http://dx.doi.org/10.5012/bkcs.2012.33.10.3387

Separation of Two Amino Acids by Microemulsion Bulk Liquid Membrane

Alireza Salabat* **and Fereshteh Dehghani Sanij**

Department of Chemistry, Faculty of Science, Arak University, P.O. Box 38156-8-8349, Arak, Iran
**E-mail: a-salabat@araku.ac.ir*
Received April 26, 2012, Accepted July 20, 2012

In this research work the potentialities of microemulsion bulk liquid membrane for the selective transport of L-tryptophan (L-Trp) and L-tyrosine (L-Tyr) are investigated at 298.15 K. Reversed micelle formed by sodium bis(2-ethylhexyl)sulfosuccinate (AOT) in dichloroethane, was used as mobile carrier to transport amino acids between a source and a receiving aqueous phase. The effects of pH, surfactant concentration and initial amino acid concentration on the extraction efficiency and transfer rate of the amino acids were studied. It is verified that for a mixture of two amino acids, L-Trp can be extracted selectively by using this type of the bulk liquid membrane with optimized condition.

Key Words : Amino acids, AOT, Liquid membrane, Reversed micelle, Microemulsion

Introduction

Recently, liquid membranes have been developed owing to several advantages such as ease of operation and scale-up, low energy consumption, low cost of operation, continuous and high selectivity factors.[1-4] Liquid membranes have also a high potential application in analytical chemistry and in mimetic systems of biological membranes.[5] Liquid membranes may be broadly classified into three following types: bulk liquid membranes (BLM), emulsion liquid membranes (ELM) and support liquid membranes (SLM). In general, the bulk liquid membrane system can be defined as a water-immiscible liquid phase (membrane phase) and a carrier molecule between two liquid phases. They are called source (donor or feed) and receiving (stripping or acceptor) phases. The source phase contains the chemical species to be transported through liquid membrane and the receiving phase is an aqueous solution with an initial composition depending on the type of transport intended to be studied. The carrier, alter the guest-permeability and facilitate the selective diffusion across the liquid membrane.[6-8] Advantages of bulk liquid membranes include high degree of phase separation which implies smaller extraction equipment, very small amount of organic solvent and high transport fluxes.[1] In recent years, there have been several reports of liquid membranes involving a new type of mobile carrier, namely reverse micelles or microemulsion globules.[9,10] Microemulsions represent a major class of organized assemblies of surfactants in solution and can largely be viewed as the compartmentalized liquids. The simplest, and best understood microemulsions, are ternary systems made up of water, oil (usually an alkane) and a single surfactant. The type of dispersion formed depends primarily on the volume fractions of water and oil, so that both water-in-oil (w/o) and oil-in-water (o/w) microemulsions may be formed. Water-in-oil microemulsions (reverse micelles) consist of nanometer-sized domains of water, stabilized by a monolayer of the surfactant, dispersed in the continuous oil phase. Sodium bis(2-ethylhexyl)sulfosuccinate, called Aerosol-OT or AOT is a well known surfactant for obtaining microemulsion systems. Amino acids can be solubilized in the water core, or the interface between water and surfactant layer, depending on their electric charge.[11]

In the present work, for our interest in the separation of amino acids and biological compounds, the amino acid extraction through the bulk liquid membrane system was investigated. The extraction efficiency and transfer rate of two amino acids, L-Trp and L-Tyr, through the membrane was studied. In order to define the liquid membrane process that enables the separation objective, the influence of several parameters has been investigated. The results indicated that these two amino acids can be separated by the liquid membranes under optimal experimental conditions.

Experimental

Reagents. Sodium bis(2-ethylhexyl)sulfosuccinate (Fig. 1) was obtained from Acrose Company with purity of 96%. Sodium chloride (GR, min 99.5%), sodium tetraborate (GR, min 99%), sodium hydroxide (GR, min 99%) and hydrochloric acid (37%) were purchased from Merck and used for the preparation of buffer solutions. L-tryptophan (> 99%), L-tyrosine (> 99%) and 1,2-dichloroethane were also purchased from Merck. Structure of these amino acids is shown in Figure 1. All chemicals were employed without further purification. Triply distilled water was used throughout the experiments.

Experimental Procedures. The extraction experiments were carried out by using a U-shaped glass tube,[12] as shown in Figure 2. Typically 25 mL of the liquid membrane, which consists of a clear water-in-oil microemulsion dispersion of AOT in dichloroethane with different molar ratios, were placed in the thermostated U-tube (298.1 ± 0.1 K). The source and receiving phase were carefully and simultaneously

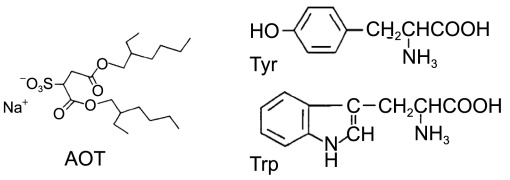

Figure 1. The chemical structures of compounds used throughout the experiments.

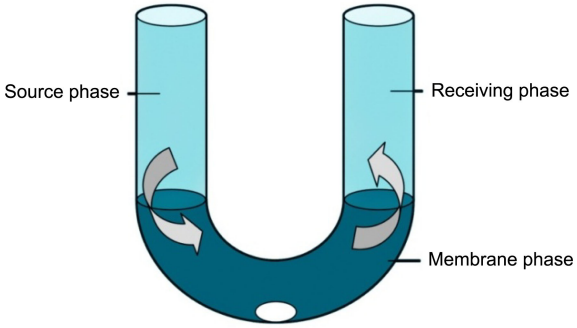

Figure 2. The device employed in amino acids extraction with liquid membrane.

inserted into the U-tube. The source phase contains 10 mL of amino acid aqueous solution with pH between 1.8-5, having $[Na^+] = 0.10$ mol L^{-1}, present in one arm (left in Fig. 2). For all experiments the aqueous receiving phase contain 10 mL of a sodium tetraborate buffer solution at pH=10, which is present in the other arm (right in Fig. 2). The liquid membrane was magnetically stirred at 200 rpm.

The concentration of the amino acids in the receiving phase was determined by UV-Vis measurements with a double beam Perkin Elmer Lambda 15 UV-Vis spectrophotometer. L-Trp and L-Tyr were detected at 275.7 nm and 272.4 nm band absorption, respectively (Fig. 3). The weaker UV absorption band of them was chosen because the strongest band at 220 nm is overlapped with AOT UV absorption band. Each experiment was repeated three times at least and the results were reported as the average value. The pH was measured by a digital Metrohm 691 pH-meter with glass electrode and saturated calomel electrode. The pH of aqueous solution was adjusted by hydrochloric acid and sodium hydroxide. Blank experiments were performed for reference in the absence of carrier. No detectable extraction of amino acid through liquid membrane was found, suggesting that the extraction of amino acid to liquid membrane is fulfilled by the carrier.

The water content of the microemulsion liquid membrane phase was determined by Kyoto mks-210 Karl Fischer

Table 1. The values of W for microemulsion liquid membrane phase

[AOT] (mol L^{-1})	W
0.005	7.3
0.03	10.3
0.05	12.0

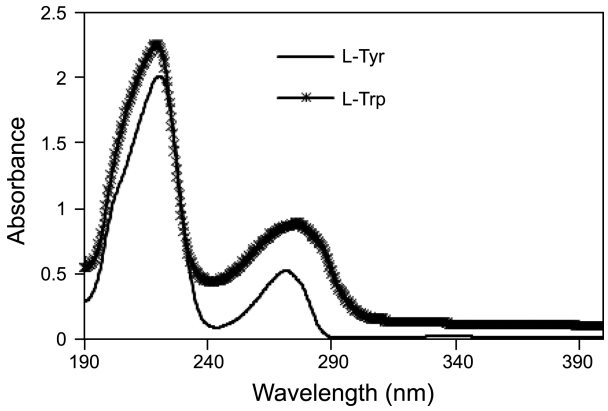

Figure 3. UV-Vis spectra of L-Trp and L-Tyr.

instrument. The water capacity of the membrane depends on surfactant concentration. Usually the water content shown by the W value, the molar ratio of water to surfactant ($W = [H_2O]/[AOT]$). Karl Fisher results showed that W is increased with increasing AOT concentration. The results reported in Table 1.

The extraction efficiency refers to the fraction of amino acid that is extracted into the receiving phase and is defined mathematically as[13]:

$$E = \frac{C_r V_r}{C_s V_s} \times 100 \quad (1)$$

where C_r and C_s are the amino acid concentrations in receiving and source phase, respectively. V_r and V_s are the phase volumes. The process of diffusion in liquid membrane is governed by Fick's first law of diffusion. The flux values can be calculated from the following equation.[14]

$$J_m = \frac{C_r V_r}{At} \quad (2)$$

where A and t are effective area of membrane (m^2) and transport time in second, respectively.

Results and Discussion

The extraction mechanism of amino acid from source phase to receiving phase and the role of AOT in this process are given schematically in Figure 4. At the interface between the aqueous source phase (I) and the organic membrane phase, an anionic surfactant (AOT^-) binds the cationic form of an amino acid (AA^+) *via* electrostatic interaction. The cationic form of an amino acid (AA^+), from reverse micelles, was extracted to the receiving phase (II) by an exchange reaction with Na^+. The electrostatic effect in the process of amino acid solubilization in reverse micelles is important. In these extraction experiments, the electrostatic interaction between the anionic AOT head group and the $R\text{-}NH_3^+$ group of AA^+ can occur in the aqueous pool of AOT reverse micelles, since the ion-pair AOT^-AA^+ have to be solvated by water to be effectively stabilized.[15-17] This mechanism of transport process can be represented as follows.[18]

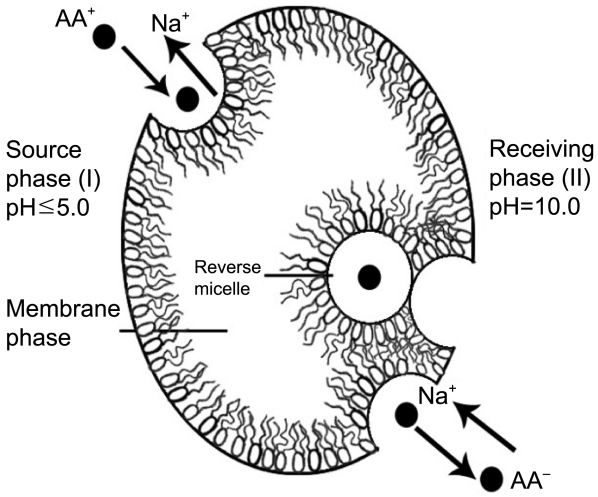

Figure 4. Mechanism of amino acids extraction by microemulsion liquid membrane.

$$AA^{+}_{(aq)(s)} + (Na^{+}AOT^{-})_{(s)|LM} \rightleftarrows Na^{+}_{(aq)(s)} + (AA^{+}AOT^{-})_{(s)|LM}$$

$$(AA^{+}AOT^{-})_{(s)|LM} \rightleftarrows (AA^{+}AOT^{-})_{(r)|LM}$$

$$Na^{+}_{(aq)(r)} + (AA^{+}AOT^{-})_{(r)|LM} \rightleftarrows (Na^{+}AOT^{-})_{(r)|LM} + AA^{+}_{(aq)(r)}$$

$$AA^{+}_{(aq)(r)} + OH^{-}_{(aq)(r)} \rightleftarrows AA^{-}_{(aq)(r)} + H_2O_{(aq)(r)}$$

Where the subscripts (s) and (r) represent the source and receiving phases, respectively; (s)|LM and (r)|LM symbolized the interfaces between the source or receiving phases and the liquid membrane.

Effect of Surfactant Concentration. Surfactant plays a vital rule in maintaining the stability of microemulsion. The concentration of AOT in the membrane phase was varied from 0.005 to 0.05 mol L^{-1} and the results for extraction efficiency and flux of the amino acids were shown in Figures 5 and 6. As can be seen in the most cases by increasing AOT concentration in the liquid membrane the extraction efficiency and flux is decreased. The extraction loss at high AOT concentration may be related to the uptake mechanism in the source phase. The microscopic partitioning of the amino acids between the amphiphile layer and the water pool becomes less when AOT concentration or the size of the water pool increases (Table 1).

Effect of pH. The ionization state of the amino acids in the source phase can be changed by changing the pH. The influence of different pH values, 1.8, 3 and 5 in the source phase on extraction was examined. The extraction efficiency and flux were calculated with an initial L-Trp and L-Tyr concentration of 4×10^{-3} mol L^{-1} at different pH. As can be seen from Figures 5 and 6 the extraction efficiency and flux of L-Trp and L-Tyr is affected by the pH of the source phase. Surprisingly, this effect is completely different for L-Trp and L-Tyr. As shown in Figure 5, the extraction efficiency and flux for L-Trp decreases with increasing of the pH. Whereas the results in Figure 6 indicate that the extraction efficiency and flux of L-Tyr increase with increasing of the pH. It should be pointed out that, this trend for the effect of pH is not general for all AOT concentrations. The optimum conditions for extraction of these two amino acids have been found to be as follows: pH=1.8 in the source phase and

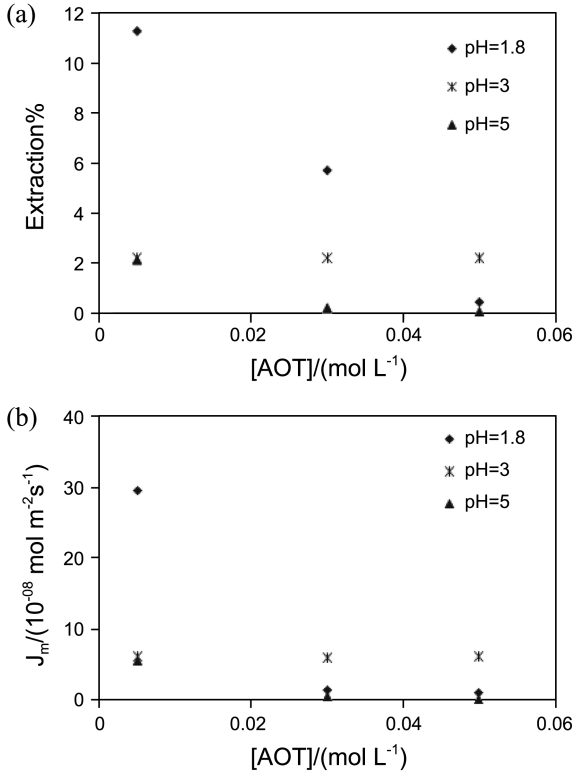

Figure 5. Effect of pH in the source phase and surfactant concentration on the (a) extraction efficiency and (b) flux of L-Trp.

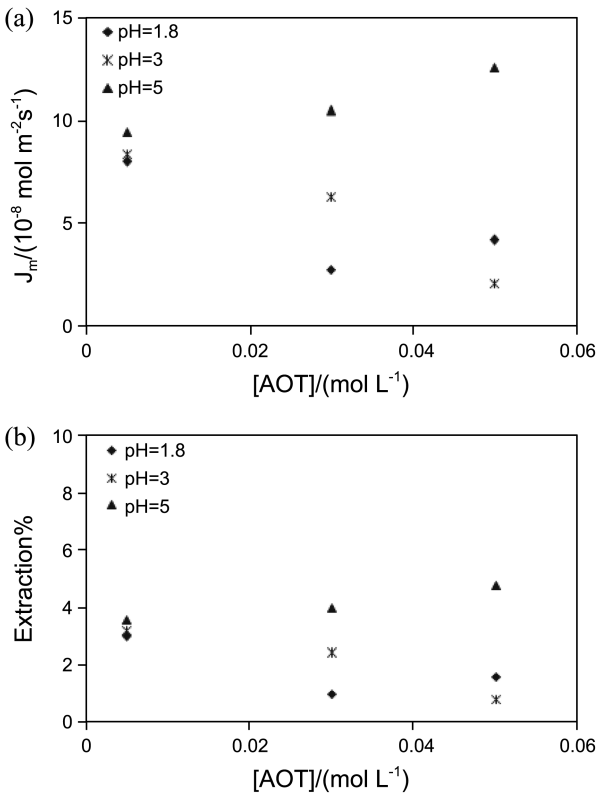

Figure 6. Effect of pH in the source phase and surfactant concentration on the (a) extraction efficiency and (b) flux of L-Tyr.

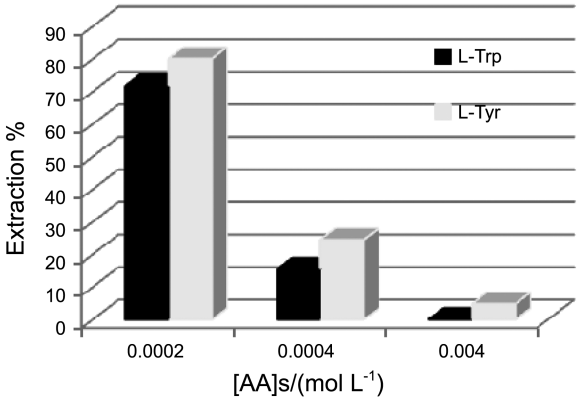

Figure 7. Effect of initial amino acid concentration on the extraction efficiency for [AOT] = 0.05 mol L^{-1} and pH=5.

0.005 mol L^{-1} AOT in the membrane, for L-Trp; pH=5 in the source phase and 0.05 mol L^{-1} AOT in the membrane, for L-Tyr.

Effect of Initial Amino Acid Concentration. The effect of the initial amino acid concentration on the extraction efficiency and flux has been also investigated at a constant concentration of AOT. The concentration of amino acids was changed from 2×10^{-4} to 4×10^{-3} mol L^{-1}. The results obtained at [AOT] = 0.05 mol L^{-1} and pH=5 of source phase, are shown in Figure 7. The experimental results show that the extraction efficiency, for two amino acids is increased with decreasing initial amino acids concentration.

Separation of Two Amino Acids. For the final step, we have examined the potentialities of the reverse micellar carriers to achieve the separation of amino acids having different hydrophobicities. We performed separation of two amino acids by the microemulsion liquid membrane at the optimized condition. As can be seen from Figure 7, the selective extraction occurred at higher amino acids concentration. Then the start point is same concentration 0.004 mol L^{-1} of two amino acids in the source phase. The results showed that for a mixture of the amino acids, L-Trp can selectively extracted to receiving phase at pH=1.8 and [AOT] = 0.005 mol L^{-1} and L-Tyr at pH=1.8 and [AOT] = 0.05 mol L^{-1} conditions.

Conclusion

In this research we have examined the specific ability of AOT reverse micelle membrane to separate two amino acids L-Trp and L-Tyr. It was shown that the extraction depends on the pH of the source phase, surfactant concentration and initial amino acid concentration in the source phase. It is verified that the suitable parameters for extraction of L-Trp would be pH of 1.8 in the source phase and AOT concentration of 0.005 mol L^{-1} in the membrane phase and for L-Tyr, pH of 5 and AOT concentration of 0.05 mol L^{-1}. The results obtained here indicate that the selective separation of two amino acids L-Trp and L-Tyr with optimized condition is possible. The results also suggest further possibilities for optimal separation of other biological species containing these two amino acids by means of this type of liquid membranes.

References

1. Muthiac, L.; Mutihac, R. *J. Inclusion Phenom. Macrocycl Chem.* **2007**, *59*, 177.
2. Tondre, C.; Hebrant, M. *J. Mol. Liq.* **1997**, *72*, 279.
3. Mohagheghi, E.; Alemzadeh, I.; Vossoughi, M. *Sep. Sci. Technology* **2008**, *43*, 3075.
4. Bringas, E.; San Roman, M. F.; Irabien, J. A.; Ortiz, I. *J. Chem. Technol. Biotechnol.* **2009**, *84*, 1583.
5. Oshima, T.; Inoue, K.; Furusaki, S.; Goto, M. *J. Membr. Sci.* **2003**, *217*, 87.
6. Bucci, R.; Canepari, S.; Cardarelli E.; Girelli, A. M.; Pietrodangelo, A.; Valiente, M. *Sep. Sci. Technology* **2004**, *39*, 3821.
7. Araki, T.; Tsukube, H. *Liquid Membranes: Chemical Applications*; CRC Press: Boca Raton, FL, 1990.
8. Hebrant, M.; Tondre, C. *Anal. Sci.* **1998**, *14*, 109.
9. Paul, B. K.; Moulik, S. P. *Curr. Sci.* **2001**, *80*, 990.
10. Kaur, A.; Vohra, D. K. *Indian J. Chem. Technol.* **2010**, *17*, 133.
11. Stobbe, H.; Yunguang, X.; Zihao, W.; Jufu, F. *Biotechnol. Bioeng.* **1997**, *53*, 267.
12. Verkuijl, B. J. V.; Minnaard, A. J.; de Vries, J. G.; Feringa, B. L. *J. Org. Chem.* **2009**, *74*, 6526.
13. Poliwoda, A.; Ilczuk, N.; Wieczorek, P. P. *Sep. Purif. Technol.* **2007**, *57*, 444.
14. Joshi, P.; Joshi, N.; Sharma, U. *Indian J. Biochem. Biophys.* **2006**, *43*, 323.
15. Leodidis, E. B.; Bommarius, A. S.; Hatton, T. A. *J. Phys. Chem.* **1991**, *95*, 5943.
16. Leodidis, E. B.; Hatton, T. A. *J. Phys. Chem.* **1990**, *94*, 6411.
17. Leodidis, E. B.; Hatton, T. A. *J. Phys. Chem.* **1990**, *94*, 6400.
18. Xun, F.; Junling, L.; Ying, M.; Li, Z.; Debao, W.; Zhengshui, H. *Colloids Surf. A: Physicochem. Eng. Aspects* **2001**, *179*, 1.