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Abstract: An aqueous two-phase system (ATPS) was presented with hydrophilic ionic liquid 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) and NaH_2PO_4 aqueous solution in this paper. The partitioning behavior of penicillin G in the ATPS was investigated. Concentrations of NaH_2PO_4, penicillin G, and [Bmim]Cl were evaluated to determine their effects on the partition coefficient and extraction yield of penicillin G. It was found that both of partition coefficient and extraction yield strongly depended on the concentration of [Bmim]Cl, penicillin and NaH_2PO_4. A high extraction yield of 93% was achieved with the following

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parameters: $NaH_2PO_4 \cdot 2H_2O$ 40% (wt%), penicillin 45000 $\sim$ 50000 u/ml, [Bmim]Cl 20 $\sim$ 21% (wt%). The [Bmim]Cl/NaH_2PO_4 system was also applied in a real filtration of penicillin G fermentation broth and the extraction yield was averaged at 91.5%. It is worthy noting that the working pH value of ATPS was at the range of 5 $\sim$ 6, no emulsification and protein denaturation could be observed.

Keywords: Ionic liquid, extraction, aqueous two-phase system, penicillin, emulsification

INTRODUCTION

Room temperature ionic liquids (RTILs) have aroused increasing interest of chemists and engineers as replacements for volatile organic solvents (VOSs). They also stimulated scientific curiosity, process development in research, and industrial applications. RTILs, salts that exist as liquid at ambient temperature, comprise of relatively larger organic cations and inorganic anions (1). They have unique chemical and physical properties, for example, high thermal stability (2, 3), no detectable vapor pressure (4, 5), etc. Some of the properties can be tailored by suitable combinations of cations and anions for specific requirements in the applications such as synthesis (6, 7), separation (8–10) and so on. Therefore, the ionic liquids are also called "designer solvents." Some previous studies have indicated that hydrophobic RTILs could effectively extract organic compounds (11) and antibiotics such as amoxicillin, ampicillin, and erythromycin (12, 13). However, the extraction yield and partition coefficient of antibiotics were dependent on pH value of the system as the traditional extraction system, and high pH value or low pH value finally resulted in degradation of antibiotics.

Gutowski group's research once demonstrated an aqueous two-phase system (ATPS) formed with hydrophilic RTIL 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) and K_3PO_4, which consisted of an upper RTIL-rich phase and a lower K_3PO_4-rich phase (14). The ATPS contained one organic salt (hydrophilic RTIL) and one inorganic salt (phosphate). It is different from the conventional ATPS reported by Guan's group (15), in which a higher extraction yield of 93.67% and partition coefficient of 58.39 could be obtained when penicillin G was extracted from its fermentation broth using polyethyleneglycol PEG 2000/$(NH_4)_2SO_4$ system. ATPS has attracted more and more attention because of mild operating conditions, and is becoming a promising separation method that has potential applications in the extraction and purification of biological active compounds (16, 17). Our early research showed that the ATPS with [Bmim]BF_4 and NaH_2PO_4 could give a high extraction yield of 93.7% for extracting penicillin G from aqueous solution at a higher pH value 4 $\sim$ 5 against butyl acetate extracting penicillin at pH 2 (18). The higher pH value

$4 \sim 5$ made penicillin less degradable, and no emulsification and protein denaturation occurred. But, $[Bmim]BF_4/NaH_2PO_4$ has fluorine-containing anion which is unstable in aqueous phase, especially in acid environment. It is urgent to develop non-fluorinated RTILs ATPS for penicillin G extraction.

In this paper, hydrophilic ionic liquid [Bmim]Cl was used to form a ATPS with NaH_2PO_4 aqueous solution for the extraction of penicillin G. The partitioning behavior of penicillin G in the ATPS was investigated. Several important operating parameters, including the concentrations of [Bmim]Cl, NaH_2PO_4, penicillin G, and pH value were studied to optimize the penicillin extraction conditions. The $[Bmim]Cl/NaH_2PO_4$ ATPS was also evaluated in the extraction of penicillin G from its industrial filtration of fermentation broth.

MATERIALS AND METHODS

Materials

Penicillin G potassium (99.5%) and the filtration of penicillin G fermentation broth were kindly supplied by the North China Pharmaceutical Group Corporation. $NaH_2PO_4 \cdot 2H_2O$ was purchased from Beijing Hongxing Chemical Plant (99%). Ionic liquid [Bmim]Cl was synthesized and purified according to the literature procedure (1, 13, 19).

Instrumental Analysis

The concentration of penicillin G was determined by a HP Series 1100 HPLC with a Zorbax Stablebond C18 column (4.6 mm $\times$ 250 mm; 3.6 μm) and a diode array detector. The mobile phase was the mixture of an acetate buffer/phosphate buffer/acetonitrile (3:25:125 v/v/v) at a flow rate of 1.1 ml/min. The pH values were measured using Mettler Toledo MP225. The volume of the solution was measured with a precision at ± 0.05 ml.

ATPS Extraction

An aqueous solution of penicillin G potassium, RTIL [Bmim]Cl and $NaH_2PO_4 \cdot 2H_2O$ was added to a beaker one by one, and stirred completely at room temperature for 5 min, then the mixture was poured into a cylinder. After the two phases were clearly separated, the volumes of both phases were recorded. The concentration of penicillin G in each phase was determined by HPLC. And also the pH value was recorded.

Extraction Yield

The extraction yield (Y) and partition coefficient (K) can be described by following formulations, respectively.

$$Y = \frac{C_{RTIL} \times V_{RTIL}}{C_{Initial} \times V_{Initial}}$$

Where C_{RTIL} and V_{RTIL} were the concentration of penicillin and the volume in the upper phase of ATPS, $C_{Initial}$ and $V_{Initial}$ were the concentration of penicillin and the volume in the initial penicillin aqueous solution, respectively.

$$K = C_{RTIL}/C_{Salt}$$

C_{RTIL} was the concentration of penicillin in the upper phase; C_{Salt} was the concentration of penicillin in the lower phase, respectively.

The ATPS was formed by the penicillin aqueous solution, [Bmim]Cl and $NaH_2PO_4 \cdot 2H_2O$. The mass of the initial penicillin aqueous solution, [Bmim]Cl and $NaH_2PO_4 \cdot 2H_2O$ were expressed by W_{aq}, W_{RTIL} and W_{Salt}. The units of concentration of penicillin G are unit/ml (u/ml) as the industry used, one billion units penicillin G potassium are 0.628 kg.

RESULTS AND DISCUSSION

Guan's group reported that penicillin could be extracted from its aqueous solution efficiently in PEG 2000/$(NH_4)_2SO_4$ ATPS without adjusting pH value to 2 against the traditional extraction using butyl acetate as extracting agent. They pointed out that the partitioning of penicillin strongly depended on the composition of ATPS (15). Therefore, the concentration of ionic liquid, phosphate, and penicillin should play very important roles in the formation of the new ATPS and its extraction performance of penicillin in this study.

Effect of Concentration of Ionic Liquid

In fact, ionic liquids not only act as the "polymer" in the formation of ATPS, but also is a kind of an organic salt. Therefore, ionic liquids should play important roles on the partitioning behavior of penicillin G in [Bmim]Cl/$NaH_2PO_4 \cdot 2H_2O$ ATPS. Figure 1 showed the influence of [Bmim]Cl concentration on partition coefficient and extraction yield. When the percentage of [Bmim]Cl varied from 16.6% to 25.0% (wt%), pH value of the system changed a little from 5.5 to 6.2, but the partition coefficient decreased from 139 to 69 distinctly. The maximum extraction yield was ca. 93.4% in the range of RTIL [Bmim]Cl concentration from 20% to 21%. A good extraction

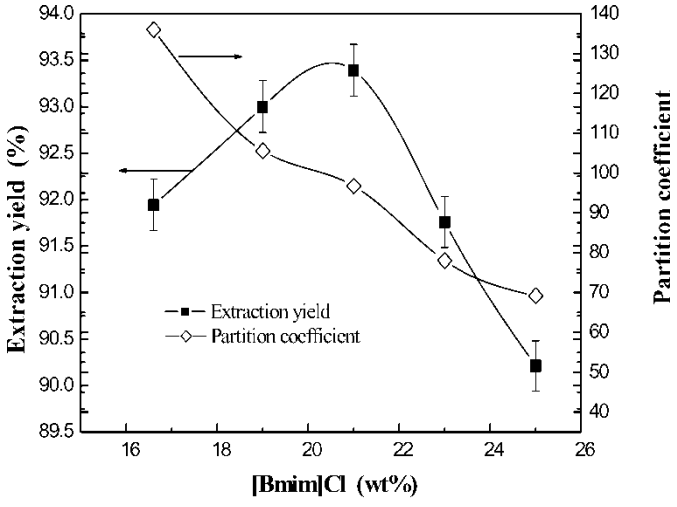

Figure 1. Effects of [Bmim]Cl concentration on partition coefficient and extraction yield (penicillin 49869 u/ml, W_{aq}:W_{Salt} = 2:1).

performance could be achieved with yield more than ca. 90% by [Bmim]Cl/NaH_2PO_4 ATPS in this study. Hence, the partition coefficient and extraction yield were apparently dependent on the composition of RTIL in the ATPS. [Bmim]Cl/$NaH_2PO_4 \cdot 2H_2O$ ATPS gave a similar extraction yield as [Bmim]BF_4/$NaH_2PO_4 \cdot 2H_2O$ ATPS (18) and PEG 2000/$(NH_4)_2SO_4$ ATPS (15), but it had a much higher partition coefficient than PEG 2000/$(NH_4)_2SO_4$ system (15).

Guan pointed out that repulsion between polymers and biomolecules could be enhanced with the increase of the "polymer" molecular weight, which resulted in the decrease of the partition coefficient of biomolecule in traditional ATPS (15). Additionally, penicillin G exists as carboxylate in aqueous solution at pH between 5 and 6, and the penicillin carboxylate dissolves more easily in a hydrophilic environment. According to what Rogers reported, the upper phase should become more hydrophobic with an increase of the concentration of [Bmim]Cl, which resulted in less penicillin G dissolved in the upper phase (14). Hence, the partition coefficient and extraction yield could decrease with the dosage of [Bmim]Cl more than ca. 21%. This is exactly analogous to what was observed in the formation of PEG/salt ATPS (14).

Effect of Concentration of Phosphate

The mixture of a penicillin G aqueous solution and [Bmim]Cl was a homogeneous solution at room temperature. After $NaH_2PO_4 \cdot 2H_2O$ was

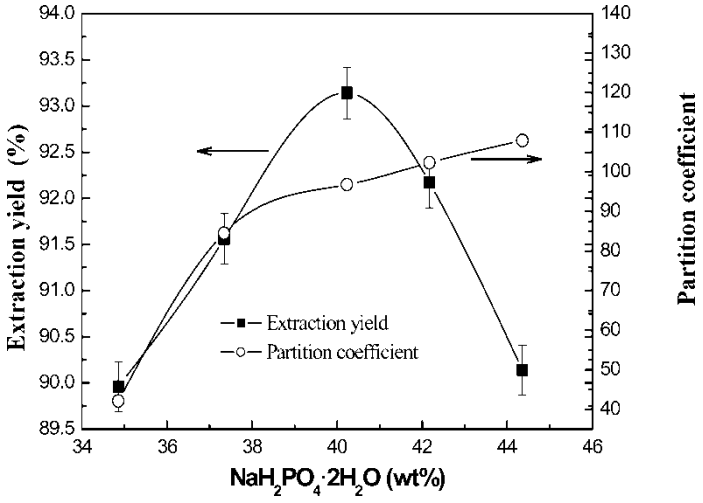

Figure 2. Effects of $NaH_2PO_4 \cdot 2H_2O$ concentration on partition coefficient and extraction yield (penicillin 49869 u/ml, W_{aq}:$W_{\mathrm{RTIL}} = 2{:}1$)

introduced into the above mixture, an aqueous two-phase system was formed. The effects of $NaH_2PO_4 \cdot 2H_2O$ on partition coefficient and extraction yield were shown in Fig. 2. The results indicated that the partition coefficient and the extraction yield were closely related to the concentration of $NaH_2PO_4 \cdot 2H_2O$. When the concentration of $NaH_2PO_4 \cdot 2H_2O$ increased from 34.9% to 44.4% (wt%), the ATPS solution pH just changed from 6.2 to 5.7, but the partition coefficient increased sharply from 42 to 108. The extraction yield of penicillin increased from 90.0% to 93.2% and then decreased with the increase of $NaH_2PO_4 \cdot 2H_2O$ concentration. The optimal yield of 93.2% was achieved when the concentration of $NaH_2PO_4 \cdot 2H_2O$ was ca. 40.0%.

Rogers pointed out that the salting-out effect of phosphate resulted in the formation of ATPS (14). It is evident that the extraction yield was not proportional to the partition coefficient which was attributed to the volume changes of upper phase. Figure 3 gave out the volume of the upper phase in ATPS. It was noted that the volume of the upper phase decreased with the increase of the $NaH_2PO_4 \cdot 2H_2O$ concentration in ATPS, which resulted in the increasing of partition coefficient. It is due to the water-structuring nature of the $[H_2PO_4]^-$ anion that results in the volume variations of upper and lower phases.

Effect of Concentration of Penicillin G

With the addition of penicillin G potassium into [Bmim]Cl/$NaH_2PO_4 \cdot 2H_2O$ ATPS, most of the penicillin could be extracted to RTIL-rich upper

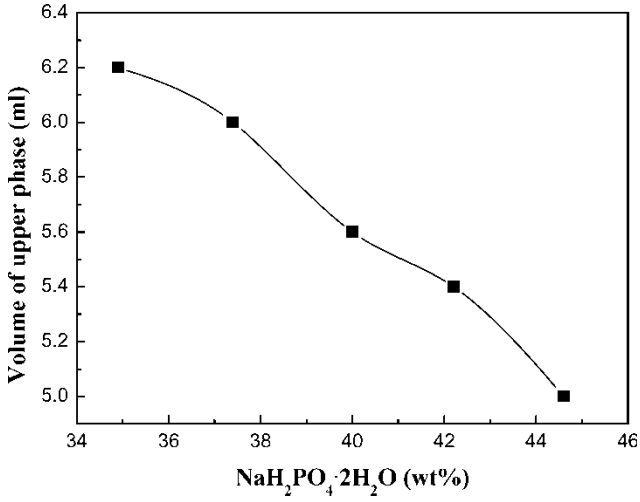

Figure 3. Effects of $NaH_2PO_4 \cdot 2H_2O$ concentration on volume of upper phase (penicillin 49869 u/ml, W_{aq}:$W_{RTIL} = 2{:}1$).

phase. Figure 4 showed the influence of penicillin concentration on the extraction performance. The result indicated that the extraction yield first increased and then decreased with the increase of the concentration of penicillin G. There was an optimal extraction result with 93% yield

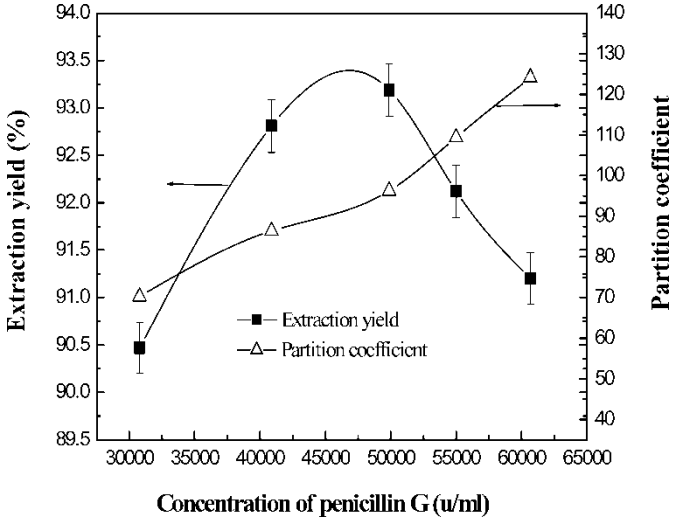

Figure 4. Effects of penicillin G concentration on partition coefficient and extraction yield (W_{aq}:W_{RTIL}:$W_{Salt} = 2{:}1{:}2$).

when the concentration of penicillin G was ca. $45000 \sim 50000$ u/ml in [Bmim]Cl/NaH_2PO_4 ATPS. Whatever the concentration of penicillin G was (30796 u/ml–60680 u/ml), it could get the extraction yield to be greater than 90.5%. However, the pH value was kept at 5.9 independent of the concentration of penicillin G. For the influence of penicillin concentration on the partition coefficient, the partition coefficient increased significantly from 49 to 124 while the penicillin concentration rose from 30796 u/ml to 60680 u/ml.

Extraction of Penicillin G from its Filtrated Fermentation Broth

The above experiment results indicated that the novel ATPS composed of [Bmim]Cl/$NaH_2PO_4 \cdot 2H_2O$ could be used for extracting penicillin G efficiently from its aqueous solution. In order to explore the possibility of ATPS application on the extraction of penicillin in a real industrial process, the [Bmim]Cl/$NaH_2PO_4 \cdot 2H_2O$ ATPS was applied for penicillin G extraction from its filtration of the fermentation broth. The filtration of penicillin broth was taken and used directly from the industrial manufacture workshop.

Table 1 presented the experimental conditions and results, and indicated that penicillin G could be extracted with an average yield higher than 90% by RTIL ATPS. Importantly, the working pH in ATPS changed a little vs the pH of the filtration, and almost kept at ca. 6.0. The partitioning of penicillin was performed in the ATPS with ionic liquid [Bmim]Cl at pH value from 5.8 to 6.0, which was completely different from the penicillin extraction by butyl acetate at pH 2.0. It is well-known that a serious emulsification would occur between the organic phase and the aqueous phase when the organic solvent mixed completely with the filtration of penicillin fermentation broth due to the protein denaturalization at pH 2.0. However, there was a clear interface between the upper phase and the lower phase in [Bmim]Cl/NaH_2PO_4 after 15 min by RTIL ATPS extraction as Fig. 5 displayed.

Table 1. Extraction yield of penicillin G from filtratation of fermentation broth

Batch	Concentration of penicillin(u/ml)	pH of filtration	pH of ATPS[a]	Yield (%)
1	23524	5.6	5.9	89.7
2	24569	5.6	5.9	91.1
3	25194	5.5	5.8	92.7
4	32165	5.7	6.0	92.5
Average				91.5

[a]ATPS containing 20% [Bmim]Cl, 40% $NaH_2PO_4 \cdot 2H_2O$.

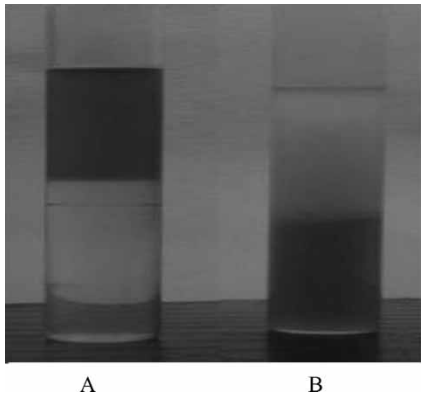

Figure 5. Emulsification phenomenon in different extraction systems (A: [Bmim]Cl/NaH_2PO_4, B: Butyl acetate).

CONCLUSION

In this study an aqueous two-phase system (ATPS) was obtained from hydrophilic ionic liquid 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) and $NaH_2PO_4 \cdot 2H_2O$. The ATPS can efficiently extract penicillin G from its aqueous solution and its filtration of fermentation broth at pH $5 \sim 6$ without emulsification and protein denaturation. The partition coefficient and extraction yield are strongly dependent on the concentration of NaH_2PO_4, penicillin and [Bmim]Cl. The salting-out effect of $[H_2PO_4]^-$ resulted in the formation of ATPS. The repulsion of the ionic liquid to the penicillin molecule led to the decrease of partition of penicillin in upper phase of ATPS. The optimal extraction yield was achieved under the following operation conditions: $NaH_2PO_4 \cdot 2H_2O$ 40% (wt%), penicillin $45000 \sim 50000$ u/ml, [Bmim]Cl $20 \sim 21\%$ (wt%).

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