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# Ultrasonic-assisted extraction of sinomenine from *Sinomenium acutum* using magnetic ionic liquids coupled with further purification by reversed micellar extraction

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ABSTRACT

Magnetic ionic liquids (MILs) have the same features as conventional ionic liquids (ILs), moreover they possess the potentiality of being recovered under the action of permanent magnetic field. Thus, MILs based on imidazolium cations and iron(III) anions were used for the ultrasonic-assisted extraction (UAE) of sinomenine (SIN) from *Sinomenium acutum* for the first time in this study. The extraction conditions were investigated in the single factor experiments, then the major factors of MIL concentration, liquid/solid ratio, and ultrasonic irradiation time were optimized by response surface methodology (RSM). The extraction yield reached 10.57 mg/g under the optimized conditions. The crude MIL extract of SIN was further purified by reversed micellar extraction (RME) using AOT/isooctane system. The recovery and the purity of SIN reached 81.3% and 82.6%, respectively after purification by RME. This hybrid method of MIL-UAE and RME used for the extraction and purification of SIN was effective, rapid, and potential for scale-up.

## 1. Introduction

Sinomenine [full name: (9α,13α,14α)-7,8-didehydro-4-hydroxy-3,7-dimethoxy-17-methyl-morphinane-6-one, abbreviated as SIN in this work], is a bioactive alkaloid extracted from the root and stem of *Sinomenium acutum* [1,2]. Owing to its pharmacological properties of anti-inflammation, immune-modulating, immunosuppression, anti-angiogenesis, and decompression *etc.*, SIN can be used for curing various diseases, especially the rheumatic diseases, arthromyodynia and some other inflammatory disorders [3–5].

Ionic liquids (ILs) are considered as "green" solvents in recent years, they are composed entirely of ions and defined as molten salts with a melting point around or below 100 °C [6]. ILs have the advantages of low flammability, low vapour pressure, good solubility extractability, high thermal and chemical stabilities, wide electrochemical window, and high conductivity [7–10]. Therefore, ILs were widely used in solvent extraction [11], dissolving cellulose [12], $CO_2$ capture [13], catalytic reactions [14], and cell electrolyte [15]. ILs can be good alternatives to the conventional volatile organic compounds (VOCs) in the extraction field. Many studies had reported the use of ILs for the solid/liquid extraction of bioactive compounds from the natural plant resources, such as *trans*-resveratrol from *Rhizma Polygoni Cuspidati* [16], puerarin from *Radix Puerariae Lobatae* [17], secoisolariciresinol diglucoside from flaxseed [18], chlorogenic acid from *Eucommia ulmoides* leaves [19], lipids from microalgae [20], and phycobiliproteins from *Gracilaria* sp. [21].

Magnetic ionic liquids (MILs) are mainly composed of high-spin $d^5$ iron(III) anions in the form of tetrachloro- or tetrabromoferrate (III) and various cations [22,23]. MILs not only have similar properties to common ILs, but also exhibit strong response to external magnetic fields, thus they have the potentiality to be recovered [24]. Recently, MILs were applied for the extraction of phenols from aqueous solution [25] and DNA from aqueous solution [26], or dispersive liquid–liquid microextraction for determination of triazine herbicides [27] in the extraction field, but their application in the extraction of active compounds from natural plants has not been reported.

The ultrasonic or microwave are the most widely assistant methods benefiting the extraction. Both microwave-assisted extraction (MAE) and ultrasonic-assisted extraction (UAE) can make the solution penetrate into the plant tissue more easily, as a results, to reduce the solvent consumption and shorten the extraction time, ultimately to raise the extraction yield [28–31]. Although the extraction using ILs is effective,

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few studies reported the further purification of target compounds and isolation of ILs after the solid/liquid extraction. Various methods were used for the purification of alkaloids, including high-speed counter-current chromatography (HSCCC) [32], macroporous resin separation [33], ion exchange chromatography [34], preparative liquid chromatography [35], and aqueous two-phase extraction [36]. Although almost all the chromatographic methods were effective, high cost or long processing time will restrict their large-scale application. Aqueous two-phase extraction was a primary one-step method for the extraction and purification of active compounds, however, to obtain products with high purity, more processing procedures were needed.

Reversed micellar extraction (RME) is a promising liquid–liquid extraction method for the separation and purification of various proteins and enzymes, such as lysozyme and myoglobin [37], soybean protein [38], inulinase [39], kallikrein [40], lipase [41], *etc*. RME is a two-step purification technique, one forward extraction and the other backward extraction. In the first step, majority biomolecules were extracted into the reverse micelles phase; while in the second step, biomolecules were released from the reverse micelles to a fresh stripping phase [42]. Recently, RME was also developed for the extraction and purification of active ingredients from natural plant resources. Li et al. used an AOT/heptane reverse micellar system to extract tea polysaccharides, 34% forward recovery and nearly 100% of backward recovery were achieved [43]. Zhang et al. used a SDBS/isooctane/n-octanol reverse micelles system for the purification of alkaloids extracted from *Sophora flavescens* Ait., the relative purity of total alkaloids reached 88.75% (w/w) [44].

In this work, MILs were developed for the UAE of SIN from *S. acutum* for the first time, and RME based on AOT/isooctane system was used for further purification of SIN. The conditions of MIL concentration, liquid/solid ratio, and ultrasonic irradiation time were optimized by RSM in the UAE procedures. The factors of type of reverse micellar system, surfactant concentration, and water content ($W_0$) of system were also investigated in the RME procedures.

## 2. Materials and methods

### 2.1. Materials and reagents

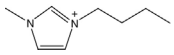
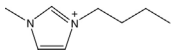
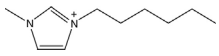
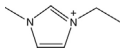
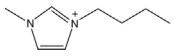
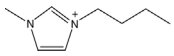
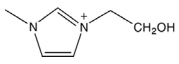
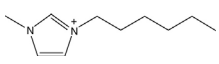
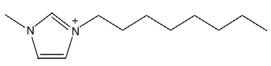
The *S. acutum* sample was purchased from the drugstore, then smashed to powder and sieved by 80 mesh particle size. The SIN standard (purity > 98% by HPLC) was purchased from National Institutes for Food and Drug Control (Beijing, China). HPLC grade methanol was obtained from TEDIA Company, Inc. (Fairfield, OH, USA). The ILs used in this study were synthesized according to the reported literatures [24,45]. The chemical structures of the ILs are shown in Table 1. Four surfactants of bis (2-ethylhexyl) sulfosuccinate sodium salt (AOT), sodium lauryl sulfate (SLS), sodium dodecyl sulfonate (SDS), and sodium dodecyl benzene sulfonate (SDBS) were purchased from Alladin Reagent Co., Ltd. (Shanghai, China). Other reagents were of analytical grade and used without further treatment. Water purified by an OLABO water purification system (model Honest DZG-303A, Xin Bei Xi Bio-Technology Co. Ltd, Jinan, China) was used to prepare the sample solutions.

### 2.2. Ultrasonic-assisted extraction of SIN

To a tube, *S. acutum* powder and MILs solution were added. The IL solutions with different concentration were prepared by dissolving the IL into the water. Then the tube was fixed in an ultrasonic instrument (model KQ-5200DE from Kunshan Ultrasound Co. Ltd., Kunshan, Jiangsu, China) for the extraction of SIN in a certain time. The electric power was 200 W, generators frequency was 40 kHz, and operating temperature was 25 °C. Then centrifugation was performed at 7000 × *g* for 10 min using a Cence® centrifuge (model TGL-16M, Hunan Xiangyi Laboratory Instrument Development Co., Ltd., Changsha, China) after extraction. The supernatant was separated with the sediment at the bottom of tube being discarded. The concentration of SIN in the MIL extract was quantified by HPLC. The extraction yield ($Y$) of SIN was calculated according to Eq. (1).

$$\text{Extraction yield (mg/g)} = \frac{\text{Mass of SIN determined (mg)}}{\text{Mass of } \textit{Sinomenium acutum} \text{ powder (g)}} \quad (1)$$

**Table 1**
Chemical structures of the ILs used in this study.

| Ionic liquids (full name) | Cations | Anions |
|---|---|---|
| [$C_4$mim]Br (1-butyl-3-methyl-imidazolium bromide) |  | $Br^-$ |
| [$C_4$mim]Cl (1-butyl-3-methyl-imidazolium chloride) |  | $Cl^-$ |
| [$C_6$mim]Br (1-hexyl-3-methyl-imidazolium bromide) |  | $Br^-$ |
| [$C_2$mim]$FeCl_3Br$ (1-ethyl-3-methyl-imidazolium bromotrichloroferrate) |  | $FeCl_3Br^-$ |
| [$C_4$mim]$FeCl_3Br$ (1-butyl-3-methyl-imidazolium bromotrichloroferrate) |  | $FeCl_3Br^-$ |
| [$C_4$mim]$FeCl_4$ (1-butyl-3-methyl-imidazolium tetrachloroferrate) |  | $FeCl_4^-$ |
| [$C_2$OHmim]$FeCl_4$ (1-ethoxyl-3-methyl-imidazolium tetrachloroferrate) |  | $FeCl_4^-$ |
| [$C_6$mim]$FeCl_3Br$ (1-hexyl-3-methyl-imidazolium bromotrichloroferrate) |  | $FeCl_3Br^-$ |
| [$C_8$mim]$FeCl_3Br$(1-octyl-3-methyl-imidazolium bromotrichloroferrate) |  | $FeCl_3Br^-$ |

### 2.3. Reversed micellar extraction of SIN

The surfactant AOT was used for the RME of SIN, and the procedures were as follow: firstly, a certain amount of AOT was dissolved in the isooctane and transferred into a tube. Then a certain amount of water was added to the tube. Lastly, the tube was agitated for 2 h using a magnetic stirrer (IKA Labortechnick, Germany), then placed on the table for 12 h at room temperature. A transparent AOT/isooctane reversed micellar system was prepared. The water content ($W_0$) of the reversed micellar system was defined in Eq. (2).

$$W_0 = \frac{n_{H_2O}}{n_{AOT}} \quad (2)$$

where $n_{H_2O}$ is the molar mass of water, and $n_{AOT}$ is the molar mass of AOT. The definition of water content ($W_0$) for the other three surfactants was the same as AOT.

To a tube, the aqueous phase (MIL extract) and AOT/isooctane reverse micellar system were added at equal volumes. The tube was agitated for 20 min, then transferred to a separating funnel and placed for complete phase-separation at room temperature. The top phase (AOT/isooctane micellar system) was separated and then absolute