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Distribution of Puerarin in Aqueous Two-Phase EOPO/K_2HPO_4/Water Systems

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The partition behaviour of puerarin in ethylene oxide-propylene oxide random copolymer (EOPO)/K_2HPO_4/water systems was investigated. The optimum conditions were EOPO (SDP30) 28 %, K_2HPO_4 24 %, temperature-induced condition was 70 °C and 0.5 h. Yield of puerarin could reach 42.26 %. Based on the modified model $\ln K/R^2 = A^* + b^*(w''-w') + c^*(w''-w')^2$, partition coefficients of puerarin in EOPO/K_2HPO_4 aqueous two-phase systems were correlated. It was found that the correlation of the experimental data was quite satisfactory.

Key Words: Aqueous two-phase system, Temperature-induced phase separation, Puerarin, Ethylene oxide-propylene oxide random copolymer (EOPO), Thermodynamic model.

INTRODUCTION

Pueraria lobata (Willd.) Ohwi is a traditional Chinese medicinal herb. Its extract is rich in isoflavones[1], puerarin (daidzein 8-C-glucoside). It has been experimentally demonstrated that puerarin can improve blood circulation, prevent cardiovascular diseases, control alcoholism and treat arrhythmia[2,3]. Various separation methods such as solvent extraction, adsorption, chromatography, crystallization, macroporous resin have been used, but most of them are time consuming, low recovery, pollution or high cost[4-6].

Aqueous two-phase extraction (ATPE) is a system in that two immiscible phases are formed when polymers such as poly(ethylene glycol) (PEG) are mixed with dextran, other polymers or salts in particular concentration. The compound in aqueous two-phase extraction system has an equilibrium distribution depending on its own surface properties such as charge and hydrophobicity and on the physicochemical properties of the two phase, which can be manipulated by adjusting the factors such as the polymer molecular mass and concentration, type of phase forming salt, salt concentration, ionic strength and pH. Recently aqueous two-phase extraction has been widely used for protein separation[7,8] for it has the potential to produce a concentrated and purified product in one step when compared to the number of steps involved in conventional bioseparation process. Aqueous two-phase extraction is a simple separation process and it offers gentle nontoxic environments for biomolecules. It is cost effective and can be scaled up easily, so it is especially suitable for the practical production.

Aqueous two-phase partitioning coupled with temperature-induced phase separation is a quick, easy and inexpensive bench-top technique for extracting and purifying technic. This technique can also be readily up-scaled for commercial use. An aqueous solution of the ethylene oxide-propylene oxide random copolymer UCON 50-HB-5100 was successfully used to extract ecdysone and 20-hydroxyecdysone from the common spinach plant[9].

EXPERIMENTAL

Puerarin used for analysis standards were purchased from the Chinese Institute of Drugs and Biological Products Identification. Commercial puerarin (purity 93 %) was purchased from Xuancheng Baicao Technology Industry Co. Ltd. (China), it was used in our solvent extraction experiments. Ethylene oxide-propylene oxide random copolymer SDP35 (EO/PO = 1:1 mol M_r: 3000) hydroxide value: 35.22, cloud point: 54.2 °C; SDP28 (EO/PO = 1:1 mol M_r: 4000) hydroxide value: 26.44, cloud point: 47.5 °C; SDP30 (EO/PO = 1:1.5 mol M_r: 4000) hydroxide value: 28.08, cloud point: 41.3 °C, were purchased from Weier Chemical Company (China). All other reagents are of analytical grade.

Preparation of the aqueous two-phase system

Primary aqueous two-phase partition system: All polymer concentrations were calculated as mass percentages. Aqueous two-phase extraction were prepared by mixing stock solution of EOPO, K_2HPO_4 and water 25 g and the puerarin 0.0500 g to obtain a total. The primary phase systems were kept at room temperature (25 °C). Phase systems were separated by centrifugation at 2000 rpm for 5 min. The puerarin-rich upper phase was removed and isolated in a separate container.

Temperature-induced phase separation: Container with upper puerarin-rich phase was placed in a water bath for 0.5 h to allow temperature-induced phase separation to occur. The lower water-buffer phase was removed and isolated. Prior to analysis. Because of increased viscosity, the EOPO phase was diluted by a factor of 10 prior to analysis.

Quantification: Results are defined by the partition coefficient K and the distribution ratio Y. Primary aqueous two-phase partition system

$$Y_1 = \frac{(G - C_1V_1)}{G}$$

Temperature-induced phase separation

$$R = \frac{V_t}{V_b}$$

$$K = \frac{C_t}{C_b}$$

$$Y_2 = \frac{1}{(1+RK)}$$

$$Y = Y_1Y_2$$

C1: The concentration of partitioned substances in the lower phase at primary aqueous two-phase partition system, mg/mL. V_1: The volume of the lower phase in primary aqueous two-phase partition system, mL. G: The mass of puerarin, mg. R: Volume ratio. V_t: The volume of the upper phase in temperature-induced phase separation, mL. V_b: The volume of the lower phase in temperature-induced phase separation, mL. K: The partition coefficient. C_t: The concentration of partitioned substances in the upper phase at temperature-induced phase separation, mg/mL. C_b: The concentration of partitioned substances in the lower phase at temperature-induced phase separation, mg/mL. Y_1: Percentage yield of puerarin in the upper phase at primary aqueous two-phase partition system. Y_2: Percentage yield of puerarin in the lower phase at temperature-induced phase separation. Y: Percentage yield of puerarin in the process.

Detection and analysis: Concentrations of puerarin were analyzed by UV at 249 nm, calibration curves of puerarin showed good linearity ($r^2 > 0.999$) over the range from 0.2-8.9 mg the regression was $y = 0.01291x - 0.00105$, where x is the absorbance at 249 nm, y is the concentration of puerarin.

RESULTS AND DISCUSSION

Separation puerarin with different EOPO: In different ethylene oxide-propylene oxide random copolymer EOPO SDP28, SDP30, SDP35 aqueous two-phase systems, EOPO (28 %, w/w), K_2HPO_4 (32 %, w/w), the tests show that they form twice two-phase systems. In presence of puerarin, the cloud point of EOPO is enhanced. Because of viscosity, EOPO SDP28/K_2HPO_4/water system was difficult to remove and analysis, so we didn't proceed further. In EOPO SDP35/K_2HPO_4/water system, temperature- induced phase separation occurred in the range of 92-97 ºC. Water evaporated quickly in this temperature, so it was not suitable for puerarin to separate in lower water-buffer phase. However, in EOPO SDP30/K_2HPO_4/water system, the operating temperatures used in this study are in the range of 70-75 ºC, which ensures the occurrence of the cloud point and phase separation.

Effects of concentration of EOPO on partitioning: The variation of the puerarin extraction efficiency was studied within EOPO SDP30 concentration range from 22-32 % (w/w). Six different concentration combination of EOPO SDP30 and K_2HPO_4 (24 %, w/w)/water system were studied. In primary aqueous two-phase partition system separation, above 70 % of puerarin was partitioned into upper (EOPO) phase. Compare with puerarin in primary aqueous two-phase partition system separation and temperature-induced phase separation, temperature-induced phase separation was important to improve the distribution ratio. Fig. 1 shows the effect of EOPO SDP30 concentration to partition coefficient.

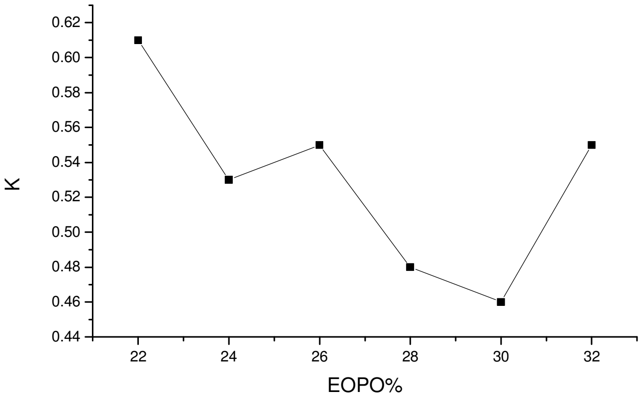

Fig. 1. Effect of EOPO SDP30 concentration to partition coefficient

In case of micelle formation from nonionic surfactants, the tendency for the alkyl chains to avoid contact with water is balanced by the hydration and space required for the poly(ethylene oxide) chains[10]. Mixed micelle formation depends on the nonionic surfactant concentrations and on the balance between these factors. Fig. 1 showed that in temperature-induced phase separation, when the concentration of EOPO SDP30 is lower, due to the salting out effect of K_2HPO_4, more water goes to the dilute phase, puerarin likes in upper phase. When the concentration of EOPO SDP30 is higher, viscosity of upper phase is increased, and thus difficult for puerarin to transfer to the lower phase. So in temperature-induced phase separation, partition coefficient is minimum. When EOPO SDP30 (30 %, w/w), the minimum of partition coefficient is 0.46. Volume ratio is summarized in Fig. 2. The optimum condition was EOPO SDP30 (28 %, w/w), K_2HPO_4 (24 %, w/w), yield of puerarin could reach 42.26 %.

Effects of concentration of K_2HPO_4 on partitioning: The six different concentration combination of K_2HPO_4 and EOPO SDP30 (28 %, w/w)/water system were studied. Fig. 4 shows the effect of K_2HPO_4 concentration to partition coefficient. Fig. 5 shows the effect of K_2HPO_4 concentration to volume ratios. Fig. 6 shows the effect of K_2HPO_4 concentration to percentage yield of puerarin in the process. The optimum condition was EOPO SDP30 (28 %, w/w), K_2HPO_4 (24 %, w/w), yield of puerarin could reach 42.26 %.

EOPO SDP30/K_2HPO_4 aqueous two-phase coupled with temperature-induced phase separation thermodynamic model: In EOPO SDP30/K_2HPO_4 aqueous two-phase

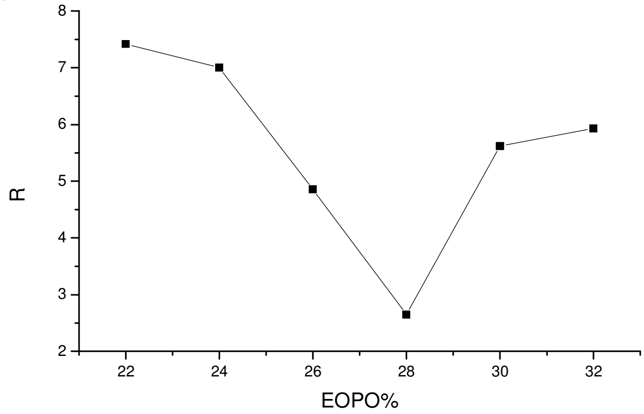

Fig. 2. Effect of EOPO SDP30 concentration to volume ratio

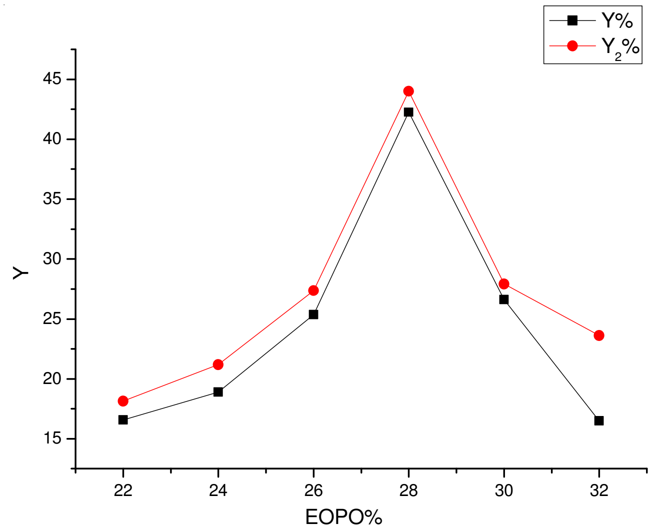

Fig. 3. Effect of EOPO SDP30 concentration to percentage yield of puerarin in the process

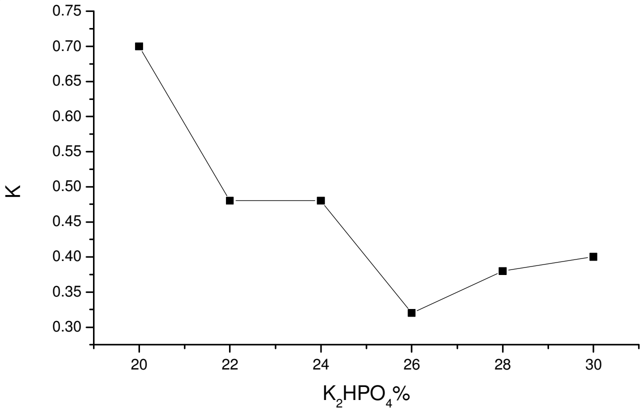

Fig. 4. Effect of K_2HPO_4 concentration to partition coefficient

coupled with temperature-induced phase separation, partition coefficient can be described by Diamond-Hsu equation (eqn. 1), but the experimental results don't fit the equation well. So we modify the equation, R (correlation coefficient) > 0.99 in the new equation (eqn. 2), accuracy is improved.

$$\ln K = A(w_1'' - w_1') + b(w_1'' - w_1')^2 \qquad (1)$$

A,b: Diamond-Hsu equation parameter, w_1': upper phase mass percentages, w_1": lower phase mass percentages

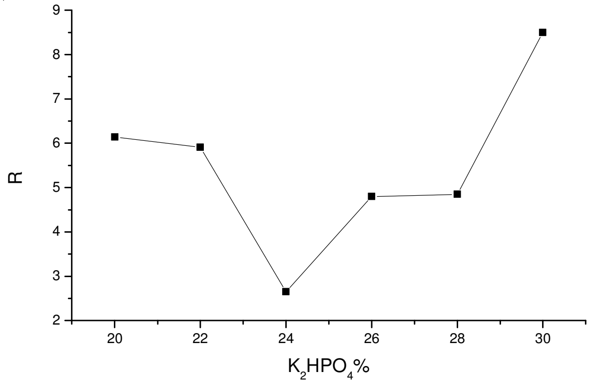

Fig. 5. Effect of K_2HPO_4 concentration to volume ratios

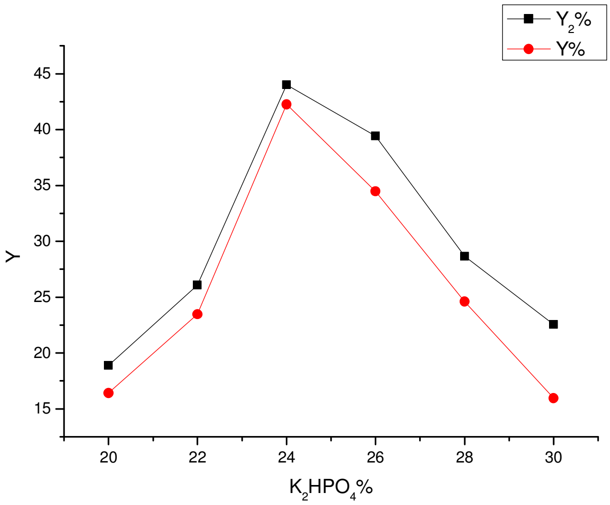

Fig. 6. Effect of K_2HPO_4 concentration to percentage yield of puerarin in the process

$$\ln\frac{K}{R^2} = A^* + b^*(w'' - w') + c^*(w'' - w')^2 \qquad (2)$$

A*, b*, c* modify the equation parameter,w", w' lower, upper phase mass percentages in temperature-induced phase separation

$$R = \frac{V_t}{V_b}$$

$$K = \frac{C_t}{C_b}$$

$$RK = \frac{V_t}{V_b} \cdot \frac{C_t}{C_b}$$

$$w'' = \frac{1}{RK+1}$$

$$w' = \frac{RK}{RK+1}$$

$$w'' - w' = \frac{1}{RK+1} - \frac{RK}{RK+1} = \frac{1-RK}{1+RK} \qquad (3)$$

R (correlation coefficient)

$$R = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}} \qquad (4)$$

SD (standard deviation)

$$SD = \sqrt{Var}$$

$$Var = \frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2 \quad (5)$$

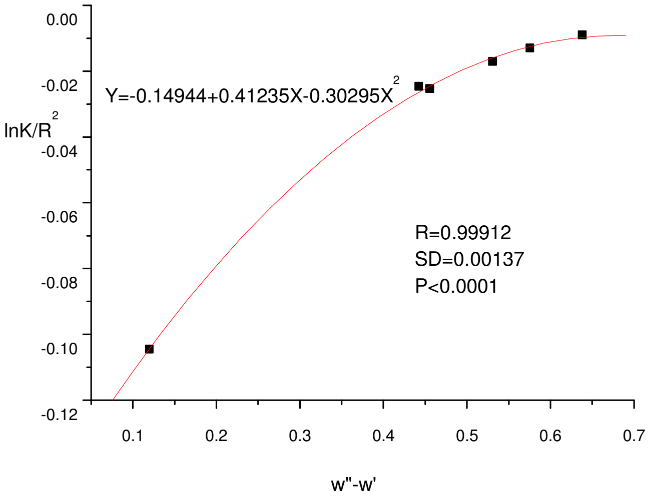

Fig. 7. Relationship between the experimental results and modified equation

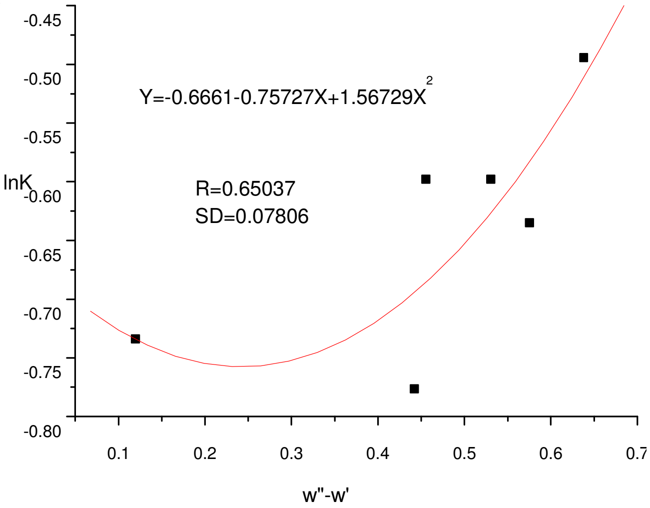

Fig. 8. Relationship between the experimental results and Diamond-Hsu equation

Conclusion

Puerarin could be separated in the solution of EOPO/K_2HPO_4/water systems. The advantage of temperature-induced phase separation is that slight chemical and copolymer EOPO can be recycled. The extraction is a relatively simple, rapid and inexpensive. The additional benefit of this procedure is that it can scaled up easily. The experimental results fit Diamond-Hsu equation, but the results can be fitted well in modified equation $\ln K/R^2 = A^* + b^*(w''-w') + c^*(w''-w')^2$.

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