Application of Deep Eutectic Solvents in Hybrid Molecularly Imprinted Polymers and Mesoporous Siliceous Material for Solid-Phase Extraction of Levofloxacin from Green Bean Extract

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Deep eutectic solvents (DES) are potential ecofriendly surfactants for the preparation of materials. In this study, both molecularly imprinted polymers (MIPs) and mesoporous siliceous materials (MSMs) were modified by betaine-based DES. Six materials were employed as solid phase extraction (SPE) adsorbents for the rapid purification of levofloxacin. The DES-based materials showed better selective adsorption than the conventional materials. The adsorption curves of DES-MIP showed superior molecular recognition ability and binding capability for levofloxacin compared to the other materials. The limit of detection and limit of quantitation of the method were 0.01 and 0.03 μg/mL for levofloxacin, respectively. The method recoveries at three spiked levels were 97.2 – 100.2% for DES-MIP, with an RSD <1.8%. DES-MIP showed the highest selective recovery (95.2%) for levofloxacin from the green bean extract, and could remove the interferent effectively.

Keywords Deep eutectic solvents, molecularly imprinted polymers, mesoporous siliceous material, solid-phase extraction, levofloxacin

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the analysis to simulate a natural system. The DES (synthesized with betaine and ethylene glycol)-modified MIPs (DES-MIP), MIPs (without DES) and NIPs (without DES and template) were prepared in an identical procedure. DES-based mesoporous siliceous and conventional MSMs prepared by hydrothermal synthesis. After synthesis, the prepared materials were characterized by scanning electron microscopy (SEM), Brunauer-Emmett-Teller (BET) analysis and Fourier transform infrared spectroscopy (FT-IR). All the materials and comparative C18 adsorbents were employed as the SPE adsorbent for the rapid purification of extracts.

### Experimental

#### Chemicals and reagents

Levosofacin (>98.0%), betaine (>98.0%), ethylene glycol (>99.5%) and 1,3,5-trimethylbenzene (TMB, 99%) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 3-Aminopropyltriethoxysilane (APTES 99%) and 2,2-azobisisobutyronitrile (AIBN 98%) were supplied by Duksan Pure Chemicals Co., Ltd. (Ansan, Korea). Methacrylic acid (MAA 98%), ethylene glycolmethacrylate (EGDMA 98%), poly(ethylene glycol)-block-propylene glycol-block-ethylene glycol (PEG-PPG-PEG, 98%), methanol (>99.9%), ethanol (>99.9%), acetonitrile (>99.9%), and terephthaosilane (TEOS, 98%) were acquired from Alfa Aesar (Heysham, England). Hydrochloric acid (HCl, 36%) was obtained from Kodaq Co., Ltd. (Siheung, Korean). All other organic solvents and inorganic reagents were acquired from Duksan Pure Chemicals Co., Ltd. (Ansan, Korea). Distilled water was filtered using a vacuum pump and filter (HA-0.45, both from Millipore, USA) prior to use. All samples were filtered (MFS-25, 0.2 μm TF, Whatman, USA) before being injected into the HPLC system.

#### Instrumentation and conditions

Fourier transform infrared spectroscopy (FT-IR, Perkin Elmer, USA) was used to examine the functional groups of the six materials using a pressed KBr tablet in the range 400 – 4000 cm⁻¹. The morphological evaluation was carried out by field emission-SEM (FE-SEM, S-4200, Hitachi, Ontario, Canada). The pore sizes and surface area of these materials were determined from the adsorption and desorption branches of the N₂ sorption isotherms, respectively, according to a simplified Broekhoff-de Boer method (BdB-FHH). HPLC analysis was performed using a Younglin HPLC system equipped with an M930 solvent delivery pump (Younglin, Korea) and a UV detector (Younglin, Korea). The analytical column was purchased from RStech. Co., Korea (150 × 4.6 mm i.d., C₁₈, 5.0 μm). The mobile phase was 0.05 M NaH₂PO₄-ACN (82:18 v/v, pH 3) and its flow rate was set to 1.0 mL min⁻¹. The detection wavelength of the detector was set to 294 nm and the injection volume was 10 μL.

#### Preparation of DESs

In this study, betaine-based DES were synthesized by a heating method. The eutectic mixtures (ratio 1:2:1) consisting of a hydrogen-bond acceptor (betaine) and hydrogen bonding donor (ethylene glycol) with accurately calculated amounts of water were stirred at 100°C until an even, colorless liquid had formed.

#### Synthesis of the polymers based on DESs

Table 1 and Fig. 1 present the scheme of MIPs preparation.

| Formulation Chemical | Chemical | NIP | MIP | DES-MIP |
|----------------------|----------|-----|-----|---------|
| Monomer APTES-MAA/mmol | 3.3 | 3.3 | 3.3 |
| Crosslinking agent EGDMA/mmol | 25 | 25 | 25 |
| Initiator AIBN/mmol | 0.3 | 0.3 | 0.3 |
| — TEOS/mL | 1 | 1 | 1 |
| — DES/mmol | — | — | 15 |
| Template Levofloxacin/mmol | — | 1 | 1 |

First of all, APTES (6.4 mmol) and MAA (8.1 mmol) were heated together at 60°C for 24 h to synthesize the monomer APTES-MAA. Subsequently, the template (levofloxacin, 1 mmol) and APTES-MAA (3.3 mmol) were added to methanol (12 mL), and the emulsion was sonicated for 10 min until they were fully dissolved and then stored at 4°C in the dark for 1 h. TEOS (1.0 mL, 4.48 mmol) after alcoholysis, EGDMA (25 mmol) and AIBN (0.3 mmol) were then added to the solution. In the next step, 1.5 mmol of betaine-based DES was then added (conventional MIPs do not require this step). After deoxygenating the solution with bubbling nitrogen for 10 min, the mixture was polymerized at 60°C for 24 h. After polymerization, the resulting bulk polymers were ground and sieved with a 0.054-mm aperture sieve, and the smallest particles of polymers were removed by sedimentation in acetone. The polymers were washed in a Soxhlet apparatus successively with MeOH-HAc (9:1, v/v) and MeOH for 24 h to remove the template, and dried under reduced pressure. The efficiency of this procedure was checked by HPLC. Non-imprinted polymers (HNIPs synthesized in the absence of template) were prepared and treated in an identical manner.

#### Synthesis of MSMs based on DES

As shown in Table 2, the MSMs were prepared using the hydrothermal polymerization method. In a typical preparation, 6 g of triblock copolymer Pluronic P123 (BASF) was dissolved in an acidic solution (15 mL of HCl (37%) and 100 mL of H₂O). A total of 6 g of 1,3,5-trimethylbenzene (TMB) was then added and the resulting solution was heated to 37 - 40°C with vigorous stirring for 2 h. A total of 13.8 mL of tetraethoxysilane (TEOS) was added and stirred for 5 min. The solution was transferred to an autoclave and aged at 40°C for 20 h under quiescent conditions. A total of 1.5 mmol of betaine-based DES was then added (conventional MSMs do not require this step), and the mixture was aged at 100°C for a further 24 h. The precipitate was filtered, washed with water and ethanol, and dried. The resulting white powder was calcined at 900°C in a muffle furnace for 6 h.

#### Absorption capacity of the materials

In the static adsorption test, 30 mg of DES-MIP was placed in a tapered plastic centrifuge tube with a stopper containing 10.0 mL of MeOH solution with levofloxacin at concentrations of 5-500 μg/mL, respectively. The mixtures were shaken mechanically for 12 h at room temperature with a horizontal shaker and separated for centrifugation at 6000 rpm for 15 min. The concentration of analyte in the solution was analyzed by HPLC. DES-MIP were weighed and suspended in 1.0 mL of a levofloxacin solution of 50 μg/mL. The mixtures were shaken mechanically for 1, 2, 4, 6, 8, 10 and 12 h at room temperature. The used MIP, MSM, and DES-MSM were used repeatedly in the above experiment.
Characterization of materials

The morphological microstructures of these materials were observed by FE-SEM. A high-temperature heat treatment at 900°C eliminated the micropores in the materials, as evidenced by the t-plots of the N₂ sorption studies. Molecular structure characterization of the polymer was performed by FT-IR using the KBr pellet method. In the FT-IR disk preparation process, 1 mg of sample material was ground together with 200 mg KBr to produce tablets. The percentage of sample to KBr was 0.5%. The FT-IR measurement range was controlled from 4000 to 400 cm⁻¹.

Purification of levofloxacin from the green bean extract

A 2.0-g sample of green bean was added to 20 mL of DI-water with stirring at 80°C for 1 h. The supernate was cooled to room temperature and filtered through a 0.45-μm membrane prior to the SPE procedure. To simulate the natural systems sample, 500 ng of levofloxacin was added to 10 mL of the green bean extract (Fig. 2). A 200-mg sample of these materials was added to six empty SPE cartridges and frits were placed at the lower and upper ends to avoid polymer loss.

The levofloxacin sample (1.0 mL) was loaded into the DES-SPE columns, and washed with DI-water (1.0 mL). The analyte was eluted from the columns using MeOH-HAc (9:1, v/v, 1.0 mL). The MeOH-HAc elution was collected at a constant volume (1.0 mL) for further HPLC analysis.

Table 2 Preparation of mesoporous siliceous materials

| Chemical          | MSM | DES-MSM |
|-------------------|-----|---------|
| PEG-PPG-PEG/g     | 6   | 6       |
| TMB/g             | 6   | 6       |
| TEOS/mL           | 13.8| 13.8    |
| DES/mmol          | —   | 15      |

Fig. 1 Schematic illustration of molecular imprinting material formation.

Fig. 2 Chromatogram of green bean extract with levofloxacin (50 ng/mL). Column, C₁₈ (150 × 4.6 mm i.d.); mobile phase, 0.05 M NaH₂PO₄-ACN (82:18 v/v, pH 3); detector, UV (294 nm); injection volume, 10 μL; flow rate, 1 mL/min.
Results and Discussion

Preparation of materials

Selection of the monomer is a crucial step for the preparation of a polymer, and APTES-MAA was used as the hybrid functional monomer for the preparation of MIPs in this study. The structures of APTES and MAA show that there are different functional groups in their molecules. C=C of MAA can be cross-linked with crosslinking agent by thermal initiated free radical polymerization in the presence of AIBN, while –COOH can be the functional group on the surface of polymer to form hydrogen bond with template, and it could also dehydrate with silicon hydroxyl; silicate ester bond of APTES can release silicon hydroxyl and make APTES firmly embedded in the silica matrix from condensation of TEOS, while –NH2 could be the functional group on the surface of polymer to form hydrogen bond with template. MSMs were prepared using the hydrothermal polymerization method. Therefore, APTES-MAA had been applied in this study as a functional monomer. In this study, both the MIPs and MSMs were modified by DES. In view of a DES composed of a salt with a hydrogen donor, the DES not only has hydrophobic and π-π interactions with the copolymer, but also hydrogen-bonding interactions with the –OH groups of the copolymer. Based on the flexible structure of DES containing both an organic group and an anion, DES might be a potential surfactant in the preparation of materials. Many functional groups from DES were observed over the surface of the DES based particles.DES formed hydrogen bonds with the surface hydroxyls of particles. Therefore, the adsorption efficiency might benefit substantially by such particle aggregation. A betaine-based DES was applied successfully to the synthesis of MIPs and MSMs.

As shown in Fig. 3, these new materials will have different adsorptivity in the SPE procedure. The rate of levofloxacin loss using the C18 adsorbent was much higher than with the other five. In three of the polymers, DES-MIP had the lowest loss rate, and the loss rate of NIP was higher than DES-MIP and MIP. On the other hand, the loss rate of the DES-MSM was also lower than conventional MSM. Overall, the DES-modified materials had better selective adsorption than the conventional materials. The adsorption of MIPs was better than that of the MSMs. Overall, the C18 sorbent was found to be unsuitable for the adsorption of levofloxacin.

Characteristics of materials

Figure 4 shows SEM images of five proposed materials. Both of the macrostructures of MSM (Fig. 4a) and DES-MSM (Fig. 4b) were similar. Many spherical particles were observed. NIP (Fig. 4c), MIP (Fig. 4d) and DES-MIP (Fig. 4e) were not spherical particles, but the structure was similar to cotton shape. On the other hand, these materials also showed some differences. The mean particle size and surface morphology of these new materials were not the same. These minor modifications in synthesis led to a significant change in particle morphology. Many small pores and functional groups were observed over the surface of the DES-based particles. Therefore, the adsorption capacity and selectivity might benefit substantially by such particle aggregation.

In the present study, a high-temperature heat treatment at 900°C eliminated the micropores in the materials, as evidenced by the t-plots of the N2 sorption studies. The popular sorption and desorption isotherms at the relative pressures were used to characterize the surface area, pore size and pore volume of these materials. The five kinds of sample were prepared using this approach, and pore size and total pore volume were characterized by N2 sorption isotherms (Table 3). In addition, the surface area, pore size and pore volume of MSM and DES-MSM...
material were much larger than those polymers. MSM had the largest surface area and porous volume among these materials. DES-MSM had the largest average pore size. On the other hand, the surface area and porous volume of three polymers were similar. But DES-MIP showed the largest average pore size. From the above, it was found that these materials had different adsorption capacity.

Use of FT-IR spectra has become a common technique to investigate material conformation because it can provide abundant information on the structure. The FT-IR spectra show two regions, the functional group region (4000 – 1330 cm$^{-1}$) and fingerprint region (1330 – 400 cm$^{-1}$). As shown in Fig. 5, the fingerprint regions of MSM and DES-MSM were similar. Therefore, the main structure of these MSMs was similar. NIP, MIP and DES-MIP have a similar fingerprint region. It was not difficult to see that these were the same type of materials. The functional group region was slightly different between traditional materials and DES-based materials, because betaine-based DES had been applied in the synthesis of the materials. Figure 5 shows there were obvious functional group peaks at 1550, 3430 and 3450 cm$^{-1}$ in the FT-IR spectra of the DES-MSM and DES-MIP, respectively. Therefore, C=\(\text{N}\) (1645 – 1500 cm$^{-1}$), –OH (3500 – 3200 cm$^{-1}$) and N–H (3500 – 3300 cm$^{-1}$) were on the surface of the materials. These functional groups should be based on the modification of betaine-based DES. Betaine-based DES was connected to the surface of these materials. The conformation of materials was modified by the betaine-based DES in the synthesis process.

**Evaluation of the selective adsorption capacity of materials**

Static absorption and dynamic adsorption experiments were performed at room temperature to evaluate the binding property of these materials. Figure 6 shows that the amount of levofloxacin adsorbed by six of the materials increased with increasing concentration (5.0 – 500 \(\mu\)g/mL). DES-MIP showed the highest affinity in all these materials. The adsorption capacity of C$_{18}$ sorbent showed only small growth; the absorption by the other five materials increased until the levofloxacin concentration was 200 \(\mu\)g/mL. When the concentration was higher than 200 \(\mu\)g/mL, the adsorption capacity showed no further change. According to Fig. 7, the adsorption capacity of these five materials increases with increasing adsorption time before saturation adsorption. On the other hand, MSMs require 8 h to reach adsorption saturation, but three polymers required 10 h.

In three of the polymers, DES-MIP had the best adsorption capacity; the adsorption capacity of NIP was lower than DES-MIP and MIP. On the other hand, the adsorption capacity of the DES-MSM was better than that of the conventional MSMs.

**Validation of SPE-HPLC method**

DES-MIP was assessed as an SPE sorbent for the purification of levofloxacin, and the method under the optimized protocols was validated. The calibration curves were in the range of 0.1 – 500.0 \(\mu\)g/mL for levofloxacin. The regression equation is as follows:
\[ Y = 0.185X + 4.2176 \quad (R^2 = 0.9995) \]

\( X \) is peak area; \( Y \) is concentration. Based on a signal-to-noise ratio of 3 and 10, the limit of detection (LOD) and limit of quantitation (LOQ) of the method for levofloxacin were 0.01 and 0.03 \( \mu \)g/mL, respectively. The precision and accuracy were assessed by analyzing five replicates of spiked samples at three spiked levels on the same day and three different days \((n = 3)\); intra-assay and inter-assay precision, which is expressed as the relative standard deviation (RSD), were 2.6 and 4.0%, respectively. The method recoveries ranged from 97.2 - 100.2% for DES-MIP and 88.3 - 91.5% for MIP when the concentrations were 5, 25 and 50 \( \mu \)g/mL at the three levels, as shown in Table 4. The RSD (relative standard deviation) of DES-MIP and MIP of the intra-day and inter-day determination was less than 1.8 and 1.7%, respectively.

**Purification of levofloxacin from a green bean extract**

These six materials were used to purify levofloxacin from a green bean extract by SPE. The green bean extract was the interferent in this purification work (Fig. 3). In Figs. 8 and 9, the \( C_{18} \) sorbent could not retain levofloxacin effectively. Two MSMs could remove the interferent well, but the recovery of levofloxacin was no better than that with the polymers. Although NIP and MIP showed good selectivity, the ability to remove impurities is not as good as the MSMs. DES-MIP showed the highest selectivity recovery (95.2%) for levofloxacin, and could remove the interferent effectively.

| SPE | Spiked/ \( \mu \)g/mL | Intra-day | Inter-day |
|-----|------------------------|-----------|-----------|
|     | Recovery, % | RSD, % | Recovery, % | RSD, % |
| MIP  | 5     | 89.2 | 1.2 | 88.3 | 1.8 |
|      | 25    | 91.1 | 1.6 | 91.5 | 0.8 |
|      | 50    | 88.9 | 1.2 | 90.4 | 1.3 |
| DES-MIP | 5     | 99.8 | 0.7 | 98.2 | 1.2 |
|      | 25    | 99.5 | 1.4 | 100.2 | 1.6 |
|      | 50    | 97.2 | 1.5 | 99.4 | 1.8 |

Table 4 SPE-HPLC method recoveries \((n = 3)\) and RSD values of levofloxacin standard solution

![Fig. 8](image-url)  The recoveries of levofloxacin and the purification chromatogram of green bean extractive with levofloxacin (50 ng/mL) by MSM (a), DES-MSM (b), NIP (c), MIP (d), DES-MIP (e) and \( C_{18} \) sorbent (f). Column, \( C_{18} \) (150 x 4.6 mm i.d.); mobile phase, 0.05 M NaH₂PO₄–ACN (82:18 v/v pH 3); detector, UV (294 mm); injection volume, 10 \( \mu \)L; flow rate, 1 mL/min.
Conclusions

In this study, betaine-based DES was applied in the preparation of MIPs and MSMs. These materials had been doped for SPE packing, and characterized by FT-IR and FE-SEM. The adsorption curves of DES-MIP showed the best molecular recognition ability and binding ability for levofloxacin of all the materials tested. The DES-based materials showed better selective adsorption than the conventional materials. In summary, DES can potentially be extended with versatility to a broad scope of effective drug screening efforts in clinical laboratories.

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References

1. B. Tang and K. H. Row, Monatsh. Chem., 2013, 144, 1427.
2. X. Li and K. H. Row, J. Sep. Sci., 2016, 39, 3505.
3. H. Zhao and G. Baker, J. Chem. Technol. Biotechnol., 2013, 88, 3.
4. A. P. Abbott, G. Capper, D. L. Davies, R. K. Rasheed, and V. Tambyrajah, Chem. Commun., 2003, 70.
5. A. P. Abbott, G. Capper, and S. Gray, ChemPhysChem, 2006, 7, 803.
6. A. L. Demain, Appl. Microbiol. Biotechnol., 1999, 52, 455.
7. M. R. Jo, H. J. Lee, T. S. Lee, K. Park, E. G. Oh, and P. H. Kim, Food Sci. Biotechnol., 2011, 20, 823.
8. B. Huerta, S. Rodríguez-Mozaz, and D. Barceló, Anal. Bioanal. Chem., 2012, 404, 2611.
9. F. J. Schenck and P. S. Callery, J. Chromatogr. A, 1998, 812, 99.
10. S. L. Lin, C. Y. Lo, and M. R. Fuh, J. Chromatogr. A, 2012, 1246, 40.
11. C. I. Kang, J. H. Song, S. H. Kim, D. R. Chung, K. R. Peck, T. M. So, and P. R. Hsueh, Eur. J. Clin. Microbiol. Infect. Dis., 2014, 33, 55.
12. T. Kemmei, S. Kodama, H. Fujishima, A. Yamamoto, Y. Inoue, and K. Hayakawa, Anal. Chim. Acta, 2012, 709, 54.
13. M. A. Soliman, J. A. Pedersen, and I. H. (Mel) Suffet, J. Chromatogr. A, 2004, 1029, 223.
14. H. B. Lee, T. E. Peart, and K. L. E. Kaiser, J. Chromatogr. A, 1996, 738, 91.
15. M. Sillanp, J. Sorvari, and M. L. Sihvonen, Chromatographia, 1996, 42, 578.
16. T. Muhammad, L. Cui, W. Jide, E. V. Piletska, A. R. Guerrero, and S. A. Piletsky, Anal. Chim. Acta, 2012, 709, 98.
17. N. Liang, P. Huang, X. Hou, Z. Li, L. Tao, and L. Zhao, Anal. Bioanal. Chem., 2016, 408, 1701.
18. X. Hu, Q. Cai, Y. Fan, T. Ye, Y. Cao, and C. Guo, J. Chromatogr. A, 2012, 1219, 39.
19. F. Gosetti, U. Chiuminatto, E. Mazzucco, E. Robotti, G. Calabrese, M. C. Gennaro, and E. Marengo, J. Chromatogr. A, 2011, 1218, 6308.
20. P. Spégel, L. Schweitz, and S. Nilsson, Anal. Bioanal. Chem., 2002, 372, 37.
21. I. A. Nicholls, O. Ramstrom, and K. Mosbach, J. Chromatogr. A, 1995, 691, 349.
22. X. Li and K. H. Row, Chromatographia, 2015, 78, 1321.
23. G. Li, W. Wang, Q. Wang, and T. Zhu, J. Chromatogr. Sci., 2016, 54, 271.
24. X. Li, Y. R. Lee, and K. H. Row, Chromatographia, 2016, 79, 375.
25. W. Tang, G. Li, K. H. Row, and T. Zhu, Talanta, 2016, 152, 1.
26. H. Yan, M. Wang, Y. Han, F. Qiao, and K. H. Row, J. Chromatogr. A, 2014, 1346, 16.

Fig. 9 The recoveries of levofloxacin.