Research Article

Partitioning of Cephalexin in Ionic Liquid Aqueous Two-Phase System Composed of 1-Butyl-3-Methylimidazolium Tetrafluoroborate and ZnSO_4

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Ionic liquid aqueous two-phase system (ILATPS) was applied in the extraction and separation of hydrosoluble antibiotics. The partitioning behavior of cephalexin (CEX) in 1-butyl-3-methylimidazolium tetrafluoroborate ($[\text{Bmim}]\text{BF}_4$)- ZnSO_4 aqueous two-phase system was studied by the partitioning parameter of the extraction efficiency. The effect of the volume of $[\text{Bmim}]\text{BF}_4$, the concentration of ZnSO_4 , temperature, pH, and the volume of ZnSO_4 solution was discussed concretely. When the volume of $[\text{Bmim}]\text{BF}_4$ was 2 mL and the concentration of ZnSO_4 was 35%, the extraction efficiency of CEX could reach 92.64% with pH unadjusted. The effect of the volume of $[\text{Bmim}]\text{BF}_4$ on the extraction efficiency was higher than that of the concentration of ZnSO_4 . The temperature influenced not only the formation of aqueous two-phase system but also the extraction efficiency of CEX. The target was found to be preferentially extracted to the $[\text{Bmim}]\text{BF}_4$ -rich phase at the pH below 4.3. The partition of CEX to the top phase was enhanced by increasing the volume of $[\text{Bmim}]\text{BF}_4$, the concentration of ZnSO_4 , and temperature; however, the partition of CEX to the top phase increased by decreasing the pH.

1. Introduction

For its gentle conditions, high biocompatibility and capacity and high extraction yield, aqueous two-phase extraction (ATPE), a novel liquid-liquid extraction technique, has been widely applied in the separation, concentration, and purification of biomolecules, such as proteins, nucleic acids, enzymes, antibodies, and antibiotics [1–6]. Aqueous two-phase system (ATPS) consists of two immiscible aqueous solutions including two incompatible polymers or one polymer and one salt above a certain critical concentration. The main problems of these polymer-based ATPSs are high viscosity of the polymer and the difficulty to isolate the extracted molecules from the polymer phase by back extraction.

In recent years, ionic liquids (ILs) composed of an organic cation and either an organic or inorganic anion have received more and more attention owing to their special features such as nonvolatility, nonflammability, good solubility, and

tunable physical and chemical properties. Some ILs can form ATPSs with concentrated solutions of salts, and these ionic liquid-salt aqueous two-phase systems (ILATPSs) have many advantages, such as low viscosity, gentle biocompatible environment which is much suitable for extraction of bioactive substance, short process time, and high extraction efficiency. Furthermore, proteins [7, 8], amino acids [9], polyphenolic compounds [10], anionic dyes [11], and biomolecules [12–14] have been successfully separated and concentrated by the ILATPSs.

Cephalexin (CEX, Figure 1) is hydrosoluble cephalosporins antibiotics. CEX is an amphoteric compound, and its isoelectric point was 4.3 [15]. The conventional methods currently used to extract CEX include liquid-liquid extraction [16], ultrafiltration [17], deproteination by organic solvent [18], solid-phase extraction [19, 20], and liquid membrane extraction [21, 22]. However, these traditional methods increase the total analysis time; using poisonous volatile

FIGURE 1: The structure of cephalexin (the isoelectric point, $pI = 4.3$).

organic solvents or sample recovery is not always satisfactory. ATPE [23, 24], an alternative method, seems to be highly warranted from economic points of view. And in general, PEG-salt ATPS was employed for CEX extraction. Up to now, there have been few reports on using IL-based ATPS to extract CEX. Guo et al. [25] studied the partition of CEX by 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim]BF₄)-MgSO₄ ILATPS. In this study, the feasibility of extracting CEX by [Bmim]BF₄-ZnSO₄ ILATPS was first reported. In an acidic environment, the CEX was easy to be extracted to the [Bmim]BF₄-rich phase. In this paper, ZnSO₄, an acid salt, was chosen as the phase-separation salt. The factors of the volume of [Bmim]BF₄, the concentration of ZnSO₄, temperature, pH, and the volume of ZnSO₄ solution affecting the partition of CEX were investigated.

2. Experimental

2.1. Reagents and Instruments. [Bmim]BF₄ was purchased from Chengjie Chemical Co., Ltd. (Shanghai, China) with a quoted purity of greater 0.99 mass fraction. ZnSO₄ of analytical grade with a minimum mass fraction purity of 99.5% and methanol of HPLC grade were procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The standard sample of cephalexin was obtained from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All chemicals were used without further purification. The stock solution of CEX was prepared at a concentration of 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ and stored at 4°C in a refrigerator. Standard working solutions of CEX were prepared by appropriately diluting the stock solution. All the solutions were prepared using deionized water throughout the entire experiments.

The BS124S electron balance (Beijing Sartorius instrument Co., Ltd., Beijing, China) was used for weighting. The pH was measured by a digital pH meter (Shanghai LIDA Instrument Factory, China). The Anke TDL-4 centrifuge (Shanghai, China) was used for centrifuging. The temperature was controlled by a thermostatic water bath (Henan, China). An Agilent 1200 HPLC (Agilent, USA) equipped with a quaternary pump and an ultraviolet-visible (UV) detector was used for analysis of extraction products. The

instrument control and data processing were actualized by using Agilent ChemStation software.

2.2. Aqueous Two-Phase Extraction. Aqueous two-phase extraction experiments were implemented by mixing the stock solutions of CEX and ZnSO₄ in 10 mL graduated tubes. The concentrations of ZnSO₄ studied were 20~46%, and the mass of CEX in it was 100 μg . The pH of the mixed solutions was adjusted by adding hydrochloric acid and ammonia water into the stock solutions. Then different volumes of [Bmim]BF₄ (1~5 mL) were added to the above mixed solutions. After plenary mixing, the mixture was centrifuged at 2,000 rpm for 15 min and then placed into a thermostatic water bath at $35 \pm 0.05^\circ\text{C}$ for 30 min to reach thorough phase separation. The volumes of the top and bottom phases were recorded precisely.

The partitions of CEX between the two phases were characterized by extraction efficiency (E) and volume ratio (R). The partitioning parameters were calculated by the following equations:

$$E = \frac{C_t V_t}{m_s} \times 100\% \quad (1)$$

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

where C_t was the equilibrium concentration of CEX in the top phase, m_s was the mass of CEX initially added, and V_t and V_b were the volumes of the top and bottom phases, respectively.

2.3. Analytical Method. After equilibrium was reached, the top phase was directly injected to HPLC without any treatment. An analytical reversed-phase column (Eclipse XDB-C18 column, 250 mm $\times$ 4.6 mm, 5 μm , serial no. G1314B) was used for chromatographic separations. The ratio of mobile phase of methanol and water was 23:77 at the flow rate of 1.0 $\text{mL}\cdot\text{min}^{-1}$ and the column temperature of 25°C. The injected volume was 20 μL and the column effluent was monitored at a wavelength of 261 nm. The calibration curve for CEX obtained in the range of 0.10~100 $\mu\text{g}\cdot\text{mL}^{-1}$ was $A = 26.1428284 \times C - 2.4398705$ ($R = 0.99999$), where C was the concentration of CEX ($\mu\text{g}\cdot\text{mL}^{-1}$) and A was the peak area.

3. Results and Discussion

3.1. Effect of the Volume of [Bmim]BF₄ and the Concentration of ZnSO₄. The extraction efficiency of CEX in [Bmim]BF₄-ZnSO₄ ILATPS at different volumes of [Bmim]BF₄ and concentrations of ZnSO₄ was listed in Figures 2 and 3. In Figure 2, different volumes of [Bmim]BF₄ were added to 3 mL CEX solutions containing different amount of ZnSO₄. When the volume of [Bmim]BF₄ was 1 mL, the minimal concentration of ZnSO₄ which can form ATPS was 20%. When the concentration of ZnSO₄ was 38% and the volume of [Bmim]BF₄ was higher than 3 mL, or when the concentration of ZnSO₄ was 44% and the volume of [Bmim]BF₄ was greater than 2 mL, there was precipitation of ZnSO₄ generated. The extraction efficiency of CEX was influenced by the combined

FIGURE 2: The influence of the volume of [Bmim]BF₄ on the extraction efficiency (*E*, %) of CEX at different concentrations of ZnSO₄.

FIGURE 3: The influence of the concentration of ZnSO₄ on the extraction efficiency (*E*, %) of CEX.

impacts of the volume of [Bmim]BF₄ and the concentration of ZnSO₄. When the volume of [Bmim]BF₄ was 3 mL and the concentration of ZnSO₄ was 20%, the extraction efficiency of CEX was about 90%; nevertheless, when the volume of [Bmim]BF₄ was 2 mL and the concentration of ZnSO₄ was 38%, the extraction efficiency of CEX has already reached 90%.

As shown in Figure 2, when the concentration of ZnSO₄ was a fixed value, the extraction efficiency of CEX increased with the volume of [Bmim]BF₄ increasing. The hydration between salt ions and water molecules leads to the phase-forming salt dissolving in the bottom phase; meanwhile, the amount of free water molecules in the bottom phase reduces,

and it results in the exclusion of [Bmim]BF₄ and target. The amount of water molecules in the [Bmim]BF₄-rich phase rose with the increase of the volume of [Bmim]BF₄, and due to the good water solubility of CEX, the amount of CEX which can transfer to the top phase also increased.

The impact of salt on the partition of CEX in the [Bmim]BF₄-ZnSO₄ ILATPS is largely as a result of the salting-out effect of salt on the liquid-liquid equilibrium of ATPS. For instance, in Figure 2, when the volume of [Bmim]BF₄ was 1 mL, the extraction efficiency of CEX improved with the concentration of ZnSO₄ adding. Figure 3 showed the influence of the concentration of ZnSO₄ on the extraction efficiency of CEX when the volume of [Bmim]BF₄ was 2 mL. The extraction efficiency of CEX achieved the maximum value (92.64%) when the concentration of ZnSO₄ was 35%. Then with the growth in the concentration of ZnSO₄, the extraction efficiency slightly decreased. Similarly, from Figure 2, when the volume of [Bmim]BF₄ was 2 mL or 3 mL, the extraction efficiency of CEX was obviously the same trend. When the salt solution reached equilibrium concentration in the bottom phase, redundant salt will remove to the top phase as salt concentration increased. The salt dissolving in the top phase combined with water molecules through hydration, so the amount of free water molecules in the top phase reduced, leading to a small part of CEX retransferring to the bottom phase. Therefore, high concentration of salt was not conducive to the extraction of the target. From Figures 2 and 3, when the concentration of ZnSO₄ was a fixed value and the volume of [Bmim]BF₄ was from 1 mL to 5 mL, the extraction efficiency of CEX obviously increased, especially when the volume of [Bmim]BF₄ was from 1 mL to 2 mL. However, when the volume of [Bmim]BF₄ was a fixed value, the growth of extraction efficiency of CEX slowly increased with the increase of salt concentration. So the effect of the volume of [Bmim]BF₄ on the extraction efficiency of CEX was greater than that of the concentration of ZnSO₄.

3.2. Effect of Temperature. The influence of temperature on the extraction efficiency of CEX was discussed from 15°C to 55°C when the volume of [Bmim]BF₄ was 2 mL and the concentration of ZnSO₄ was 35%. After equilibrium reached, the changes of the volume of the top phase and the extraction efficiency of CEX were described in Figure 4. The extraction efficiency of CEX increased in the range of 15~35°C. The volumes of the [Bmim]BF₄-rich phase always enlarged as the temperature rose, and it means that more and more water molecules were transferred to the top phase. Therefore, the extraction efficiency of CEX should have increased from 15°C to 55°C. But at the temperature of 45°C, the extraction efficiency began to decline. This was because the CEX was unstable at relatively high temperature. At 55°C, more CEX was decomposed, and the extraction efficiency of CEX decreased to 64%. It can be seen that the temperature is an essential factor affecting the partition of CEX in [Bmim]BF₄-ZnSO₄ ILATPS.

3.3. Effect of the Volume of ZnSO₄ Solution. At the pH without adjusting, the influence of the volume of ZnSO₄

FIGURE 4: The influence of temperature on the extraction efficiency (E , %) of CEX and the volume of the top phase (V_t): ■, E (%); ▲, V_t (mL).

FIGURE 5: The influence of pH and the volume of ZnSO_4 solution on the extraction efficiency (E , %) of CEX: square, 1 mL $[\text{Bmim}]\text{BF}_4$ + 35% ZnSO_4 ; circle, 2 mL $[\text{Bmim}]\text{BF}_4$ + 35% ZnSO_4 ; triangle, 1 mL $[\text{Bmim}]\text{BF}_4$ + 46% ZnSO_4 ; star, pH = 1, 1 mL $[\text{Bmim}]\text{BF}_4$ + 35% ZnSO_4 ; diamond, pH = 1, 2 mL $[\text{Bmim}]\text{BF}_4$ + 35% ZnSO_4 .

solution on the extraction efficiency of CEX was discussed. There were three ILATPSs, respectively, forming by different volumes of ZnSO_4 solution in the same ZnSO_4 concentration with the same volume of $[\text{Bmim}]\text{BF}_4$. In detail, the three ILATPSs were composed of 35% ZnSO_4 + 1 mL $[\text{Bmim}]\text{BF}_4$, 35% ZnSO_4 + 2 mL $[\text{Bmim}]\text{BF}_4$, and 46% ZnSO_4 + 1 mL $[\text{Bmim}]\text{BF}_4$ with 100 μg CEX, respectively. From Figure 5, with the volume of the bottom phase (salt-rich phase) reducing, which also means the volume ratio increasing, the extraction efficiency of CEX always increased in the three ILATPSs. For example, when the volume of $[\text{Bmim}]\text{BF}_4$ was 1 mL and the concentration of ZnSO_4 was 35%, different volumes of ZnSO_4 solution with 100 μg CEX formed ATPSS by adding $[\text{Bmim}]\text{BF}_4$. The larger the volume of the bottom

phase is, the more water molecules it can get. Then CEX was more inclined to dissolve in the ZnSO_4 -rich phase, and the extraction efficiency decreased.

3.4. Effect of pH. Cephalixin is an amphoteric compound possessing amino group and carboxyl group. The ionization states of CEX molecules vary with pH in the aqueous solution [26, 27]. It is positively charged below its isoelectric point of 4.3 and negatively charged above pH 4.3 [15]. The pH of ZnSO_4 solutions is around 3.0. In this experiment, the pH from 1.0 to 5.0 was investigated. When the pH was adjusted to 6.0, there was sediment emerging in the bottom phase. The results showed that the extraction efficiency of CEX decreased from 99.31% to 91.22% at pH 1.0~4.0; then it immediately declined to 17.48% at pH 5.0. At pH values below 2.6, the predominant form of CEX was cationic. The interaction between the CEX cationic and H^+ in the bottom phase was the mainly driving force for extraction. At pH < 2.6, almost all of the CEX was extracted to the top phase. Then with the pH rising, the extraction efficiency slightly descended. At pH 5.0 which is higher than the isoelectric point of CEX, it is negatively charged. And then it was more inclined to remain in the ZnSO_4 -rich phase. In Figure 5, when the volume of $[\text{Bmim}]\text{BF}_4$ was 1 mL, the extraction efficiency of CEX increased with the volume of ZnSO_4 solution decreasing at pH 1.0. In comparison with the same ATPSSs of unadjusted pH, the extraction efficiency of CEX at pH 1.0 was much higher when the volume ratio was similar. When the volume of $[\text{Bmim}]\text{BF}_4$ was 2 mL, the extraction efficiency had little difference which was all greater than 98% at pH 1.0. So the lower the pH is, the higher extraction efficiency the CEX has.

4. Conclusions

In this paper, the $[\text{Bmim}]\text{BF}_4$ - ZnSO_4 ILATPS was applied to separate hydrosoluble antibiotics and the partitioning behavior was discussed at great length. The distribution of CEX in the ILATPS was influenced by the volume of $[\text{Bmim}]\text{BF}_4$, the concentration of ZnSO_4 , temperature, pH, and the volume of ZnSO_4 solution. Without pH adjusted, the impact of $[\text{Bmim}]\text{BF}_4$ and ZnSO_4 on the extraction efficiency of CEX was as follows: the volume of $[\text{Bmim}]\text{BF}_4 >$ the concentration of ZnSO_4 . CEX was preferentially extracted to the $[\text{Bmim}]\text{BF}_4$ -rich phase at the pH below its isoelectric point of 4.3. At pH 1.0, the extraction efficiency of CEX increased with the increase of the volume of $[\text{Bmim}]\text{BF}_4$ and the decrease of the volume of ZnSO_4 solution; however, the extraction efficiency had little difference at different volumes of ZnSO_4 solution when the volume of $[\text{Bmim}]\text{BF}_4$ was 2 mL.

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