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Enhanced solubilization and extraction of hydrophobic bioactive compounds using water/ionic liquid mixtures

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Water is an ideal green solvent for the solubilization and separation of valued-added bioactive compounds in various chemical and biological processes, partly because water has a minimal impact on the environmental and few safety issues. However, many bioactive compounds exhibit hydrophobic properties, which leads to limited solubility in water and substantially hinders the development of green separation technologies using aqueous media. In this study, we construct a family of new water/ionic liquid (IL) mixtures with amphiphilic, anionic functional long-chain carboxylate ionic liquids (LCC-ILs) for the solubilization and extraction of hydrophobic bioactive compounds (HBCs). The LCC-ILs integrate both weak polarity and strong hydrogen-bonding basicity and, more importantly, have excellent lipophilicity while still being water-miscible; therefore, the water/LCC-IL mixtures exhibit extremely high solubilities for various HBCs. The quantitative solubilities (g/g) of various HBCs, including tocopherol, perillyl alcohol, rutin, and ginkgolides, are as high as 1.46, 0.71, 0.39 and 0.43, respectively, at 35°C in water/LCC-IL mixtures, which are the highest solubilities in aqueous solutions ever reported. The water/LCC-IL mixtures also exhibit excellent performance for the extraction of tocopherols from biomass with a yield of two to 12 times larger than with common solvents. The microscopic solvent properties and dissolution mechanism were investigated. Nano-micelles were observed when tocopherol was dissolved in water/LCC-IL mixtures, and their dissolution ability was dependent on the alkyl chain length and the concentration of LCC-ILs. These results demonstrated the considerable potential of water/LCC-ILs mixtures as promising green solvents for the solubilization and separation of HBCs.

Introduction

Bioactive products from bioresources are expected to play an important role as a major resource in the development of food additives and new drugs[1] and have attracted increasing attention from researchers. Water is certainly one of the most abundant and important substances in the world,[2] which makes it a good medium for the separation and processing of value-added bioactive compounds. Doubtlessly, water is an ideal "green" solvent for minimizing both the environmental impact and the safety problems which result from the use of toxic and volatile organic solvents in chemical processes.[3] Unfortunately, many bioactive compounds exhibit hydrophobic properties, which leads to their limited solubility in water and substantially hinders the development of green separation methods using aqueous solvents.[4]

Recently, ionic liquids (ILs) have emerged as promising solvents capable of dissolving a wide range of biomass feedstocks[5] due to their broad liquid temperature range, multiple solvation interactions, and enhanced hydrogen bond (H-bond) basicity and H-bonding interactions.[6] They are solvents that minimize the environmental footprint in reactions and separations because of their unique properties, such as negligible vapour pressure, non-flammability, and high thermal stability.[7] In addition, ILs are regarded as designable solvents because their structure and properties can be tailored using various combinations of different cations and/or the anions, which allows their properties to be tuned.[8] However, ILs usually have relatively high viscosities and polarities due to their charged structures and strong anion-cation interactions.[9] Thus, the solubilization and extraction of weakly polar bioactive compounds using common ILs are unsatisfactory.[10] Additionally, the high production costs associated with ILs greatly reduces their potential industrial applications.[11]

Using IL-water mixtures as alternative solvents for the selective extraction of bioactive compounds from natural sources has been reported in recent years and has been demonstrated to be an effective approach to solving the problems of high viscosities and production costs in certain cases.[12] The introduction of water into hydrophilic ILs can significantly decrease the viscosity of the IL phase,[13] and the IL-water mixtures exhibit satisfactory extraction efficiency for polar bioactive compounds, such as carbohydrates[13c,14], alkaloids[15], polyphenols[16,17], saponins[17], and others[18]. The primary hydrophilic ILs investigated for bioactive

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† Electronic Supplementary Information (ESI) available: Data Fig. S1–S6 showing the molecular structures of ILs used in this study, solubilities of α-tocopherol in $[P_{4444}][C_{11}H_{23}COO]$ aqueous solutions with higher concentration, and effects of LCC-IL concentration and solid to liquid ratio on extraction yield. See DOI: 10.1039/x0xx00000x

compound extraction are 1-alkyl-3-methylimidazolium and quaternary ammonium salts with chloride, bromine, and acetate as anions. Nevertheless, the extraction of weakly polar hydrophobic bioactive compounds (HBCs) by an aqueous solution of ILs has rarely been reported due to the limited solubility of hydrophobic compounds in aqueous solutions.[19] Although ILs with long alkyl chains on the cation have demonstrated enhanced solubilities for bioactive compounds,[15b,20] their weak affinity to water, relatively high melting point and correspondingly large viscosity as an IL-water mixture has limited their application.

Herein, we construct a family of water/IL mixtures using amphiphilic, anionic functional long-chain carboxylate ILs (LCC-ILs, see Fig. S1, ESI for their molecular structures) for the solubilization and extraction of hydrophobic compounds. Long-chain carboxylic acids have proven to be good facilitator for transport of hydrophobic drugs in human body,[21] but immiscible with water. The LCC-ILs have weak polarity, strong H-bond basicity, and excellent lipophilicity while still being water-miscible. Therefore, the corresponding LCC-IL aqueous solutions exhibited extremely high solubilities for various HBCs and excellent extraction performance for typical HBCs, including tocopherols. The microscopic solvent properties and dissolution mechanisms of the LCC-IL aqueous solutions were investigated in this work using Kamlet-Taft solvatochromic parameters and dynamic light scattering (DLS). Their extractive performance was also evaluated in this work with tocopherols as typical HBCs.

Results and discussion

Dissolution of hydrophobic bioactive compounds in water/LCC-ILs mixtures: solubilities and structural features

A class of anionic functional LCC-ILs with a tetrabutylphosphonium cation ($[P_{4444}][C_nH_{2n+1}COO]$, n= 7, 9, 11, 13, and 15) were prepared via neutralization of a tetrabutylphosphonium hydroxide aqueous solution with long-chain fatty acids.[22] Tetrabutylphosphonium is selected as cation because of its good hydrophilicity. The tetrabutylphosphonium-based ILs common exhibit stronger H-bond basicity and lower viscosity compared with other ILs with the same anion due to its weak anion-cation interaction.[22,23] The long-chain carboxylate anions are expected to have strong H-bond basicity and excellent lipophilicity.[10c,22] LCC-ILs have proven to be excellent solvents for hydrophobic drug-like molecules,[22b] but unfortunately also demonstrate an excessive increase in viscosity after dissolution. We found that all of the prepared LCC-ILs were fully miscible with water at room temperature because of their amphiphilic properties. The water/LCC-IL mixtures demonstrate low viscosity over a wide concentration range, strong H-bond basicity, and weak dipolarity/polarizability (for a detailed discussion see the next section), indicating great potential for the solubilization and extraction of hydrophobic compounds. The dissolution performance of prepared water/LCC-IL mixtures was first investigated using α-tocopherol (Fig. 1a) as a typical hydrophobic bioactive compound.[24] The solubility of α-tocopherol in room temperature water is very low (20.9×10^{-6} g/g).[25] It is interesting to note that LCC-IL aqueous solutions exhibited an excellent

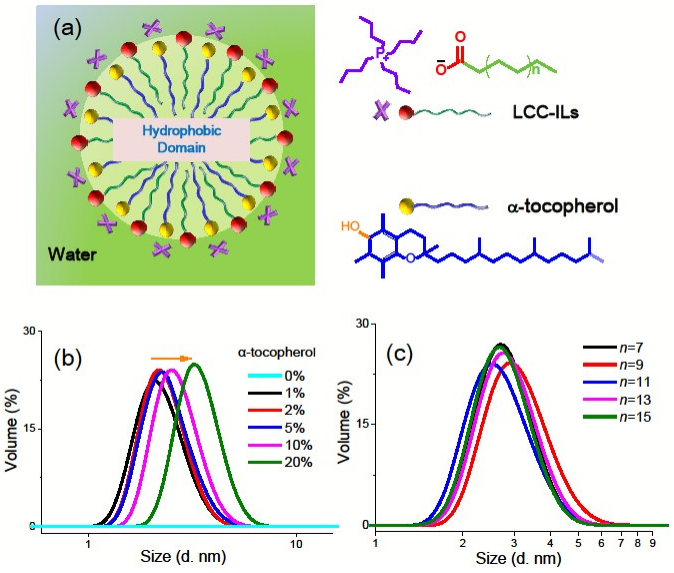

Fig. 1 (a) Schematic representation of LCC-ILs micelles containing hydrophobic α-tocopherol molecules and Chemical structure of LCC-ILs and α-tocopherol molecule. (b) DLS images of 40 wt% $[P_{4444}][C_{11}H_{23}COO]$ aqueous solution with the amount of α-tocopherol from 0 to 20%. (c) DLS images of 40 wt% LCC-ILs with different length of anionic alkyl chain (n is the carbon number of alkyl) after dissolving 10% α-tocopherol.

dissolution ability for α-tocopherol. The solubility (with solubility defined as the mass ratio of solute to solvent) of α-tocopherol in a water/$[P_{4444}][C_{11}H_{23}COO]$ mixture (15/85, mass ratio, simply denoted as 15 wt%; hereafter all weight percents given refer to ILs) at 40°C may reach 0.096, which is almost 4 orders of magnitude larger than in pure water and 1500-fold larger than in ethanol-based aqueous solutions.[25] For comparison, as shown in Fig. 2, the solubility of α-tocopherol in commonly used water-hydrophilic IL mixtures is very small; e.g., in [BMIm]Cl/water, the solubility is 0.00012, which is almost 800-fold lower than in a water/$[P_{4444}][C_{11}H_{23}COO]$ mixture at the same conditions. Although enhanced solubilities were observed in aqueous solutions containing ILs with long alkyl chains in the cationic group (for $[C_{12}MIm]Cl$: 0.044, for $[C_{12}MIm][CH_3COO]$: 0.036), the water/LCC-IL mixture still demonstrated a significantly higher solubility for

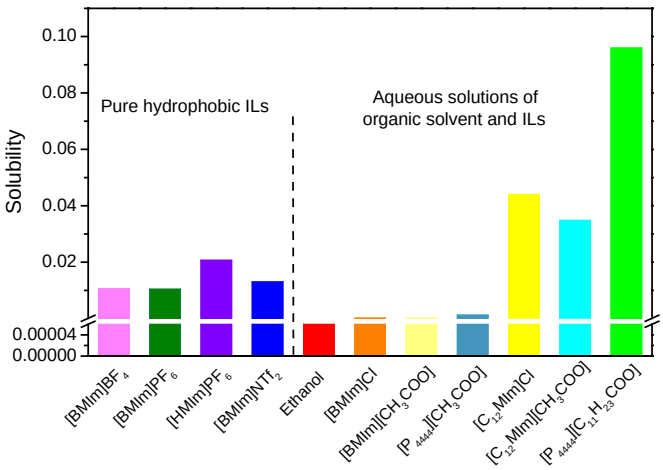

Fig.2 Solubility of α-tocopherol in pure hydrophobic ILs and aqueous solutions containing 15 wt% ethanol and ILs at 40°C (for full names of ILs and their structures see material section and Fig. S1, S2, ESI).

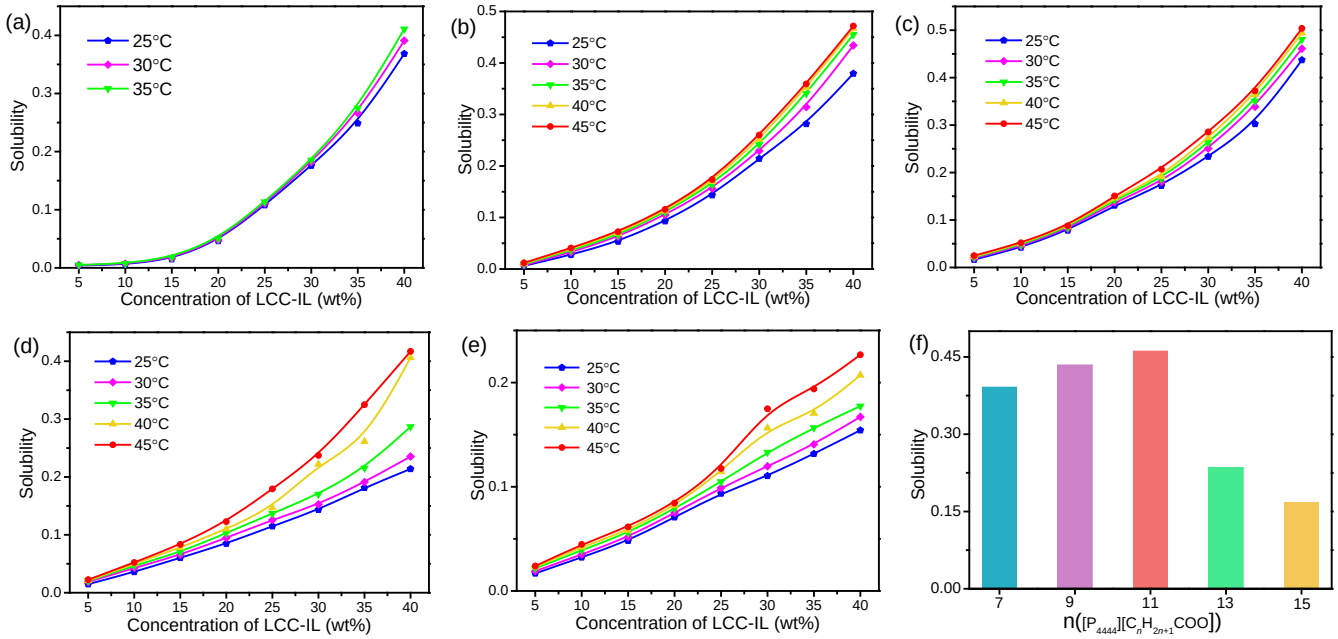

Fig. 3 Solubility of α-tocopherol in water/LCC-ILs mixtures. (a) $[P_{4444}][C_7H_{15}COO]$, (b) $[P_{4444}][C_9H_{19}COO]$, (c) $[P_{4444}][C_{11}H_{23}COO]$, (d) $[P_{4444}][C_{13}H_{27}COO]$, (e) $[P_{4444}][C_{15}H_{31}COO]$ as IL's concentration from 5 to 40 wt% at a temperature range of 25 to 45°C (the line between points is provided as a guide to the eye); (f) solubility comparison of α-tocopherol with different *n* of LCC-ILs (*n* is the carbon number of alkyl; mass concentration of LCC-ILs *x*=40 wt%, temperature T=30°C).

tocopherols (0.096). We also measured the solubility of α-tocopherol in neat common hydrophobic ILs, and the results (Fig. 2) show moderate solubilites (0.01-0.02). The solubility of α-tocopherol in water/LCC-IL mixtures is also four- to nine-fold larger than in pure, hydrophobic ILs.

DLS was employed in this work to explore the underlying dissolution mechanism of the mixed system. In contrast to the case of cationic functional long-chain ILs,[10d,26] no ordered aggregation structures, such as micelles or liquid crystals, were observed in LCC-IL aqueous solutions over the IL's mass concentration range from 5 to 95 wt% (Fig. 1b). Interestingly, micelles emerged when a small quantity of α-tocopherol was dissolved in the LCC-IL aqueous solution. As shown in Fig. 1b, with an increasing concentration of α-tocopherol in the water/$[P_{4444}][C_{11}H_{23}COO]$ (40 wt%) mixture, the size of the micelles increased from 2.24 to 3.44 nm, indicating that some of the α-tocopherol was incorporated into the micellar core. According to the DLS results and the molecular characteristics of tocopherols and LCC-ILs, we discerned a possible dissolution mechanism (Fig. 1a). The anionic group on the LCC-IL forms a strong H-bond with the hydroxyl group in α-tocopherol due to the strong ability of the carboxylate anion to accept H-bond donor; additionally, strong van der Waals interactions exist between the long alkyl chain of the LCC-ILs and the hydrophobic moiety of α-tocopherol, leading to the formation of highly ordered aggregation structures. As shown in Fig. 1c, all of the water/LCC-IL mixtures formed micellar structures following the addition of 10% α-tocopherol to the 40 wt% LCC-IL aqueous solutions. Also shown in Fig. 1c, all of the systems investigated in this work ($[P_{4444}][C_nH_{2n+1}COO]$, *n*= 7, 9, 11, 13, and 15) have aggregation structures whose size varied from 2.70 to 3.13 nm.

The solubility data of α-tocopherol in different LCC-ILs over a concentration range of 5 to 40 wt% and a temperature range of 25 to 45 °C are presented in Fig. 3a-e. This figure compiles the direct experimental results of the alkyl-chain length (*n*), the LCC-IL's mass concentration (*x*, wt%), and the temperature (T) versus *y*, which is the mass ratio of α-tocopherol in solution. As observed in Fig. 3f, the solubility of α-tocopherol in water/LCC-ILs is significantly affected by variations in the anionic alkyl chain length. Evidently, $[P_{4444}][C_{11}H_{23}COO]$ occupies the optimal alkyl chain length. The solubility of α-tocopherol in five water/LCC-IL systems generally decreased according to the following sequence: $[P_{4444}][C_{11}H_{23}COO]$ > $[P_{4444}][C_9H_{19}COO]$ > $[P_{4444}][C_7H_{15}COO]$ > $[P_{4444}][C_{13}H_{27}COO]$ > $[P_{4444}][C_{15}H_{31}COO]$, all under the same conditions, indicating the important role of the length of alkyl chains on dissolution ability. The solubility increases with IL anion alkyl chain length from *n*=7 to 11, while for *n*≥13, the solubility does not continue to increase. For example, the longest two alkyl chain lengths of 13 and 15 lead to a drastic decrease of the solubility of α-tocopherol. This effect could be probably attributed to the excessive aggregation and/or microscopic phase separation in water/LCC-ILs when *n*≥13. The hydrophobic section of α-tocopherol was attracted to the alkyl chains of the anions via van der Waals interactions, and the hydrophilic phenolic hydroxyl formed H-bond with the carboxyl groups of the anions. The synergistic effects led to an excellent solubility. However, overlong alkyl chain of LCC-IL caused reduced dipolarity ($\pi^*[P_{4444}][C_7H_{15}COO]$ = 0.967, $\pi^*[P_{4444}][C_{15}H_{31}COO]$ = 0.868 and $\pi^*[P_{4444}][C_{15}H_{31}COO]$ = 0.815) and intrinsically inclined to self-aggregate in water and improves the phase separation,[15b,27] which decreased the interaction between the IL anion and α-tocopherol, thus lowering α-tocopherol solubilities. Therefore, an appropriate anion alkyl chain length would be preferable for solubilization.

In addition, unlike systems with $n \geq 9$, it is notable that the transparent α-tocopherol saturated aqueous solution of $[P_{4444}][C_7H_{15}COO]$ became turbid when the temperature reached 40°C, indicating the key role of the length of alkyl chains on systematic thermodynamic stability. Furthermore, the solubility of α-tocopherol in LCC-IL aqueous solutions increased steeply as *x* rose from 5 to 40 wt%, but increasing the temperature from 25 to

45°C only minimally increased solubility, indicating the great importance of LCC-IL concentration on the dissolution ability and the relative stability of these micellar systems over a specific temperature range. Therefore, the optimal solubility can be balanced by modulation of either the concentration of ILs or the alkyl chain length of the LCC-ILs. As a result of the cooperative solubility effects of LCC-ILs in water, the solubilization enhancement of the mixture could transcend the extremely high solubilities currently found in conventional organic solvent-water and IL-water solutions. The highest solubility of α-tocopherol, which reached 0.50 at 45°C, was found in a 40 wt% $[P_{4444}][C_{11}H_{23}COO]$ aqueous solution. To the best of our knowledge, this is the highest solubility of α-tocopherol in a mixed aqueous solution that has been reported.[25] We also measured the solubility of α-tocopherol in water/$[P_{4444}][C_{11}H_{23}COO]$ with higher concentrations of ILs (*x*=50-70 wt%) at 25-40°C; the solubility of α-tocopherol, reaching 1.56, was 70- to 120-fold larger than in pure, common, hydrophobic ILs (for detailed data, see Fig. S3, ESI).

The water/LCC-IL mixtures exhibited excellent dissolubility for other bioactive compounds which are sparingly soluble in aqueous solutions. We also determined the solubility of perillyl alcohol, rutin and ginkgolide homologues in these water/LCC-IL mixtures. The same operational conditions were maintained throughout all experiments, namely a $[P_{4444}][C_{11}H_{23}COO]$ concentration between 5 and 70 wt% and a dissolution temperature of 35°C. The data presented in Fig. 4 are consistent with the general rule that the solubilities of solutes increase with increasing concentrations of ILs. We found that α-tocopherol and perillyl alcohol were miscible with pure $[P_{4444}][C_{11}H_{23}COO]$ at ambient temperature. The solubilities of rutin and ginkgolides in $[P_{4444}][C_{11}H_{23}COO]$ were larger than 0.25 and 0.30 respectively at 25°C; however their accurate values were unavailable because the systems turned gelatinous with rutin or ginkgolindes dissolving in pure $[P_{4444}][C_{11}H_{23}COO]$ (Fig. S6). Notably, even at very low concentrations of LCC-IL, for example at 5 wt%, the quantitative solubilities of perillyl alcohol (0.025), rutin (0.015) and ginkgolide homologues (0.013) in $[P_{4444}][C_{11}H_{23}COO]$ aqueous solutions improved significantly, over 60- to 220-fold larger than their solubilities in pure water (0.00012, 0.00024, 0.000068, respectively). Choi[28] previously reported that a deep eutectic solvent had shown a remarkable ability to solubilize ginkgolide (0.21). It is worth noting that the solubility of ginkgolide in a (*x*=70 wt%) $[P_{4444}][C_{11}H_{23}COO]$ aqueous solution is as high as 0.43, which is 2 times larger than Choi.[28] To our knowledge, this is also the highest solubility data ever reported. Therefore, this class of novel water/LCC-ILs mixtures represents great progress towards the goal of solubilizing bioactive compounds compared with existing solvents. Our work showed that HBCs with both strong H-bond donor (e.g., hydroxyl) and hydrophobic segment (e.g., long alkyl group, phenyl, and multi-heterocyclic ring), including phenols derivatives, terpenoids, and flavonoid glycosides, generally have very high solubility in LCC-IL aqueous solvents, which is crucially significant for the development of green separation technologies.

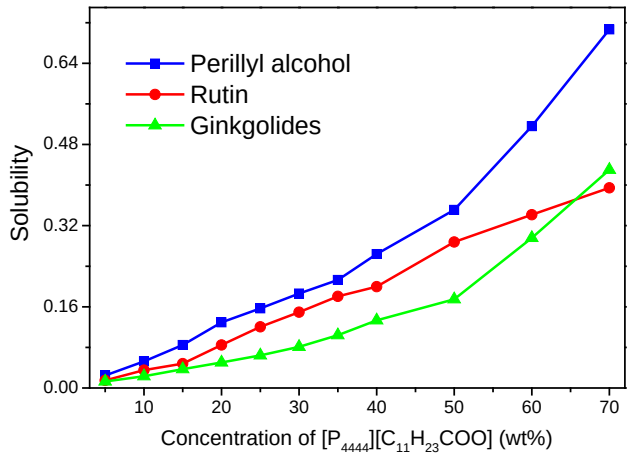

Fig. 4 Solubility of perillyl alcohol, rutin, and ginkgolide homologues in water/$[P_{4444}][C_{11}H_{23}COO]$ mixtures with IL's concentration from 5 to 70 wt% at 35°C. The line between points is provided as a guide to the eye.

Tocopherol extraction from soybean flour using an IL aqueous solution

Considering the notable solubilization ability of LCC-IL aqueous solutions for HBCs, it is expected that the water/LCC-IL mixture will show great potential in the extraction of lipo-soluble compounds such as tocopherols, which are commonly extracted by using volatile organic solvents, including ethanol, chloroform, methylene chloride and ethyl acetate.[29]

The soybean flour used in this study was screened by particle size using 100-mesh stainless steel sieves and subsequently dried at 65 °C under a vacuum for 12 h. Soybean is a globally significant crop which contains a wide variety of compounds of biological interest

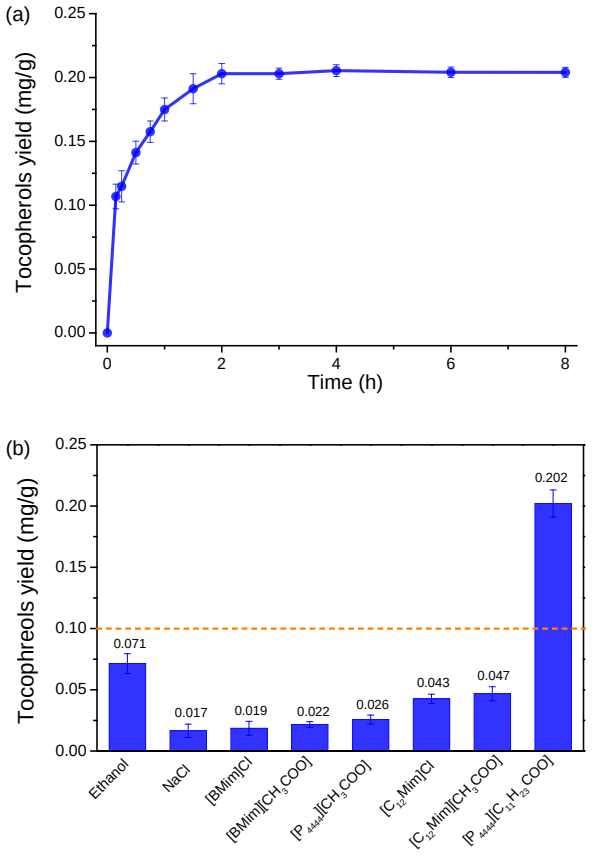

Fig. 5 Yield of tocopherols extracted from soybean flour (T = 40°C, S/L ratio = 1:20): (a) yield of tocopherols extracted as a function of time (t); (b) yield of tocopherols extracted with different extractants of the *x* = 20 wt% and t = 2h.The long-dashed orange line, yield=0.10, is provided for simpler comparison of data.

already isolated and identified and is also one of the main sources of naturally occurring tocopherols. In this work, water/[P_{4444}][$C_{11}H_{23}COO$] mixtures were used as solvents to extraction of tocopherols from soybean flour because of their relatively high solubility for tocopherols. All of the extraction experiments were carried out under the same mild operating conditions, namely, a temperature of 40°C and a stirring rate of 400 rpm. The effects of the LCC-IL concentration and the soybean-solvent mass ratio on the extraction yield of tocopherols were firstly investigated. The results (Fig. S4) indicate that tocopherols yield (mg of pure tocopherols extracted per g of dry soybean flour used) increases sharply with increasing IL's concentration at the range of 0.1-1.0M (5-50 wt%); however the growth on yield becomes slow after the IL's concentration over 0.4M (20 wt%). Additionally, as presented in Fig. S5, the yield generally increased with the decreasing solid to liquid (S/L) ratio; but, higher solvent didn't obtain significantly higher yields while led an extra waste. In view of these, a concentration of 20 wt% and a soybean-solvent ratio of 1:20 is a reasonable choice for effective extraction. Extraction samples were analyzed over a time range of 0.15 to 8 h in order to ascertain the optimal extraction time. The data presented in Fig. 5a indicates that extraction time significantly influenced tocopherols yield, which confirms that the yield increases over time; but after 2 h of extraction, the yield remained approximately steady at 0.204 mg/g.

For comparison, we also performed an extraction of tocopherols with pure water (below detection limit) and with aqueous solutions of ethanol, NaCl, [BMIm]Cl, [BMIm][CH_3COO], [P_{4444}][CH_3COO], [C_{12}MIm]Cl and [C_{12}MIm][CH_3COO]. The results in Fig. 5b show that using an aqueous solution of [P_{4444}][$C_{11}H_{23}COO$] as the extractant obtained the highest tocopherol yield (20.2 mg/100 g), which was 5 to 12 times higher than with other aqueous solutions as extractants. Therefore, the water/LCC-IL mixture is an effective solvent for the separation of tocopherols.

Physico-chemical properties of water/LCC-IL mixtures

The microscopic solvent properties and viscosity of the LCC-IL aqueous solutions were studied in order to elucidate the physico-chemical characteristics of the mixtures. In our previous work, we verified that LCC-ILs have a relatively high H-bond basicity compared to common ILs.[22] Although LCC-ILs are always liquid at room temperature, they usually have a higher viscosity than other ILs (*e.g.* the viscosity of [P_{4444}][$C_{11}H_{23}COO$] is 429.9 mPa·s while the viscosity of [BMIm]NTf_2 is 49.2 mPa·s at 25°C), which remains an obstacle to their use in practical applications.[30]

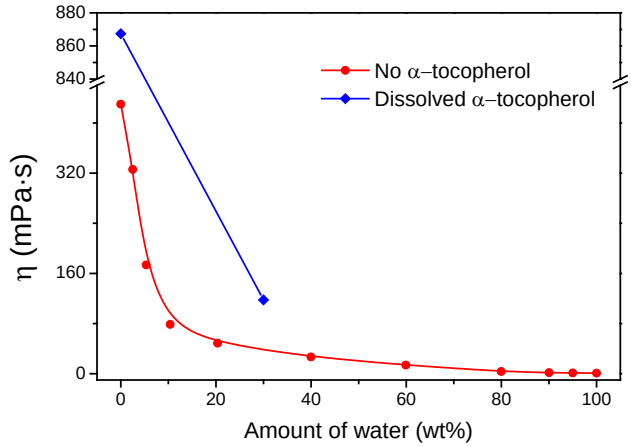

Fig. 6 Values of viscosity (η, mPa·s) for the water/[P_{4444}][$C_{11}H_{23}COO$] mixtures plotted against the mass fraction of water (red cycle) and for the solutions of 30% α-tocopherol dissolved in neat [P_{4444}][$C_{11}H_{23}COO$] and water/[P_{4444}][$C_{11}H_{23}COO$] binary mixtures with a water content of 30 wt% (blue diamond) at 25°C. The line between points is provided as a guide to the eye.

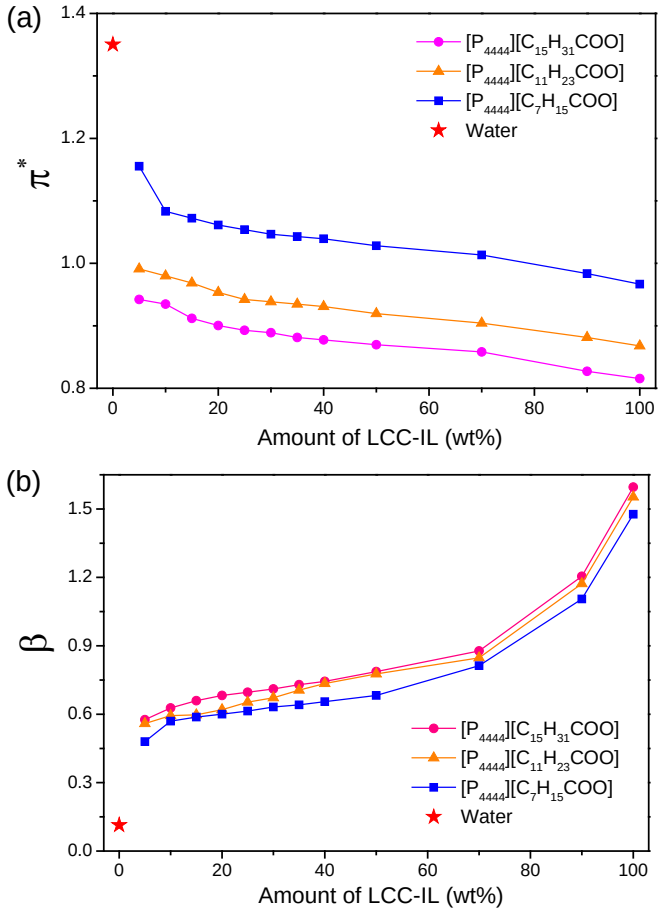

Fig. 7 Dependence of dipolarity/polarizability (π*) and hydrogen-bond basicity (β) of water/LCC-ILs mixtures on the mass fraction of LCC-ILs at 25°C. The line between points is provided as a guide to the eye.

The viscosities of [P_{4444}][$C_{11}H_{23}COO$] aqueous solutions were measured. Not surprisingly, the presence of water significantly reduced the viscosity of these mixtures. As the data indicated in Fig. 6, the viscosity of a [P_{4444}][$C_{11}H_{23}COO$] aqueous solution generally decreased with the addition of water. The viscosity of the [P_{4444}][$C_{11}H_{23}COO$] phase decreased from 429.9 mPa·s to 38.7 mPa·s after adding 30 wt% water. More importantly, despite the fact that the solution viscosity doubled after dissolving 30% α-tocopherol in neat [P_{4444}][$C_{11}H_{23}COO$], a significant 7-fold reduction in the viscosity of the mixture was observed when 30 wt% water was present. These findings demonstrate that moderate concentrations of water in binary mixtures generate comparable α-tocopherol-dissolution abilities while offering a lower viscosity than that of neat [P_{4444}][$C_{11}H_{23}COO$].

The solvatochromic probe analysis method has been broadly used for determining the microscopic solvent properties of pure liquid or liquid mixtures, as various solvatochromic parameters can

(π^*) and hydrogen-bond basicity (β) are two widely used Kamlet-Taft parameters,[6a] which were studied for the aqueous solutions of LCC-ILs in this work.

As shown in Fig. 7, the microscopic solvent properties of LCC-IL aqueous solution can be tailored by changing the concentration of IL in solution. Pure water has a large polarity (π^*=1.35) and a very low H-bond basicity (β=0.11). The π^* values of the water/LCC-IL mixtures decreased quickly with increasing concentrations of LCC-IL (Fig. 7a), indicating the significant role of LCC-IL on reducing dipolarity/polarizability. Consequently, these water/LCC-IL mixtures greatly enhanced the affinity for weakly polar bioactive compounds as compared with pure water, which was expected. In contrast to the findings for the π^* values, a positive correlation was found between the β values of mixtures and the concentration of LCC-ILs (Fig. 7b). The H-bond basicity values of the mixtures exceeded 1.0 when the mass concentration of LCC-ILs was greater than 0.80, which was notably higher than for pure water and even for many common ILs (*e.g.*, [BMIm]NTf_2 0.24). These H-bond basicity values would be highly conducive to the dissolution of the H-bond donor bioactive compounds mentioned above *via* H-bonding interaction.[22b,31]

As discussed earlier, it is clear that mixing LCC-ILs and water effectively reduces the viscosity of the IL while simultaneously finely tailoring the solvent properties of the aqueous mixture. Therefore, the mixture of water/LCC-IL is a promising solvent for the solulibization and separation of HBCs.

Conclusions

We constructed a family of water/IL mixtures using water-miscible, lipophilic, amphiphilic, anionic functional LCC-ILs for the solubilization and extraction of hydrophobic compounds. The new LCC-IL aqueous solution exhibited excellent lipophilicity and strong H-bond basicity, while maintaining a low viscosity over a wide range of IL concentrations. The water/LCC-IL mixtures demonstrated extremely high solubilities for various hydrophobic bioactive compounds with solubilities for α-tocopherol, perillyl alcohol, rutin and ginkgolide homologues up to 1.46, 0.71, 0.39 and 0.43, respectively, at 35°C using water/[P_{4444}][$C_{11}H_{23}COO$] mixtures (*x*=70 wt%). The solubility in these mixtures is significantly higher than in pure water, pure common hydrophobic ILs, and aqueous solutions of ethanol and common ILs under the same conditions. We found that α-tocopherol formed ordered nano-micelles in water/LCC-IL mixtures whose solubility was strongly depended on the alkyl chain length and LCC-IL concentration. Additionally, aqueous solutions of LCC-ILs with enhanced dissolution ability were utilized as extractants for the first time in the extraction of hydrophobic tocopherols under mild conditions from bioresources, *e.g.,* soybean flour. Compared with aqueous solutions of common ILs and traditional organic solvents, an aqueous solution of LCC-ILs has an extraction yield 2 to 12 times larger than in other solutions. Our results demonstrate the considerable potential of water/LCC-IL mixtures as promising green solvents for the efficient solubilization and separation of hydrophobic bioactive compounds and also provide inspiration for the design of new aqueous media for other chemical processes.

Experimental

Material

Tetrabutylphonium hydroxide (40% in water) was product of TCI, decanoic acid (>99%), dodecylic acid (>98%), myristic acid (>98%), palmitic acid (>97%), DL-α-tocopherol (≥96%, GC) and rutin (>98%) were purchased from Aladdin Reagent, perillyl alcohol (≥98%, HPLC) was product of Kaimei Essence & Flavor Co., Ltd. (China), the high-purity tocopherol homologues ((+)-α, (+)-δ, (±)-β), perillyl alcohol and rutin, used as standard in several quantitative and qualitative techniques, were obtained from Sigma and used as received. Three ginkgolides mixture samples were all purchased from Xuzhou Daguanyuan Co., Ltd. (China): contains 32.4% ginkgolide A (GA), 53.0% ginkgolide B (GB) and 11.4% ginkgolide C (GC). The ginkgolide standards, GA (>98%), GB(>98%) and GC (>98%), were purchased from Chengdu Mansite Pharmaceutical Co. Ltd. (China). Methanol and acetonitrile were of HPLC grade and obtained from TEDIA (USA). Tetrahydrofuran (THF) was also of HPLC grade and obtained from Merck (Germany). Ethanol, sodium chloride and phosphoric acid were of analytical grade and obtained from Sinopharm Chemical Reagent Group Co. Ltd and used without purification. The deionized water was obtained from the Wahaha Group Co. Ltd. The conventional ILs used in this study were purchased from Lanzhou Green-chem ILS, LICP, CAS, China, including 1-butyl-3-methylimidazolium chloride ([BMIm]Cl, 99%), 1-butyl-3-methylimidazolium acetate ([BMIm][CH_3COO], 99%), 1-dedocyl-3-methylimidazolium chloride ([C_{12}MIm]Cl, 99%) and 1-dedocyl-3-methylimidazolium acetate ([C_{12}MIm][CH_3COO], 99%), with water contents of these ILs below 0.6% (mass fraction).

Soybean flour was purchased at a local market in Hangzhou, Zhejiang, China. They were kept in sealed plastic bags below 10°C until use. The samples were further screened according to the particle size by means of 100 mesh stainless steel sieves. The biomass samples were further dried at 65°C under a vacuum for 12h.

Methods

Synthesis of LCC-ILs. [P_{4444}][$C_nH_{2n+1}COO$] (*n*= 7, 9, 11, 13, 15) were synthesized according to a procedure similar to a previously reported method.[19] Tetrabutylphonium hydroxide and long chain carboxylic acid were mixed in a 100 mL round-bottom flask in a 1:1 molar ratio and then reacted at approximately 40°C for 24 h. Finally, in order to remove any moisture present, the product was distilled under vacuum at 55°C for 12 h. The purity of the [P_{4444}][$C_nH_{2n+1}COO$] was ascertained by an ^{1}H NMR spectrum in $CDCl_3$ and was determined to be ≥99 wt%. The water content of all synthetic LCC-ILs, after the drying procedure, was <0.5% as determined by Karl-Fischer titration.

Solubility determination by static method. The solubilities of α-tocopherol, perillyl alcohol, rutin and ginkgolide homologues were determined by static method. A desired amount of IL with a given mass ratio of water to IL was added quickly into a prepared vial. The vial was sealed and connected to a temperature controller. When the temperature of the system reached the desired value, an excessive amount of solute was added to the mixture. The temperature of the solute and solvent was kept constant with the use of an oil-bath and a magnetic stir bar for 72 h. After standing for

an additional 12 h, samples were taken by an injector, microfiltered through a 0.45μm standard, and then analyzed by high performance liquid chromatography (HPLC).

Extraction of tocopherols. All the aqueous solutions were prepared gravimetrically within 10^{-4}g (using an analytical balance Mettler Toledo ME204E). A precisely weighed 200 mg sample of soybean flour was added to 4.0 g of the extraction solvent in a 10 mL specific sealed glass vial. Several aqueous solutions of the extraction solvent at the same solid–liquid ratio and temperature were used.

The extractions were carried out in a commercial IKA instrument which was able to both stir and maintain the temperature within ±0.1 °C. In all experiments, the stirring was kept constant at 500 rpm, with a temperature of 40°C.

After the extractions, the overall solution and extract were rapidly cooled by cold water and then centrifuged at a speed of 7200 rpm. The liquid supernatant was dissolved in ethanol and filtered under vacuum using a 0.45μm cellulose membrane to remove the insolubes. After filtration, the extracted liquid solution was quantified with HPLC.

The total amount of tocopherols in the dry soybean flour was calculated according to the weight of pure tocopherols extracted divided by the total weight of dry biomass used. At least three individual samples were prepared for each condition, and three samples of each aqueous phase were quantified, therefore allowing us to determine the average extraction yield and corresponding standard deviation.

HPLC analysis. 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 × 250 mm, 5μm) was used for for separation. For tocopherol homologues, the mobile phase was methanol and water (96/4, v/v) and the flow rate was 1 mL/min. The detection of tocopherols was performed at 292 nm. The column temperature was 40°C. For perillyl alcohol, the mobile phase was acetonitrile and water (40/60, v/v) and the flow rate was 1 mL/min. To confirm whether the impurities have interferential UV absorption at the peak position of tocopherols, recovery test was performed. Quantitative δ-, β-, and α-tocopherol standard solutions were mixed with a certain amount of extracted sample and diluted to 2 ml for three times. The recovery was 98.90%, 101.97% and 102.25% with the RSD of 3.82%, 3.86% and 1.29%, respectively.

For perillyl alcohol, the mobile phase was acetonitrile and water (40/60, v/v) and the flow rate was 1 mL/min. The detection of perillyl alcohol was performed at 210 nm. The column temperature was 30°C. For rutin, the mobile phase was acetonitrile and 0.1 wt% phosphoric acid (19/81, v/v) and the flow rate was 1 mL/min. The detection of rutin was performed at 360 nm. The column temperature was 30°C. For ginkgolide homologues, the mobile phase was methanol-water-THF (4 : 15 : 2, v/v/v) and the flow rate was 1 mL/min. The detection of ginkgolide homologues was set at 220 nm. The column temperature was 35°C.

Solvatochromic studies for the water/LCC-ILs. The Kamlet-Taft parameters, hydrogen-bond basicity (β) and dipolarity/polarizability (π^*) were measured by solvatochromic experiments, using 4-nitroaniline and *N*,*N*-diethyl-4-nitroaniline as probes. Appropriate amounts of probe molecules were added to the LCC-IL aqueous solution samples and were then mixed thoroughly. Next, each sample was transferred into a quartz colorimetric cell with a 2 mm light-path length. The maximum absorption wavelength (λ_{max}) was recorded at 25°C by (UV-2550) UV-vis absorption measurements. Every sample was repeated at least six times and the average value was taken. The Kamlet-Taft parameters dipolarity/polarizability (π^*) and the hydrogen bonding basicity (β) were calculated by using equations (1) and (2):

$$\pi^* = 8.649 - 0.314\nu(1)_{max} \quad (1)$$

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

where $\nu(1)_{max}$ and $\nu(2)_{max}$ are the wave number at which the maximum absorption of *N*,*N*-diethyl-4-nitroaniline and 4-nitroaniline, respectively, were found in the sample.

Viscosity measurement of the water/LCC-ILs. A Brookfield DV-II Cone/Plate viscometer was employed to perform the viscosity experiments. The temperature was maintained at 25±0.1°C by a Julabo F12 circulating thermostatic water bath. The viscosity of the individual samples was determined at least three times for each sample. The repeatability of viscosity values could be measured within 1%.

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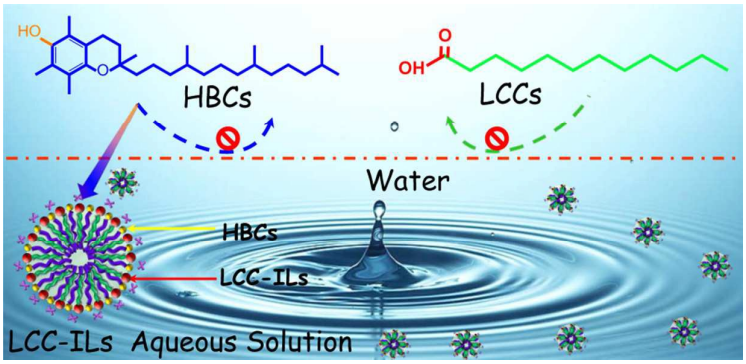

The developed water/LCC-IL (long-chain carboxylate ionic liquids) mixtures exhibited extremely high solubilities for various hydrophobic bioactive compounds (HBCs) and excellent extraction efficiency for tocopherols.