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Separation of curcuminoids using ionic liquid based aqueous two-phase system coupled with *in situ* dispersive liquid–liquid microextraction

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ABSTRACT

An aqueous two-phase extraction system (ATPS) combined with an *in situ* dispersive liquid-liquid microextraction (DLLME) method using imidazolium ionic liquids (ILs) for the separation of curcuminoids is developed. The influence of structure of IL, the type of metathesis reagents, and the back extraction agents on the extraction efficiency is investigated. 2.0 mg of curcuminoids are extracted by an IL ATPS composed of 0.4 g 1,3-diethylimidazolium iodine (EeimI), 0.6 g potassium hydrogen phosphate, 1.0 g water. Then the bis[(trifluoromethyl)sulfonyl]imide lithium ($LiNTf_2$) aqueous solution is added to the EeimI-rich phase of the ATPS. The water-immiscible ionic liquids, 1,3-diethylimidazole bis[(trifluoromethyl)sulfonyl]imide ($EeimNTf_2$), forms by the metathesis reaction. The *in situ* DLLME is triggered simultaneously and further purifies the curcuminoids. 92% of EeimI transforms into $EeimNTf_2$ and thus the $Eeim^+$ cation is used for twice in this method. Finally, 0.1 mol/L NaOH aqueous solution is used as the back extraction reagent. The curcuminoids precipitate is achieved with 93% of recovery when the aqueous solution is adjusted to pH 3.0. This ATPS-DLLME method is successfully applied to the separation of curcuminoids from *Curcuma Longa* (0.96 ± 0.02% of extraction yield, a purity of > 51% with respect to the total dry mass of the product).

1. Introduction

When the aqueous concentrations of two polymers, one polymer and one salt, or two salts reach a critical value, spontaneous phase separation takes place and aqueous two-phase system (ATPS) is formed. ATPS is a promising alternative for traditional organic-water solvent extraction system attributing to none use of volatile organic compounds, excellent selectivity, easy scale-up and continuous operation mode [1,2]. Ionic liquids (ILs) consist of organic cations and organic/inorganic anions. The intrinsic non-molecular natures of ILs give rise to unique physico-chemical properties [3]. Ionic liquid based aqueous two-phase system (IL ATPS) shows some advantages in comparison with typical polymer based ATPS, such as little emulsion formation, quick phase separation, high extraction efficiency [4,5]. IL ATPS had been applied in extraction and purification of biomolecules including proteins [6], antibiotics [7], alkaloids [8,9], drugs [10], and small organic molecules [11].

Considering that most IL of the IL ATPS distributes in the IL rich phase and the hydrophilicity/hydrophobicity of ILs is tunable by varying the type and structure of the IL anions, it is possible to convert the quantitative IL of IL rich phase into a water immiscible IL by anionic metathesis reaction. Thus the IL of IL ATPS is recovered from aqueous solution by transforming into another hydrophobic IL with same cations and different anions. Similar metathesis reaction has been utilized to develop an IL based *in situ* dispersive liquid–liquid microextraction method (DLLME). In the *in situ* DLLME method, homogeneously dispersed fine drops of hydrophobic IL are generated and functionalized as the extractant phase. High enrichment factors, low extraction times are obtained ascribe to the high contact surface between phases. The *in situ* DLLME has been applied on the separation of metal ions [12–14], polycyclic aromatic hydrocarbons [15,16], DNA [17], insecticides [18], et al.

Curcuminoids are natural polyphenolic compounds and extracted from *Curcuma. Longa*. This spice shows strong antioxidant, anti-inflammatory, anti-bacterial, and anti-carcinogenic activities and has been widely used in traditional medicine [19], pharmaceutical [20], food [21], cosmetic industries [22], etc. Curcuminoids

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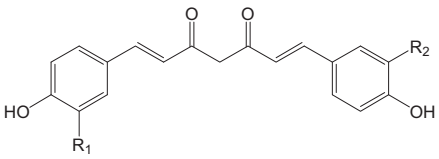
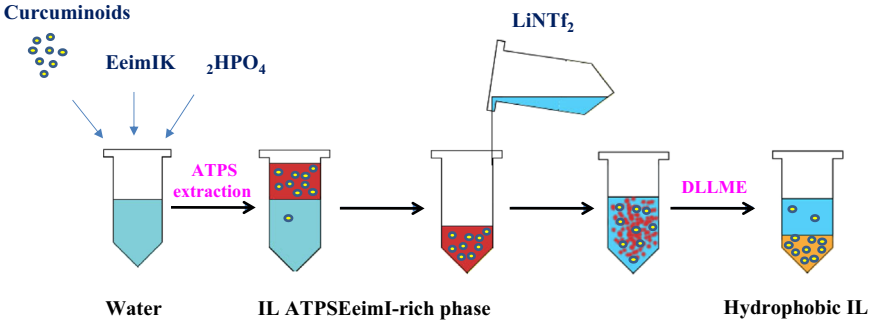


	R_1	R_2
Curcumin	OCH_3	OCH_3
Demethoxycurcumin	H	OCH_3
Bisdemethoxycurcumin	H	H

Scheme 1. Molecular structures of curcuminoids.



Scheme 2. IL ATPS extraction coupled with *in situ* DLLME for separation of curcuminoids.

has three components called as curcumin, demethoxycurcumin and bisdemethoxycurcumin. The chemical structures of the three compounds are shown in Scheme 1.

In the present work, an IL ATPS extraction coupled with an *in situ* DLLME is established to separate and purify curcuminoids from the dry powder of *Curcuma Longa*. The separation procedure is illustrated in Scheme 2. *C. longa* dried powder is extracted with IL ATPS and the curcuminoids transfered into the IL-rich phase. $LiNTf_2$ aqueous solution is then added into the IL-rich phase to trigger the metathesis reaction and *in situ* DLLME. The influence of structure of IL, the type of metathesis reagents, and the back extraction agents on the extraction efficiency is investigated.

2. Experimental methods

2.1. Chemicals and reagents

The ILs with purity over 99% (1,3-Diethylimidazole bromine (EeimBr), 1,3-dioctylimidazole bromine (OoimBr), 1,3-dimethylimidazole iodine (MmimI), 1,3-diethylimidazole iodine (EeimI), 1,3-dibutylimidazole iodine (BbimI)), and the metathesis reagents with purity over 98% (lithium bis[(trifluoromethyl)sulfonyl]imide ($LiNTf_2$), potassium hexafluorophosphate (KPF_6)) are purchased from Shanghai Cheng Jie Chemical Co. LTD. Potassium hydrogen phosphate (K_2HPO_4), a mixture of curcuminoids (curcumin, demethoxycurcumin, and bis-demethoxycurcumin) with the purity over 98% are obtained from Sino-pharm Chemical Reagent (Shenyang, China) and used as received. Other chemicals employed are at least of analytical reagent grade. Deionized water of 18 $M\Omega\,cm^{-1}$ is used throughout the experiments. *Curcuma Longa* powder with particle size of 250 μm is purchase from Beijing Biotopped Science & Technology Co. Ltd.

2.2. Instrumentation

The concentrations of the IL in the top and bottom phase are determined by measuring the absorbance at 225 nm with the UV–vis spectrometer (U-3900, Hitachi, Ltd., Japan). The contents of K_2HPO_4 in both phases are determined by measuring the absorbance at 766.5 nm with the flame atomic absorption spectrophotometer (TAS-990, Beijing Purkinje General Instrument Co., Ltd., China). Circular dichroism (CD) spectra are obtained on a MOS-450 (Bio-Logic, France) automatic recording spectropolarimeter in a wavelength range of 190–240 nm.

The concentrations of the curcuminoid standards are determined by HPLC. The HPLC system is comprised of a pump (L-2130, Hitachi, Ltd., Japan) with solvent cabinet, a column oven (HT-230A), UV/Vis detector (-2455) and computer software (D-2000). The separation is carried out using a reversed phase C18 Diamonsil column (5 μm, 250 × 4.6 mm, Dikma Technologies Inc., China). The mobile phase is acetonitrile:methanol:water (v/v/v)=40:15:45 and the pH value of water is 3.0 adjusted by glacial acetic acid. The flow rate is set at 1.5 mL/min and detection wavelength is 425 nm. Sample of 20 μL is injected onto the column. As for the real sample of *C. longa*, the gradient elution and 250 nm of the detection wavelength are used in order to investigate the purity of curcuminoids. The profile of the gradient elution is: (A) acetonitrile; (B) methanol and (C) water (pH 3.0), 0–13 min, 40% A, 15% B, 45% C; 13–35 min, 40–100% A, 15–0% B, 45–0% C; 35–45 min, 100–40% A, 0–15% B, 0–45% C.

2.3. Preparation of phase diagrams

Phase diagrams are determined by cloud point titration method at 298 ± 0.5 K. 0.2 g ILs is added to a 2 ml centrifuge tube containing 0.2 g water. A K_2HPO_4 solution is put into the tube drop by drop and shaken until the mixture became turbid and form a two-phase system. The water is then added to make the system a clear single-phase again. The above process is repeated to obtain enough data for the phase diagram. The percentage deviation of weight fraction is within an average error of ± 2%.

2.4. IL ATPS extraction procedure

0.4 g EeimI, 0.6 g K_2HPO_4 and 2.0 mg curcuminoids are put into a 2 mL centrifuge tube, and 1 mL deionized water is then added. The mixture is vortexed for 3 min to ensure the dissolve of the salt and the transfer of curcuminoids into IL-rich phase. Then centrifugation is performed at a rate of 8000 rpm for 5 min. The volumes of IL-rich phase and salt-rich phase are recorded. The concentrations of curcuminoids in salt-rich phase are determined by HPLC.

The extraction efficiency (E_1) of curcuminoids for the ATPS extraction is calculated based on the following equation: