Selective Urea Transport by Macrocyclic Carriers through a Supported Liquid Membrane

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Synthetic metallomacrocyclic receptor molecules (**1**–**4**) transport urea through a supported liquid membrane by encapsulation of the guest in the molecular cavity. The ring size of the macrocycle has a large effect on the rate of transport; carriers that have the optimal complementary shape for complexing urea are the most effective. The membrane stability is improved by the enhanced hydrophobicity of receptors with binaphthyl (**5**,**6**) or calixarene (**7**,**8**) moieties. The binaphthyl carrier **5** shows a selective transport of urea versus $KClO_4$ and *N*-methylurea in competition experiments. A mathematical model for the diffusion-limited, carrier-mediated transport of neutral compounds through a supported liquid membrane describes the fluxes in single transport well. From this model the extraction constants as well as the diffusion coefficients of the complexes can be calculated. Binaphthyl carrier **5** shows the same extraction constant for urea as calixsalophene carrier **7**. The higher urea flux for the former carrier is caused by a faster diffusion. The fluxes in competition experiments can be accurately predicted from K_{ex} and D_m values obtained in single transport experiments, which show that for binaphthyl carrier **5** the extraction constant for urea is 10 times higher than for *N*-methylurea.

Macrocyclic receptor molecules like crown ethers and calixarenes have been used for the selective transport of cations through bulk and supported liquid membranes.[1,2] Although neutral compounds have been transported by macrocyclic carriers through aqueous or organic bulk liquid membranes, to the best of our knowledge transport of neutral compounds by *macrocyclic carriers* through *supported liquid membranes* has not been reported.[3] Only recently, examples of assisted transport of neutral compounds through supported liquid membranes are known.[3-7] Yoshikawa et al. have employed the formation of a covalent bond between the carrier and the guest to transport amines.[4,5] Pirkle and Doherty have used a lipophilic amino ester for the enantioselective transport of amino esters of amides across a swollen silicone rubber.[6] Teramoto et al. have used $AgNO_3$ as a selective carrier in an aqueous supported liquid membrane to separate benzene from cyclohexane.[7] However, in none of these experiments *macrocyclic receptors* were used.

The selective urea removal is of great importance in medicine. Synthetic (macrocyclic) receptor molecules have not yet been used for this purpose because high extraction constants as well as high partition coefficients of the carrier are required to obtain a high and stable transport and most of the receptor–urea complexes known so far are too weak.[8,9] In searching for macrocyclic receptors that complex urea and can be used as selective carriers in supported liquid membranes, our group has developed crown ethers with intraannular acidic groups (COOH, SO_3H) which result in a strong interaction with urea.[10,11] However, these receptor molecules have very low partition coefficients P ($\log P < 1$), can only be used at low pH, and are therefore not suitable as carriers in supported liquid membranes.[12-14] Recently,[15] we have shown that incorporation of an electrophilic uranyl cation as a binding site in a macrocyclic receptor gives excellent receptors for polar neutral compounds.[21,22] These *metallomacrocycles* complex urea both by coordination of the urea carbonyl to the

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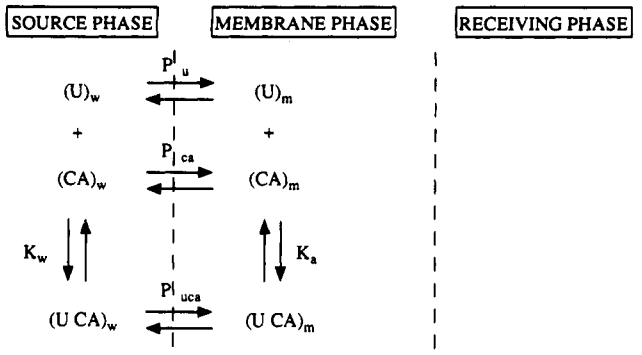

Figure 1. Schematic representation of equilibria at the source–membrane interface describing the transport of neutral compounds.

uranyl cation which is complexed by a salophene moiety, and by hydrogen bonding between the NH_2 functions of urea and the oxygen atoms of the polyethylene glycol moiety of the host. These compounds are however only poorly soluble in most solvents. Therefore, these receptors have been further modified by replacing a benzene ring of the salophene unit by a *cis*-1,2-cyclohexyl moiety to obtain more soluble receptors[23] or by incorporating a binaphthyl or a calix[4]arene group to enhance the solubility as well as the hydrophobicity.[24] In this paper the transport behavior of these compounds with neutral molecules, in particular urea, is described including the selective transport of urea versus salts and other neutral compounds. A mathematical model is used to describe the selective, carrier-mediated transport of neutral compounds through this type of membrane.

Model Description

Model Description for the Transport of One Neutral Compound. A mathematical model describing the carrier-mediated transport of neutral compounds through a supported liquid membrane was developed in analogy with the model describing the transport of free ions.[2] The different equilibria that can be defined at both interfaces of the membrane are shown in Figure 1 for the source phase interface. The same equations hold for the receiving phase interface. The rate-determining step of the transport process is assumed to be the diffusion of the complex through the membrane,[1,2,12] which means that it can be described by Fick's first law. Furthermore, the following assumptions are made: (i) there is thermodynamic equilibrium at the interfaces, (ii) initial transport (the complex concentration gradient over the membrane remains constant during the transport process), (iii) there are only 1:1 host–guest complexes, and (iv) a constant total carrier concentration throughout the membrane. The blank transport (transport in absence of carriers) of neutral compounds through an organic phase cannot be neglected and the total flux (J_{tot} at $t = 0$ in mol cm^{-2} h^{-1}) is the sum of the blank flux (J_{blank}) and the assisted flux (J_{ass}) according to Fick's first law:

$$J_{tot} = J_{ass} + J_{blank} = D_c d^{-1} [UCA]_m + D_u d^{-1} [U]_m \quad (1)$$

In eq 1 D_c is the diffusion coefficient of the complex (cm^2 h^{-1}), D_u is the diffusion coefficient (cm^2 h^{-1}) of the neutral molecule U (urea), d is the membrane thickness (cm), $[UCA]_m$ is the complex concentration in the membrane at the source phase interface (mol cm^{-3}), and $[U]_m$ is the concentration of free urea at the source phase interface (mol cm^{-3}). The blank flux can be measured as a function of the urea concentration in the source phase when no carrier is present in the membrane. This model derives an equation for the assisted transport. The assisted flux is derived as follows.

With the equilibria described in Figure 1, the complex concentration in the membrane at the source phase interface $[UCA]_m$ can be expressed as a function of the association constant in the membrane (K_a), the association constant in water (K_w), the partition coefficient of the carrier (P_{ca}), and the partition coefficient of urea (P_u). These are defined in eqs 2–5 in which m denotes the membrane phase at the source phase interface, w the aqueous phase, and [CA] the free carrier concentration. The extraction constant of urea is defined as the product of the association constant in the membrane phase (K_a) and the partition coefficient of free urea (P_u):

$$K_a = \frac{[UCA]_m}{[U]_m [CA]_m} \quad (2)$$

$$K_w = \frac{[UCA]_w}{[U]_w [CA]_w} \quad (3)$$

$$P_{ca} = \frac{[CA]_m}{[CA]_w} \quad (4)$$

$$P_u = \frac{[U]_m}{[U]_w} \quad (5)$$

$$K_{ex} = \frac{[UCA]_m}{[U]_w [CA]_m} = P_u K_a \quad (6)$$

Using the mass balance of the carrier, Fick's first law, and the assumptions mentioned above, the following equation relates the assisted flux to the parameters from eqs 2–6 (see Appendix 1):

$$\frac{[CA]_m^0}{J_{ass} d} = \frac{F}{D_m} + \frac{H}{D_m K_{ex} [U]_w^0} \quad (7)$$

in which:

$$F = 1 + \frac{1}{R_r P_{ca}}$$

$$H = 1 + \frac{1}{R_r P_{ca}} + \frac{1}{R_s P_{ca}} + \frac{K_w}{R_s P_{ca}} [U]_w^0$$

$$R_r = \frac{V_m}{V_r} \quad R_s = \frac{V_m}{V_s}$$

V stands for volume (cm^3) and s and r denote the source and the receiving phase, respectively. When the carrier does not leach to the aqueous phases ($P_{ca} > 10^5$) for the system used) and complexation in the aqueous phases can be neglected, F and H are approximately 1. From eq 7 it follows that the assisted flux can be expressed as

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$$J_{ass} = \frac{D_m K_{ex} [U]_w^0 [CA]_m^0}{d(H + FK_{ex}[U]_w^0)} \quad (8)$$

When the flux is measured as a function of the concentration of urea in the source phase, $[CA]_m^0 J_{ass}^{-1}$ can be plotted versus $([U]_w^0)^{-1}$, and D_m can be calculated from the intercept and K_{ex} from the slope of the linear correlation that is obtained according to eq 7. These values of K_{ex} and D_m can be used as starting values for an iteration procedure that optimizes eq 8 by least-square analysis for all data obtained by variation of $[U]_w^0$ and $[CA]_w^0$. This model for the transport of neutral compounds through supported liquid membranes resembles the model describing the transport of ion pairs published by Izatt et al.[25]

Model Description for the Transport of Two Neutral Compounds. The competitive, assisted transport of two neutral compounds U and N through a supported liquid membrane can also be described by this model assuming a 1:1 complexation. The extraction constants (eq 6) can now be defined as

$$K_{ex,u} = \frac{[UCA]_m}{[U]_w [CA]_m} = P_u K_{a,u} \quad (9)$$

$$K_{ex,n} = \frac{[NCA]_m}{[N]_w [CA]_m} = P_n K_{a,n} \quad (10)$$

This means that

$$\frac{[UCA]_m}{[NCA]_m} = \frac{K_{ex,u}}{K_{ex,n}} \frac{[U]_w}{[N]_w}$$

Using the mass balance of the carrier, the assumptions that there is initial transport only and that the total carrier concentration is constant throughout the membrane, the assisted flux of neutral compound U in competition experiments can be expressed as (see Appendix 2)

$$J_{ass,u} = \frac{D_{m,u} K_{ex,u} [U]_w^0 [CA]_m^0}{d(H' + FK_{ex,u}[U]_w^0 + FK_{ex,n}[N]_w^0)} \quad (11)$$

in which

$$H' = 1 + \frac{1}{R_r P_{ca}} + \frac{1}{R_s P_{ca}} + \frac{K_{w,u}[U]_w^0 + K_{w,n}[N]_w^0}{R_s P_{ca}}$$

In the same way the expression for the flux of the second compound can be derived:

$$J_{ass,n} = \frac{D_{m,n} K_{ex,n} [N]_w^0 [CA]_m^0}{d(H' + FK_{ex,u}[U]_w^0 + FK_{ex,n}[N]_w^0)} \quad (12)$$

From eqs 9–12 it follows that

$$\frac{J_{ass,u}}{J_{ass,n}} = \frac{D_{m,u}}{D_{m,n}} \frac{K_{a,u}}{K_{a,n}} \frac{P_u}{P_n} \frac{[U]_w^0}{[N]_w^0} \quad (13)$$

This means that the transport selectivity in competition experiments is determined by the diffusion constants of the complexes, the association constants in the membrane phase, the partition coefficients of the neutral guests, and the concentrations of the neutral compounds in the source phase. The extraction and diffusion constants are known from the experiments using one neutral compound only; all the other parameters are also known. When only one compound is transported ($[N]_w^0 = 0$), eq 11 simplifies to the relation for transport of one neutral compound (eq 8).

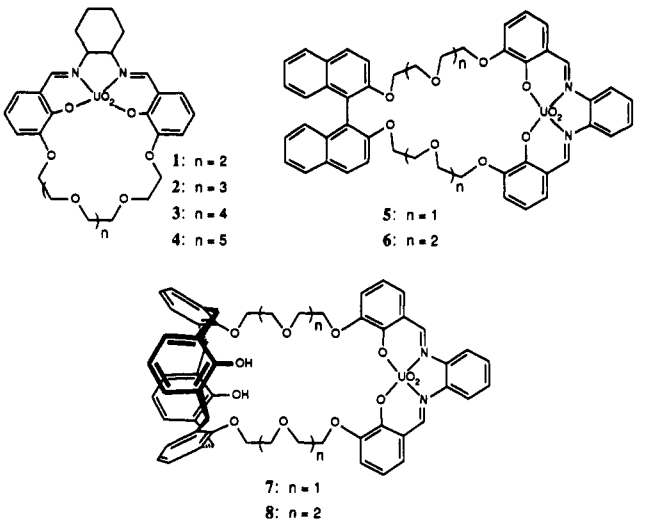

Figure 2. Metallomacrocycles used as urea carriers in a supported liquid membrane.

Results and Discussion[3]

Transport of Urea by Metallomacrocycles. Compounds 1–4 (Figure 2) were prepared from the corresponding dialdehydes and *cis*-1,2-cyclohexanediamine in methanol after which the uranyl cation was incorporated.[23] These receptors show a ring size selective complexation of urea in organic solvents.[23] The complexes of **2** and **3** with urea are the most stable complexes reported between a neutral monometallic receptor and a neutral guest. The results of solid–liquid and liquid–liquid extractions are in line with the K_a data.[23] Therefore, these receptors might be suitable to transport urea through a supported liquid membrane.

We found that the partition coefficient of the carriers 1–4 is rather low and therefore crown ether salophene derivatives have been modified with a binaphthyl (**5** and **6**) or a calix[4]arene (**7** and **8**) function in order to obtain a hydrophobic and selective urea carrier. Compounds **5–8** were prepared from the corresponding dialdehydes and 1,2-benzenediamine in THF according to ref 22, analogous to the cyclohexyl salene derivatives 1–4.[24]

These compounds were used as carriers in a supported liquid membrane composed of a carrier solution in *o*-nitrophenyl *n*-octyl ether (NPOE) immobilized in a porous polymeric support (Accurel) to investigate the relationship between the ring size of the metallomacrocycle and the rate of urea transport. The membrane separates the aqueous urea containing source phase (sp) from the aqueous receiving phase (rp) which initially contains no urea. The urea transport was monitored after 24 h (unless stated otherwise) by UV/vis analysis at 435 nm of a complex formed between urea and *p*-(*N*,*N*-dimethylamino)benzaldehyde (added to a sample of the receiving phase), following literature procedures.[26-29]

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Table I. Total Urea Fluxes[a] through a Supported Liquid Membrane Using Different Metallomacrocyclic Carriers

carrier	carrier concn (mM)	flux (10^{-8} mol cm^{-2} h^{-1}) with no. of replacements[b] 0	1	2
-	-	1.6	-	-
1	6.9	2.3	-	-
2	6.0	21	11	6.8
	2.8	12	-	-
3	6.1	8.6	-	-
4	2.8[c]	6.2	-	-
5	6.0	22	23	23
6	6.0	22	22	21
7	5.9	18	18	18
8	6.0	15	-	-

[a] $[Urea]^0_w$ = 1 M. [b] Replacements of the receiving phase after 24 h. [c] Maximum concentration in NPOE.

It was found that when the urea concentration in the receiving phase was measured as a function of time, using carrier 5 (6.0 mM in NPOE) and a 1 M urea source phase, a linear correlation was obtained. This implies that the urea flux is constant over at least 24 h.

Table I shows that the urea fluxes with the cyclohexyl receptors (1–4) strongly depend on the ring size of these carriers. Compound 1 gave a total urea flux comparable to the blank flux in experiments where no carrier is used, while the larger rings transport urea much better, especially compound 2 (n = 3). With 2 the total urea flux is 13 times higher than the blank flux and also higher than fluxes obtained using comparable concentrations of compounds 1 and 3. Compound 4 is poorly soluble in NPOE[30] and shows also a lower flux (using a 2.8 mM solution compared to the same concentration of 2). The different rates of transport are in line with extraction and complexation data. CPK-models show that the cavity of 1 is too small to encapsulate urea and the best fit for compound 2.[23] The hydrophobic carriers 5–8 encapsulate urea well and hardly any influence of the ring size was observed.

Table I also shows that upon replacing the receiving aqueous phase once or twice the flux for the cyclohexyl carriers decreases because they leach out to the aqueous phases. This is not observed for the more hydrophobic binaphthyl (5,6) or calix[4]arene (7,8) modified carriers. In these cases the urea fluxes are comparable to those obtained with carrier 2 and a stable membrane is obtained.

Urea transport measurements were also performed using blood plasma (pretreated with sodium citrate to prevent coagulation) as the source phase. In kidney dialysis, an excess of urea is removed from the patient's blood by a dialysis membrane. However, blood is much more lipophilic than water since it contains proteins and lipophilic components. This means that when using carrier-mediated transport through supported liquid membranes and blood as a source phase, the hydrophobicity of the carriers must even be higher than when using an aqueous source phase. To verify whether urea can be transported from blood using a supported liquid membrane, an experiment was performed using blood plasma containing 50 mM urea (concentration of urea in blood of a patient suffering from kidney failure) as the source phase and a 6.0 mM solution of binaphthyl carrier 5 in NPOE. A total urea flux of 20 × 10^{-8} mol cm^{-2} h^{-1} was obtained when blood plasma was used as the source phase. The blank transport of urea using an Accurel/NPOE membrane without 5 was <1 × 10^{-8} mol cm^{-2} h^{-1}. The receiving aqueous phase was analyzed by HPLC in case of the assisted transport, to verify if other components from the blood plasma were also transported through the membrane. No significant amounts of other polar neutral compounds could be detected.

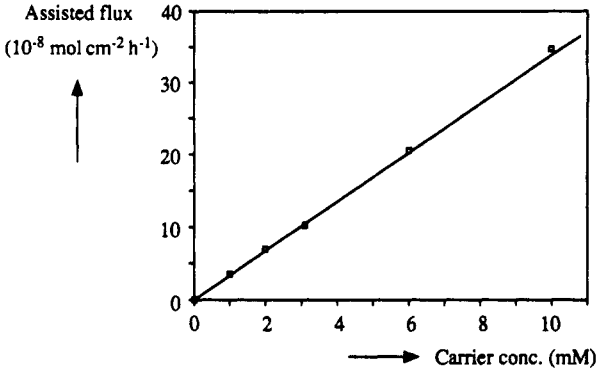

Figure 3. Influence of binaphthyl 5 concentration on the assisted urea flux through a supported liquid membrane, $[urea]^0_w$ = 1 M; the line drawn is calculated according to the model and the symbols are calculated values.

The performance of the supported liquid membranes was compared to the urea transport through a commercially available dialysis membrane (Cuprophan, d = 10 μm). In this case a urea flux of 9 × 10^{-5} mol cm^{-2} h^{-1} was observed (using a 50 mM solution of urea in *water* as the source phase) compared to a total flux of 13 × 10^{-8} mol cm^{-2} h^{-1} using a 6.0 mM solution of binaphthyl carrier 5 in NPOE/Accurel. The membrane thickness of the dialysis membrane is, however, smaller (10 μm compared to 100 μm for Accurel). The transport through a dialysis membrane is much less selective since separation is mainly based on molecular weight instead of specific interactions. The carrier-assisted transport might be improved by using thinner support materials and increasing the solubility of the carrier.

Determination of D_m and K_{ex}. To verify the assumption of diffusion-limited transport, the membrane thickness was varied by placing two membranes, clamped together, in the membrane setup (using a 6 mM solution of binaphthyl carrier 5 in NPOE and a 1 M urea source phase). In this case a total urea flux of 12 × 10^{-8} mol cm^{-2} h^{-1} was obtained compared to a total urea flux of 22 × 10^{-8} mol cm^{-2} h^{-1} using one membrane. This clearly shows the influence of the membrane thickness and therefore confirms the assumption of diffusion-limited transport. In order to verify the proposed transport mechanism for the flux (eq 8) the urea flux was measured as a function of the carrier concentration of the binaphthyl receptor 5 in the membrane phase, using a 1 M urea source phase. Figure 3 shows that there is a linear increase in flux with the carrier concentration as the model predicts.

The urea concentration in the source phase was varied to determine the diffusion coefficient and extraction constant of the binaphthyl carrier 5·urea complex using a 6 mM solution in NPOE. Figure 4 shows that the flux strongly increases with the urea concentration at lower concentrations and reaches a plateau value at higher urea

(29) To verify this method the urea concentration was determined in samples of known concentration using UV/vis-spectroscopy as well as urea determinations by titration using urease. The results obtained from both methods agree very well; in all cases the deviation is less than 5%.

(30) The solubility in NPOE of some of the other metallomacrocycles used was also quite low. Therefore, in most cases the maximum concentration is about 6 mM while (much) higher concentrations have been used for other types carriers.

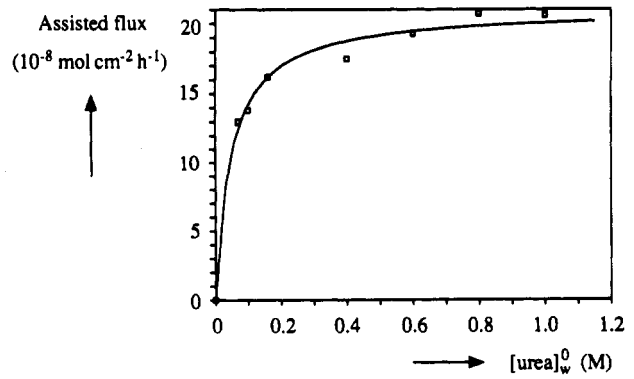

Figure 4. Influence of the urea concentration in the source phase on the assisted flux through a supported liquid membrane using binaphthyl carrier **5** ($[carrier]^0_m$ = 6.0 mM); the line drawn is calculated according to the model and the symbols are measured values.

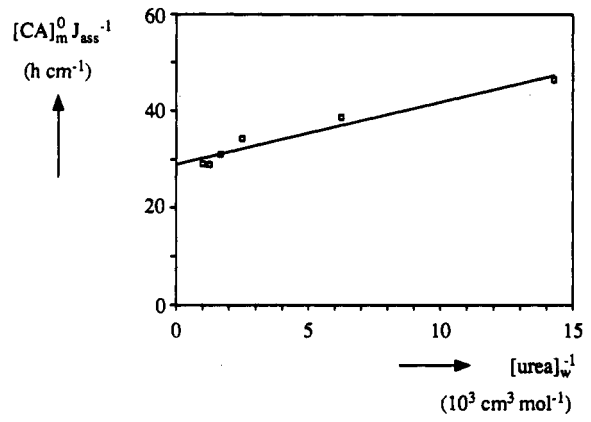

Figure 5. Graphical method used for the determination of the diffusion and extraction constant for the binaphthyl **5**·urea complex in NPOE.

Table II. Diffusion and Extraction Constants for Metallomacrocycles 5 and 7 with Urea in NPOE

complex	D_m (cm² h⁻¹)	K_{ex} (M^{-1})	R^2
5·urea[a]	3.5×10^{-4}	20	0.932
7·urea[a]	2.2×10^{-4}	22	0.965

[a] K_w = 0 in all cases.

concentrations. A plot of $[CA]^0_m (J_{ass})^{-1}$ versus $[urea]_w^{-1}$ shows the expected linear correlation (see Figure 5). An iteration procedure using Fick's first law (eq 8) was applied to obtain accurate values for D_m and K_{ex} (see Table II).

The same procedure was followed to determine the diffusion coefficient and extraction constant of the calixsalophene carrier **7** (using a 10 mM carrier solution) with urea. The assisted urea flux as a function of the urea concentration in the source phase is shown in Figure 6. Also in this case the flux strongly increases with the urea concentration at lower concentrations and reaches a plateau value at higher urea concentrations.

The calculated values for D_m and K_{ex} (Table II) show that the lower urea fluxes for the calixsalophene carrier **7** compared to the binaphthyl carrier **5** are caused by a slower diffusion of the larger calixarene derivative. The extraction constants for carriers **5** and **7**, which have a similar cavity size, are nearly equal.

Knowing the diffusion coefficients and the extraction constants, the percentage of carrier complexed at the source phase interface can be calculated. Using a 6 mM solution of binaphthyl carrier **5**, 67% of the carrier is

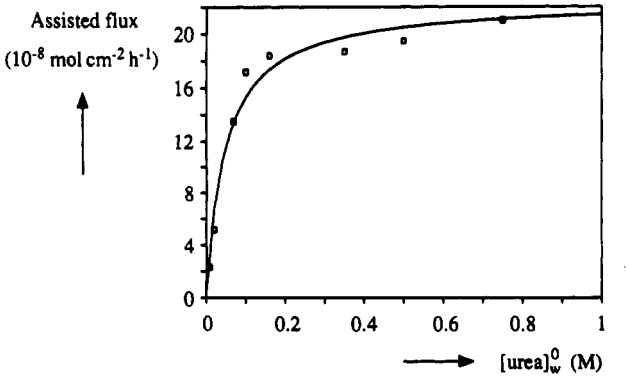

Figure 6. Influence of the urea concentration in the source phase on the assisted flux through a supported liquid membrane using calixsalophene carrier **7** ($[carrier]^0_m$ = 10 mM); the line drawn is calculated according to the model and the symbols are measured values.

Table III. Influence of $KClO_4$ Transport on the Total Urea Flux through a Supported Liquid Membrane Using Binaphthyl Carrier 5[a]

source phase	receiving phase	total urea flux (10⁻⁸ mol cm⁻² h⁻¹)	K^+ flux (10⁻⁸ mol cm⁻² h⁻¹)
0.1 M urea–0.1 M $KClO_4$	water	17	0.8
0.1 M urea	0.1 M $KClO_4$	17	0.7
0.1 M urea	water	17	–
0.1 M $KClO_4$	water	–	0.9

[a] $[Carrier]^0_m$ = 6.0 mM.

complexed using a 0.1 M urea source phase solution and 92% using a 0.5 M urea solution. For a 10 mM calixsalophene **7** solution these figures are 69 and 92%, respectively. This clearly shows that the plateau values observed for urea transport with these carriers can be explained by a full loading of the carrier. This means that increasing the urea concentration in this range does not result in an increased flux. With these diffusion coefficients and extraction constants the observed fluxes can be simulated very well (see Figures 3, 4, and 6).

Competitive Transport. In complexation studies the metallomacrocyclic receptors **2–4** were found to be selective for urea compared to other neutral compounds; the association constants found were much lower than observed for urea.[23] The following stability order in acetonitrile was found: urea > *N*-methylurea > formamide ≃ acetamide > acetone = 0. To study the selectivity of the urea carriers in membrane transport, experiments were performed using a mixture of urea and a salt or a mixture of urea and another neutral compound in the source phase. This was done using a 6.0 mM solution of binaphthyl carrier **5** (chosen for its high hydrophobicity) in NPOE. Firstly, the influence of $KClO_4$ on the transport was studied. Table III shows that no transport of $KClO_4$ occurs, neither in single nor in competition experiments.

To study the selectivity of carrier **5** for other neutral compounds, experiments were performed using aqueous solutions of formamide, acetamide, thiourea, *N*-methylurea, 1,1-*N*,*N*-dimethylurea, 1,3-*N*,*N*′-dimethylurea, and creatinine as the source phase (uric acid was insufficiently soluble in water to measure any transport). In this case the concentrations in the receiving phase were determined using HPLC because the *p*-(*N*,*N*-dimethylamino)benzaldehyde, used to determine the urea concentration by UV/vis-spectroscopy, can also form a complex with these

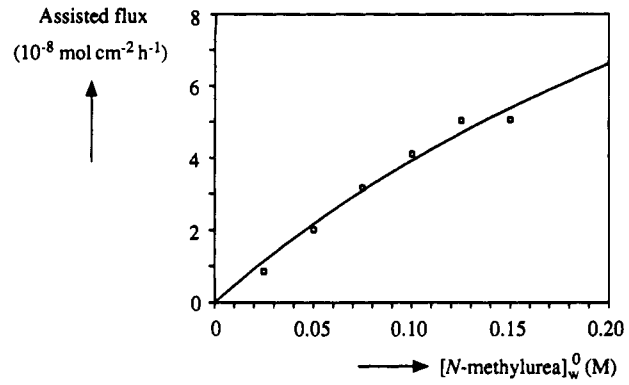

Figure 7. Influence of the *N*-methylurea concentration in the source phase on the assisted flux through a supported liquid membrane using binaphthyl carrier **5** ($[carrier]_m^0$ = 6.0 mM); the line drawn is calculated according to the model and the symbols are measured values.

derivatives and this method can therefore not be used in competition experiments. Thiourea and formamide had the same retention time as urea and could therefore not be studied. All other compounds gave high blank fluxes (using source concentrations of 0.1–1 M), in most cases higher than the carrier-mediated urea transport, except for *N*-methylurea. Therefore, the urea/*N*-methylurea selectivity of carrier **5** (6 mM in NPOE) was studied.

Firstly, the diffusion coefficient and the extraction constant for the **5**·*N*-methylurea complex were determined in separate experiments by varying the concentration in the source phase, using *N*-methylurea only (carrier concentration of 6 mM). The assisted *N*-methylurea flux as a function of the source phase concentration is shown in Figure 7. In this case no plateau value was observed. From plots of $[CA]_m^0 (J_{ass})^{-1}$ versus $[N\text{-methylurea}]_w^{-1}$, D_m and K_{ex} could be calculated using the same procedure as for urea transport. The diffusion coefficients of the urea and *N*-methylurea complexes of the binaphthyl carrier **5** are equal within experimental error. Therefore, the same diffusion coefficient is used for both complexes (3.5×10^{-4} cm^2 h^{-1}). The extraction constant for *N*-methylurea (2.3 M^{-1}) is lower than for urea (20 M^{-1}) which shows that indeed binaphthyl carrier **5** complexes urea selectively (the partition of *N*-methylurea from water to NPOE is higher than that of urea as was shown by a higher blank flux). Using these data the percentage of carrier complexed at the source phase interface was calculated using a 6 mM solution of binaphthyl carrier **5** in NPOE. Only 20% of the carrier is complexed when the *N*-methylurea concentration in the source phase is 0.1 M and 57% with 0.5 M *N*-methylurea; this explains why no plateau value is observed.

The transport selectivity in combined experiments was studied using a source phase of 0.1 M urea and 0.1 M *N*-methylurea. Assisted urea and *N*-methylurea fluxes of 14×10^{-8} mol cm^{-2} h^{-1} and 1.7×10^{-8} mol cm^{-2} h^{-1}, respectively, were found, which means that carrier **5** indeed transports urea selectively. Using the calculated diffusion coefficients and extraction constants, the transport model predicts a urea flux of 13×10^{-8} mol cm^{-2} h^{-1} and a *N*-methylurea flux of 1.5×10^{-8} mol cm^{-2} h^{-1} which is in good agreement with the observed fluxes. The transport selectivity (ratio of fluxes in combined experiments = 8.2) agrees well with the extraction selectivity ($K_{ex,u}/K_{ex,n}$ = 8.7).

Conclusions

Neutral compounds like urea can be transported by macrocyclic carriers through supported liquid membranes. High fluxes can be obtained using metallomacrocyclic carriers, which have a strong interaction with urea via H-bonds as well as coordination of the urea carbonyl to the uranyl cation, and possess a cavity in which the guest molecule fits well. The membrane stability can be improved by using carriers modified with hydrophobic groups like binaphthyl or calixarene units. Using binaphthyl carrier **5** urea can even be transported from blood plasma. The transport through a commercially available haemodialysis membrane (Cuprophan) is faster but less selective. Binaphthyl salophene carrier **5** shows a selective transport of urea versus $KClO_4$ and *N*-methylurea in competition experiments. A diffusion-limited, carrier-mediated transport mechanism accurately describes the fluxes obtained. The model shows that at higher concentrations of urea in the source phase, using binaphthyl carrier **5** and calixsalophene carrier **7** the fluxes reach a plateau value because of full loading of the carrier at the source phase interface. The carrier-mediated transport through supported liquid membranes can be further improved by using thinner support materials, other membrane solvents, and higher carrier concentrations.

Experimental Section

Materials. The synthesis of the compounds **1**–**8** has been described elsewhere or will be published in the near future.[23,24] Urea, *N*-methylurea, 1,1-*N*,*N*-dimethylurea, 1,3-*N*,*N*′-dimethylurea, thiourea, formamide, acetamide, creatinine, uric acid, *p*-(*N*,*N*-dimethylamino)benzaldehyde and potassium perchlorate were obtained from Janssen Chimica and were used as received. The polymeric film Accurel was obtained from Enka Membrana. *o*-Nitrophenyl *n*-octyl ether was obtained from Fluka and was used without further purification. The Cuprophan dialysis membranes (thickness 10 μm in the dry state) were taken from a Lundia 10 haemodialysis module which was a kind gift of Gambro, Breda. Blood plasma was kindly supplied by the bloodbank Enschede. To 0.5 L of blood plasma was added 70 mL of an aqueous solution of 0.30% citric acid, 2.63% sodium citrate, 0.22% sodium biphosphate, 3.19% glucose, and 275 mg/L of adenine. Sodium citrate was added to prevent coagulation.

Transport Experiments. The transport experiments were carried out in a permeation cell consisting of two identical cylindrical compartments (half-cell volume: 50 mL; effective membrane area: 12.4 cm^2). Details of this cell have been described previously.[1] The supported liquid membrane consisted of a thin microporous polypropylene film (Accurel; thickness d = 100 μm, porosity 64%) immobilizing the solution of carrier in *o*-nitrophenyl *n*-octyl ether (NPOE). In case of variation of the membrane thickness, two membranes clamped together were placed between the two compartments. In case Cuprophan dialysis membranes were used, the membranes were first cleaned ultrasonically in a 0.9% wt aqueous NaCl solution to remove glycerol residues that might be incorporated in the membrane, after which they were rinsed several times with doubly distilled and deionized water. After carefully wiping the membrane with a tissue, it was placed in the membrane setup. Aqueous solutions of different neutral guests were used as the source phase. Aqueous potassium perchlorate solutions or mixtures of potassium perchlorate and urea were used as a source phase to study the transport selectivity towards potassium cations. When blood plasma was used as a source phase, 40 mM of urea was added to the plasma to bring the total urea concentration to 50 mM, since the typical urea level in blood is assumed to be about 10 mM. In most cases doubly distilled and deionized water was used as

the receiving phase, except when the influence of the counter-transport of potassium cations was studied; in that case a 0.1 M $KClO_4$ solution was used. The measurements were performed at 25 °C and at least in duplicate. Samples of the receiving phase were taken after 24 h (unless stated otherwise) and the concentrations of transported species were determined by UV/vis spectroscopy, HPLC, or urease measurements. The standard deviation in the transport experiments is about 15%.

Urea Determination by UV/vis Spectroscopy. A stock solution of *p*-(*N*,*N*-dimethylamino)benzaldehyde was prepared by dissolving 8 g in 400 mL of ethanol (99.5%) and 40 mL of concentrated HCl. Small amounts of aqueous urea solutions of known concentration were added to 10 mL of this stock solution, and the solution was diluted with bidistilled and deionized water until the total volume was 25 mL. The UV/vis absorption was measured at 435 nm using a Uvikon 930 spectrophotometer, a blank solution of 10 mL of stock solution, and 15 mL of doubly distilled deionized water as a reference. In this way a calibration curve was obtained for urea concentrations from 0.1 to 2 mM. After 24 h of transport (unless stated otherwise) 15 mL of the receiving phase was added to 10 mL of the stock solution and the concentration was determined by measuring the absorption at 435 nm and using the calibration curve. In case of the dialysis membranes the urea concentrations in the receiving phase were very high and only 5 mL was added to 10 mL of stock solution after which 10 mL of doubly distilled and deionized water was added.

Urea Determination Using Urease. The coulometric titration equipment consisted of a coulometric titrator from Tacussel, type TT700, a glass electrode EA109 Metrohm, a reference electrode R112 Electrofact, a pH meter type 742 Knick, and a cation-exchange membrane from Ionics. The titration vessel was filled with 40 mL of 0.5 M Na_2SO_4 and 1 mL of urease solution (10 mg/mL H_2O), and the pH was adjusted to 7.50. After the introduction of the sample containing $0.1–7.0 \times 10^{-6}$ moles of urea, also adjusted to pH = 7.50, the coulometric titration was started. When the pH value of 7.50 was reestablished, the amount of acid generated was read from the coulometric titrator.

Analysis of the Receiving Phase Using HPLC. When using urea derivatives, other neutral compounds, or mixtures of neutral compounds, the receiving phase was analyzed after 24 h (unless stated otherwise) by HPLC using a Varian 2510 HPLC pump, a Varian variable 2550 UV detector measuring the absorption at 196 nm, a Valco injector with a 10-μL injection loop, and a LiChrospher 100 RP-18 5u column with an inner diameter of 4 mm and a length of 125 mm. The data were analyzed using a Hitachi D2500 Chromato-Integrator. Calibration curves were made for the different compounds used from which the concentration of the compounds in the receiving phase could be calculated. In case of mixtures of neutral compounds, two columns in series were used to obtain a better separation.

Appendix 1

The mass balance of the carrier is given by

$$V_m[CA]^0_m = 0.5V_m\{[CA]_{m,s} + [UCA]_{m,s} + [CA]_{m,r} + [UCA]_{m,r}\} + V_s\{[CA]_{w,s} + [UCA]_{w,s}\} + V_r\{[CA]_{w,r} + [UCA]_{w,r}\} \quad (14)$$

When we assume that the total carrier concentration is constant throughout the membrane:

$$[CA]_{m,s} + [UCA]_{m,s} = [CA]_{m,r} + [UCA]_{m,r}$$

and using the assumption of initial transport:

$$[U]_{w,s} = [U]^0_{w,s}$$

$$[UCA]_{m,r} = 0$$

$$[UCA]_{w,r} = 0$$

$$[U]_{w,r} = 0$$

and the mass balance of the carrier, we can relate $[UCA]_m$ to the parameters in eqs 2–6:

$$[CA]^0_m = F[UCA]_m + H\frac{[UCA]_m}{K_{ex}[U]^0_w} \quad (15)$$

Appendix 2

The mass balance of the carrier is given by

$$V_m[CA]^0_m = 0.5V_m\{[CA]_{m,s} + [UCA]_{m,s} + [NCA]_{m,s} + [CA]_{m,r} + [UCA]_{m,r} + [NCA]_{m,r}\} + V_s\{[CA]_{w,s} + [UCA]_{w,s} + [NCA]_{w,s}\} + V_r\{[CA]_{w,r} + [UCA]_{w,r} + [NCA]_{w,r}\} \quad (16)$$

When we again assume that (i) the total carrier concentration is constant throughout the membrane:

$$[CA]_{m,s} + [UCA]_{m,s} + [NCA]_{m,s} = [CA]_{m,r} + [UCA]_{m,r} + [NCA]_{m,r}$$

(ii) there is initial transport only:

$$[U]_{w,s} = [U]^0_{w,s}$$

$$[N]_{w,s} = [N]^0_{w,s}$$

$$[UCA]_{m,r} = [NCA]_{m,r} = 0$$

$$[UCA]_{w,r} = [NCA]_{w,r} = 0$$

$$[U]_{w,r} = [N]_{w,r} = 0$$

and using the association constants of both compounds in the membrane and the aqueous phases, the partition coefficients of both guest compounds and of the carrier, and the mass balance of the carrier, it follows that

$$[CA]^0_m = F[UCA]_m\left(1 + \frac{K_{ex,n}[N]^0_w}{K_{ex,u}[U]^0_w}\right) + \frac{H'[UCA]_m}{K_{ex,u}[U]^0_w} \quad (17)$$

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