

# Ionic liquid-based aqueous two-phase system, a sample pretreatment procedure prior to high-performance liquid chromatography of opium alkaloids

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## Abstract

An ionic liquid, 1-butyl-3-methylimidazolium chloride ([C<sub>4</sub>mim]Cl)/salt aqueous two-phase systems (ATPS) was presented as a simple, rapid and effective sample pretreatment technique coupled with high-performance liquid chromatography (HPLC) for analysis of the major opium alkaloids in *Pericarpium papaveris*. To find optimal conditions, the partition behaviors of codeine and papaverine in ionic liquid/salt aqueous two-phase systems were investigated. Various factors were considered systematically, and the results indicated that both the pH value and the salting-out ability of salt had great influence on phase separation. The recoveries of codeine and papaverine were 90.0–100.2% and 99.3–102.0%, respectively, from aqueous samples of *P. papaveris* by the proposed method.

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**Keywords:** Aqueous two-phase systems; Ionic liquid; Extraction; Opium alkaloids

## 1. Introduction

Opium is partially dried latex obtained from opium poppy, cultivated mainly in Asia, South America and parts of Europe. The analysis of opium is important for legal and intelligence purpose since it contains the major alkaloids, such as papaverine, codeine, thebaine, morphine and narcotine. Several analytical methods, such as gas chromatography (GC) [1], high-performance liquid chromatography (HPLC) [2] and capillary electrophoresis (CE) [3], have been reported for the determination of these opium alkaloids. Quantitative analysis of trace level of opium alkaloids is still a significant challenge demanding a rapid and effective sample preparation procedure prior to analysis. Currently, two principal methods have been used for sample pretreatment: liquid–liquid extraction (LLE) [4] and solid-phase extraction (SPE) [5–7]. However, LLE usually requires some poisonous volatile organic solvents. SPE is a method with good purification and concentration effects, but it requires solvent des-

orption step with traditional volatile organic solvents and the pretreatment processes are relatively time-consuming. Sometimes sample recovery is not always satisfactory. Therefore, the development of simple and environmental friendly pretreatment methods is of great interest.

Typical aqueous two-phase systems (ATPS) are generated by mixing aqueous solutions of two structurally different polymers or by mixing one polymer with certain salts at high concentration [8,9]. ATPS is a simple and environmentally friendly separation system by minimizing consumption of organic solvents harmful to the environment. The partition behavior of analytes can be controlled and optimized with a judicious choice of phase system, pH for separation, and composition of salts. With the use of ATPS, one can simultaneously carry out purification, extraction and enrichment. Another advantage of ATPS is that these systems are suitable for biological samples because each phase contains 70–90% water, which means that biomolecules will not be denatured. ATPS has been used for the separation of biomolecules, such as cells, organelles, membrane fractions and proteins [9–14]. Rogers et al. [15] reported that ATPS are suitable for the separation of small organic molecules in industrial and environmental application. Pan et al. [16] extracted and

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chromatographically separated geniposide from gardenia fruit and proved that ATPS is useful for industrial preparation of geniposide. Agasøster [14] used ATPS to extract hydrophilic drugs from blood. However, most of phase-formation polymers in conventional ATPS form an opaque solution with high viscosity during phase separation, which might make following determination difficult.

Recently, room temperature ionic liquids (RTILs) have received extensive attention for their potential use as green solvents and possible replacement for traditional volatile organic solvents in many applications [17–19]. Rogers and co-workers [20] reported that some hydrophilic ionic liquids (IL) form aqueous two-phase systems when contacting with concentrated solutions of water-structuring salts. In the present study, this new ATPS, formed by 1-butyl-3-methylimidazolium chloride ( $[C_4mim]Cl$ ) with adding salts, was successfully applied to extract opium alkaloids in *Pericarpium papaveris*. Compared with conventional ATPS, both phases in the employed  $[C_4mim]Cl/K_2HPO_4$  system are clear and fluid aqueous solutions, making this IL/salts ATPS easily coupled with HPLC. Thus, an ionic liquid-based aqueous two-phase system has been developed as a new pretreatment strategy for the analysis of opium alkaloids in *P. papaveris*.

## 2. Experimental

### 2.1. Apparatus and reagents

Standards of codeine and papaverine were obtained from National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. Standard stock solutions (100  $\mu g/ml$ ) of these compounds were prepared in water. IL (1-butyl-3-methylimidazolium chloride,  $[C_4mim]Cl$ , 99.5%) was purchased from Fluka, Switzerland.  $[C_4mim]Cl$  (20%, w/w) was prepared in water for the stock solution. *P. papaveris* were obtained from Beijing Tongrentang Drugstore, Beijing, China. HPLC-grade methanol was purchased from Tianjin Shield Company, Tianjin, China. All the other chemicals were analytical grade reagents (Beijing Chemicals, Beijing, China), and all the solutions were prepared from deionized water.

### 2.2. Preparation of phase diagrams

Phase diagrams were determined by the cloud-point method [10]. Briefly, 1.0 g  $[C_4mim]Cl$  was put into a 25 ml centrifugal tube. A salt solution of known concentration was then added dropwise to the test tube until turbidity and a two-phase system was formed. The composition of this mixture was noted. Then, water was added dropwise to the tube to get a clear one-phase system and more salt solution was added again to afford a two-phase system. The composition of this mixture was noted and so on.

### 2.3. Preparation of aqueous two-phase systems

1.0 or 2.0 g of  $[C_4mim]Cl$  solution (20%, w/w), salt and the standard solution of codeine and papaverine were added into a

5 ml centrifugal tube. And actually, both the spiked and the real aqueous samples of *P. papaveris* followed the same procedures as that of the standard. The mixture was diluted to the mark with water and mixed thoroughly, and two clear phases were formed after about two minutes. Twenty microlitres of the top phase was aspirated by a 50  $\mu l$  microsyringe and directly injected into the HPLC system for analysis.

### 2.4. Chromatographic conditions

The HPLC equipment included an Agilent 1100 Series IsoPump, an Agilent 1100 Series UV detector. A WinChrom-GPC18 (250 mm  $\times$  4.6 mm, 5  $\mu m$ , Serial No. GP040523) reversed-phase column was employed for chromatographic separation, with the mobile phase of 0.5% ammonium acetate: 1% triethylamine:methanol (v/v, 49:1:50) at the flow-rate of 0.8 ml/min [21]. The injection volume was 20  $\mu l$  and the column effluent was monitored at the wavelength of 240 nm.

## 3. Results and discussion

### 3.1. Phase diagrams

Phase diagrams were used to characterize the phase systems, and herein different salts were tested for the formation of ATPS with  $[C_4mim]Cl$  as shown in Fig. 1. Results show that ATPS can be formed by adding appropriate amount of alkali or alkaline salts, such as KOH, NaOH,  $K_3PO_4$ ,  $K_2HPO_4$ ,  $K_2CO_3$  or  $Na_2HPO_4$  to aqueous solution of  $[C_4mim]Cl$ , while adding acidic or neutral salts, such as  $KH_2PO_4$ ,  $(NH_4)_2SO_4$ , NaCl or KCl can not drive  $[C_4mim]Cl$  solution to separate into two phases. The tendency of salts to form aqueous two-phase systems in the mixtures with  $[C_4mim]Cl$  is related to the pH values of the systems induced by adding salt, which implied that the pH changes in the  $[C_4mim]Cl$ -salt two-phase system affected the polar interaction in the phases. Among the salts that can cause phase separation, four types were chosen to determine the phase diagrams of IL-salt-water systems (Fig. 2). As shown in Fig. 2, the abilities of the salts studies for phase separation followed the order of  $K_2CO_3 \approx K_3PO_4 \approx K_2HPO_4 > KOH$ , accordant with that of salting-out ability of anion. Here,  $K_2HPO_4$  was chosen in the following studies. One reason was that  $K_2HPO_4$  led to effective phases isolation between  $[C_4mim]Cl$  and salt enriched solutions, and the other was that  $K_2HPO_4$ , compared with other phase-forming salts, resulted in an appropriate pH to determine most of the opium alkaloids.

### 3.2. Effect of salt and IL concentration on partitioning

The distribution behaviors of drugs between two phases were characterized by the extraction efficiency ( $E$ ) and phase ratio ( $R$ ).

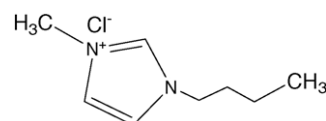


Fig. 1. Structure of  $[C_4mim]Cl$ .

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