Enhancement of Analyte Concentration Based on a Feed/Receiving Volume Effect in Uphill Membrane Transport

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The enhancement of the concentration of analytes by a feed/receiving volume effect in coupled membrane transport was both theoretically and experimentally investigated in view of the designs of uphill transport membrane sensors and novel preconcentration techniques in general. Theoretical equations for the feed/receiving volume effect were derived for the membrane diffusion-limited co-transport and counter transport cases. The theoretical results coincided well with the volume effect observed experimentally for some representative liquid membrane systems, in which the concentration of an analyte in the receiving solution was found to increase upon decreasing the volume of the receiving (inner filling) solution. The complexation reaction of an analyte in the receiving solution was found to be useful as an additional driving force for further enhancing the analyte concentration by coupled transport.

Keywords Uphill transport, feed/receiving volume effect, membrane sensor

The uphill (active) transport of chemical species by carrier-incorporated liquid-membranes has been used for the separation of analytes and for a new mode of mass transfer in chemical sensors, i.e., uphill transport membrane sensors. The most interesting feature of uphill membrane transport is, as pointed out earlier, that if the volume of the receiving (inner filling) solution is purposely made smaller than that of the feed (sample) solution, the concentration of an analyte transported in the receiving solution is significantly amplified: the equilibrium concentration of the analyte in the receiving solution becomes much higher than its initial concentration in the feed solution. Such an enhancement of the concentration of analytes has been experimentally observed for some liquid membrane systems.

The two most common modes of uphill transport are coupled transport (i.e., co-transport (symport) and counter transport (antiport)). In coupled transport, the transport of a charged solute (primary ion) against its concentration gradient across a liquid membrane containing a carrier is driven by a simultaneous transport of another charged solute (pumping ion) present in excess either in the feed or receiving solution. A chemical reaction, such as a redox or complexation reaction, in the receiving solution that modifies the carrier-mediated flux of the primary ion at the outer membrane/solution interface can also be used as an additional driving force. Some mechanistic models for the coupled transport have been proposed to describe the steady state fluxes of two solutes simultaneously diffusing across carrier-incorporated liquid membranes. Described an equation for the diffusion-limited transport and predicted that the amount of solute transported in the receiving solution during a given time period changes with the bulk extraction parameters. They also described an equation through which the feed/receiving volume effect for a nearly complete accumulation of solute in the receiving solution can be predicted. Their equation, however, can only be applied to the limited case in which the concentration of the pumping ions as an external driving force is sufficiently high to attain a nearly complete transport of the solute.

In the present work we describe in a more general manner the theoretical aspects of the feed/receiving volume effect of the membrane diffusion-limited uphill transport for partial and nearly complete accumulation of the solute, and compare the theoretical results with several experimentally observed transport behaviors.

Theoretical

If a liquid membrane containing an electroneutral carrier is interposed between two aqueous solutions, i.e., feed and receiving solutions, a solute in the feed solution can be transported into the receiving solution across the liquid membrane. We first consider a co-transport model in which a solute (primary ion M⁺) does not enter the membrane in the free form, but can enter only through the formation of an electroneutral...
ion-pair complex with a pumping ion $X^-$ according to

$$M^+ + X^- + N(m) \rightleftharpoons MNX(m). \quad (1)$$

The chemical species that can exist in the membrane phase are the ion-pair complex (MNX) and the free carrier (N). Therefore, with progress regarding transport, linear concentration gradients of the two species result within an unstirred membrane. It will be further assumed that the carrier remains only in the membrane phase. Hence, the total concentration $C_{N,tot}$ of the carrier is given by

$$C_{N,tot} = C_N(m) + C_{MNX}(m), \quad (2)$$

where the concentrations ($C(m)$) refer to the membrane phase.

The equilibrium parameters ($K_{ex}'$ and $K_{ex}''$) of reaction (1) for the two membrane/solution interfaces (') and (') are identical to the bulk equilibrium constant ($K_{ex}$) for the case of carrier-mediated transport of a single salt MX.

$$K_{ex}' C_M C_X C_N(m) = C_{MNX}(m), \quad (3a)$$

$$K_{ex}'' C_M C_X C_N'(m) = C_{MNX}'(m). \quad (3b)$$

Assuming transport to be membrane-diffusion controlled, the steady state fluxes of the two solutes (MNX and N) diffusing across the unit area of the liquid membrane (thickness: $d$) are given by

$$J_{MNX} = \frac{D}{d} [C_{MNX}(m) - C_{MNX}(m)] \quad (4)$$

and

$$J_N = \frac{D}{d} [C_N(m) - C_N'(m)]. \quad (5)$$

with an identical diffusion constant ($D$) for species MNX and N. This constraint is not serious, since the carrier molecule is usually large compared to the substrate ions; the MNX and N are thus of nearly equal size. The flux equation can be transformed into

$$J_{MNX} = J_N \quad (6)$$

At equilibrium, where $J_{MNX} = J_N = 0$, it holds that

$$\frac{C_{MNX}'}{C_{MNX}} = \frac{C_X}{C_N}. \quad (7)$$

Setting the initial concentrations of the primary and pumping ions in the feed solution (volume $V_f$) $*C_M^* = *C_X^* = 0$ and those of the receiving solution ($V_r$) $*C_M = *C_X = 0$, the equilibrium concentrations can be expressed as

$$C_{MNX}' = \frac{*C_M - n}{V_f}, \quad (8)$$

$$C_X' = \frac{*C_X - n}{V_f}, \quad (9)$$

$$C_{MNX}'' = \frac{n}{V_r}, \quad (10)$$

and

$$C_X'' = \frac{n}{V_r}, \quad (11)$$

where $n$ is the number of moles of each solute transported up to the diffusion equilibrium. By introducing these relations into Eq. (7) and rearranging it, one obtains a quadratic equation with respect to $n$, the solution of which gives

$$n = \frac{-(*C_M + *C_X) V_r + \sqrt{(*C_M + *C_X)^2 V_r^2 + 4(V_f^2 - V_r^2) *C_M *C_X}}{2(V_f/V_r - V_r/V_f)} \quad (12)$$

for the concentration of the primary ion $M^+$ in the receiving solution,

$$C_{MNX}'' = \frac{-(*C_M + *C_X) + \sqrt{(*C_M + *C_X)^2 + 4(V_f/V_r - 1) *C_M *C_X}}{2(V_f/V_r - V_r/V_f)} \quad (13)$$

This equation describes the equilibrium concentration of an analyte transported into the receiving solution as a function of the relative volume of the receiving solution to that of the feed solution and the initial concentrations of the primary and pumping ions.

For the case of a counter transport of a charged solute ($I^+$) by a carrier bearing a net charge, it is necessary that the transport of another solute ($J^+$) is exchanged against the transported solute $I^+$ according to

$$J^+ + IN(m) \rightleftharpoons I^+ + JN(m). \quad (14)$$

In this case, the equilibrium parameter ($K_{ij}$) characterizing the interfacial reaction represents the relative affinity of the primary ion ($I^+$) and the pumping ion ($J^+$) for the carrier (N). Under the same assumptions as for the co-transport case, one obtains a flux equation that describes transport up to the diffusion equilibrium:

$$J_i = J_j \quad (15)$$

At diffusion equilibrium ($J_i = J_j = 0$), the following relation holds:
When the initial concentrations (*C) of the two solutes in the feed and receiving solution are given by

\[ *C_i > 0, \quad *C_j = 0 \]  

and

\[ *C_i^* > 0, \quad *C_j^* > 0, \]  

the equilibrium concentration can be expressed as

\[ C_{i,eq} = \frac{*C_i - n}{V_f}, \]  
\[ C_{j,eq} = \frac{*C_j + n}{V_r}, \]  
\[ C_{i,eq} = \frac{n}{V_f}, \]  
\[ C_{j,eq} = \frac{*C_j^*}{V_r}. \]  

Combining these equations with Eq. (16) gives, for the number of moles (n) of the primary ions transported in the receiving solution,

\[ n = \frac{V_f * C_i * C_j^*}{*C_i^* + (V_f/V_r)(*C_i^* + *C_j^*)}, \]  

and for the concentration of the primary ion,

\[ C_{i,eq} = \frac{*C_i - n}{V_f}. \]  

Based on this equation, the feed/receiving volume effect can be calculated for the counter transport system of 1:1 coupling.

If the receiving solution contains a complexing agent (L) that forms a complex with a primary ion (M⁺) according to

\[ M^+ + L \rightleftharpoons ML^+, \]  

the equilibrium of the interfacial reaction can be expressed as, for the simple co-transport case,

\[ MNX(m) + L \rightleftharpoons ML^+ + X^- + N(m). \]  

In this case the interfacial equilibrium parameters for the (') and (*) phases are no longer identical. The boundary concentration of the MNX species at the membrane/solution interface (') is changed by reaction (24). By a similar treatment as mentioned above for the co-transport system, taking Eqs. (24) and (25) into consideration, one obtains a relation at the transport equilibrium,

\[ C_{ML,eq} = K \times \frac{C_{X,eq}^*}{C_{i,eq}}, \]  

with the equilibrium parameter K:

\[ K = \frac{k_{ML} C_L K_{ex}}{K_{ex}^*}. \]  

Here, *C_i is the concentration of a ligand (L) in the receiving solution, *C_i^* and *C_x^* are the equilibrium constants of the reaction (1) at the two boundaries(') and (*), respectively, and k_{ML} is the equilibrium constant of reaction (24). By introducing initial conditions for the transport of the solute M (*C_i^* > *C_j^* > 0 and *C_{x,eq}^* = *C_x = 0), one finally obtains for a value for the equilibrium concentration of the primary ion (M⁺) in the receiving solution:

\[ C_{ML,eq} = \frac{-K(*C_i^* + *C_j^*) + \sqrt{K^2(*C_i^* + *C_j^*)^2 + 4K(V_f/V_r)^2}}{2V_f/V_r - K V_r/V_f} \]  

### Experimental

**Reagents**

Bis[(benzo-15-crown-5)-4'-methyl]pimelate, abbreviated hereafter as bis(benzo-15-crown-5), (Dojindo Laboratories, Kumamoto, Japan), dibenzoylmethane and stearic acid (Wako Chemicals Co., Tokyo, Japan), o-nitrooctylphenyl ether (Dojin Laboratories, Kumamoto, Japan) and poly(vinyl chloride) (Wako Chemicals Co.) were used as received. Other reagents used were all of G.R. grade. Milli-Q water (Millipore, Bedford, U.S.A.) was used throughout the experiments.

**Apparatus**

A metal furnace flameless atomic absorption spectrophotometer SAS 727 (Seiko, Tokyo, Japan) was used for the determination of Cu²⁺, Na⁺ and K⁺ ions. A TOA (Tokyo, Japan) glass electrode pH meter (HM60S) was used for measuring the pH of solutions. Voltammograms were recorded with a Fuso (Tokyo, Japan) potentiostat (Model 312) in conjunction with a Riken Denshi (Tokyo, Japan) X-Y recorder (F-30P).

**Preparation of co-transport liquid membrane system for K⁺ ions**

A polytetrafluoroethylene-type membrane filter (diameter 47 mm, pore size 0.50 µm, Toyo Roshi, Tokyo, Japan) was impregnated with an o-dichlorobenzene solution containing 2.5×10⁻³ M bis(benzo-15-crown-5). The supported liquid membrane was attached to the body of an ammonia-sensitive electrode (Denki Kagaku Keiki, Tokyo, Japan) with an O-ring. The inner compartment of the body was filled with a 10 mM lithium acetate-HCl buffer solution (pH 3.0) (0.5-
2.0 ml) as the receiving solution. The electrode body was immersed in a feed solution (130 ml) containing 10 mM lithium hydroxide, $1 \times 10^{-4}$ M KCl and 1.5 mM lithium picrate. The expected transport process for K$^+$ ions through the supported liquid membrane, thus prepared, is shown in Fig. 1a. The amount of K$^+$ ions transported in the receiving solution was determined by atomic absorption spectrophotometry (AAS).

**Preparation of counter transport liquid membrane system for Na$^+$ ions**

The uphill transport system for Na$^+$ ions is shown in Fig. 1b. 1-Pentanol solution (50 ml) containing 1 mM stearic acid as a carrier was interposed between an aqueous 0.05 (or 0.01) M NaOH feed solution (50 ml) and an aqueous 0.05 M HCl receiving solution (0.5 – 3.0 ml) containing 0.05 (or 0.01) M NaCl. Though both the feed and receiving solutions were stirred with a magnetic stirrer, the liquid membrane phase was unstirred. An increase in the concentration of Na$^+$ ions in the receiving solution was obtained by AAS measurements.

**Preparation of the counter transport liquid membrane system for Cu(II) ions**

A PVC-supported liquid membrane was prepared as follows: 5 mg of dibenzoylmethane was dissolved in a mixture of 20 mg of o-nitrophenyloctyl ether and 7 ml of tetrahydrofuran. The tetrahydrofuran was evaporated overnight in a Petri dish (diameter: 7 cm). A 15-mm diameter piece cut from the PVC-supported liquid membrane was attached to the body of an ammonia-sensitive electrode (Denki Kagaku Keiki, Tokyo, Japan). In the inner compartment was placed a 3 M hydrochloric acid solution (0.3 – 2 ml) as the receiving solution. The feed solution was a 1 mM acetate buffer solution (pH 5) (50 ml) containing $2 \times 10^{-4}$ M copper(II) sulfate. The amount of Cu(II) transported into the receiving solution was determined by both AAS and voltammetry with a glassy carbon (GC) electrode. In the latter case, a three-electrode configuration was employed: a GC electrode used as a working electrode was placed in the receiving solution. A platinum wire used as an auxiliary electrode and a saturated calomel electrode used as a reference electrode were immersed in the feed solution. Voltammetric measurements were carried out in the d.c. mode at a scan rate of 10 mV s$^{-1}$.

**Results and Discussion**

A comparison of the theoretical results with the experimental transport behavior

The time-resolved co-transport of K$^+$ ions across a bis(benzo-15-crown-5)-incorporated liquid membrane is shown in Fig. 2. In this transport, picrate ions present in excess in the feed solution pump the K$^+$ ions into the receiving solution according to the expected process shown in Fig. 1a. The establishment of equilibrium of the transport can be seen as a levelling off of the amount (moles) of transported K$^+$ ions with respect to time. Interestingly, the equilibrium time becomes shorter, i.e., less than 5 min, if the volume of the receiving solution is decreased down to 0.5 ml. This is due to the fact that the amount (moles) of transported K$^+$ ions, up to the diffusion equilibrium, decreased with a decrease in the volume of the receiving solution. These results agree with a theoretical prediction based on Eq. (12). The equilibrium concentration of K$^+$ ions in the receiving
solution was plotted against the volume of the receiving solution, as shown in Fig. 3. The result shows an enhancement of the concentration of K+ ions by decreasing the volume of the receiving solution. The feed/receiving volume effect was calculated based on Eq. (13) using identical parameters as those used for the transport experiment. The theoretical result is also shown in Fig. 3. It is clear that the calculated volume effect agrees well with the experimentally observed one in the sense that the calculated and observed relative change in the concentration of K+ ions in the receiving solution coincides each other. The absolute amount (moles) of transported K+ ions found in the receiving solution is much smaller than that calculated from Eq. (12). This is reasonable, since an actual system may lose its driving force due to unexpected side reactions as well as a significant amount of K+ ions remaining as an ion-pair complex in the liquid membrane; the calculated equilibrium moles, n, thus needs a correction of this amount.

The time-resolved counter transport of Na+ ions from the feed solution into different volumes of receiving solution with a stearic acid-incorporated liquid membrane is shown in Fig. 4. When the volume of the receiving solution was larger than 2 ml, the transport reached its equilibrium within 24 h. However, when the volume is less than 1 ml, the amount of transported Na+ ion decreased with a transport time longer than 9 h. This is because the pH of the receiving solution increases from the initial pH value (pH 0.7) to pH around 7 (after 24 h), owing to the consumption of protons by counter transport. Therefore, in this case,
the amount of Na⁺ ions at a transport time of 9 h was regarded as an approximate equilibrium concentration of Na⁺ ions. Figure 5 shows the equilibrium concentration of Na⁺ ions in the receiving solution plotted against the volume of the receiving solution for different initial concentrations of Na⁺ ions in the feed solution. The results show that the equilibrium concentration is significantly enhanced by decreasing the volume of the receiving solution. Figure 5 also shows the feed/receiving volume effect calculated from Eq. (23). The theoretical curves agree well with the experimentally observed volume effect. It can also be seen that the volume effect is more significant if the concentration of Na⁺ ions is lower. This effect is also predicted from Eq. (23), which shows that the equilibrium concentration of an analyte in the receiving solution depends not only on the relative volume of the receiving solution to that of the feed solution, but also on the initial concentrations of the primary and pumping ions.

Figure 6 shows the enhancement of the equilibrium concentration of Cu(II) upon decreasing the volume of the receiving solution in the uphill transport system shown in Fig. 1c. The observed dc current due to a reduction of the Cu(II) transported into the receiving solution showed an enhancement of the current with a decrease in the volume of the receiving solution. This result obviously indicates the potential usefulness of this approach for the amplification of a signal if the electrode is used as a Cu(II) ion uphill transport membrane sensor. In this transport, two moles of protons in the receiving solution are consumed by the uphill transport of one mole of Cu(II) ions. Therefore, Eq. (23), which describes the volume effect for a 1:1 coupled transport system, is not allowed to be applied to this system. What is interesting in this type transport is whether the degree of amplification by the feed/receiving volume effect for the 1:2 uphill transport system is the same extent or not as for the 1:1 coupled transport system. The experimentally observed volume effect for Cu(II) transport showed an almost identical curve as that for the Na⁺ ion transport system. This suggests that the degree of amplification by the volume effect is almost to the same extent for both the 1:1 and 1:2 counter transport systems.

**Theoretical predictions**

Amplification of the concentration of an analyte according to the feed/receiving volume effect for different modes of uphill transport: the co-transport, counter transport and co-transport plus complexation was calculated based on Eqs. (13), (23) and (28), respectively, using identical transport parameters, except that for the last case additional complexation parameters were used. The results are shown in Fig. 7. Though the volume effect for counter transport is much larger than for co-transport, the amplification for a co-transport system is significantly enhanced by additionally supplying the complexation energy as a source of the external driving force. Similarly, the degree of amplification for counter transport can also be enhanced by complexation reactions of the primary ion transported in the receiving solution (the data are not shown here). These results suggest that the complexation reaction in the receiving solution acts as an additional driving force for coupled transport. This phenomenon will also be useful in designing uphill transport membrane sensors with an enhanced amplification ability.

In Fig. 8 is shown the calculated equilibrium concentration of the primary ion in the receiving solution as a function of the volume of the receiving solution.
function of its initial concentration. The results show that the concentration dependence is not linear, especially at higher concentrations of the primary ion, approaching that of the pumping ion. At very low initial concentrations of the primary ion (less than $10^{-6}$ M), the equilibrium concentrations for both the co-transport and counter transport systems are almost identical. This region may correspond to the case reported by Morf et al.\(^1\) where a nearly complete accumulation of the solute in the receiving solution was assumed, according to

\[ C_{eq}^{co-transport} = \frac{V_r}{V_f} C_s. \]

\[ C_{eq}^{counter transport} = \frac{V_r}{V_f} C_s. \]

This suggests that a linear calibration curve with uphill transport membrane sensors can only be obtained in the limited concentration range of an analyte, i.e., very low concentration relative to that of the pumping ions.

The interesting feature of the feed/receiving volume effect is that, according to Eqs. (12) and (22), the amount (moles) of the primary ions transported into the receiving solution decreases upon decreasing the volume of the receiving solution. This indicates that the equilibrium time of uphill transport and, consequently, the response time of an uphill transport membrane sensor, becomes shorter if the volume of the receiving (inner) solution is decreased. Of course, for such a membrane diffusion-limited transport, other factors, such as the area and thickness of the liquid membrane, also affect the equilibrium time of transport. In addition, a thinner liquid membrane can decrease the diffusion time of the primary ions within the membrane. Therefore, for shortening the response time of an uphill transport membrane sensor without any loss of the amplification factor, the sensor should be designed so that the electrode assembly has a smaller volume of inner solution and a larger area of thin liquid membrane at the same time. Cell fabrication and experimental work are now in progress in order to examine to what extent the time for equilibrium (response time) can be shortened.

The present paper described theoretical equations used to predict the feed/receiving volume effect for the membrane diffusion-limited coupled transport. The theoretical results agree fairly well with the actually observed volume effect for several liquid membrane systems, though the theoretical model is rather simple. The equations predict the degree of enhancement of the concentration of an analyte in terms of the experimental parameters, i.e., volumes of the receiving and feed solutions, the initial concentrations of primary and pumping ions and the bulk equilibrium constants, which are important in view of designing uphill transport membrane sensors and of novel preconcentration techniques in general. For sensor development, it is recommended that the incorporation of counter transport systems is much preferable to co-transport systems, unless the volume effect for co-transport systems is improved by supplying additional driving forces, such as a complexation reaction in the receiving solution.

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References


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