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Novel Gas-assisted Three-liquid-phase Extraction System for Simultaneous Separation and Concentration of Anthraquinones in Herbal Extract☆

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ABSTRACT

Gas-assisted three-liquid-phase extraction (GATE), which has the advantages of both three-liquid-phase extraction and solvent sublation, is a novel separation technique for separation and concentration of two organic compounds into different phases in one step. This highly effective and economically applicable method has been developed for separating emodin and rhein from herbal extract. In a GATE system composed of butyl acetate/PEG4000/ammonium sulfate aqueous solution, influence of various parameters including gas flow rate, flotation time, salt concentration, initial volume of PEG and butyl acetate was investigated. Within 50 min of 30 $ml \cdot min^{-1}$ nitrogen flow, removal ratio of emodin and rhein from aqueous phase could be over 99% and 97%, respectively. Mass fraction of emodin in the BA phase and rhein in the PEG phase could reach 97% and 95%, respectively. It is demonstrated that gas bubbling is effective for partitioning of emodin and rhein into butyl acetate and PEG phase respectively, and dispersed PEG and butyl acetate could be captured from the aqueous solution. Experimental results show that GATE could be an effective and economical technology for concentration and separation of co-existed products in medicinal plants.

1. Introduction

Anthraquinones are the major biologically active ingredients of some most popular traditional medicinal herbs, *e.g.* Rhubarb and Polygonum cuspidate. Previous pharmacology studies have demonstrated that anthraquinones have various bioactivities, such as antitumor, antifungal, antiplasmodial, antiviral and virucidal activity [1–5]. Recently, various technologies have been reported for the separation of anthraquinones from medicinal plant, such as ultrasonic extraction, supercritical CO_2 extraction, high-speed countercurrent chromatography, and aqueous two-phase extraction [6–11]. Although some exciting results have been achieved with these methods, some inevitable shortcomings limit their further applications, including sophisticated equipment, harsh condition, high energy consumption and cumbersome procedure. Besides, few studies have been reported concerning the separation of co-existing anthraquinones from each other.

Three-liquid-phase extraction system (TES), composed of three coexisting liquid phases, is a promising alternative to the traditional solvent extraction because of its outstanding advantages [12,13]. The physicochemical properties of three co-existing phases in TES are tunable by manipulating various factors and it is easy to scale-up, so that TES provides potential to separate two or more target compounds selectively through one-step process [14]. TES has been successfully applied to concentration of natural products [15,16], antibiotic separation [17,18], phenolic wastewater treatment [19–22] and multimetal separation [23–30]. However, in a TES process, the intensive agitation leads to loss of organic solvent and polymer and creates secondary contamination for aqueous solutions, which may reduce the separation efficiency of objective chemical compounds. In our previous report, TES was improved by combination with solvent sublation, and the new technique was named gas-assisted three-liquid-phase extraction (GATE) [31]. With the assistance of ascending gas stream, GATE could reduce the consumption of organic solvents and polymers and give higher concentration coefficient comparing with TES. Therefore, it is more efficient, environment friendly and economically applicable in the extraction of natural product.

In this work, GATE is applied to isolate two coexisting anthraquinones in the simulated herbal extract. Because of their good surface activity, highly similar structures and properties (Fig. 1), emodin and rhein are selected as the target compounds for separation and concentration. In a GATE system composed of butyl acetate/PEG4000/ammonium sulfate aqueous solution, influence of various parameters including gas flow rate, flotation time, salt concentration, initial volume of PEG and butyl acetate is investigated. In the GATE process, organic phase,

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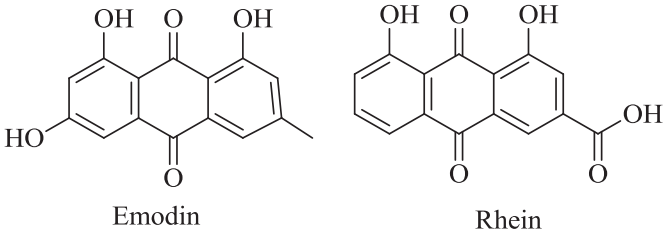

Fig. 1. Chemical structures of emodin and rhein.

polymer phase and salt aqueous solution phase coexist in the column equipment as shown in Fig. 2. As the ascending of gas bubbles, emodin and rhein dissolved in the aqueous phase can be extracted and concentrated into the organic phase and the polymer phase, respectively.

2. Materials and Methods

2.1. Chemicals and apparatus

Polyethylene glycol (PEG) 4000 was purchased from China National Pharmaceutical Group Corporation. Emodin and rhein crude powder were purchased from Shaanxi Sciphar Hi-tech Industry Co. Ltd., China, and the standards of emodin and rhein (purity > 97% by HPLC) were purchased from National Institute for Food and Drug Control, China. Water was of HPLC grade. Butyl acetate (BA), ammonium sulfate and other chemicals were of analytical reagent grade purchased from Beijing Chemical Reagent Co., Ltd., China.

A pH meter (pH211, HANNA, Italy) was used to adjust the pH of the solution. For transfer convenience, stock solutions of ammonium sulfate with mass concentration of 40% and polymers with mass concentration of 50% were prepared. Simulated herbal stock solutions were prepared with purchased crude powder of emodin and rhein. Concentrations of emodin and rhein were approximately 30 ppm and were determined by HPLC (HP1100 Agilent Technologies, U.S.) with ultraviolet spectrometer at 254 nm as detector. The analysis was performed with a C18 Zorbax ODS column (4.6 mm I.D. × 150 mm, 5 μm, Agilent, U.S.) at 20 °C in thermostat. The mobile phase consisted of methanol and 1.0% aqueous acetic acid solution and a 20 μl sample was ejected into the column with a gradient elution of 75%–80% methanol at 0–15 min at a flow rate of 1.0 ml·min^{-1}. Both calibration curves showed satisfactory linearity over the concentration range of (1–100) × 10^{-6} with correlation coefficients ≥0.9999.

Fig. 2 is the schematic diagram of the experimental device. The glass column is equipped with a G4 sintered glass sparger (pore size 3–4 μm). The inner diameter of the column is 36 mm and the length is 450 mm. Three ports are open at different parts along the column to obtain samples of the three phases.

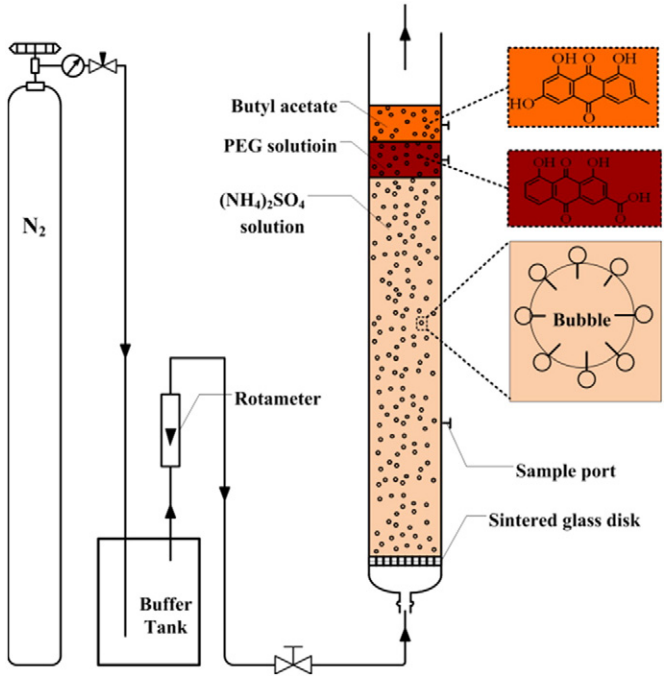

Fig. 2. Schematic diagram of gas-assisted three-liquid-phase extraction system.

2.2. Optimization of different parameters

Based on our preliminary study, the optimum pH of the separation of emodin and rhein is 8.0. PEG 4000 is the most favorable polymer and BA is the best organic extractant considering the toxicity, volatility and cross-solubility. Therefore, PEG 4000 with 50% (mass) water (for transfer convenience) and BA was used to construct the polymer phase and organic solvent phase in this study. The initial pH of all the systems was set at 8.0. Concentrations of emodin and rhein were determined by HPLC. Various parameters of GATE such as gas flow rate, gas bubbling time, concentration of ammonium sulfate, initial volume of PEG phase and BA phase were optimized. All the initial volumes of aqueous phase were 320 ml, and all the separation processes were carried out at room temperature.

2.3. Separation procedure

The aqueous phase of the GATE was prepared by mixing of 40% (mass) ammonium sulfate solution, simulated herbal extract and water in a 500 ml beaker. Under magnetic stirring, solution pH was adjusted to 8.00 ± 0.05 by adding sulfuric acid and sodium hydroxide solution.

In order to quantify the separation and concentration efficiency, the removal ratio of target compound from aqueous phase (E_i) and mass fraction of compound i in the three different phases ($W_{i,p}$) are defined as

$$E_i = \left(1 - \frac{C_i}{C_{i,0}}\right) \times 100\% \quad (1)$$

$$W_{i,p} = \frac{C_{i,p} \times V_p}{C_{i,0} \times V_0} \times 100\%. \quad (2)$$

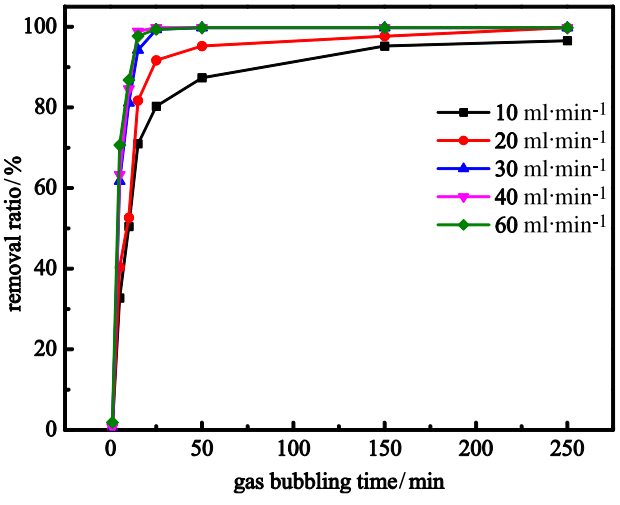

Fig. 3. Effect of gas bubbling time on the removal ratio of emodin from aqueous phase (pH = 8.0; salt concentration = 20%; V_{PEG} = 40 ml; V_{BA} = 40 ml).