}^3$; [CA] = $1.3 \times 10$ mol $\text{dm}^{-3}$.]

where $[\text{Glu}]_{M,i}$ is the concentration of *i*-Glu adsorbed in the membrane, $k_D$ denotes the adsorption constant, and [*i*-Glu] is the concentration of *i*-Glu in the solution equilibrated with the membrane. For the nonlinear isotherm, the concentration of D-Glu adsorbed in *Z*-D-Glu imprinted membrane (Figure 3) and that of L-Glu in *Z*-L-Glu one (Figure 4) can be represented by the following equation:

$$[\text{Glu}]_{M,j} = k_D[j\text{-Glu}] + nK_C[\text{CA}][j\text{-Glu}]/(1 + K_C[j\text{-Glu}])$$

where *n* is the ratio of the maximum amount of *j*-Glu adsorbed on the chiral recognition site to the amount of single repeating unit of D-glucose ring in the membrane; $K_C$ is the affinity constant between *j*-Glu and the recognition site, and [*j*-Glu] denotes the *j*-Glu concentration in the solution equilibrated with the membrane. The isotherms in Figures 3 and 4 were drawn using the parameters determined for best fit. From Figures 3 and 4 it can be concluded that the chiral recognition site in the membrane exclusively recognized the isomer that has the same absolute configuration as that of the print molecule, and the corresponding isomer was not incorporated in the recognition site.

### Electrodialysis

Electrodialysis of racemic Glu was studied as one of the applications of the present molecularly imprinted CA membranes to membrane separation. Enantioselective electrodialysis of racemic Glu is shown in Figure 5 as a function of applied potential difference. The total flux values through both membranes were linearly proportional to the applied potential difference $\Delta E$. Over levels of 12.5 V of $\Delta E$, enantioselective permeation was scarcely observed. However, the separation factor of $\alpha_{D/L}$ or $\alpha_{L/D}$ increased with the decrease in $\Delta E$, and below 2.5 V of $\Delta E$ the separation factors for both membranes reached 2.3, which was equal to their adsorption selectivities. The optimized potential difference can make it possible to attain enantioselective permeation, which should be reflected by their adsorption selectivities. However, the relatively high potential difference was too much for enantioselective permeation to be reflected in the adsorption selectivity and, as a result, the permselectivity of the membrane decreased with the increase in the applied potential difference. Finally, permselectivity reached unity at over 12.5 V of $\Delta E$. It also is of interest to permeate D- and L-Glu simultaneously from a racemic feed solution. That is, the feed side, middle chamber (M), of the sketch in Figure 6, is laid out in between two permeate sides. The L-Glu permselective membrane, which was imprinted by *Z*-L-Glu and recognized L-Glu in preference to D-Glu, was mounted between the L and the M sides, and the D-Glu permselective membrane was mounted between the M and R sides. The time transport curves for dual direction enantiselective electrodialysis are shown in Figure 6. As can be seen, L-Glu was transported preferentially to the L side and the D-Glu was transported simultaneously to the R side.

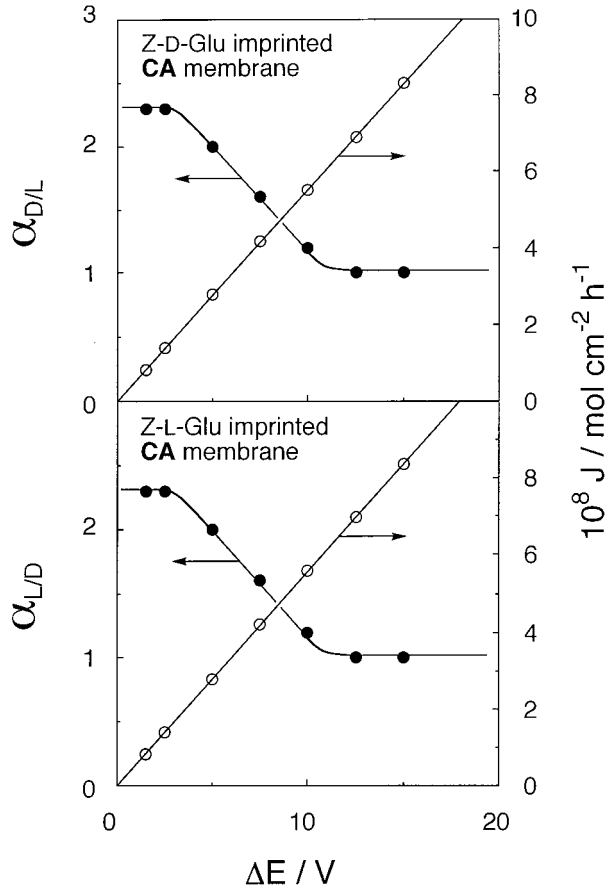


**Figure 5** Influence of the difference in applied potential on enantioselective electrodialysis of D-Glu and L-Glu. [(*Z*-D-Glu)/(CA) = (*Z*-L-Glu)/(CA) = 0.5; [Ac-D-Trp] = [Ac-L-Trp] = 1.0 mmol dm$^{-3}$.]

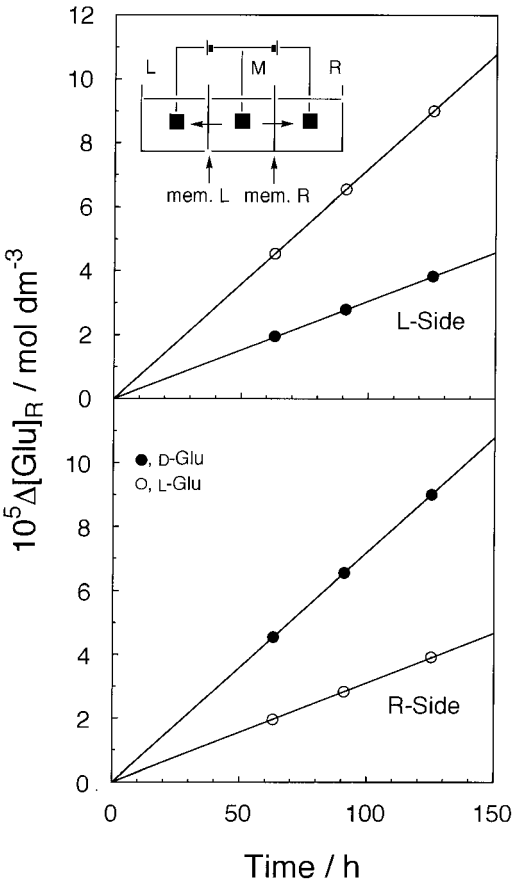


**Figure 6** Time transport curves of D-Glu and L-Glu by electrodialysis at $\Delta E$ = 2.5 V through molecularly imprinted CA membranes. [(*Z*-D-Glu)/(CA) = (*Z*-L-Glu)/(CA) = 0.5.]

## CONCLUSIONS

Molecularly imprinted polymeric membranes were prepared from CA by applying the alternative molecular imprinting technique. The *Z*-D-Glu imprinted polymeric membranes thus obtained showed adsorption selectivity toward D-Glu and vice versa. The affinity constants between Glu and the chiral recognition site for two kinds of membranes were determined to be $3.1 \times 10^3$ $mol^{-1}$ $dm^3$ from the adsorption isotherm of D-Glu or L-Glu in the molecularly imprinted CA membranes. Electrodialysis of the racemic amino acids shows the possibility that permselectivity directly reflects its adsorption selectivity. The present study suggests that the molecularly imprinted CA membranes has potential to attain the optical resolution of amino acids.

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