



Separation of berberine from *Coptidis rhizoma* using ionic liquid modified magnetic nanosilica

Xiaoman Li, Xinlong Jiang, Yuning Cui, Minglei Tian

College of Chemistry and Environmental Engineering, Yangtze University, Jingzhou, Hubei, China

Abstract

An ionic liquid modified magnetic nanosilica chain was prepared and used to separation berberine from *Coptidis Rhizoma*. To increase the efficiency, the adsorption amounts of a serial ionic liquid modified magnetic nanosilica was investigated with berberine standard solution. At 25°C and pH=3.0, the Fe₃O₄@IL@Sil-C obtained the highest adsorption amount in 3.0 h. Then the sorbent was applied in extraction solution of *Coptidis Rhizoma* and 0.03 mg/mL of berberine can be separated. The recoveries and RSD were in the range of 92.6-99.8% and 4.6-7.8% highlighting the accuracy of this sorbent.

Keywords: ionic liquid, magnetic nanosilica, berberine, *Coptidis rhizoma*

Introduction

Berberine is a quaternary ammonium isoquinoline alkaloid which was normally extracted from natural plants such as *Coptidis Rhizoma* and so on. These plants are widely used as traditional herbal medicines to treat infection, diabetes, even tumor [1]. After extracting berberine from plants, it can be used alone or combined with other drugs to treat various clinical diseases with exert different pharmacological effects [2]. Malhotra *et al.* summarized development of berberine and the necessary of isolation of berberine from the plant [3]. Furthermore, Chu *et al.* proved that berberine showed enough antimicrobial activity against microbial communities when the concentration of berberine was higher than 32.0 µg/mL [4]. Hence, to increase the bioactivity and bioavailability of berberine, its purity should be increased.

In order to isolate the berberine, various methods were reported. Patel reviewed several extraction methods, such as supercritical fluid, pressurized hot water, ultrahigh pressure, and microwave assisted solvent, to extract berberine from natural plants [5]. Furthermore, sorbents with higher efficiency were used to separate and purify the berberine from extracted solution. Base on the adsorption mechanism, various sorbents were developed especially magnetic sorbents because they could rapidly isolate the berberine from other substances by using an external magnet [6].

In Meng's research, molecularly imprinted polymers were made on the surface of Fe₃O₄ nanoparticles by using berberine as template. The sorbent could separate alkaloids in herbs and rat plasma, and especially for berberine the limit of detection was 0.01 ng/mL [7]. Jiang *et al.* used aptamer-functionalized Fe₃O₄ magnetic nanoparticles-based solid-phase extraction method to extract berberine in *Cortex phellodendri* extracts, and the purity of berberine was as high as 98.7% [8]. Aghaie and Hadjmoham used p-naphtholbenzein to modified Fe₃O₄ sorbent to obtain a hydrophobic surface and to improve the extraction efficiency, and lower limit of quantification of 0.5 and 0.6 µg/L were obtained for plasma and urine samples [9].

However, the natural plant extracted solution contains huge numbers of substances. In order to increase the selectivity, the sorbents need further modification. In this research,

ionic liquids (ILs) were selected. ILs are well-known green reaction media with excellent chemical properties, such as hydrophilicity/hydrophobicity with different cations or anions, miscibility with several inorganic/organic solvents [10, 11]. When the IL groups were modified on the surface of sorbents, they can provide a range of chemical bonds (hydrogen, ionic, π-π, *et al.*) to increase the interaction between sorbents and target compounds [12].

On the other hand, there are no reports of the separation of berberine by a modified magnetic sorbent combined with IL. In this research, imidazole ionic liquid modified magnetic nanosilica was prepared and applied to separate berberine from the herbal plant *Coptidis Rhizoma*.

Materials and methods

1. Materials and instruments

Tetraethyl orthosilicate, FeCl₃·6H₂O, FeCl₂·4H₂O aqueous ammonia, (3-chloropropyl)-trimethoxysilan, imidazole were purchased from Aladdin Inc. (Shanghai, China) and all grades of purity were higher than 98.0%. Organic solvents such as ethanol, toluene *et al.* were supported by Beilian Company (Tianjing, China) and purities were higher than 99.0%. Ultrapure water was produced by a purification machine (UPH-I-5, Youpu, China) and all organic solvents should be filtered before use. Berberine was supplied by Yuanye Biotechnology (Shanghai, China). The herbal plant *Coptidis Rhizoma* was purchased from a local market. The extracted solution was obtained by dipping 20.0g of *Coptidis Rhizoma* powder in ethanol for 24h.

All samples were analyzed by HPLC (LC3000, CXTH, Beijing, China) using a ODS-AP column (4.6 × 150 mm, 5 µm, Dalian Elite, China). The mobile phase, flow rate, UV wavelength, injection volume and column oven temperature were acetonitrile/water/phosphoric acid (25.0:75.0:0.5, v/v/v), 0.8 mL/min, 360.0 nm, 10.0 µL and 35°C, respectively.

2. Preparation of ionic liquids modified magnetic nanosilica

The preparation of nanosilica and IL modified magnetic sorbents were used the following steps, as shown in Fig. 1. First of all, nanosilica was synthesized. 6.0 mL of tetraethyl

orthosilicate was dissolved in 60.0 mL of ethanol, then 40.0 mL of water and 5.0 mL of aqueous ammonia were added. The solution was stirred for 20.0 h under 25°C. The obtained white nanosilica (Nanosil) powder was washed by water and ethanol until pH=7.0.

200.0 mL of water was added in a flask under the protection of nitrogen, 19.5 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 6.7 g of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ were dissolved in water with a mechanical stirring device, then 20.0 mL of aqueous ammonia was slowly dripped into the solution. After 30.0 min, the obtained dark Fe_3O_4 powder was washed by water. After full drying, 10.0 g of Fe_3O_4 was added in a beaker with 100.0 mL of ethanol and 40.0 mL of water. Under continuous stirring, 6.0 mL of tetraethyl orthosilicate and 5.0 mL of aqueous ammonia were added and after 12.0 h, microsphere magnetic nanosilica ($\text{Fe}_3\text{O}_4@Sil$), a light black powder, was obtained.

Microsphere magnetic nanosilica chain was synthesized with assistance of magnetic field. The same as the previous step, the Fe_3O_4 powder was obtained and 10.0 g of it was added in a beaker with 100.0 mL of ethanol and 40.0 mL of water. A magnetic field was applied around the beaker, 6.0 mL of tetraethyl orthosilicate and 5.0 mL of aqueous ammonia were added with a facile stirring, after 12.0 h magnetic nanosilica chain ($\text{Fe}_3\text{O}_4@Sil-C$) was obtained.

After all, 5.5 g of $\text{Fe}_3\text{O}_4@Sil$ and $\text{Fe}_3\text{O}_4@Sil-C$ were added into two different flasks. In each flask, 150.0 mL of toluene and 9.0 mL of (3-chloropropyl)-trimethoxysilan were added and the mixture was heated to 100°C for 6.0 h, then 10.0 g of imidazole was added into flask and after 8.0 h, two IL modified sorbents $\text{Fe}_3\text{O}_4@IL@Sil$ and $\text{Fe}_3\text{O}_4@IL@Sil-C$ were obtained.

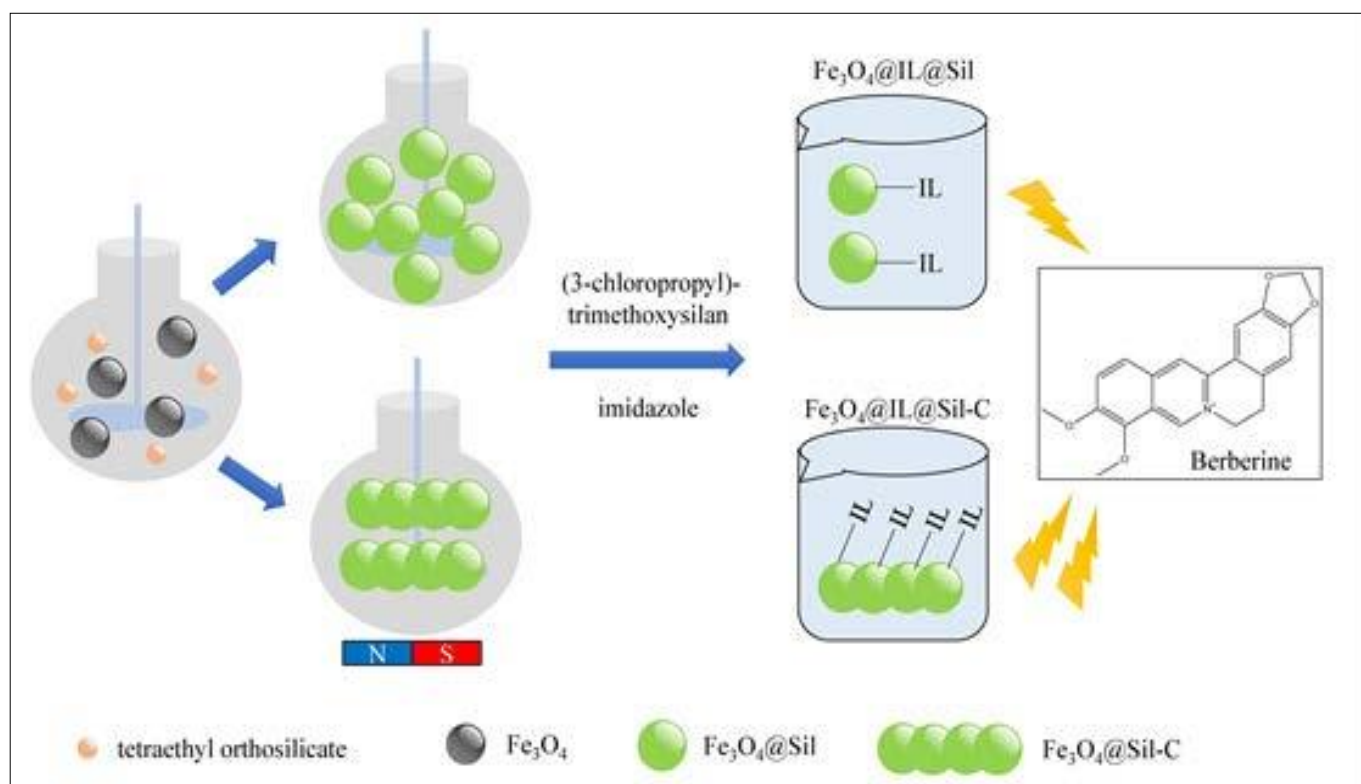


Fig 1: Preparation of nanosilica, magnetic sorbents and ionic liquid modified sorbents.

3. Adsorption study in tube with different conditions

The maximum adsorption amount of each sorbent was tested with a high concentration of berberine standard solution (0.06mg/mL in methanol). A 1.0 mL of the berberine standard solution was placed into the tube with 0.03 g of the 5 sorbents. All tubes were shaken at 25 °C, and after 24.0 h tubes were centrifuged and the supernatant solutions were filtered for HPLC analysis.

Then, in order to optimize the adsorption condition, three major conditions such as adsorption time (in the range of 1.0-7.0 h), adsorption temperature (in the range of 15-45 °C), and pH of solution (in the range of 3.0-11.0), which affected the adsorption capacity efficiently were evaluated and analyzed by HPLC.

4. Preparation of herbal plant extraction sample

The herbal plant *Coptidis Rhizoma* was ground to powder and 0.5 g of it was dipped into 10.0 mL of ethanol at room

temperature for 24.0 h. The supernatant solution was filtrated and stored in glass bottle for further use.

Results & Discussion

1. Characterization

The characteristics of all obtained sorbents were analyzed by FT-IR (Nicolet 6700, Thermo Fisher, Waltham, USA) in the range of 400-4000 cm^{-1} with a scan rate of 20 scans/min. In the FT-IR spectra (Fig. 2), $\text{Fe}_3\text{O}_4@IL@Sil$ and $\text{Fe}_3\text{O}_4@IL@Sil-C$ showed the appearance of peaks nearby 1572.3 cm^{-1} which is the finger print region of IL groups, and the finger print region of the Cl^- around 690.0 cm^{-1} also belongs to these two sorbents. Nanosilica exhibits a conspicuous peak in the range of 3200-3600 cm^{-1} and it belongs to the finger print region of the -OH group. In other four sorbents, the -OH peak is obviously decreased indicating the -OH group was replaced by other groups.

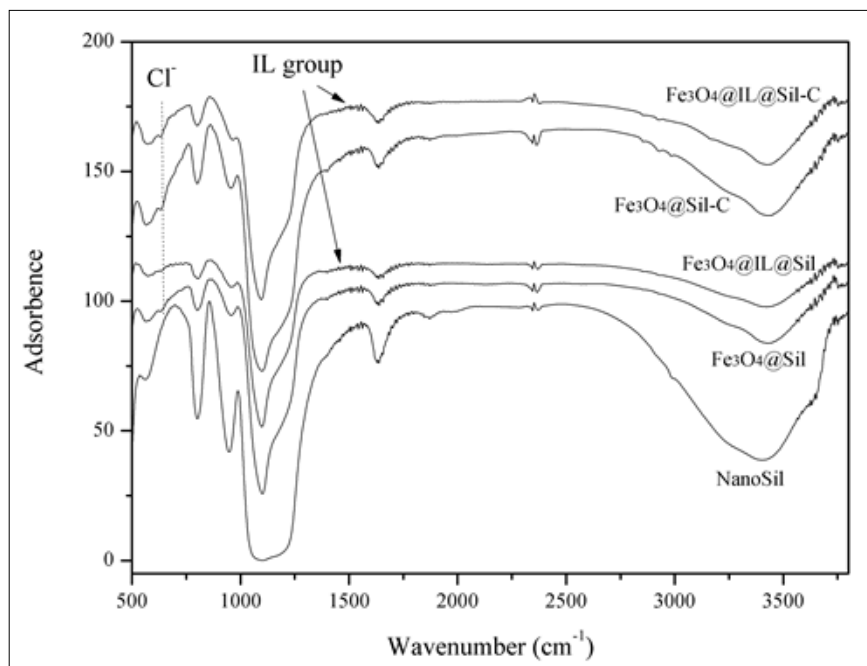


Fig 2: FT-IR of five sorbents.

2. Study of the maximum adsorption amounts

The maximum adsorption is used to explain the relationship between the sorbents and berberine at a fixed temperature. The results were shown in Fig. 3. Comparing the nanosil, $\text{Fe}_3\text{O}_4@Sil$ and $\text{Fe}_3\text{O}_4@Sil-C$, on the surface of these three sorbents only $-OH$ groups can interact with berberine using a weak hydrogen bond, so the adsorption amount was low. However, microsphere structure of $\text{Fe}_3\text{O}_4@Sil$ and chain structure of $\text{Fe}_3\text{O}_4@Sil-C$ provided more space and surface

area to interact with berberine, so the adsorption amounts of these two were slightly increased.

After modification of IL group, two more major interactions such as $\pi-\pi$ bond (between imidazole group in IL and carboatomic ring in berberine) and electrostatic force (between Cl^- in IL and N^+ in berberine) obviously increased the adsorption amounts. Hence, $\text{Fe}_3\text{O}_4@IL@Sil$ and $\text{Fe}_3\text{O}_4@IL@Sil-C$ could be applied for further tests.

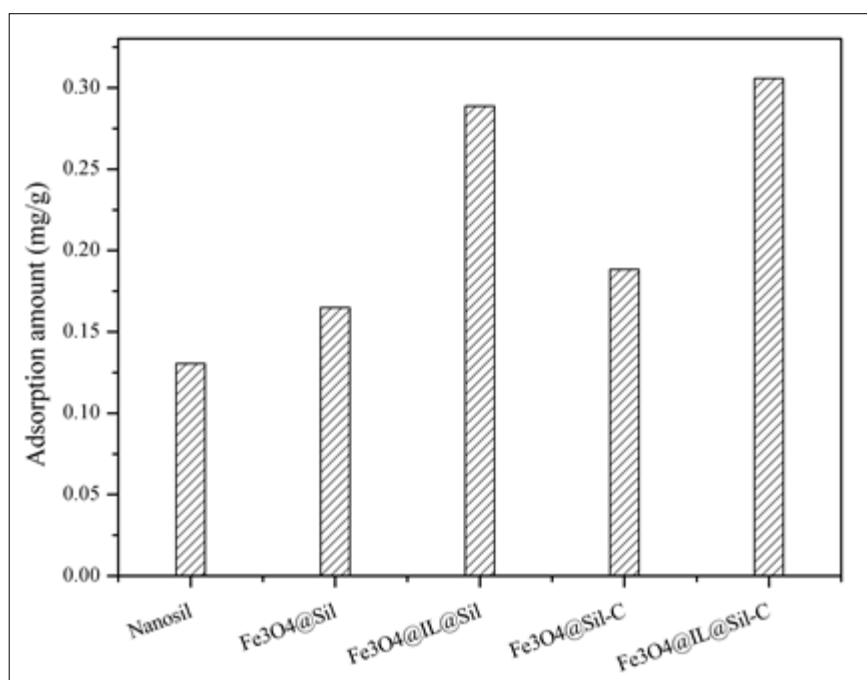


Fig 3: Maximum adsorption amounts of five sorbents.

3. Optimization of variable conditions

Fig. 4 showed the adsorption amounts with different time on $\text{Fe}_3\text{O}_4@IL@Sil$ and $\text{Fe}_3\text{O}_4@IL@Sil-C$ at 25°C . The adsorption amounts were increased with time increasing and there was no obviously increasing after 3.0 h for both

sorbents. Then different temperatures were evaluated. As shown in Fig. 5, the adsorption amounts increased with increasing temperature until 25°C . When the temperature was higher than 30°C the adsorption amounts decreased rapidly.

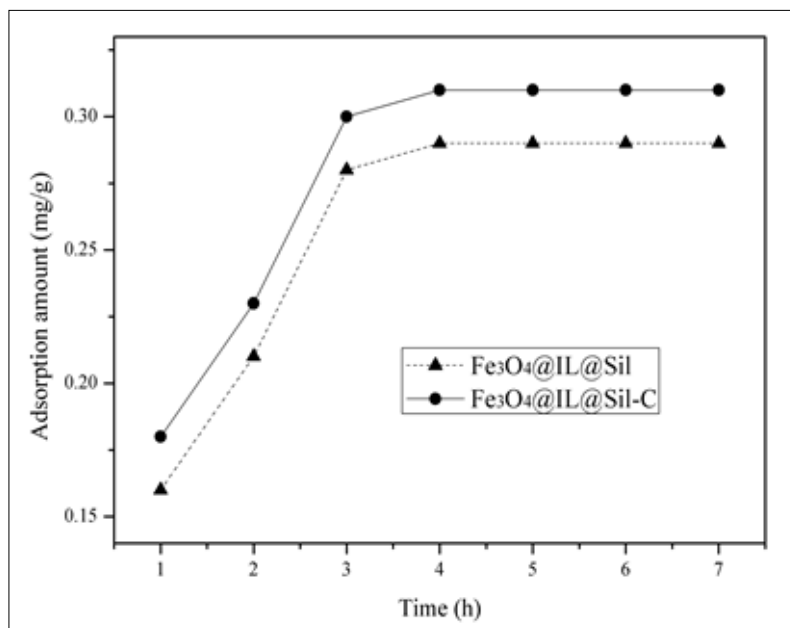


Fig 4: Effect of adsorption amounts with different time on Fe₃O₄@IL@Sil and Fe₃O₄@IL@Sil-C at 25°C.

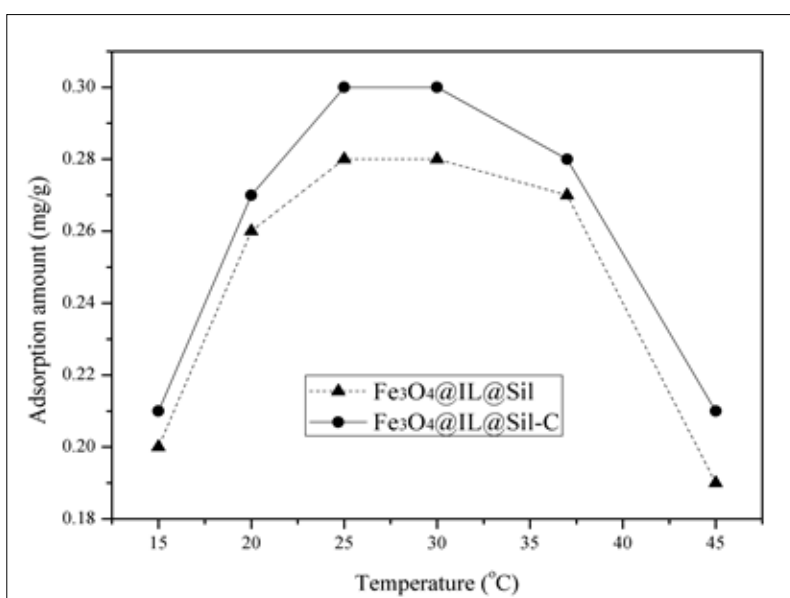


Fig 5: Effect of adsorption amounts with different temperature on Fe₃O₄@IL@Sil and Fe₃O₄@IL@Sil-C for 3.0 h.

From Fig. 4 and 5, the results revealed that Fe₃O₄@IL@Sil-C had higher adsorption amount than Fe₃O₄@IL@Sil, the phenomenon can be explained that nanochain structure provides a shearing force in dynamic magnetic field to induce sufficient and effective adsorption [13]. Hence, Fe₃O₄@IL@Sil-C was selected for further application. Finally, the adsorption amount of Fe₃O₄@IL@Sil-C at

different pH was evaluated. The result in Fig. 6 showed that with increasing of pH, the structures of sorbent and berberine became unstable, so the adsorption amount decreased dramatically.

Hence, Fe₃O₄@IL@Sil-C would be applied as the optimized sorbent to separate berberine from real sample for 3.0 h at 25°C in acidic environment.

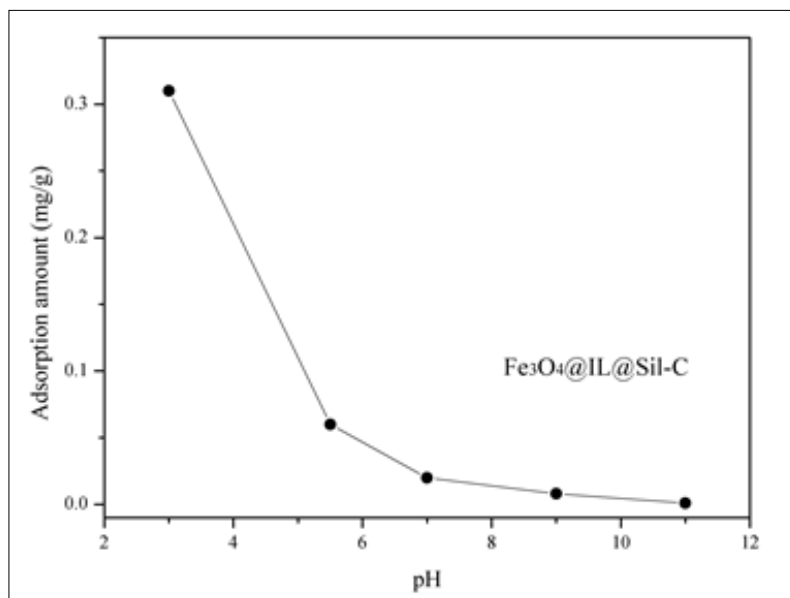


Fig 6: Effect of pH on $\text{Fe}_3\text{O}_4@\text{IL}@\text{Sil-C}$ at 25°C for 3.0 h.

4. Validation

The linearity and LOD of the analysis condition were validated first. The calibration curves were constructed using the chromatographic peak areas measured at seven increasing berberine concentrations from 0.001-0.3 mg/mL. Good linearity of the calibration curves was obtained and the correlation equation was $y=3.2 \times 10^{-7}x-18625$ ($R^2=0.99$) (y is the peak area and x is the concentration in solution). The LOD and LOQ were 2.3×10^{-4} mg/mL and 8.4×10^{-4} mg/mL, respectively, which were based on signal-to-noise ratios of three (LOD) and ten (LOQ). A certain concentration was then spiked into the *Coptidis Rhizoma* extracted solvent and analyzed to calculate the recoveries. The accuracy and precision of the method were evaluated by assays of repeatability calculated as the RSDs with

$\text{Fe}_3\text{O}_4@\text{IL}@\text{Sil-C}$ process five times over a five-day period. Good recoveries of 92.6-99.8 % and RSDs of 4.6-7.8 % were obtained. These results mean that the sorbent is reliable for the extraction sample.

5. Application of sorbent in real extraction solution

0.5 g of $\text{Fe}_3\text{O}_4@\text{IL}@\text{Sil-C}$ was mixed with 10.0 mL of extraction solution of *Coptidis Rhizoma*, and the mixture was shaken at 25°C for 3.0 h. Then a magnet was closed to the mixture to gather the sorbent and the concentration of berberine in solution was analyzed by HPLC (Fig. 7). Under the optimized conditions, 0.03 mg/mL of berberine can be separated from extraction solution of *Coptidis Rhizoma* by $\text{Fe}_3\text{O}_4@\text{IL}@\text{Sil-C}$.

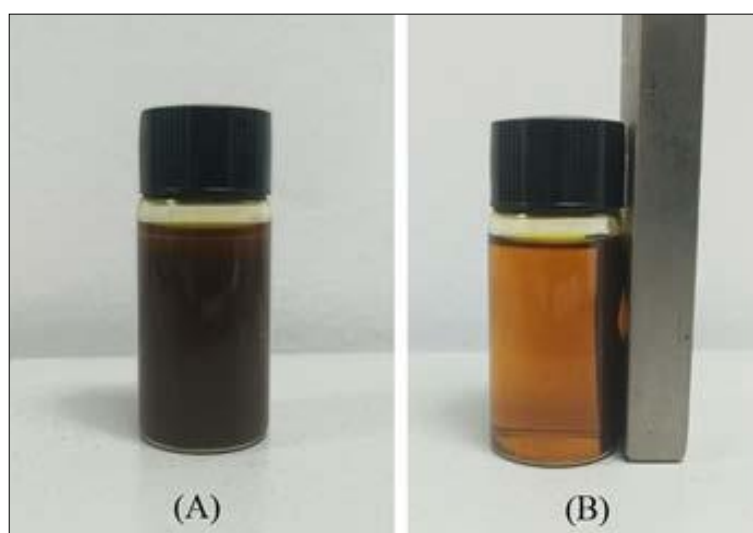


Fig 7: (A) $\text{Fe}_3\text{O}_4@\text{IL}@\text{Sil-C}$ was mixed with extraction solution of *Coptidis Rhizoma*, and (B) the sorbent in the mixture was gathered by a magnet.

Conclusion

In the study, a serial of ionic liquid modified magnetic nanosilica was prepared, and the chain structure exhibited the highest adsorption amount for berberine. Combined with HPLC, the $\text{Fe}_3\text{O}_4@\text{IL}@\text{Sil-C}$ was successfully served as a

sorbent to adsorb berberine. Using the optimized conditions, good recoveries of 92.6-99.8 % and RSDs of 4.6-7.8 % were obtained with the LOD and LOQ were 2.3×10^{-4} mg/mL and 8.4×10^{-4} mg/mL. The sorbent was applied to separate berberine from extraction solution of *Coptidis*

Rhizoma. and as a result, 0.03 mg/mL of berberine can be obtained indicating that Fe₃O₄@IL@Sil-C is an excellent alternative sorbent and has great application potential for separation of berberine in natural herbal plants.

References

- Zhang Y, Ma J, Zhang W. Berberine for bone regeneration: Therapeutic potential and molecular mechanisms. *Journal of Ethnopharmacology*,2021:277:114249.
- Liu X, Tan J, Cheng S, Chen Z, Ye J, Zheng J, Xu F, Zhang W, Liao Y, Yang X. Comparative transcriptome analysis provides novel insights into the molecular mechanism of berberine biosynthesis in *Coptis chinensis*. *Scientia Horticulturae*,2022:291:110585.
- Malhotra B, Kulkarni GT, Dhiman N, Joshi DD, Chander S, Kharkwal A *et al.* Recent advances on *Berberis aristata* emphasizing berberine alkaloid including phytochemistry, pharmacology and drug delivery system. *Journal of Herbal Medicine*,2021:27:100433.
- Chu M, Zhang M, Liu Y, Kang J, Chu Z, Yin K *et al.* Role of Berberine in the treatment of methicillin-resistant *Staphylococcus aureus* Infections. *Scientific Reports*,2016:6:24748.
- Patel P. A bird's eye view on a therapeutically 'wonder molecule': Berberine. *Phytomedicine Plus*,2021:1(3):100070.
- Qian J, Kai G. Application of micro/nanomaterials in adsorption and sensing of active ingredients in traditional Chinese medicine. *Journal of Pharmaceutical and Biomedical Analysis*,2020:190:113548.
- Meng J, Zhang W, Bao T, Chen Z. Novel molecularly imprinted magnetic nanoparticles for the selective extraction of protoberberine alkaloids in herbs and rat plasma. *Journal of Separation Science*,2015:38(12):2117-2125.
- Jiang L, Chen B, Chen B, Li X, Liao H, Zhang W *et al.* Aptamer-functionalized Fe₃O₄ magnetic nanoparticles as a solid-phase extraction adsorbent for the selective extraction of berberine from *Cortex phellodendri*. *Journal of Separation Science*,2017:40(14):2933-2940.
- Aghaie A, Hadjmoham MR. Fe₃O₄@p-Naphtholbenzein as a novel nano-sorbent for highly effective removal and recovery of Berberine: Response surface methodology for optimization of ultrasound assisted dispersive magnetic solid phase extraction. *Talanta*,2016; 156-157:18-28.
- Fang L, Tian M, Yan X, Xiao W, Row KH. Dual ionic liquid-immobilized silicas for multi-phase extraction of aristolochic acid from plants and herbal medicines. *Journal of Chromatography A*,2019:1592:31-37.
- Fang L, Xie J, Lin L, Tian M, Row KH. Multi-phase extraction of ephedrine from *Pinellia ternata* and herbal medicine using molecular imprinted polymer coated ionic liquid-based silica. *Phytochemical Analysis*,2020:31:242-251.
- Fang L, Tian M, Row KH, Yan X, Xiao W. Isolation of aristolochic acid I from herbal plant using molecular imprinted polymer composited ionic liquid-based zeolitic imidazolate framework-67. *Journal of Separation Science*,2019:42:3047-3053.
- Wan L, Song H, Chen X, Zhang Y, Yue Q, Pan P *et al.* A magnetic-field guided interface coassembly approach to magnetic mesoporous silica nanochains for osteoclast-targeted inhibition and heterogeneous nanocatalysis. *Advanced Materials*,2018:30:1707515.
- Raj Bhan Saket, MK Bhatnagar. Adsorption of lead (II) from aqueous solution by fly ash. *International Journal of Chemistry Studies*, Volume 4, Issue 1, 2020, Pages 25-28