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Dispersive solid-phase extraction of berberine using ionic liquid modified magnetic sorbent

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Abstract
An ionic liquid modified magnetic sorbent was prepared and used in dispersive solid-phase extraction method to isolate berberine. The adsorption efficiency was optimized under various conditions. In berberine standard ethanol solution, the modified magnetic sorbent obtained the highest adsorption amount within 7 h at 30ºC. When the sorbent and method were applied in isolation of berberine from natural plant extracted solution, the LOD (4.8×10^{-4} mg/mL), LOQ (1.6×10^{-3} mg/mL), the recoveries (87.8-99.6%) and RSDs (8.6-9.9%) proved the accuracy of this sorbent.

Keywords: dispersive solid-phase extraction, berberine, ionic liquid, magnetic, adsorption

Introduction

Bioactive compounds extracted from natural plants are widely used in whole world. For example, in order to infection, diabetes, even tumor [1], berberine as an ammonium isoquinoline alkaloid was used alone or combined with other drugs to treat various clinical diseases [2]. Chen *et al*. applied berberine has a potential role in improving the intestinal microbiome of cultured fishes [3]. Cao *et al*. proved that berberine has strong antiviral activity against different viruses [4]. Kang *et al*. revealed that berberine can achieve synergism effect increasing the intracellular concentration of other medicine [5]. According to Malhotra's research, berberine should be isolated from natural plants for further application [6]. Hence, various extraction 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 [7].

On the other hand, several new extraction methods such as solid phase extraction (SPE), such as matrix solid-phase dispersion (MSPD), dispersive solid-phase extraction (DSPE), solid-phase microextraction (SPME), *et al*. DSPE as one of the alternative techniques has been applied in several areas. Neely *et al*. summarized several applications of DSPE to analyze drugs, toxic alkaloids and pesticides in foods. The recoveries were in a wide range between 63% and 148%. The DSPE method was proven to be an efficient and reliable procedure for the extraction and analysis [8]. Shi *et al*. develop an amorphous DSPE with hydrogenated phosphatidylcholine to obtain the crystalline berberine [9].

However, the solution from DSPE needs more treatment such as centrifugation or filtration before analysis. In order to decrease the analysis time and simplify the separation process, magnetic solid phase extraction (MSPE) was applied. It used magnetic sorbent which is easy to be collected by an external magnetic field without further treatment. Yin *et al*. summarized MSPE in sample preparation for the analysis of phytochemical compounds in plants, biological samples and herbal preparations [10].

Combining the advantages of DSPE and MSPE, the new method magnetic dispersive solid phase extraction (MDSPE) was applied in bioactive compounds extraction. Karami-Osboo *et al*. used MDSPE to extract aflatoxins and the recoveries in the range of 72.0-95.0% showed the proper accuracy of the method [11]. Aghdam *et al*. used carbonized cellulose-ferromagnetic nanocomposite in MDSPE to isolate plasticizers in aqueous samples and the relative recoveries were found to be in the range of 89.0-101.0% [12]. Aghaie and Hadjmoham used p-naphtholbenzein to modified Fe_3O_4 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 [13].

In this research, MDSPE with a magnetic sorbent was used to isolate berberine from the natural plant *Coptidis Rhizoma*. However, the natural plant extracted solution contains various substances and the selectivity of traditional magnetic sorbent such as Fe_3O_4 is not high enough. In order to increase the selectivity, ionic liquid (IL) as a sorbent modifier was applied. IL is known as a green solvent with excellent chemical properties, such as hydrophilicity/hydrophobicity, miscibility with several inorganic/organic solvents, *et al*. When it was modified on the surface of sorbents, it can provide a range of chemical bonds (hydrogen, ionic, π-π, *et al*.) to increase the interaction between sorbent and target compounds [14]. Hence, the IL modified magnetic sorbent (IL-Fe_3O_4) was prepared and the adsorption efficiency was optimized. Finally, the extraction efficiency of MDSPE with IL-Fe_3O_4) was proved by this research.

Materials and methods

1. Materials and instruments

$FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$ aqueous ammonia, (3-chloropropyl)-trimethoxysilan, and imidazole were purchased from Aladdin Inc. (Shanghai, China) and all grades of purity were higher than 98.0%. Ethanol and other organic solvents (99.0%) were supported by Beilian Company (Tianjing, China). 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 20.0 g of *Coptidis Rhizoma* powder in ethanol for 24h.

All samples were analyzed by HPLC (LC3000, CXTH, Beijing, China) using the ODS-AP column (4.6 × 150.0 mm, 5.0 μm, Dalian Elite, China). The mobile phase, flow rate, UV wavelength, injection volume and column oven temperature were acetonitrile/water (25.0:75.0, v/v), 0.8 mL/min, 360.0 nm, 10.0 μL and 35◦C, respectively.

2. Preparation of ionic liquid modified magnetic sorbent

The preparation of IL modified magnetic sorbents were used the following steps. First of all, 100.0 mL of water was added in a flask under the protection of nitrogen, 9.8 g of $FeCl_3 \cdot 6H_2O$ and 3.5 g of $FeCl_2 \cdot 4H_2O$ were dissolved in water with a mechanical stirring device, then 10.0 mL of aqueous ammonia was slowly dripped into the solution. After 30.0 min, the Fe_3O_4 as a light black powder was obtained and washed by water until the pH=7.0. After full drying, 3.0 g of Fe_3O_4, 100.0 mL of toluene and 6.7 mL of (3-chloropropyl)-trimethoxysilan were added in a flask. The temperature of the flask was increased to 100 ºC and after 8 h the imidazole (6.0 g) was added. After the mixture was stirred for another 8h, the IL modified magnetic sorbent IL-Fe_3O_4 was obtained.

3. Adsorption study in tube with different conditions

The maximum adsorption amount of each sorbent was tested with a high concentration of berberine (0.06 mg/mL) in different solvents. A 3.0 mL of the berberine standard solution was placed into a tube with 0.05 g of sorbent and shaking at 25ºC for 10 h, a magnetic bar was applied to collect the sorbent and the solution was filtered for HPLC analysis. Then, in order to optimize the adsorption condition, three major conditions such as adsorption time (in the range of 1-24 h), adsorption temperature (in the range of 15-60 ºC), 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 2.5 g of it was dipped into 20.0 mL of ethanol at room temperature for 24 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. 1), IL-Fe_3O_4 showed more appearance of peaks than Fe_3O_4. On IL group, imidazole showed the finger print peak around 1397.5 cm^{-1}, Cl^- showed the finger print peak at 638.5 cm^{-1} and the peaks in the region of 2853.1-2990.0 cm^{-1} belong to the C-H bonds. The results in Fig. 1 revealed that the IL group was successfully immobilized on the sorbent with a satisfactory magnetic field response.

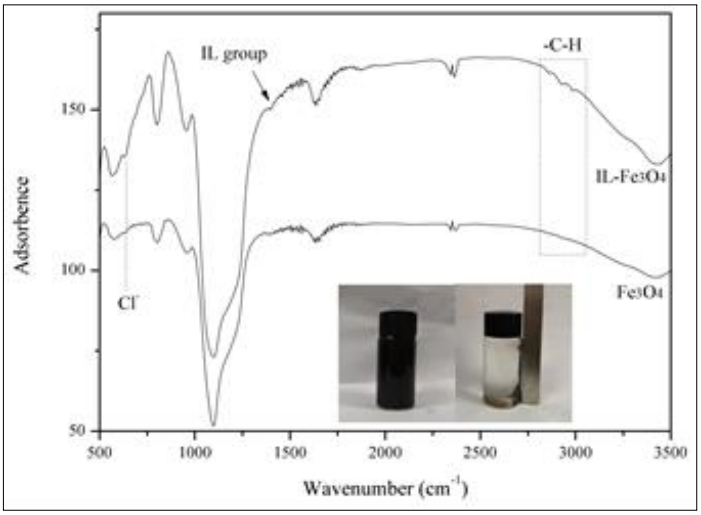

Fig 1: FT-IR of two sorbents.

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. 2. Comparing the Fe_3O_4 and IL-Fe_3O_4, there were three major interactions between the IL group and berberine such as hydrogen bond (between imidazole group in IL and ether bond in berberine), π-π bond (between imidazole group in IL and carboatomic ring in berberine) and electrostatic force (between Cl^- in IL and N^+ in berberine). Hence, the IL-Fe_3O_4 sorbent showed much higher adsorption amount. Furthermore, the polarity and hydrogen bond of water were stronger than organic solvents, but the solubility of berberine in water was the lowest. Also, the weak hydrogen bond of ethanol had lower influence on adsorption amount. So, the IL-Fe_3O_4 sorbent in ethanol can adsorb more berberine.

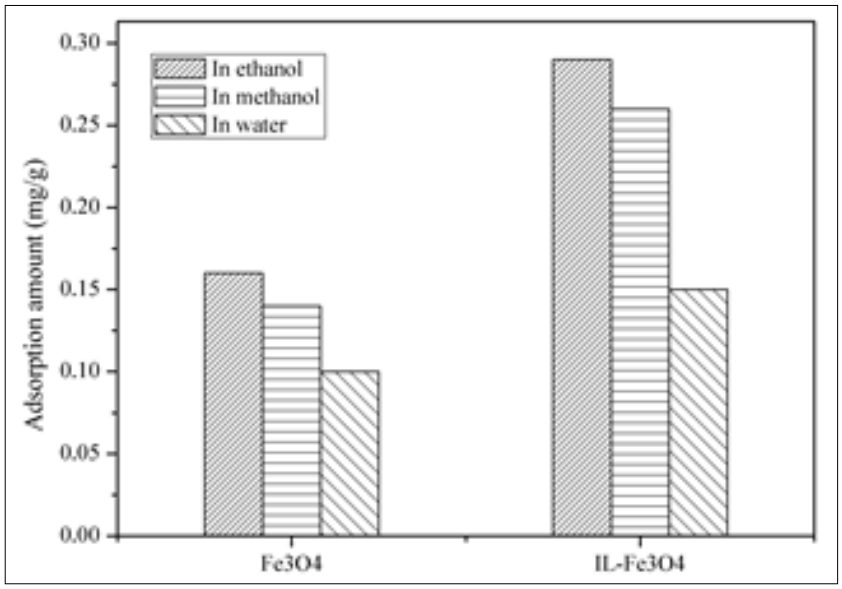

Fig 2: Comparison of maximum adsorption amounts of two sorbents in different solutions.

3. Optimization of variable conditions

Fig. 3 showed the adsorption amounts with different time on Fe_3O_4 and IL-Fe_3O_4 at 25ºC. The adsorption amounts were increased with time increasing and there was no obviously increasing after 7 h for IL-Fe_3O_4. After 7 h, the adsorption amount of Fe_3O_4 was slightly increased but the capability was quite lower than IL-Fe_3O_4.

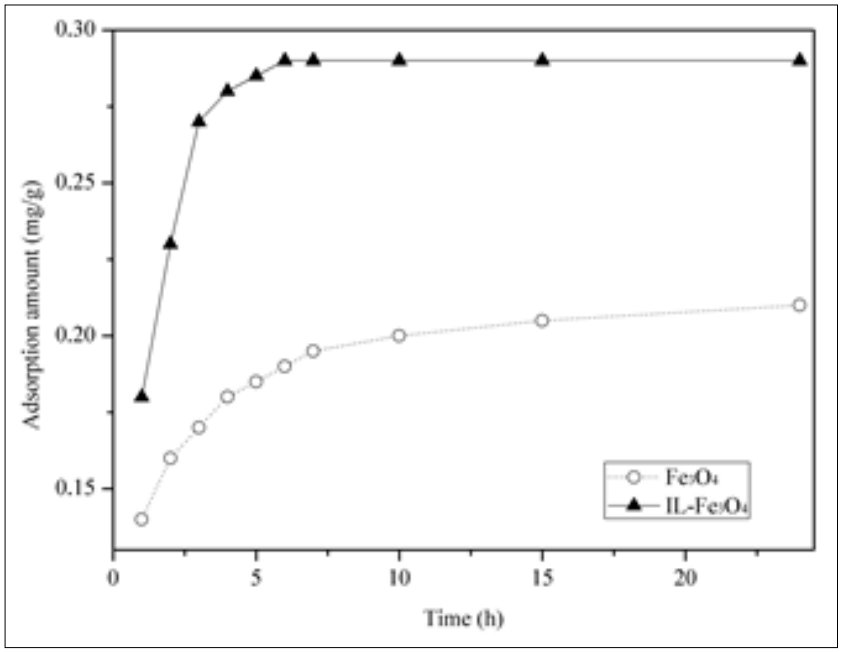

Fig 3: Effect of adsorption amounts with different time on Fe_3O_4 and IL-Fe_3O_4 at 25ºC.

Then different temperatures were evaluated. As shown in Fig. 4, the adsorption amounts increased with increasing temperature until 30ºC. When the temperature was higher than 30ºC the adsorption amounts decreased rapidly.

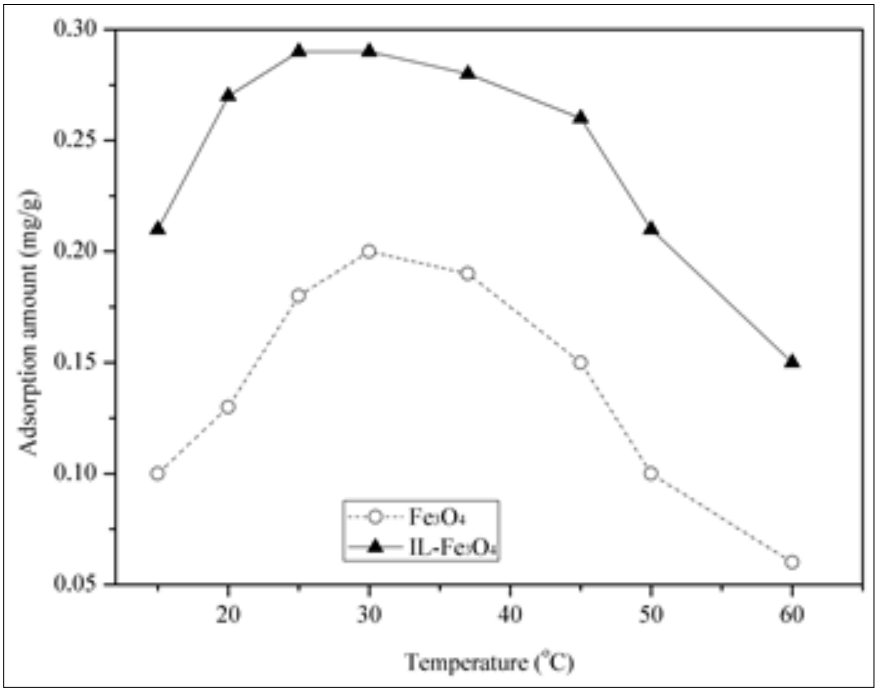

Fig 4: Effect of adsorption amounts with different temperature on two sorbents for 7 h.

Finally, the adsorption amount of Fe_3O_4 and IL-Fe_3O_4 at different pH was evaluated and the results were shown in Fig. 5. The structural stability of Fe_3O_4 was quite unstable in acidic environment so it showed a low adsorption ability. With increasing of pH, the molecular stability of berberine decreased, so the adsorption amount decreased dramatically. But for IL-Fe_3O_4, the IL group covered the surface of sorbent and stabilized the molecular of berberine, so the stabilities of both sorbent and berberine were increased and the pH of solution had no obviously negative effect on adsorption ability in a range of 6-9.
Hence, IL-Fe_3O_4 was selected for as the potential sorbent for further extraction of berberine from real sample solution.

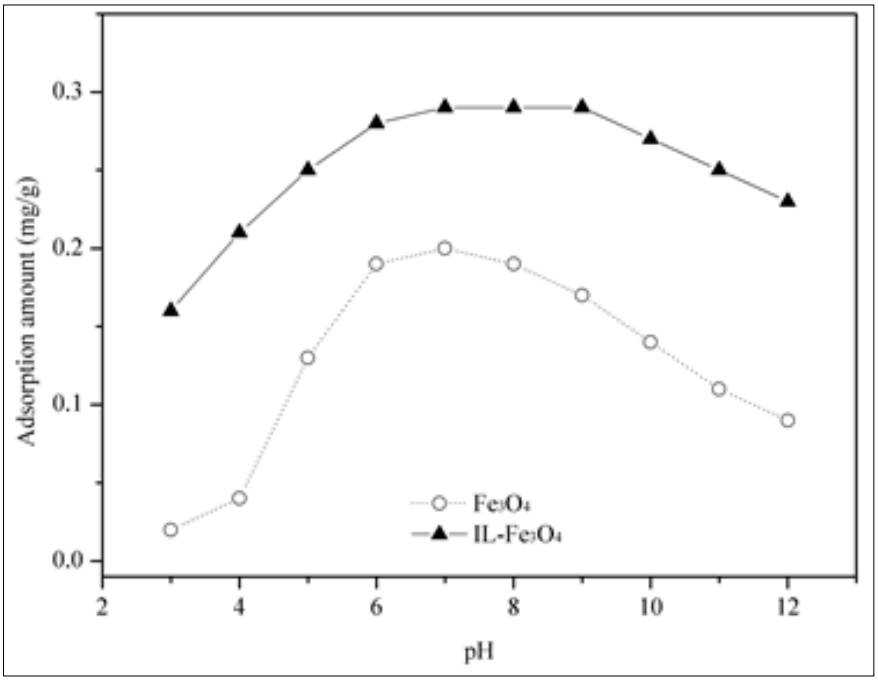

Fig 5: Effect of pH on Fe_3O_4 and IL-Fe_3O_4 at 30°C for 7 h.

4. Application of IL-Fe_3O_4 in real extracted solution
0.5 g of IL-Fe_3O_4 was mixed with 10.0 mL of extracted ethanol solution of *Coptidis Rhizoma*, and the mixture was shaken at 30°C for 7 h. Then a magnetic bar was applied to collect the sorbent and moved it into another bottle for desorption. All solutions were analyzed by HPLC and Fig. 6 showed the chromatograms of three steps. Under the optimized conditions, the IL-Fe_3O_4 can efficiently extract and separate berberine from extracted ethanol solution.

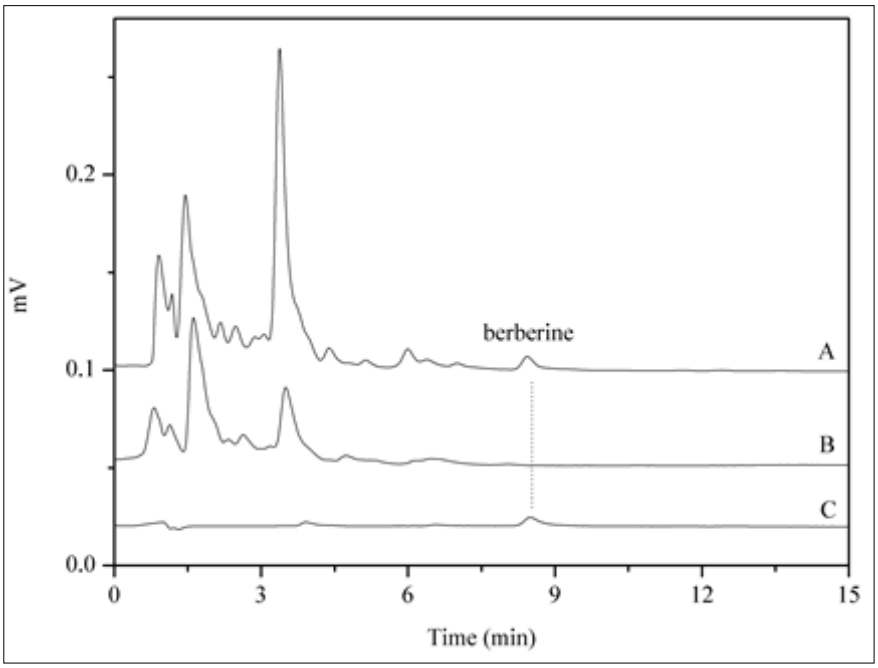

Fig 6: Chromatograms of dispersive solid-phase extraction of berberine from *Coptidis Rhizoma* extract. (A) Extracted *Coptidis Rhizoma* by ethanol, (B) After dispersive solid-phase extraction, (C) desorption of berberine from sorbent.

5. Validation

The linearity and LOD of the analysis condition were validated first. The calibration curves were constructed using the chromatographic peak areas measured at ten increasing berberine concentrations from 0.002-0.2 mg/mL. Good linearity of the calibration curves was obtained and the correlation equation was y=125280x+525 (R^2=0.91) (y is the peak area and x is the concentration in solution). The LOD and LOQ were 4.8×10^{-4} mg/mL and 1.6×10^{-3} 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 solution and analyzed to calculate the recoveries. The accuracy and precision of the method were evaluated by assays of repeatability calculated as the RSDs with IL-Fe_3O_4 process five times over a five-day period. Good recoveries of 87.8-99.6% and RSDs of 8.6-9.9% were obtained. These results mean that the sorbent is reliable for the extraction sample.

Conclusion

In the study, the results proved the higher adsorption efficiency of the ionic liquid modified magnetic sorbent. In ethanol solution, the highest adsorption amount was obtained on IL-Fe_3O_4 at 30°C for 7 h. When the IL-Fe_3O_4 sorbent was applied in dispersive solid- phase extraction of berberine, the LOD and LOQ (4.8×10^{-4} mg/mL and 1.6×10^{-3} mg/mL) proved that the method had good accuracy, the recoveries of 87.8-99.6% and RSDs of 8.6-9.9% proved that the sorbent had excellent efficiency to isolate berberine from extraction solution. The results indicated that IL-Fe_3O_4 as an alternative sorbent had great application potential for isolation of berberine from other natural plants.

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