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Short communication

Selective recovery of amino acids by aqueous two-phase electrophoresis

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Abstract

Aqueous two-phase systems (ATPSs) formed by spontaneous phase separation of different water-soluble polymers are bio-compatible. Aqueous two-phase electrophoresis, coupling traditional solvent extraction with electrophoresis, is a novel separation technique and provides a successful method for separating mixtures of biomolecules. In this work, the selective recovery of amino acids by aqueous two-phase electrophoresis was examined with dextran–polyethylene glycol–water as a working system. The experiment results show that glutamic acid can be separated from phenylalanine and tryptophan successfully. The influences of the electrophoresis time, field strength, and phase volume ratio were studied in an intermittent separation equipment.

Keywords: Aqueous two-phase system; Electrophoresis; Amino acids

1. Introduction

Aqueous two-phase systems (ATPSs) are formed by spontaneous phase separation of different water-soluble polymers such as polyethylene glycol (PEG) and dextran (DEX). Such partitioning systems have many desirable characteristics. Composed mainly of water, they are biologically friendly; amino acids may be stabilized by the phase forming polymers, and rapid intraphase transport allows for short processing time [1–3]. In addition, the process is easily scalable and can be operated continuously [4].

Two-phase electrophoresis (TPE), coupling traditional extraction with electrophoresis, is a novel separation method [5]. The TPE technique is similar to electrophoresis and electrodialysis on one hand, and to traditional extraction on the other. In this technique, there are two distinct liquid phases within the separation device to nullify the harmful effects of convection. One of the phases contains the mixture to be separated and the other acts as a solvent to remove the components separated [6]. An electric field perpendicular to the phase interface is imposed on the system, so oppositely charged particles should move into different phases. With the migration of the charged particles, the original partition balance can be changed and hence the separation is effected. With these improvements, TPE provides stability against convection mixing and enables product to be isolated.

Aqueous two-phase electrophoresis (ATPE), as its name implies, integrates aqueous two-phase separation with electrophoresis. Although the two phases are electrically conductive, few applications with electric field imposed on these systems have been made. Levine and Bier [7] have studied the electrophoretic mobility of a protein in an ATPS by using a U-tube electrophoresis device, and they noted an impediment to electro-separations by applying a field to the system. Marando and Clark [8,9] have used the DEX–PEG–water system to separate mixtures of hemoglobin and albumin. The experimental result shows a significant improvement on the separation obtained by partitioning in the same two-phase system with no applied field under the same conditions.

In previous research, our laboratory examined the separation effect of organic acids [10,11] and dyestuffs [12,13] by TPE. From these studies, it can be shown that purification can be enhanced with electric field applied comparing with traditional extraction. Investigations on the purification of single amino acid by ATPE were also done. The conclusion can be safely drawn that ATPE is very effective for the separation and purification of charged molecules, such as amino acids.

Amino acids are amphoteric and have net charges when pH of the solution is not equal to their isoelectric points. It is known that amino acids will be positively charged when pH value is lower than their isoelectric points, or else charged

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negatively. Consequently, amino acids can be charged differently by controlling pH of the solution at a specified value and will move into different phase with electric field applied.

In this study, we have examined the selective recovery of amino acids by ATPE. The selective recovery of amino acids by this new technique was investigated in DEX–PEG–water system. The influence of factors such as separation time, field strength and initial solute concentration was studied in an intermittent separation equipment. The principle of separating amino acids by ATPE was discussed at the same time.

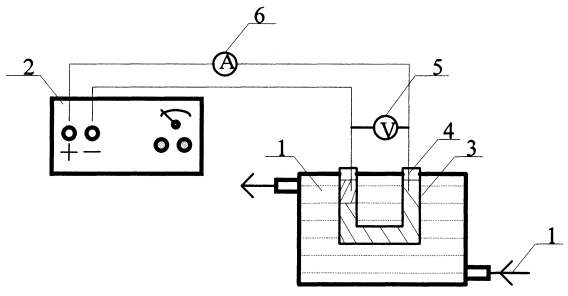
2. Materials and methods

Glutamic acid, tyrosine, phenylalanine and tryptophan were purchased from Chinese Medicine, 6000 average molecular weight PEG was from Tianjin Xinya Industry and Trade Corporation and 185,000 average molecular weight DEX was obtained from Sigma Chemical. Deionized water was prepared in our laboratory. All other chemicals were of analytical grade. ATPSs were prepared by adding 8.0 g DEX and 8.5 g PEG to 100 ml water. The initial pH of the systems was about 6.8.

The concentration of amino acids was determined using HPLC. A C18 reverse phase column, 200 mm long and 2.1 mm in diameter, was employed. Mobile phase was composed of 20 mM sodium acetate, 0.018% TEA, pH 7.2, 0.3% THF. A small volume of sample solutions was introduced into a fluid flow by using a sample injector. The concentration of the sample in effluent was monitored with an ultraviolet detector.

The experiment apparatus is shown in Fig. 1. The main equipment, U-tube, is made of glass and its inner diameter is 10 mm. Using U-tube, rather than a straight tube, could avoid the bad effect of convection mixing, brought out by the electrolysis gas bubbles. In order to control the experiment temperature at 25°C, water-bath is used to remove the heat produced by the electrode reactions. The two electrodes are made of platinum wire of 0.5 mm in diameter, and the distance between the two electrodes is 10 cm. The volume ratio (bottom phase/top phase) is 3. The electrodes are inserted into different phases. The electric field strength is determined by the output voltage of the electrophoresis instrument DYY-III.

For each experimental run, the ATPS's pH is adjusted with the buffer of 0.12 N NaAc and 1.6 N HAc. The pH value of the buffer is 3.6. The amino acids are separated by traditional solvent extraction at first, i.e., to partition the solutes in the ATPS by shaking for 12 h and settling enough time (2 days) with no electric field applied. The clarified top phase and bottom phase are isolated by an extraction funnel. The recovery of the amino acids by conventional extraction can be obtained by analyzing the concentration of the extract phase before electrophoresis. Then some volume (12 ml) of bottom phase is introduced into one leg of the U-tube, and 4 ml top phase is loaded into the other. The electric field is applied as soon as the system is loaded. Under a specified voltage and different time, concentrations of the top phase and bottom phase are analyzed by HPLC. The influence of time on the effective partition coefficient and recovery ratio can be calculated by the concentration of different time. The influences of other factors such as voltage and volume ratio can be obtained by analyzing concentration similarly.



1. Water-bath 2. Electrophoresis apparatus 3. U-tube electrophoresis equipment 4. Electrode 5. Voltmeter 6. Amperemeter

Fig. 1. Intermittent TPE apparatus.

3. Results and discussion

3.1. Influence of electric field on two aqueous phase electrophoresis

Some spectrograms of HPLC are reported here to prove that no new component was produced during the separation by ATPE. Figs. 2–5 show the effect of separation was improved with electric field applied undoubtedly. The peaks in the spectrograms are of glutamic acid, tyrosine (chosen as a referential amino acid in quantitative analysis), phenylalanine and tryptophan, respectively. The results without

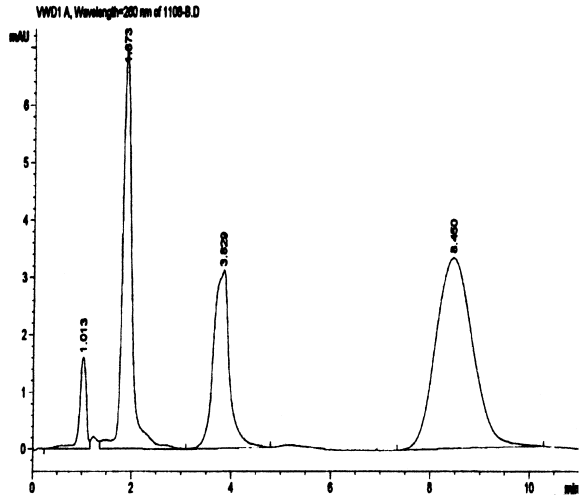


Fig. 2. Spectrogram of top phase before electrophoresis.

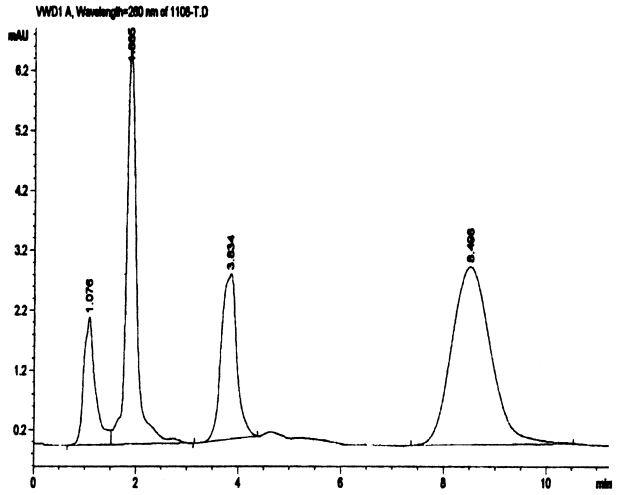


Fig. 3. Spectrogram of bottom phase before electrophoresis.

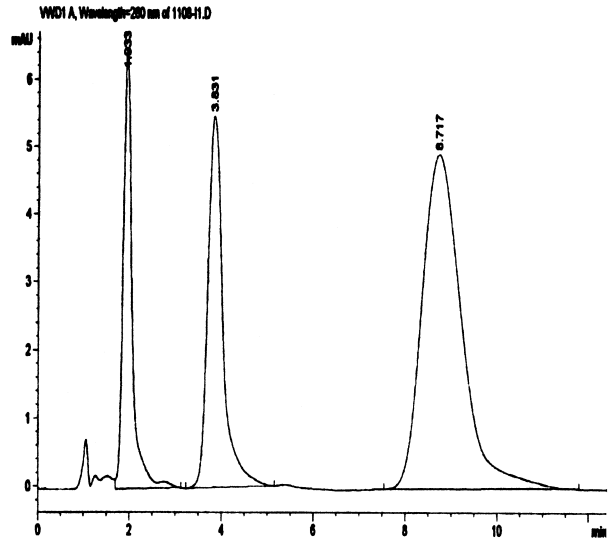


Fig. 4. Spectrogram of top phase after electrophoresis.

electric field are shown in Figs. 2 and 3 where it can be seen that the three amino acids distribute almost evenly in two phases before electrophoresis under these conditions. It can also be seen that upon application of field, amino acids can be directed into either the top or the bottom phase, i.e., selective recovery of amino acids can be obtained by ATPE. The reason for this phenomenon is that the pH value of the system is 3.90, which is larger than the isoelectric point of glutamic acid and lower than that of phenylalanine and tryptophan. During the experiment, the anode was inserted into the bottom phase and the cathode into the top phase. Consequently, amino acids that was charged differently and moved into different phases in a specified external electric field, i.e., glutamic acid moved to the bottom phase while phenylalanine and tryptophan moved to the top phase.

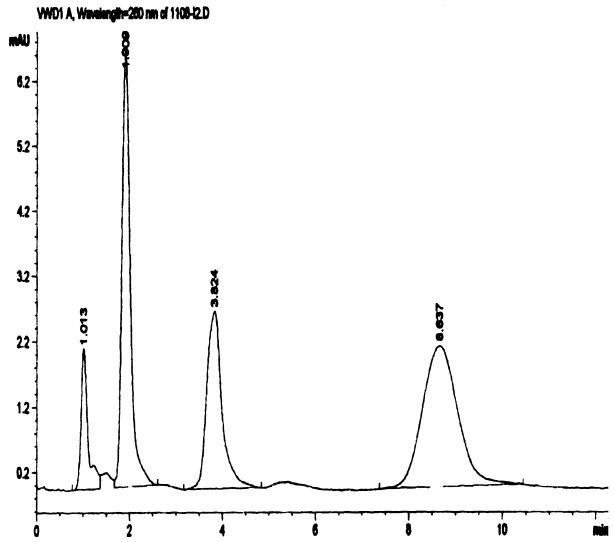


Fig. 5. Spectrogram of bottom phase after electrophoresis.

3.2. Influence of time

With volume ratio (top phase/bottom phase = 1/3), 60 V/cm applied, experiments were conducted by changing the electrophoresis time. The influence of the electrophoresis time on the separation effects of amino acids can be obtained by analyzing the concentrations under different time.

The experimental results are shown here in terms of concentration, an effective partition coefficient and recovery ratio. Several terms must be defined before discussing these results. The effective partition coefficient, K, is defined as the concentration of a species in the extract phase divided by that in the other phase after a specified field was applied for a specified time. The recovery ratio is defined as the percent of the total amino acid in the feed recovered in the extract phase. Results of standard partitioning experiments in the absence of an applied electric field, indicated by the values at time is zero, are presented in these figures for comparison with the ATPE.

Figs. 6–8 show that the separation was improved obviously with increasing time as indicated by the values of concentration and effective partition coefficients. Fig. 6 indicates that glutamic acid concentrated further in the bottom phase while phenyalanine and tryptophan concentrated in the top phase. It can also be seen from Figs. 7 and 8 that the effective partition coefficient of glutamic acid decreased from 0.96 to 0.34, and 90% glutamic acid was enriched in the bottom phase after 240 min. Under the same conditions, the effective partition coefficients of phenylalanine and tryptophan increased from 0.60 to 1.99 and from 0.58 to 2.36, respectively, and more phenylalanine and tryptophan were focused in the top phase.

The explanation for this phenomenon is that with the cathode inserted into the upper phase, more time in the applied

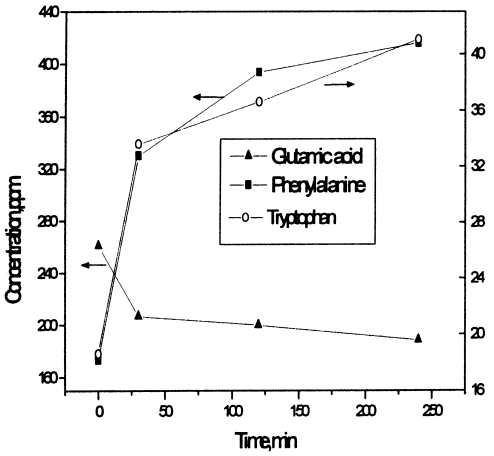


Fig. 6. Influence of time on the concentration.

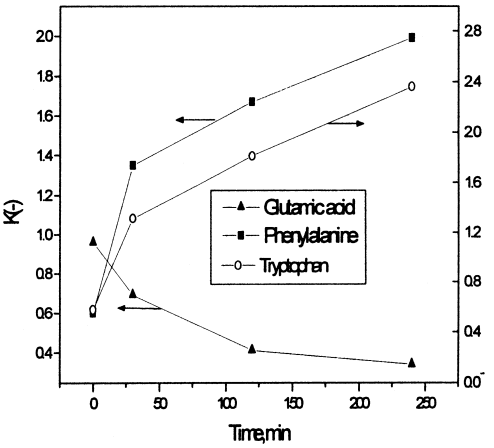


Fig. 7. Influence of time on the effective partition coefficient.

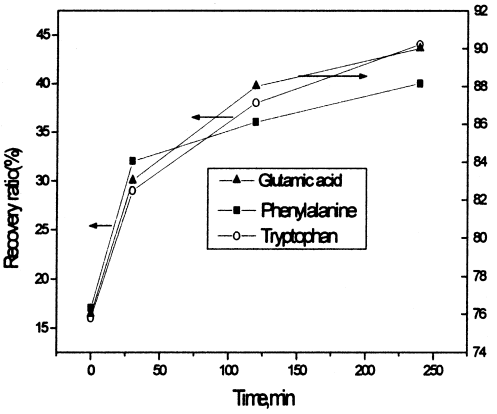


Fig. 8. Influence of time on the recovery ratio.

field allowed more of the negatively charged amino acids to move across the phase interface into the bottom phase and positively charged into the top phase. Consequently, for a given velocity of each amino acid molecule, longer time would allow more amino acid to move far enough to cross the interface.

3.3. Influence of electric field strength

With other conditions unchanged, experiments were also conducted by changing the electric field strengths. The influence of the electric field strength on the separation effects of amino acids can be obtained by analyzing the concentrations under different electric field strengths.

The Run I of Table 1 shows the results in the absence of the electric field. Comparison of Run II with Run III indicates the effect of increasing electric field strength on the separation of amino acids. It can be seen that with volume ratio (top phase/bottom phase $= 1/3$), 60 V/cm applied for 300 min, nearly all glutamic acid concentrated in the bottom phase. Under the same conditions, the effective partition coefficients of phenylalanine and tryptophan increased from 0.83 to 2.53 and from 0.89 to 2.64, respectively, indicating that more and more phenylalanine and tryptophan concentrated in the top phase.

The explanation for this phenomenon is that the larger voltage leads to the stronger force of the charged particles in electric field, and hence the solutes are easier to overcome the impeding of the interface to enter into another phase. Meanwhile the impetus for amino acids to move in each phase is increased too. Consequently, there are more solutes transferring across the interface and the recovery ratio is improved.

3.4. Influence of volume ratio

With the other experimental conditions unchanged, experiments of different phase volume ratios were conducted. The influence of phase volume ratios on the separation effects of phenylalanine and tryptophan can be obtained by analyzing the concentrations of different phase volume ratio.

The results shown in Table 2 demonstrate that the top phase concentration and effective partition coefficient of glutamic acid decreased with the electric field applied, while that of phenylalanine and tryptophan increased significantly. It can also be seen that the recovery of the desired amino acid was improved with the phase volume ratio increased.

Comparison of system II with system III indicates that the difference of phase volume ratio did not make the effective partition coefficients change much when a specified electric field was applied for enough time. The main reason for this is that with a specified electric field and enough separation time allowed, a balance partition of amino acids in the working system can be achieved, which also has been proved by work with other systems in our lab. The amino acids having crossed the interface stay mainly near the interface and hence the concentration of amino acids near the interface increases with time. So an inverse electric field will form with increasing time, which will counteract the migration of amino acids. Furthermore, the higher concentration near the interface of the top phase facilitates the inverse concentration diffusion, and hence there are less amino acids across the interface. Therefore, the effective partition coefficients

Table 1
Influence of electric field strength on the separation process for Glu, Phe and Trp

Run		Field (V/cm)	Time (min)	Concentration (ppm)			*K* (–)			Recovery (%)		
				Glu	Phe	Trp	Glu	Phe	Trp	Glu	Phe	Trp
I	Top phase	–	–	260.5	106.9	15.0	1.05	0.83	0.89	74	22	23
	Bottom Phase			247.4	128.8	16.9						
II	Top phase	40	300	169.9	212.0	26.8	0.52	2.03	2.27	85	40	43
	Bottom Phase			329.5	104.3	11.8						
III	Top phase	60	300	–	231.7	28.0	≈0	2.53	2.64	100	46	47
	Bottom phase			354.5	91.2	10.6						

Table 2
Influence of volume ratio on the separation process for Glu, Phe and Trp

Run[a]		Field (V/cm)	Time (min)	Concentration (ppm)			K (–)			Recovery (%)		
				Glu	Phe	Trp	Glu	Phe	Trp	Glu	Phe	Trp
I	Top phase	–	–	222.1	93.2	8.7	0.89	0.91	0.92	–	–	–
	Bottom phase			248.8	102.4	9.5						
II	Top phase	60	300	129.3	213.0	19.3	0.44	3.09	3.60	87	51	54
	Bottom phase			296.4	68.9	5.36						
III	Top phase	60	300	142.6	158.0	14.4	0.42	3.95	3.56	76	75	73
	Bottom phase			338.5	40.0	4.05						

[a] System I: system in absence of the electric field; system II: system with the phase volume ratio 1/3; system III: system with the phase volume ratio 3/4.

almost kept unchanging in the systems of different volume ratio with enough separation time. The more volume of the extract phase makes the more amino acid enriched in the extract phase. Consequently, the percent of the total amino acid in the feed recovered in the extract phase increased, i.e., the recovery ratio is improved.

4. Conclusion

ATPE, using a PEG/DEX ATPS as a medium for electrophoretic transport, is an effective separation method for small amphoteric biomolecules, such as amino acids. Amino acids were transported into either the PEG-rich top phase or the DEX-rich bottom phase depending on the amino acids net charge and electrode placement. Increase in the electrophoresis time, the applied electric field and the phase volume ratio with other parameters held constant all result in further directing amino acids into one of the phases. Selective recovery of amino acids was achieved by directing oppositely charged amino acids into different phases. The conclusion can be drawn that a highly efficient separation of amino acids can be obtained by changing the operating conditions, such as prolonging time or increasing the electric field strength.

Acknowledgements

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References

[1] P.F. Fauquex, H. Hustedt, M.R. Kula, Phase equilibration in agitated vessels during extractive enzyme recovery, J. Chem. Techol. Biotechnol. 35B (1985) 51–59.
[2] K.H. Kroer, H. Hustedt, S. Granda, M.R. Kula, Technical aspects of separation using aqueous two-phase systems in enzyme isolation processes, Biotechnol. Bioeng. 20 (1978) 1967–1988.
[3] R. Kuboi, T. Hasegawa, K. Yamahara, I. Komasawa, G. Johansson, Selective recovery of proteins by control of their surface properties utilizing PEG-bound affinity ligands, J. Chem. Eng. Jpn. 31 (4) (1998) 618–625.
[4] J.R. Luther, C.E. Glatz, Genetically engineered charge modifications to enhance protein separation in aqueous two-phase system: electrochemical partitioning, Biotechnol. Bioeng. 44 (1994) 147–153.
[5] J.J. Stichlmair, J. Schmidt, R. Proplesch, Electro-extraction: a novel separation technique, Chem. Eng. Sci. 47 (12) (1992) 3015–3022.
[6] K.J. Ptasinski, P.J.A.M. Kerkhof, Electric field driven separations: phenomena and applications, Sep. Sci. Technol. 27 (8/9) (1992) 995–1021.
[7] M.L. Levine, M. Bier, Electrophoretic transport of solutes in aqueous two-phase systems, Electrophoresis 11 (1990) 605–611.
[8] M.A. Marando, W.M. Clark, Two-phase electrophoresis of proteins, Sep. Sci. Technol. 28 (8) (1993) 1561–1577.
[9] W.M. Clark, Electrophoresis enhanced extractive separation, Chemtech. 22 (7) (1992) 425–429.
[10] J.G. Liu, G.S. Luo, S.L. Zhai, A development of two-phase electrophoresis technology, Mod. Chem. Ind. (China) 19 (8) (1999) 8–11.
[11] W.B. Jiang, G.S. Luo, Y.Y. Dai, A study on the separation of organic acid by electro-extraction, in: Proceedings of the Ninth National Conference of Chemical Engineering — NCCE, Vol. 8, Qingdao, China, 1998.
[12] G.S. Luo, W.B. Jiang, Y.C. Lu, S.L. Zhu, Y.Y. Dai, Two-phase electrophoresis separation of dyestuffs from dilute solution, Chem. Eng. J. 73 (2) (1999) 137–141.
[13] G.S. Luo, M.J. Yu, W.B. Jiang, S.L. Zhu, Y.Y. Dai, Electroextraction separation of dyestuffs, Sep. Sci. Technol. 34 (5) (1999) 781–791.