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Measurement and Correlation of Partition Coefficients of Glycyrrhizic in Aqueous Two-phase EOPO/K_2HPO_4 Systems

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The partition behaviour of glycyrrhizic ammonium salt in ethylene oxide-propylene oxide random copolymer (EOPO)/K_2HPO_4/water systems was investigated. The optimum conditions were EOPO L64 28 %, K_2HPO_4 32 %, phase separation temperature was 63 ºC, yield of glycyrrhizic ammonium salt could reach 68.4 %. Based on the modified model $\ln K/R = A^* + b^*(w'' - w') + c^*(w'' - w')^2$, partition coefficients of glycyrrhizic in aqueous two-phase extraction system of 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, Glycyrrhizic ammonium salt, Ethylene oxide-propylene oxide random copolymer, Thermodynamic model.

INTRODUCTION

Licorice (glycyrrhiza uralensis fisch) is the most popular medicinal herb used in traditional Chinese medicine. The main active ingredient is glycyrrhizic acid (GA), which has been shown to have various therapeutic activities, *e.g.*, antiviral, antiinflammatory and anticancer and it can also be used as food additives-sweeter[1,2]. There is very low concentration of active ingredients in the medical plant. 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.

Aqueous two-phase systems (ATPS) widely used to separate and purify bimolecular, are usually composed of aqueous solution of two biologically inert and immiscible polymers, *e.g.*, polyethylene glycol (PEG) (upper phase) and dextrin or hydroxypropyl starch (lower phase)[3,4].

Aqueous two-phase partitioning coupled with temperature-induced phase separation is a quick, easy and inexpensive bench-top technique for extracting and purifying glycyrrhiza acid from raw material. 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[5].

EXPERIMENTAL

Glycyrrhizic acid monoammonium salt used for analysis standards were purchased from the Chinese Institute of Drugs and Biological Products Identification. Commercial glycyrrhizic acid monoammonium salt (purity 97 %) was purchased from Gansu Technology Industry Co. Ltd. (China), it was used in our initial solvent extraction experiments. ethylene oxide-propylene oxide random copolymer EOPO L64 (Mt2879), L62 (Mt2500), L61 (Mt2000) were purchased from Wanen 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 system were prepared by mixing stock solution of ethylene oxide-propylene oxide random copolymer (EOPO), K_2HPO_4 and water 25 g and the glycyrrhizic acid monoammonium salt 0.400 g to obtain a total. The primary phase systems were kept at room temperature (25 ºC). Phase systems were separated by centrifugation at 2000 rpm for 5 min. The GA-rich upper phase was removed and isolated in a separate container.

Temperature-induced phase separation: Container with upper GA-rich phase was placed in a water bath for 15 min 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

$$Y_1 = (G-C_1V_1)/G$$

Temperature-induced phase separation

$$R = V_t/V_b \qquad K = C_t/C_b \qquad Y_2 = 1/(1 + RK) \qquad Y = Y_1Y_2$$

C_1: 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 glycyrrhizic acid monoammonium salt, mg; R: volume ratios; 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 glycyrrhizic acid monoammonium salt in the upper phase at primary aqueous two-phase partition system; Y_2: percentage yield of glycyrrhizic acid monoammonium salt in the lower phase at temperature-induced phase separation; Y: percentage yield of glycyrrhizic acid monoammonium salt in the process.

Detection and analysis: UV analysis was applied to determine the content of glycyrrhizic acid. Glycyrrhizic acid concentration was calculated using the standard sample of glycyrrhizic acid monoammonium salt as the calibration standard. A good linear relationship was obtained over the range of 0.0015-0.0204 mg/mL and the regression was y = 15.900862x - 0.004816 ($r^2 > 0.999$), where y is the absorbance at 254 nm, x is the concentration of glycyrrhizic acid monoammonium salt and R is the regression coefficient.

RESULTS AND DISCUSSION

Glycyrrhizic acid extraction in the testing solution with different EOPO: In different ethylene oxide-propylene oxide random copolymer EOPO L64 (Mt2879), L62 (Mt2500), L61 (Mt2000) aqueous two-phase systems coupled with temperature-induced phase separation, the tests showed that EOPO L61/K_2HPO_4/water and EOPO L62/K_2HPO_4/water didn't separate two phases in temperature-induced phase separation. Because the cloud point of EOPO L61 or EOPO L62 was low, only 17 and 24.4 ºC, but the cloud point of EOPO L64 was 59 ºC, actually phase separation temperature was 63 ºC.

Effects of EOPO concentration: In this section, 12 different concentration combination of EOPO L64 and K_2HPO_4 (24 %, w/w)/water system were studied. In primary aqueous two-phase partition system separation, above 80 % of glycyrrhizic acid was partitioned into upper (EOPO) phase. Compare with glycyrrhizic acid monoammonium salt 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 L64 concentration to partition coefficient. The optimum condition was EOPO L64 (28 %, w/w), K_2HPO_4 (24 %, w/w), yield of glycyrrhizic ammonium salt could reach 60.5 %.

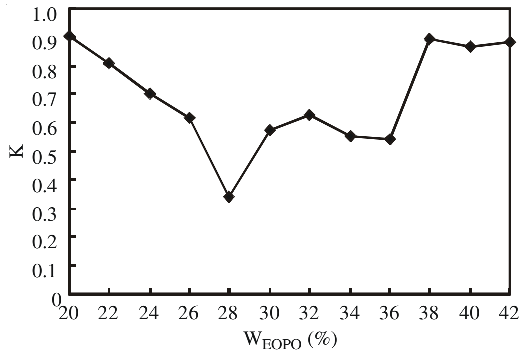

Fig. 1. Effect of EOPO L64 concentration to partition coefficient

Effects of salt concentration: In this section, 11 different wconcentration combi-nation of K_2HPO_4 and EOPO L64 (28 %, w/w)/water system were studied. Fig. 2 shows the effect of K_2HPO_4 concentration to partition coefficient. The optimum condition was EOPO L64 (28 %, w/w), K_2HPO_4 (32 %, w/w), yield of glycyrrhizic ammonium salt could reach 68.4 %.

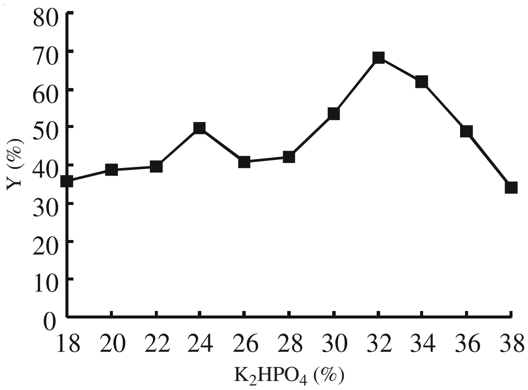

Fig. 2. Effect of K_2HPO_4 concentration to percentage yield of glycyrrhizic acid monoammonium salt in the process

EOPOL64/K_2HPO_4 aqueous two-phase coupled with temperature-induced phase separation thermodynamic model: In EOPOL64/K_2HPO_4 aqueous two-phase coupled with temperature-induced phase separation, partition coefficient can be described by Diamond-Hsu eqn. 1, Fig. 3), but the experimental results don't fit the equation well. So we modify the equation, R (correlation coefficient) > 0.99 in the new eqn. 2, Fig. 4, accuracy is improved.

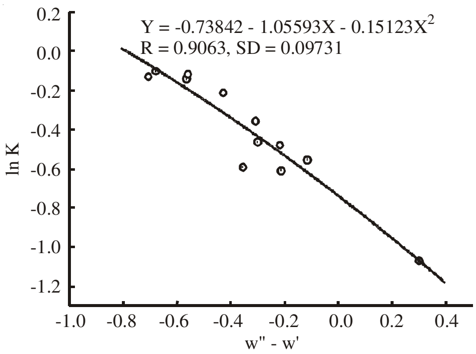

Fig. 3. Relationship between the experimental results and Diamond-Hsu equation

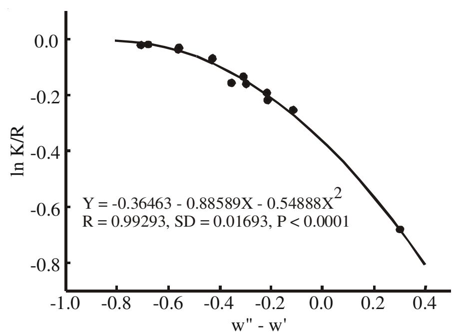

Fig. 4. Relationship between the experimental results and modified equation

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

A, b: Diamond-Hsu equation parameter, w'_1: upper phase mass percentages, w''_1: lower phase mass percentages

$$\ln K/R = A^* + b^*(w'' - w') + c^*(w'' - w')^2 \quad (2)$$

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

$$R = \frac{V_t}{V_b} \quad K = \frac{C_t}{C_b} \quad RK = \frac{V_t}{V_b} \cdot \frac{C_t}{C_b} \quad w'' = \frac{1}{RK+1} \quad w' = \frac{RK}{RK+1}$$

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

R(correlation coefficient)

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

SD(standard deviation)

$$SD=\sqrt{Var}$$

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

Conclusion

Glycyrrhizic acid 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. An added 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.

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