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Separation of Glycyrrhizic Acid Using Smart Polymer Based Aqueous Two-Phase Systems

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Abstract. In this paper, glycyrrhizic acid was separated using ethylene oxide / propylene oxide copolymer ($EO_{60}PO_{40}$) and K_2HPO_4 aqueous two-phase systems (ATPS). The influence of the content of $EO_{60}PO_{40}$ and K_2HPO_4 and temperature was studied. The results showed that the optimal separation conditions were: 30% $EO_{60}PO_{40}$, 24% K_2HPO_4, 53°C of extraction temperature, 1.5 h of extraction time. This separation process provides a simple and convenient method for extracting glycyrrhizic acid.

Introduction

Glycyrrhiza uralensis Fisch. is a kind of Chinese herbal medicine with great application value (Zeng et al. 1988). Glycyrrhizic acid (GA) is an important component in *G. uralensis* for its biological activities, such as immune-stimulating activities, anti-inflammatory and antitumor (Cherng et al. 2004; Hoever et al. 2005; Sasaki et al. 2002; Shibata 2000; Van Rossum et al. 1999).

It was reported that GA was separated by using an ion-exchange chromatographic method (Baltina et al. 1993), and 82.3% of GA was purified. Another reported method was the ultrafiltration (Pan et al. 2000) and 52.3% of GA was separated.

The aim of this present study was to develop a convenient method of separating GA from licorice extract. Smart aqueous two-phase extraction (ATPE) was used in this study to separate GA. $EO_{60}PO_{40}$ is a kind of thermosensitive polymer. When adjusting the temperature change, the phase separation of the system of $EO_{60}PO_{40}$ / K_2HPO_4 could be observed.

Experimental Methods

Materials

Licorice root was purchased from Chinese herbal medicine market. $EO_{60}PO_{40}$ was obtained from Shanghai and Sichuan trade co., LTD. Other chemical reagents were obtained from Beijing Dingguo Changsheng Biotechnology CO., LTD.

Procedures

Standard Curve Drawing

The ammonium salt of ammonium glycyrrhizinate was precisely weighted 0.0525 g, dissolved with 50% ethanol and transferred to a 50 mL volumetric flask, and then the beaker was moistened with 50% ethanol, washed for 3 times, transferred the fluid-transfer fluid into a 50 mL volumetric flask, and adding 50% ethanol to the scale line and shaking it well (short for A solution). 0 mL, 1 mL, 2 mL, 3 mL, 4 mL and 5 mL of solution A were absorbed by the pipette and added to the 50 mL volumetric flask respectively. 50% ethanol was diluted to the scale line and shaken evenly. The absorbance of a series of gradient solutions was determined by ultraviolet-visible spectrophotometer at 253 nm wavelength and 50 % ethanol as reference solution.

Preparation of Concentrated Liquorice Solution

50 g of liquorice was added to 100ml distilled water and refluxed for 1.5 h. Then the extracting solution was centrifuged at a speed of 3000 r/min for 10 min for the supernatant.

Aqueous Two-Phase Extraction System

The total mass of $EO_{60}PO_{40}$/ K_2HPO_4 system was 10 g, in which glycyrrhiza concentrate was 4 g. This system was mixed well and centrifuged for the upper and lower phases. Then the upper phase was subjected to temperature induction, the system was automatically divided into two phases, whose upper phase was $EO_{60}PO_{40}$ phase and lower phase was water phase. This water phase contained glycyrrhizic acid which could be detected at 253 nm wavelength.

Effect of EO60PO40 Mass Fraction on Extraction Results

The specific operation method was shown in Table 1 below. 4 g glycyrrhiza concentrate was added into this system.

Table 1 Aqueous two-phase extraction system different mass fraction of EO60PO40

Numbering	1	2	3	4	5
K_2HPO_4/g	2.4	2.4	2.4	2.4	2.4
$EO_{60}PO_{40}$/g	2.0	2.5	3.0	3.5	4.0
H_2O/g	5.6	5.1	4.6	4.1	3.6
$EO_{60}PO_{40}$/%	20%	25%	30%	35%	40%

Effect of K2HPO4 Mass Fraction on Extraction Results

The specific operation method was shown in Table 2 below. 4 g glycyrrhiza concentrate was added into this system.

Table 2 Aqueous two-phase extraction system different mass fraction of K_2HPO_4

Numbering	1	2	3	4	5
$EO_{60}PO_{40}$/g	3.0	3.0	3.0	3.0	3.0
K_2HPO_4/g	1.8	2.0	2.2	2.4	2.6
H_2O/g	5.2	5.0	4.8	4.6	4.4
K_2HPO_4/%	18%	20%	22%	24%	26%

Effect of Temperature on Extraction Results

For this study, the aqueous two-phase extraction system was composed as followed: 30 % $EO_{60}PO_{40}$ (3.0 g), 24 % K_2HPO_4 (2.4 g), 46% H_2O (4.6 g). Then 4 g glycyrrhiza concentrate was added into this system. The temperature gradient was shown in Table 3.

Table 3 Effect of temperature gradient on extraction results

Numbering	1	2	3	4	5
Temperature /°C	47	50	53	56	59

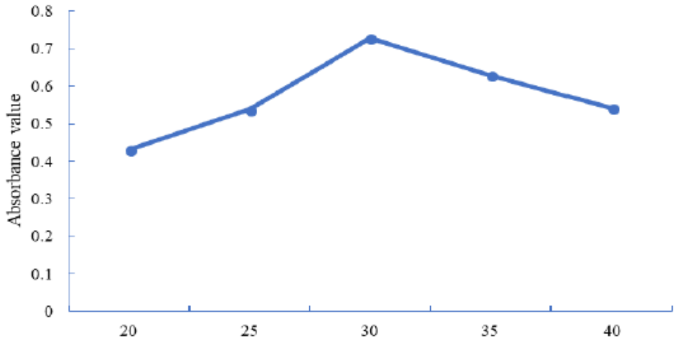
Effect of Time on Extraction Results

The extraction system was accordance with *Effect of temperature on extraction results*. The extraction temperature was 53 °C. The extraction time was 0.5h, 1h, 1.5h and 2h.

Results and Analysis

Effect of $EO_{60}PO_{40}$ Mass Fraction on Extraction Results

When the mass fraction of K_2HPO_4 was 24 %, the effect on the extraction rate of glycyrrhizic acid was investigated under the change of the $EO_{60}PO_{40}$ mass fraction. The results were shown below (Fig.1).



Mass fraction of $EO_{60}PO_{40}$ (%)

Fig. 1 Absorbance value of glycyrrhizin solution extracted by different mass fraction of $EO_{60}PO_{40}$

As shown in Fig. 1, when the mass fraction of $EO_{60}PO_{40}$ was between 20% and 30%, the absorbance value of the solution measured gradually increased. And when the mass fraction of $EO_{60}PO_{40}$ increased to 30%, the absorbance value of solution decreased gradually. Therefore, the $EO_{60}PO_{40}$ mass fraction of glycyrrhizic acid in liquorice was 30%.

Effect of K_2HPO_4 mass Fraction on Extraction Results

When $EO_{60}PO_{40}$ mass fraction was 30%, the effect of K_2HPO_4 mass fraction on the extraction rate of glycyrrhizic acid was investigated. The results were shown in Fig. 2.

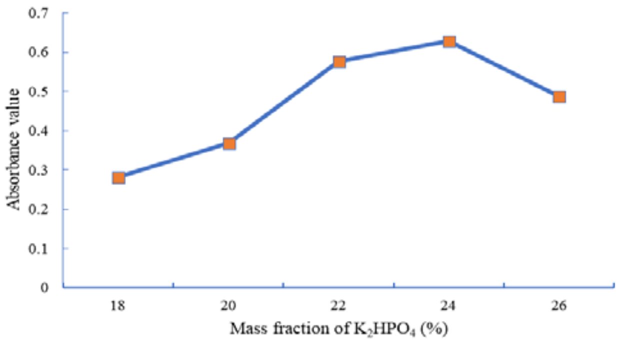


Fig. 2 Absorbance value of glycyrrhizin solution extracted by different mass fraction of K_2HPO_4

As shown in Fig. 2, when the mass fraction of K_2HPO_4 was between 18% and 24%, the absorbance value of the solution measured gradually increased. And when the mass fraction of K_2HPO_4 increased to 24%, the absorbance value of solution decreased gradually. Therefore, the K_2HPO_4 mass fraction of glycyrrhizic acid in liquorice was 24%.

Effect of Temperature on Extraction Results

When $EO_{60}PO_{40}$ mass fraction was 30% and K_2HPO_4 mass fraction was 24%, the effects of temperature changes on the extraction rate of glycyrrhizin were investigated.

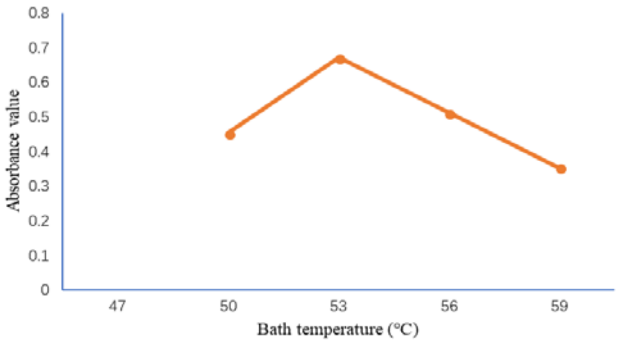


Fig. 3 The absorbance of glycyrrhizin solution at different water bath temperature

As shown in Fig. 3, when the temperature was 47 °C, the solution is mixed. When the temperature was less than 53 °C, with the increase of temperature, the absorbance of glycyrrhizic acid solutions was gradually increased. When the temperature was more than 53 °C, the absorbance glycyrrhizic acid solutions was decreased. So, the best extraction temperature was 53 °C.

Effect of Extraction Time on Extraction Results

In the conditions of $EO_{60}PO_{40}$ mass fraction 30 %, K_2HPO_4 mass fraction 24 %, extraction temperature 53 °C, the effect of extraction time on extraction efficiency was investigated.

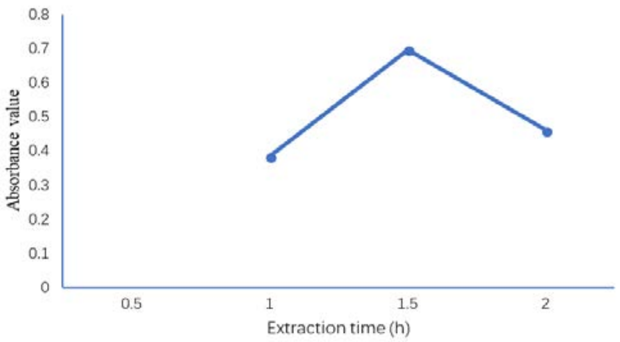


Fig. 4 The absorbance of extraction solution under different bath time

As shown in Fig. 4, when the extraction time was 1.5 h, the absorbance value of glycyrrhizin solution was the highest.

Conclusion

The conclusion was drawn by studying the smart aqueous two-phase extraction system of glycyrrhizin. The best extraction conditions were: 30 % $EO_{60}PO_{40}$, 24 % K_2HPO_4, 53 °C of extraction temperature, 1.5 h of extraction time.

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