Polymer Bulletin 40, 517–524 (1998)

Polymer Bulletin

Carboxylated polysulfone membranes having a chiral recognition site induced by an alternative molecular imprinting technique

Masakazu Yoshikawa [1], Jun-ichiro Izumi [1], Takashi Ooi [1], Toshio Kitao [1], Michael D. Guiver [2], Gilles P. Robertson [2]

[1] Department of Polymer Science and Engineering, Kyoto Institute of Technology, Matsugasaki, Kyoto 606-8585, Japan
[2] Institute for Chemical Process and Environmental Technology, National Research Council of Canada, Ottawa, Ontario K1A OR6, Canada

Received: 22 January 1998/Accepted: 20 February 1998

SUMMARY

Molecularly imprinted polymeric membranes were prepared from carboxylated polysulfone. Membranes imprinted by Z-D-Glu recognize the D-isomer in preference to the corresponding L-isomer, and vice versa. The amino acid preferentially adsorbed by the membrane was also selectively permeated by electrodialysis.

INTRODUCTION

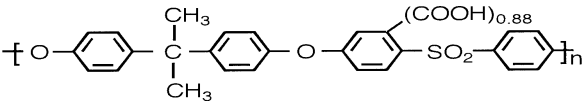
We have reported that optical resolution of racemic α-amino acids was attained by molecularly imprinted polymeric membranes, which were prepared from polystyrene resins bearing oligopeptide (1-4) or derivatives of natural polymer (5). The molecular imprinting technique, first proposed by Wulff (6), is a facile one for introducing molecular recognition sites into polymeric materials (7-9).

We have applied an alternative molecular imprinting technique to our studies (1-5) that is based on inducing "molecular memory" in a membrane substrate at the same time of gelation. An optically active imprint molecule is combined with the membrane polymer containing a functional group moiety in solution. The membrane polymer assumes a favourable conformation for the functional group to interact with the imprint molecule. Upon gelation and membrane formation, a "molecular memory" of the optical isomer is retained by the formed membrane, such that it recognizes or favours interaction with isomers of the same configuration. The present work concerns the formation of molecularly imprinted polymeric membranes obtained from an entirely non-chiral synthetic polymer having carboxyl groups. The chiral recognition ability of these imprinted polymeric membranes derived from carboxylated polysulfone was investigated.

EXPERIMENTAL

Materials

Carboxylated polysulfone, having a degree of substitution of 0.88, was prepared by the modification of polysulfone Udel P-3500 as reported previously (10).



* NRCC No. 41959

Preparation of molecularly imprinted polymeric membranes

Each polymeric membrane in the present study was prepared from tetrahydrofuran (THF) solution, containing the imprinting components. Carboxylated polysulfone acts not only as a functional polymer having hydrogen bonding sites for amino acid recognition, but also as a membrane matrix like molecularly imprinted cellulose acetate membranes (5). N-α-Benzyloxycarbonyl-D-glutamic acid (Z-D-Glu) or N-α-benzyloxycarbonyl-L-glutamic acid (Z-L-Glu) were adopted as print molecules. Mole ratios of 0.5, 1.0, and 3.0 for print molecule to unit mole of polysulfone in the membrane preparation process were studied. A 200 mg quantity of carboxylated polysulfone and a prescribed amount of print molecule, Z-D-Glu or Z-L-Glu, were dissolved in 2 cm^3 of THF. The amount of Z-Glu was 59 mg for the ratio of 0.5, 118 mg for 1.0, and 354 mg for 3.0, respectively. The THF solution thus prepared was poured into a 8.9 cm diameter flat laboratory dish, and the solvent was allowed to evaporate at ambient temperature for 24 h. The resulting membrane was further dried at 50 °C for 2 h. After drying, the print molecule was extracted from the resultant membrane by a large volume of methanol until the print molecule was scarcely detectable in methanol by UV analysis. In the present study, 82—99 % of added print molecule was leached from the membranes.

Adsorption selectivity

The molecularly imprinted polymeric membranes were immersed in a 1.0 mmol dm^{-3} racemic glutamic acid (D-/L-Glu) solution in 50 vol. % aqueous ethanol, and the mixture was allowed to equilibrate at 40 °C for 216 h. A 0.02 wt. % of sodium azide was added as a fungicide. The amount of amino acid in the supernatant subtracted from the amount initially in the solution gave the amount of amino acid adsorbed by the membrane. Quantitative measurements of aliquots of the solution at the initial stage and after 216 h were made using a high performance liquid chromatography (HPLC) instrument (JASCO PU 980) equipped with a UV detector (JASCO UV 970) and a TSKgel Enantio Ll column (150 x 4.6 (i.d.) mm) (Tosoh Corporation) with aqueous copper sulfate solution as an eluent.

Adsorption selectivity SA(ifj) is defined as

$$S_{A(ifj)} = ((i\text{-}AA) / (j\text{-}AA)) / (Ci / Cj)$$

where (i-AA) and Ci are the amount of optical isomer (i) amino acid adsorbed in the membrane and the concentration in the solution after equilibrium was reached, respectively.

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. A 0.02 wt. % of sodium azide was added as a fungicide. Quantitative analyses were done as described above.

Electrodialysis

A 50 vol. % aqueous ethanol solution of racemic Glu was placed in both chambers of the permeation cell. The concentration of racemic Glu was fixed to be 1.0 mmol dm^{-3}, as with the adsorption experiements. The electrodialysis was carried out at 40 °C with stirring, and with a constant applied voltage of 2.5 V between platinum black electrodes (10 mm square; distance between the electrodes, 65 mm for single direction electrodialysis and 90 mm for dual direction

electrodialysis). Aliquots were drawn from the permeate side at each sampling time. The amounts of D-Glu and L-Glu that permeated through the membrane (J_i, J_j) were determined on an HPLC instrument described above.

The separation factor α_{ij} is defined as the 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

The amounts of racemic Glu's and Z-Glu's adsorbed in the molecularly imprinted polymeric membranes and adsorption selectivities for the imprinted polymeric membranes are summarized in Table 1. The adsorbed amino acids are given not only in absolute amounts ($(AA)_M$ / mol), but also relative to polysulfone repeating unit ($(AA)_M$ / (PSf)). Adsorption selectivity toward print molecule or print molecule family was observed in both Z-D-Glu and Z-L-Glu imprinted polymeric membranes though the adsorption selectivities were lower compared with those by other molecularly imprinted membranes containing chiral moieties (1-5). Even though different substrates, such as Glu and Z-Glu, were adopted, the chiral recognition was attained. However, the absolute amount of each Glu adsorbed in the membrane was more than that of Z-Glu. This can be explained by the fact that the dimension of Glu is smaller than that of Z-Glu, where the amino group is protected by benzyloxycarbonyl (Z) group.

Table 1 Adsorption selectivity of amino acids from racemic mixtures in molecularly imprinted membranes

Imprinting Condition	Substrate	10^6 $(AA)_M$ / mol	$(AA)_M$ / (PSf)	$S_{A(L/D)}$
(Z-**L**-Glu) / (PSf) = 3.0	**D**-Glu	10.33	1.58	1.0
	L-Glu	10.53	1.61	
(Z-**L**-Glu) / (PSf) = 1.0	**D**-Glu	3.53	0.54	1.0
	L-Glu	3.66	0.56	
(Z-**L**-Glu) / (PSf) = 0.5	**D**-Glu	1.64	0.25	1.2
	L-Glu	1.96	0.30	
(Z-**L**-Glu) / (PSf) = 0.5	Z-**D**-Glu	1.44	0.22	1.2
	Z-**L**-Glu	1.77	0.27	
(Z-**D**-Glu) / (PSf) = 3.0	**D**-Glu	10.39	1.59	1.0
	L-Glu	10.20	1.56	
(Z-**D**-Glu) / (PSf) = 1.0	**D**-Glu	3.40	0.52	1.0
	L-Glu	3.27	0.50	
(Z-**D**-Glu) / (PSf) = 0.5	**D**-Glu	1.83	0.28	1.2
	L-Glu	1.50	0.23	
(Z-**D**-Glu) / (PSf) = 0.5	Z-**D**-Glu	1.57	0.24	1.2
	Z-**L**-Glu	1.31	0.20	

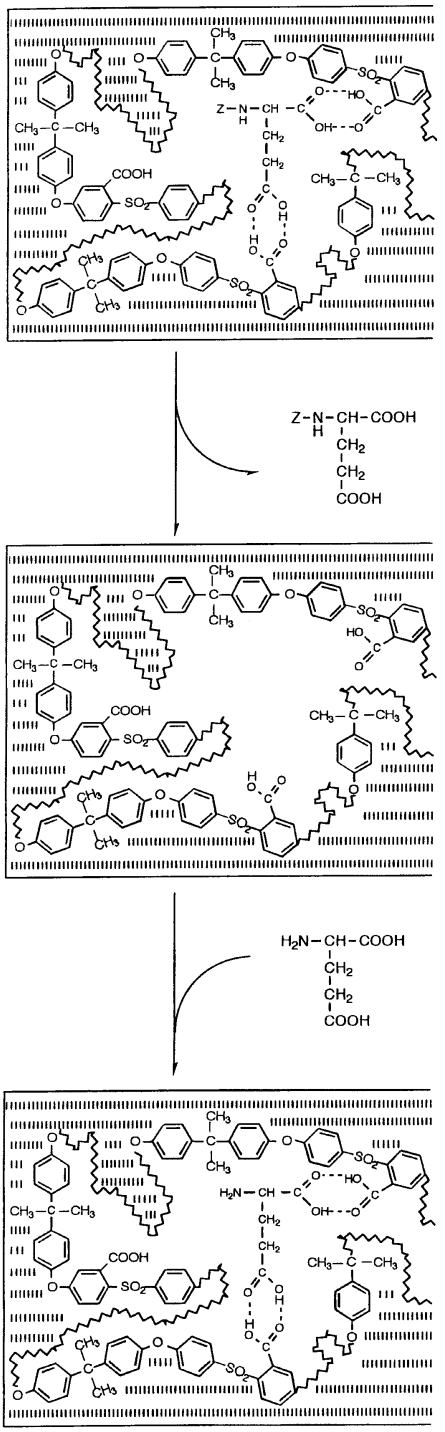


Figure 1 Tentative scheme for the formation of molecularly imprinted membrane and its chiral recognition.

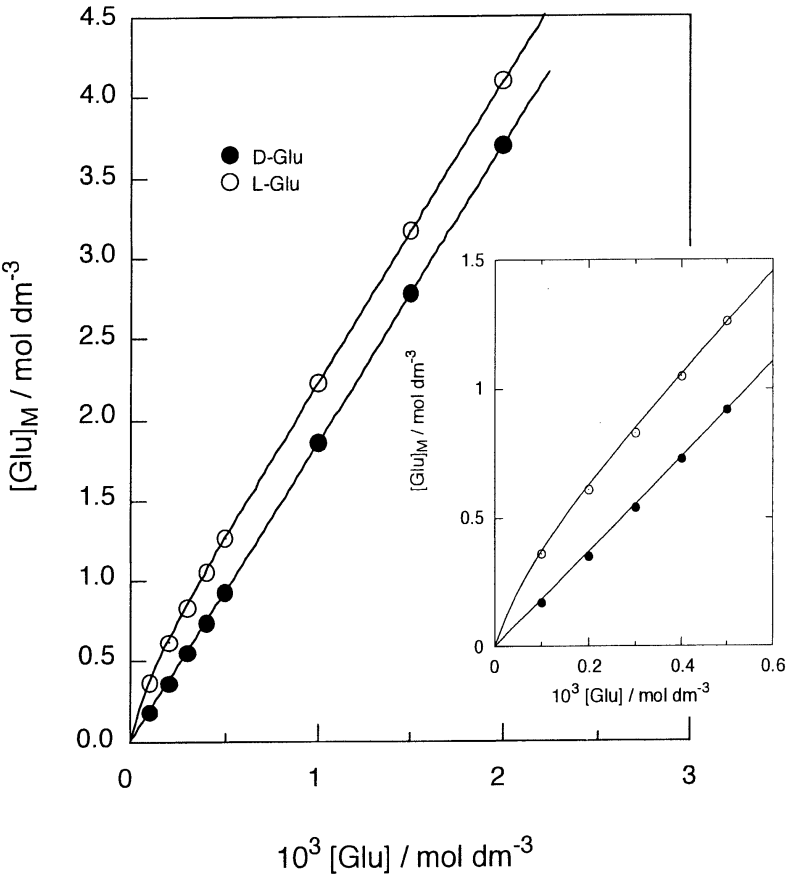


Figure 2 Adsorption isotherms of D-Glu and L-Glu in the Z-D-Glu imprinted polymeric membrane.
((Z-D-Glu) / (PSf) = 0.5; k_D = 1.7 x 10^3; [PSf] = 7.4 mol dm^{-3}; n = 5.7 x 10^{-2}; K_C = 7.9 x 10^3 mol^{-1} dm^3.)

From the results summarized in Table 1 and those reported previously (1-5), it can be said that the chiral recognition site was induced by the presence of print molecule Z-D-Glu or Z-L-Glu in the membrane preparation process. A representative scheme for the formation of molecularly imprinted polymeric membranes and the recognition of racemic amino acid mixtures are given in Figure 1. To investigate the specificity of the recognition site, adsorption isotherms of D-Glu and L-Glu in Z-D-Glu and Z-L-Glu imprinted membrane were measured and shown in Figures 2 and 3, respectively. A molecular imprinting ratio for (Z-Glu) / (PSf) was 0.5.

The adsorption isotherm of each Glu in membranes imprinted with the opposing optical isomer are straight lines passing through the origin. This implies that L-Glu in Figure 2 and D-Glu in Figure 3 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 2 and that of L-Glu in Figure 3 in the like optical isomer-imprinted membrane show a complicated profile. This implies that the

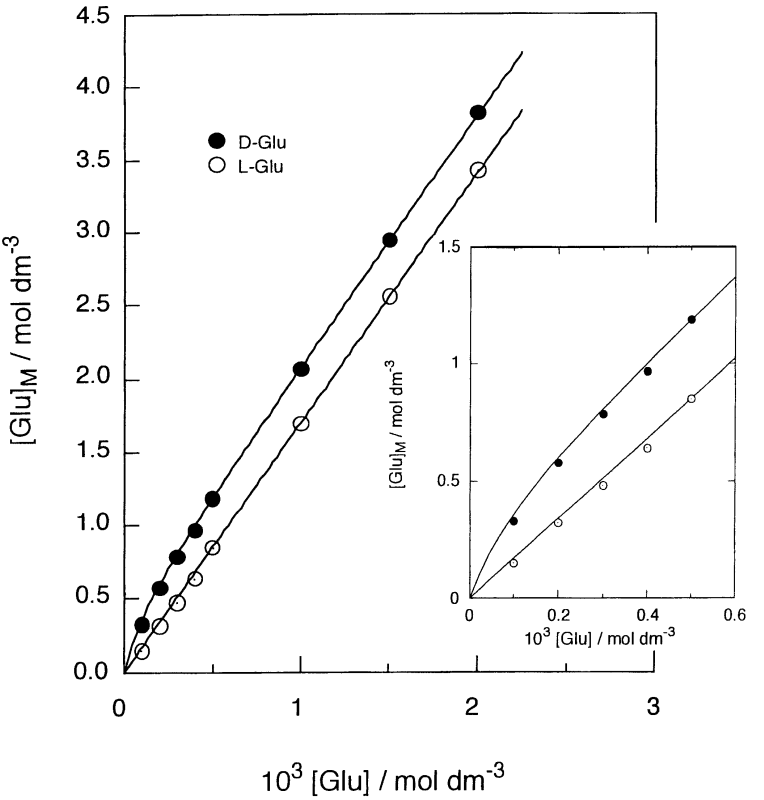


Figure 3 Adsorption isotherms of D-Glu and L-Glu in the Z-L-Glu imprinted polymeric membrane.
((Z-L-Glu) / (PSf) = 0.5; k_D = 1.8 x 10^3; [PSf] = 7.4 mol dm^{-3}; n = 5.7 x 10^{-2}; K_C = 7.9 x 10^3 mol^{-1} dm^3.)

adsorption consists of non-specific adsorption combined with adsorption on specific recognition sites toward the same print molecule family, in a comparable way to dual mode sorption of gases. (11-13). The linear isotherm for L-Glu in Figure 2 and D-Glu in Figure 3 are represented by the following equation:

$$[Glu]_{M,I} = k_D\,[I{=}Glu]$$

where $[Glu]_{M,i}$ 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 non-linear isotherm, the concentration of D-Glu adsorbed in Z-D-Glu imprinted membrane and that of L-Glu in Z-L-Glu ones can be represented by the following dual-mode equation:

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

where n is the ratio of the maximum amount of j-Glu adsorbed on the chiral recognition site to the amount of repeating PSf unit in the membrane, K_C is the affinity constant between j-Glu and the recognition site, and [D-Glu] denotes the j-Glu concentration in the solution equilibrated with the membrane. The isotherms in Figures 2 and 3 were drawn using the parameters determined for best fit. From Figures 2 and 3, it can be concluded that the chiral recognition site in the membrane exclusively recognized the isomer which has the same absolute configuration as that of the print molecule, and the corresponding isomer was not incorporated in the recognition site.

As a possible application of molecularly imprinted polymeric membranes in the chemical industry, enantioselective permeation using these membranes was studied. In the present study, the applied potential difference ΔE was fixed to be 2.5 V so that the permselectivity may directly reflect its adsorption selectivity. (2-5). The membrane imprinted by Z-D-Glu selectively permeated D-Glu, and conversely L-Glu was preferentially permeated through the membrane imprinted by Z-L-Glu. As expected, the permselectivities $\alpha_{i/j}$ for these membranes were determined to be 1.2, which were equal to the adsorption selectivities. Experimental fluxes are summarized in Table 2.

Table 2 Amino acid fluxes from racemic mixtures by single direction electrodialysis

Imprinted Membrane	Permeant	10^9 Flux / mol cm^{-2} h^{-1}
Z-D-Glu	D-Glu	5.70
	L-Glu	4.74
Z-L-Glu	D-Glu	5.46
	L-Glu	6.56
Z-D-Glu	Z-D-Glu	4.85
	Z-L-Glu	4.03
Z-L-Glu	Z-D-Glu	3.56
	Z-L-Glu	4.28

In general, the flux values for Z-Glu's were lower than those for Glu. This might be also due to the fact that the dimension of Glu is smaller than that of Z-Glu, where the amino group is protected by benzyloxycarbonyl (Z) group. This also correlates with lower observed adsorption values of Z-Glu versus Glu shown previously in Table 1. It is also of interest to permeate D- and L-isomer simultaneously from a racemic feed solution. That is, the feed side, middle chamber (M) of the sketch in Figure 4, is laid out in between two permeate sides. The L-Glu permselective membrane, which was imprinted by Z-L-Glu, was mounted between L side and M side and the D-Glu permselective membrane between M side and R side. The time transport curves for dual direction enantioselective electrodialysis are shown in Figure 4. As can be seen, L-Glu was preferentially transported to the L side and the D-Glu was simultaneously transported to the R side. This result suggests that the dual direction enantioselective electrodialysis can be used to separate racemic amino acids.

Conclusion

Molecularly imprinted polymeric membranes showing optical resolution can be prepared from non-chiral synthetic polymer, carboxylated polysulfone, by applying by an alternative molecular imprinting technique. The membrane imprinted by D-isomer recognizes D-isomer in preference to the corresponding L-isomer, and vice versa. Electrodialysis of the racemic amino acid showed that permselectivity directly reflects its adsorption selectivity.

ACKNOWLEDGEMENT

The authors are indebted to Mr. Kenji Soda for his technical assistance.

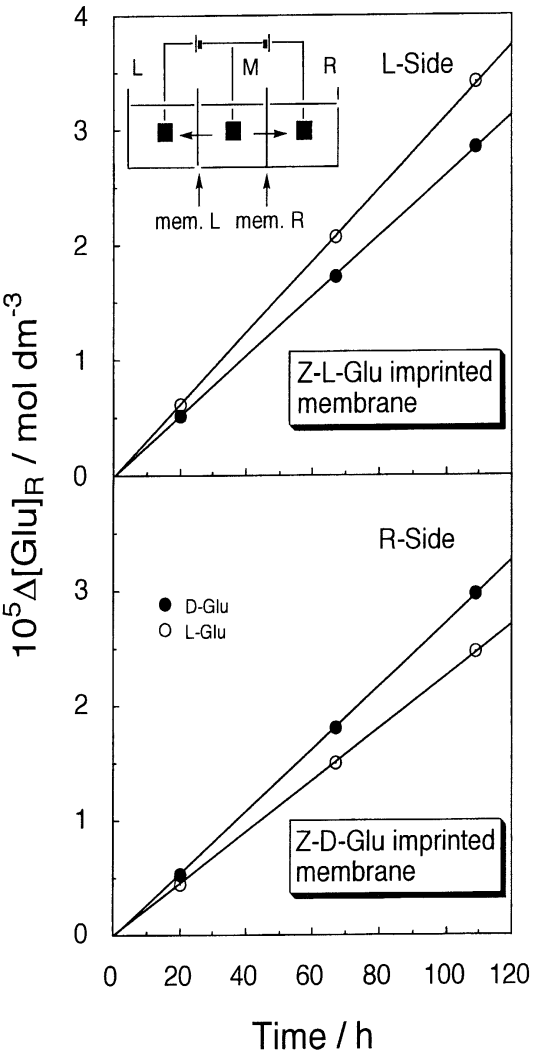


Figure 4 Time-transport curves of D- and L-Glu by dual direction electrodialysis at $\Delta E = 2.5V$.
[Membrane L, (Z-L-Glu) / (PSf) = 0.5; membrane R, (Z-D-Glu) / (PSf) = 0.5.]

REFERENCES

1. Yoshikawa M, Izumi J, Kitao T, Koya S, Sakamoto S (1995) J Membr Sci 108:171
2. Yoshikawa M, Izumi J, Kitao T, Sakamoto S (1996) Macromolecules 29: 8197
3. Yoshikawa M, Izumi J, Kitao T (1996) Chem Left 611
4. Yoshikawa M, Izumi J, Kitao T (1997) Polymer J 29: 205
5. Izumi J, Yoshikawa M, Kitao T (1997) Membrane (Maku) 22:149
6. Wulff G, Sarhan A (1972) Angew Chem 84:364
7. Mosbach K (1994) TIBS 19: 9
8. Shea K J (1994) Trends Polym Sci (1994) 2: 166
9. Wulff G (1995) Angew Chem Int Ed Engl 34: 1812
10. Guiver M D, Croteau S, Hazlett J D, Kutowy O (1990) Br Polym J 23:29
11. Koros W J, Paul D R, Rocha A A, (1976) J Polym Sci Polym Phys Ed 14: 687
12. Vieth W R, Howell J M, Hsieh J H, J Membrane Sci (1976) 1: 177
13. Paul D R (1989) Ber Bunsenges Phys Chem 83: 294