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Hydrophobic ionic liquids-assisted polymer recovery during penicillin extraction in aqueous two-phase system

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ABSTRACT

In this study, terminal modified poly(ethylene glycerol) (PEG) was employed as the phase-forming polymer to construct aqueous two-phase (ATP). After the phase equilibrium, 95.8% penicillin could be extracted into imidazole-terminal PEG-rich phase efficiently. Imidazole-terminal PEG (I-PEG) was separated from the aqueous phase containing penicillin to hydrophobic ionic liquid phase at basic pH, and a weak acidic aqueous phase was then employed to recover the I-PEG from the hydrophobic ionic liquid phase into water at pH 5.5–6. The recycle of the polymer was achieved with the aid of hydrophobic ionic liquids.

1. Introduction

Aqueous two-phase (ATP) is an efficient implement for separation of various substrates [1–5]. By introduction of two kinds of polymers (PEG and dextran) or one polymer and one salt (PEG and phosphate salt) into water, two immiscible aqueous phases could be formed [6]. Each phase of ATP contains one primary polymer with over 70% water. Due to the low-interfacial tension between the two conjugated phases, ATP facilitates the separation of biomaterials such as small molecular compounds [7], proteins [8,9] and antibiotics [10] without the problem of pH-adjustment and organic solvent pollution [11].

Despite these advantages, ATP has not found extensive industrial application. One of the reasons is that it's difficult to recover the phase-forming polymers from the aqueous phase containing extract [2,3]. As a result, the residual polymer in aqueous solution reduces the purity of products and increases the operational cost. Various methods have been developed to deal with the issue, including centrifugation [12], electrophoresis [13], and precipitation by organic solvent [14], as well as pH-sensitive copolymer [15,16] and temperature-sensitive copolymer [17–19].

In this study, imidazole-terminal poly(ethylene glycerol) (I-PEG) (Fig. 1) is used as phase-forming polymer to extract penicillin in ATP system. Hydrophobic ionic liquid [Bmim]PF_6 (1-butyl-3-methylimidazole hexafluoraphosphate) (Fig. 1) was chosen to recover the phase-forming polymer from ATP using pH difference as the driving force. Ionic liquids are room-temperature liquid salts with neglectable vapor pressure and tunable structure, which facilitates their applications in catalysis, extraction and material application [20–26]. In this study, ionic liquids could recover the imidazole-terminal PEG at mild pH with high efficiency. To our knowledge, this is the first report of applying ionic liquids to recover phase-forming materials of ATP.

2. Experimental

2.1. Materials

Bromobutane, Na_2HPO_4, imidazole, PEG 4000, thionyl chloride, A.R. were purchased from Beijing Chemical Company. KPF_6, purity >99%, from Meihao Detergent Company, Jiangsu Province. *N*-Methylimidazole, purity >99%, from Kaile Chemical Plant, Zhejiang Province. Succinic anhydride, purity >99%, purchased from Sigma–Aldrich. Penicillin G was received from North China Pharmaceutical Group as a gift. All other chemicals were purchased from commercial suppliers and were of the highest purity available.

2.2. The synthesis of ionic liquids

1:1.15 mol ratio *N*-methylimidazole and bromobutane were added into a flask and heated at 70 °C for 24 h. After cooling down, equal volume ethyl acetate was introduced to wash the sample twice. The sample was evaporated to get the product [Bmim]Br (1-butyl-3-methylimidazole bromide). [Bmim]Br was metathesis with PF_6^- anion in aqueous solution at 40 °C for 24 h. The product was extracted with CH_2Cl_2 and washed twice with water. After evaporation, the product [C_4mim]PF_6 was obtained.

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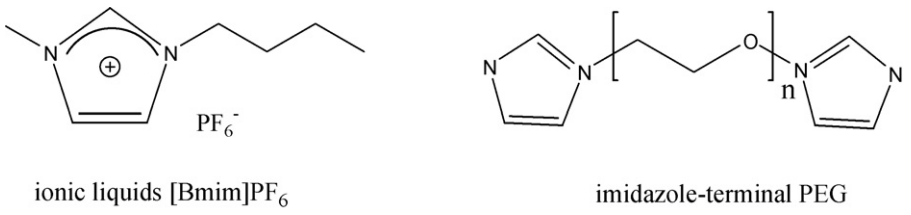

Fig. 1. Structure of ionic liquid [Bmim]PF_6 and I-PEG.

2.3. The synthesis of I-PEG and C-PEG

The method to produce functionalized PEG was according to Bückmann's reference [27]. 0.01 mol PEG 4000 was dissolved in 100 ml toluene in the presence of 50% Et_3N. 0.03 mol thionyl chloride was added dropwise in 30 min under reflux [28]. The sample was heated for 4 h. After evaporation in vacuo, the product PEG chloride was obtained (Fig. 2).

To prepare I-PEG, equal mole of PEG chloride and imidazole were dissolved in toluene, and the sample was stirred at 60 °C for 6 h. After evaporation and gel chromatography (HPLC-SEC, GF-250, 1 ml/min, CH_2Cl_2 as flux), the pure product I-PEG was obtained.

In order to prepare C-PEG (PEG with carboxylic acids terminals) for comparison, 0.01 mol PEG 4000 was dissolved in 50 ml toluene. 0.03 mol succinic anhydride was added, and the mixture was stirred for 5 h in an oil bath at 150 °C. The mixture was cooled, taken up in CH_2Cl_2, and the polymer was precipitated by diethyl ether. The product was recrystallized twice from CH_2Cl_2/ether.

2.4. Measurement of the phase diagram of ATP

The phase diagram of ATP was prepared according to the "cloud point" method [29]. Simply, a concentrated aqueous solution of polymer or ionic liquids was added dropwise into an aqueous solution containing preweighted Na_2HPO_4. The turbidity of mixture was inspected with UV (Lambda bio 40, PerkinElmer Company) at 600 nm until full phase separation happened. The concentration of polymer or ionic liquids was recorded. The procedure was repeated until a sufficient number of points for the construction of the binodal curve were obtained.

2.5. Partitioning of penicillin in ATP

0.1 g penicillin was added into 10 ml water containing certain amount of polymer (or ionic liquids) and Na_2HPO_4. The mixture was stirred well and centrifugated at 4000 rpm for 5 min. The concentration of penicillin in the polymer-rich and salt-rich phase was checked with HPLC (HP1100 system, Agilent Corporation) equipped with 250 mm × 4 mm Zorbax SB-C18 column and DAO detector at 254 nm. The mobile phase was a mixture of methanol and 0.05 M phosphate (36:64 v/v) with a flow rate at 1 ml/min. The concentration of polymer in the two phases was measured using gel chromatogram. The partitioning ratio D was calculated from Eq. (1):

$$D = \frac{\text{penicillin concentration in the polymer-rich phase}}{\text{penicillin concentration in the salt-rich phase}} \quad (1)$$

Phase transferring of polymer between [Bmim]PF_6 and aqueous phase: after phase equilibrium of ATP, the polymer-rich phase (including PEG, I-PEG and C-PEG) containing penicillin was taken out and mixed with equal-volume hydrophobic [Bmim]PF_6 phase or organic solvent (for the comparation). The pH was increased by adding $NH_3 \cdot H_2O$ solution under the inspection of pH-indicator. The system was stirred well and centrifugated at 4000 rpm for 5 min. The concentration of penicillin in the ionic liquids and water phase were checked with HPLC. The concentration of polymer in the two phases was measured through gel chromatogram. The partitioning ratio of polymer and penicillin was obtained in Eq. (2):

$$D = \frac{\text{solute concentration in ionic liquids phase (or organic solvent)}}{\text{solute concentration in water phase}} \quad (2)$$

For back-extraction of polymer from [Bmim]PF_6 phase, a fresh water phase was introduced, and its pH was lowered by phosphate

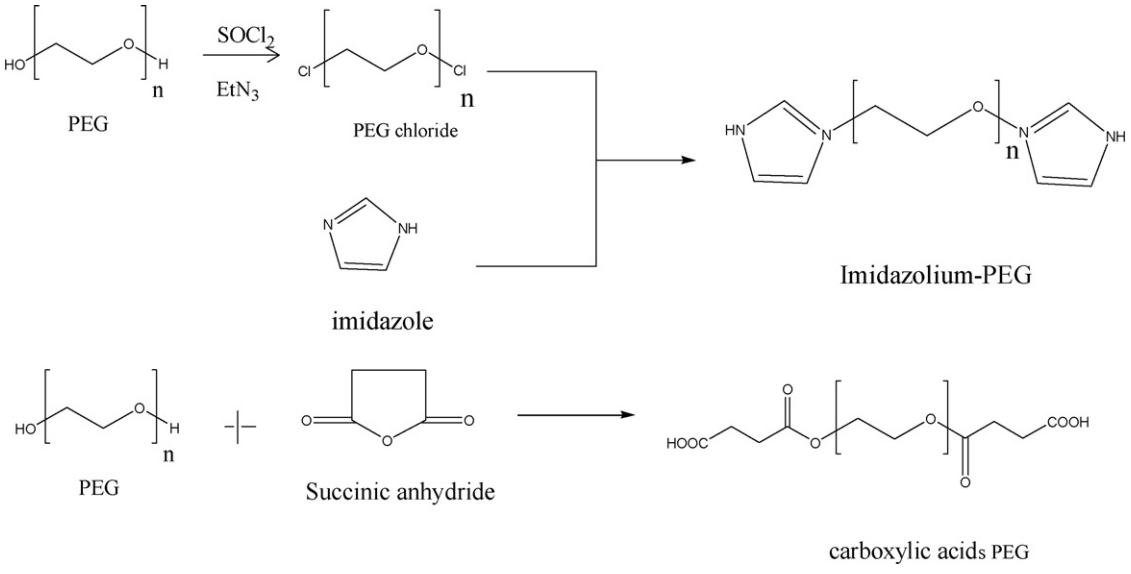

Fig. 2. Preparation of functionalized PEG.

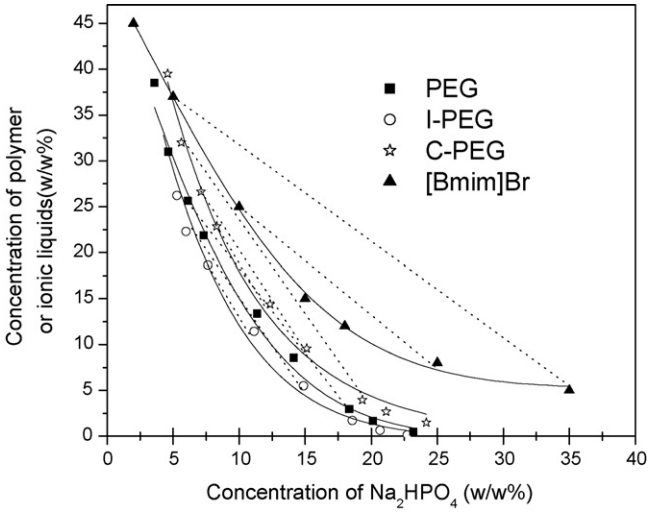

Fig. 3. Phase diagram of different ATP systems.

acid under the inspection of pH-indicator. The concentration of polymers between the two phases was inspected with gel chromatogram. The partitioning ratio of polymer and penicillin was obtained in Eq. (2).

3. Results and discussion

3.1. Phase diagram of ATP

Fig. 3 gives the phase diagram of four ATP systems with different phase-forming materials including PEG, I-PEG, C-PEG and [Bmim]Br at pH 7. The dot lines refer to the tie line of ATP systems. It is found that the phase-separation in I-PEG ATP is easier than that of PEG. This observation could be attributed to the hydrophobicity of imidazole ring at neutral pH [30] (exact analogy is *N*-alkylimidazole, which has a similar *N*-substituted group as imidazole-terminal PEG), which decreases the compatibility of I-PEG with water and facilitates its phase separation into ATP. On the contrary, the phase-separation of C-PEG ATP is postponed due to the hydrophilic nature of carboxylic acid terminals. The phase separation of [Bmim]Br ATP is much difficult comparing with other polymer systems due to the extreme hydrophilic nature of [Bmim]Br [20,26].

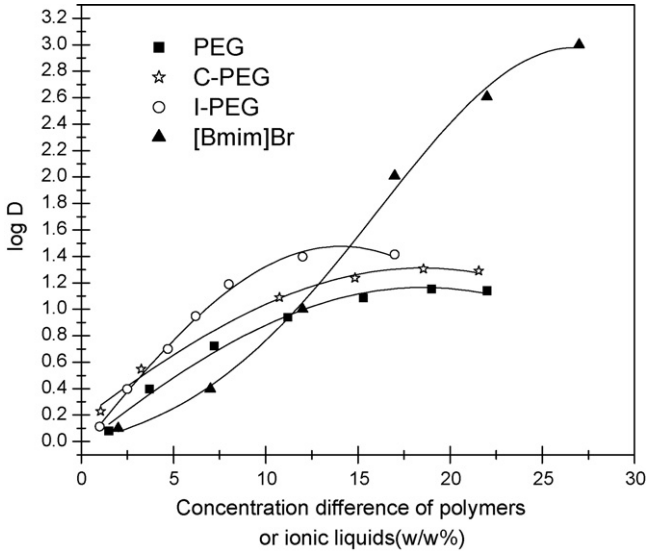

Fig. 4. Partitioning ratio of penicillin in ATP systems.

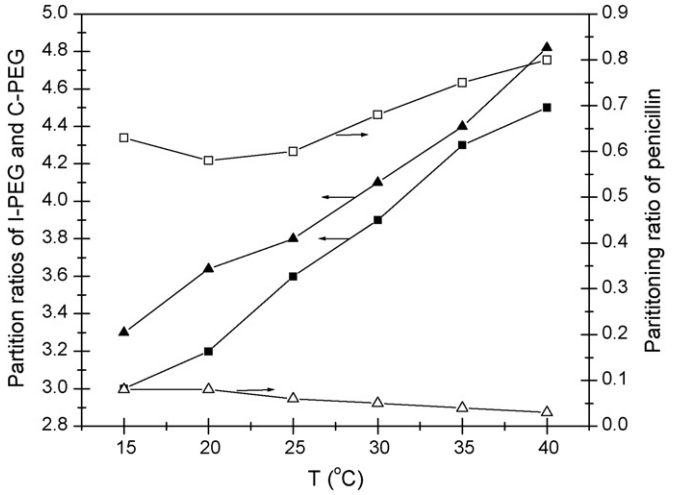

Fig. 5. Partitioning ratios of polymer and penicillin between [Bmim]PF_6 and aqueous salt solution: (■) and (□) C-PEG at pH 4; (▲) and (△) I-PEG at pH 6.

3.2. Separation of penicillin in ATP

Fig. 4 illustrates the partitioning ratio of penicillin in several ATP systems including PEG, I-PEG, C-PEG and [Bmim]Br. The *X*-axis refers to the concentration difference of phase-forming materials between the polymer (or ionic liquids)-rich phase and salt-rich phase in these ATP systems (the tie line length, TLL). The *Y*-axis represents the logarithm of partitioning ratio of penicillin in these systems. The partitioning ratio of penicillin in I-PEG system exceeds 120% over that of PEG system. This may because of the π–π strong interactions between imidazole ring of I-PEG and the aromatic ring of penicillin [31]. While for C-PEG ATP, penicillin separation efficiency is lower than that in I-PEG system due to the unfavorable electrostatic repulsion between the two components with similar charge.

The phenomena in [Bmim]Br ATP is quite different. Its partitioning ratio is lower at low-[Bmim]Br concentration difference (<15%) than other systems, but increases markedly when the concentration difference of ionic liquids exceeds 20%. It reaches 1000 at 40% concentration difference of ionic liquids. The result may be due to

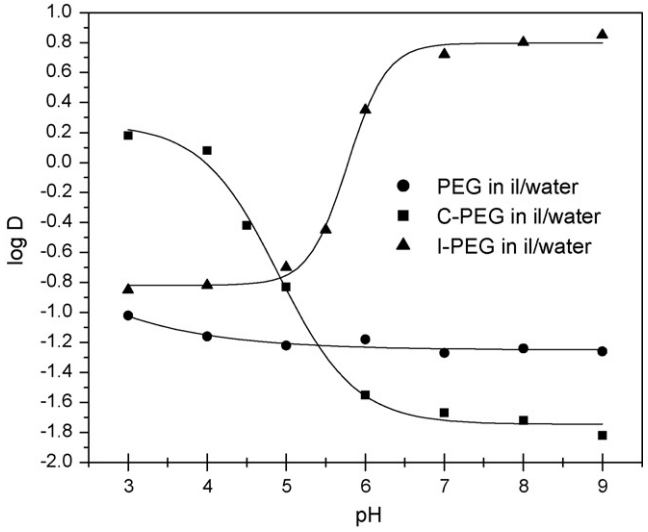

Fig. 6. Partitioning ratio of polymers between [Bmim]PF_6/water at different pH (some PEG chains are broken pH 3, and their partitioning ratio are given as the average).

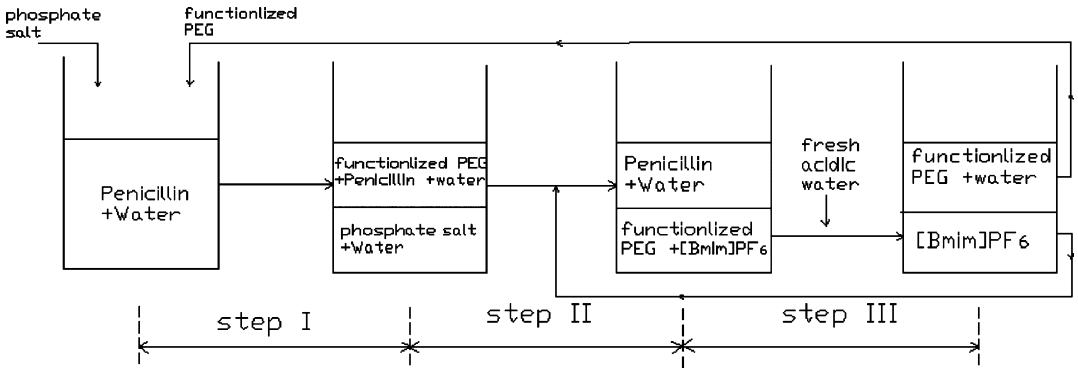

Fig. 7. Integrated processes for separation of penicillin and recovery of polymer.

the abundance of imidazole rings in ionic liquids-rich phase, which improves the affinity for penicillin molecules. Although the efficiency for penicillin separation of I-PEG ATP is inferior to that of [Bmim]Br system, it is satisfying for practical application because over 95.8% penicillin is extracted into the polymer-rich phase.

3.3. Separation of polymers into hydrophobic [Bmim]PF_6 phase

Fig. 5 shows the partitioning ratio of I-PEG and C-PEG between [Bmim]PF_6 and water phases, as well as the partitioning ratio of penicillin at different temperature. Hydrophobic ionic liquid [Bmim]PF_6 is introduced in equilibrium with the polymer-rich phase of ATP to separate the polymers from the aqueous phase containing penicillin. It can be seen from Fig. 6 that the partitioning ratio of C-PEG in [Bmim]PF_6/water system at pH 4 increases from 3.0 to 4.5 when the temperature rises up from 15 to 40 °C. This may be due to the improved hydrophobicity of PEG at high temperature [32], which increases the partitioning ratio of functionalized PEG. The I-PEG in [Bmim]PF_6/water system at pH 6 has similar separation efficiency as that in C-PEG system at pH 4. This phenomenon can be explained by the result of Huddleston et al. that hydrophobic ionic liquids phase exhibits high affinity for ionic compounds when they are neutralized [33]. For example, the distribution ratio (D) of benzoic acid is higher than 1 at pH 1.77 and 6.54, while it falls below 1 at pH 11. In contrast, aniline has $D < 1$ at pH 1.77, $D = 1$ at pH 6.54 and $D > 10$ at pH 11. The results suggest that hydrophobic ionic liquids show high pH-sensitivity to ionic compounds. As a result, I-PEG is efficiently transferred into hydrophobic ionic liquids at basic pH, leaving a clarified, penicillin-rich feed stream suitable for further purification. In addition, imidazole-containing ionic liquids show natural compatibility with the imidazole-capped I-PEG, which provides additional driving force for separation of I-PEG into ionic liquids phase.

On the other hand, the partitioning ratio of penicillin in the two systems is quite different. The partitioning ratio of penicillin in [Bmim]PF_6/water system containing I-PEG decreases from 0.08 to 0.03 when the temperature increases from 15 to 40 °C, which is in line with Visser's observation that charged substrate is hard to be partitioned into hydrophobic ionic liquids phase [34]. On the contrary, penicillin in C-PEG system has noticeable residual concentration in hydrophobic ionic liquids phase. The situation is consistent with the observation in organic solvent/water system in the presence of amine or phosphorus coextractants [35], in which penicillin could be extracted into organic solvent at pH 4–5. The imidazole groups facilitate the separation of I-PEG into ionic liquids phase at high pH, leaving penicillin in water. As a result, the two materials in ATP could be efficiently separated.

3.4. Recovery of polymer into fresh water

Fig. 6 shows the partitioning ratio of PEG, I-PEG and C-PEG between [Bmim]PF_6 and fresh water at different pH. It is found that PEG always favors the aqueous phase. As for C-PEG, phase-transfer from hydrophobic phase to water happened at higher pH (pH >5.0). On the other hand, the partitioning ratio of I-PEG in ionic liquids/water increases with pH and reaches equilibrium at pH 8–9. Imidazole is a weak base with pK_b of 6.8 (the pK_b of *N*-methylimidazolium is 6.95 [36]). Imidazole terminal makes PEG reside in hydrophobic phase at basic condition and transfer back into water phase at weak acidic condition ($D = 0.15$ at pH 5). The use of imidazole as terminal group of PEG and hydrophobic ionic liquids in equilibrium with water phase increases the pH necessary for phase-transfer of PEG. Therefore, the pH range for the recovery of the polymer in our system is relatively broad. The problem of PEG hydrolysis can be partially solved because PEG chain is unstable in acid, rather than in base [37], thus the lifespan of PEG could be prolonged.

4. Conclusions

A three-step process is constructed in this work, which contains imidazole-terminal PEG and hydrophobic ionic liquids. The details are shown in Fig. 7. In Step I, I-PEG constructs an ATP with the aid of Na_2HPO_4 salt, which extracts penicillin into the polymer-rich phase with high efficiency. In Step II, hydrophobic ionic liquids [Bmim]PF_6 phase is introduced into the polymer-rich phase of ATP, and extracts I-PEG from penicillin at pH 8–9. In Step III, I-PEG in [Bmim]PF_6 phase is recovered into a fresh water solution at pH 5.5–6. In comparison with other separation systems, the three-step process integrated functionalized polymer and ionic liquids phase. It can not only separate penicillin efficiently, but also recover and reuse the imidazole-terminal PEG at mild pH. Moreover, the integrated system is a "green" process using only recyclable PEG and ionic liquids.

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