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Salting-in counter-current chromatography separation of tanshinones based on room temperature ionic liquids

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ABSTRACT

Ionic liquids have been widely used for the extraction and separation of bioactive natural and synthetic mixtures. In this study, we provided an updated example by using an ionic liquid-based salting-in counter-current chromatography (CCC) strategy for the separation of hydrophobic tanshinones without subsequent column chromatography purification. Several ionic liquids such as 1-allyl-3-methylimidazolium chloride ([AMIM]Cl), 1-methallyl-3-methylimidazolium chloride ([MAMIM]Cl) and 1-butyl-3-ethylimidazolium chloride [BMIM]Cl could significantly decrease the partition coefficients (*K*) of tanshinones in the selected two-phase solvent composed of hexane-ethyl acetate-methanol-ionic liquid aqueous solution (5:5:6:4, *v/v*). Typically, *K* values of three target tanshinones including tanshinone I, 1,2-dihydrotanshinquione and tanshinone IIA were reduced from 3.57, 4.57 and 5.50 to 1.62, 2.33 and 3.08, respectively, by the inclusion of 10% [AMIM]Cl in the solvent system. After salting-in CCC separation, the purified tanshinones were obtained only by simple ethyl acetate extraction. In general, the current results demonstrated that the ionic liquid-based salting-in CCC may be as an alternative strategy for the optimization of CCC solvent systems and separation of lipophilic natural products.

1. Introduction

During the past three decades, ionic liquids have been proposed as promising sustainable solvents and demonstrated wide applications such as for synthesis, catalysis [1–3], extraction and separation [4,5]. Usually, ionic liquids are molten salts composed of cations and anions. They are usually liquids at temperatures below 100 °C, also called room temperature ionic liquids [1,2,6]. Due to the unique physicochemical properties of ionic liquids, such as non-volatility, non-flammability and adjustable miscibility, ionic liquids have been at the cutting edge of the most promising science and technology including analytical chemistry, synthesis, catalysis, engineering and electrochemistry [7,8]. Due to their low volatility and thermal stability, ionic liquids have been used as green solvents to replace some organic solvents in the chemical manufacturing industries, which was considered to be the key to being an economic and environmentally friendly society [9–11]. Ionic liquids were also recognized as good stabilizing media for proteins, enzymes, and nucleic acids due to their excellent ability for forming solutions with a wide range of compounds and materials [12–15]. In addition, the designability and tunability of ionic liquids allow them to act as modifiers of mobile phases and stationary phases in the separation of bioactive compounds [16]. Since Rogers and coworkers [17] have demonstrated that cellulose could be dissolved in 1-butyl-3-ethylimidazolium chloride ([BMIM]Cl), ionic liquids have led to a renaissance in the field of extraction and separation, especially for the extraction and separation of bioactive compounds from diverse origins [4,5].

Natural products play an essential role in healthcare and remain a continuing source of novel drug leads [18–20]. Extractions and separations are the key to obtaining meaningful bioactive components from natural products. However, conventional extractions, including maceration, percolation, Soxhlet extraction, and solvent extraction often require long complicated extraction periods and copious amounts of hazardous and flammable solvents [21,22]. Currently, ionic liquids, a type of green solvent, have been proposed as promising media for the extraction and separation of natural products, which could improve the extraction and separation through unique interactions between ionic liquids and bioactive components [5,23,24].

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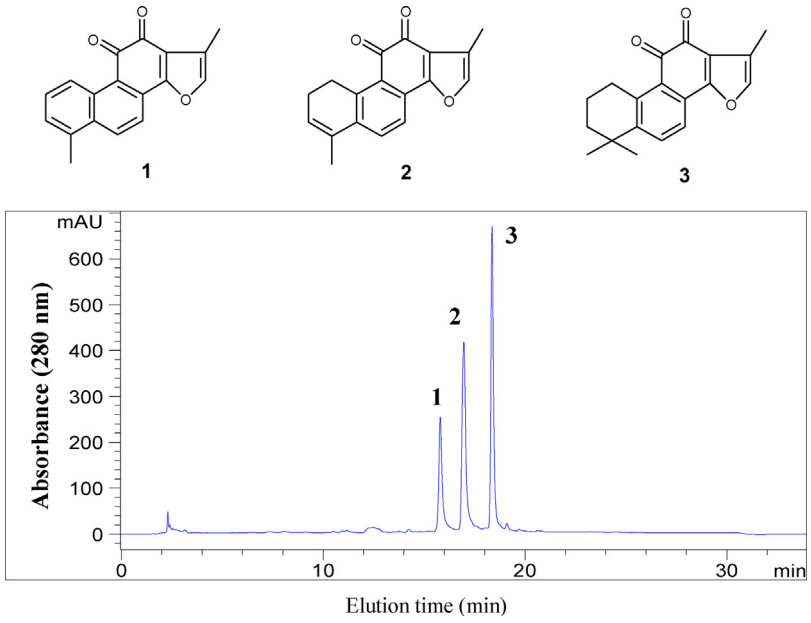

Fig. 1. HPLC analysis of the tanshinone sample: **1**, tanshinone I; **2**, 1,2-dihydrotanshinquinone; and **3**, tanshinone IIA. The column was a reversed-phase Zorbax SB-C8 (250 mm × 4.6 mm I.D., 5 μm) with a guard column (10 mm × 4.6 mm I.D., 5 μm). The system of methanol and water was used as the mobile phase in the gradient mode as follows: 0–20 min, methanol from 60% to 100% and water from 40% to 0% and 20–25 min, methanol remains 100%. The flow rate was kept at 0.8 mL/min with UV detection at 280 nm by a DAD detector. The column temperature was kept at 25 °C.

Up until now, complex natural product extracts have been separated by a number of methods, including liquid–solid and liquid–liquid isolation techniques [25,26]. Among these developed separation and purification techniques, counter-current chromatography (CCC) is a unique liquid–liquid partition chromatography technique and holds great promise in resolving complex natural products. In addition, it does not have complications resulting from a solid support matrix, such as irreversible solute adsorption, contamination, reaction and deactivation. Usually, CCC separation only relies on different partition coefficients of components in a two-phase system to separate samples [27–30]. Recent studies [31–34] have indicated that ionic liquids have salting-in or salting-out properties in the liquid-liquid partitioning of components. Therefore, we selected ionic liquids as additives to investigate their effect on the CCC separation of natural products.

Ionic liquids have been widely used in extraction and separation [35,36]. However, the removal and recovery of ionic liquids are still challenging because their non-volatile nature makes it difficult to remove or recover them through simple evaporation [4,23]. In recent years, some back-extraction methods have been used to remove ionic liquids from bioactive compounds and recycle ionic liquids [37]. In our previous work [38], we have successfully used room temperature ionic liquids as additives to separate hydrophilic arctiin by a salting-in CCC strategy. However, the similar hydrophilic physical properties between arctiin and ionic liquids made it difficult to remove ionic liquids from the target arctiin effluents. In order to obtain the purified arctiin products, a reversed phase C_{18} column had to be used after CCC separation. Therefore, we proposed that an ionic liquid-based salting-in CCC strategy may be more suitable for hydrophobic compounds. In this work, we demonstrated a new example of salting-in CCC separation through simple back-extraction to remove and recover ionic liquids. Hydrophobic tanshinones were selected as model natural products, which are well-known natural products from a famous traditional Chinese medicine danshen (the rhizome of *Salvia miltiorrhiza* Bunge) and possess anti-inflammatory, anti-tumor, and neuroprotective effects [39,40].

2. Experimental

2.1. Reagent and materials

All organic solvents for extraction and CCC separation including hexane, ethyl acetate and methanol were of analytical grade (Sinopharm Chemical Reagent Co., Shanghai, China). Water was purified by means of a water purifier (18.2 MΩ cm) (Wanjie Water Treatment Equipment Co. Ltd., Hangzhou, China) and used for the preparation of all solutions and the dilutions. The solvents used for the HPLC analysis were of chromatographic grade. Acetonitrile and methanol were purchased from Tedia, USA. The tanshinone standards and samples used for CCC separation were prepared by our previous preparation process [41,42]. In short, the dried rhizomes of *S. miltiorrhiza* Bunge, purchased from Huqingyutang, Hangzhou, China, were extracted by 95% ethanol, and then, a normal-phase silica gel column was applied to separate tanshinones and eluted by petroleum ether (60–90 °C):ethyl acetate (1:1, *v/v*) to enrich the lipophilic tanshinone fractions. Danshen (the rhizome of *Salvia miltiorrhiza* Bunge) contains a large number of components, including tanshinones, diterpenoid quinones and hydrophilic phenolic acids [39,40,43]. After fractionation, three prominent tanshinones including tanshinone I (**1**), 1,2- dihydrotanshinquinone (**2**) and tanshinone IIA (**3**) were enriched (Fig. 1) and further used as the CCC samples.

The ionic liquids 1-allyl-3-methylimidazolium chloride ([AMIM]Cl), 1-methallyl-3-methylimidazolium chloride ([MAMIM]Cl) and [BMIM]Cl were synthesized in our lab [38]. In brief, 0.5 mol of 1-methylamidizole and 0.62 mol of excessive halogenated hydrocarbons including chloropropene, allyl chloride, and 1-chlorobutane were reacted with 12 h at 55 °C, and then, ethyl acetate was used to remove unreacted reagents. According to the polar differences between ionic liquids and impurities, a reversed-phase C_{18} column chromatography was selected to remove minor impurities in the ionic liquids with a methanol-water linear gradient. The structures of purified ionic liquids were further confirmed by electrospray ionization tandem mass

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