



## Partitioning Behavior of Tetracycline in Hydrophilic Ionic Liquids Two-Phase Systems

Jingyu Pang, Changri Han, Yanhong Chao, Lei Jing, Haiyan Ji, Wenshuai Zhu, Yonghui Chang & Huaming Li

To cite this article: Jingyu Pang, Changri Han, Yanhong Chao, Lei Jing, Haiyan Ji, Wenshuai Zhu, Yonghui Chang & Huaming Li (2015) Partitioning Behavior of Tetracycline in Hydrophilic Ionic Liquids Two-Phase Systems, Separation Science and Technology, 50:13, 1993-1998, DOI: 10.1080/01496395.2015.1018436

To link to this article: <http://dx.doi.org/10.1080/01496395.2015.1018436>



Accepted online: 23 Apr 2015.



Submit your article to this journal [↗](#)



Article views: 14



View related articles [↗](#)



View Crossmark data [↗](#)

# Partitioning Behavior of Tetracycline in Hydrophilic Ionic Liquids Two-Phase Systems

Jingyu Pang,<sup>1,2</sup> Changri Han,<sup>1</sup> Yanhong Chao,<sup>2</sup> Lei Jing,<sup>2</sup> Haiyan Ji,<sup>3</sup> Wenshuai Zhu,<sup>3</sup> Yonghui Chang,<sup>1</sup> and Huaming Li<sup>3</sup>

<sup>1</sup>Key Laboratory of Tropical Medicinal Plant Chemistry of Education, Hainan Normal University, Haikou, P. R. China

<sup>2</sup>School of Pharmacy, Jiangsu University, Zhenjiang, P. R. China

<sup>3</sup>School of Chemistry and Chemical Engineering, Jiangsu University, Zhenjiang, P. R. China

Ionic liquids-based aqueous two-phase system (ILs-ATPS) offers an alternative approach for the extraction of tetracycline (TC) through their partitioning between two phases. The procedure was composed of 1-butyl-3-methylimidazolium halide ([Bmim]X (X = Cl, Br)) and K<sub>2</sub>HPO<sub>4</sub> using a liquid-liquid extraction technique. The influence factors on partition behavior of TC were optimized systematically, including the quality of salts and IL, the amount of TC, pH value, and temperature. The maximum extraction efficiency of TC could reach 99%. The interactions between the ATPS and TC have been examined by isothermal titration calorimetry. Extraction of antibiotics TC using ILs-ATPS could achieve nearly complete extractions in a single-step by way of the proper tailoring of their concentrations in the aqueous media.

**Keywords** ionic liquid; aqueous two-phase; tetracycline; extraction

## INTRODUCTION

For its low-cost, gentle condition, for being recyclable and biocompatible, aqueous two-phase extraction (ATPE), a novel liquid-liquid extraction technique, has been used to establish bioprocesses for the primary recovery and partial purification of biomolecules, such as antibiotics (1), proteins (2), and enzymes (3). By introduction of polymer/polymer or polymer/salt above a certain concentration, two immiscible aqueous phases could be formed. Compared with conventional extraction methods, ATPE are more environmentally benign without using harmful volatile organic solvent in the whole process.

Received 17 August 2014; accepted 9 February 2015.

Address correspondence to Changri Han, Key Laboratory of Tropical Medicinal Plant Chemistry of Education, Hainan Normal University, Haikou 571158, P. R. China. E-mail: [hchr116@126.com](mailto:hchr116@126.com) or Yanhong Chao, School of Pharmacy, Jiangsu University, Zhenjiang 212013, P. R. China. E-mail: [chaoyh@ujs.edu.cn](mailto:chaoyh@ujs.edu.cn)

Color versions of one or more of the figures in the article can be found online at [www.tandfonline.com/lsst](http://www.tandfonline.com/lsst).

It has been reported that ionic liquids (ILs) can form aqueous two-phase system (ATPS) in the presence of inorganic salts (4), and these ILs-ATPS have received much attention owing to their “green” characteristics, such as a negligible volatility at ambient conditions, high thermal and chemical stabilities, and a large solubilization ability for a wide variety of compounds (5, 6). These new ATPS had been successfully used to separate small molecular antibiotics, as shown in Table 1.

Tetracycline (TC, Fig. 1a), a family of polyketide antibiotics produced by Streptomyces genus of actinobacteria, was first isolated in 1948 (15) and differed from the existing antibiotics due to the broad spectrum for antimicrobial activity and low toxicity. Diverse separation methods such as solvent extraction, ion-exchange, chromatography, crystallization, or a combination of these methods have been used for the purification of antibiotics. Solvent extraction, due to its effectiveness and economical nature, is by far the most widely used for the separation technique of antibiotics. By now TC has been separated successfully using several IL-ATPS extraction systems, such as [Omim]Br/anionic surfactant (1), [Bmim]BF<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> (16), [Bmim]BF<sub>4</sub>/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (17), IL/Na<sub>2</sub>CO<sub>3</sub> (18), and [Omim]BF<sub>4</sub>-NaH<sub>2</sub>PO<sub>4</sub> (19). However, the main effecting parameters on the partitioning behavior using ILs-ATPS as well as the IL types were still lack.

In this paper, the feasibility of extracting TC by [Bmim]X (X=Cl, Br)-K<sub>2</sub>HPO<sub>4</sub> ATPS was reported. The structure of [Bmim]X (X=Cl, Br) was shown in Fig. 1b. The schematic diagram of separating TC in this ILs-ATPS was proposed in Fig. 2. The main objectives of this work were:

- extraction of TC into ILs-rich phase;
- optimization of the extraction conditions, such as the amount of IL, K<sub>2</sub>HPO<sub>4</sub>, and TC, the solution pH and the temperature;
- analysis of the main interaction for the extraction process.

TABLE 1  
Separation of antibiotics by ATPS

Antibiotics	Aqueous two-phase systems	Extraction efficiency(%)	References
Oxytetracycline	[Omim]Br/anionic surfactant	97.8	(1)
Oxytetracycline	[Bmim]BF <sub>4</sub> /(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	99.0	(7)
Chloramphenicol	[C <sub>4</sub> mim]Cl/K <sub>2</sub> HPO <sub>4</sub>	95.0	(8)
Sulfadiazine	[Bmim]BF <sub>4</sub> /(NH <sub>4</sub> ) <sub>3</sub> -Citrate	98.3	(9)
Tilmicosin	[C <sub>4</sub> mim]Cl/K <sub>2</sub> HPO <sub>4</sub>	95.8	(10)
Acetylspiramycin	[Bmim]BF <sub>4</sub> /NaH <sub>2</sub> PO <sub>4</sub>	90.1	(11)
Azithromycin	[Bmim]BF <sub>4</sub> / Na <sub>2</sub> CO <sub>3</sub>	90.9	(12)
Penicillin G	[C <sub>4</sub> mim]Cl/NaH <sub>2</sub> PO <sub>4</sub>	93.0	(13)
Roxithromycin	[Bmim]BF <sub>4</sub> /Na <sub>2</sub> CO <sub>3</sub>	90.7	(14)

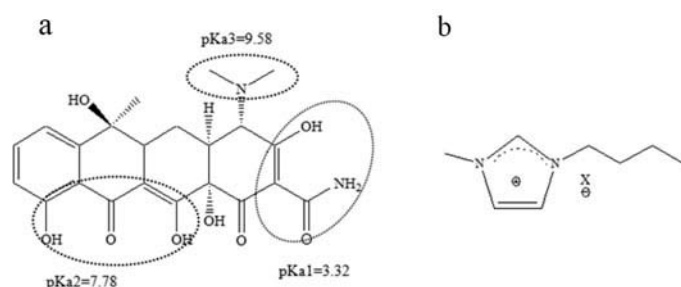


FIG. 1. The molecular structures of tetracycline (a) and [Bmim]X(X=Cl, Br) (b).

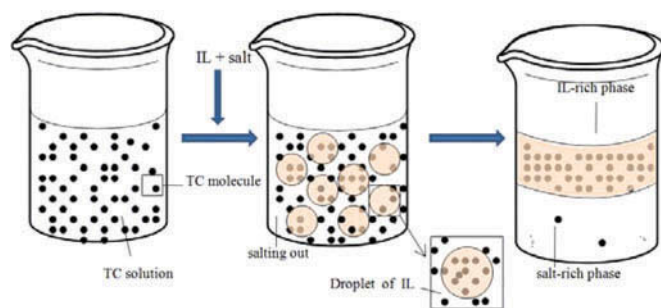


FIG. 2. The schematic diagram for the separation of TC in ILs-ATPS composed of [Bmim]X(X=Cl, Br)-K<sub>2</sub>HPO<sub>4</sub>.

## EXPERIMENTAL

### Instruments and Reagents

UV-2401PC UV-vis spectrophotometer (Beijing Purkinje General Instrument Company, Ltd., Beijing, China), SK5200H ultrasonic wave cleaner (Shanghai Branch Leads Supersonic Instrument Limited Company), and vortex mixer (Jintan Instrument Manufacturing Co., Ltd.)

[Bmim]X(X = Cl, Br) was commercially obtained from Chengjie Chemical Co., Ltd. (Shanghai, China), quoted purity

99%. Tetracycline was obtained from Sigma-Aldrich Chemical Co. (99% purity). K<sub>2</sub>HPO<sub>4</sub> was purchased from Kermel Chem. Ltd., with a purity of  $\geq 99.0\%$ . Other reagents were analytical grade.

### Preparation of the Phase Diagram for [Bmim]X(X=Cl, Br)-K<sub>2</sub>HPO<sub>4</sub> System

The binodal curves were determined by the cloud-point method (4). [Bmim]X (X=Cl, Br) were weighed into a 10 mL tube. A K<sub>2</sub>HPO<sub>4</sub> solution of known mass fraction was added dropwise to the tube till the appearance of a turbid solution, followed by dropwise addition of water until the formation of a clear and limpid solution. The procedure above was repeated to obtain sufficient data to construct the phase diagram. The temperature was controlled at  $25 \pm 0.1^\circ\text{C}$  by water thermostat.

### Extraction Process

A given amount of IL, K<sub>2</sub>HPO<sub>4</sub>, and TC aqueous solution was added into a graduated glass tube. The volume of the glass tube was calibrated before use. The mixture was shaken vigorously for 30 min to attain equilibrium by vortex mixer and the temperature of the system was controlled at  $298.15 \pm 0.05\text{ K}$  with a DC-2006 low temperature thermostat. The phase separation quickly occurred after cessation of the shaking process. Then, a centrifuge operated at 4000 rpm was used to run for a period of 5 min in each test to ensure a complete phase separation. After the volume of the top and bottom phases was recorded, the samples collected from both phases were diluted and analyzed against blanks containing the same phase composition but without TC to avoid interference from phase components (20). The TC concentrations in both phases were determined using UV-vis spectrophotometer at 355 nm. The calibration curve of TC was:  $A = 0.029 C + 0.0028$  ( $R^2 = 1$ ), where A is the absorbance of TC, and C is the concentration of TC (mg/L). The linearity for analyzing

TC was in the concentration range of 0-100 mg/L. The RSD was less than 0.5% ( $n = 5$ ).

The partition coefficients ( $K$ ) of the TC between the phases were calculated by

$$K = \frac{C_t}{C_b} \quad (1)$$

where  $C_t$  and  $C_b$  were equilibrium concentrations of TC in the IL-rich top phase and the phosphate-rich bottom phase, respectively.

Phase volume ratio ( $R$ ) was defined as the volume ratio of the top phase to the bottom phase:

$$R = \frac{V_t}{V_b} \quad (2)$$

where  $V_t$  and  $V_b$  were the volume of the top phase and the bottom phase, respectively.

Extraction efficiency ( $E$ ) of TC could be calculated by using the following equation (21):

$$E = \frac{C_t V_t}{C_t V_t + C_b V_b} = \frac{KR}{1 + KR} \quad (3)$$

### Isothermal Titration Calorimetry (ITC)

Experiments were carried out at 25°C using a Microcal VP-ITC microcalorimeter (MicroCal Inc., Northampton, MA). Each solution was degassed and thermostated before titration. The sample cell was loaded with 1.436 mL of TC solution (50  $\mu$ M) and the reference cell contained Milli-Q grade water. A syringe was filled with the mixture (2 mM IL + 5 mM  $K_2HPO_4$ ). An initial injection of 2  $\mu$ L followed by 28 injections of 10  $\mu$ L and its stirring speed was fixed at 307 rpm. The interval between injections was 210 s to guaranty the equilibrium in each titration point. The heat released or absorbed upon each injection was measured, and the data were plotted as of integrated quantities. The molar enthalpy change for the binding,  $\Delta H$ ; the binding stoichiometry,  $n$ ; the affinity constant,  $K_{af}$  and the molar entropy change,  $\Delta S$  were calculated with MicroCal ORIGIN 8.0 software supplied with the instrument.

## RESULTS AND DISCUSSION

### Phase Diagrams of ATPS

Phase diagrams data were basic for the separation process and the subsequent efficiency. These curves can illustrate the composition of IL and salts required to form a two phase system. The results were shown in Fig. 3. And it is clear that the curve of [Bmim]Br-  $K_2HPO_4$  system was closer to the axis origin, which indicated that this system possesses stronger phase-forming ability.

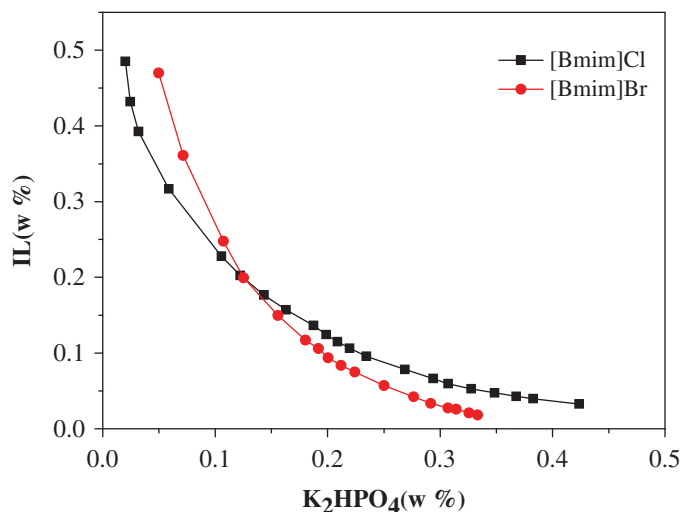


FIG. 3. Phase diagram for the [Bmim]X(X=Cl, Br)- $K_2HPO_4$  system at 25 °C.

### Effect of $K_2HPO_4$ Amount

The influence of  $K_2HPO_4$  amounts on the extraction efficiency of TC were investigated in [Bmim]X(X = Cl, Br)- $K_2HPO_4$  systems and the result was shown in Fig. 4. The extraction efficiency increased with the increasing of  $K_2HPO_4$  mass. When the  $K_2HPO_4$  amount was up to 2.4 g, the maximal TC extraction efficiencies of 99.5% and 99.3% in [Bmim]Cl- $K_2HPO_4$  and [Bmim]Br- $K_2HPO_4$  systems were both obtained. This was exactly analogous to the formation of IL-salt ATPS. This phenomenon was probably due to the solvophobic interaction. Kosmotropic (i.e., water-structuring) salts increase the dielectric constant of the aqueous phase, thus forcing the low dielectric  $[Bmim]^+$  cations to the upper phase with concurrent transfer of halogen anions. We can summarize the formation of ATPS of this type by considering the use of other water-structuring salts to salt-out the IL ions from aqueous solution (22). [Bmim]Cl should be a chaotropic salt and thus could be salted-out by a kosmotropic salt to form the IL-ATPSs (23, 24). Similarly, [Bmim]Cl was formed by metathesis and concentrated in the upper phase of an ATPS produced on addition of KCl to aqueous  $[Bmim]_2HPO_4$ .

### Effect of [Bmim]X(X=Cl, Br) Amount

In fact, IL not only acts as the “polymer” in the formation of ATPS, but also a kind of organic. Therefore, IL should play important roles on the extraction efficiency of TC. Figure 5 demonstrated the effect of [Bmim]X(X = Cl, Br) amount to extraction efficiency. When the amount of IL increased from 0.3 g to 0.45 g, the extraction efficiency of TC increased to around 99%. And then extraction efficiency dropped gradually when adding more IL above 0.45 g. It was observed that [Bmim]Br- $K_2HPO_4$  ILs-ATPS showed higher TC extraction efficiency than [Bmim]Cl- $K_2HPO_4$  system, but the influence degree of IL amount to the TC extraction efficiency was also

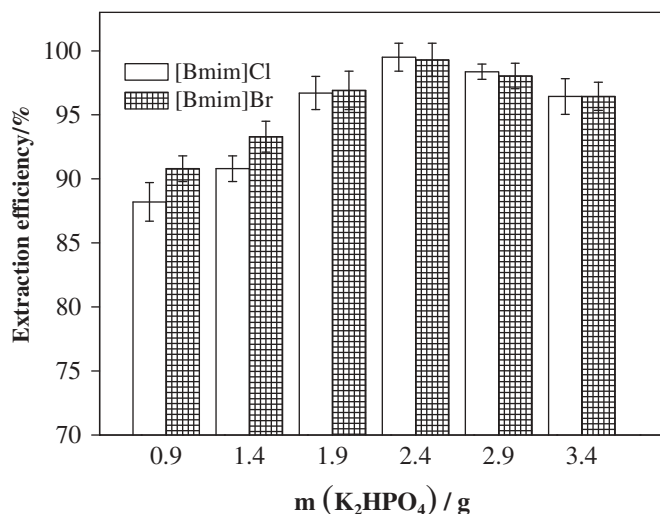


FIG. 4. Effect of K<sub>2</sub>HPO<sub>4</sub> amount to the extraction efficiency of TC. Conditions: 0.8 mL TC solution, 0.45 g [Bmim]X (X=Cl, Br), room temperature, initial pH (6.38).

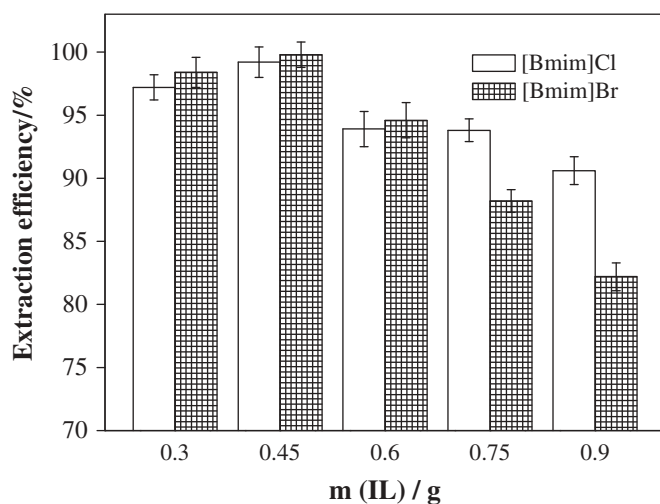


FIG. 5. Effect of [Bmim]X (X=Cl, Br) amount to the extraction efficiency of TC. Conditions: 0.8 mL TC solution, 2.4 g K<sub>2</sub>HPO<sub>4</sub>, room temperature, initial pH (6.38).

higher for [Bmim]Br-K<sub>2</sub>HPO<sub>4</sub> than that of [Bmim]Cl-K<sub>2</sub>HPO<sub>4</sub>. A large amount of hydrophilic IL existing in the IL-rich phase can also interact with water molecules, which will influence the solubility of target in the top phase. The existing IL decreased the solubility of target in the top phase. And TC extraction efficiency of [Bmim]Br-K<sub>2</sub>HPO<sub>4</sub> ILs-ATPS declined more noticeable than that of [Bmim]Cl-K<sub>2</sub>HPO<sub>4</sub>.

#### Effect of TC Amount

The influence of TC amount on the extraction efficiency was investigated with the conditions of 0.45 g IL and 2.4 g

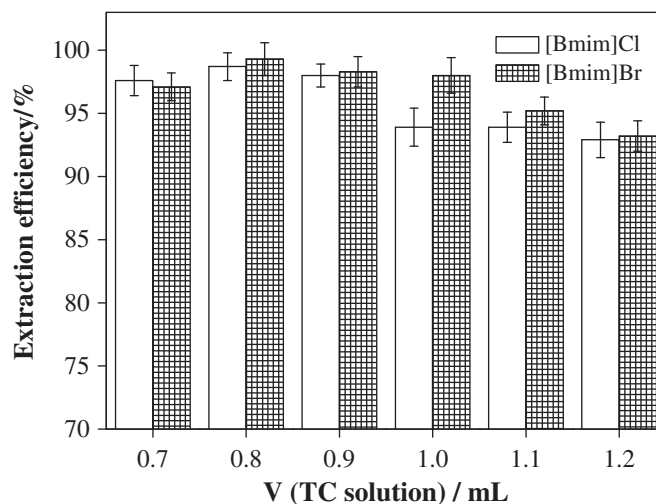


FIG. 6. Effect of TC solution volume (100 mg/L) to the extraction efficiency. Conditions: 2.4 g K<sub>2</sub>HPO<sub>4</sub>, 0.45 g [Bmim]X (X=Cl, Br), room temperature, initial pH (6.38).

K<sub>2</sub>HPO<sub>4</sub>. It was evident from Fig. 6 that the extraction efficiency kept on above 93% when the volume of TC solutions was below 1.2 mL. But adding more TC solution of 1.3 mL into [Bmim]Cl-K<sub>2</sub>HPO<sub>4</sub> or [Bmim]Br-K<sub>2</sub>HPO<sub>4</sub> systems, the extraction efficiency reduced significantly from 92.9% to 65.9%, or from 93.2% to 66.9%, respectively. As the volume of TC solution increased, TC had an affinity with IL at the beginning, thus the extraction efficiency increased. However, when the excessive TC was added, TC would accumulate at the interface, which resulted in the decreasing of TC extraction efficiency (25).

#### Effect of pH

The extraction efficiency of TC was studied as a function of the pH of the extraction buffer, and the results were shown in Fig. 7. As TC was stable in acid condition, so the range of pH 3.0-8.0 was chosen to study the pH dependence of TC extraction. As illustrated in Fig. 7, the efficiency increased gradually as the pH changed from 3.0 to 8.0. It is well known that the charged characteristics of antibiotics were greatly affected by the pH value of aqueous solutions. TC can appear as one of the three forms in different pH conditions: cationic when pH < pK<sub>a1</sub>(3.30), zwitterionic when pK<sub>a1</sub>(3.30) < pH < pK<sub>a2</sub>(7.68), and anionic when pH > pK<sub>a2</sub> (26). More and more anionic proportion of species will exist in aqueous solutions with the increase of pH. Therefore, it is deduced that the electrostatic interactions between charged groups of TC and [Bmim]<sup>+</sup> of ILs-ATPS played an important role for the partitioning of TC into IL-rich phase and caused the extraction efficiency dependent on pH.



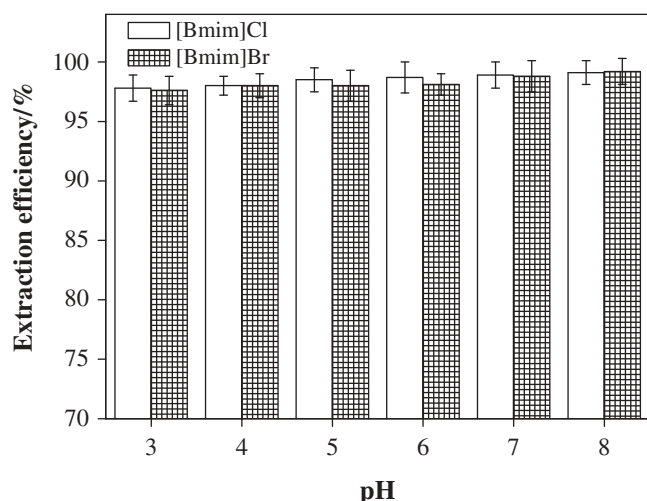


FIG. 7. Effect of pH to the extraction efficiency of TC. Condition: 2.4 g  $K_2HPO_4$ , 0.45 g [Bmim]X(X=Cl, Br), 0.8 mL TC solution, room temperature.

### Effect of Temperature

The influence of temperature on the extraction efficiency of TC in [Bmim]Br- $K_2HPO_4$  / [Bmim]Cl- $K_2HPO_4$  systems were investigated in the range of 25-65°C, respectively. The changes of the extraction efficiency and partition coefficient with the change of temperatures were shown in Fig. 8. The optimal temperature for [Bmim]Cl- $K_2HPO_4$  and [Bmim]Br- $K_2HPO_4$  ILs-ATPS to extract TC were 35°C and 45°C, respectively. The extraction efficiency of TC could reach about 99% for [Bmim]Br- $K_2HPO_4$ /[Bmim]Cl- $K_2HPO_4$  systems at their optimal temperatures. The volumes of the IL-rich phase always increased as the temperature increased, and more and more water molecules were transferred to the top phase, their role as solvents to TC was enhanced. But above the optimal temperature, both the extraction efficiency and partition coefficient began to decline, which may lie in that TC was unstable at relatively high temperature.

### Thermodynamics Study by ITC

The ITC measurements give information on the stoichiometry and affinity constant as well as enthalpy changes in the interacting molecules. The thermodynamic parameters of the main ILs +  $K_2HPO_4$ /TC interaction were listed in Table 2. The positive  $\Delta H$  values indicated that the extraction was an endothermic process. The negative  $\Delta G$  values mean that the interaction process was spontaneous. And [Bmim]Br- $K_2HPO_4$  showed a little higher affinity for the TC than [Bmim]Cl- $K_2HPO_4$ . These thermodynamic signatures were agreement with the experimental results.

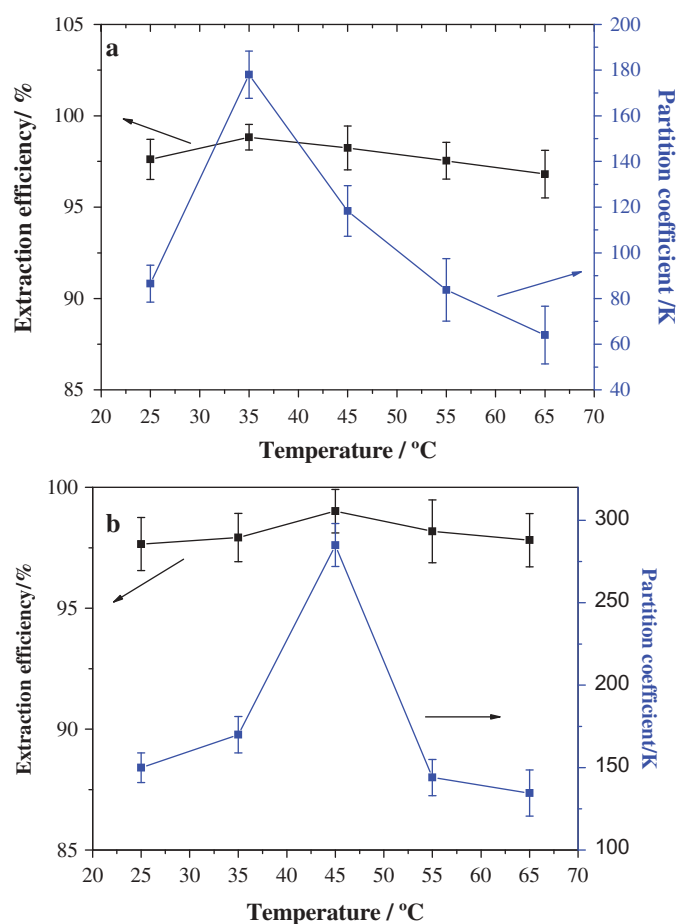


FIG. 8. Effect of temperature to the extraction efficiency of TC: (a) [Bmim]Br- $K_2HPO_4$  ATPS (2.4 g  $K_2HPO_4$  + 0.45 g [Bmim]Br + 0.8 mL TC solution, pH=8); (b) [Bmim]Cl- $K_2HPO_4$  ATPS (2.4 g  $K_2HPO_4$  + 0.45 g [Bmim]Cl + 0.8 mL TC solution, pH=8)

### CONCLUSIONS

The [Bmim]X(X = Cl, Br)- $K_2HPO_4$  ILs-ATPS were successfully used for the separation of TC. And the partitioning behaviors were also discussed at great length. The extraction efficiency of TC in the ILs-ATPS was influenced by the amount of salt, ionic liquid and TC, the solution pH and the temperature. The optimal condition for extraction of TC was 0.45 g IL, 2.4 g  $K_2HPO_4$ , and 0.8 mL TC (100 mg/L, pH 8.0) at 45°C (for [Bmim]Cl- $K_2HPO_4$ ) or 35°C (for [Bmim]Br- $K_2HPO_4$ ). The maximum extraction efficiency for TC in [Bmim]Cl- $K_2HPO_4$  and [Bmim]Br- $K_2HPO_4$  systems can be reached 99.3% and 99.8%, respectively, at their optimal conditions. The measure of ITC denoted that the ATPS extraction of TC was an endothermic and spontaneous process. It is believed that the information obtained here is useful for the understanding of the partitioning behavior of antibiotics in ILs-ATPS.

TABLE 2  
Affinity constant ( $K_{af}$ ), number of binding sites ( $n$ ) and thermodynamic parameters corresponding to the interaction [Bmim]X(X = Cl, Br)- $K_2HPO_4$  between and TC.

IL+ $K_2HPO_4$ /TC	$K_{af}(10^5M^{-1})$	$n$	$\Delta H(kJ/mol)$	$\Delta G(kJ/mol)$	$\Delta S(J/K mol)$
[Bmim]Cl+ $K_2HPO_4$ /TC	0.28	0.56	6.7	-25.5	107.6
[Bmim]Br+ $K_2HPO_4$ /TC	0.36	0.55	22.6	-26.0	162.4

## FUNDING

The authors are grateful for financial support by the National Nature Science Foundation of China (Nos. 21166009, 21266007, 21376111) and the Natural Science Foundation of Jiangsu Province (Nos. BK20130513, BK20131207).

## REFERENCES

- Yang, X.; Zhang, S. H.; Yu, W.; Liu, Z. L.; Lei, L.; Li, N.; Zhang, H. Q.; Yu, Y. (2014) Ionic liquid-anionic surfactant based aqueous two-phase extraction for determination of antibiotics in honey by high-performance liquid chromatography. *Talanta*, 124: 1–6.
- Gai, Q. Q.; Qu, F.; Zhang, T.; Zhang, Y. K. (2011) Integration of carboxyl modified magnetic particles and aqueous two-phase extraction for selective separation of proteins. *Talanta*, 85(1): 304–309.
- Li, H. X.; Li, Z.; Yin, J. M.; Li, C. P.; Chi, Y. S.; Liu, Q. S.; Zhang, X. L.; Urs, W. B. (2012) Liquid-liquid extraction process of amino acids by a new amide-based functionalized ionic liquid. *Green Chem.*, 14(6): 1721–1727.
- Gutowski, K. E.; Broker, G. A.; Willauer, H. D.; Huddleston, J. G.; Swatloski, R. P.; Holbrey, J. D.; Rogers, R. D. (2003) Controlling the aqueous miscibility of ionic liquids: Aqueous biphasic systems of water-miscible ionic liquids and water-structuring salts for recycle, metathesis, and separations. *J. Am. Chem. Soc.*, 125: 6632–6633.
- Freire, M. G.; Claudio, A. F. M.; Araujo, J. M. M.; Coutinho, J. A. P.; Marrucho, I. M.; Canongia Lopes, J. N.; Rebelo, L. P. N. (2012) Aqueous biphasic systems: A boost brought about by using ionic liquids. *Chem. Soc. Rev.*, 41(14): 4966–4995.
- Hallett, J. P.; Welton, T. (2011) Room-temperature ionic liquids: solvents for synthesis and catalysis. 2. *Chem. Rev.*, 111(5): 3508–3576.
- Wang, Y.; Xu, X. H.; Han, J.; Yan, Y. S. (2011) 25 Separation/enrichment of trace tetracycline antibiotics in water by [Bmim]BF<sub>4</sub>-(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> aqueous two-phase solvent sublation. *Desalination*, 266(1-3): 114–118.
- Jiang, D. Y.; Han, J.; Yu, C. L.; Li, Y. F.; Wang, L.; Yan, Y. S. (2013) Extraction and determination of chloramphenicol in meat using 1-butyl-3-methylimidazolium chloride- $K_2HPO_4$  aqueous two-phase system coupled with HPLC. *Asian J. Chem.*, 25(15): 8579–8583.
- Li, Y. F.; Han, J.; Yan, Y. S.; Chen, B.; Zhang, G. C.; Liu, Y.; Sheng, C. G. (2013) Simultaneous extraction and determination of sulfadiazine and sulfamethoxazole in water samples and aquaculture products using [Bmim]BF<sub>4</sub>/(NH<sub>4</sub>)(3)C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> aqueous two-phase system coupled with HPLC. *J. Iran. Chem. Soc.*, 10(2): 339–346.
- Pan, R.; Shao, D. J.; Qi, X. Y.; Wu, Y.; Fu, W. Y.; Ge, Y. R.; Fu, H. Z. (2013) Extraction of trace tilmicosin in real water samples using ionic liquid-based aqueous two-phase systems. *Water Sci. Technol.*, 67(8): 1671–1677.
- Wang, Y.; Han, J.; Xie, X. Q.; Li, C. X. (2010) Extraction of trace acetylspiramycin in real aqueous environments using aqueous two-phase system of ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate and phosphate. *Cent-Eur. J. Chem.*, 8(6): 1185–1191.
- Han, J.; Wang, Y.; Kang, W. B.; Li, C. X.; Yan, Y. S.; Pan, J. M.; Xie, X. Q. (2010) Phase equilibrium and macrolide antibiotics partitioning in real water samples using a two-phase system composed of the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate and an aqueous solution of an inorganic salt. *Microchim. Acta*, 169(1-2): 15–22.
- Liu, Q. F.; Yu, J.; Li, W. L.; Hu, X. S.; Xia, H. S.; Liu, H. Z.; Yang, P. (2006) Partitioning behavior of penicillin G in aqueous two phase system formed by ionic liquids and phosphate. *Sep. Sci. Technol.*, 41(12): 2849–2858.
- Li, C. X.; Han, J.; Wang, Y.; Yan, Y. S.; Xu, X. H.; Pan, J. M. (2009) Extraction and mechanism investigation of trace roxithromycin in real water samples by use of ionic liquid-salt aqueous two-phase system. *Anal. Chim. Acta*, 653(2): 178–183.
- Duggar, B. M. (2011) Aureomycin: a product of the continuing search for new antibiotics. *Ann. N. Y. Acad. Sci.*, 1241, 163–169.
- Ma, C. H.; Wang, L.; Yan, Y. S.; Che, G. B.; Yin, Y. S.; Wang, R. Z.; Li, D. Y. (2009) Extraction of tetracycline via ionic liquid two-phase system. *Chem. Res. Chin. Univ.*, 25(6): 832–835.
- Wang, Y.; Xu, X. H.; Han, J.; Yan, Y. S. (2011) Separation/enrichment of trace tetracycline antibiotics in water by [Bmim]BF<sub>4</sub>-(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> aqueous two-phase solvent sublation. *Desalination*, 266(1-3): 114–118.
- Marques, C. F. C.; Mourao, T.; Neves, C. M. S. S.; Lima, A. S.; Boal-Palheiros, I.; Coutinho, J. A. P.; Freire, M. G. (2013) Aqueous biphasic systems composed of ionic liquids and sodium carbonate as enhanced routes for the extraction of tetracycline. *Biotechnol. Prog.*, 29(3): 645–654.
- Soto, A.; Arce, A.; Khoshkbarchi, M. K. (2005) Partitioning of antibiotics in a two-liquid phase system formed by water and a room temperature ionic liquid. *Sep. Purif. Technol.*, 44(3): 242–246.
- Pei, Y. C.; Wang, J. J.; Wu, K.; Xuan, X. P.; Lu, X. J. (2009) Ionic liquid-based aqueous two-phase extraction of selected proteins. *Sep. Purif. Technol.*, 64(3): 288–295.
- Tan, Z. J.; Li, F. F.; Xu, X. L. (2012) Isolation and purification of aloë anthraquinones based on an ionic liquid/salt aqueous two-phase system. *Sep. Purif. Technol.*, 98: 150–157.
- Claudio, A. F.; Ferreira, A. M.; Shahriari, S.; Freire, M. G.; Coutinho, J. A. (2011) Critical assessment of the formation of ionic liquid-based aqueous two-phase systems in acidic media. *J. Phys. Chem. B*, 115(38): 11145–11153.
- Pei, Y. C.; Wang, J. J.; Liu, L.; Wu, K. (2007) Liquid-liquid equilibria of aqueous biphasic systems containing selected imidazolium IL and salt. *J. Chem. Eng. Data*, 57: 2026–2031.
- Mohammed, Taghi, Z. M.; Hamzehzadeh, S. (2007) Liquid-liquid equilibria of aqueous two-phase systems containing 1-butyl-3-methylimidazolium bromide and potassium phosphate or dipotassium hydrogen phosphate at 298.15 K. *J. Chem. Eng. Data*, 52: 1686–1692.
- Fan, J. P.; Cao, J.; Zhang, X. H.; Huang, J. Z.; Kong, T.; Tong, S.; Tian, Z. Y.; Zhu, J. H.; Ouyang, X. K. (2012) Extraction of puerarin using ionic liquid based aqueous two-phase systems. *Sep. Sci. Technol.*, 47(12): 1740–1747.
- Zhao, Y. P.; Gu, X. Y.; Gao, S. X.; Geng, J. J.; Wang, X. R. (2012) Adsorption of tetracycline (TC) onto montmorillonite: Cations and humic acid effects. *Geoderma*, 183-184: 12–18.