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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_4mim]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 desorption 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 viscidity 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 μg/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 μl 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 × 4.6 mm, 5 μm. 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 μl 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).

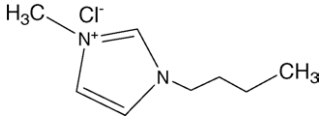

Fig. 1. Structure of [C_4mim]Cl.

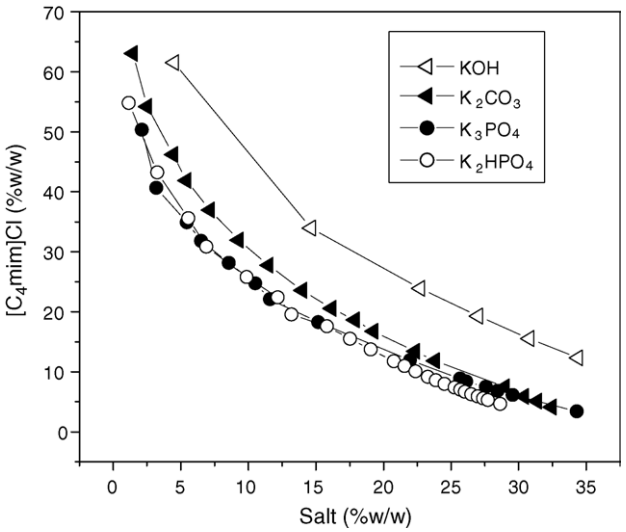

Fig. 2. Phase diagrams for the [C4mim]Cl/salt/water systems at 22 °C. In the region above the binodial curve, the system is separated into two phases, below the binodial curve, the system is a homogeneous phase.

The parameters E and R were defined as follows:

$$E = \frac{C_t V_t}{C_b V_b + C_t V_t}$$

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

C_b, C_t, V_b and V_t are the drug concentration and volumes in bottom and top phase, respectively. The extraction efficiency E is the ratio of drugs in top [C4mim]Cl enriched phase and total drugs adding to the system. Fig. 3 shows the effects of [C4mim]Cl and K_2HPO_4 concentration on the partition behavior of codeine and papaverine. Ten micrograms codeine and 5 μg papaverine were added and the following experiments for optimization were carried out at the same condition. In systems containing 0.2 g [C4mim]Cl, an ATPS was formed when K_2HPO_4 amount was over 3.4 g. The extraction efficiencies of codeine and papaverine were almost invariable when K_2HPO_4 amount increase from 3.6 to 4.4 g. The average extraction efficiencies of codeine and papaverine in this range were 81.7 and 84.9, respectively. Salt deposited when K_2HPO_4 amount is over 4.8 g. In systems containing 0.4 g [C4mim]Cl, when K_2HPO_4 ranged from 2.8 to 3.6 g, the average extraction efficiencies of codeine and papaverine were 83.0% and 90.0%, respectively.

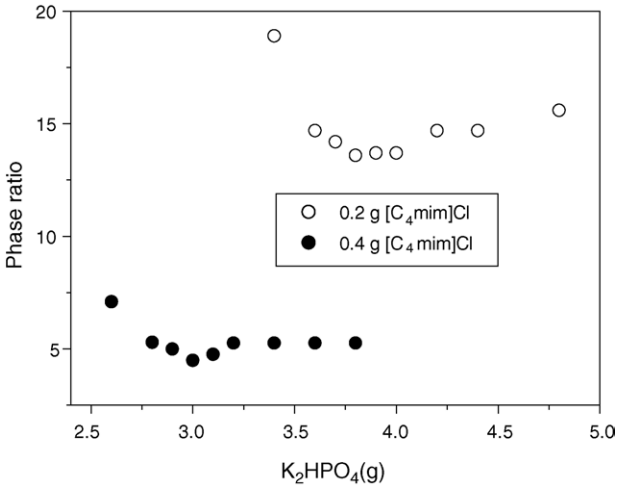

Fig. 4. Effects of [C4mim]Cl and K_2HPO_4 concentration on phase ratios.

K_2HPO_4 concentration has important influence on phase ratio. As can be seen in Fig. 4, the phase ratio decreased first and then increased with the increasing salt concentration, which showed that the salting-out ability of K_2HPO_4 is closely correlated with the concentration of K_2HPO_4. Fig. 5 showed that with the increasing concentration of K_2HPO_4, more [C4mim]Cl was driven into the upper phase where the enriched [C4mim]Cl existed, resulting in the decreasing phase ratio and the increasing maximum absorbance value from about 0.86–1.20 in the upper phase. After the minimum R, increasing K_2HPO_4 resulted in phase ratio increase due to more water assembled to K_2HPO_4 enriched bottom phase. Considering the extraction efficiencies of codeine, papaverine and enrichment factors of the system and low consumption of [C4mim]Cl, the optimum condition for

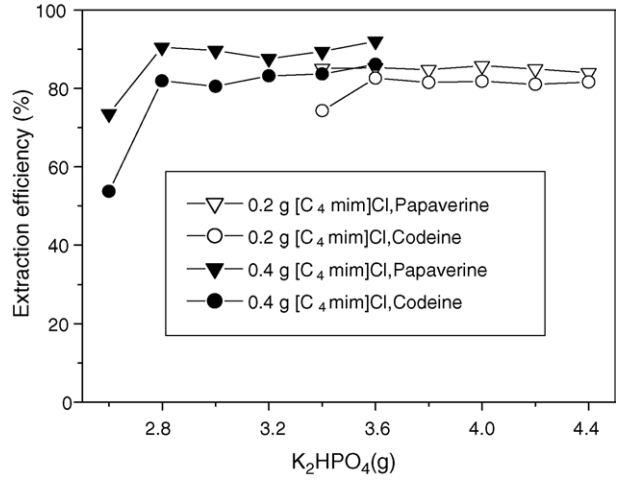

Fig. 3. Effects of [C4mim]Cl and K_2HPO_4 concentration on extraction efficiencies of codeine and papaverine.

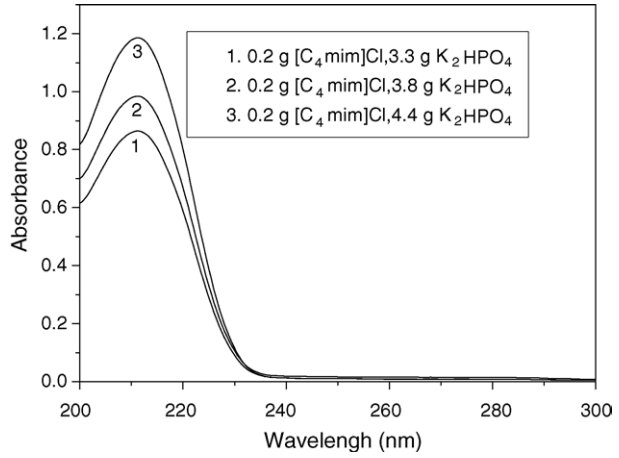

Fig. 5. Absorbance spectra of [C4mim]Cl in top phase for various concentrations of K_2HPO_4 (water as blank).

Table 1
Extraction efficiency, linearity, limits of detection and reproducibility in the analysis of codeine and papaverine by ATPS and HPLC

Analyte	Average extraction efficiency ($\bar{E}$ (%), $n = 6$)	Linearity (μg/ml)	Detection limit (μg/ml)	Reproducibility (R.S.D. (%), $n = 6$)
Codeine	81.1	0.1–30.0	0.03	2.1
Papaverine	84.9	0.2–20.0	0.02	3.8

Table 2
Analytical results (mean ± S.D., $n = 3$) for codeine and papaverine in water samples of *Pericarpium papaveris*

Added (μg/ml)		Codeine		Papaverine	
Codeine	Papaverine	Determined (μg/ml)	Recovery (%)	Determined (μg/ml)	Recovery (%)
0.00	0.00	5.96 ± 0.14		1.20 ± 0.05	
0.50	0.25	6.47 ± 0.08	100.2 ± 1.2	1.44 ± 0.02	99.3 ± 1.3
1.00	0.50	6.86 ± 0.10	90.0 ± 1.5	1.69 ± 0.05	99.4 ± 2.9
5.00	2.50	10.9 ± 0.22	99.3 ± 2.0	3.76 ± 0.03	102.0 ± 0.8

codeine and papaverine extraction was 3.8 g K_2HPO_4 and 0.2 g [C_4mim]Cl in 5 ml solution.

3.3. Effect of temperature on partitioning

Since temperature affects the phase characters of traditional poly(ethylene glycol) (PEG)/salt ATPS and stringent control of temperature is recommended in all partitioning experiments, the effect of temperature on distribution behavior of codeine and papaverine in [C_4mim]Cl/K_2HPO_4 systems was also investigated in this study. With the temperature increasing from 15 to 55 °C, the extraction efficiencies of codeine and papaverine were almost invariable while the phase ratio increased. In this system, higher temperatures result in more [C_4mim]Cl redissolved into K_2HPO_4 enriched bottom phase. Accordingly, [C_4mim]Cl concentration in top phase decreased. But the decrease of [C_4mim]Cl in top phase does not affect the extraction efficiencies of drugs. This new extraction system can afford a wide temperature range for extraction of codeine and papaverine.

3.4. Method validation

The parameters, such as linearity, reproducibility, limit of detection and extraction efficiencies of two drugs were determined from a spiked solution of analytes under the above-optimized conditions. The results were listed in Table 1. A series of drugs were added to systems containing 0.2 g [C_4mim]Cl and 3.8 g K_2HPO_4. After phase separation, the drug concentration in the [C_4mim]Cl phase was measured by HPLC method as described in Section 2.4. Linear ranges of codeine and papaverine were 0.1–30.0 and 0.2–20.0, respectively. The reproducibility was studied from six replicated experiments for spiked solution of 2.0 μg/ml codeine or 1.0 μg/ml papaverine. Sensitivity was evaluated by the limit of detection (LOD, the real sample concentration with a signal-to-noise ratio of at 3) (see Table 1). The average extraction efficiencies for codeine and papaverine were 81.1% and 84.9% with the relative standard deviations (R.S.D.) of 2.1% and 3.8%, correspondingly.

To verify the accuracy of the method, the developed method was applied to the determination of the recoveries of codeine and papaverine in aqueous samples of *P. papaveris*. A 1.0 g power of *P. papaveris* was mixed with 50 ml water. After boiling for 4 h, the mixture was filtrated with a 0.45 μm filter and used directly for further experiment. Two millilitres of filtrate was added to optimum aqueous two-phase system. Twenty microlitres of the top phase was directly injected for proposed HPLC method to determine codeine and papaverine. The recoveries were determined from spiked 0.25–5.00 μg/ml codeine and papaverine. The recoveries of codeine and papaverine are in the range of 90.0–100.2% and 99.3–102.0%, respectively (see Table 2).

To further verify the accuracy, the developed method was applied to analyze codeine and papaverine in *P. papaveris*. The results were compared with those achieved from LLE extraction experiments (Table 3) following the published LLE extraction method [22]. The experimental results were really satisfactory to apply this method to fulfill the determination tasks. Fig. 6 showed the chromatograms of *P. papaveris* sample after ATPS extraction and methanol LLE extraction, respectively. This experiment further demonstrated the accuracy of our method.

Table 3
Determination data (mean ± S.D., $n = 3$) of codeine and papaverine in *Pericarpium papaveris* sample

Sample	Found by present method		Found by LLE-HPLC	
	Codeine (μg/g)	Papaverine (μg/g)	Codeine (μg/g)	Papaverine (μg/g)
Pericarpium papaveris	625 ± 15	60 ± 2.5	623 ± 32	65 ± 2.0

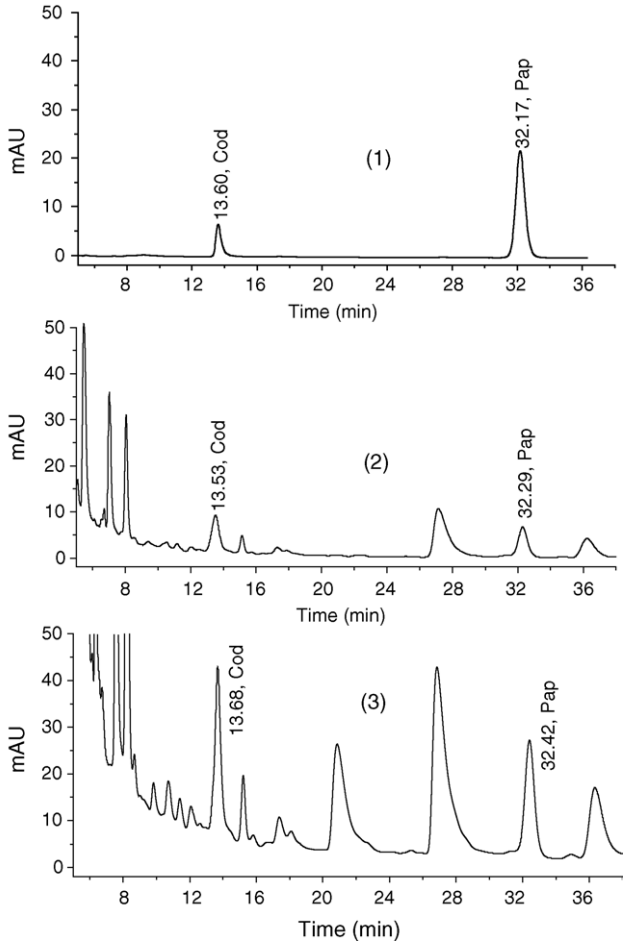

Fig. 6. Chromatogram of (1) standard solution of 10 μg/ml codeine (Cod) and 4 μg/ml papaverine (Pap); (2) methanol LLE of *Pericarpium papaveris* sample; (3) ATPS extraction of *Pericarpium papaveris* sample.

4. Conclusion

[C_4mim]Cl-based aqueous two-phase technique in combination with HPLC is demonstrated to be a new method for the determination of opium alkaloids in *P. papaveris*. Compared with the conventional polymer-salt-water systems, such as PEG/K_2HPO_4 ATPS, the extraction efficiencies of [C_4mim]Cl/K_2HPO_4 ATPS were stable in a wide temperature range. Moreover, this system had relative lower viscosity and was convenient to be coupled with HPLC. This ATPS technique was less time-consuming and used nonvolatile solvent, which were distinct advantages over the conventional SPE. The developed method was proved highly efficient and fast for the separation and enrichment of hydrophobic drugs in aqueous samples, and would be greatly potential in coupling with other instruments, such as FIA and CE, etc. and might open up new possibility in the concentration and separation of other drugs from biological samples.

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