

Multiphase extraction of ephedrine from *Pinellia ternata* using bionic liquid-modified polymer

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Multiphase extraction (MPE) was applied as a developed, convenient and efficient method in separation of ephedrine from *Pinellia ternata*. Firstly, in order to increase the adsorption efficiency, bionic liquid-modified polymer was created. Comparing the effects of all sorbents under variables conditions, the highest amount of 5.8 mg/g can be adsorbed on dual imidazole ionic liquid modified polymer (Im-Im-Poly) in methanol/water (70:30, v/v) solution at 25°C within 30.0 min. Then the Im-Im-Poly was applied in MPE, after 7 times repetition of extraction, around 1.0 mg/g of ephedrine from *Pinellia ternata* was detected. After washing by water, ethanol and methanol, and elution by methanol/acetic acid (99.0:1.0, v/v), ephedrine was successfully separated.

Keywords: multiphase extraction, bionic liquid, polymer, adsorption, ephedrine, *Pinellia ternata*.

INTRODUCTION

Pharmaceutical bioactive compounds from natural plants or plant extracts are widely used all over the world. Although these compounds are applied in herbal medicines¹, not all of them are safe. Some bioactive compounds can resist microorganisms², even some are toxic for living animals and human body at high concentrations^{3,4}. One of the latest researches proved that a specific bioactive compound is implicated in multiple cancer types especially in upper tract urothelial cancers of human⁵. Ephedra alkaloids are one type of bioactive compounds in several plant. These sympathomimetic amines have been used for the treatment of asthma and bronchitis in several over-the-counter cold medicines^{6,7}. However, the misuse of ephedrine is quite danger, over the 1.0 mg/kg of ephedrine could reduce the heart rate and blood pressure of rate obviously.

In this case, ephedrine in plant extract which will be used as herbal medicine needs to be detected and excess of it have to be removed. Several researchers separated ephedrine from real samples using solid phase extraction (SPE) with designed sorbents. Georgescu et al. synthesized a molecular imprinted polymer (MIP) using thiophene as functionalized monomer with ethylenedioxi-thiophene. Ephedrine as the template was responsible for electrochemical properties of electrodes based on the polymers⁸. Arezou Taghvi and Hamed Hamishehkar prepared carbon coated magnetic nanoparticles (C/MNPs) in SPE to separate ephedrine from urine samples with a recovery of 98.7%⁹. Zhong et al. prepared a MIP with myricetin as functional monomer in SPE and the recovery of myricetin was 92.7% from *Ampelopsis grossedentata* extracts¹⁰. Deng et al. applied monolithic MIP fiber in SPE to recognition of ephedrine in serum and urine samples. The recoveries and relative standard deviations (RSDs) for sample analysis were found in the range of 91.0–104.0% and 3.8–9.1%, respectively¹¹.

However, in previous reports, ephedrine should be firstly extracted from plants to solvent and then be adsorbed, also large amount of organic solvent was needed. In order to simplify the process, multi-phase extraction (MPE) with developed sorbent was introduced. MPE is an improved method based on SPE cartridges, with ap-

propriate solvents the bioactive compound was extracted and transferred to the sorbent, and then interferences and bioactive compounds on the sorbent will be separated by washing and elution steps. In MPE, the properties of the sorbent are crucial for the performance of separation. To increase the separation efficiency, sorbents need functional modification. Ionic liquids (ILs) are one of the most excellent modifications. ILs are well-known green reaction medias with excellent chemical properties in the fields of analytical chemistry, sample preparation, organic synthesis, extraction and separations^{12–14}. Their hydrophobicity, miscibility with several inorganic/organic solvents, and π - π interactions between functional groups allow ILs to be applied widely as sorbents modifications^{15,16}. And it was proved that IL based sorbent had high adsorption efficiency on organic substances¹⁷.

Hence, in order to efficiently separate the ephedrine, sorbents in MPE needs to be designed. The separation ability of bionic liquid sorbent, such as bifunctional ionic liquid-based silicas, was approved in SPE of bioactive compound¹⁸. So in this research, bionic liquid-modified polymer was created and extraction, washing and elution solvents of MPE were evaluated. With the optimized condition, ephedrine in natural plant *Pinellia ternata* was detected and separated. The results are expected to develop the new method and extend their potential application in the separation and purification of ephedrine in herbal medicines.

EXPERIMENTAL

Chemicals

Glycidyl methacrylate, p-divinylbenzene, benzoyl peroxide, sodium dodecyl sulfate, heptane, 3-chloropropylamine, 1,4-dichlorobutane, imidazole, methyl imidazole and ethylimidazole were purchased from Aladdin Inc. (Shanghai, China). HPLC grade of acetonitrile were from CINC High Purity Solvents Co. Ltd. (Shanghai, China). Ephedrine was from Yuanye Biotechnology (Shanghai, China). Organic solvents such as methanol, ethanol, triethylamine were obtained from 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 were filtered before use. Dried *Pinellia ternata* was purchased from a local pharmacy and ground to powder.

Synthesis process

First of all, microporous polymer as the substrate was prepared. 3.0 mL of glycidyl methacrylate as monomer, 5.0 mL of p-divinylbenzene as crosslink and 3.0 mL of heptane as porogen were mixed in 50.0 mL of ethanol. 0.5 g of sodium dodecyl sulfate was dissolved in 100.0 mL of water. After the two solutions were stirred under nitrogen environment for 30.0 min, benzoyl peroxide as the initiator was added. The temperature of the mixed solution was increased to 80°C for 12 h. The obtained polymer particle (Poly) was washed by 50.0 mL of water and 100.0 mL of ethanol. Then, in a flask, 3.0 g of Poly and 3.9 g of 3-chloropropylamine were well mixed in 80.0 mL of ethanol, and 3-chloropropyl modified polymer (Cl-Poly) was synthesized after 8 h at 80°C. After fully dried, the Cl-Poly was modified by following steps in Fig. 1.

Mono IL-modified Poly (Im-Poly, MIm-Poly or EIm-Poly) were obtained by synthesizing 0.5 g of Cl-Poly with 0.7 g of imidazole or methylimidazole or ethylimidazole in 80.0 mL of ethanol at 80°C for 12 h.

In another way, 0.5 g of Cl-Poly, 0.7 g of imidazole, 1.2 g of triethylamine and 80.0 mL of ethanol were stirred in a flask for 8 h at 80°C to produce the imidazole-modified Poly (Imidazole-Poly). All obtained Imidazole-Poly was then mixed with 3.8 g of 1,4-dichlorobutane in 80.0 mL of ethanol and heated to 80°C. After 8 h, chlorinated IL-modified Poly (Cl-Im-Poly) was obtained. Finally, Cl-Im-Poly was synthesized with 0.7 g of imidazole or methylimidazole or ethylimidazole in 80.0 mL of ethanol for 8 h at 80°C. After washing with 50.0 mL of methanol and drying at 80°C for 12 h, three bionic liquid-modified polymers (Im-Im-Poly, MIm-Im-Poly and EIm-Im-Poly) were obtained.

Apparatus

The characteristics of blank and modified polymers 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. Scanning electron microscope (SEM) data were obtained by JSM-7600F (JEOL, Japan) instrument of 15 kV. All samples were analyzed by a HPLC (LC3000, CXTH, Beijing, China) with a TC-C18 column (4.6×250 mm, 5 μm, Agilent, USA). The mobile phase, flow rate, UV wavelength, injection volume and column oven temperature were acetonitrile/water/triethylamine/phosphoric acid (5.0:94.8:0.1:0.1, v/v/v/v), 1.0 mL/min, 264.0 nm, 5.0 μL and 35°C, respectively.

Comparison of adsorption efficiency on sorbents in tube

In order to obtain the adsorption efficiency on sorbents, 0.5 g of blank polymer, three mono IL-modified Polys and three bionic liquid-modified polymers were mixed with 3.0 mL of eight concentrations (in the range of 0.0001–1.4 mg/mL) of ephedrine methanol solution in sealed tube. After shaking for 2 h at 25°C the concentrations of unadsorbed ephedrine in solutions were analyzed by HPLC. The adsorption efficiencies were obtained by Eq. (1):

$$E(\%) = \frac{(C_0 - C_1) \times V}{C_0 \times V} \times 100\% \quad (1)$$

where E (%) represents the adsorption efficiency, C_0 and C_1 (mg/mL) are the initial concentrations and concentrations after adsorption, respectively. V (mL) is the solution volume and m (g) represents the weight of sorbents.

Then the effect of conditions such as adsorption time (within 60.0 min), methanol/water ratio (from 100.0:0.0 to 0.0:100.0, v/v), and temperature (from 4°C to 60°C) on adsorption abilities were evaluated using 0.5 g of sorbent with 3.0 mL of 1.0 mg/mL of ephedrine solution.

Procedure of multiphase extraction

Firstly, the extraction solvent should be selected. *Pinellia ternata* was grinded to powder and 5.0 g of it were respectively dipped into 20.0 mL of different solvents (water, methanol, ethanol and methanol/water (70.0:30.0, v/v)) for 3 h at 25°C. Then the MPE was processed as shown in Fig. 2. Bionic liquid-modified polymer as the sorbent (0.5 g) was packed at the bottom of the empty

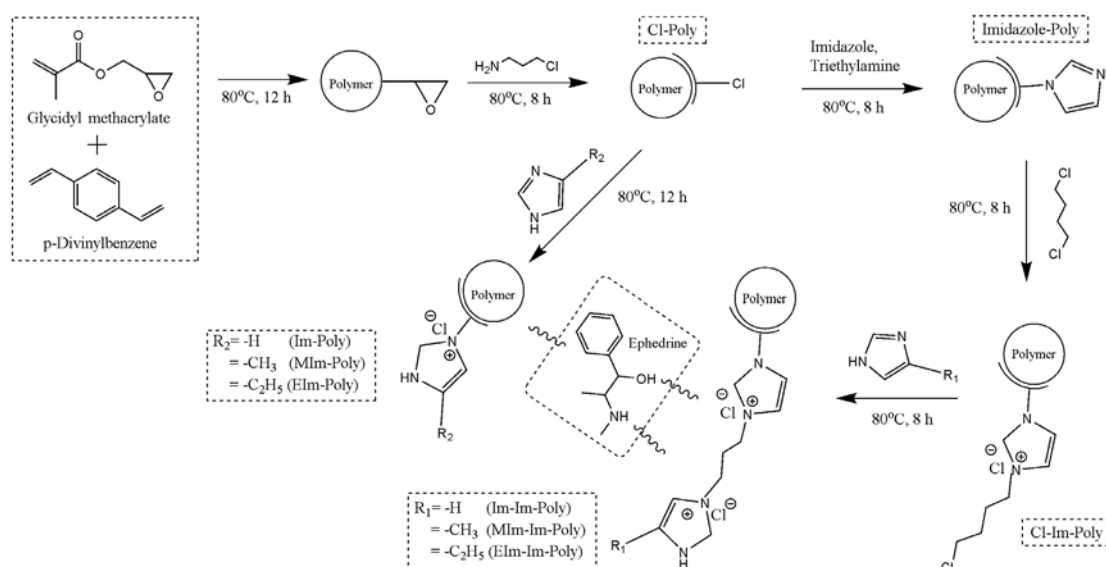


Figure 1. Synthesis process of blank polymer, Im-Poly, MIm-Poly, EIm-Poly, Im-Im-Poly, MIm-Im-Poly and EIm-Im-Poly

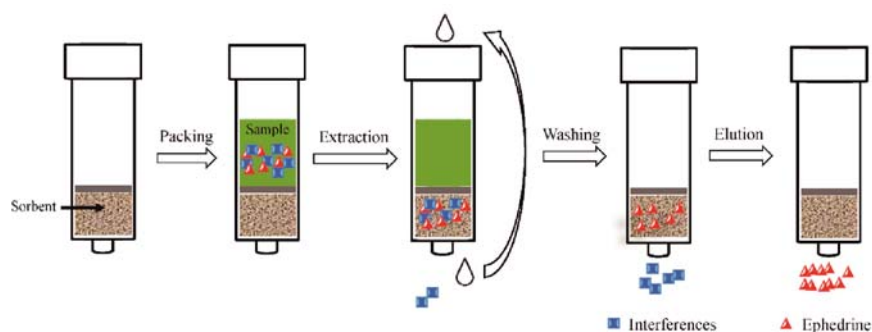


Figure 2. Process of multiphase extraction

cartridge. 5.0 g of *Pinellia ternata* powder packed on top of the sorbent. Extraction solvent was then poured into the cartridge to extract and remove all substances from plant powder to the sorbent. During this period, the sorbent adsorbed all substances with different intensities of chemical bonds. Then the filtered solvent was collected and poured into cartridge for repeated extraction over several times. After extraction, the sorbent was then washed to remove any interference and finally ephedrine was eluted.

RESULTS AND DISCUSSION

Characteristic

In Fig. 3, both the vibrations of $-\text{CH}_2$ and Cl are around 706.8 cm^{-1} . However, the intensity of $-\text{CH}_2$ is much weaker than Cl. So the band at 706.8 cm^{-1} was belong to $-\text{CH}_2$ group, and higher intensity of the bands on IL-modified polymers were from Cl. The increased intensity of the band at 993.6 cm^{-1} which belong to $-\text{C}-\text{N}$ bond proved that more imidazole groups were immobilized. Except blank polymer, all IL immobilized polymers showed two bands at 1485.7 cm^{-1} and 1508.6 cm^{-1} which were the finger print region of IL groups. Moreover, the vibration range of the $-\text{C}-\text{H}$ group in

alkanes is $2850\text{--}3000\text{ cm}^{-1}$. Therefore, the band between 2850.0 cm^{-1} and 3000.0 cm^{-1} was assigned to the carbon chain on the IL group, and the intensity of the bands increased proved that more $-\text{C}-\text{H}$ on IL groups were immobilized^{19, 20}. All the bands in FT-IR spectrogram proved that the IL groups were immobilized on blank polymer. More confirmation was shown in Fig. 4, the SEM of Im-Im-Poly (C and D) clearly showed the porous structure of IL groups immobilized on surface of blank polymer (A and B) with different scales.

Study and optimization of adsorption sorbents

Figure 5 showed the adsorption efficiencies of ephedrine on all sorbents, and the efficiencies on three bionic liquid-modified polymers were higher than other sorbents. The results can be explained according to the structure of ephedrine. The hydrophobic benzene ring in ephedrine can interact with IL by $\pi-\pi$ interaction. The low polar methyl group can interact with carbon chain on IL. $-\text{OH}$ and $-\text{NH}$ groups can be hydrolyzed to create strong hydrogen bond and ionic interaction. Moreover, more binding points and an additional carbon chain on bionic liquid groups promoted the adsorption efficiency.

In addition, with the concentration of ephedrine solution increasing, the maximum adsorption amounts of all sorbents were obtained (blank polymer: 0.5 mg/g ,

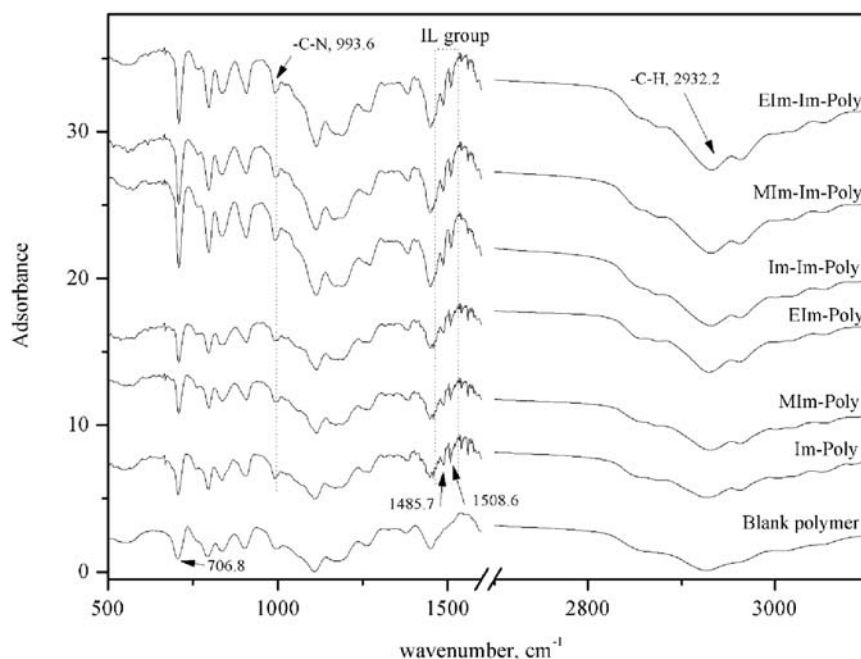


Figure 3. FT-IR of ionic liquid-modified and bionic liquid-modified polymers

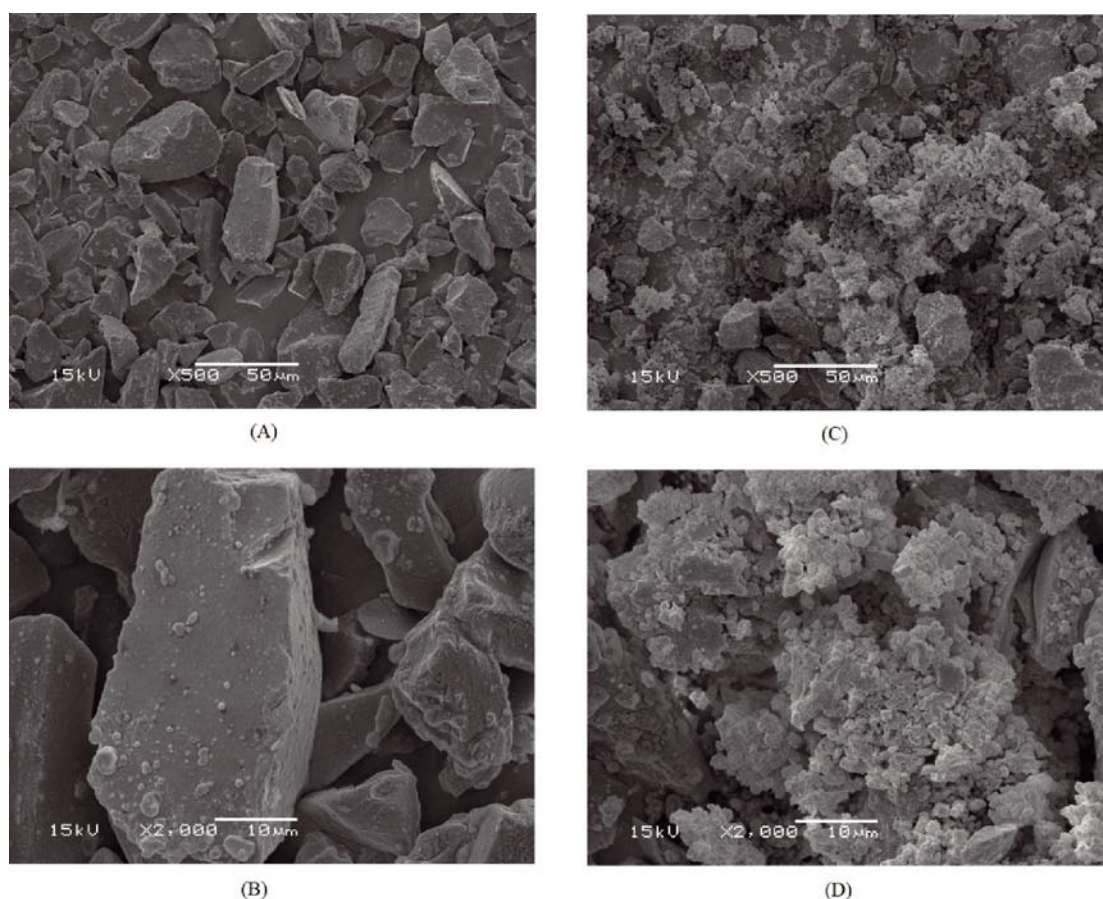


Figure 4. SEM of blank polymer (A, B) and Im-Im-Poly (C, D)

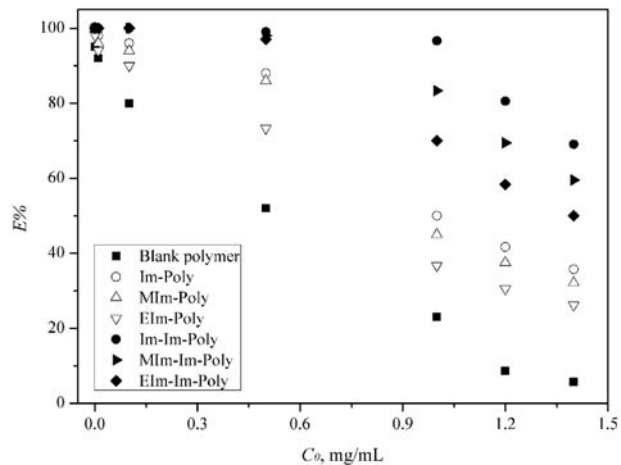


Figure 5. Adsorption efficiencies of ephedrine on all sorbents. Experiment conditions: 0.5 g of sorbents with 3.0 mL of 0.0001-1.4 mg/mL of ephedrine methanol solution, 2 h adsorption at ambient temperature

Im-Poly: 3.0 mg/g, MIm-Poly: 2.7 mg/g, EIm-Poly: 2.2 mg/g, Im-Im-Poly: 5.8 mg/g, MIm-Im-Poly: 5.0 mg/g and EIm-Im-Poly: 4.2 mg/g). The differences among three bionic liquid-modified polymers could be explained that the hydrophobic property of the outer layer IL increased with carbon chain length increasing, it may cover the IL groups and block chemical bonds. So the Im-Im-Poly obtained the highest adsorption efficiency.

The adsorption amounts on all modified polymers with different times were shown in Fig. 6 except the blank Poly which the adsorbed amount was quite lower than the others. The adsorbed amounts of ephedrine on MIm-Poly and EIm-Poly were saturated at 20.0

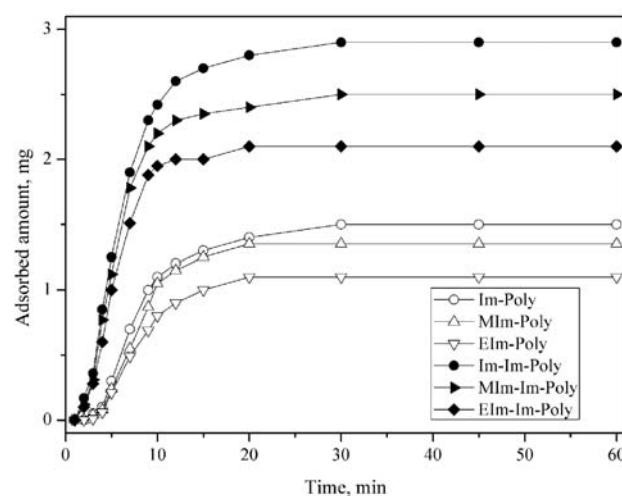


Figure 6. Adsorption amount of ephedrine on all sorbents with different times. Experiment conditions: 0.5 g of sorbents with 3.0 mL of 1.0 mg/mL of ephedrine methanol solution at ambient temperature

min, and Im-Poly at 30.0 min. This because the longer carbon chain on IL increased the hydrophobic property which was reduced the interaction between ephedrine and sorbents. When bionic liquids were immobilized, higher adsorbed amounts were obtained because of the increasing binding points. Im-Im-Poly showed the lowest hydrophobic property among the three bionic liquids-modified polymers, so it obtained the highest adsorbed amount. The saturated adsorption times of Im-Im-Poly was increased to 30.0 min, so it was suggested as the optimized adsorption time.

Normally the adsorption ability would be affected under different temperature, however pure methanol

was volatilized under high temperature. So considering the efficiency and volatility of solvents, methanol/water (v/v) solution with different ratio was evaluated on three bionic liquid-modified polymers. Fig. 7 showed that the adsorption abilities kept on a high level when ratio of methanol in water was 70% for Im-Im-Poly, 80% for MIm-Im-Poly and 90% for EIm-Im-Poly. EIm-Im-Poly had a high hydrophobic property because of the long carbon chain, its adsorption ability would be decreased in a high ratio of water solution.

Comparing the results in Figs. 5–7, Im-Im-Poly with the highest adsorption ability was investigated under different temperature in methanol/water (70.0:30.0, v/v). The adsorption ability increased when the temperature increasing to 25°C. However, the structure of Im-Im-Poly may unstable when temperature was higher than 40°C to cause more desorption than adsorption (Fig. 8). In summary, Im-Im-Poly in methanol/water (70.0:30.0, v/v) at 25°C was selected as the optimized adsorption condition.

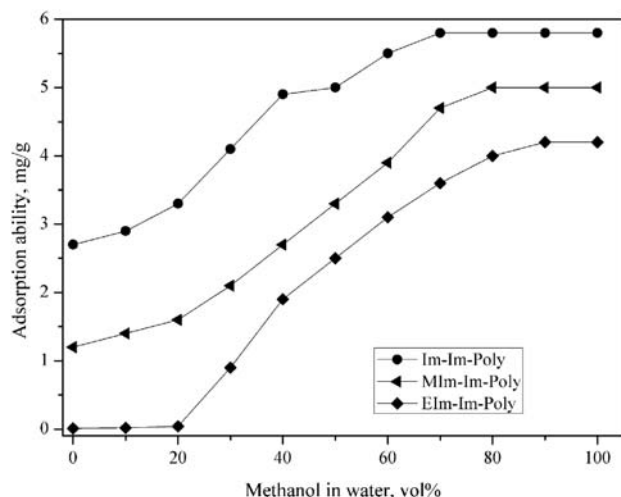


Figure 7. Adsorption abilities of Im-Im-Poly, MIm-Im-Poly and EIm-Im-Poly in methanol/water (v/v) solution. Experiment conditions: 0.5 g of sorbents with 3.0 mL of 1.0 mg/mL of ephedrine solution, 30.0 min adsorption at ambient temperature

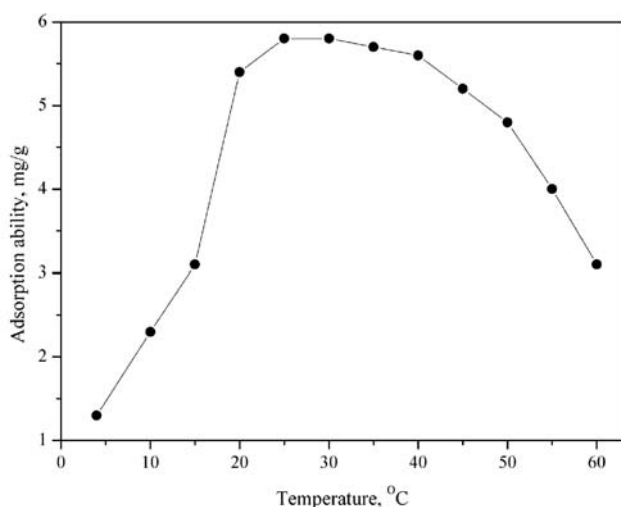


Figure 8. Adsorption abilities of Im-Im-Poly under different temperature. Experiment conditions: 0.5 g of sorbents with 3.0 mL of 1.0 mg/mL of ephedrine in methanol/water (70.0:30.0, v/v) solution, 30.0 min adsorption

Multiphase extraction of ephedrine from *Pinellia ternata*

The extraction solvent was confirmed firstly. Among four extraction solvents, methanol could extract the highest amount of ephedrine (1.1 mg/g from *Pinellia ternata*), however the amount in methanol/water (70.0:30.0, v/v) was 4.7% less than methanol. Considering methanol/water (70.0:30.0, v/v) was the optimized adsorption solvent, so it was used in extraction process.

According to the restricted volume of the cartridge, 2.0 mL of methanol/water (70.0:30.0, v/v) was used at 25°C. During the extraction process, the amount extracted in each repetition was measured. After 7 repetitions, there was no increase in the amount of ephedrine in the extract. The sample powder was then removed and dipped into the extraction solvent for several hours, no ephedrine was detected, showing that 7 repetitions were considered optimal.

The effects of the washing and elution solutions were then assessed. When 2.0 mL of water, ethanol and methanol were used as washing solvents, large amounts of hydrophilic and organic interferences were removed. Therefore, the volume of washing solvents needed to be increased until no more interference was detected, and according the results 4.0 mL, 4.0 mL and 8.0 mL of water, ethanol and methanol were decided. Finally, methanol/acetic acid (99.0:1.0, v/v) with strong elution ability was applied and 4.0 mL of it can elute all ephedrine from Im-Im-Poly and Fig. 9 shows that the separation was successful.

Finally, the accuracy and precision of the MPE method with Im-Im-Poly as the sorbent were evaluated by assays of repeatability calculated as the relative standard deviations (RSDs) 5 times over a 5-day period. The RSDs of 2.55–4.41% proved that MPE with Im-Im-Poly showed a good accuracy in separation of ephedrine from *Pinellia ternata* extract.

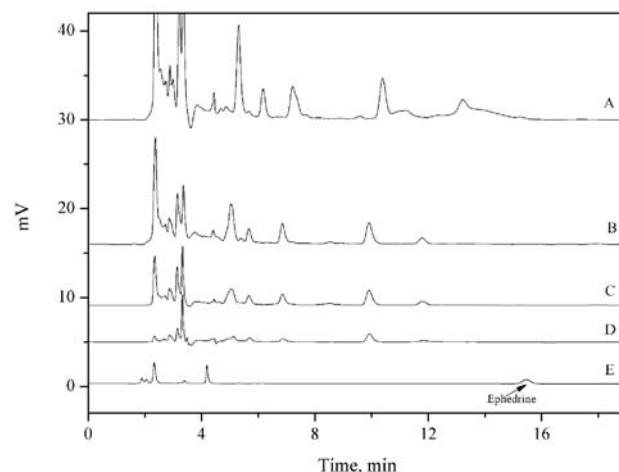


Figure 9. Chromatograms of washing and elution step in MPE. (A) Extract from *Pinellia ternata* by methanol/water (70.0:30.0, v/v); (B) Washing by 4.0 mL of water; (C) Washing by 4.0 mL of ethanol; (D) Washing by 8.0 mL of methanol; (E) Elution by 4.0 mL of methanol/acetic acid (99.0:1.0, v/v). Chromatographic conditions: a TC-C18 column with acetonitrile/water/triethylamine/phosphoric acid (5.0:94.8:0.1:0.1, v/v/v/v) as the mobile phase. Flow rate, UV wavelength, injection volume and column oven temperature were, 1.0 mL/min, 264.0 nm, 5.0 μ L and 35°C

Table 1. Comparison of the proposed methods with previous studies

Sorbent	Target	Method	Sample	Adsorption amount (mg/g)	RSD (%)	Ref
C/MNPs	Ephedrine and methamphetamine	SPE	Urine	0.02	3.2–9.7	10
MIP	Ephedrine and pseudoephedrine	SPME	Urine and serum	–	3.8–9.1	12
DGT	Ephedrine and methamphetamine	Adsorption	Surface water	2.7	1.3–8.9	21
MIP	Ephedrine and pseudoephedrine	SPE	<i>Ephedrae herba</i>	5.7	–	22
MIP	Ephedrine	SPE	Chinese Ephedra	4.2	2.7–3.3	23
Bionic liquid-modified polymers	Ephedrine	MPE	<i>Pinellia ternata</i>	5.8	2.6–4.1	This work

The proposed method was compared with previous studies on ephedrine (Table 1). Taghvimi and Hamishehkar synthesized a carbon coated magnetic nanoparticles (C/MNPs) and applied it in SPE to separation methamphetamine and ephedrine from urine samples⁹. Deng et al. applied the capillary electrophoresis (CE) method coupled with monolithic molecular imprinted polymer (MIP) fiber based solid phase microextraction (SPME) to determination of ephedrine and pseudoephedrine¹¹. Zhang et al. prepared diffusive gradients in thin films (DGT) to detect methcathinone and ephedrine in surface river water. The adsorbed amount was detected as 2.7 mg/g with RSD in the range of 1.3–8.9%. Tian et al. synthesized molecularly imprinted co-polymers with ephedrine as the template and applied the sorbent in SPE to adsorb ephedrine. Dong et al. used MIP with methacrylic acid as the monomer to separate ephedrine from one natural plant. Overall, the sorbents developed in the present study exhibited higher adsorption efficiency and the MPE method was a simpler that achieved better separation performance.

CONCLUSION

In this study, bionic liquid-modified polymers were created and applied as sorbents in MPE method to separate ephedrine from *Pinellia ternata* extract which was used as herbal medicine to treat cough. Im-Im-Poly with 5.8 mg/g of the largest adsorption amount was selected as the optimized sorbent. And in methanol/water (70.0:30.0, v/v) at 25°C Im-Im-Poly obtained more excellent adsorption performance than other sorbents (mono IL-modified Poly, MIm-Im-Poly and EIm-Im-Poly). When it was applied in MPE, 2.0 mL of methanol/water (70.0:30.0, v/v) was used as extraction solvent for 7 times repetition. All interferences were washed by water, ethanol and methanol, and the adsorbed ephedrine was removed with high purity. The provide experimental data revealed that MPE with Im-Im-Poly was an excellent potential method, and the successful separation of ephedrine from herbal plants indicated that the proposed method could be applied in pharmaceutical purpose.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 51503020) and by National Research Foundation of Korea grant funded by the Korean Government (No. NRF-2019R1A2C1010032).

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