Surface-active Ionic Liquids for Micellar Extraction of Piperine from Black Pepper

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We present the application of ionic liquid-aqueous micellar solutions as isolation media for the pharmaceutically active ingredient piperine from black pepper. Several surface-active ionic liquids including a biodegradable betaine derivative were used for the extraction of piperine, and a strong correlation between extraction yield and the critical micelle concentration of the respective ionic liquid was found. A scaled strategy for the isolation of piperine was developed that allowed recovery and recycling of the aqueous ionic liquid micellar solution for five runs without any loss in extraction efficiency.

Key words: Ionic Liquids, Micelles, Active Ingredient Isolation, Piperine, Biomass Processing, Sustainable Chemistry

Introduction

Biomass feedstocks provide a unique and indispensible source for drug development and manufacturing: Current estimations of the pharmaceutical market indicate that approximately a third of all drugs currently on the market is derived from natural products [1]. Piperine ((2*E*,4*E*)-5-(1,3-benzodioxol-5-yl)-1-(1-piperidinyl)-,2,4-pentadien-1-one (**1**), a common alkaloid that is responsible for the pungent taste of pepper, is naturally occurring in *Piper nigrum* (6 % – 9 %), in *Piper longum* fruits (4 %) and in *Piper retrofractum* (4 % – 5 %) [2]. Apart from its nutraceutical uses, it has attracted attention for its inhibitory influence on enzymatic drug biotransforming reactions in the liver. The pharmacological properties of piperine are widespread: Piperine exhibits not only antifungal [3], antidiarrhoeal [4], anti-inflammatory [5], 5-lipoxygenase and cyclooxygenase-1 inhibitory activities [6], but also serves as a positive $\mathrm{GABA_A}$ receptor [7, 8].

As most plant-derived active ingredients, piperine is typically isolated from cultivated plant material using excessive refluxing with volatile organic solvents such as ethanol, toluene or chlorinated hydrocarbons [9, 10]. This extraction process is not only time consuming and requires large amounts of often toxic solvents, but suffers from co-extraction of undesired by-products such as gums, polysaccharides or essential oils. Additional purification steps are thus required making the isolation and purification of a natural product a large obstacle in any cost-efficient and sustainable drug manufacturing process.

In the past years, ionic liquids (ILs, salts with melting point $< 100\,^\circ\mathrm{C}$) have emerged as alternative solvents for biomass processing, as they provide unique dissolution properties for biomass feedstocks. Based on the disruption of the intermolecular hydrogen-bonded network up to ~ 30 wt.-% cellulose can be solubilized in ionic liquids that are typically composed of imidazolium head groups. This can be highly beneficial for the extraction of valuable ingredients from biomass, as the tunability of ionic liquids allows designing a tailor-made ionic liquid for a specific extraction problem. While the use of ionic liquids for biomass dissolution such as wood [11 – 15], cellulose [16 – 19], lignin [20], chitin [21 – 24], silk fibroin [25], wool keratin [26], starch and zein protein [27], cotton and bamboo [28], chitosan [21], and cork biopolymers [29] has attracted tremendous interest, less attention has been paid to the isolation of valuable ingredients using ionic liquids. Pioneering work was performed by Lapkin *et al.* in a study comparing conventional solvents, ionic liquids, supercritical carbon dioxide and fluorous solvents for the extraction of the anti-malaria drug artemisinin from crude *Artemisia annua* seeds [30]. This strategy has spread, and successful examples for the application of ionic liquids as extraction media include the isolation of the pharmaceutically active steroid betulin from birch bark [31], suberinic material from cork [32], the bioactive alkaloid *S*-(+)-glaucine from plant material of *Glaucium flavum* Crantz (Papaveraceae) [33, 34], the flavonoid rutin [35], and the actives senkyunolide I, senkyunolide H and Z-ligustide [36], or shikonin and β,β'-dimethylacrylshikonin [37] from traditional Chinese medical plants. Additionally, valuable essential oils, *e. g.* orange oil or rosmary oil could be directly distilled from the ionic liquid media, thus allowing a simple isolation of the pure volatile fragrance components and a complete recovery of the ionic liquid [38 – 40]. An elegant strategy was also developed by the group of MacFarlane, who used distillable ionic media for the isolation of hydrolyzable tannins [41]. In some cases, the role of the ionic liquid is not limited to the function as mere solvent, but it can directly take part in the further transformation towards a drug intermediate, as we could previously show for the reactive dissolution of star anise with Brønsted-acidic ILs that gave direct access to an important intermediate for the manufacturing of the anti-influenza drug Tamiflu™ [42].

While ionic liquids often proved to be superior to conventional solvents in terms of extraction yield and purity, their usage is still associated with a major drawback: Ionic liquids are relatively expensive – at least compared to conventional organic solvents – which may restrict their large-scale application as a bulk solvent. Our search for a compromise between economic and environmental criteria got us interested in the use of aqueous-ionic liquid systems as reaction media for synthesis and catalysis [43]. Recent investigations on the behavior of ionic liquids in the presence of water showed that certain ionic liquids can form aggregates in aqueous solution [44 – 47]. This is the case for long-chain 1-alkyl-3-methyl-imidazolium chloride salts [$C_n$mim]Cl with $n = 8 - 18$, where the apolar side chain stays in contact with that of other cations and micelles are formed. While the physicochemical properties of 1-alkyl-3-methyl-imidazolium chlorides and bromides are reasonably well explored, so that critical micelle concentration values (CMC) are available, applications remain limited. Yet we could show that these micellar systems can be efficiently applied for synthesis and found enhanced reaction rates for Diels-Alder reactions compared to the rates in neat water. A similar effect was reported by Coutinho *et al.* who found increased enzyme activity of Candida antarctica lipase B (CaLB) in aqueous solutions of the ionic liquid 1-decyl-3-methyl-imidazolium chloride, [C_{10}mim]Cl [48].

The concept of aqueous-ionic liquid systems as extraction media has been only sparingly explored, although promising work on the cellulose pretreatment with aqueous solutions of ionic liquids has been reported by Welton *et al.* [49]. Cao *et al.* investigated ultrasound-assisted extraction by ILs for the isolation of piperine from white pepper [50]. Different aqueous solutions of short-chain 1-alkyl-3-methyl-imidazolium bromides [$C_n$mim]Br with $n = 3$ and $n = 4$ were used in rather high concentration of 5 M. A strong influence of the anion was observed, as the extraction efficiency decreased in the order $BF_4^- > Br^- > H_2PO_4^- > PF_6^-$ which was explained by a reduced hydrophilicity and water miscibility [50]. Although [C_4mim]Br performed significantly better than [C_3mim]Br, longer chain lengths of the alkyl imidazolium backbone were not investigated. Recently Coutinho *et al.* published an elegant strategy for the isolation of caffeine from guaraná seeds using aqueous IL solutions [51].

Only few papers reported the exploration of the extraction of active ingredients with surface-active ionic liquids. Yao *et al.* extracted different tanshinones, namely tanshinone I, tanshinone IIA and cryptotanshinone from *Salvia miltiorrhiza bunge*. Based on bromide anions, the chain length of the 1-alkyl-3-methyl-imdazolium cation was varied, as tanshinones are lipophilic compounds. While [C_8mim]Br failed to extract the active ingredients, the extraction yield increased dramatically for tanshinone I and tanshinone IIA when the chain length was increasing from C_{10} to C_{16} [52]. Microwave-assisted micellar extraction was also successfully applied by Lin *et al.* in 2012 using an aqueous two-phase system (ATPS) consisting of [$C_n$mim]BF_4/NaH_2PO_4 for the active ingredients hyperin and isoquercitrin from *Apocynum venetum*; however, better results were obtained with the butyl side chain rather than with the surface-active 1-methyl-3-octyl-imidazolium tetrafluoroborate [53].

In this paper, we show that IL-water micellar systems might provide novel and high-yield but yet cost-efficient extraction media for active ingredients. For the extraction of piperine from ground black pepper, we have investigated micellar solutions of several surface-active ionic liquids as extraction media and provide a scaled strategy for the isolation of piperine allowing recovery and recycling of the aqueous-ionic liquid micellar solution.

Table 1. Influence of extraction conditions on the extraction yield of piperine from black pepper using a 50 mM solution of [C_{12}mim]Cl in water.

Entry[a]	Conditions	Piperine (wt.-%)[b,c]
1	1 h, 5 wt.- %	3.42 ± 0.38
2	3 h, 5 wt.- %	3.74 ± 0.19
3	24 h, 5 wt.- %	4.64 ± 0.30
4	3 h, 1 wt.- %	3.86 ± 0.27
5	3 h, 5 wt.- %	3.74 ± 0.19
6	3 h, 10 wt.- %	2.93 ± 0.11

[a] Performed with 50.0 ± 0.1 mg ground black pepper in 950 μL of ionic liquid solution at 25 °C; [b] yield was determined *via* HPLC using phenol as internal standard; [c] average of three independent experiments (mean ± STD, $n = 3$).

Results and Discussion

We have chosen a set of surface-active ionic liquids based on common 1-alkyl-3-methyl-imidazolium chlorides [$C_n$mim]Cl **2**, **3** and **8** (Fig. 1) with alkyl chain lengths varying from $n = 10$ to $n = 14$. As the inherent toxicity and biodegradability of long-chain imidazolium salts might restrict their application, we also included a surface-active betaine-derived ionic liquid **9** that has been reported to be biodegradable (Fig. 1).

In an initial screening, we developed an HPLC strategy that allowed quantifying piperine in the presence of the aqueous ionic liquid systems to quickly identify optimum conditions for the extraction process. When investigating the influence of extraction time using a 50 mM [C_{12}mim]Cl solution in water at 25 °C, we found that with increasing extraction time, extraction efficiency also increased (Table 1, entries 1 – 3). As a compromise between time demand and extraction yield, we decided to work with an extraction time of 3 h as already 3.74 wt.- % of piperine per black pepper dry weight have been extracted within that time. When the ratio between biomass and ionic liquid solution was further investigated, we found only a slight difference between 1 % (w/v) and 5 % (w/v) biomass in ionic liquid-containing solution. However, with a higher biomass loading of 10 % (w/v) the ex-

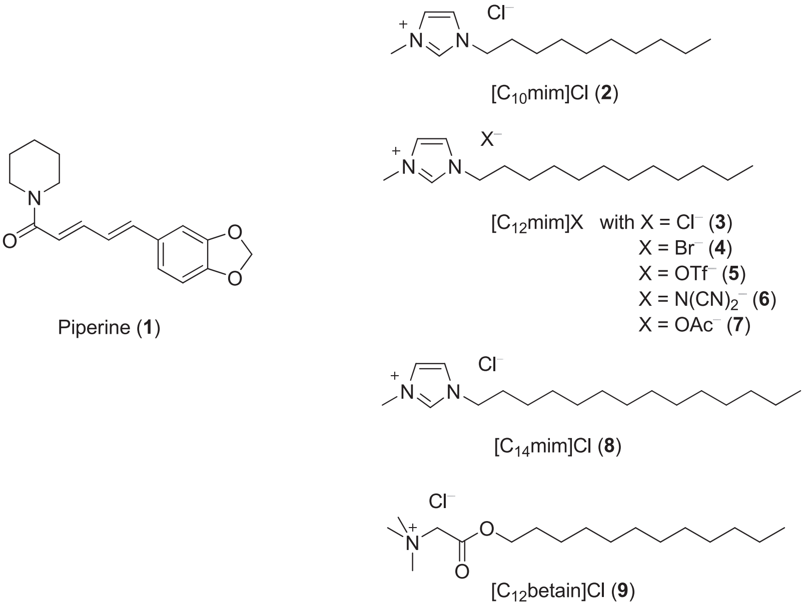

Fig. 1. Structures of piperine (**1**) (left) and ionic liquids **2** – **9** used in this study (right).

traction efficiency decreased significantly, which might be related to mixing problems (Table 1, entries 4 – 6).

Based on the optimum conditions, we turned our attention towards the influence of different concentrations of [C$_{12}$mim]Cl in water. We found an interesting correlation of the CMC of the respective ionic liquid with the extraction yield of piperine: At low concentrations of $\leq$ 10 mM, only small amounts of piperine $<$ 0.20 wt.- % could be extracted, which is only slightly higher than the extraction yield that was obtained with pure water under similar conditions. However, as soon as the concentration of [C$_{12}$mim]Cl reached the CMC (15 mM) a significant increase in the extraction efficiency was observed [54 – 56] (Fig. 2).

A similar pattern was observed when expanding the range of ionic liquids. For solutions of [C$_{10}$mim]Cl (**2**), [C$_{14}$mim]Cl (**8**) as well as the biodegradable [C$_{12}$betaine]Cl (**9**) the extraction efficiency increased dramatically at concentrations higher than CMC of the respective ionic liquid. For all ionic liquids, the extraction yield eventually leveled off at concentrations higher than 100 mM, and up to $\sim$ 4 wt.- % of piperine could be extracted. We found that the increase in the extraction efficiency can be correlated with the chain length and found an increase occurring in the order [C$_{14}$mim]Cl (**8**) < [C$_{12}$mim]Cl (**3**) ≈ [C$_{12}$betaine]Cl (**9**) < [C$_{10}$mim]Cl (**2**). This again is in accordance with literature data [43], as a linear relationship between the logarithm of CMC and the number of carbon atoms n is found for 1-alkyl-3-methyl-imidazolium-based ionic liquids [C$_n$mim]Cl. In comparison to the large effect of the chain length in the cation, the anion in the surface-active ionic liquids [C$_{12}$mim]X had less influence on the extraction efficiency. With the exception of the triflate anion (X = OTf, **5**) that gave only moderate yields of 2.28 wt.- %, all other anions including halides (X = Cl$^-$ or Br$^-$, **4**), dicyanamide (X = N(CN)$^-$, **6**) or acetate (X = OAc$^-$, **7**) were suitable for the extraction and gave comparable yields between 3.20 and 3.74 wt.- % (Table 2).

When comparing the extraction performance of micellar solutions of 1-alkyl-3-methyl-imidazolium-based ionic liquids, we found that these solutions can compete with different conventional organic solvents reported in literature. The volatile and toxic solvents such as chloroform, methanol and toluene gave only slightly higher yields of 3.81 – 4.10 wt.- %, whereas the environmentally more benign butyl acetate extracted around 3.10 wt.- % and gave the lowest yield among the organic solvents screened here (Fig. 3).

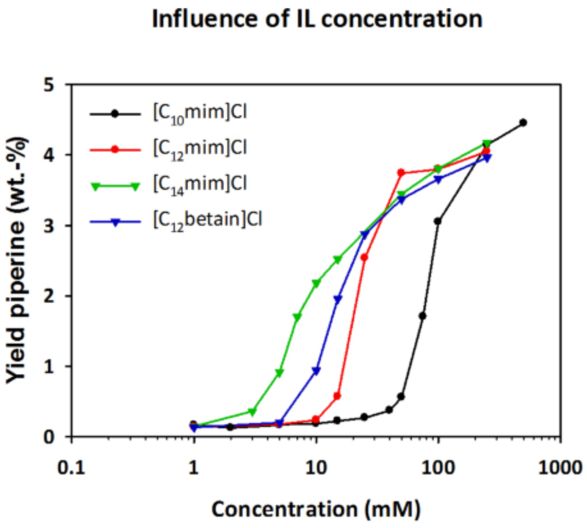

Fig. 2 (color online). Influence of the concentration of surface-active ionic liquids in water on the extraction of piperine from black pepper.

Table 2. Influence of different anions on the extraction yield of piperine from black pepper using a 50 mM solution of [C$_{12}$mim]X in water.

Entry[a]	Ionic liquid solution	Piperine (wt.-%)[b,c]
1	50 mM [C$_{12}$mim]OTf (**5**)	2.28 ± 0.19
2	50 mM [C$_{12}$mim]Cl (**3**)	3.74 ± 0.19
3	50 mM [C$_{12}$mim]Br (**4**)	3.08 ± 0.13
4	50 mM [C$_{12}$mim]N(CN)$_2$ (**6**)	3.28 ± 0.33
5	50 mM [C$_{12}$mim]OAc (**7**)	3.35 ± 0.45

[a] Performed with 50.0 ± 0.1 mg ground black pepper in 950 μL of ionic liquid solution at 25 °C; [b] yield was determined *via* HPLC using phenol as internal standard; [c] average of three independent experiments (mean ± STD, $n = 3$).

The importance of the surface-activity of the applied ionic liquid solution is obvious when the extraction yield is compared with the yield of a 50 mM solution of NaCl in water: Only 0.06 wt.- % could be isolated here, which is even below the extraction yield with pure water, that reached 0.11 wt.- %. The influence of aqueous ionic liquid solutions compared to pure water is also visible from electron microscopy that was performed on the recovered biomass after extraction. Although micellar solution of 1-alkyl-3-methyl-imidazolium based ionic liquids [C$_n$mim]Cl did not completely dissolve biomass as we observed in our previous studies [31] with neat ionic liquids, we

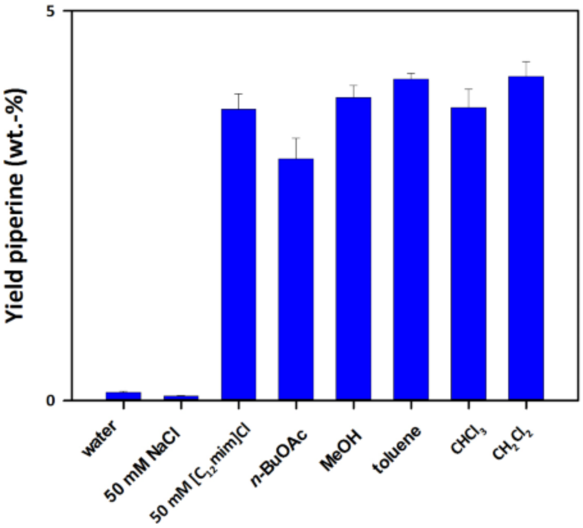

Fig. 3 (color online). Comparison of IL and conventional solvents.

observed some changes in biomass morphology that could not be observed when pure water was used as extraction media (Fig. 4). A similar effect was observed by Coutinho *et al.* who investigated the extraction of caffeine from guaraná seed and reported an increase in the ratio of broken cells to intact cells in the presence of ionic liquid/aqueous mixtures, although the biomass was not completely dissolved [51].

Table 3. Extraction efficiencies of pure ionic liquids [C_{10}mim]Cl, [C_{12}mim]Cl and [C_{14}mim]Cl.

Entry[a]	Ionic liquid	Piperine (wt.-%)[b,c]
1	[C_{10}mim]Cl	3.74 ± 0.28
2	[C_{12}mim]Cl	3.57 ± 0.28
3	[C_{14}mim]Cl	3.52 ± 0.28

[a] Performed with 50.0 ± 0.1 mg ground black pepper in 950.0 ± 5.0 mg of pure ionic liquid at 80 °C; [b] yield was determined *via* HPLC using phenol as internal standard; [c] average of three independent experiments (mean ± STD, $n = 3$).

Consequently, it seems that complete solubilization of the biopolymer matrix is not always necessary for efficient extraction of the valuable ingredients: When comparing the extraction yields of aqueous solution of the ionic liquids [$C_n$mim]Cl with those obtained with pure ionic liquids, we found that the micellar systems can be a superior alternative to the neat ionic liquid (Table 3, entries 1–3). It should be noted that the extraction with pure ionic liquids had to be performed at 80 °C as [C_{12}mim]Cl and [C_{14}mim]Cl were solid at room temperature. Even at this temperature, viscosity problems occurred with longer alkyl chains, and the extraction efficiencies decreased. Only [C_{10}mim]Cl, which is liquid at room temperature gave yields comparable to those of aqueous micellar solution.

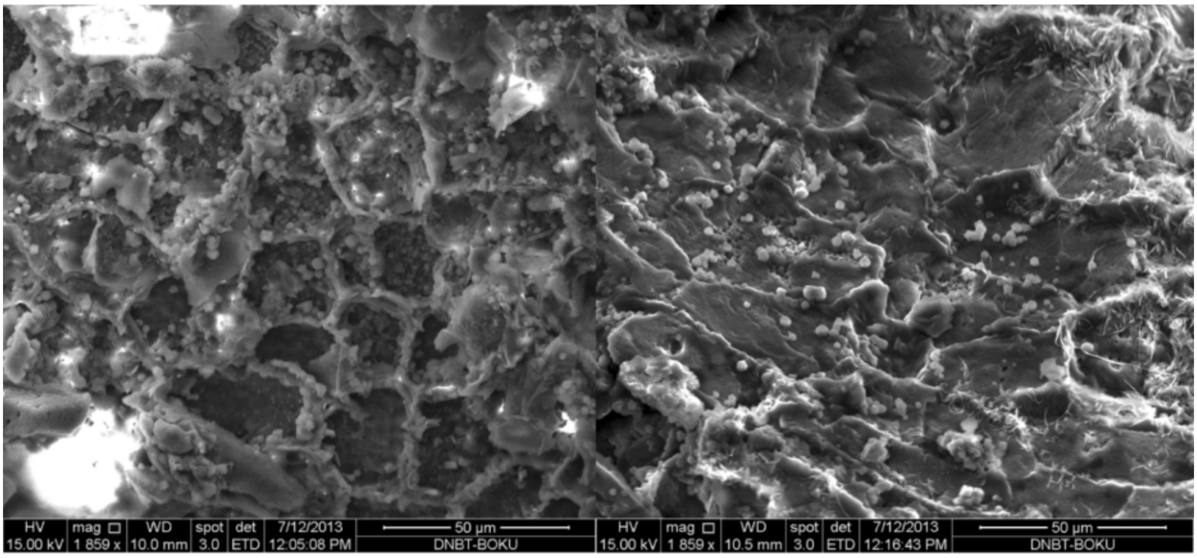

Fig. 4. Electron microscopy of residual pepper after extraction with water (left) and with aqueous ionic liquid micellar solution (right).

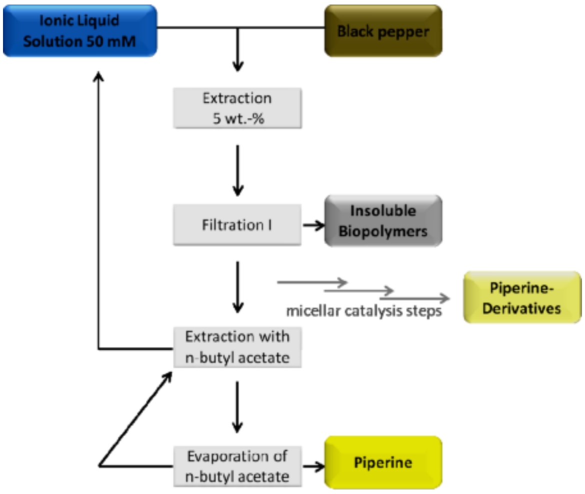

Fig. 5 (color online). Scale-up strategy for the isolation of piperine from black pepper.

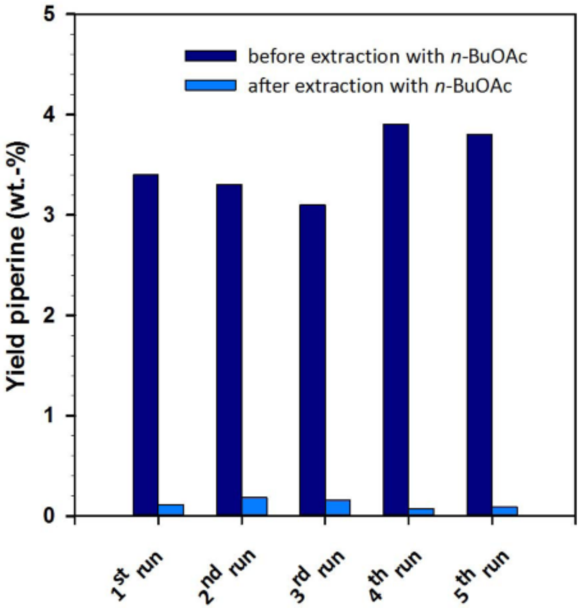

Fig. 6 (color online). Results of the scale-up and recycling.

Scaled procedure and reusability of the extraction media

Once the optimum extraction conditions were identified, we turned our attention towards a scaled extraction process allowing not only the isolation of piperine, but also the recovery of the micellar ionic liquid solution (Fig. 5).

As previously optimized we worked in a 5% (*w*/*v*) solution of ground black pepper. After extraction of 1.00 g ground black pepper with 19 mL of a 50 mM solution of [C_{12}betaine]Cl in water for 3 h at room temperature, the remaining biomass was removed *via* filtration. Once the biomass was removed, the micellar solution was extracted with a small amount of environmentally benign butyl acetate. We found that after extraction with only 5 mL butyl acetate piperine was nearly quantitatively separated leaving less than 0.2 wt.-% piperine in the ionic liquid micellar solution. This allowed not only a quick and clean separation of piperine in excellent purity, but also the recovery of the aqueous solution containing the ionic liquid that could be directly used for four additional runs without any loss in performance (Fig. 6).

Alternatively, the micellar solution containing piperine can be directly subjected to any consecutive synthetic modification towards piperine derivatives, which will be the object of our future studies.

Conclusion

Aqueous-ionic liquids micellar solutions are efficient extraction media for the isolation of active ingredients and can overcome common problems of conventional organic solvents. Based on the isolation of piperine from black pepper, we have demonstrated that the extraction efficiency of various aqueous-ionic liquid micellar solutions strongly depends on the CMC of the respective ionic liquid. Based on a biodegradable betaine derivative, a simple and scalable isolation procedure was developed, which allowed separation of piperine from the extraction media and the recycling of the aqueous solution for four times without loss in the extraction efficiency.

Experimental Section

Commercially available reagents and solvents were used as received from Sigma Aldrich unless otherwise specified. Ionic liquids [C_{10}mim]Cl, and [C_{12}mim]Cl [C_{12}mim]Br and [C_{14}mim]Cl were prepared from freshly distilled *N*-methylimidazole and alkyl chloride or alkyl bromide according to the literature [57]. Solid [C_{12}mim]Cl and [C_{14}mim]Cl were repeatedly crystallized from THF until colorless crystals were obtained. Ionic liquids [C_{12}mim]OTf [58], [C_{12}mim]N$(CN)_2$ [59], [C_{12}mim]OAc [60] have previously been reported in literature and were prepared *via* metathesis reaction of [C_{12}mim]Cl with the corresponding silver salt of the anion. All ionic liquids were dried for at least 24 – 48 h at 50 °C and 0.01 mbar before use and were stored under argon.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 400 spectrometer at 400 and 100 MHz, respectively, using the solvent peak as reference. J values are given in Hz. ^{13}C NMR spectra were run in proton-decoupled mode.

For the determination of piperine a Phenomenex Luna 10 μm C18 100 A column (250 × 4.60 mm) was used with $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ 55/45 as solvent and a flow of 1 mL min^{-1}; detection was done at 271 nm, at 30 °C column oven temperature, 4 °C tray temperature. Retention times were 4.07 min for phenol and 9.2 min for piperine. Standard calibrations were performed for aqueous and organic extraction.

Dodecyl 2-chloroacetate

Dodecanol (14.18 g, 76.10 mmol) and chloroacetyl chloride (11.17 g, 98.90 mmol) were dissolved in 50 mL of anhydrous dichloromethane under argon and chilled to 0 °C. Triethylamine (10.00 g, 98.90 mmol) was added dropwise. The solution was stirred at room temperature over night until TLC indicated full conversion. The solution was diluted with 50 mL of water and extracted with dichloromethane. The combined organic layers were successively washed with 2 N HCl, saturated NaHCO_3 and brine. The solution was dried over Na_2SO_4, filtered and evaporated to dryness. Dodecyl 2-chloroacetate was obtained as a light-yellow oil in 95 % yield and used as obtained. – ^{1}H NMR (CDCl_3): $\delta = 0.88$ (3H, t, $J = 6.4$ Hz, $\text{-C}_{11}\text{H}_{22}\text{-C}\mathit{H}_3$), 1.26 (18H, m, $\text{-C}_2\text{H}_4\text{-C}_9\mathit{H}_{18}\text{-CH}_3$), 1.67 (2H, t, $J = 6.6$ Hz, $\text{-CH}_2\text{-C}\mathit{H}_2\text{-C}_{10}\text{H}_{21}$), 4.06 (2H, s, $\text{Cl-C}\mathit{H}_2\text{-CO}$), 4.19 (2H, t, $J = 0.7$ Hz, $\text{-C}\mathit{H}_2\text{-C}_{11}\text{H}_{23}$). These data were in accordance with the literature [61].

*2-(Dodecyloxy)-N,N,N-trimethyl-2-oxoethanaminium chloride, [C$_{12}$betaine]Cl (**9**)*

Dodecyl 2-chloroacetate (5.39 g, 20.51 mmol) was dissolved in 15 mL anhydrous THF. A solution of trimethylamine in THF (102.5 mmol) was added dropwise at room temperature. After stirring over night the precipitate was collected *via* filtration and washed with anhydrous THF and anhydrous diethyl ether. After drying *in vacuo* (2×10^{-2} mbar) overnight, [C$_{12}$betaine]Cl (**9**) was obtained as colorless crystals in 88 % yield. – ^{1}H NMR (CDCl_3): $\delta = 0.81$ (3H, t, $J = 7.0$ Hz, $\text{-C}_{11}\text{H}_{22}\text{-C}\mathit{H}_3$), 1.19 (18H, m, $\text{-C}_2\text{H}_4\text{-C}_9\mathit{H}_{18}\text{-CH}_3$), 1.58 (2H, t, $J = 6.7$ Hz, $\text{-CH}_2\text{-C}\mathit{H}_2\text{-C}_{10}\text{H}_{21}$), 3.60 (9H, s, $\text{N-(C}\mathit{H}_3)_3$), 4.10 (2H, t, $J = 7.0$ Hz, $\text{-C}\mathit{H}_2\text{-C}_{11}\text{H}_{23}$), 5.01 (2H, s, $\text{Cl-C}\mathit{H}_2\text{-CO}$). These data were in accordance with the literature [62].

Extraction procedure for IL solutions

A suspension of black pepper in aqueous IL solution (1 wt.-%: 10.0 mg pepper, 990 mg IL solution; 5 wt.-%: 50 mg pepper, 950 mg IL solution; 10 wt.-%: 100 mg pepper, 900 mg IL solution) was stirred at 25 °C for 3 h. A sample of 100 μL was taken from the supernatant and diluted to 5 mL with the IL solution. An aliquot of 1 mL was taken, mixed with 200 μL of internal standard (60.0 mg phenol in 100 mL of water) filtered over a 0.2 μm syringe filter and measured immediately *via* HPLC. Results are based on three independent experiments.

Scaled procedure for the isolation of piperine

Ground black pepper (1.000 g) was stirred with 19 mL of a 50 mM solution of [C$_{12}$betaine]Cl in water at 25 °C for 3 h. After filtration the solution was extracted 3 times with a total volume of 5 mL of *n*-butyl acetate. Samples of 100 μL were taken from the aqueous layer, and HPLC measurements carried out before and after extraction with butyl acetate to quantify the amount of piperine in the micellar solution. The remaining micellar solution was directly used for the next extractions step without further purification.

For isolation of piperine, the combined organic layers were dried over Na_2SO_4, filtered and evaporated to dryness. – ^{1}H NMR (CDCl_3): $\delta = 1.59 - 1.63$ (6H, m, $\text{N-CH}_2\text{-C}\mathit{H}_2\text{-C}\mathit{H}_2\text{-C}\mathit{H}_2\text{-CH}_2\text{-}$), 3.54 – 3.64 (4H, m, $\text{N-C}\mathit{H}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-C}\mathit{H}_2\text{-}$), 5.98 (2H, s, $\text{O-CH}_2\text{-O}$), 6.43 (1H, d, $J = 15.1$ Hz, N-CO-CH), 6.72 – 6.99 (5H, m, Ph-*H*, Ph-C*H*-C*H*), 7.35 – 7.54 (1H, m, N-CO-CH-C*H*). These data are in accordance with the literature [63]. Results are based on two independent experiments.

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[1] D. G. I. Kingston, *J. Nat. Prod.* **2010**, *74*, 496 – 511.
[2] V. G. Gaikar, G. Raman, US6365601B1, **2002**.
[3] H. M. D. Navickiene, A. C. Alecio, M. J. Kato, V. S. Bolzani, M. C. M. Young, A. J. Cavalheiro, M. Furlan, *Phytochemistry* **2000**, *55*, 621 – 626.
[4] S. Bajad, K. L. Bedi, A. K. Singla, R. K. Johri, *Planta Med.* **2001**, *67*, 284 – 287.
[5] A. M. Mujumdar, J. M. Dhuley, V. Deshmukh, P. H. Raman, S. R. Naik, *Jpn. J. Med. Sci. Biol.* **1990**, *43*, 95 – 100.
[6] J. R. Stohr, P. G. Xiao, R. Bauer, *J. Ethnopharmacol.* **2001**, *75*, 133 – 139.
[7] J. Zaugg, I. Baburin, B. Strommer, H.-J. Kim, S. Hering, M. Hamburger, *J. Nat. Prod.* **2010**, *73*, 185 – 191.

[8] S. Hering, T. Erker, T. Schwarz, I. Baburin, D. Schellmann, WO2011080313A1, **2011**.

[9] L. Marion in *Alkaloids – Chemistry and Physiology*, Vol. 6, (Ed.: R. H. F. Manske), Academic Press, New York, **1960**, pp. 31–36.

[10] H. Staudinger, H. Schneider, *Ber. Dtsch. Chem. Ges. B* **1923**, *56B*, 699–711.

[11] D. A. Fort, R. C. Remsing, R. P. Swatloski, P. Moyna, G. Moyna, R. D. Rogers, *Green Chem.* **2007**, *9*, 63–69.

[12] I. Kilpelaeinen, H. Xie, A. King, M. Granstrom, S. Heikkinen, D. S. Argyropoulos, *J. Agric. Food Chem.* **2007**, *55*, 9142–9148.

[13] N. Sun, M. Rahman, Y. Qin, M. L. Maxim, H. Rodriguez, R. D. Rogers, *Green Chem.* **2009**, *11*, 646–655.

[14] M. Zavrel, D. Bross, M. Funke, J. Buechs, A. C. Spiess, *Bioresour. Technol.* **2009**, *100*, 2580–2587.

[15] A. Brandt, J. P. Hallett, D. J. Leak, R. J. Murphy, T. Welton, *Green Chem.* **2010**, *12*, 672–679.

[16] R. P. Swatloski, S. K. Spear, J. D. Holbrey, R. D. Rogers, *J. Am. Chem. Soc.* **2002**, *124*, 4974–4975.

[17] A. Pinkert, K. N. Marsh, S. Pang, M. P. Staiger, *Chem. Rev.* **2009**, *109*, 6712–6728.

[18] H. Wang, G. Gurau, R. D. Rogers, *Chem. Soc. Rev.* **2012**, *41*, 1519–1537.

[19] S. Zhu, Y. Wu, Q. Chen, Z. Yu, C. Wang, S. Jin, Y. Ding, G. Wu, *Green Chem.* **2006**, *8*, 325–327.

[20] Y. Pu, N. Jiang, A. J. Ragauskas, *J. Wood Chem. Technol.* **2007**, *27*, 23–33.

[21] H. Xie, S. Zhang, S. Li, *Green Chem.* **2006**, *8*, 630–633.

[22] S. Yamazaki, A. Takegawa, Y. Kaneko, J.-I. Kadokawa, M. Yamagata, M. Ishikawa, *Electrochem. Commun.* **2009**, *11*, 68–70.

[23] Y. Wu, T. Sasaki, S. Irie, K. Sakurai, *Polymer* **2008**, *49*, 2321–2327.

[24] Y. Qin, X. Lu, N. Sun, R. D. Rogers, *Green Chem.* **2010**, *12*, 968–971.

[25] D. M. Phillips, L. F. Drummy, D. G. Conrady, D. M. Fox, R. R. Naik, M. O. Stone, P. C. Trulove, L. H. C. De, R. A. Mantz, *J. Am. Chem. Soc.* **2004**, *126*, 14350–14351.

[26] H. Xie, S. Li, S. Zhang, *Green Chem.* **2005**, *7*, 606–608.

[27] A. Biswas, R. L. Shogren, D. G. Stevenson, J. L. Willett, P. K. Bhowmik, *Carbohydr. Polym.* **2006**, *66*, 546–550.

[28] C. He, Q. Wang, J. Sun, X. Chen, CN101225552A, **2008**.

[29] H. Garcia, R. Ferreira, M. Petkovic, J. L. Ferguson, M. C. Leitao, H. Q. N. Gunaratne, K. R. Seddon, L. P. N. Rebelo, P. C. Silva, *Green Chem.* **2010**, *12*, 367–369.

[30] A. A. Lapkin, P. K. Plucinski, M. Cutler, *J. Nat. Prod.* **2006**, *69*, 1653–1664.

[31] A. K. Ressmann, K. Strassl, P. Gaertner, B. Zhao, L. Greiner, K. Bica, *Green Chem.* **2012**, *14*, 940–944.

[32] R. Ferreira, H. Garcia, A. F. Sousa, M. Petkovic, P. Lamosa, C. S. R. Freire, A. J. D. Silvestre, L. P. N. Rebelo, C. S. Pereira, *New J. Chem.* **2012**, *36*, 2014–2024.

[33] M. G. Bogdanov, I. Svinyarov, R. Keremedchieva, A. Sidjimov, *Sep. Purif. Technol.* **2012**, *97*, 221–227.

[34] M. G. Bogdanov, I. Svinyarov, *Sep. Purif. Technol.* **2013**, *103*, 279–288.

[35] H. Zeng, Y. Wang, J. Kong, C. Nie, Y. Yuan, *Talanta* **2010**, *83*, 582–590.

[36] C. Yansheng, Z. Zhida, L. Changping, L. Qingshan, Y. Peifang, U. Welz-Biermann, *Green Chem.* **2011**, *13*, 666–670.

[37] Y. Xiao, Y. Wang, S. Gao, R. Zhang, R. Ren, N. Li, H. Zhang, *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2011**, *879*, 1833–1838.

[38] K. Bica, P. Gaertner, R. D. Rogers, *Green Chem.* **2011**, *13*, 1997–1999.

[39] T. Liu, X. Sui, R. Zhang, L. Yang, Y. Zu, L. Zhang, Y. Zhang, Z. Zhang, *J. Chromatogr. A* **2011**, *1218*, 8480–8489.

[40] Y. Zhai, S. Sun, Z. Wang, J. Cheng, Y. Sun, L. Wang, Y. Zhang, H. Zhang, A. Yu, *J. Sep. Sci.* **2009**, *32*, 3544–3549.

[41] S. A. Chowdhury, R. Vijayaraghavan, D. R. MacFarlane, *Green Chem.* **2010**, *12*, 1023–1028.

[42] A. K. Ressmann, P. Gaertner, K. Bica, *Green Chem.* **2011**, *13*, 1442–1447.

[43] K. Bica, P. Gaertner, P. J. Gritsch, A. K. Ressmann, C. Schroeder, R. Zirbs, *Chem. Commun.* **2012**, *48*, 5013–5015.

[44] J. Bowers, C. P. Butts, P. J. Martin, M. C. Vergara-Gutierrez, R. K. Heenan, *Langmuir* **2004**, *20*, 2191–2198.

[45] S. Dorbritz, W. Ruth, U. Kragl, *Adv. Synth. Catal.* **2005**, *347*, 1273–1279.

[46] Z. Miskolczy, K. Sebok-Nagy, L. Biczok, S. Goekturk, *Chem. Phys. Lett.* **2004**, *400*, 296–300.

[47] I. Goodchild, L. Collier, S. L. Millar, I. Prokes, J. C. D. Lord, C. P. Butts, J. Bowers, J. R. P. Webster, R. K. Heenan, *J. Colloid Interface Sci.* **2007**, *307*, 455–468.

[48] S. P. M. Ventura, L. D. F. Santos, J. A. Saraiva, J. A. P. Coutinho, *Green Chem.* **2012**, *14*, 1620–1625.

[49] A. Brandt, M. J. Ray, T. Q. To, D. J. Leak, R. J. Murphy, T. Welton, *Green Chem.* **2011**, *13*, 2489–2499.

[50] X. Cao, X. Ye, Y. Lu, Y. Yu, W. Mo, *Anal. Chim. Acta* **2009**, *640*, 47–51.

[51] A. F. M. Claudio, A. M. Ferreira, M. G. Freire, J. A. P. Coutinho, *Green Chem.* **2013**, *15*, 2002–2010.

[52] K. Wu, Q. Zhang, Q. Liu, F. Tang, Y. Long, S. Yao, *J. Sep. Sci.* **2009**, *32*, 4220 – 4226.

[53] X. Lin, Y. Wang, X. Liu, S. Huang, Q. Zeng, *Analyst* **2012**, *137*, 4076 – 4085.

[54] M. Blesic, M. H. Marques, N. V. Plechkova, K. R. Seddon, L. P. N. Rebelo, A. Lopes, *Green Chem.* **2007**, *9*, 481 – 490.

[55] S. O. A. El, P. A. R. Pires, T. Abdel-Moghny, E. L. Bastos, *J. Colloid Interface Sci.* **2007**, *313*, 296 – 304.

[56] J. Luczak, J. Hupka, J. Thoeming, C. Jungnickel, *Colloids Surf. A* **2008**, *329*, 125 – 133.

[57] B. F. Gilmore, G. P. Andrews, G. Borberly, M. J. Earle, M. A. Gilea, S. P. Gorman, A. F. Lowry, M. McLaughlin, K. R. Seddon, *New J. Chem.* **2013**, *37*, 873 – 876.

[58] H. Tokuda, K. Hayamizu, K. Ishii, M. A. B. H. Susan, M. Watanabe, *J. Phys. Chem. B* **2004**, *108*, 16593 – 16600.

[59] C. Jork, C. Kristen, D. Pieraccini, A. Stark, C. Chiappe, Y. A. Beste, W. Arlt, *J. Chem. Thermodynam.* **2005**, *37*, 537 – 558.

[60] Z. Guo, B.-M. Lue, K. Thomasen, A. S. Meyer, X. Xu, *Green Chem.* **2007**, *9*, 1362 – 1373.

[61] F. H. Abdel-Salam, A. G. El-Said, *J. Surfactants Deterg.* **2011**, *14*, 371 – 379.

[62] H. Ljusberg-Wahren, D. Lundberg, K. Holmberg, WO2005044237A1, **2005**.

[63] P. S.-W. Leung, Y. Teng, P. H. Toy, *Org. Lett.* **2010**, *12*, 4996 – 4999.