Green Chemistry

Cite this: *Green Chem.*, 2012, **14**, 2617

www.rsc.org/greenchem

PAPER

High performance separation of sparingly aqua-/lipo-soluble bioactive compounds with an ionic liquid-based biphasic system†

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Received 20th April 2012, Accepted 10th July 2012
DOI: 10.1039/c2gc35614g

Separation of high value bioactive compounds is a viable route to make full use of the biomass resources and improve the profitability. However, the sparing aqua- and lipo-solubility of the bioactive compounds makes their separation really challenging. Considering that ionic liquids show good solubility of biomass and could easily form biphasic systems with organic solvents, an ionic liquid (IL)-based biphasic system consisting of ionic liquid, water and ethyl acetate is proposed in this study. Ginkgolide homologues were selected as model compounds to evaluate its practicality. Adequate distribution coefficients, relatively high extraction capacity and selectivity were obtained with the novel biphasic system. The improved distribution coefficients of the ginkgolides are mainly attributed to the multiple interactions between ginkgolide and IL, which were confirmed by means of quantum chemistry calculations. Moreover, the effect of the interactions between ginkgolides and the extraction solvent on the selectivity coefficient was studied by measuring the Kamlet–Taft parameters of the extraction solvent. Based on the results of fractional extraction, which was simulated by calculation and validated by experiments, as well as the comparison of organic solvent consumption, the employed IL-based extraction would be a valid and clean method as an alternative to chromatographic methods for separating bioactive compounds in large-scale operations. It is noteworthy that the amount of organic solvents consumed with this method was supposed to be less than 1/11 of the most widely used chromatographic method.

Introduction

Bioactive products from biomass have contributed greatly to the development of food additives and new drugs.[1,2] However, the currently available separation and purification techniques are unsatisfactory: although chromatographic separation is the most popular method for the fine resolution of structural similar mixtures, it is subjected to high volatile organic solvent consumption.[3,4] Liquid–liquid extraction is an economic consideration in industrial separations, nevertheless, the attempts to establish an efficient liquid–liquid extraction method for bioactive compounds is strongly limited by their sparing solubility in water or weakly polar solvents,[5] which usually comprises one phase of traditional biphasic systems.

Unlike the cases in traditional solvents, biomass compounds exhibit extraordinary solubility in ionic liquids (ILs), which are capable of virtually all possible types of interactions with solutes.[6–8] In addition, the high cohesive energy makes it easier for ILs to form biphasic systems with organic solvents, and the structures of ILs could be tailored for specific applications.[9–11] The above mentioned unique properties reveal that ILs possess great potential for being good extractants in extractive separations of bioactive products. Up to now, most studies have focused on utilizing ILs to leach bioactive components from natural resources,[12,13] attempts to separate structurally similar compounds with ILs are rare: mainly lipo-soluble compounds such as essential oils and tocopherols have been investigated.[14–16]

A representative class of sparingly aqua-/lipo-soluble bioactive compounds is ginkgolide homologues (Fig. 1).[17] They are reported to be partly responsible for the neuromodulatory properties of *Ginkgo biloba* extracts, the best selling herbal product all around the world.[18] As GB was proven to be the most potent antagonist of the platelet-activating factor (PAF) receptor,[19] it has been the focus of numerous research projects and separating the ginkgolide homologues is of great importance and really challenging.[19–21] As far as we know, chromatography is still a widely used separatory method.[22–24]

Therefore, in this study, we focus on developing a high performance IL-based liquid–liquid extraction method as an alternative to chromatography for the fine resolution of sparingly

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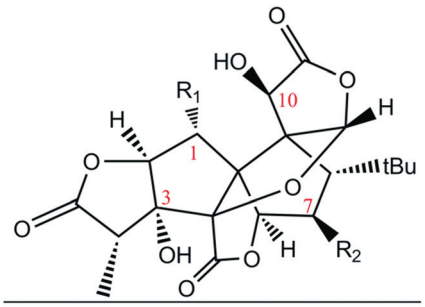
†Electronic supplementary information (ESI) available: Phase equilibrium procedure and the experimental tie-lines of [EMIm]BF_4 + water + ethyl acetate at 303.2 K (Fig. S1). See DOI: 10.1039/c2gc35614g



Compound	R_1	R_2
ginkgolide A (GA)	H	H
ginkgolide B (GB)	OH	H
ginkgolide C (GC)	OH	OH

Fig. 1 Molecular structure of GA, GB and GC.

aqua-/lipo-soluble bioactive compounds, where ginkgolide homologues were selected as model compounds to evaluate its practicability. A novel immiscible biphasic system was investigated, and the influence of the structure and concentration of IL in the extraction solvent, the initial concentration of ginkgolides, as well as the temperature on extractive separation of ginkgolides were studied, followed by simulation and experimental verification of fractional extraction, as well as back extraction and regeneration of the IL. Moreover, quantitative calculations on hydrogen bonding interactions between ginkgolides and the IL were performed and Kamlet–Taft parameters of the extraction solvent were determined to further explore the extraction mechanism.

Experimental

Materials

The ILs used in this study were purchased from Lanzhou Greenchem. ILS (LICP. CAS. China), including tetraethylammonium chloride ($N_{2,2,2,2}Cl$, 99%), 1-ethyl-3-methylimidazolium chloride ([EMIm]Cl, 99%), 1-(2′-hydroxylethyl)-3-methylimidazolium chloride ([HOEtMIm]Cl, 99%), 1-ethyl-3-methylimidazolium bromide ([EMIm]Br, 99%), *N*-ethylpyridinium bromide ([EPy]-Br, 99%), 1-ethyl-3-methylimidazolium tetrafluorophosphate ([EMIm]BF_4, 99%), 1-ethyl-3-methylimidazolium acetate ([EMIm]OAc, 98%), *N*-butylpyridinium bromide ([BPy]Br, 99%), *N*-hexylpyridinium bromide ([HPy]Br, 99%), *N*-octylpyridinium bromide ([OPy]Br, 99%), with water contents of these ILs below 0.5% (mass fraction). Three ginkgolides mixture samples were all purchased from Xuzhou Daguanyuan Co., Ltd (China): one contains 64.9% GA, 24.0% GB and 8.2% GC, one contains 58.3% GA and 40.5% GB, and another contains 20.0% GA and 71.9% GB. The ginkgolide standards, GA (>98%), GB (>98%) and GC (>98%), were purchased from Chengdu Mansite Pharmaceutical Co. Ltd. (China). Ethyl acetate and methanol were of HPLC grade and obtained from TEDIA (USA). Tetrahydrofuran (THF) was also of HPLC grade and obtained from Merck (Germany). Acetonitrile, *n*-hexane, *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and *n*-butanol were of analytical grade and obtained from Sinopharm Group Co. Ltd (China). The purified water was obtained from the Wahaha Group Co. Ltd (China).

Extraction equilibrium procedure

Water content of the IL was measured with a Karl Fisher titrator before preparing the extraction solvent. A known amount of ginkgolide powder was dissolved in ethyl acetate and an equal volume of this solution and the extraction solvent were moved into an Erlenmeyer flask. The flask was shaken vigorously in a thermostatic rotary shaker under a speed of 200 rpm and a set temperature (±0.1 K). As preliminary experiments showed that the distribution equilibrium was achieved after a 20 min shaking period, a 2 h shaking period was chosen for the phase contacting experiment. After shaking, the flask was allowed to settle for at least 3 h at the same temperature. Then, samples were taken from both phases by syringes without disturbing the phase boundary and diluted with methanol for the high performance liquid chromatography (HPLC) analysis.

The distribution coefficient (D_i) of solute i is calculated according to eqn (1),

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

where C_i^{e} and C_i^{r} refer to the mass fractions of solute in the extraction phase and the raffinate phase, respectively. The selectivity of solute i to solute j ($S_{i/j}$) is calculated according to eqn (2),

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

The extraction equilibrium experiments were repeated at least three times and the relative uncertainties of the distribution coefficients were less than 5%.

HPLC analysis

The concentration of ginkgolides A, B and C were analyzed by HPLC.[25] The HPLC system included a Waters 1525 binary HPLC pump, a Waters 717 plus autosampler, a Waters thermostat and a Waters 2487 dual absorbance UV detector. A Waters Symmetry C_{18} column (4.6 mm × 150 mm, 5 μm) was used for separation of the homologues. The mobile phase was methanol–water–THF (4 : 15 : 2, v/v/v) and the flow rate was 1 mL min^{-1}. The UV detector was set at 220 nm. The column temperature was 308.2 K. The ginkgolides in the samples were identified and quantified by direct comparison with reference standards.

Solvatochromic probe analysis

The Kamlet–Taft parameters were measured according to the literature procedure.[26,27] Probes 4-nitroaniline (1) and *N*,*N*-diethyl-4-nitroaniline (2) were dissolved in dichloromethane, respectively. Appropriate amounts of the solution was added to a small vessel and the dichloromethane was evaporated under vacuum at 293–308 K for 1–2 h to completely remove the solvent. After that, a certain amount of IL–diluent mixture was added to the

vessel, stirred thoroughly and then transferred into a quartz colorimetric cell. The cell was placed in a Shimadzu 2550 UV-vis spectrophotometer and the maximum absorption wavelength was recorded. The UV-vis scanning was repeated at least five times for each measurement and the average value was taken as the final one. The uncertainty of maximum absorption wavelength was within ±0.5 nm. The Kamlet–Taft parameters dipolarity/polarizability (π^*) and the hydrogen bonding basicity (β) were determined using eqn (3) and (4), respectively,

$$\nu(1)_{\max} = 27.52 - 3.182\pi* \quad (3)$$

$$\nu(2)_{\max} = 1.035\nu(1)_{\max} - 2.80\beta + 2.64 \quad (4)$$

where $\nu(1)_{\max}$ and $\nu(2)_{\max}$ are the wavelengths of maximum absorbance of dissolved *N*,*N*-diethyl-4-nitroaniline and 4-nitroaniline, respectively.

Computational details

All geometric optimizations were performed with the Gaussian03 program[28] at the B3LYP/6-31+G(d, p) theoretical level. [EPy]Br, exhibited good separation results for the ginkgolide homologues, and was selected as the model IL. Preliminary geometry optimizations of [EPy]Br, GA and GB were performed, respectively. The input configurations of GA and GB were produced from published configurations of GB and modifications of these.[29] Then the preliminary optimized geometries were used for optimization on GA–[EPy]Br and GB–[EPy]Br pairs starting from various initial configurations. All optimized configurations were verified as minimum energy configurations without imaginary frequencies by full calculation of the Hessian and a harmonic frequency analysis. Interaction energies were corrected for the basis set superposition error (BSSE) by means of the counterpoise method of Boys and Bernardi.[30] The AIM analysis was performed with the AIMAll program[31] to provide the electron density of ginkgolides and a deeper insight into the interactions between ginkgolide and [EPy]Br.

Fractional extraction

Fractional extraction experiments were carried out on a centrifugal extractor with four theoretical plates. Thereby the extraction and scrubbing sections of a fractional extraction with eight theoretical plates was verified separately. The centrifugal rotational speed was adjusted to 2970 rpm, and no entrainment was found visually in both the extraction and the scrubbing phases. The feed stock and extraction solvent were prepared according to the calculation results, and their flow rates were controlled by an HPLC pump and a wriggle pump, respectively. The flow ratio was in accordance with the calculation conditions. After the extraction system reached equilibrium, the outputs of each theoretical plate were sampled for HPLC analysis. The extraction and raffinate phases were collected, respectively. The GB product in the raffinate phase was obtained *via* rotary evaporation to remove ethyl acetate, and was then washed with water four times to remove the IL, and was followed by vacuum drying at 343.2 K for 12 h. The IL in the extraction phase was reused according to the following procedure.

Regeneration of used IL

The regeneration of the used IL and back extraction of ginkgolides from the extraction phase were carried out using a dilute-swing effect. Firstly, ethyl acetate solution (total ginkgolides concentration of 6.0 mg mL^{-1}) was contacted with an equal volume of [EPy]Br aqueous solution (60 wt%) according to the previously mentioned extraction equilibrium procedure at 303.2 K (±0.1 K). After that, the extraction phase and raffinate phase were separated. Then, two volumes of water were added to the extraction phase, and the obtained solution was washed by 1/10 volume of ethyl acetate four times. The used ethyl acetate was collected. The remaining [EPy]Br solution was distillated under reduced pressure vacuum at 333.2 K by rotary evaporation to remove some water, and the mass fraction of [EPy]Br was adjusted to 60% by adding the appropriate content of water for the next extraction equilibrium.

Results and discussion

Extractive separation of ginkgolide homologues with and without IL as extractant

In general, ethyl acetate is a low-toxic solvent and has good solubility for large amounts of sparingly aqua-/lipo-soluble compounds.[24,32,33] However, it could hardly form an immiscible biphasic system with conventional solvents except for water, which shows poor solubility to those compounds. Unlike conventional solvents, ILs are reported to form biphasic systems with organic solvents more easily, and those ILs with halide anions are reported to be good solvents and separation media for biomasses, thus ethyl acetate was selected as the stock solvent and IL as the extractant. In addition, water was used as the diluent to tune the physical and chemical properties of the extraction solvent. Phase equilibriums of [EMIm]Br–water–ethyl acetate and [EPy]Br–water–ethyl acetate (Fig. 2) suggest that no IL was detected in the ethyl acetate-rich phase and mutual solubilities of the studied biphasic systems are rather low. Then, this immiscible biphasic system was employed for the separation of the ginkgolide homologues and various ILs were studied.

For comparison purposes, the distribution data of ginkgolides in conventional biphasic systems, ethyl acetate-/*n*-butanol–water and acetonitrile-/DMF-/DMSO–*n*-hexane, were determined. The results listed in Table 1 reveal that all the distribution coefficients for GA and GB are below 0.08, and the values are even closer to zero for the biphasic systems containing *n*-hexane, where no ginkgolide was detected in the *n*-hexane-rich phase after extraction equilibrium. However, the distribution values obtained in IL-contained biphasic systems are at least one order of magnitude higher than those in the IL-absent biphasic systems. For example, distribution coefficients of GA and GB are 0.021 and 0.015 in ethyl acetate–water biphasic system while those are 0.46 and 0.27 in the ethyl acetate/[EMIm]Br–water biphasic system, where the mole fraction of [EMIm]Br in the extraction solvent was 12.6%. This phenomenon indicates that there are strong interactions between the ginkgolides and the IL, which drive ginkgolides into the IL-rich phase. The hydrogen bonding interactions between ginkgolides and the IL were further confirmed by the computational study. Fig. 3 shows the

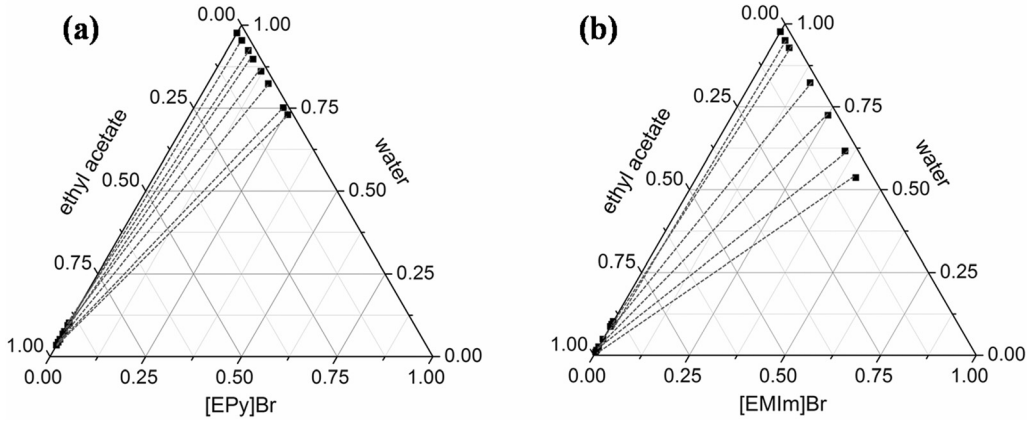


Fig. 2 Experimental tie-lines of the ternary systems at 303.2 K: (a) [EPy]Br + water + ethyl acetate; (b) [EMIm]Br + water + ethyl acetate.

Table 1 Extractive separation of ginkgolide homologues with various biphasic systems at 303.2 K[a]

	Distribution coefficient			Selectivity	
Biphasic system	GA	GB	GC	GA/GB	GC/GB
Acetonitrile–*n*-hexane[b,c]	<0.001	<0.001	—	—	—
DMF–*n*-hexane[b,c]	<0.001	<0.001	—	—	—
DMSO–*n*-hexane[b,c]	<0.001	<0.001	—	—	—
Ethyl acetate–water[c]	0.021	0.015	—	1.4	—
n-butanol–water[c]	0.075	0.059	—	1.3	—
$N_{2,2,2,2}$Cl/water–ethyl acetate	0.40	0.31	3.7	1.3	12
[EMIm]Cl/water–ethyl acetate	0.33	0.21	2.0	1.6	9.3
[HOEtMIm]Cl/water–ethyl acetate	0.14	0.084	0.75	1.7	8.9
[EMIm]Br/water–ethyl acetate	0.46	0.27	1.7	1.7	6.5
[EPy]Br/water-ethyl acetate	0.41	0.24	1.6	1.7	6.6
[EMIm]BF_4/water–ethyl acetate	1.0	0.63	1.3	1.6	2.0
[EMIm]OAc/water–ethyl acetate[c]	1.2	1.8	—	0.7	—

[a] The initial concentration of ginkgolides in ethyl acetate (mg mL^{-1}): GA 6.49, GB 2.40 and GC 0.82. The initial mole fraction of IL in the extraction solute was 12.6%. The volume ratio was 1 : 1. [b] The concentrations of GA, GB and GC in the *n*-hexane-rich phase were below the HPLC detection limits. [c] Distribution coefficient of GC was not given for the reason that the peak area of GC in one phase was too small and it was difficult to determine the distribution coefficient of GC accurately.

optimized structures of the ginkgolide-IL complexes. It can be seen that explicit hydrogen bonding interactions are formed between the ginkgolide and both the cation and anion of the IL, the hydroxyl groups on the ginkgolides are more apt to form hydrogen bonding with the anion of the IL. For instance, the distance of O–H···Br between GA and the anion in the GA–[EPy]-Br complex is 2.308 Å, and the values are 2.210 Å and 2.267 Å for those in the GB–[EPy]Br complex. The above mentioned distances are all less than the sum of the van der Waals radii of H (1.20 Å) and Br (1.85 Å).[34] AIM analysis on the optimized geometries of GA–[EPy]Br and GB–[EPy]Br complexes show that the corresponding electron density at the bond critical point are 0.0247, 0.0265 and 0.0303, respectively, which are all within the hydrogen bonding range. Besides the moderate distribution coefficients, the selectivity coefficients of GA to GB are also higher when the IL was used as the extractant. These results confirm the fact that ILs truly play a key role in the extractive separation of ginkgolide homologues.

It is also found in Table 1 that the distribution coefficients of ginkgolides in the studied biphasic systems generally followed a decreasing order, GC > GA > GB, which was in accordance with the order of polarity and hydrogen bonding acidity of the ginkgolide homologues. As shown in Fig. 1, GC bears four hydroxyl groups on the cage skeleton, while the number for GA and GB are two and three, respectively. Thus, the polarity and hydrogen bonding acidity of GC is higher than those of GA and GB. For GB, however, the hydroxyl group at C_1 readily forms intramolecular hydrogen bonding with the adjacent hydroxyl group at C_{10},[23,29] causing the weakest polarity and hydrogen bonding acidity of GB among the three homologues.

Effect of the IL's structure on the extractive separation of GA and GB

The IL's anion has been reported to have a large influence on the IL's properties .[35] To estimate the influence of the IL's anion, we investigated a selection of ILs with 1-ethyl-3-methylimidazolium cations and varying anions (Table 1). Unlike all the other ILs, the distribution coefficient of GA is lower than that of GB in the biphasic system containing [EMIm]OAc under the same conditions. One explanation is that the increased basicity of the acetate anion makes it more efficient at disrupting the intramolecular hydrogen bonding than Cl^-.[36] It is also seen that the selectivities for the homologues obtained with Cl^-- and Br^--based ILs are higher than for the BF_4^--based IL. Such phenomena may be not caused by the relatively weak hydrogen bonding basicity of the BF_4^--based IL, but may be caused by different mutual solubilities of these biphasic systems. The phase equilibrium data listed in Fig. 2 and S1† reveals that the [EMIm]BF_4/water–ethyl acetate biphasic system exhibits higher mutual solubilities than the biphasic system containing [EPy]Br. The impacts of the mutual solubilities of the biphasic system on the distribution

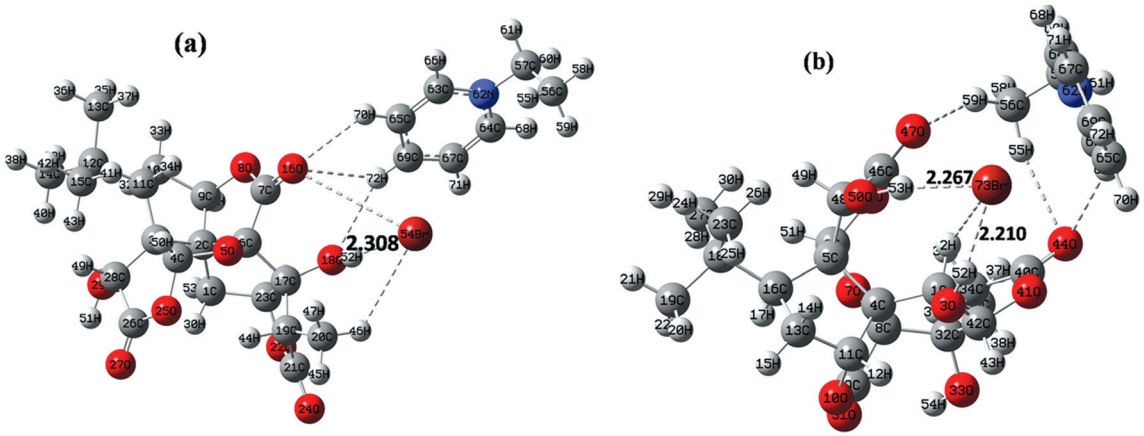


Fig. 3 Optimized structures of GA–[EPy]Br (a) and GB–[EPy]Br (b) complexes at B3LYP/6-31+G(d, p) level. Dashed lines imply possible hydrogen bonding between ginkgolide and [EPy]Br with interatomic distance in angstroms.

coefficients of ginkgolide homologues influences the selectivities of the ginkgolides.

The effect of the IL's cation was also taken into account. The data provided in Table 1 reveals that the pyridinium-based IL and the ammonium-based IL show better separation efficiencies for GA/GB and GC/GB pairs than the others with the same anion, respectively. Furthermore, the effect of alkyl chain length of Br^--based pyridinium ILs on the separation of ginkgolide homologues was also studied. As shown in Fig. 4, the distribution coefficients of ginkgolide homologues are elevated and the selectivity of GA to GB decreases with the increase of the chain length. On one hand, as it is known that when the ILs' anions are identical, increasing the cation's alkyl chain length enhances the hydrophobicity of the ILs.[37] The elevated distribution coefficients of ginkgolides in more hydrophobic ILs could possibly be ascribed to the fact that more hydrophobic ILs can solubilize ginkgolides more easily through hydrophobic interaction between the cations of the IL and the lactones and tetrahydrofuran rings, as well as the *tert*-butyl groups in the ginkgolides. On the other hand, higher mutual solubilities of the biphasic system were observed with IL with longer alkyl chain lengths, which also influences the extraction results.

Additionally, it is worth noting that almost contrary trends were observed for the selectivity of GA/GB and GC/GB: the value for the GC/GB pair declines while that for the GA/GB pair increases slowly from $N_{2,2,2,2}Cl$ to [EPy]Br. As the structural differences between GC and GB lies in an additional hydroxyl group exhibiting hydrogen bonding acidity, increasing the hydrogen bonding basicity of the extractant would facilitate the selective separation of the GC/GB pair. While for GA/GB pairs, although the intramolecular hydrogen bonding interaction still exists in GB, its bond strength is likely to be influenced by the high hydrogen bonding basicity of the extraction solvent, leading to a decreasing trend of selectivity value.

Effect of the concentration of IL on the extractive separation of GA and GB

An [EPy]Br aqueous solution was used as the extraction solvent to evaluate the influence of IL concentration on the distribution

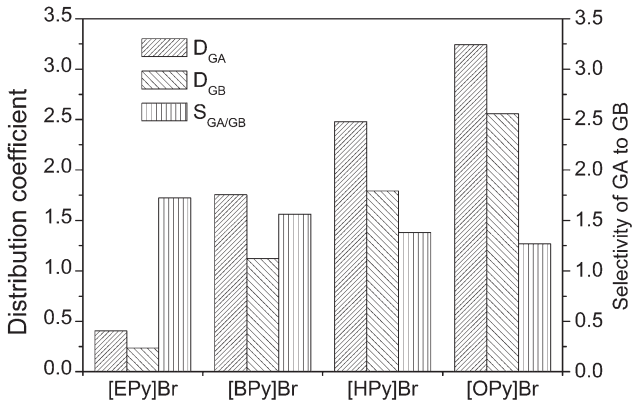


Fig. 4 Effect of the alkyl chain length attached to the IL's cation on the distribution coefficient of GA and GB, as well as selectivity of GA to GB. The initial concentration of GA and GB was 3.5 and 2.4 mg mL^{-1}, respectively. The initial mole fraction of IL in the extraction solute was 12.6%. The volume ratio of the two phases was 1 : 1. The temperature was 303.2 K.

behavior. Distribution coefficients of GA and GB, as well as the selectivity of GA to GB, are plotted against the initial mole fraction of [EPy]Br in the extraction solvent (Fig. 5). The distribution data provided revealed that the distribution coefficients of both ginkgolides increase slowly when the mole fraction of [EPy]Br in the extractive solvent is low, and then went up significantly in higher [EPy]Br mole fractions, climbing to 6.7 and 4.2 for GA and GB respectively as the mole fraction of [EPy]Br was 31.6%. As discussed above, ILs are able to interact with ginkgolides through various interactions, therefore, the increasing amount of [EPy]Br in the extraction phase enhances the interaction strength between ginkgolides and the extraction solvent, which in turn increases the distribution coefficients of the ginkgolides.

It was also seen in Fig. 5 that the selectivity coefficient of GA to GB increases with increasing of the mole fraction of [EPy]Br in the low IL mole fraction region and then decreases, showing a maximum at about x_{IL} = 12.6%. The Kamlet–Taft parameters of the [EPy]Br–water solutions were determined to explain the

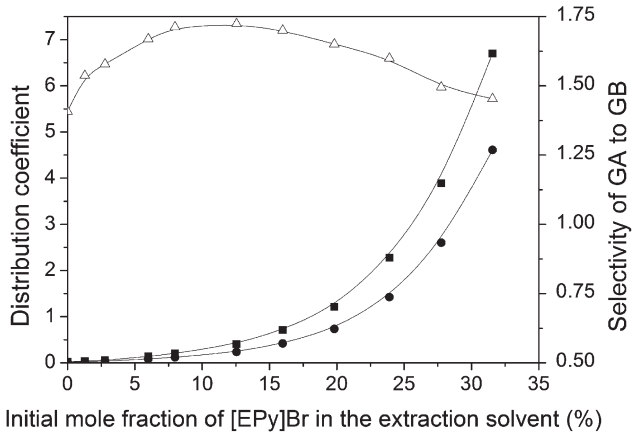


Fig. 5 Effect of the mole fraction of [EPy]Br in the extraction solvent on distribution coefficients of (■) GA and (●) GB as well as (Δ) selectivity of GA to GB. The diluent was water. The initial concentration of GA and GB was 3.5 and 2.4 mg mL^{-1}, respectively. The volume ratio of the two phases was 1 : 1. The temperature was 303.2 K.

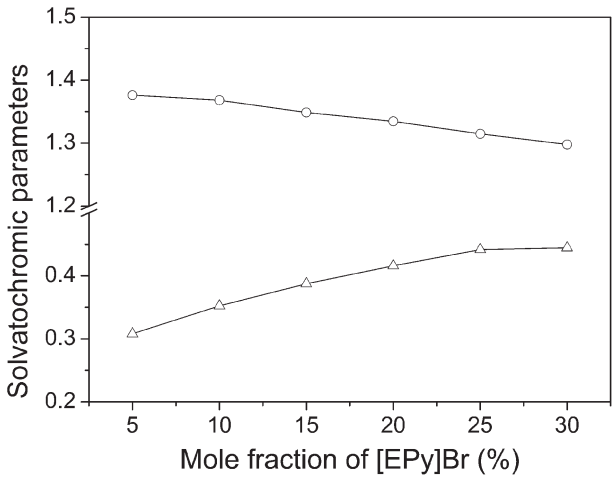


Fig. 6 The interaction parameters (○) dipolarity/polarizability and (Δ) hydrogen bonding basicity *versus* mole fraction of [EPy]Br in the aqueous solution.

maximum selectivity value (Fig. 6): the dipolarity/polarizability of the solution decreases slowly while the hydrogen bonding basicity increases with increasing concentrations of [EPy]Br. As decreasing dipolarity/polarizability and increasing hydrogen bonding basicity of the extractant solvent have contrary influence on the selectivity of GA to GB, the presence of a maximum in the selectivity coefficient against the mole fraction of [EPy]Br is reasonable.

Effect of the concentration of ginkgolides and temperature on the extractive separation of GA and GB

The total concentration of ginkgolides, up to 30 mg mL^{-1}, were examined to evaluate the extraction capacity of the [EPy]Br/water–ethyl acetate biphasic system. The equilibrium concentrations of ginkgolides in the extraction phase *versus* those in the raffinate phase are plotted in Fig. 7. It is seen that the equilibrium

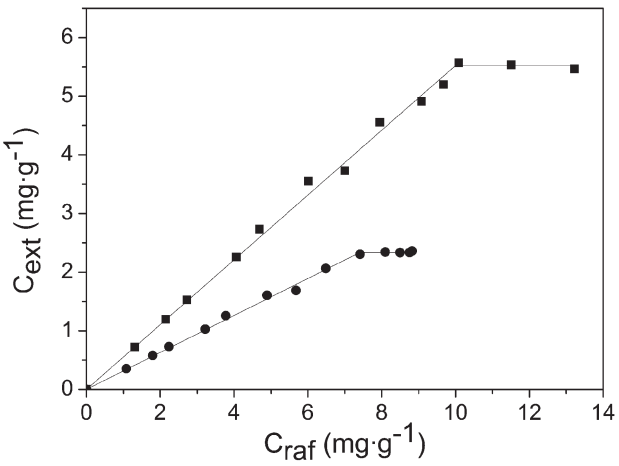


Fig. 7 The concentration of ginkgolide in the extraction phase (C_{ext}) *versus* that in the raffinate phase (C_{raf}): (■) GA, (●) GB. The mole fraction of [EPy]Br in the extraction phase was 12.6%. The volume ratio of the two phases was 1 : 1. The temperature was set at 303.2 K.

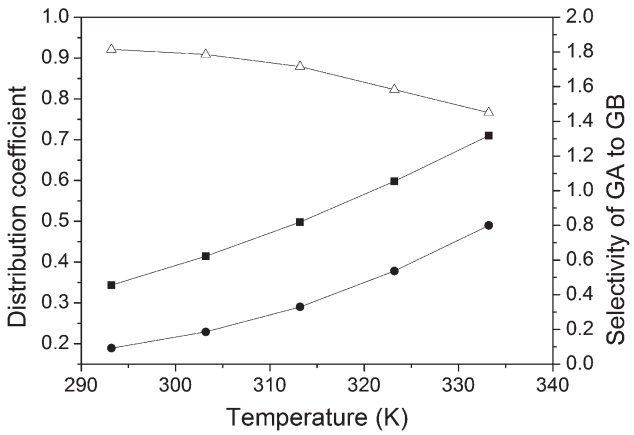


Fig. 8 Effect of temperature on extraction data of the ginkgolides: (■) distribution coefficient of GA, (●) distribution coefficient of GB, (Δ) selectivity of GA to GB. [EPy]Br was used as the extractant and water was the diluent. Mole fraction of [EPy]Br in the extraction solute was 12.6%. The initial concentration of GA and GB was 3.5 and 2.4 mg mL^{-1}, respectively. The volume ratio of the two phases was 1 : 1.

concentrations of ginkgolides in the extraction phase increase linearly with the concentrations in the raffinate phase below the initial concentration of ginkgolides at 25.3 mg mL^{-1}, and then remain constant at values of 5.52 and 2.34 mg mL^{-1} for GA and GB at high concentrations, showing the saturation of ginkgolides in the extraction phase. These phenomena once again demonstrates that the IL/water–ethyl acetate biphasic system has enough extraction capacity for the sparingly aqua-/lipo-soluble bioactive compounds.

The distribution data, as well as the selectivity of GA to GB, obtained with the [EPy]Br/water–ethyl acetate biphasic system from 293.2 K to 333.2 K are presented in Fig. 8. It is seen that the distribution coefficients of both ginkgolides increase while the selectivity coefficient slightly decreases with the increase of temperature, revealing that room temperature would be appropriate for the separation process.

Fractional extraction

With the aim to analyze the possibility of separating ginkgolide homologues in large-scale manufacturing processes, fractional extraction was simulated by calculations and verified experimentally. During the calculation, the mass balance and mass transfer of the ginkgolides were taken into consideration, other factors such as mutual solubilities of the biphasic systems and heat balance were ignored, and also, the distribution coefficients of the ginkgolides were considered as constants. The distribution values of ginkgolides used for the calculations were under the condition that the mole fraction of [EPy]Br was 12.6% in the extraction solvent at 303.2 K. The results plotted in Fig. 9 reveal that GB could be separated from the ginkgolide homologues with high purity and recovered under optimized conditions. For example, the calculated purity and recovery of GB are 98.9% and 91.6% under the flow conditions feed (F) : extraction solvent (ES) : scrubbing solvent (SS) = 0.075 : 1 : 0.275, where the extraction and scrubbing stages are 30 and 10, respectively.

Furthermore, two multistage extraction experiments under different flow ratios were performed to validate the calculation results. Under the flow ratio F : ES : SS at 0.15 : 0.3 : 1, the relative mass fraction of GB in the ginkgolides A and B mixture increased from 75.0% in the feed stock to 87.4% in the product, whereas the calculated purity was 84.7%. Plots of the calculated and experimental relative mass fractions of GB in the ginkgolides A and B mixture *versus* the stage number (Fig. 10) clearly show that comparable experimental data were obtained. The GB product was purified and HPLC analysis revealed that the residual IL and ethyl acetate were both below the HPLC detection limit. The relative mass fraction and recovery values for GB under the flow ratio of 0.15 : 0.3 : 1 were 91.5%, with the calculation value of 93.3%. As the experimental results agree well with the calculated ones, it can be inferred that both high purity and high recovery of GB could be achieved by fractional extraction under optimum experimental conditions.

Organic solvent consumption: comparison of fractional extraction and chromatography

Considering environmental and economic reasons, reducing the consumption of organic solvents is crucial for a clean separation process. Besides superiority over conventional liquid–liquid extractions, IL-based extraction is also expected to offer an environmentally benign alternative of the most commonly used chromatographic method in the separation of structurally similar compounds. Thus, the consumption of organic solvents throughout the IL-based extraction procedure is compared with a typical preparative chromatographic separation process[24] in preparing the GB product with high purity.

For the convenience of comparison, the ginkgolide mixture containing GA, GB and GC was selected as the starting material, out of consideration that the distribution coefficients of these three ginkgolides are known in the present work. The relative

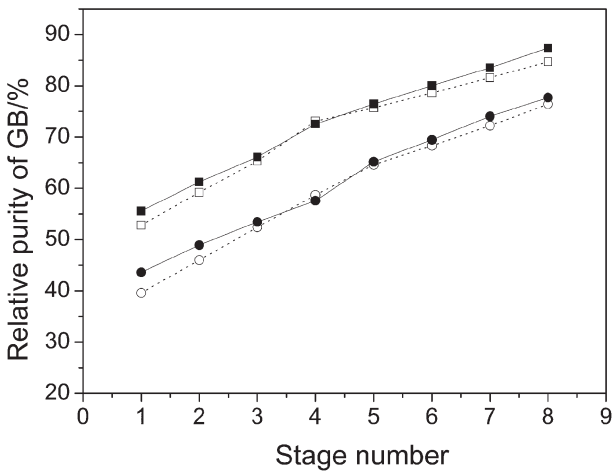


Fig. 10 The calculated (dotted lines) and experimental (solid lines) purity of GB in the extraction and raffinate phases at different stages: (■) raffinate phase, (●) extraction phase. The stages were numbered toward the flow direction of the light phase.

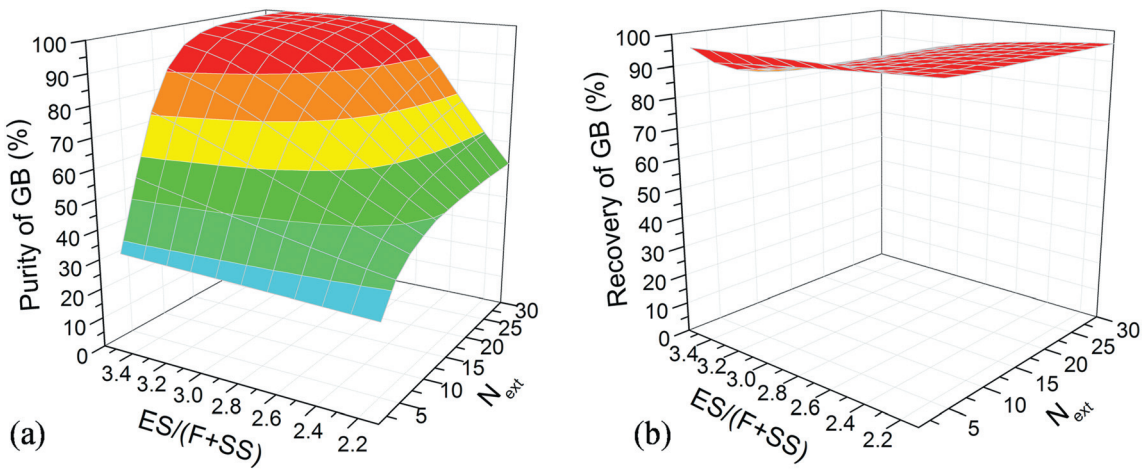


Fig. 9 Simulation of multi-stage fractional extraction: calculated purity (a) and recovery (b) of GB *versus* the flow ratio (ES/(F + SS), ES, F and SS represent the volume flow rate of the extraction solvent, feed and scrubbing solvent, respectively) and the extraction stages of the extraction section (N_{ext}). The IL was [EPy]Br and the distribution coefficient of the ginkgolides was obtained from Table 1. For the scrubbing section, the equilibrium stages was set at 10, the scrubbing solvent was ethyl acetate, and the SS/ES ratio was set at 1 : 0.275.

Table 2 Comparison of the organic solvent consumption by using chromatography and IL-based extraction methods

	Organic solvent (L g^{-1} GB)				
Separation method	Ethyl acetate	Petroleum ether (b.p. 40–60°C)	Methanol	IL (kg g^{-1} GB)	Water (L g^{-1} GB)
Chromatography[a]	9.71	2.52	0.03	0	0
IL-based extraction[b]	1.06	0	0	1.04	3.84

[a] The operating conditions of preparative chromatography developed in ref. 24 were as follows: the stationary phase was silica impregnated with 6.5% NaOAc, the mobile phase was gradient from petroleum ether–ethyl acetate 30 : 70, *via* petroleum ether–ethyl acetate 27 : 73, petroleum ether–ethyl acetate 20 : 80, 100% ethyl acetate to ethyl acetate–methanol 98 : 2. For a single chromatography operation, 500 mg of ginkgolides solution was injected and baseline separation was achieved after 46 fraction numbers (1024 mL of elution solvent). The column was regenerated for a subsequent separation by flushing with 4 column volumes of solvents (petroleum ether–ethyl acetate, 30 : 70). [b] The conditions for calculating the organic solvent consumption utilizing [EPy]Br/water–ethyl acetate system are as shown below: the concentration of total ginkgolides in the feed stock was 25 mg mL^{-1}, the flow ratio of F : ES : SS ratio was 0.09 : 1 : 0.28, and the theoretical plates for the extraction and scrubbing sections was all 30. Regeneration of IL was carried out by adding two volumes of water to the extraction solvent, followed by a countercurrent extraction of 10 theoretical plates with ethyl acetate (IL solution : ethyl acetate = 1 : 10, v/v).

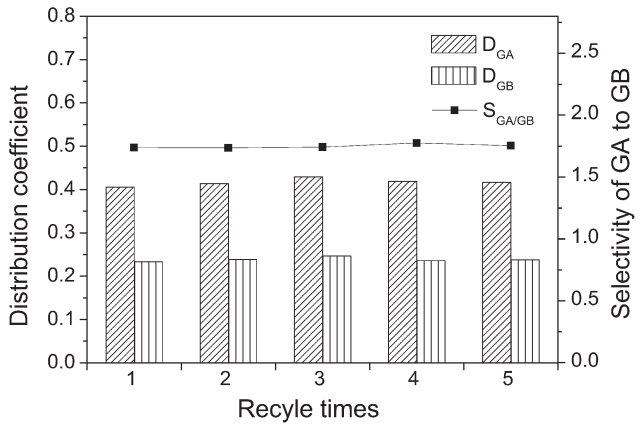


Fig. 11 Distribution coefficient and selectivity of ginkgolides *versus* the recycle times. The IL was [EPy]Br, and its mole fraction in the extraction was 12.6%. The initial concentration of ginkgolides was 6 mg mL^{-1}. The temperature was 303.2 K.

mass fractions of GA, GB and GC in the mixture are 34.1%, 27.7% and 38.2%, respectively, which are in accordance with those in the literature which reported the chromatography method.[24] The purity and recovery of GB in the product were supposed to be 98% and 100% for the chromatography method, while the values were 98.2% and 98.0% for the IL-based extraction.

The consumption amounts the organic solvent, water and IL are listed in Table 2. It is seen that the amount of organic solvents consumed with the chromatography method was 12.26 L g^{-1} GB, which is more than 11-fold of the values obtained by the IL-based extraction (1.06 L g^{-1} GB). The reduction of organic solvents consumption in the IL-based extraction method could possibly be attributed to two main reasons: one is the use of an IL as a substitute of organic solvent, which acts as an extractant in the extraction system; the other is that the injection of feed stock for fractional extraction is continuous while it is intermittent for chromatography.

Regeneration of used IL

The ability to recycle the ILs is important for practical use and for increasing the "greenness" of the procedure. Given the influence of IL concentration on the distribution behavior of ginkgolides, it is expected that the IL can be regenerated from the extraction phase by means of the dilute-swing effect. The HPLC analysis of the reused IL showed that the residue of ginkgolides was below 0.6 mg g^{-1} in the IL aqueous solution, which is negligible compared with the initial concentration of ginkgolides at 6 mg mL^{-1}. The results in Fig. 11 show that comparable distribution coefficients and selectivity of the ginkgolides were obtained within the 5 studied cycles, revealing that the reused IL remains high efficient for ginkgolide separation.

Conclusion

In this work, an IL-based liquid–liquid extraction, as an economic alternative to chromatography processes in large-scale manufacturing of sparingly aqua-/lipo-soluble bioactive compounds was proposed. For this purpose, a novel biphasic system consisting of ethyl acetate, IL and water was utilized, with ginkgolide homologues as model compounds. As compared with IL-absent conventional organic biphasic systems, the IL-containing ones showed adequate distribution coefficients, high selectivity and extraction capability for the ginkgolide homologues. The experimental results reveal that the structure of the IL influences the distribution behavior of the ginkgolides; the improved distribution coefficients were related to the multiple interactions between the ginkgolides and the ILs, including hydrophobic interactions, hydrogen bonding interactions, *etc.*; the mutual solubilities of the biphasic system and the mutual effect of dipolarity/polarizability and hydrogen bonding basicity of the extraction solvent may play key roles in determining the selectivity of GA to GB. Simulation and experimental verification of the multistage fractional extraction suggests that GB, with high purity and recovery, could be achieved under optimized conditions. Compared with the commonly used chromatography method, the consumption of organic solvents would be greatly reduced. Besides, the IL could be regenerated and maintains comparable separation efficiency. These results suggest that the employed IL-based extraction method is an efficient and clean process for the separation of sparingly aqua-/lipo-soluble compounds.

Acknowledgements

The authors are grateful for the financial support from the National Natural Science Foundation of China (no. 20936005, 21076175 and 21076178) to this work. The authors gratefully acknowledge Prof. Haoran Li at Department of Chemistry, Zhejiang University, China for providing the Gaussian 03 program and helpful guidance.

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