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Selective Separation of Zwitterionic Phospholipid Homologues with Functional Ionic Liquids as Extractants

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Natural zwitterionic compounds are widely used as surfactants, drugs and food additives. However, obtaining high-purity zwitterions from biomass remains challenging because of the presence of structurally similar homologues in plants. Here, we developed a novel extraction method to separate zwitterionic phospholipid homologues using ionic liquids (ILs) as extractants. A large distribution coefficient and excellent separation selectivity for phosphatidylcholine (PC) were achieved with hydroxyl-functionalized and carboxyl-functionalized ILs as the extractants. An effective IL-cosolvent extraction strategy was employed in this work to reduce the consumption of IL and improve the extraction efficiency. Additionally, the underlying extraction mechanism was explored using *ab initio* calculations and dynamic light scattering. The results indicated the existence of multiple hydrogen-bonding interactions between the IL and both the negative and positive moieties of the zwitterion, and the formation of micelles in the IL-cosolvent mixture was also observed. In addition, the effects of the structure and concentration of ILs and the temperature on extraction performance were investigated, and the feasibility of recovery of ILs by electrodialysis was evaluated.

Introduction

Zwitterions are neutral compounds having formal unit electrical charges of opposite sign. They are sometimes referred to as inner salts. This combination of oppositely charged moieties grants the compounds unique physicochemical properties, such as self-assembly behaviour and ultra-hydrophilicity.[1] Zwitterionic compounds have been widely used as surfactants, drugs, food additives and catalysts. In general, zwitterions are obtained from natural products and chemical syntheses. Natural zwitterions are usually extracted from biomass. For example, zwitterionic phospholipids are separated from soybean or egg,[2] and betaine is obtained from the roots, stems and leaves of beets, sunflower seeds, etc.[3] The zwitterions extracted from natural resources are considered as healthy and green substances, which makes them more easily accepted as drug and food additives. However, the production of high-purity zwitterionic compounds suffers from a long-standing problem that the product of interest often appears in a mixture with various structurally similar homologues, resulting in numerous difficulties in separation processes. Therefore, there is a great need to develop efficient methods for the separation of zwitterionic homologues.

A representative class of natural zwitterionic compounds consists of phospholipid homologues from soybeans or eggs, including phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Fig. 1). Among those homologues, PC was reported to possess various biological activities, such as regulating serum lipid levels, protecting the heart and enhancing memory.[2] Therefore, PC is known as the third nutrient in addition to protein and vitamins, and almost 90% of the exogenous choline of the human body is provided by PC.[4] PC is also used extensively as a natural emulsifier, wetting agent and baking improver.[5]

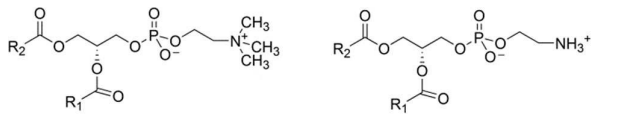

Fig. 1 The structures of PC and PE. (R_1 and R_2 refer to C_{14}-C_{20} saturated or unsaturated fatty acids.)

Thus far, several methods for the separation of phospholipid homologues have been developed, such as low-pressure column chromatography, simulated moving bed chromatography, membrane separation and supercritical fluid extraction.[6,7] Chromatographic technologies are feasible methods for the separation of phospholipid homologues. However, these methods bear the drawback of limited capacity, high cost and large consumption of solvents. The purity of PC produced by membrane separation and supercritical fluid extraction is moderate due to their insufficient molecular recognition ability and limited number of theoretical plates. Therefore, the

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development of an efficient and economical method for the separation of phospholipid homologues remains challenging.

Ionic liquids (ILs) have drawn much attention in the past decade due to their unique properties, such as negligible vapour pressure, high thermal and chemical stability, and the feasibility of structural and functional tunability to meet the requirements for specific tasks, which leads them to be regarded as green solvents.[8] Furthermore, ILs can easily form biphasic systems with water or weak polar organic solvents because of their relatively high cohesive energy.[9,10] Therefore, ILs have been widely applied in various extraction separation processes as replacements for organic solvents, such as liquid-liquid extraction,[11-16] biphasic aqueous extraction,[17,18] membrane extraction,[19-22] etc. In those processes, ILs generally demonstrate improved selectivity over traditional organic extractants because of their multiple solvation interactions, enhanced hydrogen-bond basicity and hydrogen-bonding interaction.[23-27] Until now, IL-based extraction has been successfully used in the separation of various compounds, including macromolecules,[28-30] biomolecules and drugs,[31,32] metal ions,[33-35] organic solutes (e.g., aromatics, aliphatics, phenols, gas and organic acids),[36-41] etc, but their application in the extraction of zwitterions has rarely been reported,[42,43] and the selective separation of zwitterionic homologues was still unexplored.

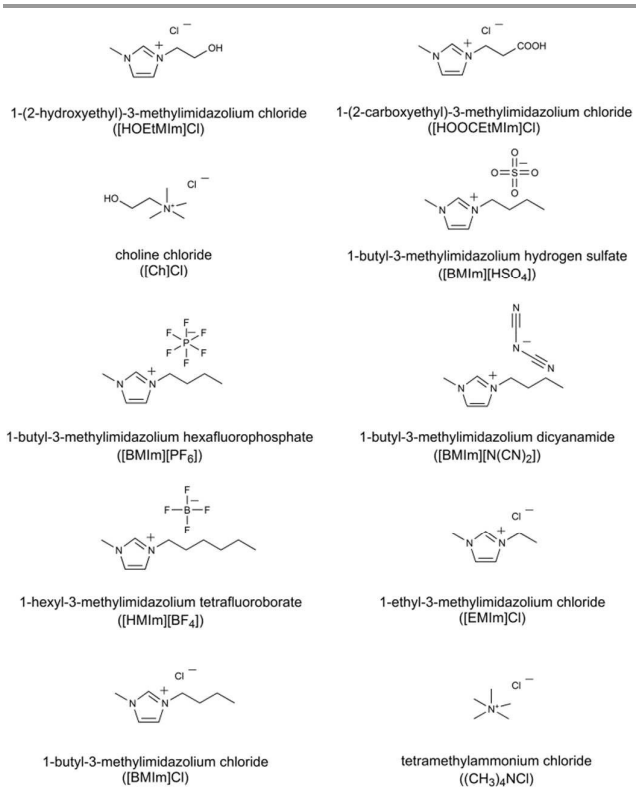

Fig. 2 ILs used in this study.

Herein, we developed a novel extractive method for the separation of phospholipid zwitterionic homologues with ILs as extractants (Fig. 2). Interestingly, we found that the hydroxyl-functionalized ILs presented excellent extraction performance and the cation and anion of hydroxyl-functionalized ILs can participate in selective H-bonding with the negative and positive moieties of phospholipid zwitterions, respectively; more importantly, the functional ILs can distinguish the minor differences among zwitterionic homologues and the targeted PC could be preferentially separated from the mixture of phospholipid homologues. The effects of the structure and concentration of ILs and the temperature on extraction performance were investigated. In addition, the extraction mechanism was analysed using *ab initio* calculation and dynamic light scattering (DLS).

Materials and Methods

Materials

The ILs used in this study were purchased from Lanzhou Green Chemistry and Catalysis, LICP, CAS (China), including 1-(2-hydroxyethyl)-3-methylimidazolium chloride ([HOEtMIm]Cl, 99%), 1-carboxyethyl-3-methylimidazolium chloride ([HOOCEtMIm]Cl, 98%), 1-butyl-3-methylimidazolium hydrogen sulfate ([BMIm]HSO_4, 97%), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIm]PF_6, 99%), 1-butyl-3-methylimidazolium dicyanamide ([BMIm]$N(CN)_2$, 98%), 1-hexyl-3-methylimidazolium tetrafluoroborate ([HMIm]BF_4, 99%), 1-ethyl-3-methylimidazolium chloride ([EMIm]Cl) and 1-butyl-3-methylimidazolium chloride ([BMIm]Cl). The ILs had been treated by the oil pump for 8 h and then were put into the vacuum freeze-drying oven for 48h under the pressure of 1 Pa to remove water before using as the extractants and the water contents of these ILs were below 0.5% (mass fraction). Phosphatidylcholine (PC, 98%) was purchased from the Lipoid Group (Germany). *N*-hexane, methanol, *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and methanoic acid were of analytical grade and were obtained from Sinopharm Group Co. Ltd (China). The diols, 1,2-propanediol of analytical grade, 1,3-propanediol (98%), 1,3-butanediol (99%) and choline chloride ([Ch]Cl, 98%), were purchased from Aladdin (China). 2,3-Butanediol (98%) and tetramethylammonium chloride (>98.0%) were obtained from J&K Chemical (China). Other chemicals (analytical grade) were all commercially obtained and used without further purification. The phospholipid raw materials were purchased from Beijing Meiyasi Biotechnology Co., LTD.

Methods

Extraction equilibrium procedure. The extraction experiments were carried out using procedures similar to those reported in the literature.[30,36,44] A known amount of real sample of soybean phospholipids was dissolved in *N*-hexane (15 mg/mL), and aliquots of this solution were mixed with an equal volume of extraction solvent in an Erlenmeyer flask. The flask was shaken for 2 h in a thermostatic rotary shaker at 220 r/min and then settled for 2 h at the same temperature. The shaking time was sufficient for extraction equilibrium. Samples were

taken by pipette without disturbing the phase boundary. The light phase was diluted with *N*-hexane and the heavy phase was diluted with ethanol and *N*-hexane (1/1, v/v) for the HPLC analysis. The extraction equilibrium experiments were repeated three times, and the relative uncertainties of the distribution coefficient were within 5%.

HPLC analysis. The HPLC system consisted of an autosampler, a Waters Sunfire silica gel column (5 μm, *Φ*3.9 mm×150 mm), a Waters 1525 binary pump and a Waters 2487 dual *λ* absorbance detector. The mobile phase was a mixture of isopropanol, water and *N*-hexane (42/5/53, v/v/v). The detection of PC was performed at 205 nm. The column temperature was 35 °C. The distribution coefficient (*Di*) and the selectivity of solute *i* to solute *j* were calculated according to eqs (1) and (2),

$$\boldsymbol{D_i = C_i^e / C_i^r} \quad (1)$$

$$\boldsymbol{S_{i/j} = D_i / D_j} \quad (2)$$

where $\boldsymbol{C_i^e}$ and $\boldsymbol{C_i^r}$ refer to the mass fraction of solute in the extraction phase and in the raffinate phase, respectively.

Dynamic light scattering. A Malvern Zetasizer Nano ZSP was employed to measure the size of the micelles in solution. Each measurement was repeated three times to ensure accuracy.

Quantum chemistry calculation. The geometry optimization calculations were performed using Gaussian 09 software.[45-47] Hybrid density functional theory (DFT) incorporating Becke's three-parameter exchange functional with Lee, Yang and Parr's (B3LYP) correlation functional and the 6-31+G(d,p) basis set was employed.[27] Because ILs are composed of cations and anions, the relative positions of the cations and anions should be considered when optimizing the structure of ILs. When we defined the initial configuration of ILs, the anion was placed in multiple regions around the cation, and then each possible structure was optimized using Gaussian 09 software. The vibrational frequencies of the optimized configurations of the ILs were calculated to confirm that a stable configuration was obtained. After comparing the heats of formation of all stable structure, the configuration that had the lowest heat was the optimal one for the IL.[48,49]

Results and Discussion

Extraction Separation of Phospholipids Using Organic Solvents and ILs as Extractants

In this work, hexane was selected as the non-polar phase because of its low toxicity and good solubility for phospholipids, while a series of polar organic solvents and ILs were chosen as extractants. In particular, because PC has a strong H-bonding acceptor in the P=O group, several functional ILs with carboxyl and hydroxyl groups were selected for the separation of phospholipid homologues to improve the H-bonding donor ability of the ILs. Meanwhile, several common imidazole-based ILs were also selected to further demonstrate the results. We also selected several polar organic solvents to compare the extraction performance of organic solvents and ILs.

The distribution coefficients of PC (D_{PC}) and PE (D_{PE}) and the selectivity of PC to PE ($S_{PC/PE}$) in hexane-organic solvent biphasic systems have been determined using a real sample of soybean phospholipids powder, and the results are presented in Fig. 3.

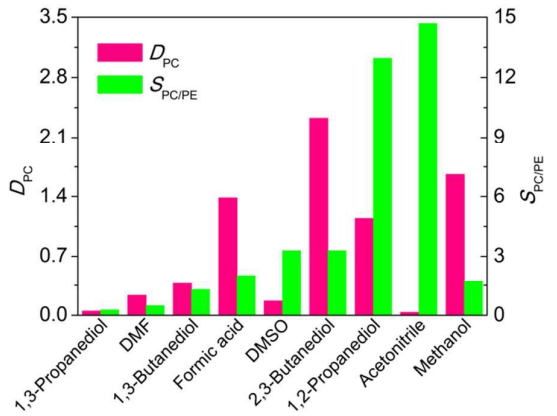

Fig. 3 Distribution coefficients and selectivities of PC to PE in hexane-organic solvents biphasic systems.

As the results in Fig. 3 illustrate, it is obvious that PC exhibited higher values of distribution coefficients than PE in all of the extractants except for DMF and 1,3-propanediol, indicating that PC was selectively separated from PE by liquid-liquid extraction. Among all of the organic extractants, formic acid (D_{PC}=1.38), 1,2-propanediol (D_{PC}=1.14) and 2,3-butanediol (D_{PC}=2.33), had relatively high D_{PC} values, while the D_{PC} values in polar aprotic solvents, including acetonitrile, DMSO and DMF, were less than 0.23. This finding is partially attributed to the relatively strong H-bonding acidity of formic acid, 1,2-propanediol and 2,3-butanediol, which is helpful in forming selective H-bonding interaction with PC. In terms of the selectivity of PC to PE, the $S_{PC/PE}$ values obtained for 1,2-propanediol and acetonitrile were significantly higher than those obtained for the other organic extractants. For example, the $S_{PC/PE}$ values were up to 12.96 and 14.69 for 1,2-propanediol and acetonitrile, respectively, while that of 1,3-propaneidol was only 0.27, which is far less than the former values. It is worth noting that although 1,2-propanediol and 1,3-propanediol both have two hydroxyl groups, their $S_{PC/PE}$ values were entirely different, indicating that the position of the hydroxyl group had a crucial influence on the extraction separation. We will discuss the underlying mechanism in the following section.

The separation performances of ILs were also evaluated in this work. The extraction data were presented in Fig. 4. First, four imidazolium-based ILs with different anions were chosen to extract phospholipids. However, as shown in Fig. 4, the values of D_{PC} in all of the common imidazolium-based ILs with different anions were less than 0.34. The result is probably because phospholipids are weak polar compounds while the polarity of ILs is very strong,[50,51] which contradicts the solvation of PC. Therefore, considering the strong H-bonding acceptor ability of PC, several ILs with H-bonding donors, such as carboxyl-functionalized ILs and hydroxyl-functionalized ILs,

were utilized to separate PC and PE. However, the viscosity of those functional ILs are so large that the ILs are in the solid state even at room temperature, which limits the mixing and transport properties of extraction processes. Thus, strong polar organic solvents, such as methanol, DMF and 1,2-propanediol, were used as cosolvents to reduce the viscosity of the functionalized ILs, and the mixture of cosolvents and ILs were utilized as composite extractants (Fig. 4).

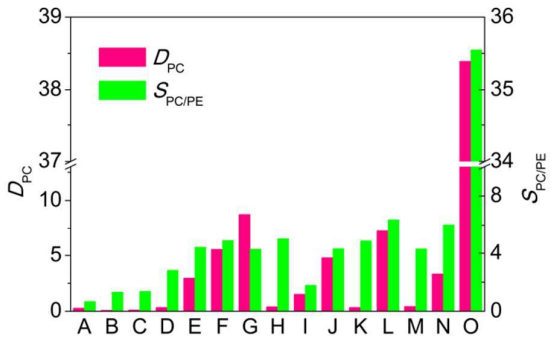

Fig. 4 Distribution coefficients and selectivities of PC to PE in hexane-IL or hexane-IL-cosolvent biphasic systems. (The mole concentration of ILs in organic solvents was 0.05. A: [BMIm][HSO_4]; B: [BMIm][$N(CN)_2$]; C: [HMIm][BF_4]; D: [BMIm][PF_6]; E: $(CH_3)_4NCl$-methanol; F: [EMIm]Cl-methanol; G: [BMIm]Cl-methanol; H: [HOOCEtMIm]Cl-1,2-propanediol; I: [HOOCEtMIm]Cl-DMF; J: [HOOCEtMIm]Cl-methanol; K: [Ch]Cl-1,2-propanediol; L: [Ch]Cl-methanol; M: [HOEtMIm]Cl-1,2-propanediol; N: [HOEtMIm]Cl-DMF; O: [HOEtMIm]Cl-methanol)

It is very interesting that the hydroxyl-functionalized IL-cosolvent mixture and the carboxyl-functionalized ILs-colvents mixture demonstrated excellent extraction performance. For example, the D_{PC} was surprisingly 38.39 and the $S_{PC/PE}$ reached up to 35.55 when using a [HOEtMIm]Cl-methanol mixture (the mole fraction of IL is 5%) as the extractant, which were 33.56 and 2.7 times larger than those of 1,2-propanediol, respectively. Compared with the carboxyl-functionalized ILs, the extractants of the hydroxyl-functionalized IL-cosolvent mixture showed superior extraction performance. Specifically, while the D_{PC} was over 38 when using a 5% [HOEtMIm]Cl/methanol mixture as the extractant, that of PE did not reach 2, indicating that the majority of PC were extracted into the IL phase and the majority of PE remains in the hexane phase. Non-functional imidazole-based ILs with chloride anion, [BMIm]Cl and [EMIm]Cl, showed better extraction performance than common ILs with other anions, but the D_{PC} and $S_{PC/PE}$ were still lower than [HOEtMIm]Cl. The results indicated that both the chloride anion and hydroxyl influenced the interactions between PC, PE and ILs. Besides, the extraction data with ILs/methanol mixture as the extractants were better than the previous extractants.[5,52] The D_{PC} of methanol/water mixture was below 1, which was significantly lower than ILs-methanol systems (D_{PC} of 5% [HOEtMIm]Cl/methanol equaled 38.39), indicating that the addition of ILs can notably enhance the extraction performance. Furthermore, the D_{PC} and $S_{PC/PE}$ of pure methanol were 1.66 and 1.72, respectively, which were significantly lower than those of ILs-methanol mixture, due to the large mutual solubility of methanol and hexane. Therefore IL was necessary for an effective extraction. Consequently, it is reasonable to believe that PC could be selectively separated from PE by liquid-liquid extraction using IL-cosolvent as an extractant and the hydroxyl-functionalized imidazole ILs with chloride anion displayed excellent extraction performance.

However, recent studies indicate that the toxicity of imidazolium-based ILs should not be ignored.[53] Therefore, we consider an analogue of [HOEtMIm]Cl, choline chloride ([Ch]Cl), which is nontoxic and biocompatible and widely included in the nutrition facts of children's food due to its biological activity.[54] Though the melting point of [Ch]Cl was high, it is still been classified as IL by many researchers.[55] As shown in Fig. 4, [Ch]Cl also demonstrated satisfactory separation efficiency, with a D_{PC} value of 7.21 and a $S_{PC/PE}$ value of up to 6.38 when using a 5% [Ch]Cl/methanol mixture as the extractant. In addition, the extraction experiment of tetramethylammonium chloride ($(CH_3)_4NCl$) and methanol mixture as the extractant was also conducted, with a D_{PC} value of 2.95 and a $S_{PC/PE}$ value of 4.41. The difference between [Ch]Cl and $(CH_3)_4NCl$) was that the [Ch]Cl had hydroxyl group and the results indicated that the hydroxyl group was beneficial for the separation of PC and PE.

Therefore, this class of hydroxyl-functionalized IL-cosolvent extractants made great progress toward better extraction efficiency compared with common organic solvents and common ILs. This is of crucial significance for the development of energy-efficient extraction technologies because the consumption of solvents and the number of theoretical plates required for a certain task can be significantly decreased with the larger distribution coefficient and selectivity of the extractants.[11,56]

Effect of the Concentration of ILs on Extraction Separation

The effects of the concentrations of [HOEtMIm]Cl and [Ch]Cl in methanol on extraction efficiency were investigated, and the results are shown in Fig. 5. It was clear that both D_{PC} and $S_{PC/PE}$ showed great dependence on the concentration of ILs. The D_{PC} values increased in the first stage and then decreased. The D_{PC} value reached a maximum, which was nearly 12, when the mole fraction of [Ch]Cl was 0.04. Conversely, a downward tendency for D_{PE} was found over the whole range of IL concentrations. Therefore, the $S_{PC/PE}$ reached a maximum of 9.13 when the mole fraction of [Ch]Cl was 0.05. As shown in Fig. 5(b), D_{PC} first increased then decreased with the increase in the [HOEtMIm]Cl content in the IL/methanol mixture and D_{PE} decreased slightly, which was in agreement with the results from the use of [Ch]Cl/methanol as the extractant. Because of the similar structure of these two ILs, the results are reasonable. When the mole fraction of [HOEtMIm]Cl equaled 0.05, the D_{PC} value reached a maximum that was almost 38.38, and that of PE was 1.08.

The presence of a maximum in a plot of the distribution coefficient against the IL concentration has also been observed in similar IL-mediated liquid–liquid extraction processes, and it was generally attributed to the synergistic effect of IL and cosolvent.[57,58] Hence, this finding confirmed that even a small amount of IL in the IL/methanol mixture could effectively improve the separation efficiency. This is very important for the

development of industrially attractive extraction technology because of the lower consumption of relatively expensive ILs.

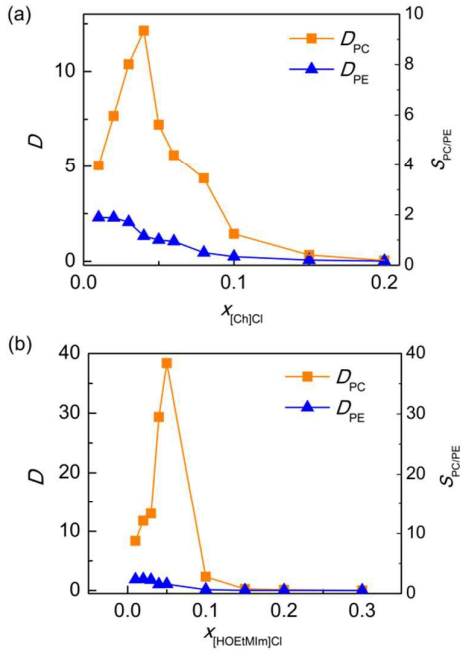

Fig. 5 Distribution coefficients of PC and PE at different mole ratios of ILs and methanol at 30 °C. (a) [Ch]Cl-methanol; (b) [HOEtMIm]Cl-methanol. The initial concentration of phospholipids in the hexane phase was 15 mg/mL.

Effect of Temperature on Extraction Separation

Experiments were also performed at different extraction temperatures. The distribution data of PC and PE in the [Ch]Cl-methanol-hexane biphasic system at different temperatures are presented in Fig. 6. It can be seen that the D_{PC} value declined gradually while the D_{PE} value increased slightly as the temperature increased. This was possibly because the H-bonding was more stable at lower temperatures, which was beneficial for the formation of micelles (for a detailed discussion, see the next section). Conversely, the mutual solubilities of the IL-methanol-hexane ternary system increased with the increase in temperature, which probably led to the worsened extraction performance.

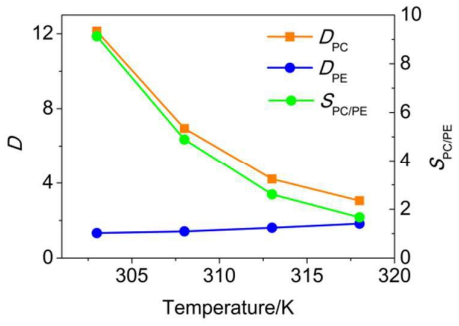

Fig. 6 Distribution data of PC and PE with [Ch]Cl-methanol (4/96, mol/mol) as the extractant at different temperatures. The initial concentration of the phospholipids in the hexane phase was 15 mg/mL.

Extraction Mechanism

The ability of PC to form self-assembled structures in polar solvents is already known.[59-61] The interactions of a range of organic solvents and imidazolium-based ILs with phospholipid compounds have been explored by molecular dynamics simulations and potential of mean force calculations.[62] In this work, DLS was employed to determine whether aggregate structures were present in the extractant phase. As shown in Fig. 7, when PC was dissolved in solvents, the size of aggregate structure observed using DLS in pure DMSO and 1,3-propanediol were 1 nm and 1.2 nm, respectively. However, the sizes of the aggregates of PC in [HOEtMIm]Cl and [Ch]Cl were 7.5 nm and 10.5 nm, respectively, which were much larger than those in DMSO and 1,3-propanediol, indicating stronger aggregation in IL-based extractants. 1,3-Propanediol is structurally similar to 1,2-propanediol. However, the size of the PC micelle in 1,3-propanediol was 1.2 nm, indicating that 1,3-propanediol could not induce a large amount of micelles in the solution. Likewise, the result of the DMSO also reveals that aggregation of PC cannot form. However, the size of the aggregates in 1,2-propanediol was approximately 25 nm, suggesting that large amounts of micelles were formed in 1,2-propanediol, which might explain the better extraction efficiency of 1,2-propanediol compared to that of 1,3-propanediol. Besides, the results in Fig. 7 (b) revealed that the addition of ILs in methanol can enhance the extraction efficiency.

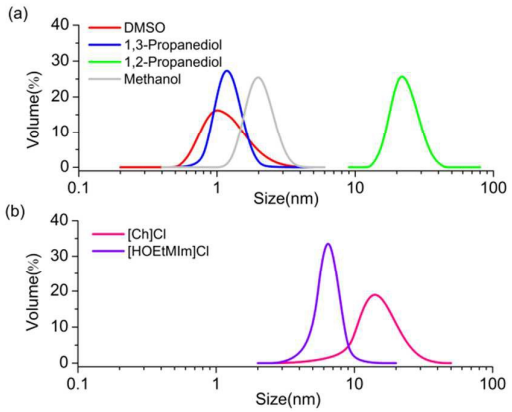

Fig. 7 DLS images of extractant phase: (a) DMSO, 1,3-propanediol, 1,2propanediol and methanol; (b) [HOEtMIm]Cl-methanol and [Ch]Cl-methanol (5/95, mol/mol).

For a better understanding of the underlying extraction mechanism of [Ch]Cl, *ab initio* calculations were carried out to investigate the micro-interaction between PC or PE and [Ch]Cl. The optimized structures of the IL-PC and IL-PE complex are shown in Fig. 8 (The structures of PC and PE were simplified to the phosphate head group). IL resided on the zwitterion side of PC of PE. The negatively charged chloride ion was located between two ammonium cations. The distance from Cl^- to the nitrogen atom in the ammonium moiety of PC (Fig. 8 (a): Cl_{32}-N_1) was 4.17 Å, and the distance from Cl^- to the central nitrogen atom of [Ch]Cl (Fig. 8 (a):Cl_{32}-N_{33}) was 4.03 Å, which were very close, indicating the presence of a strong interaction

between the anion of [Ch]Cl and PC. Conversely, as shown in Fig. 8 (a), the P=O group of PC formed multiple hydrogen-bonding interactions with the H atoms in the choline cation. The distances to the H atoms of the choline group of [Ch]Cl, H_{48} and H_{36}, were 2.25 Å (Fig. 8 (a):C_{46}-H_{48}•••O_{23}) and 2.32 Å (Fig. 8 (a):C_{34}-H_{36}•••O_{23}), respectively (the sum of the van der Waals radius of H and O atoms is 2.72 Å). In particular, the hydroxyl group of [Ch]Cl as a hydrogen-bond donor formed a very strong interaction with the negative P=O group of PC (the O_{53}-H_{52}•••O_{22} distance was 1.89 Å). In addition, as shown in Fig. 8 (b), the P=O group of PE also formed multiple hydrogen-bonding interactions with the H atoms in the choline cation of [Ch]Cl. However, the distances among the same atoms of [Ch]Cl and P=O group of PE were 2.71 Å (Fig. 8 (b): C_{22}-H_{24}•••O_{11}), 2.36 Å (Fig. 8 (b): C_{34}-H_{36}•••O_{11}) and 1.96 Å (Fig. 8 (b): O_{41}-H_{40}•••O_{11}), respectively, which of PC were 2.32 Å (Fig. 8 (a): C_{34}-H_{36}•••O_{23}), 2.25 Å (Fig. 8 (a): C_{46}-H_{48}•••O_{23}) and 1.89 Å (Fig. 8 (a): O_{53}-H_{52}•••O_{22}), respectively. The hydrogen-bonding distances between PC and [Ch]Cl were shorter than that between PE and [Ch]Cl, indicating the hydrogen-bonding between PC and [Ch]Cl was stronger. On the other hand, we obtained the interaction energy between PC or PE and [Ch]Cl by *ab initio* calculations. The absolute value of the interaction energy between PC and [Ch]Cl was 42.61 kJ/mol, which was larger than the absolute value of the interaction energy between PE and [Ch]Cl (22.22 kJ/mol), indicating that the interaction between PC and [Ch]Cl was stronger than that between PE and [Ch]Cl. Combining the results of the DLS experiments (Fig. 7) and *ab initio* calculations (Fig. 8), the strong hydrogen bonding interactions between PC and [Ch]Cl are probably in favour of the formation of the aggregation structure, such as micelles, in the IL-cosolvent mixture. Therefore, the extensive hydrogen bonding is probably beneficial for the extraction of PC and the formation of micelles.

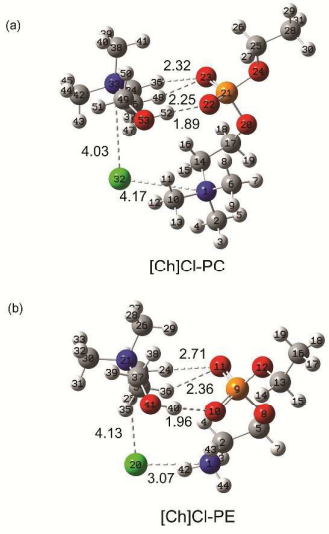

Fig. 8 The optimized geometries of (a) PC and [Ch]Cl, (b) PE and [Ch]Cl at the HF/6-31 G(d,p) level. The dark gray, light gray, orange, red, blue and green atoms represent C, H, P, O, N and Cl atoms, respectively. The dotted lines represent hydrogen-bonding interactions and electrostatic attraction, with interatomic distances in angstrom.

Recovery of ILs

The recovery of ILs is necessary for practical application of the IL-mediated extraction processes. However, phospholipids are high-boiling-point solutes that create barriers for separating those compounds from ILs. ILs are salts that can dissociate into separate anions and cations, whereas phospholipids are undissociated zwitterions. Therefore, it is possible to recover ILs using electrodialysis.[63] The principle of electrodialysis was presented in Supporting Information Fig. S1.[64] The solution of [Ch]Cl and methanol flowed into the diluting compartment, where the ILs dissociated into a cation and an anion under an electric field, and the ions then entered different compartments through an ion exchange membrane. The conductivity of the [Ch]Cl/methanol mixture is plotted against the time of electrodialysis in Fig. 9. It is obvious that the conductivity of the methanol solution declined sharply, indicating that choline chloride was almost removed within 20 minutes.

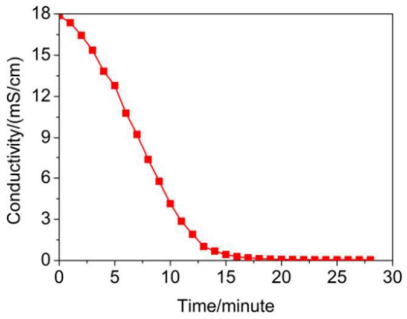

Fig. 9 The conductivity of the [Ch]Cl-methanol mixture (4/96, mol/mol).

Conclusion

In conclusion, we developed for the first time an efficient extractive method for the separation of phospholipid zwitterionic homologues with common imidazole-based ILs and hydroxyl-functionalized ILs as extractants. The extraction performances of various ILs along with organic solvents were evaluated. Hydroxyl-functionalized ILs demonstrated larger distribution coefficients and better separation selectivity than organic solvents for the separation of phospholipid homologues. The experimental results revealed that the IL concentration in the extractants had a significant effect on the distribution of PC. The D_{PC} and $S_{PC/PE}$ values reached maxima of 38.38 and 35.52, respectively, when the mole fraction of [HOEtMIm]Cl in methanol was 0.05. More importantly, [Ch]Cl is a nontoxic and biocompatible hydroxyl-functionalized IL that also showed satisfactory extraction performance, with D_{PC} and $S_{PC/PE}$ reaching 12.14 and 9.13, respectively (4% [Ch]Cl/methanol). The *ab initio* calculations indicated the presence of multiple H-bonding interactions between the hydroxyl group and anion of the ILs and the negative and positive moieties of phospholipid zwitterions, which probably promoted the formation of micelles in the IL-methanol mixture and then led to a selective separation of PC from the phospholipid zwitterionic homologues. As a class of "designable" solvents, ILs have high

potential for use in the separation of zwitterionic compounds of various structures.

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