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E. Mohagheghi[a]; I. Alemzadeh[a]; M. Vossoughi[b]
[a] BBRC, Chemical and Petroleum Engineering Department, Sharif University of Technology, Azadi Ave., Tehran, Iran [b] Institute of Nanoscience and Nanotechnology (INST),

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Study and Optimization of Amino Acid Extraction by Emulsion Liquid Membrane

E. Mohagheghi,[1] I. Alemzadeh,[1] and M. Vossoughi[2]
[1]BBRC, Chemical and Petroleum Engineering Department, Sharif University of Technology, Azadi Ave., Tehran, Iran
[2]Institute of Nanoscience and Nanotechnology (INST)

Abstract: A batch extraction of an essential amino acid, phenylalanine, from an aqueous solution of different concentrations by an Emulsion Liquid Membrane (ELM) was developed using D2EHPA as a cationic carrier, Span 80 as the surfactant, paraffin, and kerosene as the diluents, and HCl as the internal electrolyte. All effective parameters such as the initial pH of the aqueous external phase, the electrolyte concentration in the aqueous internal phase, carrier, and surfactant concentration in the emulsion, the volume ratio of the organic to aqueous internal phase (Roi), the volume ratio of the W/O emulsion to the aqueous external phase (Rew) and time were examined and optimized using the Taguchi method. Applying the Taguchi method to analyze the experimental results, the effects and contribution of each of the factors on the extraction efficiency were obtained. The results obtained from the experiments illustrated that with a stable emulsion, by optimizing all the effective parameters, a considerable amount of phenylalanine can be extracted in a short time with an acceptable ratio of swelling and breakage.

Keywords: Emulsion liquid membrane, optimization, phenylalanine, surfactant, Taguchi method

INTRODUCTION

Amino acids are of high importance in foods, animal feeds, and pharmaceuticals. The production of amino acids, which are the main structural components of proteins and enzymes, has been significantly increased.

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Address correspondence to M. Vossoughi, BBRC, Department of Chemical Petroleum Engineering, Sharif University of Technology, Azadi Ave., Tehran, 11365-8639, Iran. E-mail: vosoughi@sharif.ir

They can be obtained by biosynthesis or from protein hydrolysis, but their separation from fermentation broths or protein hydrolysis is rather difficult.

Amino acids dissociates in aqueous solution, forming characteristic ionic species as a function of pH of the solution. They are positively charged at pH < 3, has no charge at neutral pH (3–9), and is negatively charged at pH > 9. Therefore their solubility in non-polar solvents is very low (1). Cell removal (by centrifugation or membrane filtration), ion exchange, evaporation, and crystallization at the isoelectric point are the techniques generally used to separate amino acids from fermentation broths or protein hydrolysis. However, these methods are not often acceptable because for example ion exchange generates a lot of waste liquor for regeneration and much energy is wasted in evaporation. In addition, liquid-liquid extraction of amino acids is only possible by adding some extractants to the organic phase such as phosphoric acid derivatives, high molecular weight quaternary aliphatic amines, or crown-ethers. Furthermore, none of the above methods are capable to do the separation and concentration at the same time (2–4).

Emulsion Liquid Membrane (ELM) can be a promising alternative method since it has many advantages compared to the conventional methods. Its advantages including energy saving, simultaneous selective separation, and concentration, higher fluxes, and low capital, and the operational cost has introduced it as a novel effective method for separating various bio-products from dilute solutions (4,5). ELM was first developed by Li in 1986 (6) and used for separation and concentration of amino acids by Thien et al. in 1988 (7).

Emulsion liquid membrane is prepared by emulsion making between two immiscible liquid phases (organic and aqueous phases) in advance and then dispersing the prepared emulsion into the third liquid phase by mild agitation. In this system the liquid membrane isolates two miscible liquid phases which are internal phase capsulated by the membrane phase and the external phase (continuous phase) in which the material to be extracted is solubilized. The solute of interest is separated and concentrated in the internal phase by being transported through the liquid membrane. A schematic diagram of a liquid emulsion membrane system is shown in Fig. 1.

The mechanism by which amino acids are transported through the membrane from the exterior aqueous phase into the interior aqueous phase is carrier facilitated transport. In this mechanism a carrier in anionic or cationic form is added to the membrane phase to solubilize amino acids into the organic phase. Thien et al. (7) used an anionic carrier (a tricapryl quaternary ammonium salt) and could obtain three times the initial concentration of L-phenylalanines; however, the process in

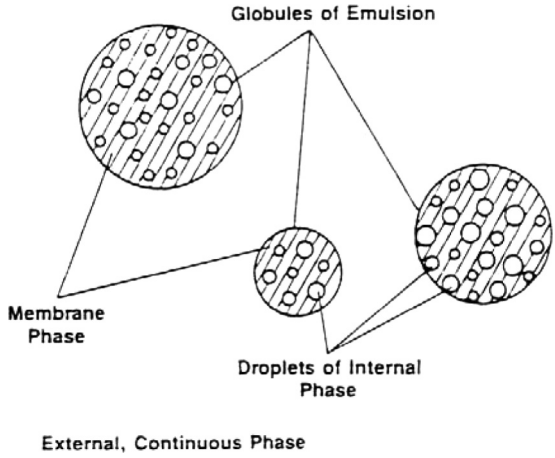

Figure 1. A schematic diagram of a liquid emulsion membrane system (8).

which the amino acid was concentrated in its anionic form was disturbed by the presence of other anionic material such as inorganic anions like sulfate and phosphate used as mineral sources for the microorganisms. In addition, the surface of the microorganism is charged negatively and this could result in fouling the membrane interface, therefore the removal of the cells before the process was necessary. In order to solve this problem, Itoh et al. and Hong et al. (2,7) tried the ELM method using a cationic carrier, a di-2ethylhexyl phosphoric acid (D2EHPA) in which amino acids should exist in their cationic forms.

As it is shown schematically in Fig. 2 the D2EHPA first exists as a carrier/proton complex. When the carrier reaches the interface between the external and membrane phases, an ion exchange reaction takes place and the carrier makes a complex with Phe^+. The cation/Phe^+ complex then diffuses through the membrane to the interface between the internal and membrane phases. Another ion exchange reaction takes place. Due to the extremely low pH in the internal phase, the cation/Phe^+ complex must release the Phe^+ and the carrier is immediately protonated. These processes are repeated and the Phe is thus separated and concentrated in the internal phase (2).

In the present study a batch ELM process using D2EHPA was applied in order to investigate the effects of various experimental parameters on the application of ELM for L-phenylalanine extraction. The experimental conditions were optimized in order to obtain the best system performance.

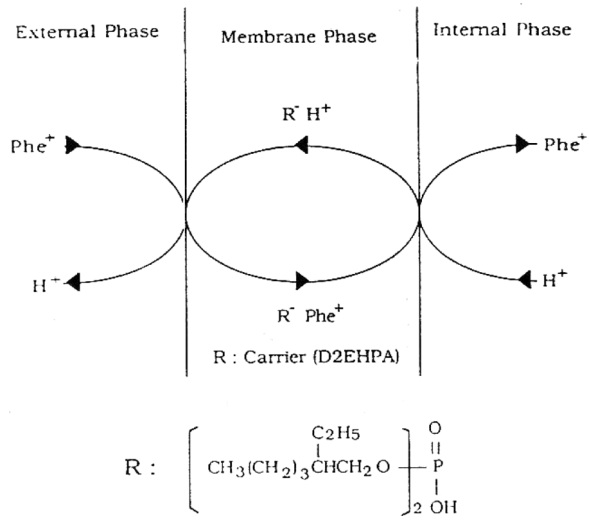

Figure 2. A schematic diagram of the transport mechanism for Phenylalanine (2).

EXPERIMENTAL

Reagents

All chemicals were used as supplied without further purification. Paraffin with density of 0.82–0.84 g/cm^3 at 20°C and viscosity of 25–26 cP at 40°C was supplied by Rose Polymer Co. Kerosene was of commercial grade and used without any pretreatment. Sorbitanemonooleate (SPAN 80) used as surfactant to stabilize the emulsion and di(2-ethylhexyl) phosphoric acid (D2EHPA) used as the carrier in the oil phase of emulsion were purchased from Merck Co. Sodium hydroxide (NaOH), HCl, and other materials of reagent grade were supplied also by Merck Co. L-Phenylalanine (Phe) was purchased from ROTH, Germany. Ninhydrine for manual amino acid determination was used as supplied by Merck Co. and was of analytical grade.

Equipments

GGS 27C made by BOSCH (Germany) could provide quite an intense shear (12000 to 27000 rpm) to make a stable emulsion. It was equipped with a turbine impeller. To disperse the emulsion into the external aqueous phase a mixer providing maximum agitation of 800 rpm was used

with the same impeller as that for emulsifying. The mixer was supplied by Shimi Fann Co. Extraction experiments were carried out in a cylindrical glass container of 10 cm diameter and 14 cm height equipped with four round glass baffles with 8 mm diameter.

Disposable sanitary syringes were used to take samples during the extraction. A laboratory centrifuge was used to separate emulsion and water phase.

Spectronic 21D-UV/Vis spectrophotometer was used to direct measuring absorbance of Phe concentration in 258 nm and absorbance of the complex of ninhydrine with Phe in 570 nm.

Heating the emulsion in a boiling bath also applied to do the demulsification.

Procedure

All the experiments were conducted at ambient temperature and were carried out in the following succession.

Emulsions were prepared by blending all membrane components including paraffin, kerosene, surfactant, and carrier in advance and then slowly adding the interior phase solution containing hydrochloride acid under turbulent mixing at 21000 rpm. Hydrochloride acid was added as an electrolyte supplying required driving force for extraction. Time of mixing for all emulsions was 7 min. Roi shows the volume ratio of the organic to aqueous phase.

Batch separations were carried out in a baffled glass vessel at ambient temperature. A measured amount of emulsion was dispersed in the external phase containing aqueous Phe solution under mild agitation of 200–400 rpm. pH was adjusted by adding sulfuric acid and sodium hydroxide. Rew shows the volume ratio of W/O emulsions to aqueous external phase. During the process pH was adjusted in the predetermined pH.

5 ml samples were taken throughout the experiment at predetermined time intervals by sanitary syringes and centrifuged at 3000 rpm immediately and then filtered in order to separate the external phase from the emulsion phase for analysis.

Phenylalanine concentration in the external phase separated in every sample was measured by direct absorbance measuring in 258 nm and colorimetric assay by ninhydrine in 570 nm.

After certain contact time, system stopped and left in rest for about 30 min. Then the emulsion and the external phase were separated and the emulsion was broken by heating in a boiling bath or passing through the electrostatic coalescer made in laboratory scale. The internal phase was

collected and analyzed for phenylalanine concentration. The membrane phase also could be used again to make the emulsion.

DESIGN OF EXPERIMENTS (DOE) BY TAGUCHI METHOD

DOE is a powerful statistical technique for determining the optimal factor settings of a process and thereby achieving improved process performance, reduced process variability, and improved manufacturability of products and processes. Basic phases in applying the Taguchi experimental design technique to the project are the planning of the project, designing the experiments, conducting the experiments, analyzing the results, and confirming the predicted results (9,10).

Planning of the Project

To plan the project, a wide study was carried out to identify the most effective factors in amino acid extraction by emulsion liquid membrane. Effective factors and their levels were selected according to the previous works done by other scientist and the results obtained from preliminary experiments done in the project. Table 1 shows the factors and their levels.

Levels of electrolyte concentration in the internal phase and the pH in the external phase were selected based on the fact that in D2EHPA facilitated transport pH difference between the external and the internal phases which should be kept as high as possible to provide the required

Table 1. Effective factors and their levels

No.	Factors	Unit	Level 1	Level 2	Level 3
1	HCl concentration in internal phase	Mol/lit	1	1.6	2.4
2	pH of external phase	–	2	3	4
3	Carrier concentration in membrane phase	v/v%	10	15	20
4	Volume ratio of emulsion phase to external phase (Rew)	–	0.2	0.3	0.4
5	Volume ratio of membrane phase to internal phase (Roi)	–	1	1.5	2
6	Surfactant concentration in membrane phase	v/v%	2	3	5
7	Time of extraction	Min	5	10	15

driving force of the extraction. So based on the mechanism of the extraction described in section 1, pH must be kept low in the internal phase and high in the external phase in order to prevent the carrier from being protonated, but since the carrier is the cationic type, Phe should exist as cation to be separable and this is possible at pH < 3 (2). So based on the above discussion, levels of pH and HCl concentration in the internal phase were selected as observed in Table 1.

It should be considered that since Phe^+ exchanges for H^+ when it is transported into the internal phase, pH gradually decreases so to obtain a greater amount of the Phe transport, pH should be kept constant. This was done by addition of a strong NaOH solution during the experiments.

To select suitable levels of Rew, its effect on mass transfer and stability of the membrane were studied. Higher Rew leads to more amount of emulsion which in turn results in more globules dispersed in the external phase, so the mass transfer area supplied per unit volume of external phase increases when Rew increases. However, more mass transfer area also increases water transport through the membrane. Furthermore, when there are more globules in the same volume of the external phase, the collision of those increases which will cause entrapment of the external phase fluid within the colliding globules. Both of the stated phenomena would result in the higher amount of swelling and breakage (14–16). These effects, along with a series of preliminary tests, led to the levels indicated in Table 1. It should be also taken into consideration that phase inversion that is the conversion of multiple emulsion of W/O/W into simple emulsion of W/O usually takes place at Rew = 0.55–0.6 (16), so in order to avoid this phenomena, in these experiments maximum amount of Rew was chosen as 0.4.

In order to investigate the effect of the volume ratio of oil to the internal phase (Roi) on the extraction, three levels of 1, 1.5, and 2 were chosen. Bigger Roi means smaller volume of internal phase, less number of water droplets, and therefore less internal mass transfer area. On the other hand, smaller Roi results in a bigger capacity of the internal phase for Phe and less resistance against mass transfer due to the thinner membrane thickness (4,15). So based on the stated facts, published works and preliminary tests above levels were selected.

To select the levels of surfactant concentration, the following competitive effects were investigated. In order to prepare a stable emulsion preventing breakage of the emulsion and leakage of Phe, a suitable amount of surfactant is required (2,15). But mass transfer of Phe decreases when the surfactant concentration increases due to the higher viscosity of the membrane (4). In addition, Span 80 is capable of solubilizing and transfer water through reversed micelles into the internal phase so a considerable rate of swelling will result (13,16). Considering all the

above aspects, doing simple stability tests by leaving the emulation at room temperature for at least 3 days and breaking the emulsion by heating in boiling bath, three levels of 2, 3, and 5% were selected.

In order to select the levels of time, some preliminary tests were done to find out approximately how much time is required to reach a considerable amount of extraction and also how long the emulsion can remain stable. The preliminary tests showed that one of the main advantages of ELM is the fast extraction which can be achieved in a few minutes.

The carrier concentration in the membrane phase was selected based on the reaction between the carrier and Phe which is as follows (4):

$$A(NH_3^+)COOH + \overline{2(HR)_2} \Leftrightarrow \overline{A(NH_3R_2H)COOH\,.\,(HR)_2} + H^+$$

where $(HR)_2$ and $A(NH_3{}^+)COOH$ represent the D2EHPA dimmers and Phenylalanine respectively and the overbar refers to the organic phase. So the amount of the carrier in the membrane phase must be enough to react with Phe^+. But it should be considered that D2EHPA increases the viscosity of the membrane and due to its structure, which is similar to a surfactant (Fig. 2), is able to solubilize water in small aggregates such as dimers and can increases the osmotic swelling which increases the breakage of the membrane, although its capacity for water uptake is considerably smaller than the surfactant (13).

Composition of the membrane phase i.e. the volume ratio of paraffin to kerosene was selected 70/30 v/v% which could make a stable emulsion with a good rate of mass transfer.

Agitation speed was selected 300 rpm which resulted in good mixing and the least breakage of the globules of emulsion. This was chosen based on the reports in other related literatures and preliminary works done. Solution of phenylalanine to be extracted by emulsion liquid membrane was selected 5 g/lit.

Designing Experiments

In order to design the experiments Qualitek 4 (QT4), software of Taguchi, was used to ease the analysis. An L_{18} orthogonal array with 8 columns and 18 rows was designed by the software. The basis of designing the array in the Taguchi method is the degree of freedom of factors and the total degree of freedom which are calculated as follows:

DOF of a factor: (number of levels of a factor)–1
Total DOFs: (number of results)–1

Table 2. Experimental layout using L18 orthogonal array

	Factor level						
Experiment number	Concentration of HCl	pH	Concentration of Carrier	Rew	Roi	Concentration of Surfactant	Time
1	1	1	1	1	1	1	1
2	1	2	2	2	2	2	2
3	1	3	3	3	3	3	3
4	2	1	1	2	2	3	3
5	2	2	2	3	3	1	1
6	2	3	3	1	1	2	2
7	3	1	2	1	1	2	3
8	3	2	3	2	2	3	1
9	3	3	1	3	3	1	2
10	1	1	3	3	3	2	1
11	1	2	1	1	1	3	2
12	1	3	2	2	2	1	3
13	2	1	2	3	3	3	2
14	2	2	3	1	1	1	3
15	2	3	1	2	2	2	1
16	3	1	3	2	2	1	2
17	3	2	1	3	3	2	3
18	3	3	2	1	1	3	1

In this study DOF of each factor is 2 and the total degree of freedom is 14 (7*2). The degree of freedom for the orthogonal array should be greater than or at least equal to those for the process parameters. The selected array has 17 degree of freedom and can handle three-level factors. The experimental layout for the extraction is shown in Table 2.

Conducting the Experiments

Eighteen experiments were performed according to the L18 orthogonal array. The amount of extraction was calculated by measuring the Phe concentration in the external phase after extraction. The results of each experiment are shown in Table 3.

Analyzing

Results (percent of extraction) collected from the experiments were analyzed in order to obtain information about the new design condition and

Table 3. Experimental results for percent of extraction

Experiment No.	Percent of Extraction
1	34.2
2	46.2
3	49.1
4	62.3
5	64
6	29.6
7	47.9
8	50.2
9	53.7
10	38.8
11	35.1
12	37
13	61.7
14	34.2
15	60.7
16	38.8
17	59.4
18	58.9

an estimate of the improvement. It was done by Qualitek 4 software. Analysis was done in two parts. Initially this was performed by simple analysis to produce average effects of factor in order to determine the best design condition and influence of factors. Calculations in this part involve only simple arithmetic (addition and division) operations. The second type of analysis is ANOVA analysis which is discussed in section 3.5.

The quality characteristic (QC) which is the sense of desirability of the result was selected as bigger-the-better because we intended to achieve the highest amount of extraction possible in this study. The average effect of every factor at different levels was calculated then by averaging the results of one factor at a time at every level as can be observed in Table 4.

In order to investigate the effect of each factor on the extraction of Phenylalanine it is useful to discuss each factor separately as follows.

Effect of Electrolyte Concentration in the Internal Phase

The results from three different HCl concentrations between 1 M and 2.4 M are shown in Fig. 3. As can be observed, when hydrogen concentration in the internal phase was increased from 1 to 1.6 M, the average

Table 4. Average effects of factors

No.	Factors	Level 1	Level 2	Level 3
1	HCl (M)	40.066	52.083	51.483
2	pH	47.283	48.183	48.166
3	Carrier (v/v%)	50.899	52.616	40.116
4	Rew	39.983	49.199	54.45
5	Roi	45.35	49.016	49.266
6	Surfactant (v/v%)	43.649	47.1	52.883
7	Time	51.133	44.183	48.316

effect of the factor increased from 49.1% to 52.1% which represents an increase in Phe extraction. This is due to the greater driving force provided by the hydrogen gradient across the membrane. However, further increase in HCl concentration from 1.6 to 2.4 M did not lead to a considerable change. This is due to the effects of the osmotic gradient on membrane instability which leads to emulsion breakage and thus leakage of Phe into the external phase as described in section 3.1.

Effect of Carrier Concentration in the Membrane Phase

As Fig. 4 illustrates, an increase in carrier concentration in the membrane phase from 10% to 15% resulted in a slight rise in average effect, that means an increases in Phe extraction rate which represents the increased ability of Phe to permeate the membrane phase via the carrier/Phe^+ complex. However, a sharp fall can be observed by increasing the

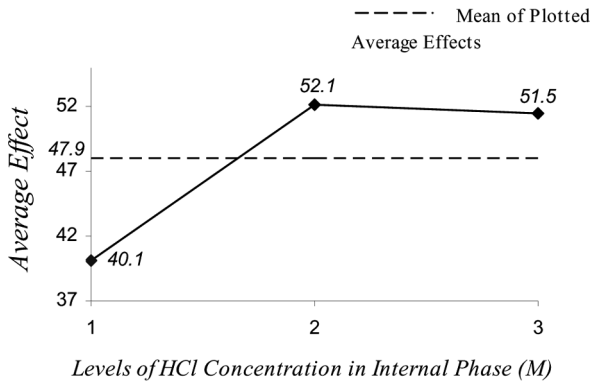

Figure 3. Average effect of HCl concentration on Phe extraction.

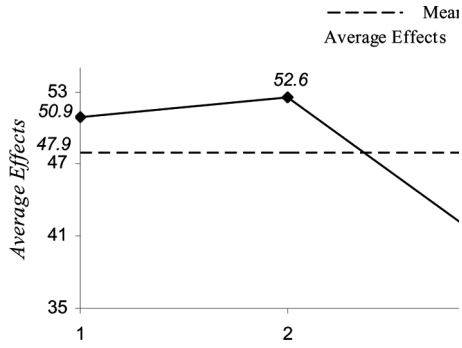

Figure 4. Average effect of carrier concentration on Phe extraction.

concentration to 20%. Based on the discussion in section 3.1., this decrease was expected because of the considerable increase in membrane viscosity which restricted mass transfer of Phe through the membrane.

Effect of the External Phase pH

As can be seen in Fig. 5, experiments were evaluated in three different pH's. When pH increased from 2 to 3, Phe extraction enhanced. However, more increase in pH to 4 did not cause any significant changes and the average effect remained almost the same. Two opposite effects of pH on the extraction as discussed in section 3.1 met each other at pH = 3 in this project which is well in accordance with the discussion stated.

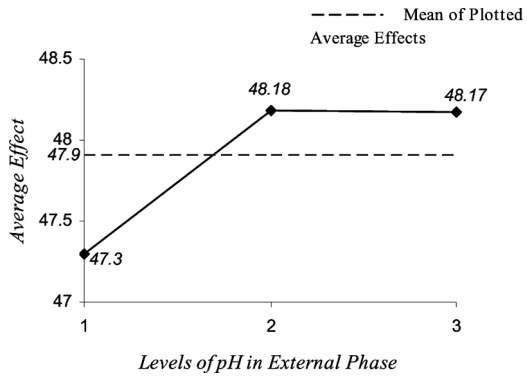

Figure 5. Average effect of pH on Phe extraction.

Effect of Rew

Figure 6 illustrates the average effect of Rew on Phe extraction. When Rew increased from 0.2 to 0.3 a sharp rise in the average effect was caused. However, further increase to 0.4 caused less increase in the average effect than before. This behavior was expected due to the effect of swelling and breakage as discussed in section 3.1.

Effect of Roi

In order to investigate the effect of volume ratio of oil to internal phase on the extraction degree, three levels of 1, 1.5, and 2 were chosen. As can be observed in Fig. 7, an increase in Roi from 1 to 1.5 resulted in a sharp rise in the average effect. This can be explained by the more stable emulsion due to the higher concentration of the surfactant at the interface of the membrane/aqueous phases and in the bulk membrane phase. Therefore, despite the effect of higher concentration of the surfactant on the swelling, more stable emulsion overweighed all mentioned effects in section 3.1. Further increase in Roi to 2, however, did not give such a drastic increase in average effect as observed before. The reason obviously is relating to the all effects mentioned in section 3.1.

Effect of Surfactant Concentration in the Membrane Phase

Figure 8 shows the average effect of Span 80 concentration on Phe extraction. As can be observed in Fig. 8, three levels of 2, 3, and 5% of surfactant concentration were investigated and according to the results

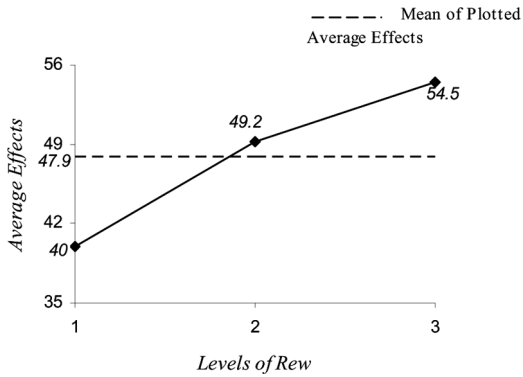

Figure 6. Average effect of Rew on Phe extraction.

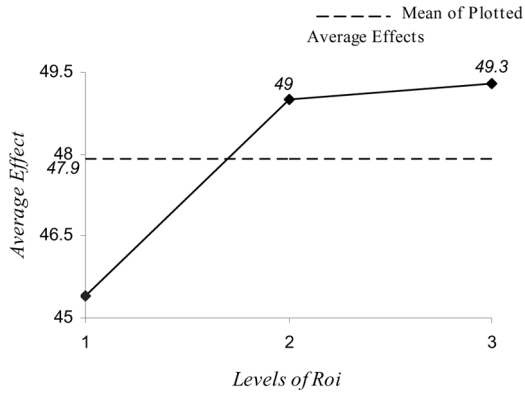

Figure 7. Average effect of Roi on Phe extraction.

achieved, the effect of the surfactant concentration on the stability of the membrane was the predominant effect. Therefore, when Span 80 concentration increased up to 5%, the Phe extraction increased significantly and almost consistently. Consequently concentration of 5% was the optimum concentration which could form the most stable emulsion.

Effect of Time

As Fig. 9 shows, the changing time of the process from 5 to 10 min led to less amount of Phe extraction due to an increase in the instability

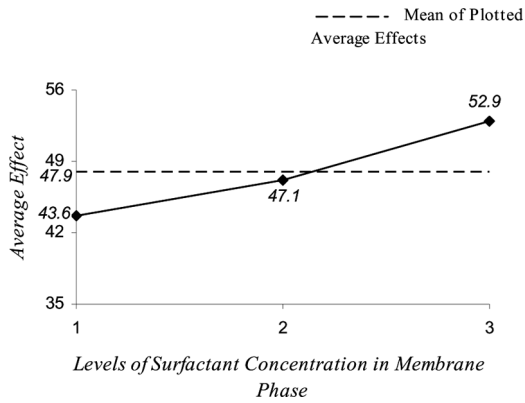

Figure 8. Average effect of surfactant concentration on Phe extraction.

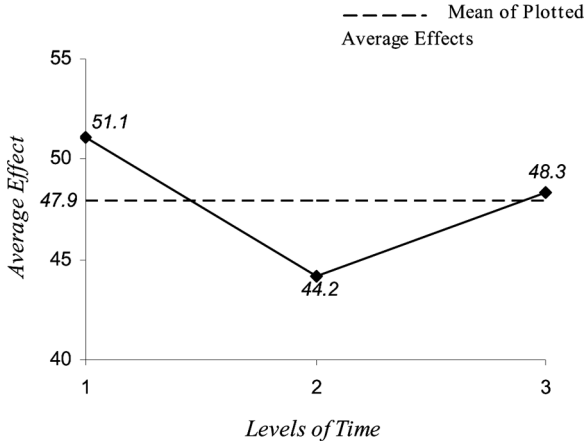

Figure 9. Average effect of time on Phe extraction.

of the emulsion. However, with further increase we encountered an unexpected result. Based on different effects of duration of the process discussed in section 3.1, it can be inferred from Fig. 9 that when the contact time increased from 10 min to 15 min, the amount of Phe extracted was high in that it could overcome the Phe leakage due to the instability of the emulsion. However, the rate of Phe transfer after 15 min still could not reach the amount achieved in 5 min. It should be also taken into consideration that although increasing the contact time might increase the net amount of the Phe transported into the internal phase, the increased amount of swelling dilutes the internal phase and negates the target of the process which is to obtain a concentrated solution of phenylalanine.

Analysis of Variance (ANOVA)

The main objective of ANOVA is to extract from the results how much variation each factor causes relative to the total variation observed in the result. Table 5 illustrates the results of ANOVA.

The second column shows the number of DOFs of the items calculation which was described in section 3.2. The DOF for the error term must be calculated after independently calculating the DOFs for the factors and the total as follows:

$$DOF_e\text{: (total DOFs)}-\text{(total of all factor DOFs)}$$

Table 5. ANOVA Table

No.	Factors	DOF	Sums of Squares	Variance	F-Ratio	Pur Sum	Percent
1	HCl (M)	2	550.199	275.099	13.219	508.577	22.346
2	pH	2	3.179	1.589	0.076	0.000	0.000
3	Carrier (v/v%)	2	550.952	275.476	13.237	509.331	22.379
4	Rew	2	643.586	321.793	15.462	601.964	26.449
5	Roi	2	57.694	28.847	1.386	16.073	0.706
6	Surfactant (v/v%)	2	261.206	130.603	6.275	219.584	9.648
7	Time	2	146.639	73.319	3.523	105.017	4.614
	Other/Error	3	62.433	20.910			13.858
	Total	17	2,275.891				100.000%

The third column shows the sum of squares. The total sum of squares was calculated independently first using the following formulation:

$$S_T = \sum_{i=1}^{N} Y_i^2 - C.F$$

N is the number of the total experiments, Y_i is the result of every experiments and $C.F.$ is the correction factor which was calculated as follows:

$$C.F = \frac{\left(\sum_{i=1}^{N} Y_i\right)^2}{N}$$

The factor sums of squares were calculated next, one factor at a time:

$$S_A = \frac{A_1^2}{N_{A_1}} + \frac{A_2^2}{N_{A_2}} + \cdots - C.F$$

A_1 is the sums of the results when the level of factor is 1.

N_{A1} is the total number of experiments in which level 1 of factor A is present.

The sum of squares for the error term was calculated as follows:

$$S_e = S_T - (S_A + S_B + \cdots)$$

Variance or Mean Squares was calculated by the following formulation:

$$V_A = \frac{S_A}{DOF_A}$$

The fifth column shows F-Ratio which was calculated by dividing variance of the factor by variance of the error:

$$F_A = \frac{V_A}{V_e}$$

Pure sum of squares which is shown in the sixth column of Table 5 was calculated as follows:

$$S'_A = S_A - (V_e \times DOF_A)$$

and percent influence of the factors (P%) was calculated by comparing the pure sums of squares of the factors with the total sum of squares:

$$P_A = \frac{S'_A}{S_T}$$

The total percent influence, shown at the bottom of the P column in Table 5 is always set to 100%. The percent influence of the error term again was calculated in the same manner: the sum of all factor influences subtracted from the total.

$$P_e = 100 - (P_A + P_B + \cdots)$$

Based on the percent of influence of each factor (P%) shown in the table of ANOVA, Rew, carrier concentration in the membrane phase, HCl concentration in the internal phase, surfactant concentration in the membrane phase and time are the significant factors respectively affecting the performance characteristics. Roi and pH are of less importance. The influence of pH is calculated 0.000.

Since pH has the least influence with Pure Sum (S) and Percent of influence (P) of 0.000, it was pooled. Pooling is the process of ignoring a factor once it is considered insignificant. It is done by combining the influence of the factor with that of the error term. Pooling is strongly recommended because in deciding which factors are important and which are not, we should try to minimize the chance of calling something important when it is not.

After one factor is identified for pooling, ANOVA always starts by establishing the new values for the error term by ignoring the percentage of all factors identified for pooling (treat pooled factors as if they are not there). With the revised error values, the F-Ratio and Pure sums can now be recalculated. Table 6 illustrates the new ANOVA results. As can be observed, F-Ratios, Pure sums, and Percents are different from those in previous table before pooling pH. Error term also has become less.

Rew is the most significant factor affecting the extraction process with the percent of influence of 27.125% and Roi is the least significant

Table 6. ANOVA Table after pooling pH

No.	Factors	DOF	Sums of Squares	Variance	F-Ratio	Pur Sum	Percent
1	HCl (M)	2	550.199	275.099	20.964	523.954	23.021
2	PH	(2)	(3.179)		POO	LED	0.000
3	Carrier (v/v%)	2	550.952	275.476	20.999	524.708	23.055
4	Rew	2	643.586	321.793	24.522	617.341	27.125
5	Roi	2	57.694	28.847	2.198	31.450	1.381
6	Surfactant (v/v%)	2	261.206	130.603	9.952	234.961	10.323
7	Time	2	146.639	73.319	5.587	120.394	5.289
	Other/Error	5	65.612	13.122			9.806
	Total:	17	2,275.891				100.00%

factor with P = 1.38%. Figure 10 depicts the column diagram of every factor's contribution in Phenylalanine extraction. As can be observed, Rew and Roi have the most and the least contribution respectively.

Error term represents the collective influence of all factors not included in the study, uncontrollable factors (noise factors) plus any experimental error if present. It is not a matter of how well the experiment was conducted.

The optimum levels of significant factors and the performance expected at the optimum condition are shown in Table 7.

The confidence interval (C.I) was calculated using ANOVA values. The C.I calculates the lower and upper limits of confidence level.

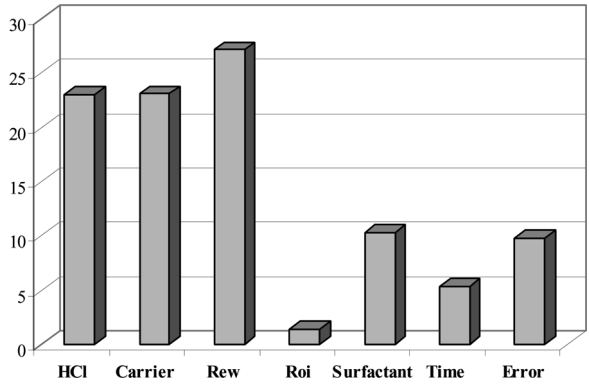

Figure 10. Column diagram of every factor's contribution in Phenylalanine Extraction.

Table 7. Optimum levels of factors and expected performance at the optimum condition

No.	Factors	Level Desc.	Level	Contribution
1	HCl (M)	1.6	2	4.205
2	Carrier (v/v%)	15	2	4.738
3	Rew	0.4	3	6.572
4	Roi	2	3	1.388
5	Surfactant (v/v%)	5	3	5.005
6	Time	5	1	3.255

Total Contribution From all Factors 25.162.
Current Grand Average Of Performance 47.877.
Expected Result At Optimum Condition 73.040.

Confidence level can vary between 80 and 95% generally. In this study confidence levels of 90 and 95% were chosen, then the confidence interval was calculated using the following formulation:

$$\text{Confidence interval (C.I.)} = \pm\left[\frac{F(1, n_2) \times V_e}{N_e}\right]^{0.5}$$

Where $F(1, n_2)$ is the F value from F table for factor DOF and error DOF at the confidence level desired, V_e the variance of the error term (from ANOVA), and N_e the effective number of replications:

$$N_e = \frac{\textit{total number of resultsor } S/N}{\textit{DOF of mean}(\textit{always} = 1) + \textit{DOF of all factors included in estimating the mean performance at optimum condition}}$$

Confidence intervals on optimum performance for two confidence levels of 90% and 95% are $+/-5.592$ and $+/-5.759$ respectively. The C.I calculates the lower and upper limits of this value, within which mean performance of the actual test samples should fall. The confidence level expressed as a percentage indicates how often the performance is expected to exceed the value estimated.

Confirming Predicted Results

As Fig. 11 depicts, the Phe concentration could decrease from 5 g/l to 1.2 g/l in 5 min which indicates extraction percent of 75.3. it was within the confidential interval for both 90% and 95% confidential levels.

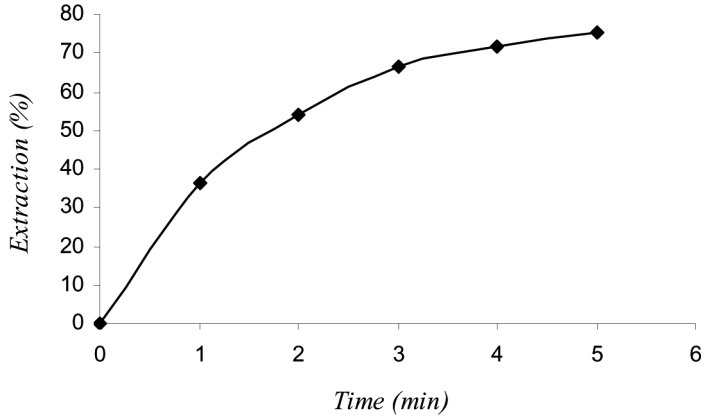

Figure 11. Phenylalanine extraction in optimum condition Time = 5 min, Span 80 = 5 v/v%. Roi = 2, Rew = 0.4, D2EHPA = 15 v/v%, HCl = 1.6 M, Phe conc. = 5 g/l, Agitation speed = 300 rpm.

CONCLUSION

The application of the Emulsion Liquid Membrane to extract amino acids from a synthetic solution was investigated. Phenylalanine was selected as the solute to be extracted. D2EHPA was used as the carrier, HCl solution as the internal reagent, and Span 80 as the emulsifier.

According to the design of experiment which was done by the Taguchi method, the influence of electrolyte (HCl) concentration in the internal phase, surfactant concentration in the membrane phase, carrier concentration in the membrane phase, volume ratio of W/O emulsion to the aqueous external phase (Rew), volume ratio of the membrane phase to the internal aqueous phase (Roi), pH of the external phase and time was investigated and based on the experimental results and the analysis of variance done by the Taguchi method, the following conclusions can be drawn:

- Rew has the most influence on the process performance. Increasing Rew can make a considerable rise in the rate of extraction. However, swelling and breakage of the emulsion also become more serious with increasing Rew due to the more globules collision.
- Carrier Concentration is the second significant factor. When carrier concentration increases, the amount of Phe transferred into the internal phase increases due to the more carrier molecule available to form Carrier/Phe^{+} Complex. However, when it reaches 15%, which can be

introduced as a critical amount, further increase will not result in more extraction, but will cause significant decrease in extraction by increasing the membrane instability.

- HCl concentration in the internal phase also affects the extraction of Phe by providing the driving force for mass transfer. When the HCl concentration increases, extraction rate increases, however, it also has considerable effect on swelling by increasing the pressure gradient between the internal and the external phases.
- Surfactant concentration has the main effect on emulsion stability which is of high significance in the ELM process. However, it should be taken into consideration that increasing the surfactant concentration increases membrane viscosity which can increase resistance against mass transfer and also can enhance water transport by forming reversed micelles.
- Selecting the suitable duration of the process is also important in terms of providing longer time for Phe transfer and increasing the risk of swelling and breakage. Therefore, achieving a considerable amount of extraction in as short a time as possible is preferable.
- Roi has little influence on the performance. When Roi increases, the emulsion stability increases due to increasing of viscosity and availability of more surfactant molecules in the interface of membrane/internal phases. On the other hand, mass transfer resistance and swelling also increase when Roi increases. However, this is not considerable up to Roi = 1.5, after that we can observe a significant decrease in the rate of increase of the average effect.
- pH has the least influence on the performance. Therefore in order to obtain better analysis by the Taguchi method pH can be regarded as an insignificant factor. Regarding pH as an insignificant factor means that the optimum condition prescribed by analysis does not require any particular level of pH and even in case of specifying a nominal working level for this factor; we no longer need to specify tight tolerance on its specified levels.

Using the Taguchi method, the optimum levels of significant factors and the expected amount of Phe extraction at the optimum condition were identified. The predicted amount of extraction (73%) could be achieved by confirmation experiment at optimum condition.

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