Extraction Equilibria of Amino Acids and Dipeptides in Various Organic Solutions

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Experimental data on the partitioning of amino acids (tryptophan, phenylalanine) and dipeptides (tryptophan−leucine, phenylalanine−leucine) between the aqueous feed phase and the organic solvent phase are presented. The organic solvent phase consists of a carrier, sodium di-2-ethylhexyl sulfosuccinate/quaternary ammonium salt/dioleyl phosphoric acid, dissolved in (*Z*)-9-octadecen-1-ol (an organic solvent). The equilibrium distribution coefficient for extractions is determined by varying the following experimental conditions: feed solution pH, initial feed phase concentration, and composition of organic phase. The partition coefficient for the stripping process is also determined by varying the stripping agent (sodium hydroxide, sodium chloride, sodium carbonate), the concentration, and the pH of the stripping solution. The extraction and stripping reaction coefficients are calculated by combining the experimental data and the kinetic expressions for interfacial reactions. The variation of the values of the coefficients with the solute type (i.e. amino acid or dipeptide) and with the operating conditions of the system di-2-ethylhexyl sulfosuccinate in (*Z*)-9-octadecen-1-ol is presented.

1. Introduction

Reactive extraction systems are formed by dissolving a carrier in an organic solvent and loading the resulting solution on a polymeric membrane support. The efficiency of these systems is determined by the partitioning characteristics of the "target" molecules and their ability to form a complex with the carrier and the effective diffusivity of the solute−carrier complex in the organic solvent (Hano et al., 1995; Cascaval et al., 1996; Ersoz et al., 1995; Deblay et al., 1990). A few research groups have focused on the thermodynamic and kinetic properties of some organic−aqueous systems, especially determination of equilibrium partition coefficients and extraction/stripping rate constants (Cohen et al., 1995; Ziova et al., 1996; Caselli and Mangone, 1992; Titinger et al., 1997a,b). Despite the increase in the amount of research in this field, there is a lack of published experimental data for the partitioning of model solutes onto potentially useful extraction systems.

Amino acids and peptides are important bioproducts and can be recovered by liquid membrane-based processes, which have potential advantages over the conventional ion-exchange and liquid−liquid extraction processes. Although there are many reports of the superiority of the liquid membrane separations, the extraction behavior of these solutes has not been investigated in detail. Therefore, the aim of this paper is to generate relevant data for the partitioning of the model amino acids (tryptophan (Trp), phenylalanine (Phe)) and dipeptides (tryptophan−leucine (Trp−Leu), phenylalanine−leucine (Phe-Leu)) in various organic solutions. Special attention is given to the (*Z*)-9-octadecen-1-ol (commercially known as oleyl alcohol) system with a sodium di-2-ethylhexyl sulfosuccinate carrier (commercially known as Aerosol OT (AOT)).

It is worth mentioning that the organic solutions containing surfactants (e.g. AOT) could form reverse micelles or microemulsions if the solutions were shaken vigorously (Cardoso et al., 1996; Wang et al., 1995; Leodidis and Hatton, 1990a,b). These forms were not reported when tri-*n*-octylmethylammonium chloride (TOMAC) was used as the surfactant (Hano et al., 1991). In our experiments gentle shaking was used and these aggregates were not observed.

The importance of the AOT−oleyl alcohol system has been demonstrated by its effectiveness in the removal of hydrophobic solutes from dairy industry process solutions (Hossain and Stanley, 1996). The partition coefficient values under different conditions will help us design and improve the performance of this membrane-based recovery process. A mathematical model is also developed to calculate the reaction coefficients of the extraction and stripping processes.

2. Modeling of Equilibrium

All amino acids with uncharged residues exist in three forms in aqueous solution, according to the following reactions in equilibrium:

$$\underset{(A^+)}{NH_3^+-CHR-COOH} \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} \underset{(A^\pm)}{NH_3^+-CHR-COO^-} \underset{-H^+}{\overset{+H^+}{\rightleftharpoons}} \underset{(A^-)}{NH_2-CHR-COO^-} \quad (1)$$

The dissociation constants for systems of low concentration can be defined by the following equations

$$K_1 = (C_{A^\pm})(C_{H^+})/(C_{A^+})_{eq} \quad (2)$$

$$K_2 = (C_{A^-})(C_{H^+})/(C_{A^\pm})_{eq} \quad (3)$$

where C_{A^+}, $C_{A^\pm}$, and C_{A^-} are the concentrations of the cation, zwitterion, and anion forms of the amino acid, respectively, and C_{H^+} is the concentration of hydrogen ion.

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The form of the amino acid depends on the pH of the aqueous solution relative to the pK (defined as p$K = -\log K$) values. At a pH < (pK_1 + pK_2)/2, the solute is predominantly in cation form and is capable of undergoing reaction with a negatively charged carrier.

The dissociation reactions of a dipeptide in water are similar to that in eq 1.

$$NH_3^+-CHR_1-CO-NH-CHR_2-COOH \underset{H^+}{\overset{-H^+}{\rightleftharpoons}} NH_3^+-CHR_1-CO-NH-CHR_2-COO^- \quad (4a)$$

$$NH_3^+-CHR_1-CO-NH-CHR_2-COOH^- \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} NH_2-CHR_1-CO-NH-CHR_2-COO^- \quad (4b)$$

For the above reactions, the dissociation constants can be expressed similarly as in eqs 2 and 3, respectively, for COOH and NH_3 groups with the amino acid values replaced by peptide concentrations.

The total concentration of amino acid at low pH is related as

$$(C_{A_T}) = (C_{A^+}) + (C_{A^\pm}) + (C_{A^-}) \quad (5)$$

Using the equilibrium relationship eq 2, C_{A^+} is obtained as

$$(C_{A^+}) = (C_{A_T})/(1 + K_1/[C_{H^+}]) \quad (6)$$

At the interface of the organic phase and the aqueous solution, the following reaction takes place where amino acid ions react with the negative ions of the carrier to form a complex in the organic solution. During this ion-exchange reaction, a solution cation is exchanged for a sodium cation from the carrier solution. The reaction proceeds according to

$$(A^+)_{fi} + (Na^+AOT^-)_{fo} \rightleftarrows (Na^+)_{fi} + (A^+AOT^-)_{fo} \quad (7)$$

where fi and fo represent the aqueous feed and organic phases, respectively, at the feed−organic interface. The extraction equilibrium coefficient K_E can be defined as

$$K_E = \frac{[C_{Na^+}]_{fi}[C_{A^+AOT^-}]_{fo}}{[C_{A^+}]_{fi}[C_{Na^+AOT^-}]_{fo}} \quad (8)$$

where C_{Na^+}, $C_{Na^+AOT^-}$, and $C_{A^+AOT^-}$ are the concentrations of sodium, carrier, and carrier complex, respectively. It is noted that the carrier concentrations are in the whole volume (carrier + solvent).

The distribution coefficient, D_E, is defined as the ratio of the concentration of solute in the organic phase over that in the aqueous phase at equilibrium and can be expressed as follows:

$$D_E = \frac{[C_{A^+AOT^-}]_{fo}}{(C_{A_T})_{fi}} \quad (9)$$

It is difficult to measure accurately the concentration of solute−carrier complex in the organic phase. This can be calculated by knowing the initial and equilibrium concentrations of the solute in the aqueous phase (assuming negligible adsorption at the interface).

Substituting for $(C_{A_T})_{fi}$ from eq 6 and for C_{A^+} from eq 8, a relationship between D_E and K_E is obtained:

$$D_E = K_E \frac{[C_{Na^+AOT^-}]_{fo}}{(C_{Na^+})_{fi}} \Big/ \left[1 + \frac{K_1}{C_{H^+}}\right] \quad (10)$$

All concentrations in the above equations can be calculated or measured; therefore, by determining the distribution coefficient at constant pH for various AOT concentrations, the value of K_E can be calculated, if K_1 is known for the solutes.

During stripping with an aqueous solution, the decomplexation of the solute−carrier complex takes place and the amino acid solute is released according to the reaction

$$(A^+AOT^-)_{so} + (Na^+)_{si} \rightleftarrows (A^+)_{si} + (Na^+AOT^-)_{so} \quad (11)$$

where so and si represent the organic and strip phases at the strip−organic interface, respectively.

This reaction is characterized by a stripping equilibrium coefficient, K_S and is expressed as

$$K_S = [C_{A^+}]_{si}[C_{Na^+AOT^-}]_{so}/[C_{A^+AOT^-}]_{so}[C_{Na^+}]_{si} \quad (12)$$

A distribution coefficient at the strip−LM interface is defined by the following equation

$$D_S = \frac{[C_{A^+AOT^-}]_{so}}{[C_{A_T}]_{si}} \quad (13)$$

This equation can be expressed in terms of K_S and Na^+, H^+, and Na^+AOT^- concentrations, as shown here

$$D_S = \frac{1}{K_S} \frac{[C_{Na^+AOT^-}]_{so}}{[C_{Na^+}]_{si}} \Big/ \left[1 + \frac{K_1}{(C_{H^+})_{si}}\right] \quad (14)$$

By determining D_S and C_{Na^+} experimentally at various strip pH values, K_S values can be obtained. Rearranging eq 14 and taking the log on both sides, we obtain

$$\log_{10}(D_{ss}) = \log(K_{ss}^2 K_1) - \text{pH} \quad (15)$$

where

$$D_{ss} = \frac{C_{H^+}}{D_s} \quad \text{and} \quad K_{ss} = K_s \frac{[C_{Na^+AOT^-}]_{so}}{(C_{Na^+})_{si}} \quad (16)$$

A plot of the left-hand side of eq 15, that is, $\log(D_{ss})$ against pH, will yield a straight line from which K_S can be calculated.

3. Materials and Methods

3.1. Chemicals. Aerosol OT (AOT), product no. 560454R, was from BDH lab supplies, oleyl alcohol (85%, technical grade) was from Aldrich Chemical Co. Inc., USA, and the quaternary ammonium salt (commercially know as Aliquat 336) was from Henkel, Australia. DOLPA was a gift from Professor M. Goto, Kyushu University, Faculty of Engineering, Hakozaki, Japan. The chemicals H_3PO_4, CH_2-COONa, Na_2HPO_4, $NaH_2PO_4H_2O$, NaCl, NaOH, and HCl were supplied by BDH lab supplies, Poole, England. Phenylalanine−leucine, phenylalanine, tryptophan, and tryptophan−leucine were supplied by Sigma Chemical Co., St. Louis, MO.

OPA reagent was made from 12.5 mL of 0.1 M sodium borate, 2.5 mL of 10% (mg/mL) sodium dodecyl sulfate (SDS), and 20 mg of OPA dissolved in 500 μL of methanol and 50 μL of mercaptoethanol. All of these reagent components were added together, the volume was made up to

25 mL with distilled water, and then the reaction mixture was mixed prior to use.

3.2. Procedure for Equilibrium Measurements of Solutes at Ambient Temperature. 3.2.1. Extraction into Organic Solution. A feed solution was prepared in acetate phosphate buffer containing a single amino acid (or dipeptide) at the desired pH and contacted with an organic solution, for example, AOT−oleyl solution at a volume ratio of 1:1, in 15 mL centrifuge tubes. The feed concentration was fixed approximately at 0.5 mM (except for the experimental study of the effect of concentration), and the feed pH range was within 1−7. The solutions in the tubes were mixed for a period of 3 h using the rotating table at a very low rpm. After the contacting time the solutions were centrifuged for 5 min at 4000 rpm to separate the two phases and obtain a clear bottom aqueous phase. There was no indication of microemulsion formation or dissolution of one phase into another. The bottom aqueous layer was removed using a pasteur pipet and analyzed for its amino acid content. The initial and final pH values of the aqueous phase were also measured.

3.2.2. Solute Recovery in a Stripping Solution. Strip solutions were prepared in phosphate buffer at various concentrations of strippant, usually Na_2CO_3 or NaCl or NaOH. A strip solution at the desired pH was added, again in a 1:1 ratio, to the AOT−oleyl alcohol solution loaded with the amino acid (retained after extraction). The pH of the strip solution was in the range 5−9.

This was mixed for 3 h using the rotating table and then again centrifuged for 5 min at 4000 rpm to obtain a clear bottom aqueous phase. The aqueous layer was removed using a pasteur pipet and analyzed for its amino acid content. The initial and final pH values were also measured.

3.3. Procedure for Equilibrium Measurements at Controlled Temperatures. These experiments were carried out in the same manner as discussed for the ambient-temperature experiments with the following change. Instead of using the rotating table for mixing, samples were attached to a rack in a water bath set to the appropriate temperature and mixed at an rpm setting of ∼70, for the appropriate length of time, and then measured as before.

3.4. Analytical Procedure. 3.4.1. Analysis of Trp and Trp−Leu Using a Spectrophotometric Method. The absorbances of the initial feed solution and the aqueous phases produced after the extraction and stripping experiments were measured using a spectrophotometer over the wavelength range 200−300 nm. Some samples had to be diluted (usually a 10× dilution) to be below the maximum absorbance. The value obtained at the wavelength 280 nm was used as the absorbance of the sample. This was converted to a concentration using a standard UV absorbance versus concentration curve. Thus, the values of concentration for initial feed, feed, and stripping solution at equilibrium were obtained. The values of organic phase concentration were calculated by difference of initial and final aqueous phase concentrations. The distribution coefficients for extraction (D_E) and stripping (D_S) were calculated by using eqs 9 and 15, respectively.

3.4.2. Analysis of Phe and Phe−Leu Using a Microplate Method. The spectrophotometric method used for analysis of Trp and Trp−Leu was also tested for Phe and Phe−Leu. However, initial trials indicated that this method was prone to interferences for these amino acids, possibly from the buffer system. Also, although measurements were simple, processing of several samples was time-consuming, so a more effective assay was needed.

Table 1. Partitioning of Trp and Trp−Leu at pH = 1−6 in AOT−Oleyl Alcohol Solution

AOT concn (% (g/g))	feed pH	concn of Trp (mM) initial	concn of Trp (mM) final	distribution coefficient (D_E)	concn of Trp−Leu (mM) initial	concn of Trp−Leu (mM) final	distribution coefficient (D_E)
	1	0.598	0.044	12.591	0.566	0.012	46.167
	2	0.614	0.042	13.619	0.566	0.022	24.727
5	3	0.587	0.052	10.288	0.536	0.019	27.211
	4	0.529	0.118	3.483	0.553	0.024	22.042
	5	0.602	0.396	0.520	0.548	0.033	15.606
	6	0.583	0.471	0.238	0.557	0.125	3.456
	7				0.533	0.211	1.526
	1	0.598	0.019	30.474	0.566	0.012	46.167
	2	0.614	0.016	37.375	0.566	0.021	25.952
10	3	0.587	0.038	14.447	0.536	0.029	17.483
	4	0.529	0.087	5.080	0.553	0.017	31.529
	5	0.602	0.278	1.165	0.548	0.027	19.296
	6	0.583	0.344	0.695	0.557	0.084	5.631
	7				0.533	0.127	3.197
	1	0.583	0.015	37.867	0.566	0.03	17.867
	2	0.567	0.019	28.842	0.566	0.042	12.476
20	3	0.562	0.042	12.381	0.536	0.034	14.765
	4	0.535	0.077	5.948	0.553	0.025	21.120
	5	0.559	0.195	1.867	0.548	0.036	14.222
	6	0.578	0.289	1.000	0.557	0.054	9.315
	7				0.533	0.077	5.922
	1	0.583	0.022	25.500	0.566	0.006	93.333
	2	0.567	0.023	23.652	0.566	0.02	27.300
40	3	0.562	0.057	8.860	0.536	0.018	28.778
	4	0.535	0.065	7.231	0.553	0.026	20.269
	5	0.559	0.158	2.538	0.548	0.038	13.421
	6	0.578	0.257	1.249	0.557	0.055	9.127
	7				0.533	0.055	8.691

Further analysis of Phe and Phe−Leu was carried out using an OPA assay adapted for use in the plate reader from one of the four methods compared in a paper by Bertrand-Harb et al. (1993). This colorimetric method utilizes the absorbance at 340 nm produced as a result of the bond between the OPA and amino acid present in the sample. Using the plate reader, a standard curve and up to 40 unknown samples can be simply and quickly analyzed in one assay.

4. Results and Discussion

The results are presented as the distribution coefficients for extraction and stripping processes as a function of (i) the feed solution pH, temperature, and compositon of some liquid membrane systems; (ii) the number of stripping solutions; and (iii) the temperature and pH of the stripping solution. The values of the distribution coefficients are compared among the solutes (amino acids and dipeptides) for various liquid membrane systems and stripping conditions.

4.1. Effects of Feed pH on Extraction in Three Organic Solutions. The effects of feed pH on the UV absorbance values of the feed solution and on the distribution coefficient (D_E) for Trp and Trp−Leu at 10%, 20%, 30%, and 40% AOT (g/g of solution) are listed in Table 1. The D_E for Trp decreases with increasing pH, and beyond pH > 2, it sharply decreases to very low values. This low value in D_E could be due to less acid ions available at higher pH. pK_1 for Trp is 2.4, and at pH > pK_1 the percentage of amino acid or peptide present as the positively charged form decreases. The D_E for Trp−Leu shows a similar decrease beyond pH 4. However, the values of D_E for Trp−Leu are a few times greater than that for Trp. The values of D_E are very small (except for the 40% AOT system) at pH > 5 because of the approach to pI (the isoelectric point; for Trp, pI is 5.7), where positively charged acid ions are minimum.

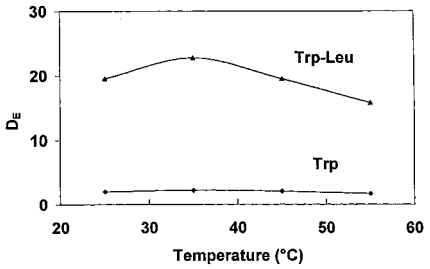

Figure 1. Values of extraction distribution coefficient of 0.5 mM Trp and Trp−Leu at various temperatures for extraction with 10% AOT (g/g) in oleyl alcohol solution.

Table 2. Partitioning of Trp and Trp−Leu at pH = 1−6 in Various Organic Solutions

DOLPA (% (g/g))	feed pH	concn of Trp (mM) initial	concn of Trp (mM) final	distribution coefficient (D_E)
10	1	0.577	0.577	0.0
	2	0.625	0.468	0.34
	3	0.599	0.292	1.05
	4	0.568	0.191	1.97
	5	0.616	0.223	1.76
	6	0.575	0.317	0.81
20	3	0.602	0.077	6.8
	4	0.568	0.074	6.7
	5	0.611	0.082	6.5

Aliquat 336 (% (g/g))	feed pH	conc of Trp (mM) initial	conc of Trp (mM) final	distribution coefficient (D_E)
10	6	0.543	0.52	0.044
	7	0.561	0.55	0.016
20	6	0.593	0.5	0.086
	7	0.561	0.49	0.14
40	6	0.543	0.386	0.41
	7	0.561	0.398	0.41

For 10−40% (g/g) Aliquat 336 in oleyl alcohol solutions, the D_E for Trp is small (about 0.02−0.4) in the pH range 6−7 and at pH < 6 the extracted quantity is not measurable. This suggests that Aliquat 336 is not a good carrier for extraction at acidic pH.

For a DOLPA−oleyl alcohol system, the D_E values are good (Table 2) but lower than those obtained with 10% (g/g) AOT in the pH range 2−6.

On the basis of the above findings and for selective separation of Trp−Leu, AOT−oleyl alcohol systems were selected for detailed study.

4.2. Effect of Feed Solution Temperature. The effect of various temperatures (25−55 °C) on D_E for Trp and Trp−Leu at 10% (g/g) AOT in oleyl alcohol at pH = 4.5 is shown in Figure 1. This effect is negligible for Trp. The effect on D_E for Trp−Leu is very small, and it changes from 21.2 (at 25 °C) to 19.3 (at 55 °C).

4.3. Effect of Feed Solution Concentration. The effect of the initial feed solution concentration (0.5−10 mM) for extractions at pH 4.5 with 10% (g/g) AOT is presented in Figure 2. The D_E for Trp is fairly constant, and for Trp−Leu, it increases up to a feed concentration of 4 mM and beyond this remains constant.

4.4. Effect of the Composition of Organic Solution. The effects of varying the AOT concentration (% g/g) in the range 10−40% at pH = 4.5 for Trp and Trp−Leu are shown in Figure 3. Beyond 20% (g/g) the values of D_E are unaffected by the increase of the AOT concentration.

4.5. Accuracy of the Results. To check the reproducibility of the results, a few sets of data for Trp and Trp−Leu at two pH values are considered (Table 3) and analyzed. It is shown that the maximum errors for the distribution coefficient are 12.6% and 20% for Trp and Trp−Leu, respectively.

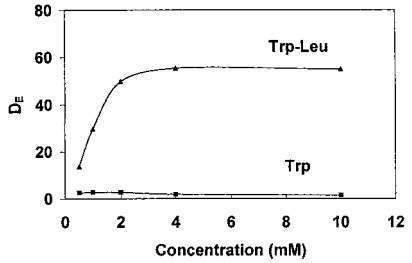

Figure 2. Effect of initial feed concentration on the extraction distribution coefficients of Trp and Trp−Leu in 10% (g/g) AOT in oleyl alcohol at pH 4.5.

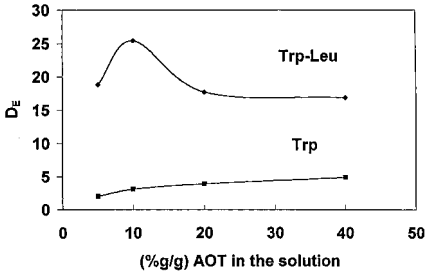

Figure 3. Effect of AOT concentration (% g/g) in oleyl alcohol on the distribution coefficient of Trp and Trp−Leu at feed pH 4.5.

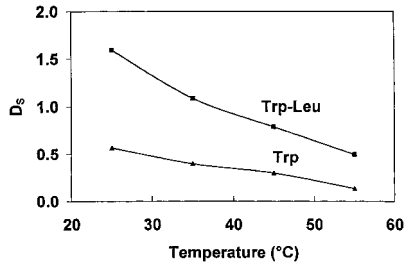

Figure 4. Effect of temperature on the distribution coefficient of Trp and Trp−Leu for stripping with 0.1 M NaCl at pH 5.5.

Table 3: Reproducibility of the Distribution Coefficients for Trp and Trp−Leu in 10% (g/g) AOT−Oleyl Alcohol Solution

concn of Trp−Leu (mM) initial	concn of Trp−Leu (mM) final	D_E (Trp−Leu)	$(D_E)_{av}$	concn of Trp (mM) initial	concn of Trp (mM) final	D_E(Trp)	$(D_E)_{av}$
0.548	0.022	23.909		0.673	0.208	2.236	
0.561	0.029	18.345	22.9	0.621	0.187	2.321	2.16
0.578	0.021	26.524		0.581	0.1999	1.92	
0.572	0.022	25		0.624	0.043	13.51	
0.551	0.025	21.04	25.12	0.572	0.032	16.88	15.46
0.546	0.018	29.33		0.624	0.039	15	

4.6. Effect of Strip pH on Stripping. The coefficients of stripping D_S for Trp between the organic phase and various stripping solutions are shown in Table 4. Solutions of 0.1 M NaCl and 0.1 M NaOH were tested for stripping of Trp from 10% (g/g) AOT solution. The values of D_S decrease with the strip pH and with the type of strippant.

4.7. Effect of Temperature on Stripping. The effect of various temperatures (25−55 °C) on the stripping of solutes (with 0.1 M NaOH solution) from loaded organic solution at pH = 4.5 is shown in Figure 4. The values of D_S vary slightly with feed pH and vary significantly with the stripping temperature.

4.8. Comparison of Distribution Coefficients. The D_E values for Phe, Trp, Trp−Leu, and Phe−Leu extractions

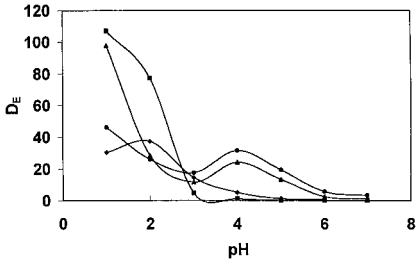

Figure 5. Comparison of extraction distribution coefficients for (a) Trp (◆), Phe (■), Trp−Leu (●), and Phe−Leu (▲).

Table 4. Stripping of Trp from 10% (g/g) AOT−Oleyl Alcohol Solution with NaCl and NaOH

feed pH	0.1 M NaCl strip pH	concn of Trp in organic solution (mM) initial	concn of Trp in organic solution (mM) final	distribution coefficient (D_S)
3.5	5	0.548	0.105	4.25
	5.6	0.547	0.205	1.67
	6.0	0.543	0.249	1.18
	6.5	0.544	0.27	1.01
4.5	5.0	0.432	0.144	2.0
	5.6	0.434	0.182	1.38
	6.0	0.431	0.214	1.01
	6.5	0.438	0.213	1.06

feed pH	0.1 M NaCl strip pH	concn of Trp in organic solution (mM) initial	concn of Trp in organic solution (mM) final	distributio coefficient (D_S)
3.5	5	0.574	0.121	3.74
	5.5	0.568	0.195	1.91
	6	0.555	0.305	0.82
	7	0.579	0.332	0.74
	9	0.569	0.322	0.77
4.5	5	0.462	0.166	1.78
	5.5	0.465	0.199	1.34
	6	0.458	0.257	0.78
	7	0.439	0.273	0.61
	9	0.433	0.259	0.67

Table 5. Comparison of Partition Coefficients for Amino Acids and Dipeptides

solute	reversed micelles/organic solution: AOT (0.05 M) in isooctane reversed micelles[a]	AOT (0.3 M) in isooctane reversed micelles[b]	AOT (0.25 M) in oleyl alcohol—this work
tryptophan	12	280 ± 10	14 ± 3
L-phenylalanine	6	90 ± 3	3.5 ± 0.5
phenylalanine−leucine	not reported	not reported	102 ± 6
tryptophan−leucine	not reported	not reported	27 ± 3

[a] Furusaki and Kishi (1990). [b] Leodidis and Hatton (1990).

with 10% (g/g) AOT are compared in Figure 5, respectively. For pH < 2.5 the D_E for Phe is much greater than those of Trp, and for pH > 3 the difference is insignificant. The D_E values of Phe−Leu are about the same as those of Trp−Leu over the pH range 2−7.

There are only a few partitioning studies for amino acids and peptides with AOT solutions. The values obtained here are compared with those reported by Furusaki and Kishi (1990) and Leodidis and Hatton (1990a,b) in Table 5. Although the experimental conditions and analytical methods are different, the values are comparable to those reported by Furusaki and Kishi (1990). The high values obtained by Leodidis and Hatton (1990a,b) could be due to the high concentrations of solutes (compared to those used in our study). The values were not reported for Trp−Leu and Phe−Leu in their studies. The values listed for other peptides, for example, Trp−Gly, Phe−Gly, and so forth (Furusaki and Kishi, 1990), are in the range 10−15.

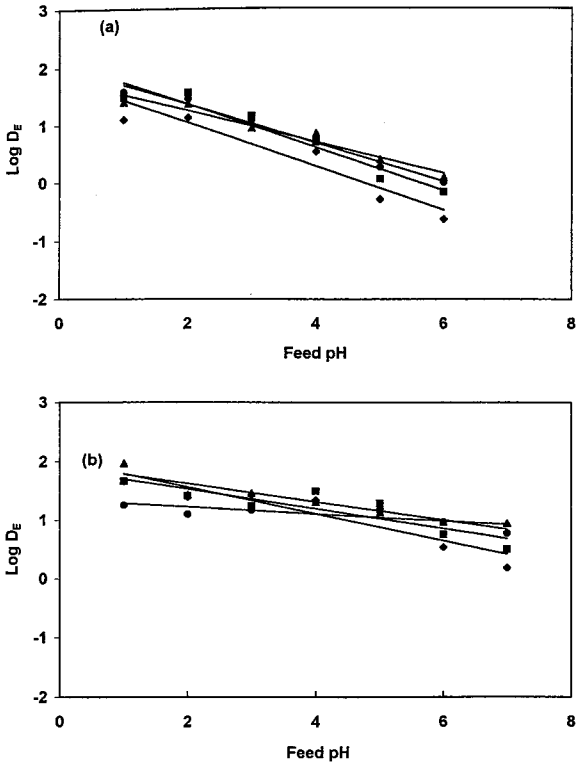

Figure 6. Plot of log(D_E) versus feed pH for (a) Trp and (b) Trp−Leu at the following AOT concentrations: 5% (◆); 10% (■); 20% (●); 40% (▲).

Table 6. Parameter Values for the Correlations of the Distribution Coefficient

membrane composition	parameter	Trp	Trp−Leu
10% AOT	*a*	−2.12	−1.88
	b	−0.38	−0.17
40% AOT	*a*	−1.80	−1.94
	b	−0.27	−0.16

The equilibrium results for stripping are not available in the literature.

4.9. Correlations for the Distribution Coefficients. The effect of feed pH (in the range 2−5) on the distribution coefficient of the solutes in 10% (g/g) AOT can be correlated by an empirical equation of the form

$$\log_{10}(D_E) = -a + b(\text{pH}) \qquad (17)$$

The values of the parameters *a* and *b* for the extraction of the solutes are listed in Table 6a. The plots of the left-hand side of eq 17 versus pH for solutes the Trp and Trp−Leu are presented in Figure 6. The data for all other solutes also show very good correlations.

4.10. Equilibrium Reaction Coefficients. The equilibrium extraction coefficient of the solutes is calculated from the slope of the plot of the experimental values of D_E and using eq 8. A plot for Trp into various AOT solutions is shown in Figure 7. The values for Trp along with the other solutes are listed in Table 7a.

The reaction coefficient for stripping K_S is obtained from the intercept of the plot of the left-hand side of eq 15 against strip pH. A plot for stripping of Trp with a 0.1 M

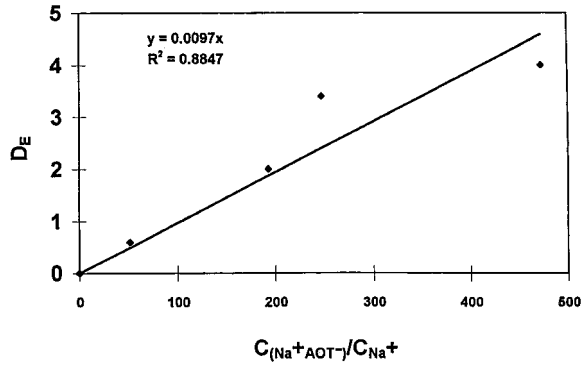

Figure 7. Plot of log(D_E) of Trp versus $C_{Na^+}/C_{(Na^+AOT^-)}$.

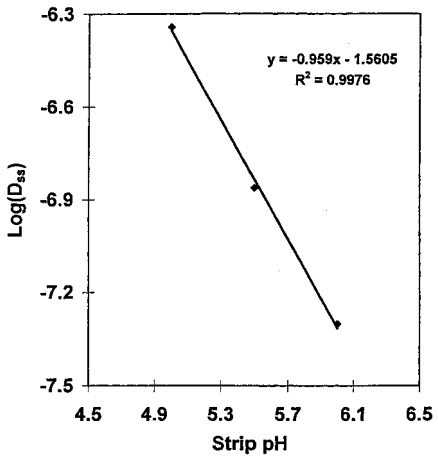

Figure 8. Plot of log(D_{SS}) of Trp versus strip solution pH.

Table 7. Values of the Interfacial Reaction Coefficients: (a) Extraction Coefficients at Feed pH 4.5 and (b) Stripping Reaction Coefficients for NaCl and Na_2CO_3 Solutions

(a) Extraction Reaction Coefficients at pH 4.5

Trp	Trp−Leu	ratio of Trp−Leu/Trp	Phe	Phe−Leu	ratio of Phe−Leu/Phe
1.3	19.47	15	1.27	13.83	10.9

(b) Stripping Reaction Coefficients

stripping solution	stripping coefficients (feed solution pH = 4.5) Trp	Trp−Leu	ratio of the coefficients for Trp−Leu/Trp
0.1 M NaCl at pH = 5.5	14.71	4.95	0.34
0.1 Na_2CO_3 at pH = 6	10.86	25.93	2.4

NaCl solution is shown in Figure 8. The values of K_S for Trp and Trp−Leu are listed in Table 7b.

5. Conclusions

Experimental results are reported for the partitioning of two amino acids and two dipeptides in a few organic solutions, with detailed data on AOT−oleyl alcohol, as this system gives excellent values. It is shown that the value of D_E (the partition coefficient for extraction) is much higher for dipeptides compared to those of amino acids. The value of D_E sharply decreases with increasing feed pH and is relatively unaffected by temperature. The value of D_E remains relatively constant for amino acids, and for dipeptides it increases with increasing solute concentration before attaining a constant value. The value of D_S (the partition coefficient for stripping) is high for lower strip pH values (i.e., 5 in this case, indicating small recovery), and it decreases sharply with the increase of strip pH (i.e., higher recovery of solute). It is also shown that the recovery is greatly affected by the stripping agent (e.g., a NaCl solution is good for amino acids and a Na_2CO_3 or NaOH solution is good for Trp−Leu).

List of Symbols

a = a constant in eq 17
A = amino acid
AOT = Aerosol OT, sodium di-2-ethylhexyl sulfosuccinate
b = a constant in eq 17
c = a constant in eq 18
C = concentration of ionic solute (mmol/mL)
D = distribution coefficient, defined in eqs 9 and 13
K = equilibrium constant, defined in eqs 8 and 12
K_1, K_2 = dissociation constants, defined in eqs 2 and 3
d = a constant in eq 18
Na = sodium
Phe = phenylalanine
Phe−Leu = phenylalanine−leucine
T = temperature (°C)
Trp = tryptophan
Trp−Leu = tryptophan−leucine
x = pH of feed solution
y = % AOT in oleyl alcohol
u = viscosity of liquid membrane
S = Shear rate

Subscripts and Superscripts

A = ionic amino acid
A_T = total amino acid
E = extraction from feed solution
fi = feed solution
fo = organic phase in contact with the feed
S = stripping from LM solution
si = strip solution
so = organic solution in contact with the strip
+ = positively charged solute
− = negatively charged solute
± = zwitterionic solute

Literature Cited

Bertrand-Harb, C.; Nicolas, M. G.; Dalgalallondo, M.; Chobert, J. M. Determination of Alkylation Degree by HCl Colorimetric Methods and Amino Acid Analysis. A comparative study. *Sci. Aliments* **1993**, *13*, 577−584.

Cardoso, M. M.; Barradas, M. J.; Carrondo, M. J. T.; Kroner, K. H.; Deckwer, W.-D.; Crespo, J. P. S. G. Extraction of Amino Acids by Reversed Micellar Transport using Liquid Membranes. *7th Proc. World Filtr. Congr.;* Budapest, Hungary, 1996; Vol. 2, p 576.

Cascaval, D.; Oniscu, C.; Tudose, R. Z. Study of Separation of Organic Compounds by Reactive Extraction. II. Study of Separation of Glutamic acid. *Bul. Inst. Politeh. Iasi, Sect. 2: Chim. Ing. Chim.* **1996**, *42*, 75−82.

Caselli, M.; Mangone, A. Phase Equilibria Involving Autoaggregate Systems: Partitioning of Amino acids Between a Water Saline Solution and an Organic Phase Containing Reverse Micelles. *Ann. Chim.* **1992**, *82*, 23−38.

Cohen, L. M.; Eiteman, M. A.; Gainer, J. L. A Model to Predict the Partition Coefficients of Amino Acids in PEG/Salt Aqueous Two-phase Systems. *Sep. Sci. Technol.* **1995**, *30*, 225−237.

Deblay, P.; Minier, M.; Renon, H. Separation of L-valine from Fermentation broths Using a Supported Liquid Membrane. *Biotechnol. Bioeng.* **1990**, *35*, 123−131.

Ersoz, M.; Vural, U. S.; Okdan, A.; Pehlivan, E.; Yildiz, S. Transport Studies of Amino Acids through a Liquid Membrane System containing Carboxylated Poly (styrene) Carrier. *J. Membr. Sci.* **1995**, *104*, 263−269.

Furusaki, S.; Kishi, K. Extraction of Amino Acids and Peptides by Reversed Micelles. In *Solvent Extraction;* Proceedings of ISEC'90, Kyoto, Japan; Sekine, T., Ed.; 1990; pp 1749−1754.

Hano, T.; Ohtake, T.; Matsumoto, M.; Kitayama, D.; Hori, F.; Nakashio, F. Extraction Equilibria of Amino Acids with Quaternary Ammonium Salt. *J. Chem. Eng. Jpn.* **1991**, *24*, 20−24.

Hano, T.; Matsumoto, M.; Kawazu, T.; Ohatake T. Separation of Di- and Tripeptides with Solvent Extraction and an Emulsion liquid membrane. *J. Chem. Technol. Biotechnol.* **1995**, *62*, 60−63.

Hossain, Md. M.; Stanley, R. A. Selective Removal of Hydrophobic Peptides from Protein Hydrolysates using a Supported Liquid Membrane Process. *Sep. Sci. Technol.* **1996**, *31*, 1443−1462.

Leodidis, E. B.; Hatton, T. A. Amino Acids in AOT Reversed Micelles. 1. Determination of Interfacial Partition Coefficients Using the Phase-Transfer Methodology *J. Phys. Chem.* **1990**, *94*, 6400−6411.

Leodidis, E. B.; Hatton, T. A. Amino Acids in AOT Reversed Micelles. 2. The Hydrophobic Effect and Hydrogen Bonding as Driving Forces for Interfacial Solubilization. *J. Phys. Chem.* **1990b**, *94*, 6411−6420.

Tintinger, R.; Zhu, J.; Grossman, C.; Maurer, G. Partitioning of Some Combination Di-(Ethylene Glycol) and Di-Potassium Hydrogen Phosphate at 293 K: Experimental Results and Predictions. *Ber. Bunsen-Ges. Phys. Chem.* **1997a,** *101*, 687−697.

Tintinger, R.; Zhu, J.; Grossman, C.; Maurer, G. Partitioning of Some Amino Acids and Low Molecular Mass Peptides in Aqueous Two-Phase Systems of Poly(ethylene glycol) and Dextran in the Presence of Small Amounts of K_2HPO_4/KH_2PO_4−Buffer at 293 K: Experimental Results and Correlation. *J. Chem. Eng. Data* **1997b**, *42*, 975−984.

Wang, W.; Weber, M. E.; Vera, J. H. Reverse Micellar Extraction of Amino acids Using Dioctyldimethylammonium Chloride. *Ind. Eng. Chem. Res.* **1995**, *34*, 599−606.

Ziova, J.; Vandak, D.; Schlosser, S.; Sturdik, E. Extraction Equilibrium of Butyric Acid with Organic Solvents. *Sep. Sci. Technol.* **1996**, *31*, 2671−2684.

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