Polyurea Bearing L-Lysinyl Residue as a Chiral Building Block and Its Application to Optical Resolution

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Abstract

Chial polyureas were prepared from L-Lys-OEt and 2,4-TDI. The optimum polyaddition conditions were determined as follows: [L-Lys-OEt] = 2.08×10^{-1} mol dm^{-3} and [2,4-TDI] = 2.50×10^{-1} mol dm^{-3} ([L-Lys-OEt]/[2,4-TDI] = 0.83). The polyuera thus obtained gave a durable self-standing membrane and it was directly converted into molecular recognition membranes by applying an alternative molecular imprinting. The D-isomer molecularly imprinted membrane adsorbed the D-isomer in preference to the L-isomer and *vice versa*. In the present study, two types of print molecule, such as Z-D-Glu and Z-L-Glu, worked as print molecules though the polyurea consisted of L-lysinyl residue. Those two types of molecularly imprinted membrane showed chiral separation ability. The membrane, which was molecularly imprinted by the D-isomer and preferentially incorporated the D-isomer into the membrane, selectively transported the D-isomer and *vice versa*.

1. Introduction

Membrane separation is regarded to be ecologically and economically competitive to other conventional separation methods, since membrane separations, excepting pervaporation, are operated without phase transition. Furthermore membrane separation is continuously operated under mild conditions. In these days, membrane separation has been applied in various areas, such as desalination by reverse osmosis (RO), production of ultrapure water by nanofiltration (NF), waste water treatment by microfiltration (MF) and ultrafiltration (UF), concentration of valuables by MF and UF, concentration and removal of ionic materials by electrodialysis (ED), gas separation for recovery of H_2, concentration of O_2, and removal of CO_2, hemodialysis, hemofiltration, and so forth.

Membrane is expected to contribute to solve problems the globe has faced; (1) environmental problems, such as, air pollution (SO_x, NO_x), volatile organic compounds (VOCs), water pollution, removal of endocrine disruptor from drinking water, and so forth, (2) energy/resource problems, such as water resources, effective utilization of biomass, fuel cell, concentration difference power generation, ocean thermal energy conversion, etc., (3) health problems, such as artificial organs, artificial kidney, artificial heart, production of pharmaceuticals, production or separation of optically pure drugs, and so forth. From this, membranes contributing toward the solution of those problems can be called "Green Membranes".

In these days, membranologists have intensively focused their attentions on urgent problems, such as production of pure water and waste water treatment, and so forth. Among problems, separation of optically pure compounds is not an urgent problem to be solved but an important one to prepare for soon coming health problems. To this end, the authors' research group studied optical resolution with membranes, such as polymeric membranes with molecular recognition sites introduced by an alternative molecular imprinting (Yoshikawa & Izumi, 2003; Sueyoshi et al., 2012) and those with chiral environment or chiral selector in a main chain (Ikeuchi et al., 2009; Hatanaka et al., 2011) or in a side chain (Mizushima et al., 2011).

In the previous study, polyurea was obtained from L-lysine ethyl ester (L-Lys-OEt) and 1,4-phenylene diisocyanate (1,4-PDI) and their chiral separation ability was studied (Hatanaka et al., 2011). There are various diisocyanates as components for polyureas (Feldman & Barbalata, 1996). Among those diisocyanates, 2,4-toluene diisocyanate (2,4-TDI) was adopted as a diisocyanate component and their optical resolution ability

was studied in the present study.

2. Method

2.1 Materials

L-Lysine ethyl ester dihydrochloride (L-Lys-OEt/2HCl) was purchased from BACHEM AG and used without further purification. 2,4-TDI was purchased form nacalai tesque and used as received. N-α-Benzylocycarbonyl-D-glutamic acid (Z-D-Glu) and N-α-benzyloxycarbonyl-L-glutamic acid (Z-L-Glu) were purchased from Watanabe Chemical Industries and used without purification. Methanol, ethanol, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), D-glutamic acid (D-Glu), and L-glutamic acid (L-Glu) were obtained from commercial sources and used as received. Dimethylactamide (DMAc) and triethylamine (TEA) were purified by the conventional methods (Riddick et al., 1986). Water purified with an ultrapure water system (Simpli Lab. Millipore S. A., Molsheim, France) was used.

2.2 General Polyaddition Procedure

Requisite amounts of chemicals were placed in a reaction flask fitted with a condenser and a thermometer. The mixture was magnetically stirred at 0°C for 18h. The resulting solution was poured into water under rapid stirring, and the precipitate product was washed with water and then with methanol and dried *in vacuo*.

2.3 Characterization of the Polyurea

The viscosity number was determined with an Ubbelohde viscometer at a concentration of 5.0×10^{-3} g cm^{-3} in DMAc/LiCl (5 wt.%) at 25°C.

The IR spectrum was recorded by using a Perkin-Elmer Spectrum GX: 64 scans at a resolution of 4 cm^{-1} were collected with a membrane, which was prepared from HFIP solution.

The ^{1}H NMR spectrum was recorded in DMF-d$_7$ using a BRUKER AV-300 with residual partially protonated solvent of DMF-d$_7$ as an internal standard ($\delta = 2.91$ (Silverstein & Webster, 1998)).

The thermal stability of the polyurea was evaluated on a Hi-Res Modulated TGA 2950 (TA Instruments) under nitrogen at heating rate of 10°C min^{-1}.

Dynamic mechanical thermal analysis measurement was carried out with a dynamic mechanical analyzer, RSA III (TA Instruments) in tension mode. The experiment was performed at a frequency of 1 Hz and a heating rate of 2.0°C min^{-1} from 80 to 200°C. The dimension of sample was $19.99 \times 7.83 \times 0.088$ mm^3.

The specific rotations were obtained with Horiba SEPA-200 polarimeter at 589 nm at ambient temperature in DMF.

2.4 Membrane Preparation

The control membrane was prepared as follows: 60.0 mg of polyurea was dissolve in 1.20 cm^3 of DMAc. The polymer solution thus prepared was poured into a flat laboratory dish (6.0 cm diameter), and the solvent was allowed to evaporate at 50°C for 2 days. The thickness of the membrane thus obtained was around 25 μm.

Molecularly imprinted polyurea membranes were also prepared. In the preparation of molecularly imprinted polymeric membranes, it was requisite to know the amount of the lysinyl residue in the polyurea, which was adopted as a candidate material. It was necessary to know the amount of the lysinyl residue in the polyurea to determine the molecular imprinting ratio, which was the mole ratio of the amount of print molecule to that of lysinyl residue in polyurea. In the case of molecular imprinting by adopting chiral print molecule, the lysinyl residue in the polyurea was expected to show a dominant role to construct chiral (molecular) recognition sites. In the present study, the polyurea was prepared by step-growth addition reaction, in other words, there was no by-product, which simplified the polymerization process, even though biuret group was also formed in addition to urea linkage during the polymerization reaction (Stevens 1990; Feldman & Barbalata, 1996). The amount of lysinyl residue in the candidate polyurea (Exp. No. 5 in Table 1) was determined to be 2.61×10^{-3} mol g^{-1}, assuming that the polyaddition reaction was quantitatively proceeded, in other words, the composition of the obtained polyurea was exactly equal to the monomer composition. About 61.0 mg (1.59×10^{-4} mol of lysinyl residue) of polyurea (Exp. No. 5 in Table 1) and 22.3 mg (7.93×10^{-5} mol) of print molecule (Z-D-Glu or Z-L-Glu) were dissolved in 1.20 cm^3 of DMAc. The imprinting ratio, the mole ratio of the amount of print molecule to that of lysinyl residue in polyurea, was fixed to be around 0.50. The solution was poured into a flat laboratory dish (6.0 cm diameter) and dried at 50°C for 2 days to evaporate the solvent. After drying, the print molecule was extracted from the resultant membrane by a large amount of 50 vol.% aqueous ethanol until the print molecule was hardly detectable in aqueous ethanol solution by UV analysis. In the present study, most

added print molecule was leached from the membrane. Thickness of the membranes thus obtained was around 26 μm for the Z-D-Glu molecularly imprinted membrane and 27 μm for the Z-L-Glu one, respectively.

2.5 Adsorption Selectivity

Adsorption selectivities for those three types of membrane were studied adopting racemic mixture of glutamic acid (Glu) as model racemates. Membranes were immersed in a racemic mixture of Glu solution, similar to the mixture studied in enantioselective membrane transport, that is, a 50 vol.% aqueous ethanol solution of racemic Glu (each concentration, 1.0×10^{-3} mol dm^{-3}), and the membranes were allowed to be equilibrated at 40°C. In the present study, the amount of racemic Glu adsorbed in the membrane was too low to be determined precisely by the aliquots of the solution after equilibrium had been reached. From this, that was determined as follows: the membrane, which had reached equilibrium with racemic Glu solution, was taken out from the immersing solution, blotted free solution adhering on the surface, and then transferred to an 50 vol.% aqueous ethanol solution to desorb the racemic Glu from the membrane. Aliquots of the solution of adsorption after equilibrium had been reached and that for desorption were used for quantitative estimation by liquid chromatography (LC) [Jasco PU-2080, equipped with a UV detector (Jasco UV-2075)], using a CHIRALPAK MA(+) column [50 x 4.6 mm (i.d.)] and aqueous copper sulfate as an eluent.

The amount of Glu in the desorption solution gave the amount of Glu adsorbed by the membrane. The adsorption selectivity $S_{A(i/j)}$ is defined as:

$$S_{A(i/j)} = ((\text{i-Glu}/\text{j-Glu})) / ([\text{i-Glu}]/[\text{j-Glu}]) \tag{1}$$

where (i-Glu) and [i-Glu] are the amount of i-Glu adsorbed in the membrane and the concentration of i-Glu in the solution after equilibrium had been reached. Hereafter, subscripts of D and L mean the D- and L-isomer, respectively.

2.6 Enantioselective Transport

A membrane, of which area being 3.0cm^2, was fixed tightly with Parafilm between two chambers of a permeation cell. The volume of each chamber was 40.0cm^3. A 50 vol.% of aqueous ethanol solution of racemic Glu was placed in the left-side chamber (L-side) and a 50 vol% aqueous ethanol solution in the right-side chamber (R-side). Each concentration of racemic Glu was fixed to be 1.0×10^{-3} mol dm^{-3}. All experiments were carried out at 40°C with stirring. An aliquot was drawn from the permeate side (R-side) at each sampling time. The amounts of D-Glu and L-Glu transported through the membrane were determined by LC as described above. The flux J (mol cm^{-2} h^{-1}) is defined as:

$$J = Q / At \tag{2}$$

where Q (mol) is the amount of transported Glu, A (cm^2) the membrane area, and t (h) is the time.

The permselectivity $\alpha_{i/j}$ is defined as the flux ratio J_i/J_j divided by the concentration ratio [i-Glu]/[j-Glu]

$$\alpha_{i/j} = (J_i/J_j) / ([\text{i-Glu}]/[\text{j-Glu}]) \tag{3}$$

Table 1. Polyaddition reaction of L-Lys-OEt/2HCl and 2,4-TDI[a]

Exp. No.	[L-Lys-OEt] /[2,4-TDI]	L-Lys-OEt/2HCl g (mol dm^{-3})	2,4-TDI g (mol dm^{-3})	DMAc cm^3	TEA cm^3	Yield g (%)	η_i/C[b] g^{-1} cm^3
1	1.00	0.7416 ($2.00 \cdot 10^{-1}$)	0.5226 ($2.00 \cdot 10^{-1}$)	13.4	1.6	0.4921 (47.08)	30.20
2	1.00	0.7416 ($2.50 \cdot 10^{-1}$)	0.5222 ($2.00 \cdot 10^{-1}$)	10.4	1.6	0.6080 (58.18)	37.47
3	1.00	0.7415 ($3.00 \cdot 10^{-1}$)	0.5222 ($3.00 \cdot 10^{-1}$)	8.4	1.6	0.6164(58.99)	35.95
4	0.83	0.6179 ($1.67 \cdot 10^{-1}$)	0.5229 ($2.00 \cdot 10^{-1}$)	13.6	1.4	0.7246(75.59)	74.26
5	0.83	0.6179 ($2.08 \cdot 10^{-1}$)	0.5226 ($2.50 \cdot 10^{-1}$)	10.6	1.4	0.8065(84.17)	80.90
6	0.83	0.6179 ($2.50 \cdot 10^{-1}$)	0.5222 ($3.00 \cdot 10^{-1}$)	8.6	1.4	0.7219(75.37)	74.96
7	0.67	0.4942 ($1.33 \cdot 10^{-1}$)	0.5229 ($2.00 \cdot 10^{-1}$)	13.8	1.2	0.6610(76.04)	50.48
8	0.67	0.4941 ($1.67 \cdot 10^{-1}$)	0.5228 ($2.50 \cdot 10^{-1}$)	10.8	1.2	0.7019(80.58)	65.71
9	0.67	0.4942 ($2.00 \cdot 10^{-1}$)	0.5223 ($3.00 \cdot 10^{-1}$)	8.8	1.2	0.6883(79.05)	66.48

[a] Polymerization temp., 0°C; polymerization time, 18h.

[b] Measured at a concentration of 5.0×10^{-3} g/cm^3 in DMAc/LiCl (5 wt.%) at 25°C.

3. Results and Discussion

3.1 Polyaddition

The scheme of polymerization of L-Lys-OEt and 2,4-TDI is shown in Figure 1. The results of polymerization of L-Lys-OEt and 2,4-TDI are summarized in Table 1. Those results are visually shown in Figure 2 for convenience. In order to search the optimum condition for polyaddition reaction, the effect of monomer concentration was studied adopting equimolar amount of each monomer (Exp. No. 1–3 in Table 1). The maximal viscosity number was given at the concentration of 2.50×10^{-1} mol dm^{-3}. The molecular weight (viscosity number) of polyurea (Exp. 1–3 in Table 1) was too low to give a self-standing membrane. From the fact that the molar ratio of monomer, [L-Lys-OEt]/[2,4-TDI], was unity, most linkage formed in the polyurea was thought to be a urea one. The introduction of long side chains would both increase the molecular weight and enhance the entanglement of polyurea chains. This would lead to the construction of durable self-standing membranes like observed in previous study (Hatanaka et al., 2011). To this end, the introduction of biuret linkage into the polyurea was attempted.

The introduction of biuret linkage was achieved by the addition of excess amount of diisocyanate monomer into the reaction mixture (Stevens, 1990; Feldman & Barbalata, 1996). The polymerization with the monomer ratios, [L-Lys-OEt]/[2,4-TDI], of 0.83 and 0.67 were studied. Those results are also summarized in Table 1 (Exp. No. 4–9) and Figure 2 as well. Among those nine kinds of polyaddition reaction, the polyurea obtained under the polymerization conditions of the monomer ratio of 0.83 and the 2,4-TDI concentration of 2.50×10^{-1} mol dm^{-3} (Exp. No. 5 in Table 1) gave the highest viscosity number (molecular weight). In the present study, this polyurea (Exp. No. 5 in Table 1) was adopted as a membrane material.

In IR spectrum, a characteristic absorption band at 1736 cm^{-1} assigned to C=O stretching band of ester and that at 1643 cm^{-1} assigned to C=O stretching band of urea were detected. The ^{1}H NMR spectrum of polyurea (Exp. No. 5 in Table 1) is shown in Figure 3. From Figure 3, it was confirmed that the carboxy protecting group, ethoxy one, was preserved in the polyurea. The IR and ^{1}H NMR spectra revealed that the expected polyurea was obtained from L-Lys-OEt and 2,4-TDI. However, the stereoregularity of the polyurea and the composition of urea and biuret linkage in the polyurea were hardly determined.
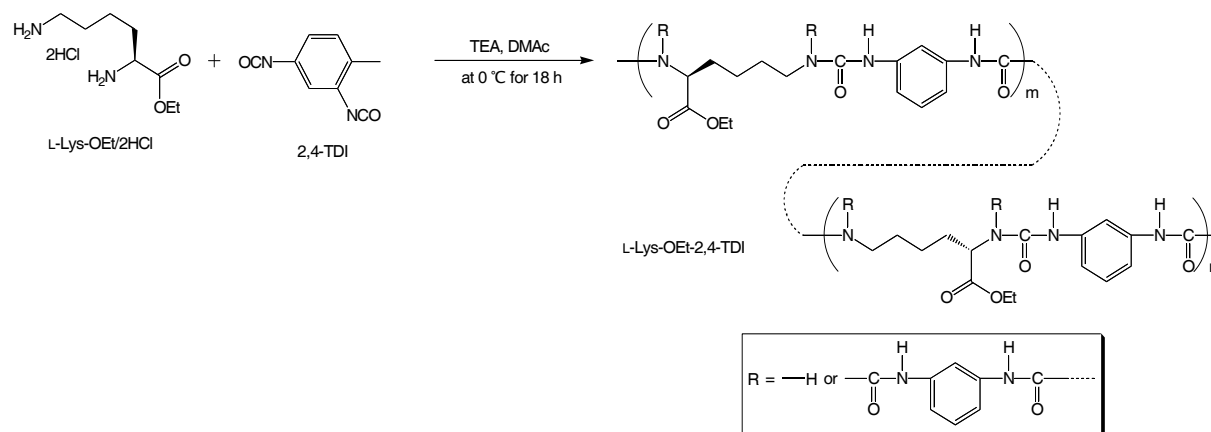

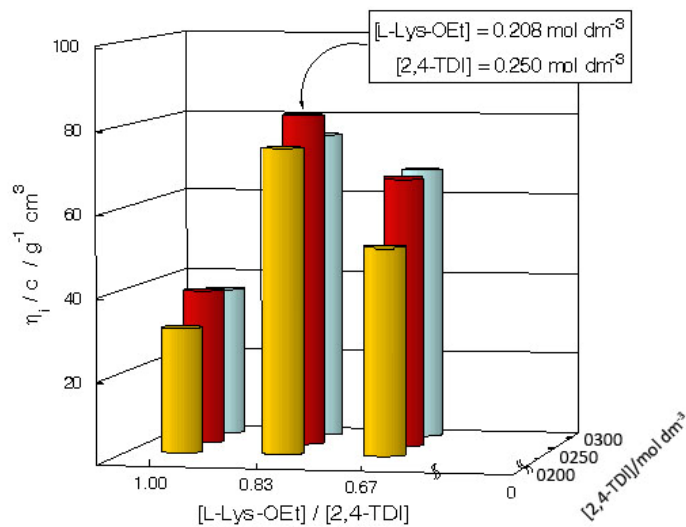Figure 1. Polymerization scheme of chiral polyurea (L-Lys-OEt-2,4-TDI)

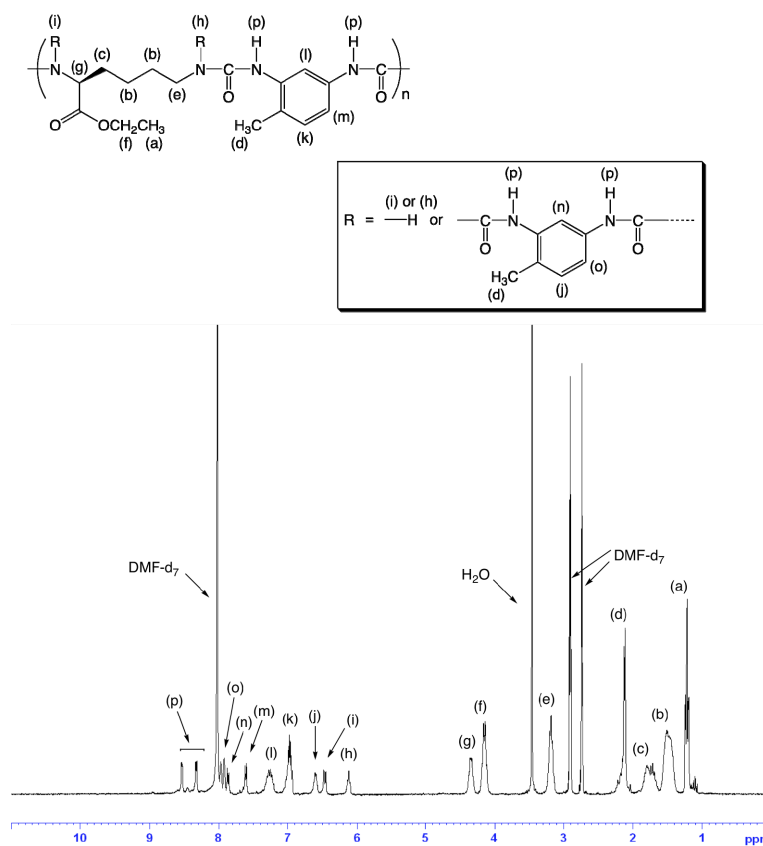Figure 2. Results of polyaddition reaction of L-Lys-OEt and 2,4-TDI

Figure 3. ^{1}H NMR spectrum of L-Lys-OEt-2,4-TDI (Exp. No. 5 in Table 1)

3.2 Characterization

Figure 4 gives thermogravimetric analysis (TGA) thermograph of polyurea (Exp. No. 5 in Table 1). From this it was concluded that thermal stability of the polyurea was not so good, which might be due to the fact that carboxy group in lysinyl residue is protected by ethoxy moiety. Comparing with the thermal stability of the previous polyurea L-Lys-OEt-1,4-PDI from L-lysine ethyl ester and 1,4-PDI (Hatanaka et al., 2011), the present urea L-Lys-OEt-2,4-TDI exhibited a higher degradation temperature of 221.4°C than the previous L-Lys-OEt-1,4-PDI (207.7°C). This might be due to the steric hindrance of the bulkiness of a methyl group in 2,4-TDI unit (Stevens, 1990; Walton & Lorimer, 2000).

Figure 5 gives the dependence of dynamic storage modulus E', dynamic loss modulus E'', and tan δ on temperature for the polyurea (Exp. No. 5 in Table 1). The signal peak of tan δ, assigned to the glass transition temperature, can be found at 155.6°C, which was lower than that of previous L-Lys-OEt-1,4-PDI (186.1°C) (Hatanaka et al., 2011). The chain flexibility of 2,4-TDI unit, which is less rigid than 1,4-PDI, might lower the glass transition temperature of the present polyurea (Stevens, 1990; Walton & Lorimer, 2000). The T_g value is more dependent on the flexibility, rather than the steric hindrance.

The optical rotation ($[\alpha]_D$) of the polyurea and the corresponding starting material of L-Lys-OEt/2HCl are given in Table 2. The results revealed that optically active polyurea was obtained. From this, the membranes from the present polyureas were expected to express chiral separation ability since there can be found chiral environment in the present polyureas.

Table 2. Specific rotations of diamine monomer and chiral polyurea

	$[\alpha]_D$
	deg cm^2 g^{-1}
L-Lys-OEt/2HCl[a]	5.6
L-Lys-OEt-2,4-TDI[b]	-5.4

[a] in DMF, c=5.00 g dL^{-1}, L=10 cm.
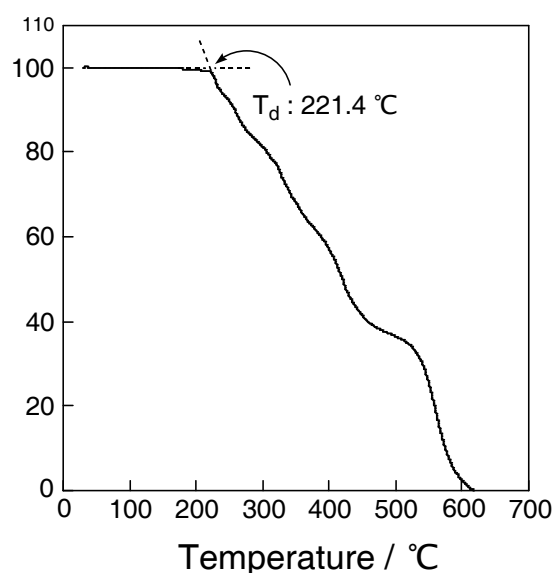
[b] in DMF, c=1.00 g dL^{-1}, L=10 cm.

Figure 4. Thermogravimetric analysis curve of L-Lys-OEt-2,4-TDI (Exp. No. 5 in Table 1)
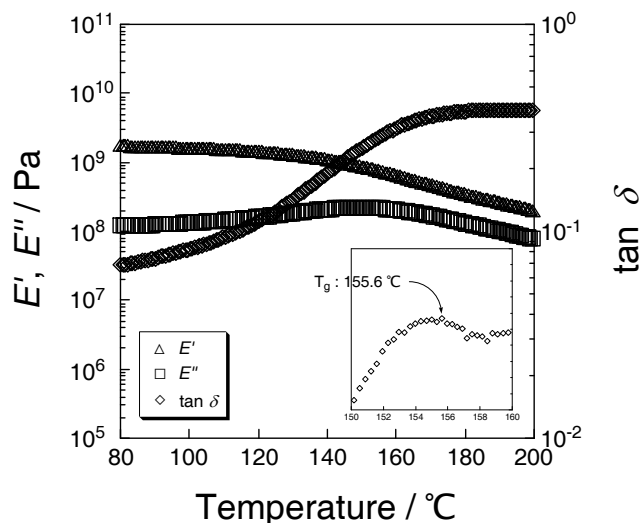
(Heating rate, 10°C min^{-1})

Figure 5. Temperature dependence of *E'*, *E''*, and tan *δ* for the chiral polyurea (Exp. No. 5 in Table 1) at a frequency of 1 Hz and a heating rate of 2.0°C min⁻¹

3.3 Adsorption Selectivity

Adsorption selectivity of the present polyurea membrane was studied adopting racemic mixture of Glu as model racemates. The results for adsorption study are summarized in Table 3. Against expectation, the polyurea membrane (control membrane) slightly showed adsorption selectivity toward L-Glu, thought it consisted of L-lysinyl residue with a chiral center.

Molecular imprinting is thought to be a facile way to introduce molecular recognition sites into materials (Piletsky et al., 1999; Haupt & Mosbach, 2000; Sellergren, 2001; Wulff, 2002; Ulbricht, 2004, Alexander et al., 2006). In the present study, conventional molecular imprinting could not be applicable since polymeric materials, polyureas, were already prepared. In such a case, an alternative molecular imprinting is an effective method to introduce molecular recognition sites into polymeric materials (Yoshikawa, 2001; Yoshikawa & Izumi, 2003; Sueyoshi et al., 2012). Adopting an alternative molecular imprinting, polymeric materials, in the present study, polyureas will be directly converted into molecular recognition materials (membranes). To this end, molecularly imprinted polymeric membranes were prepared from the present polyurea adopting N-α-protected glutamic acid, such as Z-D-Glu or Z-L-Glu, as a print molecule.

The adsorption selectivities for those two types of molecularly imprinted membrane are also given in Table 3 together with that for the control non-imprinted membrane. As expected, the membrane imprinted by Z-D-Glu adsorbed D-Glu in preference to the corresponding L-Glu and *vice versa*. In the authors' previous study, molecularly imprinted membranes from synthetic polymers, such as carboxylated polysulfone (Yoshikawa et al., 1998, 2007), polysulfone with aldehyde group (Sueyoshi et al., 2012), and polyamide (Kondo et al., 2000), the print molecule and the antipode equally worked as a print molecule; in other words, the adsorption selectivity for the membrane imprinted by the D-isomer gave nearly same selectivity expressed by that imprinted by the antipode. Contrary to this, in the present study, the adsorption selectivity of Z-D-Glu imprinted membrane was lower than that for the L-isomer molecularly imprinted one. This is due to the fact that the present polyurea consisted of L-lysinyl residue. In the extreme case, the formation of molecular recognition site was strictly dependent on the absolute configuration of polymeric materials; as an example, a tetrapeptide derivative consisting of D-amino acid residue was converted into the D-isomer recognition materials by adopting the D-isomer of print molecule and *vice versa*, and the print molecule, of which absolute configuration was opposite to that of the tetrapeptide derivative, hardly worked as a print molecule (Yoshikawa & Izumi, 2003). However, in the present study, the polyurea was flexible enough to construct molecular recognition sites toward the D-isomer by using a print molecule of D-isomer derivative and *vice versa*. The flexibility of the present polyurea was advantageous to construct D-isomer and L-isomer recognition membrane depending on the selection of print molecules, but disadvantageous to obtain higher substrate specificity in adsorption selectivity. From the results summarized in Table 3, the polyurea consisting of L-lysinyl residue was easier to form L-isomer recognition sites

than D-isomer recognition ones. These adsorption results suggested that the molecularly imprinted membranes would show chiral separation ability, which will be confirmed in the next section.

Table 3. Adsorption selectivity of three types of membrane toward racemic mixture of Glu in aqueous EtOH

Menbrane	D-Glu		L-Glu		$S_{A(D/L)}$	$S_{A(L/D)}$
	$10^5 \dfrac{mol}{g\text{-mem.}}$	$10^2 \dfrac{mol}{mol\ (Lys\text{-residue})}$	$10^5 \dfrac{mol}{g\text{-mem.}}$	$10^2 \dfrac{mol}{mol\ (Lys\text{-residue})}$		
Z-D-Glu imprinted mem.	6.46	2.45	6.10	2.34	1.06	0.94
Control mem.	6.39	2.45	6.59	2.52	0.97	1.03
Z-L-Glu imprinted mem.	7.28	2.79	8.05	3.09	0.90	1.11

3.4 Enantioselective Transport

Figure 6 shows time-transport curves of racemic mixture of Gu through two types of molecularly imprinted membrane, such as Z-D-Glu molecularly imprinted membrane (a) and Z-L-Glu molecularly imprinted one (b). As anticipated from adsorption selectivity summarized in Table 3, the control non-imprinted membrane hardly showed permselectivity, though the time-transport curves of the control non-imprinted membrane was not shown in Figure 6. Contrary to this, molecularly imprinted membranes showed chiral separation ability. The D-isomer molecularly imprinted membrane transported the D-isomer in preference to the L-isomer and *vice versa*. The permselectivity of the D-isomer molecularly imprinted membrane, $\alpha_{D/L}$, was determined to be 1.10, while that for the L-isomer molecularly imprinted one, $\alpha_{L/D}$, to be 1.09. Those permselectivities reflected their adsorption selectivities. In the present study, the interaction between the enantiomer selectively incorporated into the membrane and membrane material was not so strong, the preferentially adsorbed enantiomer was selectively transported through the membrane.
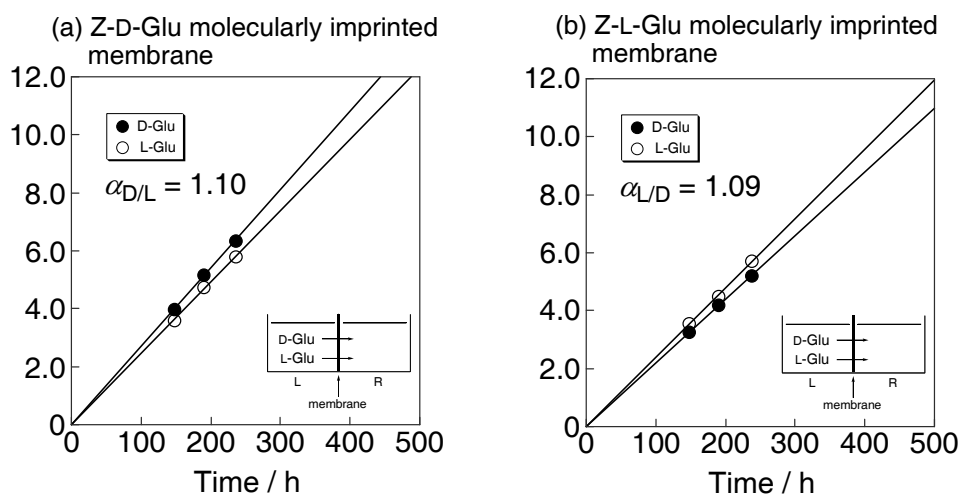

Figure 6. Time-transport curves of racemic mixture of Glu through Z-D-Glu molecularly imprinted (a) and Z-L-Glu molecularly imprinted (b) membranes at 40°C in aqueous EtOH solution adopting a concentration gradient as a driving force for membrane transport

4. Conclusions

Chial polyureas were prepared from L-Lys-OEt and 2,4-TDI. The optimum polyaddition conditions were determined as follows: [L-Lys-OEt] = 2.08 x 10^{-1} mol dm^{-3} and [2,4-TDI] = 2.50 x 10^{-1} mol dm^{-3} ([L-Lys-OEt]/ [2,4-TDI] = 0.83). The polyuera thus obtained gave a durable self-standing membrane and it was directly converted into molecular recognition membranes by applying an alternative molecular imprinting. The D-isomer molecularly imprinted membrane adsorbed the D-isomer in preference to the L-isomer and *vice versa*. In the present study, two types of print molecule, such as Z-D-Glu and Z-L-Glu, worked as print molecules though the

polyurea consisted of L-lysinyl residue. Those two types of molecularly imprinted membrane showed chiral separation ability. The membrane, which was molecularly imprinted by the D-isomer and preferentially incorporated the D-isomer into the membrane, selectively transported the D-isomer and *vice versa*.

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