Clozapine Recognition via Molecularly Imprinted Polymers; Bulk Polymerization Versus Precipitation Method

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ABSTRACT: Two clozapine (CLZ) imprinted polymers were prepared by bulk and precipitation methods. Methacrylic acid and ethylene glycol dimethacrylate (EDMA) were used as functional and crosslinker monomers, respectively. The mean diameter and particle size distribution of the imprinted (P-MIP) and nonimprinted (P-NIP) particles obtained in precipitation method were examined. A conventional batch-adsorption test was applied for characterization of CLZ–polymer interaction. Dissociation constant (K_D) and maximum binding sites (B_{max}) were calculated using Scatchard analysis. To evaluate the recognition properties of polymers, phenytoin (PTN) binding to each polymer was also studied and compared to CLZ. The imprinting factor (IF) and selectivity factor (α) were also determined for each polymer. Average diameter and polydispersity of P-MIP were 925 nm and 0.17, respectively. The data for P-NIP were 1.05 μm and 0.18. The K_D, IF, and α values calculated for P-MIP were 0.45 μM, 3.26, and 17.43, respectively. The data for imprinted polymer, prepared by bulk polymerization (B-MIP), were 14.5 μM, 1.95, and 3.67. These results demonstrated that precipitation polymerization is a more convenient, more effective, and more reproducible method than bulk polymerization for the synthesis of uniformly sized micron and submicron-imprinted polymer particles. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 3590–3595, 2011

Key words: molecular imprinting; molecular recognition; adsorption; bulk polymerization; precipitation polymerization; clozapine

INTRODUCTION

Molecularly imprinted polymers (MIPs) are tailor-made materials with high selectivity for a template and have been intensively studied in the recent years.[1,2] MIPs have been exploited for several applications such as chemical sensors,[3,4] capillary electrochromatography,[5,6] enantiomer separations,[7,8] artificial antibodies,[9–11] HPLC stationary phases,[1,12,13] and solid-phase extraction.[14–16] In general, MIPs are prepared by bulk polymerization method. However, the obtained polymer needs to be crushed, ground, and sieved, which is a laborious and time-consuming procedure. Furthermore, particles possess irregular shape and heterogeneous binding site distribution.[1,17] Thus, to avoid these drawbacks and optimize the production of MIPs, several strategies have been proposed for the preparation of the spherical MIP particles, such as suspension polymerization,[18,19] multistep swelling polymerization,[20] emulsion polymerization,[21] and precipitation polymerization.[1,17,22-25] In abovementioned methods, water is usually used as dispersive solvent. In comparison with organic solvents, the noncovalent interactions between functional monomers and template decreases in water during polymerization procedure. Precipitation polymerization is an effective and completely surfactant-free method for the production of micro and nanospheres in organic solvents, which are performed in dilute solutions.[1] Such small particles are used for capillary electrophoresis,[26] radioligand-binding assays,[27] and chemical sensors.[25] The crucial difference between bulk polymerization and precipitation method is the volume of the solvent. The precipitation method requires larger volumes of the medium in polymerization than the bulk method.[28] The submicron particles are less suitable for HPLC and SPE applications due to the high backpressure of the column packed with such small particulates.[1]

Clozapine (CLZ) is an atypical antipsychotic with a low potential for inducing extrapyramidal side

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effects, used primarily in the management of patients with schizophrenia resistant to conventional neuroleptics.[29,30] Although CLZ is effective in up to 50–60% of these patients, its wider use has been limited by the risk of agranulocytosis, which makes frequent hematological monitoring necessity.[29,31,32] On the other hand, regarding plasma levels related to therapeutic effects, optimal concentrations of CLZ in human serum are 350–600 ng/mL.[33] Because of wide pharmacokinetic variability, it has been suggested that monitoring plasma CLZ concentrations might play a useful role in clinical management.[29] In our previous work, we prepared CLZ-imprinted polymers by bulk polymerization method.[34] The polymers were characterized, and the polymerization procedure was optimized based on porogen and template/monomer ratio. In the present study, we compared bulk polymerization to precipitation method in preparation of CLZ-imprinted polymer. The polymers were characterized by particle size analysis and conventional batch adsorption experiments, and their template-binding properties were studied in comparison with blank nonimprinted polymers (B-NIPs). Dissociation constant (K_D) and maximum binding sites (B_{max}) for CLZ in each gram MIPs were determined using the Scatchard analysis method. The imprinting factor (IF) and selectivity factor (α) were also calculated for MIPs.

EXPERIMENTAL

Chemicals and materials

CLZ and phenytoin (PTN) were purchased from Tehran Chemie (Tehran, Iran), and methacrylic acid (MAA), and ethylene glycol dimethacrylate (EDMA) were obtained from Sigma-Aldrich (Milwaukee, USA). 2,2′-Azobis-isobutyronitrile (AIBN) was obtained from Acros (Geel, Belgium). All solvents used such as acetonitrile (ACN), tetrahydrofuran (THF), and methanol; acetic acid and trifluoroacetic acid were of HPLC grade. The structures of chemicals used in this study are presented in Figure 1.

Preparation of bulk polymers

CLZ (0.5 mmol) and MAA (2.5 mmol) were dissolved in chloroform (5 mL) in a glass tube. The solution was kept at 4°C for 1 h. EDMA (12.5 mmol) and AIBN (10 mg) were then added to the solution. After purging the solution with nitrogen gas in an ice bath for 5 min, the tube was sealed under nitrogen and heated, in an oven, at 60°C for 22 h. The resultant monolith-imprinted polymer (B-MIP) was crushed, ground, and sieved. The particles less than 25 μm were collected. The template was removed by washing with acetic acid–methanol (2 : 8, v/v). After

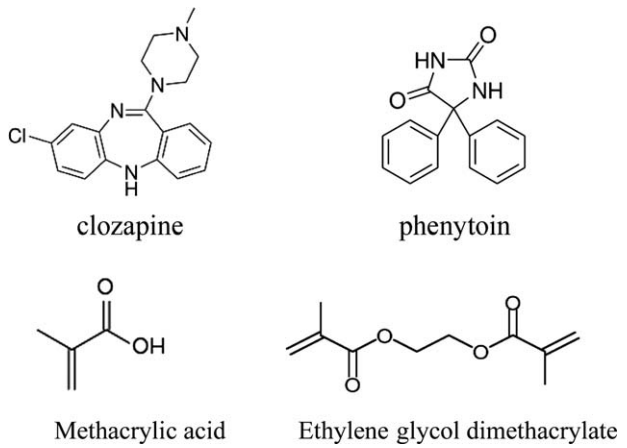

Figure 1 Structures of chemicals used in this study.

centrifugation at 3000 rpm for 10 min, the supernatant was discarded. This procedure was repeated until the CLZ could not be detected by HPLC in supernatant. Finally, the particulates were washed with methanol several times and dried for 24 h in an oven at 45°C. A blank B-NIP was synthesized, in the absence of CLZ, following the same procedure described earlier.

Preparation of polymer particles by precipitation method

The template (0.5 mmol) and MAA (2.5 mmol) were dissolved in chloroform (50 mL) in a 250-mL conical flask and was kept at 4°C for 1 h. Then, EDMA (12.5 mmol) and AIBN (10 mg) were added to the solution. The total solution was sparged with nitrogen gas in an ice bath for 5 min to remove oxygen. Finally, the flask was sealed and heated at 60°C for 22 h. The obtained imprinted particles (P-MIP) were filtered with a polypropylene membrane filter (porosity 0.45 μm; Teknokroma, Spain) and washed with acetic acid-methanol (2 : 8, v/v) successively. The washing procedure was repeated until the template could not be detected by HPLC in the extraction solvent. Finally, the particulates were washed with methanol several times and dried for 24 h in an oven at 45°C. Nonimprinted particles (P-NIP) were prepared in the same way in the absence of CLZ.

Size analysis of polymer particles

The mean diameter and particle size distribution of the P-MIP and P-NIP were examined using THF as dispersive solvent. The desired amounts of polymer particles were suspended in THF and analyzed by a Malvern Zetasizer nano ZS (Malvern, UK). Average diameter of particles and polydispersity index was reported.

Batch adsorption experiments

Dry polymer (10 mg) was incubated in a glass tube in 2 mL ACN with CLZ (2–100 μg/mL). The tubes were shaken on a horizontal shaker at room temperature for 24 h and then centrifuged at 13,000 rpm for 5 min. The concentration of CLZ in supernatant was measured by HPLC. From the known initial concentration of CLZ in the solution and its final concentration after 24 h, the number of moles of CLZ adsorbed on the polymer and the number of moles of free CLZ after equilibration could be computed.[15] Each test was carried out four times, and mean ± SD was reported.

The IF was calculated according to eq. (1):

$$IF = \frac{K_{MIP}}{K_{NIP}} \quad (1)$$

where K was the partition coefficient for each polymer and calculated in eq. (2):

$$K = \frac{bound\ CLZ/gpolymer}{[Free]} \quad (2)$$

where [*Free*] was the free unbound CLZ in solution after equilibrium and *bound CLZ* was the amount of CLZ bound to per gram dry polymer.

When the CLZ concentration was varied in solution, a Scatchard plot was constructed according to the eq. (3):

$$\frac{Bound}{[Free]} = -\frac{Bound}{K_D} + \frac{B_{max}}{K_D} \quad (3)$$

where *Bound* was the amount of CLZ bound to polymer at equilibrium; [*Free*] was the CLZ concentration at equilibrium; B_{max} was the maximum binding sites and K_D was the dissociation constant. The value of K_D and B_{max} could be calculated from the slope and intercept of straight line derived from a plot of $\frac{Bound}{[Free]}$ versus *Bound*.

To study the recognition properties of CLZ-imprinted polymers, 10 mg of dry polymers was incubated in ACN with 0.1 mg/mL PTN or CLZ at room temperature for 24 h and the number of moles of CLZ or PTN adsorbed on the polymer was calculated as described before. The selectivity factor (α) of each polymer was calculated according to eq. (4):

$$\alpha = \frac{K_{CLZ}}{K_{PTN}} \quad (4)$$

where K_{CLZ} was the partition coefficient of CLZ for each polymer and K_{PTN} was the partition coefficient of PTN for the same polymer.

HPLC analysis

Chromatographic determination of compounds was done on a Younglin (South Korea) Acme 9000 system, consisting of SP930D solvent delivery module, SDV50A Solvent Mixing Vacuum Degasser, Column Oven CTS30, UV730 Dual Wavelength UV/VIS detector set to 254 nm and ODSA C18 (4.6 × 250 mm, 5 μm) column. The data analysis was done by Autochro-3000 software. The injection volume was 20 μL, the flow rate was 0.8 mL/min, and the column temperature was fixed at 30°C. An isocratic method was used for the analysis of compounds. The mobile-phase composition was ACN-acetic acid 2% (50/50, v/v).

RESULTS AND DISCUSSION

Preparation of imprinted polymers

The precipitation method is a simpler and more convenient than bulk polymerization for preparing uniformly sized particles.[35] The polymer particles are obtained without crushing and sieving. Thus, the resultant particles are spherical and more uniformly sized in comparison with polymers obtained in bulk polymerization. The functional monomer used in this study was MAA. Because of the presence of amine groups in CLZ structure, it can easily bind this acidic monomer. In our previous study, we have successfully used MAA as a functional monomer for the preparation of lamotrigine-imprinted polymer. Lamotrigine is an anticonvulsant drug with two amine groups in its structure.[15] Therefore, we used MAA as functional monomer to promote noncovalent binding with the template molecule to obtain good selectivity and reversibility. Usually, the template/monomer ratio of less than 1 is used for the preparation of optimized imprinted polymer.[36] In our previous work, we practically found that the best CLZ/MAA/EDMA ratio is 1 : 5 : 25, and the best polymerization solvent is chloroform for the synthesis of imprinted polymer in bulk method.[34] We have also used this ratio for precipitation method and studied the imprinted polymer in comparison with imprinted one prepared in bulk polymerization.

Size analysis of particles

The crushed bulk polymers usually provide irregular shaped particles, whereas precipitation polymerization results in a nearly monodisperse population of spherical particles.[22] In this study, bulk materials were crushed and sieved for obtaining particles with dimensions <25 μm, whereas the P-MIP particles obtained in precipitation method exhibit an average diameter and polydispersity index of around 925 nm and 0.17, respectively. The data for P-NIP was 1.05 μm and 0.18. Other researchers have used precipitation method to obtain uniformly sized imprinted

polymers. In a study, nicotine-imprinted microspheres were about 4 μm in diameter.[37] In other studies, imprinted particle size values obtained in precipitation polymerization ranged from 400 nm to 5 μm.[1,17,22,38] Small particle size and polydispersity index in our study showed that precipitation polymerization is a suitable method for the preparation of submicron-imprinted polymers with narrow size distribution.

Batch adsorption measurements

In other studies, the conventional batch adsorption method has been used for the characterization of binding properties of imprinted and NIPs.[15] In this study, 10 mg of polymers was incubated in 2 mL ACN, with CLZ (2–100 μg/mL) at room temperature for 24 h. Figure 2 shows that CLZ binding to both MIPs was higher than their NIPs in all concentrations. But P-MIP had a higher affinity for CLZ in comparison with B-MIP. The calculated IF for P-MIP and B-MIP, in 100 μg/mL, was 3.26 and 1.95, respectively. Thus, the imprinting procedure was successfully happened in both polymerization methods, but the precipitation method was more effective in preparation of specific binding sites than bulk polymerization procedure.

Scatchard analysis

Figure 3 showed the Scatchard plot of CLZ binding to MIPs. From Scatchard plot [Fig. 3(a)], one dissociation constant (K_D) could be discerned for P-MIP, and Figure 3(b) showed that two K_Ds (one for high affinity and another for low-affinity-binding sites) could be calculated for B-MIP polymer. These data indicated that binding sites in P-MIP are more homogeneous than the binding sites in B-MIP. On the other hand, K_D and B_{max} values of binding sites of P-MIP were 0.45 μM and 20.6 μmol/g polymer, respectively, whereas the values for higher affinity binding sites in B-MIP were 14.5 μM and 12.78 μmol/g polymer. The K_D value obtained in precipitation method was 32 times less than K_D in bulk polymerization. This data demonstrated a significantly higher affinity of P-MIP for CLZ than B-MIP. In a study, calculated K_D values of a series of MIPs prepared in precipitation polymerization for cinchonidine ranged from 5.5 to 14.9 μM.[39] The K_D value (0.45 μM) obtained in this work was 12.2 times less than the K_D value of optimized cinchonidine MIP. It meant that CLZ binding to P-MIP was significantly stronger than cinchonidine binding to its prepared MIP. Therefore, the preparation of selective binding sites for CLZ using this method could be more successful than that for cinchonidine. The main reason for this result could be the stronger interaction of

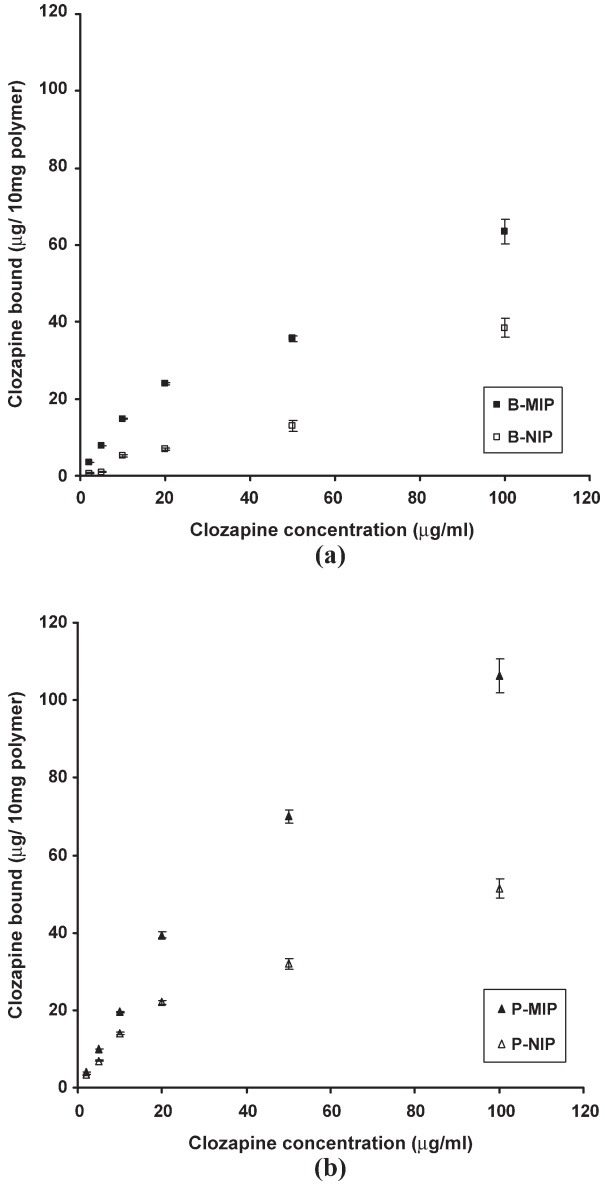

Figure 2 CLZ binding to polymers. Ten milligrams of each polymer were incubated in 2 mL ACN with CLZ. Polymers obtained in bulk method (a) and in precipitation polymerization (b).

MAA with CLZ, via hydrogen bonds, in comparison with cinchonidine during polymerization procedure. Its findings indicated that precipitation method could successfully prepare homogeneous binding sites with a high affinity for CLZ.

Recognition properties of polymers

PTN is an antiepileptic agent, and the selectivity of the MIP could help in recognizing the CLZ from other drugs. Both CLZ and PTN have H-bond donor and acceptor in their structure. Thus, the noncovalent interactions could effectively take place between MAA and these drugs. Another factor in binding to

MIPs is the spatial structure of the molecule. Their structures are completely different, and this could be the main reason for better adsorption of CLZ to MIPs in comparison with PTN. Because of their different spatial structures, PTN was selected as a chemical for studying the selectivity of CLZ-imprinted polymers. To study recognition properties of CLZ-imprinted polymers, 10 mg of polymers was incubated, in 2 mL ACN, with pheytoin (0.1 mg/mL) at room temperature for 24 h. Amount of bound PTN was calculated after equilibration as described before. The average data of PTN binding to P-NIP and B-NIP was a little more than P-MIP and B-MIP.

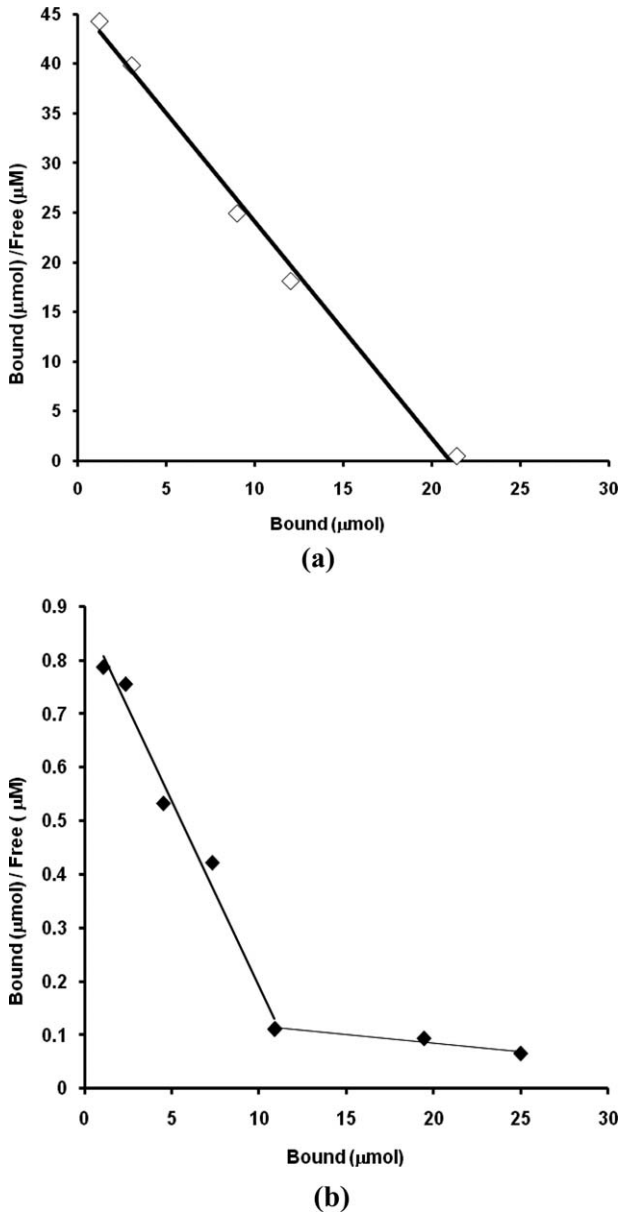

Figure 3 Scatchard plot of CLZ–polymer interaction. Ten milligrams of P-MIP (a) or B-MIP (b) were incubated with different concentrations of CLZ in ACN at room temperature.

TABLE I
Selectivity Factor of Each Polymer for CLZ

Polymer	α (Selectivity Factor)
B–MIP	3.67
B–NIP	0.87
P–MIP	17.43
P–NIP	0.86

These differences between MIPs and their NIPs in binding PTN were not statistically significant ($P > 0.05$). On the other hand, binding of PTN to P-MIP was less than B-MIP. The selectivity factors of polymers were calculated as described before and presented in Table I. The data demonstrated that the selectivity of P-MIP was 4.75 times more than B-MIP ($P < 0.001$). Thus, imprinted polymer synthesized in precipitation polymerization had more selective binding properties for CLZ than B-MIP prepared in bulk polymerization method. Therefore, monodisperse submicron-imprinted particles obtained in precipitation method had superior selective properties for CLZ.

CONCLUSIONS

In this study, two CLZ-imprinted polymers were prepared by bulk and precipitation polymerization methods. The mean diameter and size distribution of polymer particles obtained in precipitation method were examined. Binding properties of polymers were studied in a conventional batch adsorption test. Dissociation constant and maximum binding sites of CLZ–polymer interactions were determined using Scatchard analysis. Affinity of each polymer for PTN was studied and compared to CLZ. IF and selectivity factor were also determined for each polymer. Our data indicated that precipitation method was more convenient, more effective, and more reproducible than bulk polymerization. In comparison with bulk polymer, monodisperse submicron-imprinted particles obtained in precipitation method had superior selective properties for CLZ with significantly higher IF and binding capacity.

References

1. Lai, J. P.; Yang, M. L.; Niessner, R.; Knopp, D. Anal Bioanal Chem 2007, 389, 405.
2. Hart, B. R.; Shea, K. J. J Am Chem Soc 2001, 123, 2072.
3. Blanco-Lopez, M. C.; Lobo-Castanon, M. J.; Miranda-Ordieres, A. J.; Tunon-Blanco, P. Anal Bioanal Chem 2003, 377, 257.
4. Wang, P.; Hu, W.; Su, W. Anal Chim Acta 2008, 615, 54.
5. Nilsson, J.; Spegel, P.; Nilsson, S. J Chromatogr B Analyt Technol Biomed Life Sci 2004, 804, 3.
6. Lin, J. M.; Nakagama, T.; Uchiyama, K.; Hobo, T. J Pharm Biomed Anal 1997, 15, 1351.
7. Andersson, L. I.; Mosbach, K. J Chromatogr 1990, 516, 313.

8. Lu, Y.; Li, C.; Liu, X.; Huang, W. J Chromatogr A 2002, 950, 89.
9. Ramstrom, O.; Yu, C.; Mosbach, K. J Mol Recognit 1996, 9, 691.
10. Ou, J.; Tang, S.; Zou, H. J Sep Sci 2005, 28, 2282.
11. Suedee, R.; Srichana, T.; Rattananont, T. Drug Deliv 2002, 9, 19.
12. Mayes, A. G.; Andersson, L. I.; Mosbach, K. Anal Biochem 1994, 222, 483.
13. Kempe, M.; Fischer, L.; Mosbach, K. J Mol Recognit 1993, 6, 25.
14. Alexiadou, D. K.; Maragou, N. C.; Thomaidis, N. S.; Theodoridis, G. A.; Koupparis, M. A. J Sep Sci 2008, 31, 2272.
15. Mohajeri, S. A.; Ebrahimi, S. A. J Sep Sci 2008, 31, 3595.
16. Mohajeri, S. A.; Hosseinzadeh, H.; Keyhanfar, F.; Aghamohammadian, J. J Sep Sci 2010, 33, 2302.
17. Jin, Y.; Jiang, M.; Shi, Y.; Lin, Y.; Peng, Y.; Dai, K.; Lu, B. Anal Chim Acta 2008, 612, 105.
18. Hu, S. G.; Wang, S. W.; He, X. W. Analyst 2003, 128, 1485.
19. Baggiani, C.; Anfossi, L.; Baravalle, P.; Giovannoli, C.; Giraudi, G.; Barolo, C.; Viscardi, G. J Sep Sci 2009, 32, 3292.
20. Haginaka, J.; Sakai, Y. J Pharm Biomed Anal 2000, 22, 899.
21. Curcio, P.; Zandanel, C.; Wagner, A.; Mioskowski, C.; Baati, R. Macromol Biosci 2009, 9, 596.
22. Wei, S.; Molinelli, A.; Mizaikoff, B. Biosens Bioelectron 1943, 2006, 21.
23. Yan, S.; Fang, Y.; Gao, Z. Biosens Bioelectron 2007, 22, 1087.
24. Sun, H.; Fung, Y. Anal Chim Acta 2006, 576, 67.
25. Zhang, Z.; Liu, Y.; Long, Y.; Nie, L.; Yao, S. Anal Sci 2004, 20, 291.
26. De Boer, T.; Mol, R.; De Zeeuw, R. A.; De Jong, G. J.; Sherrington, D. C.; Cormack, P. A.; Ensing, K. Electrophoresis 2002, 23, 1296.
27. Bompart, M.; Gheber, L. A.; De Wilde, Y.; Haupt, K. Biosens Bioelectron 2009, 25, 568.
28. Haginaka, J. J Chromatogr B Analyt Technol Biomed Life Sci 2008, 866, 3.
29. Spina, E.; Avenoso, A.; Facciola, G.; Scordo, M. G.; Ancione, M.; Madia, A. G.; Ventimiglia, A.; Perucca, E. Psychopharmacology (Berl) 2000, 148, 83.
30. Baldessarini, R.; J; Frankenburg, F. R. N Engl J Med 1991, 324, 746.
31. Alvir, J. M.; Lieberman, J. A.; Safferman, A. Z.; Schwimmer, J. L.; Schaaf, J. A. N Engl J Med 1993, 329, 162.
32. Lieberman, J. A.; Alvir, J. M. Drug Saf 1992, 7(Suppl 1),1.
33. Hiemke, C.; Dragicevic, A.; Grunder, G.; Hatter, S.; Sachse, J.; Vernaleken, I.; Muller, M. J. Ther Drug Monit 2004, 26, 156.
34. Mohajeri, S. A.; Karimi, G.; Khansari, M. R. Anal Chim Acta 2010, 683, 143.
35. Tang, K.; Chen, S.; Gu, X.; Wang, H.; Dai, J.; Tang, J. Anal Chim Acta 2008, 614, 112.
36. Spivak, D. A. Adv Drug Deliv Rev 2005, 57, 1779.
37. Sambe, H.; Hoshina, K.; Moaddel, R.; Wainer, I. W.; Haginaka, J. J Chromatogr A 2006, 1134, 88.
38. Wang, L.; Zhang, Z.; Huang, L. Anal Bioanal Chem 2008, 390, 1431.
39. Liu, Y.; Hoshina, K.; Haginaka, J. Talanta 2010, 80, 1713.