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SEPARATION OF L-VALINE BY ANIONIC CARRIER-MEDIATED TRANSPORT IN A SUPPORTED LIQUID MEMBRANE

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Abstract – An anionic carrier-mediated separation of L-valine from the dilute aqueous solution was studied with a liquid membrane, constituted by a solution of D2EHPA (Di-2-Ethyl Hexyl Phosphoric Acid) in 1-decanol and supported by a hydrophobic microporous membrane. The equilibrium and transport rate data are reported in terms of distribution coefficients and mass transfer coefficients under the varying conditions of feed phase pH and carrier concentration. The experimental results obtained are analyzed to confirm the mechanism of the complex formation between carrier and L-valine and to describe the transport rate of L-valine through the supported liquid membrane by an appropriate theoretical model. The selectivity and the stability of the supported liquid membrane under study were also evaluated to ensure its potential application as a commercial down-stream process.

Key words: *L-Valine, Supported Liquid Membrane, D2EHPA, Mass Transfer Coefficient, Selectivity and Stability*

INTRODUCTION

Production of amino acids is carried out essentially in two main ways: chemical and biochemical synthesis. The latter is usually a microbial fermentation or an enzymatic synthesis. In the production cycle the various unit operations are used for separation, concentration and purification of amino acids. These stages account for more than 50 % or even reach up to 80 % of the total production cost. Among the various modes of operation, liquid membranes immobilized on a solid microporous support (SLM's) or in an emulsion form (ELM's) offer great potential for their characteristic to accomplish both separation and concentration in one step (see reviews by [Eyal and Bressler, 1993]).

Many papers are present in the literature on the use of liquid membranes in the emulsion form. The main disadvantage of the technique is the swelling of the membrane. This creates a dilution of the separated product in the internal phase, an increase in membrane breakage, and an increase of the stirring power required to disperse the emulsion [Itoh et al., 1990a]. In this respect, supported liquid membranes have the greatest ease of use and therefore the greatest potential for commercial application. For this kind of membrane, however, the main problems are the stability and the minimization of the diffusional resistances inside the membrane. These can be reduced by using various techniques as the combined effect of the carrier and its diluent [Molinari et al., 1989].

Liquid membrane technology, in fact, takes advantage of the active or enhanced transport by using a complexing agent (carrier) in chiral or achiral form. The first type of carrier is usually used for separation of enantiomers obtained from chemical synthesis, the second for amino acids obtained from biochemical synthesis such as fermentation [Aida et al., 1986].

Several publications have examined the separation and purification of amino acids using anion exchanger, Aliquat 336, as a carrier [Thien et al., 1988; Renon et al., 1990]. Renon studied particularly the case of L-valine permeation through an SLM. These workers pointed out that, owing to cationic nature of the carrier, the negatively charged surface of microorganisms could result in the fouling of the membrane interface, which lowered the degree of the extraction of the amino acid. In addition, their process was not suitable for a continuous process since the accumulation of chloride ions in the feed phase would impair the separation efficiency [Hong et al., 1992]. On the other hand, cation exchange extractants such as D2EHPA (Di-2-Ethyl Hexyl Phosphoric Acid) can be used to transport amino acid in the cationic form from its acidic solutions ($pH<pK_1$). And D2EHPA has low solubility (under 10 ppm even for nondilute extractant) in neutral and acidic water solutions [Huang et al., 1988]. For these reasons, D2EHPA was recently studied as a carrier in the recovery of amino acids [Itoh et al., 1990b; Termato et al., 1991; Boyadizhiev et al., 1994]. In the case of L-valine, no studies on the use of D2EHPA as a carrier and its related equilibrium and kinetic data have been reported and most of earlier studies lack analysis of kinetic data to develop an appropriate theoretical model for the transport of amino acid through the SLM.

In this work, a carrier-mediated separation of L-valine from the dilute aqueous solution has been studied with a supported liquid membrane, constituted by a solution of D2EHPA in 1-decanol and supported by a hydrophobic microporous membrane. The aims of the present work are to find the parameters that afford such a membrane high amino acid flux with sufficient stability and selectivity, and to interpret and predict the experimental equilibrium and transport rate data with an appropriate theoretical model.

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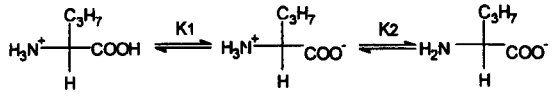
THEORY

1. Equilibria

In aqueous solutions, L-valine exists in ionic form of different charge depending on the pH value of the medium as follows:



(1)

A^+ $A^\pm$ A^-

Here A^+, $A^\pm$ and A^- are cation, zwitterion and anion of the L-valine A, respectively, and the values of dissociation constants, K_1 and K_2, are [Barret, 1985]

$$K_1 = \frac{C_{A^\pm} C_{H^+}}{C_{A^+}} = 10^{-2.32}, \quad K_2 = \frac{C_{A^-} C_{H^+}}{C_{A^\pm}} = 10^{-9.62} \tag{2}$$

L-valine can be solubilized into the organic phase after chemical complexation with an ionic carrier. When D2EHPA is used as a carrier, it can be assumed that the dimer of D2EHPA, $(\overline{HD})_2$ reacts with A^+ at the aqueous organic interface according to [Teramoto et al., 1991]

$$A^+ + 2(\overline{HD})_2 \overset{K_{eq}}{\rightleftarrows} \overline{AD(HD)_3} + H^+ \tag{3}$$

The equilibrium concentration of the complex ($C_{\overline{ADe}}$), hydrogen ion (C_{H^+e}), L-valine (C_{Ae}), cation of L-valine (C_{A^+e}) and carrier ($C_{\overline{HDe}}$) can be expressed by, respectively,

$$C_{\overline{ADe}} = C_{Ao} - C_{Ae} = x \tag{4}$$

$$C_{H^+e} = C_{H^+o} + x \tag{5}$$

$$C_{Ae} = C_{Ao} - x \tag{6}$$

$$C_{A^+e} = \frac{C_{Ae}}{1 + K_1/C_{H^+e}} \tag{7}$$

$$C^2_{\overline{ADe}} = (C_{\overline{HDo}} - 2x)^2 \tag{8}$$

and the equilibrium constant (K_{eq}) can be evaluated by

$$K_{eq} = \frac{C_{\overline{ADe}} C_{H^+e}}{C_{A^+e} C^2_{\overline{HDe}}} \tag{9}$$

The distribution coefficients of L-valine and L-valine cation, m_A and m_A^+ can be presented by, respectively,

$$m_A = \frac{C_{\overline{ADe}}}{C_{Ae}} = K_{eq} \frac{C^2_{\overline{HDe}}}{C_{H^+e} + K_1} \fallingdotseq K_{eq} \frac{C^2_{\overline{HDe}}}{K_1} \quad (\text{at } C_{H^+e} \ll K_1) \tag{10}$$

$$m_{A^+} = \frac{C_{\overline{ADe}}}{C_{A^+e}} = m_A \left(1 + \frac{K_1}{C_{H^+e}}\right) \tag{11}$$

Eq. (10) can be rearranged to the log form

$$\log\left(\frac{m_A K_1}{K_{eq}}\right) = 2\log(C_{\overline{HDe}}) = 2\log\left(C_{\overline{HDo}} - 2\frac{m_A C_{Ao}}{m_A + 1}\right) \tag{12}$$

The stoichiometry of the reaction mechanism described by Eq. (3) can be confirmed by plotting Eq. (12) with the equilibrium data at various concentrations of the D2EHPA carrier.

2. Kinetics

A schematic diagram of the transport mechanism of L-valine through a supported liquid membrane is shown in Fig. 1 and five consecutive elementary steps are considered as follows:

① Reactant (A^+) diffuses from the bulk phase to the interface through the feed side aqueous film by concentration gradient.

② A^+ is exchanged with H^+ of H^+D^- and complex (A^+D^-) formation is made at the interface.

③ Complex diffuses through liquid membrane by concentration gradient.

④ A^+ is separated from the complex by ion exchange with counterion.

⑤ A^+ diffuses from the interface to the bulk phase through the stripping side aqueous film by concentration gradient.

Concentration profile is shown in Fig. 2 and the flux of L-valine through three films in feed, liquid membrane and stripping sides can be expressed by

$$N^o = K^o(C_{A^+f} - C_{A^+s}) = k_f(C_{A^+f} - C_{A^+f\,i}) = k_{m,\overline{AD}}(C_{\overline{AD}0} - C_{\overline{ADL}}) = k_s(C_{A^+s\,i} - C_{A^+s}) \tag{13}$$

From Eqs. (11) and (13) the relationship of overall and local mass transfer resistances can be rearranged to be

$$\frac{1}{K^o} = \frac{1}{k_f} + \frac{1}{k_{m,\overline{AD}} \cdot m_{A^+}} + \frac{1}{k_s} \tag{14}$$

The hydrodynamic properties of feed and stripping sides are assumed to be equivalent each other. k_s may then be set equal to k_f and Eq. (14) can be rewritten

$$\frac{1}{K^o} = \frac{2}{k_f} + \frac{1}{k_{m,\overline{AD}} \cdot m_{A^+}} \tag{15}$$

With the geometry of an apparatus used in this study which

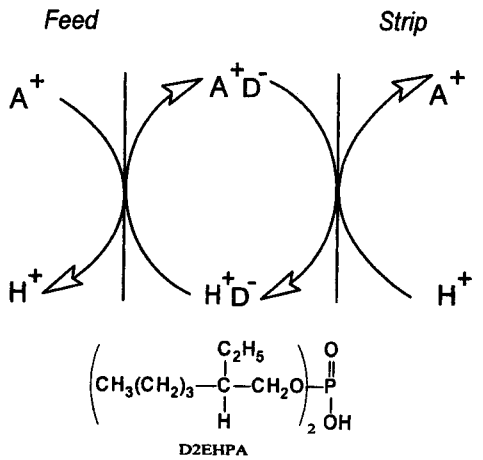


Fig. 1. Schematic diagram of the transport mechanism of L-valine.

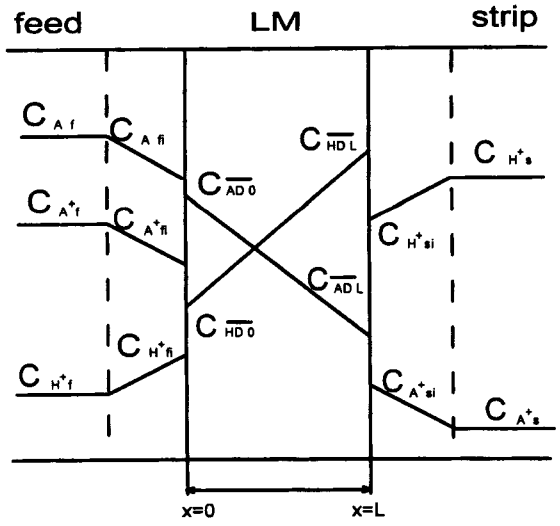


Fig. 2. Concentration profiles in an SLM.

gives equal volumes of feed and stripping sides, the mass balance of L-valine cation is written

$$-\frac{V_f}{S}\frac{dC_{A^+f}}{dt}=\frac{V_s}{S}\frac{dC_{A^+s}}{dt}=K^o(C_{A^+f}-C_{A^+s}) \tag{16}$$

Since the volume of organic extractant immobilized in the microporous polymeric membrane is small enough compared to the volumes of feed and stripping sides that it can be ignored, the initial concentration of L-valine cation (C_{A^+fo}) can be expressed as the sum of L-valine cation in the feed and stripping sides.

$$C_{A^+fo}=C_{A^+f}+C_{A^+s} \tag{17}$$

The integration of Eq. (16) with an initial condition t=0, $C_{A^+f}=C_{A^+fo}$ and Eq. (17) leads to

$$\ln\left(2\frac{C_{A^+f}}{C_{A^+fo}}-1\right)=\ln\left(2\frac{C_{Af}}{C_{Afo}}-1\right)=-\frac{2K^oS}{V_f}t \tag{18}$$

The value of K^o can be obtained experimentally from the slope of Eq. (18) in the case of the concentration of L-valine of the feed phase more than half of the initial concentration and the individual mass transfer coefficients can be obtained from Eq. (15) by plotting K^o as a function of m_A^+.

Apart from the above model, Renon [1990] characterized the L-valine transport through the SLM by its permeation rate that is deduced from

$$\ln\frac{C_A}{C_{Ao}}=-P_A\frac{S}{V_f}t \tag{19}$$

This equation is equivalent to the integrated form of Eq. (16) with overall mass transfer coefficient (K^o) replaced by permeability (P_A) when the stripping side film resistance is neglected, i.e., $C_{A^+s}=0$. The adequacy of this model shall, therefore, be checked with the SLM system under study because aqueous film resistance at the stripping side may affect the overall transport rate.

EXPERIMENTS

1-Decanol was chosen as a solvent because of its low solubility value of 0.037 g/l and high viscosity value of 11.8 cP at 20 °C [Neplenbroek et al., 1992]. It was purchased from Sigma and D2EHPA from Tokyo Kasei for being used as the organic membrane phase. L-Valine and D-(+)-glucose from Sigma were used to prepare a synthetic aqueous amino acid solution. All other chemicals were of analytical grade.

Distribution coefficients were measured as follows. 20 ml of 0.01 M solution of L-valine and the equal volume of organic phase were mixed in flasks. They were shaken at 25 °C for 12 h and settled for more than 2 h. After that, samples were taken from the aqueous phase and analyzed. Amino acid concentration in the organic phase was calculated from the difference of concentration between initial and final values. The L-valine concentration was measured using the ninhydrin reagent solution (Sigma) and by the technique of Moore [Moore et al., 1954].

Supported liquid membranes were prepared by dipping the polymeric sheets into the organic solution for about 12 h and wiping them by a soft paper. The support used was a flat sheet microporous hydrophobic polytetrafluoroethylene (PTFE) membrane (Millipore Co., Fluoropore® Membrane) of 0.5 μm pore size, 85 % porosity, and 175 μm thickness. SLM was placed between the feed and strip compartments of the cell shown in Fig. 3. Membrane surface area was 17.35 cm^2. The feed and stripping solutions, each one with 250 ml volume, were stirred with a mechanical stirrer at 300 rpm. This rate of 300 rpm was high enough to reduce the resistances in the aqueous boundary layers of the SLM. The feed phase was maintained at constant pH by controlled addition of 10.0 N KOH with a pH controller (Jenco electronics, Taiwan) and a peristaltic pump (Coleparmer, USA). Samples were taken from the feed phase for analysis. The stripping phase was HCl or KCl solution for using H^+ or K^+ as counterions.

Glucose and NaCl were chosen to simulate the impurities in the fermentation broth. The concentration of glucose was measured by Glucose Analysis Kit from Sigma (Enzymatic re-

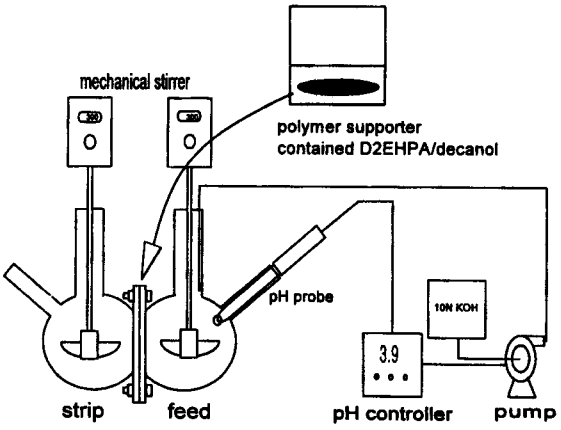


Fig. 3. Schematic diagram of experimental apparatus.

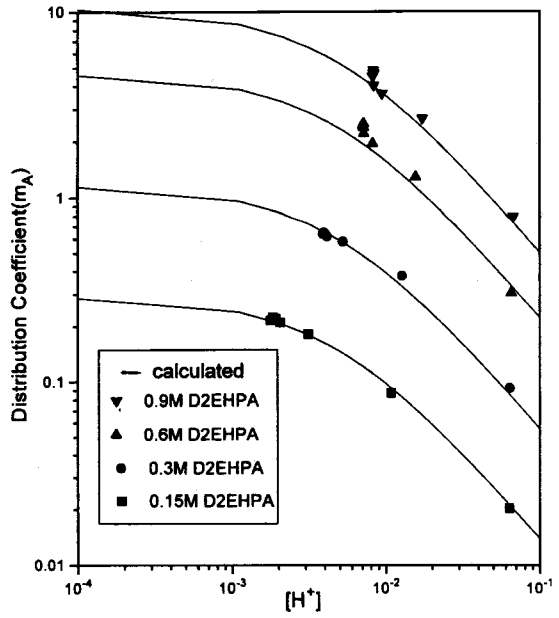


Fig. 4. The effects of the pH of the feed phase and carrier concentration on the distribution coefficient (m_A).
Aqueous phase; 0.01 M L-valine. Organic phase; D2EHPA in 1-decanol.

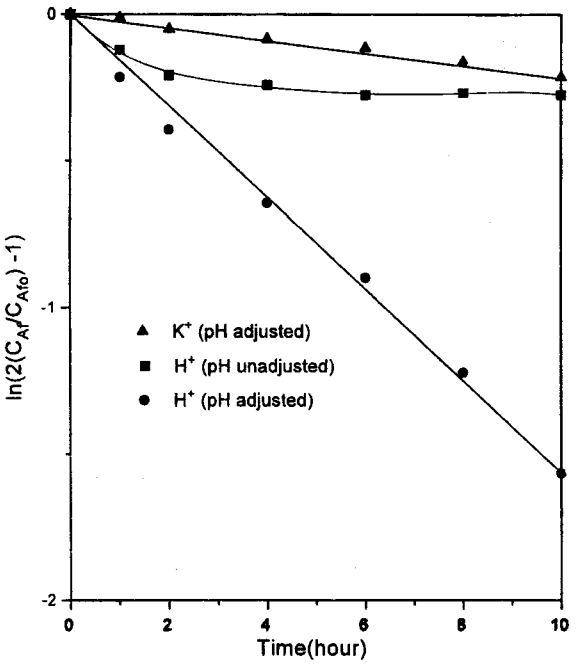


Fig. 5. Effects of counterion and pH adjustment of the feed phase on the transport rate of L-valine.
Feed phase; 0.01 M L-valine (pH 4.0 const.). LM phase; D2EHPA in 1-decanol. Strip phase; 1.0 N HCl or 1.0 N KCl.

actions with glucose oxidase and peroxidase, and spectrophotometry at 435 nm). Sodium concentration which was dissolved in the feed phase was measured by an Atomic Absorption Spectroscopy (Jarrel-Ash 82-270, USA).

For the calculation of the diffusivity, the viscosity was also measured by a rheometer (Physica rheometer model. MC 120).

RESULTS AND DISCUSSION

The distribution coefficients measured under various pH values of the feed phase are shown in Fig. 4. Since L-valine must exist as a cation to be complexed with an anion of the D2EHPA, a low pH is desirable. On the other hand, if the pH of the feed phase is too low, the weakly acidic carrier will become protonated and thus unable to transport L-valine cation. It is observed that the distribution coefficients were indeed drastically reduced below pH 4.0 of the feed phase. If the pH is therefore adjusted to a pH of 4.0 by addition of a strong KOH solution, an increase in overall L-valine transport can be expected. This effect is shown in Fig. 5, which indicates that the transport rate of L-valine with pH adjustment of the feed phase was higher than the one without pH adjustment. In fact, hydrogen ion is transfered as a counterion from the stripping phase to the feed phase and the pH of the feed phase drastically decreases without pH adjustment. Boyadizhiev [1994] used K^+ as a counterion to eliminate this shortcoming owing to the change of the pH of the feed phase. In the case that the pH of the feed phase was adjusted, the L-valine transport of using H^+ as a counterion was, however, much faster than that of using K^+ as shown in Fig. 5. This observation might be attributed to the fact that D2EHPA preferably reacts with H^+ to K^+ due to its weakly acidic character and thus the stripping stage at least partially controlled the overall process rate.

The effect of the carrier concentration on the distribution coefficient is also shown in Fig. 4. As carrier concentration increased, L-valine distribution coefficient and hydrogen ion concentration of the feed phase increased. The log-log re-

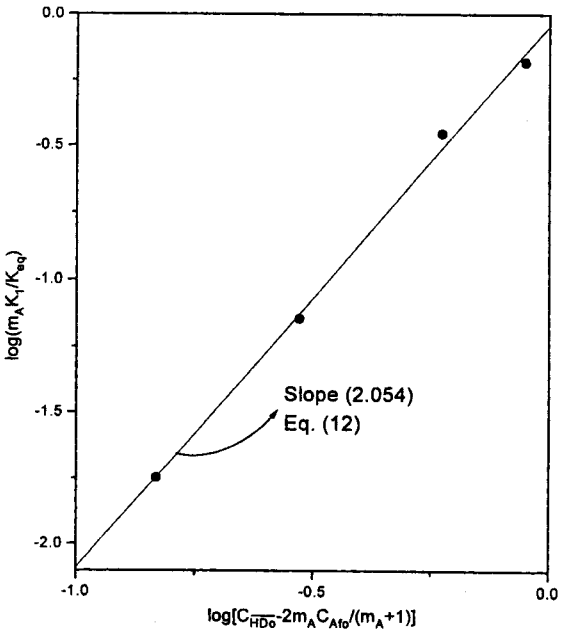


Fig. 6. Relation between carrier concentration and distribution coefficient (m_A).
Aqueous phase; 0.01 M L-valine, Organic phase; D2EHPA in 1-decanol.

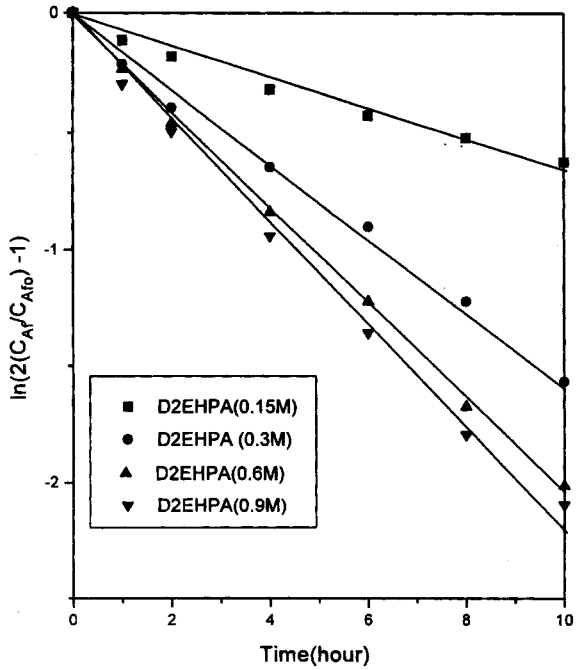


Fig. 7. Kinetics of L-valine in an SLM.
Feed phase; 0.01 M L-valine (pH 4.0 const.). LM phase; D2EHPA in 1-decanol. Strip phase; 1.0 N HCl.

lation between carrier concentration and distribution coefficient as developed in Eq. (12) was a straight line with a slope of 2 as shown in Fig. 6, experimentally confirming that 1 mole of L-valine reacted with 2 moles of D2EHPA dimer. The equilibrium constant of the complex formation was found to be 0.07 M^{-1} and the distribution coefficients calculated with this value of equilibrium constant were, as shown in Fig. 4, in good agreement with the experimental data obtained with the varying carrier concentrations.

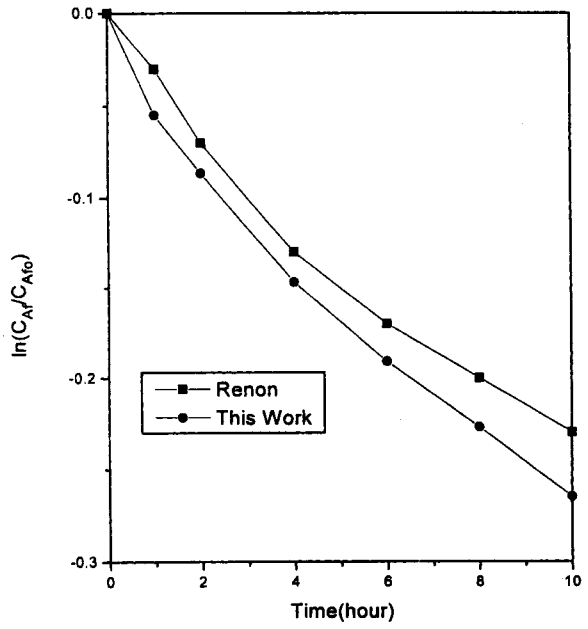


Fig. 8. Nonlinearity by Renon's model.

Table 1. Effects of carrier concentration on the distribution coefficient and the overall mass transfer coefficient at pH 4.0

$C_{\overline{AD}}$ (mol/l)	0.15 M	0.3 M	0.6 M	0.9 M
Valine distribution coefficient, m_A	0.31	1.23	4.93	11.09
Valine cation distribution coefficient, m_A^+	12.65	50.19	201.19	452.59
Overall mass transfer coefficient, K^o (M^{-1})	1.62	3.85	5.43	6.05

Charged L-valine can only permeate through the organic membrane phase via the L-valine/carrier complex in carrier-mediated transport systems. The carrier concentration thus influences the transport rate of L-valine through the variation in distribution coefficient. The effect of the carrier concentration on the L-valine transport rate is shown in Fig. 7. The data were obtained with the pH of the feed phase adjusted to a constant value of 4.0 which gives the highest distribution coefficient and represent the highest transport rates of L-valine under the given carrier concentrations. As the carrier concentration increased, transport rate of L-valine increased. Fig. 7 shows good linearities in plotting the experimental measure of the left hand side of Eq. (18) against time and the values of overall mass transfer coefficient were estimated from these plots. It is noted that the plots as shown in Fig. 8, based on Renon's model, Eq. (19), do not show linear relationship even on their experimental data. This result leads to a conclusion that the model employed in this study incorporating the aqueous film resistance of the stripping phase is more adequate to interpret and predict the experimental transport rate data.

The values of overall mass transfer coefficient obtained

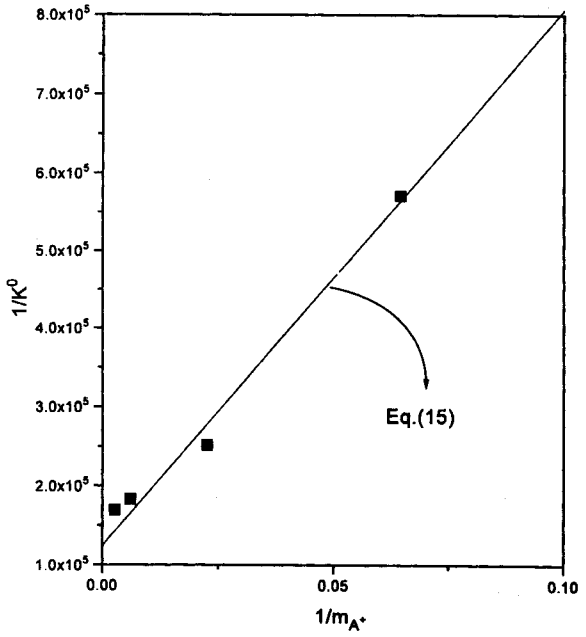


Fig. 9. Relation between overall mass transfer coefficient (K^o) and distribution coefficient (m_A^+).

Table 2. Parameters for calculation

$C_{\overline{AD}}$ (mol/l)	0.15 M	0.3 M	0.6 M	0.9 M
μ (10^{-2} Pa·sec)	1.28	1.44	1.78	2.23
$D_{\overline{AD}}$ (10^{-11} m^2/sec)	2.35	2.09	1.69	1.35
$k_{m,\overline{AD}}$ (10^{-7} m/sec)	1.51	1.34	1.08	0.87

are given in Table 1 and are plotted in Fig. 9 against m_A^+ to evaluate the values of local mass transfer coefficient of each side (k_f, k_s, $k_{m,\overline{AD}}$) according to Eq. (14). The values of k_f, k_s, $k_{m,\overline{AD}}$ were found to be 1.613×10^{-5}, 1.613×10^{-5}, 1.465×10^{-7} m/sec, respectively. The value of $k_{m,\overline{AD}}$ is considered reasonable as shown in Table 2, since it is close enough to the theoretical value of $k_{m,\overline{AD}}$ calculated from

$$k_{m,\overline{AD}} = \frac{D_{\overline{AD}}}{\delta^{ff}} \fallingdotseq \frac{D_{\overline{D}}}{\delta^{ff}} \tag{20}$$

In this calculation, the diffusion coefficient of the complex in the liquid membrane phase ($D_{\overline{AD}}$) was assumed to be as equal as that of the carrier in the form of two units of D 2EHPA dimer ($D_{\overline{D}}$), since the L-valine is much smaller than the carrier and the L-valine/carrier complex is thus considered roughly equivalent to the carrier in size, and $D_{\overline{D}}$ was calculated from Wilke and Chang's correlation [Wilke and Chang, 1955] with the solvent association parameter of 1.0 and the solute molar volume at the normal boiling point of 1,910 cm^3/gmol obtained from the literature data [Teramoto et al., 1991]. The latter value could be roughly compared with the value of 1,703 cm^3/gmol estimated from the data on atomic volumes [Treybal, 1980]. The effective thickness of the membrane (δ^{ff}) was obtained from

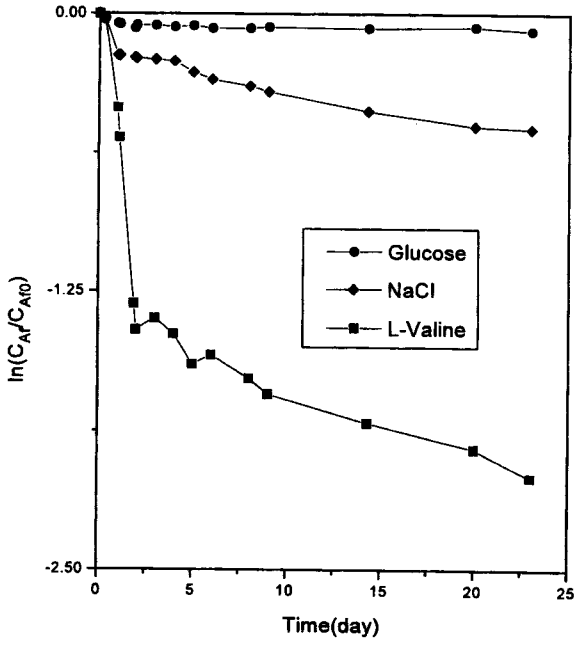


Fig. 10. Selectivity of the SLM system.
Feed phase; 0.01 M L-valine, 0.1 M glucose, 0.1 M NaCl (pH 4.0 const.). LM phase; 0.3 M D2EHPA in 1-decanol. Strip phase; 1.0 N HCl.

$$\delta^{ff} = \delta \times \tau \tag{21}$$

where δ is the thickness and τ is the tortuosity of the support. The value of τ of the present support is reported to be 1.25 by the producer.

Fermentation broths include various kinds of impurities such as carbohydrates and inorganic salts. The issue of selectivity should, therefore, be examined in investigating the use of supported liquid membranes for a commercial bioseparation operation. In this study, the selectivity of the D 2EHPA/decanol liquid membrane toward glucose and Na^+ was examined. As shown in Fig. 10, the selectivity of L-valine remained very high toward both glucose and Na^+. This seems to be resulted from the facts that glucose is a nonelectrolyte and cannot partition into the organic phase or compete for carrier and that sodium ion is more hydrophilic than L-valine. Fig. 10 also shows that the stability of the D2EHPA/decanol liquid membrane was found to be sufficient to ensure a selective transport of L-valine during a continuous run lasting more than 20 days.

CONCLUSION

L-Valine could be extracted successfully from synthetic aqueous solutions using a D2EHPA/1-decanol mixture supported by a hydrophobic microporous membrane. The selectivity and the stability of the liquid membrane system studied were sufficient to achieve one-step purification operation for the applications in downstream processing.

Experimental data show that L-valine is solubilized into 1-decanol after chemical complexation with D2EHPA dimer at a stoichiometric ratio of 1 : 2 and that L-valine distribution coefficients were drastically reduced below pH 4 of the feed phase, indicating the importance of the feed phase pH control at this value in operating the performance.

A mathematical model based on the film theory was appropriate to interpret and predict the transport rate of L-valine in terms of mass transfer coefficients. The overall mass transfer coefficients were better fit with a model incorporating the film resistance of the stripping phase rather than neglecting it, and the individual mass transfer coefficient in the liquid membrane phase itself was within a range that could be predicted by a film theory.

NOMENCLATURE

A : L-valine
$\overline{(HD)_2}$: D2EHPA dimer
$\overline{AD(HD)_3}$: complex of carrier and L-valine
C : concentration [M]
D : diffusion coefficient [m^2/sec]
H : hydrogen
k : local mass transfer coefficient [m/sec]
K^o : overall mass transfer coefficient [m/sec]
K_1 : dissociation constant [M^{-1}]
K_{eq} : equilibrium constant [M^{-1}]
m : distribution coefficient
S : interface area of the liquid membrane [m^2]

P : permeability
V : volume [m^3]

Greek Letters

δ : thickness of the polymer membrane [m]
δ^{eff} : effective thickness of the membrane [m]
τ : tortuosity of the polymer membrane

Subscripts

A : L-valine
$\underline{A^+}$: cation of L-valine
$\underline{AD}$: complex of carrier and L-valine
$\overline{D}$: two units of D2EHPA dimer
e : equilibrium state
o : initial state
f : feed phase
$\underline{H}$: hydrogen
$\overline{HD}$: D2EHPA dimer
m : liquid membrane
s : stripping phase

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