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Preparation of Molecularly Imprinted Polymer for Chiral Recognition of Racemic 1,1′-Binaphthalene-2,2′-Diamine by HPLC

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Summary. Molecularly imprinted polymers (MIPs) were synthesized by imprinting a new template—*S*-(-)-1,1′-binaphthalene-2,2′-diamine (*S*-DABN) and applied as chiral stationary phases for chiral separation of DABN racemates by high-performance liquid chromatography (HPLC). The influence of some key factors on the chiral recognition ability of MIPs, such as the type of functional monomers and porogen and the molar ratio of template to monomer, was systematically investigated. The chromatographic conditions, such as mobile phase composition, sample loading, and flow rate, were also measured. The chiral separation for DABN racemates under the optimum chromatographic conditions by using MIP chiral stationary phase (CSP) of P3, prepared with the S-DABN/MAA ratio = 1/4 and used acetonitrile (2 mL) and chloroform (4 mL) as porogen, showed the highest separation factor (2.14). Frontal analysis was used to evaluate affinity to the target molecule of MIPs. The binding sites (B_t) of MIPs and dissociation constant (K_d) were estimated as 4.56 μmol g^{-1} and 1.40 mmol L^{-1}, respectively. In comparison with the previous studies, this approach had the advantages, such as the higher separation factor, easy preparation, and cost-effectiveness, it not only has the value for research but also has a potential in industrial application.

Key Words: 1,1′-binaphthalene-2,2′-diamine, chiral recognition, HPLC, molecularly imprinted polymers

Introduction

1,1′-Binaphthalene-2,2′-diamine (DABN) *(Fig. 1)*, as a kind of expensive chiral compound, has been widely used in many metal-catalyzed [1-3], organo-catalyzed asymmetric reactions [4, 5]. Optically pure DABN is usually applied in the synthesis of asymmetric catalyst [6], which is one of the most challenging subjects for organic reaction [7]. However, the DABN enantiomer is very expensive due to the difficulty in production. Therefore, it is necessary to develop effective and inexpensive treatment methods to obtain pure enantiomer of DABN. Nishi and his colleagues [8] explored the chiral separation of DABN by capillary electrophoresis utilizing crown ethers and got the separation factor as 1.33. Monser et al. [9] synthesized chiral substituted amide, which was strongly adsorbed on to porous graphitic carbon to produce a novel type of carbon-based chiral stationary phase for separation

racemic DABN and attained the separation factor as 1.10. It is clear that these results are unsatisfactory. However, few research works focused on the study about chiral separation of racemic DABN so far. Further studies are still essential to investigate the efficient procedure to obtain single enantiomer of DABN.

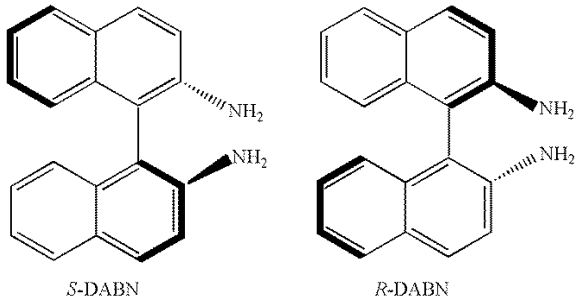

Fig. 1. The chemical structure of DABN

Molecularly imprinted technique (MIT) provides a new choice for separation of racemic DABN. In some reports about MIT, polymer materials were prepared in this way: the functional monomers initially formed complexes with the template molecules, and then the functional monomers copolymerized with the cross-linker. After polymerization, the template was removed, and three-dimensional structure cavities, which were complementary in terms of size, shape, and functional groups of the templates, were generated inside the polymers, which were capable of recognizing and specifically binding the target molecular [10, 11]. MIT has the prominent advantages of predetermination, recognition, and practicability [12]. Hence, it has attracted extensive research interest [13-17]. It was also applied in chiral recognition of racemic DABN, but the research about chiral recognition of racemic DABN by MIT is rare. Dobashi et al. [18] used the prepared molecularly imprinted polymers (MIPs) to chiral separation DABN racemates by bulk polymerization. In their report, they merely utilized chloroform as solvent, they did not adopt the method of pre-polymerization during preparation process, and they employed the usual slurry packing to fill polymer particles. Finally, they got a separation factor as 1.30 for DABN racemates. However, in our study, we used the mixed solvent of acetonitrile and chloroform as porogen, utilized pre-polymerization in polymer synthesis, and we made full use of dry pack method. We gained a higher separation factor as 2.14 at last.

In this study, we focused on the preparation of a MIPs chiral stationary phase (CSP) used for high-performance liquid chromatography (HPLC) by dry packing method. Furthermore, the effects of preparation conditions of

MIPs, including the type of functional monomers, porogen, and the molar ratio of template/monomer on chiral separation of DABN were discussed. In addition, the effects of chromatographic conditions, such as mobile phase composition, sample loading, and flow rate on the chiral separation, were also discussed. The MIPs chiral stationary phase we prepared showed higher separation factors than the previous reports. In comparison with previous studies [9, 18], our method was highly efficient, easy to prepare, and cost-effective.

Experimental

Reagents, Materials, and Instrumentation

S-DABN, *R*-DABN *(Fig. 1)* were purchased from Sichuan Tiancai Fine Chemical Company (China) and used as received. Methacrylic acid (MAA, Shenyang Chemical Reagents, 98%) and 2-vinylpyridine (2-VP, Acros Organics Factory) were distilled under reduced pressure to remove inhibitors. Acrylamide (AM, Aladin Chemical Reagents) and 2,2′-azobisisobutyronitrile (AIBN, Tianjin Chemical Reagents) were recrystallized from ethanol. Ethyleneglycol dimethacrylate (EDMA, 500 mL, Hechuang Chemical Reagents, 98.5%) was extracted for three times with 10% aqueous sodium hydroxide and dried over magnesium sulfate. Acetonitrile (ACN) and chloroform ($CHCl_3$) were of HPLC or analytical grade and used as received.

All chromatography measurements were performed by a reversed-phase HPLC system (JASCO, Japan). The ^1H NMR spectra were recorded by AVANCE-500 MHz spectrometer (Bruker, Switzerland).

Preparation of MIPs

The typical progress for preparation of the MIP CSPs was as follows: template molecule (S-DABN，0.5 mmol) and a certain quantity of a functional monomer (AM, 2-VP or MAA) were dissolved in a mixture of acetonitrile and chloroform with different volume ratios (6 mL) in a 100-mL flask. The mixture was degassed by ultrasonic device along with filling of nitrogen for 5 min. The pre-polymerization was carried out at 18°C for 12 h, then the cross-linker (EGDMA, 10 mmol) and the initiator (AIBN, 0.12 mmol) were added into the mixture. After bubbling N_2 for 5 min, the polymerization reaction was carried out in a water bath at 60°C overnight. After that, the obtained polymer monoliths were removed from the flask and crushed by a mortar and pestle to give polymer powders *(Table I)*. The polymer powders were sieved to give polymer particles with particle size <60 μm. The particles were precipitated three times in acetone to remove the fine particles of size <30 μm. Particles of 30–60 μm (0.8 g) were put into a stainless-steel

HPLC column (250 mm × 2.0 mm, i.d.) and packed by dry-packing method to give a HPLC column with MIPs CSP. The other MIP CSPs (P1–P7) were prepared by a similar method. The non-imprinted blank polymer (NIP) was prepared in the same way except the absence of *S*-DABN.

Table I. Effect of functional monomers on the chiral separation

No.	Template[a] (mmol)	Functional monomers[a] (mmol)			Cross-linker[a] (mmol)	Initiator[a] (mmol)	α[b]
	S-DABN	AM	2-VP	MAA	EGDMA	AIBN	
P1	0.5	2	0	0	10	0.12	1.62
P2	0.5	0	2	0	10	0.12	1.37
P3	0.5	0	0	2	10	0.12	2.14

[a]Preparation conditions for MIPs: The template and the functional monomers were dispersed in the mixed porogen (2 mL of acetonitrile and 4 mL of chloroform) at 18°C and standing for 12h. Then, the EGDMA and AIBN were added into the mixture and vibrated at 60°C for 12 h to give the MIPs.

[b]Chromatographic conditions: mobile phase, acetonitrile; flow rate, 0.10 mL min^{-1}; sample loading, 5 μL of 0.10 mg mL^{-1} of racemic DABN.

^{1}H NMR Experiments

A series of samples containing fixed concentrations of *S*-DABN (50 mmol L^{-1}) and functional monomers (200 mmol L^{-1}) in $CDCl_3$ were used to observe the interaction between functional monomers and *S*-DABN. ^{1}H NMR spectra were recorded at room temperature. Tetramethylsilane (TMS) was used as an internal standard. The results are shown in *Fig. 2*.

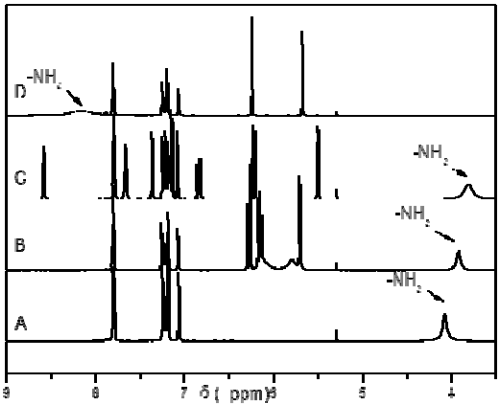

Fig. 2. The ^{1}H NMR spectra of the mixtures of S-DABN and different functional monomers in $CDCl_3$ at 25°C. (A) *S*-DABN; (B) *S*-DABN +AM; (C) *S*-DABN +2-VP; (D) *S*-DABN+MAA

MIPs–HPLC Analysis

The HPLC column was washed to remove the template molecules by using acetonitrile as eluent. A stable baseline was achieved before sample injection. All experiments were carried out at 25°C. UV absorption was detected at wavelength of 254 nm. The sample solution (5 μL) of racemic DABN in acetonitrile (0.1 mg mL^{-1}) was injected. The retention factor (k) was calculated from the equation $k = (t - t_0) / t_0$, where t is the retention time of the analyte, and t_0 is the elution time of the void marker, acetone. The separation factor was calculated from the equation $\alpha = k_2 / k_1$, where k_1 and k_2 are the retention factors of *R*-DABN and *S*-DABN, respectively. The resolution was calculated from the equation $R_s = 2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 are the retention times of *R*-DABN and *S*-DABN, and w_1 and w_2 are the baseline peak widths of *R*-DABN and *S*-DABN, respectively. The results were summarized in *Tables I, II,* and *III*.

Table II. Effect of the molar ratio of template/monomer and porogen on the chiral separation

Code	Template[a] (mmol)	Functional monomer[a] (mmol)	Porogen[a] (mL)		Retention factor[b], k		α[b]
	S-DABN	MAA	ACN	$CHCl_3$	*S*-DABN	*R*-DABN	
P4	0.5	1	2	4	0.94	0.52	1.81
P3	0.5	2	2	4	2.12	0.99	2.14
P5	0.5	3	2	4	1.23	0.83	1.48
P6	0.5	2	4	2	No resolution		–
P7	0.5	2	1	5	No resolution		–

[a]Preparation conditions for MIPs were the same as those in *Table I* except the differences in the amounts of MAA and the compositions of porogen.

[b]Chromatographic conditions were the same as those in *Table I*.

Table III. Effect of mobile phase on the chiral separation of DABN by P3 CSP[a]

Content of acetic acid in acetonitrile	Retention time (min)		Retention factor, k		α	R_s
(%)	*S*-DABN	*R*-DABN	*S*-DABN	*R*-DABN		
0	39.65	25.31	2.12	0.99	2.14	1.04
2	35.45	23.52	1.90	0.93	2.04	0.94
4	31.48	21.72	1.66	0.84	1.98	0.99
6	30.53	21.04	1.72	0.88	1.95	0.93

Chromatographic conditions: flow rate, 0.10 mL min^{-1}; sample loading, 5 μL of 0.10 mg mL^{-1} of racemic DABN.

[a]The MIPs labeled as P3 in *Tables I* and *II* was applied as CSPs in the HPLC column.

Frontal Analysis

Frontal analysis, based on the classical equilibrium theory of adsorption, had been frequently applied to elucidate template-imprinted polymer interactions and evaluate the adsorption energies and saturation capacities in the binding of templates to MIPs [19]. The dissociation constants and the number of binding sites of the MIPs were determined by frontal chromatography. The mobile phase was acetonitrile with a flow rate of 0.10 mL min^{-1}. A series of acetonitrile solution of DABN racemates (5 μL) with different concentrations (0.100, 0.125, 0.150, 0.175, and 0.200 g L^{-1}) were injected at 25°C. The effective number of binding sites (B_t) and the dissociation constant (K_d) were calculated according to this equation [20]:

$$\frac{1}{[S]_0(V-V_0)} = \frac{K_d}{[S]_0 B_t} + \frac{1}{B_t} \quad (1)$$

where $[S]_0$ is the concentration of the analyte, V is the elution volume of the analyte and calculated by peak time of *S*-DABN. V_0 is void volume of the MIPs and calculated by peak time of acetone. The experimental data were processed according to eq. (1), and $1 / ([S]_0(V - V_0))$ was plotted versus $1/[S]_0$ as shown in *Fig. 4*. B_t and K_d are calculated from the intercepts on the ordinate ($1/B_t$) and the abscissa ($-1/K_d$). The results are summarized in *Table IV*.

Table IV. Binding capacity of MIPs and NIP[a]

	B_t (μmol g^{-1})	K_d (mmol L^{-1})	Calibration equation	R^2
MIPs	4.56	1.40	$y = 219.21 + 0.29x$	0.997
NIP	1.90	2.32	$y = 526.62 + 1.22x$	0.999

Chromatographic conditions were the same as those in *Table I*.
[a]The MIPs labeled as P3 in *Tables I* and *II* were used in this HPLC column.

Results and Discussion

The results summarized in *Tables I* and *III* and *Fig. 3* revealed that DABN racemates could be chirally separated by HPLC with the MIP CSP that we prepared in this study. *Tables I* and *II* showed the effects of functional monomers, the molar ratio of template/monomer, and the porogen on the chiral separation of DABN, respectively. *Figure 3* showed the chromatograms of the chiral separation of DABN racemates by P3 CSP.

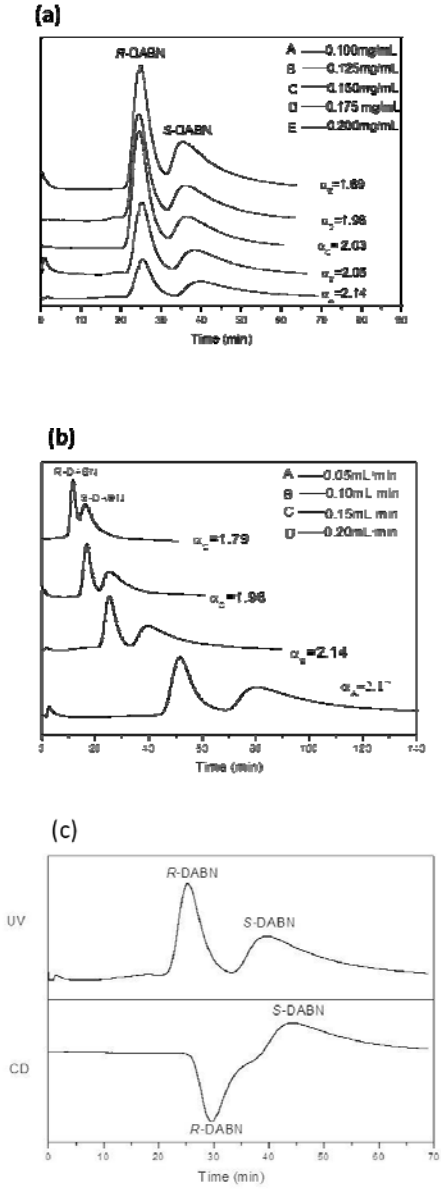

Fig. 3. Chromatograms for chiral separation of DABN racemates by using P3 CSP. (A) Effect of sample loading on chiral separation. (B) Effect of flow rate on chiral separation. (C) Chromatogram for separation of racemic DABN under the optimal chromatographic conditions. The mobile phase was acetonitrile

According to the description in experimental section, the MIPs (P1–P7) were prepared and used as CSPs in HPLC, which assessed the MIPs' performance for chiral separating racemic DABN. As shown in *Table I*, P3 (us-

ing MAA as functional monomer) had the higher separation factor (α = 2.14) than P1 and P2 (α = 1.62 and 1.37, respectively, based on AM and 2-VP). The reason might be caused by the formation of ion pairs between alkaline *S*-DABN molecules and acidic MAA molecules. This hypothesis was proved by the ^{1}H NMR analysis in *Fig. 2.*

As shown in *Fig. 2*, by adding MAA, the chemical shift of –NH_2 proton in *S*-DABN sharply changed (from 4.08 to 8.18ppm), the reason was thought to be caused by the formation of ion pairs between the substituted ammonium cations and the carboxyl anions [21]. However, by adding 2-VP and AM, only small changes of chemical shift of –NH_2 proton were observed (from 4.078 to 3.816 ppm and from 4.078 to 3.925 ppm, respectively). It may be caused by the hydrogen bonds formed between *S*-DABN and 2-VP or AM.

The molar ratio of the template to functional monomer (T/M) has been found to be important to the number and quality of MIP recognition sites [22]. A series of polymers (P3, P4, P5) were synthesized by changing the T/M ratio and applied into the chiral separation of DABN racemate. As shown in *Table II*, the results indicate that P3 CSP prepared with the T/M ratio = 1/4 showed the maximum value of separation factor (α = 2.14), and CSPs P4 prepared with the T/M ratio = 1/2 and P5 prepared with the T/M ratio = 1/6 showed the lower separation factors (α = 1.81 and α = 1.48, respectively). In the cases of CSPs prepared with the T/M ratio = 1/4 (P3, P6, and P7 in *Table II*), only P3 prepared in the porogen of acetonitrile (2 mL) and chloroform (4 mL) showed enantioselectivity for DABN isomers. P6 and P7 prepared in the porogens of acetonitrile (4 mL)/chloroform (2mL) and acetonitrile (1 mL)/chloroform (5 mL) did not show any enantioselectivity.

Previous studies had found that the mobile phase composition had a great influence on the chiral recognition properties of MIPs [23,24]. Therefore, the effect of mobile phase composition on chiral separation was necessary for the investigation. As shown in *Table III*, when the pure acetonitrile was used as the mobile phase, it showed the highest separation factor (α = 2.14). With increasing the proportion of acetic acid in mobile phase (the mixture of acetic acid and acetonitrile) from 0 to 6 vol.%, both separation factor and retention time of *S*-DABN decreased. When the mobile phase contained 6 vol.% of acetic acid, the separation factor decreased to 1.95. With increasing the acetic acid content from 0 to 6 vol.% in the mobile phase, the retention time of *S*-DABN decreased from 39.65 to 30.53 min. It should be caused by the formation of ion pairs between the free carboxyl groups of MIPs and amino groups of *S*-DABN, it also could be supported by the ^{1}H NMR spectroscopy *(Fig. 2)*.

The influences of sample loading and flow rate on the chiral recognition abilities of P3 CSP on DABN enantiomer were investigated. The results are summarized in *Fig. 3.* It was found that the separation factor decreased with increasing sample loading and flow rate. When the sample loading increased from 0.100 to 0.200 mg mL^{-1}, α decreased from 2.14 to 1.89. Similarly, the separation factor decreased from 2.17 to 1.79 with an increase of flow rate from 0.05 to 2.00 mL min^{-1}. As a result, the optimum chromatogram *(Fig. 3C)* was obtained under the chromatographic conditions that the mobile phase: acetonitrile; flow rate: 0.10 mL min^{-1}; sample loading: 0.10 mg mL^{-1}.

Binding capacity

In order to estimate the binding capacity of the MIPs that we prepared, the effective number of binding sites (B_t) and the dissociation constant (K_d) of the MIPs were calculated by the frontal analysis and compared with those of the non-imprinted blank polymer (NIP). The experimental data were processed according to eq. (1), and the plot of $1/[S]_0(V - V_0)$ versus $1/[S]_0$ was given in *Fig. 4.* The calculated B_t and K_d are shown in *Table IV*. The larger the B_t, the better the binding capacity for the target molecule, while a large K_d means low affinity to the analyte. Obviously, the B_t of the MIPs (4.56 μmol g^{-1}) was greater than that of the NIPs (1.90 μmol g^{-1}) *(Table IV)*. The lower K_d of the MIPs (1.40 mmol L^{-1}) than the K_d of the NIPs (2.32 mmol L^{-1}) indicates that the target molecules were embedded into the imprinted caves and could not be readily dissociated from the MIP due to the spatial matching. In conclusion, the MIPs had the higher binding capacity for the imprinted molecules than the NIP.

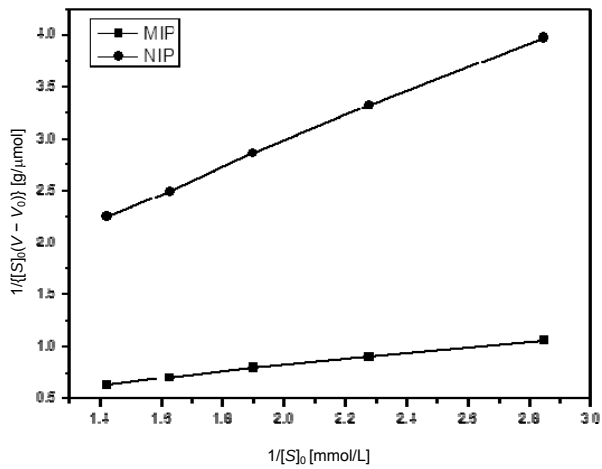

Fig. 4. Plots of $1/[S]_0(V - V_0)$ versus $1/[S]_0$ for the MIPs and NIP

Conclusions

The MIPs imprinted with *S*-DABN have been successfully prepared and used for separating DABN racemates by HPLC. The effects of preparation conditions of the MIPs and the chromatographic conditions for chiral separation of DABN by using the MIPs CSP are discussed in detail. Using the mixed solvents of acetonitrile/chloroform as porogen and the pre-polymerization approach used in preparation of the MIPs afforded the MIPs CSPs having the higher separation factors in chiral separation of DABN racemates. The optimum chromatographic conditions were that the mobile phase: acetonitrile; flow rate: 0.10 mL min^{-1}; sample loading: 0.10 mg mL^{-1}. The chiral separation for DABN racemates under the optimum chromatographic conditions by using MIP CSP of P3 were prepared with the S-DABN/MAA ratio = 1/4 and used the mixture of acetonitrile (2 mL)/chloroform (4 mL) as porogen showed the highest separation factor (2.14), and it was much higher than the separation factor reported previously. In comparison with the previous studies, this approach had the advantages, such as the higher separation factor, easy preparation, and cost-effectiveness, it not only has the value for research but also has a potential in industrial application.

Acknowledgments

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References

[1] C.J. Wang, Z.P. Xu, X. Wang, and H.L. Teng, Tetrahedron, **66**, 3702(2010)
[2] A. Sakakura, K. Suzuki, K. Nakano, and K. Ishihara, Adv. Synth. Catal., **348**, 2457 (2006)
[3] R.K. Rao, A.B. Naidu, E. A. Jaseer, and G. Sekar , Tetrahedron, **65**, 4619 (2009)
[4] G. Guillena, M.C. Hita, C. Najera, and S.F. Viozquez, J. Org. Chem., **73**, 5933 (2008)
[5] A. Sakakura, K. Suzuki, K. Nakano, and K. Ishihara, Org. Lett., **8**, 2229 (2006)
[6] K. Mikami, K. Aikawa, and Y. Yusa, J. Org. Chem., **4**, 95 (2002)
[7] K. Mikami, H. Kakuno, and K. Aikawa, Angew. Chem., **117,** 7423 (2005)
[8] H. Nishi, K. Nakamura, H. Nakai, and T. Sato, J. Chromatogr., A, **757**, 225 (1997)
[9] L.I. Monser, G.M. Greenway, and D.F. Ewing, J. Tetrahedron: Asymmetry, **7**, 1189 (1996)
[10] K. Mosbach, Trends Biochem. Sci., **19**, 9 (1994)
[11] G., Wulff, Angew. Chem. Int. Ed., **34**, 1812 (1995)

[12] T.W. Tan (Ed) Handbook of Molecular Imprinting Technique and Application, Beijing, 2010, p.12
[13] M. Kempe and K. Mosbach, Tetrahedron Lett., **36**, 3563 (1995)
[14] M. Kempe and K., Mosbach, J. Chromatogr., A, **694**, 3 (1995)
[15] L.I. Andersons and K., Mosbach, J. Chromatogr. A, **516**, 313 (1990)
[16] K. Mosbach and O. Ramström, Nat. Biotechnol., **14**, 163 (1996)
[17] Ch.-Y. Hung and Ch.-Ch. Hwang, Acta Chromatogr., **18**, 106 (2007)
[18] A. Dobashi, S. Nishida, K. Kurata, and M. Hamada, Anal. Sci., **18**, 35 (2002)
[19] O. Liseca, P. Hugob, and A. Seidel-Morgenstern, J. Chromatogr., A, **908**, 19 (2001)
[20] H. Li, L. Nie, and S. Yao, Chromatographia, **60**, 425 (2004)
[21] J. O'Mahony, A. Molinelli, K. Nolan, M. R. Smyth, and B. Mizaikoff, Biosens. Bioecetron., **20**, 1884 (2005)
[22] H.S. Andersson, J.G. Karisson, S.A. Piletsky, A.-C. Koch-Schmidt, K. Mosbach, and I.A. Nicholls, J. Chromatogr., A, **848**, 39 (1999)
[23] J. Yin, G. Yang, and Y. Chen, J. Chromatogr., A, **1090**, 68 (2005)
[24] J. Ou, L. Kong, C. Pan, X. Su, X. Lei, and H. Zou, J. Chromatogr., A, **1117**, 163 (2006)

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