Studies on the Preparation and Properties of Sol–Gel Molecularly Imprinted Polymer Based on Tetramethoxysilane and Methyltrimethoxysilane for Recognized Sulfonamides

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The synthesis of a molecularly imprinted polymer (MIP) composed of tetramethoxysilane (TMOS) and methyltrimethoxysilane (MTMOS) for recognized sulfonamides by sol–gel process has been investigated. The MIP's preparation conditions, the H₂O/Si molar ratios (*R*), pH value of competition solution, calcination temperature, and the TMOS and MTMOS molar fractions are discussed. Adsorption and selectivity in different pH solutions were determined by competition experiments between the MIP template (sulfamethazine, SMZ) and the analogue (sulfamethoxazole, SMO) using High-Performance Liquid Chromatography (HPLC). The results show that the selectivity of the MIP in competition solution with pH = 7.4 was higher than that with pH = 4.6. In addition, the second stage temperature of calcination, which was carried out at 375 °C, was obtained high selectivity. Moreover, the selectivity of the MIP (10.5) was approximately 3.5 times greater than the non-imprinted polymer (3.72) under the optimum preparation conditions.

KEY WORDS: Molecularly Imprinted Polymer / Sulfonamide / Sol–Gel / Sulfamethazine / Sulfamethoxazole / HPLC /

Molecular recognition is a phenomenon that can be envisaged as the preferential binding of a molecule to a “receptor” with high selectivity over its close structural analogues. This concept has been translated elegantly into the technology of molecular imprinting, which allows specific recognition sites to be formed in synthetic polymers through the use of various templates.^{1–5} Molecular imprinting of synthetic polymers with a specific target molecule can be done if the target resembles the template or imprint molecule used during polymerization and then removed after polymerization. Polymerization occurs when monomers carrying certain functional groups interact with the template and arrange themselves around the template into a ‘frozen’ position.^{6–9} Imprinting has been achieved by three ways; non-covalent, covalent and sacrificial spacer. Non-covalent imprinting relies on the ability of the template molecule to produce one or more strong intermolecular non-covalent interactions with the functional monomers, *e.g.*, H-bonding, electrostatic or π – π interactions. Removal of the template affords a cavity, which is complementary in size shape and functionality to the template molecule, and which contains the recognition site used for recognizing specific molecules. In covalent imprinting, the template-monomer covalent bond is formed before the polymerization of MIP. Polymerization is conducted in the usual manner but template removal and rebinding is achieved by a chemical rather than physical process.¹⁰ In the sacrificial spacer method, the pre-organized or covalent approach employs reversible covalent bonds, usually involving a prior chemical synthesis step to link the precursors to the template or to a structurally similar molecule.⁹ In other words, monomers are covalently linked to the template *via* short spacers. The spacers

are eliminated once the imprinting process is completed, giving room and suitable functional groups for non-covalent interactions with the target molecule.¹¹ Molecularly imprinted polymers have been used as chromatographic stationary phases, artificial antibodies, catalysts, sensors and drug assay tools.^{12,13}

Many common MIPs are synthesized by organic methods, involving polymerization of functional monomers and a crosslinking monomer such as acrylamide, acrylic acid and ethylene glycol dimethacrylate *etc.*^{14–16} The applications of organic MIPs are also restricted to the use of organic solvents for the dissolution of organic polymers or template.¹⁷ The preparation of MIPs in aqueous system is very challenging task because of the interference of water molecules. Non-specific interactions between the template and functional monomers or polymers may also become much weaker or even disappear in aqueous system. Thus, it is of great importance to develop imprinted materials that can be used in aqueous environment. In this study, our laboratory synthesizes a novel inorganic MIPs by the sol–gel process that can be used in aqueous environment.

In the sol–gel process, inorganic siloxane based polymers are formed by acid or alkali catalyzed hydrolysis and condensation of a series of silane monomers. As the inorganic precursors hydrolyze and condense to form siloxane bonds, a sol is formed. With time, these colloidal particles aggregate leading to the formation of a porous, three dimensional network—the gel. Inclusion of a template species results in the formation of imprinted media. The first approach to synthesizing molecular imprinted materials was based on silica gels and traces back to the early 1930s. Polyakov¹⁸ first

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reported that the silica pore structure was influenced by the presence of benzene, toluene or xylene during the drying process. The extent of silica gel adsorption of the different solvent vapors was dependent upon the structure of the solvent present during the drying process. Selectivity was suggested to arise from changes in the silica structure induced by the presence of the particular solvent. Much later, investigations of molecular imprinting technique started anew, and after the 1970s imprinted sol–gel materials have been produced. During the past decade, the sol–gel process has become a convenient approach for the preparation of stable host matrices for the development of molecularly imprinted polymers.¹⁹ He *et al.*²⁰ developed the selective imprinted amino-functionalized silica gel sorbent by combining a surface molecular imprinting technique with a sol–gel process for online solid-phase extraction-HPLC. The method was applied to the determination of three trace sulfonamides in pork and chicken muscle samples. The imprinted functionalized silica gel sorbent showed high affinity, selectivity, fast kinetics, capacity, and good site accessibility for sulfonamides. Su *et al.*²¹ synthesized the sulfamethazine-imprinted polymer on the silica surface *via* quasiliving radical polymerization. The molecularly imprinted polymer layer grafted on the silica surface was constructed by using sulfamethazine as the template, methacrylic acid as the functional monomer and ethylene dimethacrylate as a cross-linker. The sulfamethazine-imprinted polymer was used successfully for the determination of sulfamethazine in milk by HPLC analysis. In addition, the sol–gel molecularly imprinted polymers based on tetraethoxysilane (TEOS) for recognized caffeine has been developed in previous studies of our laboratory.²² In our study, by introducing pore-forming agent, lactic acid, into sol–gel process, the porosity of MIP was enhanced and the performance of MIP was thus improved. And, by introducing base catalyst in the sol–gel process, large pore volume was obtainable, and the caffeine adsorption of MIP was increased. Therefore, the sol–gel process produces extremely versatile polymer materials that have a wide variety of applications in analytical chemistry. The binding sites of material show many advantages including large adsorption, high selectivity, and more accessible sites.^{23,24}

Sulfonamides have been used as antibacterial agents for over 70 years. Sulfonamides are extensively used in veterinary practice for the treatment of various bacterial infections. Because of their use in food producing animals, the risk of occurrence of unwanted residues in edible products exists. The European Community has adopted a maximum residue level (MRL) of 100 mg/kg for sulfonamides in foodstuffs of animal origin.²⁵ Sulfamethazine (SMZ) has been shown to produce thyroid follicular tumors in rodent bioassays,²⁶ and sulfonamides can cause allergic reactions in humans.²⁷ Moreover, the development of antibiotic resistance is a continuing concern.²⁸ Sol–gel molecularly imprinted polymer utilized as artificial separator for SMZ is able to be used for the recognition and analysis of SMZ and its analogues.

The main propose of this article is to prepare a series of the molecularly imprinted polymers based on tetramethoxysilane

and silane coupling agents and to systematically investigate their competition experiments in the MIP for template and analogue. In addition, H₂O/Si molar ratios (*R*), pH value of competition solution, calcination temperature, and the TMOS and MTMOS molar fractions on MIP are also studied.

EXPERIMENTAL

Materials

Tetramethoxysilane (TMOS) (Shin-Etsu Chemical Co. Ltd. Tokyo, Japan) without purification was used as received. Methyltrimethoxysilane (MTMOS) (Shin-Etsu Chemical Co. Ltd. Tokyo, Japan) without purification was used as received. Sulfamethazine (SMZ) (SIGMA Chemical Co. St. Louis, MO) as a template was used as received. Sulfamethoxazole (SMO) (SIGMA Chemical Co. St. Louis, MO) as a structure analogous molecule was used as received. Hydrochloric acid (Scharlau Chemie S.A. Sentmenat, Barcelona) as a menstruum of sulfonamides was used as received. Ammonium hydroxide (TEDIA Co. Inc. Fairfield, USA) as an auxiliary agent to adjust the pH value, and it was used as received.

Preparation of Sol–Gel Molecularly Imprinted Polymer (MIP)

Sulfamethazine was dissolved in deionized water, and then tetramethoxysilane (TMOS) and methyltrimethoxysilane (MTMOS) were added. 0.002 mol of sulfamethazine and 750 μ L of hydrochloric acid were dissolved completely in various quantity of deionized water by stirring the solution at room temperature for 30 min, and then various ratios of tetramethoxysilane (TMOS) and methyltrimethoxysilane (MTMOS) were added to above solution. The sol–gel solution was reacted at 60 °C in the following 2 d. After the reaction was completed, the monoliths were grounded and sieved to obtain particle sizes between 53 and 88 μ m. Next, the template molecule was removed by calcination, and then the MIP was purged with deionized water. A non-imprinted polymer (NIP) was prepared in parallel and under identical conditions but in the absence of the template.

High-Performance Liquid Chromatography (HPLC) Analysis of MIP

Both the sulfamethazine and the structurally analogous molecule (sulfamethoxazole) were dissolved in water to produce a standard solution which contained 100 ppm of sulfamethazine and sulfamethoxazole respectively. The 200 mg MIP was added in 10 mL standard solution and adsorbed at room temperature for 30 min to complete the adsorption. Then, the MIP solution mixtures were centrifuged to obtain the residual solution. The above solution was injected into HPLC to investigate the residual concentration of sulfamethazine and sulfamethoxazole. The adsorption and selectivity (α) of MIP were determined by competition adsorption experiment; the quantity of adsorption (Ad_{SMZ} and Ad_{SMO}) of MIP was obtained by the difference between the original concentration (100 ppm) and the residual concentration. The selectivity (α)

was obtained by comparison the adsorption of the template (Ad_{SMZ}) and analogue (Ad_{SMO}) on MIP. The HPLC analysis was performed by Waters 510 HPLC pump equipped with a SFD UV/vis Detector S32109 (285 nm). Samples were analyzed on a 150 mm $\times$ 4.6 mm 5 μ m Hypersil HS C18 column at room temperature with a flow rate of 1.0 mL/min (mobile phase, water:methanol:acetic acid = 69:28:3, v/v).

Fourier Transform Infrared Spectroscopy (FT/IR) Analysis of the MIP

The Fourier transformed infrared spectra from pressed KBr pellets containing about 1% MIP using a JASCO FT/IR-410 spectrophotometer.

Accelerated Surface Area and Porosimetry (ASAP)

BET surface area and pore structure characteristics were determined by nitrogen adsorption-desorption isotherm used for analysis. Porosity measurements were performed with Micromeritics ASAP 2010 analyzer. All samples were degassed at 120 °C for 24 h before measurement. BET surface area and pore parameters were measured with 55-point full analysis at cryogenic temperature (77.35 K). The micropore surface area and micropore pore volume were obtained by t -plot analysis with the Harkins–Jura equation.

RESULTS AND DISCUSSION

Mechanism of the Sol–Gel Molecularly Imprinted Polymer

The molecular imprint polymers were prepared by adding the sulfamethazine (SMZ) solution dispersed along with tetramethoxysilane (TMOS) and methyltrimethoxysilane (MTMOS) within the sol–gel mixture. The sol–gel process resulted in numerous hydroxyl groups produced after the hydrolysis and condensation of the silicon alkoxides. These hydroxyl groups promoted the interaction (hydrogen-bond) between the imprinted molecule and inorganic polymer matrix.^{29–31} During the formation of molecularly imprinted polymer (MIP), the template (SMZ) was incorporated into the inorganic polymer matrix, and the imprinted cavities and specific recognition sites of the sol–gel molecularly imprinted polymer were then formed after removing the template, water, and methanol. At this time, the water and methanol were removed prior to the template, and the hydroxyl groups of matrix interacted with the template by hydrogen bond to enhance the recognition ability. The schematic diagram of the molecularly imprinted polymer preparation is shown in Figure 1.

Effect of the H₂O/Si Molar Ratios (R) on MIP

In this study, the adsorption of the template (Ad_{SMZ}) and the adsorption of analogue (Ad_{SMO}) were obtained by competition adsorption experiments. The competition experiments using HPLC were measured by various H₂O/Si molar ratios (R) to study the effects of R on adsorption and selectivity. The fundamental properties of the sol–gel molecularly imprinted polymer with various R are shown in Table I. The sample

Figure 1. Schematic diagram of molecularly imprinted polymer preparation.

codes $R_{2.5}$, R_5 , $R_{7.5}$, R_{10} , $R_{12.5}$ and R_{15} represent 2.5, 5, 7.5, 10, 12.5 and 15 of the H₂O/Si molar ratios in the sol–gel mixture, respectively. The results show that the competition experiment for Ad_{SMZ} increased with an increase in the R . In general, the R value must at the least $R = 2$ when the sol–gel mixture reactant was only the TMOS.³² In this system, the quantity of R demand was smaller ($R < 2$) while the MTMOS was added in the sol–gel mixture. Therefore, the large amount of R caused the hydrolysis and condensation reaction more completely, and more the imprinted cavities and specific recognition sites were formed. Accordingly, the more cavities were provided the greater amount of adsorption sites so the Ad_{SMZ} rose with the larger amount of R .

The BET surface area and the average pore diameter were experimented during the Accelerated Surface Area and Porosimetry (ASAP). The BET surface area and average pore diameter of the MIP also increased with increase the R . This was due to the quantity of water having played an important role in the sol–gel process. The water not only hydrolyzed the reactant while reacting, but at the same time it was a product in the condensation reaction, also playing as third party porogens.

Table I. The properties of the sol–gel molecularly imprinted polymers (MIPs) prepared by various H₂O/Si molar ratios (*R*)

Sample codes.	<i>R</i> (H ₂ O/Si)	Ad _{SMZ} (μmol/g)	Ad _{SMO} (μmol/g)	α	<i>f</i>	BET surface Area (m ² /g)	Average pore Diameter (nm)
<i>R</i> 5	5	1.61	1.03	1.56	2.52	365.7	4.40
<i>R</i> 7.5	7.5	1.71	0.70	2.43	4.13	366.5	4.89
<i>R</i> 10	10	1.82	0.60	3.03	5.52	367.7	5.51
<i>R</i> 12.5	12.5	1.83	1.21	1.51	2.76	369.2	5.82
<i>R</i> 15	15	1.92	1.29	1.49	2.86	371.8	6.05

*TMOS/(TMOS + MTMOS) = 0.5, reacted at 60 °C for 2 d, and the pH value of the competition experiment solution was 4.6.

Table II. Characterizations of MIP measured by diverse pH value

Sample codes	pH = 4.6				pH = 7.4			
	Ad _{SMZ} (μmol/g)	Ad _{SMO} (μmol/g)	α	<i>f</i>	Ad _{SMZ} (μmol/g)	Ad _{SMO} (μmol/g)	α	<i>f</i>
<i>R</i> 5	1.61	1.03	1.56	2.52	1.03	0.27	3.81	3.92
<i>R</i> 7.5	1.71	0.70	2.43	4.13	1.27	0.28	4.54	5.77
<i>R</i> 10	1.82	0.60	3.03	5.52	1.30	0.24	5.42	7.05
<i>R</i> 12.5	1.83	1.21	1.51	2.76	1.57	0.39	4.03	6.32
<i>R</i> 15	1.92	1.29	1.49	2.86	1.55	0.42	3.69	5.72

The large amount of water caused the bigger pores and the porous material with the larger BET surface area. On the other hand, the more BET surface area were provided the greater amount of adsorption sites so the Ad_{SMZ} rose with the larger quantity of *R*. But with increasing *R*, the adsorption of analogue, Ad_{SMO} was decreased firstly, and then increased. It indicated that the suitable *R* can improve the recognition ability of MIP.

In the Table I, the selectivity (α) is defined as $\alpha = \text{Ad}_{\text{SMZ}}/\text{Ad}_{\text{SMO}}$, and the imprint factor (*f*) is defined as $f = \text{Ad}_{\text{SMZ}} \times \alpha$. We expected to achieve high adsorption and high selectivity in these MIPs; hence the imprint factor is indicative of obtaining higher efficiency from Ad_{SMZ} multiplied by α . The α and *f* increased with an increase in the *R* until *R* = 10, and then decreased gradually. In other words, the better α and *f* were achieved under optimum *R* = 10, but the additional more *R* could decrease the efficiency, hence the α and *f* had a maximum value at *R* = 10.

Effect of Competition Experiment pH on MIP

Competition experiments using HPLC were performed on solutions with different pH values to study the effects of pH on adsorption and selectivity of the MIP. In this study, the pH value of the original sulfonamides solutions was pH = 4.6. Therefore, controlling the pH of the competition experiment solution to match the p*K*_a can affect the sulfonamides to change its deprotonate or protonated. The relative ability for a molecule to give up a proton (deprotonate) is measured by its p*K*_a value. When the pH of a system is equal to the p*K*_a of the compound within the system, that compound is half protonated (and half deprotonated).^{33–36} In addition, if pH values are higher than p*K*_a values of the sulfonamides this would result in mostly deprotonate of the compound, and further, the level of deprotonation that occurred for the SMZ and SMO affected the adsorption and selectivity.

The characterizations of the MIP with diverse pH for competition experiment are shown in Table II. Under pH = 4.6, most of SMZ and SMO molecules were not deprotonate and the α were greater than 1 ($\alpha > 1$). This indicated that the MIP possessed the recognition ability and the α had a maximum value at *R* = 10. In addition, we changed the pH to match the p*K*_a of SMZ (p*K*_a of SMZ was 7.4), and controlled the pH to match the p*K*_a by ammonium hydroxide (NH₄OH). Under pH = 7.4 the SMZ molecules were half deprotonated while the SMO molecules were almost deprotonated (p*K*_a of SMO was 5.9). These SMO molecules were mostly ionized and became amine salt to influence adsorption in the basic condition.³⁷ Therefore, the results which had less SMO molecules were adsorbed and the greater α and *f* were achieved. Even though the Ad_{SMZ} and Ad_{SMO} of MIP were less than that with pH = 4.6, but the α was raised to 5.42 at *R* = 10. As the results show, that the Ad_{SMZ} and Ad_{SMO} decreased with an increase in pH, but the α and *f* increased with an increase in the pH. Consequently, the α and *f* for the competition experiment solution with a pH = 7.4 were more than 2–3 times greater than the competition experiment solution with a pH = 4.6.

Effect of the Calcination Temperature Processes on MIP

The removal of the template by calcination under various elevated temperature processes was studied. The diagram of calcinations temperature profiles are shown in Figure 2. Calcination occurred when the monoliths were exposed to a three-stage temperature profile defined by fixed first stage at 200 °C for 1 h, the second stage was from 300 °C to 400 °C for 1 h, and the third stage was at 600 °C for 2.5 h. The characterization of the MIP with various second stage temperatures are shown in Table III. In this section, HPLC analysis was carried out on samples *R*10 (H₂O/Si molar ratio equals 10), and the second stage temperatures were carried out on 300 °C, 325 °C,

Figure 2. The diagram of various calcination temperature processes.

Table III. Characterization of MIP prepared by various second stage calcination temperatures

Sample codes.	Second stage temperature (°C)	Ad _{SMZ} (μmol/g)	Ad _{SMO} (μmol/g)	α	f
R10-300	300	1.30	0.24	5.42	7.05
R10-325	325	1.31	0.23	5.70	7.47
R10-350	350	1.32	0.21	6.29	8.30
R10-375	375	1.36	0.13	10.5	14.2
R10-400	400	1.51	0.70	2.16	3.26

*TMOS/(TMOS + MTMOS) = 0.5, $R = 10$, reacted at 60 °C for 2 d, and the pH value of the competition experiment solution was 7.4.

350 °C, 375 °C, and 400 °C to obtain the optimum adsorption and selectivity. The results exhibited that the α and f increased with the increase in the second stage temperature until 375 °C, and then the α and f decreased gradually. In other words, the α and f were achieved under optimum second stage temperature, but the additional second stage temperature could have decreased the efficiency. Due to the condensation reaction carried on with the hydroxyl groups by calcination, the sol-gel processes reacted more completely about 200 °C. In addition, the methyl groups of methyltrimethoxysilane (MTMOS) carried on decomposition because they became hydroxyl groups, and the hydroxyl groups reacted to each other during the condensation reaction about 400 °C.³⁸ Furthermore, the more specific recognition sites were formed when the condensation reaction was allowed to continue close to completion, hence the α and f increased with the increase of the second stage temperature in these MIP. In order to investigate this hypothesis, the Fourier Transform Infrared Spectroscopy (FT/IR) spectra of the MIP obtained form before and after calcination are shown in Figure 3. FT/IR spectra exhibited in the MIP before and after calcination were almost the same. For after calcination, characteristic absorption peaks of Si-CH₃ at 1300 cm⁻¹ disappeared. This result indicates that the methyl groups carried on decomposition because they became hydroxyl groups at 400 °C. Hence, the contention has been proved significant by the above accounts. Moreover, the second stage

Figure 3. FT/IR spectra of the MIP obtained form before (A) and after (B) calcination.

temperature was carried out on 400 °C to obtain the high adsorption, but the selectivity of SMZ was sacrificed. Therefore, the optimum second stage temperature was set at 375 °C for the present MIP.

Effect of the TMOS and MTMOS Molar Fractions on MIP

In this section, the preparation condition of the MIP with various TMOS and MTMOS fractions were studied. The fundamental properties of the sol-gel molecularly imprinted polymer with various TMOS and MTMOS fractions are shown in Table IV. The results indicate that the Ad_{SMZ} decreased with decrease of the TMOS and MTMOS fractions. This was due to the less quantity of TMOS causes the smaller BET surface area; therefore, an obviously less surface area results in fewer recognition sites and lower adsorption. In addition, the less quantity of TMOS caused the bigger pores, but the SMZ entered the specific recognition sites when the average pore size approximated to 5 nm which caused the increase of the opportunity of recognition sites bound with the template. Based on the results of the above descriptions, the greater α and f were achieved under better optimum TMOS and MTMOS molar fractions, TMOS/(TMOS + MTMOS) = 0.5.

Effect of Imprint (MIP) and Non-Imprint (NIP)

In this section, the optimum preparation conditions were carried out on $R = 10$, TMOS/(TMOS + MTMOS) = 0.5, and reacted at 60 °C for 2 d, and the second stage temperature of calcination was 375 °C. A non-imprinted polymer (NIP) was prepared in parallel and under identical conditions but in the absence of the template. The fundamental properties of the MIP and NIP are shown in Table V. Comparison of the MIP and NIP prepared under optimum conditions showed that the BET surface area of the NIP was greater than the MIP, but even then the Ad_{SMZ} of NIP was not greater than the MIP. In addition, a comparison of the MIP and NIP showed that the α of MIP (10.5) was greater than NIP (3.72), and that the f of MIP (14.2) was greater than NIP (3.46). The α and f of MIP was approximately 3.5 times greater than the NIP.

Table IV. The properties of the MIP prepared by various TMOS concentrations

Sample codes.	TMOS/(TMOS + MTMOS)	Ad _{SMZ} (μmol/g)	Ad _{SMO} (μmol/g)	α	f	BET Surface Area (m ² /g)	Average pore Diameter (nm)
R10-0.3	0.3	0.41	0.42	0.98	0.40	1.853	9.13
R10-0.5	0.5	1.36	0.13	10.5	14.2	347.9	5.28
R10-0.8	0.8	1.76	0.44	4.00	7.04	611.1	2.27

Table V. The properties of the MIP and NIP

Sample codes.	Ad _{SMZ} (μmol/g)	Ad _{SMO} (μmol/g)	α	f	BET Surface Area (m ² /g)	Average pore Diameter (nm)
MIP	1.36	0.13	10.5	14.2	347.9	5.28
NIP	0.93	0.25	3.72	3.46	380.0	7.18

*TMOS/(TMOS + MTMOS) = 0.5, $R = 10$, reacted at 60 °C for 2 d, and the pH value of the competition experiment solution was 7.4.

CONCLUSIONS

The experimental results show that sol-gel molecularly imprinted polymers were successfully prepared during our research. The results indicate that the addition of an opportune amount of water could effectively increase selectivity of the sol-gel molecularly imprinted polymer. Moreover, that controlling the pH of the competition experiment solution to match the pK_a of the template increased the adsorption and selectivity of the sol-gel molecularly imprinted polymer. In addition, the second stage temperature of calcination, which was carried out at 375 °C, the more efficiency and high selectivity of MIP was obtained. Consequently, the most significant results were that a comparison of the sulfamethazine imprinted MIP prepared under optimum conditions and the NIP showed that the selectivity of the molecular imprinted polymer was about 3.5 times greater than the selectivity of the non-imprinted polymer.

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REFERENCES

- G. Wulff, *Angew. Chem., Int. Ed.*, **34**, 1812 (1995).
- K. Mosbach and O. Ramstrom, *Biotechnology*, **14**, 163 (1996).
- N. Perez-Moral and A. G. Mayes, *Anal. Chim. Acta*, **504**, 15 (2004).
- E. Busi, R. Basosi, F. Ponticelli, and M. Olivucci, *J. Mol. Catal. A: Chem.*, **217**, 31 (2004).
- H. Y. Yan and K. H. Row, *Int. J. Mol. Sci.*, **7**, 155 (2006).
- K. Haupt and K. Mosbach, *Trends Biotechnol.*, **16**, 468 (1998).
- P. A. G. Cormack and K. Mosbach, *React. Funct. Polym.*, **41**, 115 (1999).
- R. S. Gill, M. Marquez, and G. Larsen, *Microporous Mesoporous Mater.*, **85**, 129 (2005).
- M. E. Diaz-Garcia and R. B. Laino, *Microchim. Acta*, **149**, 19 (2005).
- W. Cummins, P. Duggan, and P. McLoughlin, *Anal. Chim. Acta*, **542**, 52 (2005).
- K. Haupt, *Anal. Chem.*, **75**, 376a (2003).
- K. Haupt and K. Mosbach, *Chem. Rev.*, **100**, 2495 (2000).
- L. I. Andersson, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, **745**, 3 (2000).
- T. B. Liao, X. Tu, and S. J. Li, *Polym-Plast Technol. Eng.*, **46**, 613 (2007).
- K. Farrington, E. Magner, and F. Regan, *Anal. Chim. Acta*, **566**, 60 (2006).
- H. Yan, L. Jin, and K. H. Row, *J. Liq. Chromatogr. Relat. Technol.*, **28**, 3147 (2005).
- K. Haupt, *Chem. Commun.*, **2**, 171 (2003).
- M. V. Polyakov, *Zh. Fiz. Khim.*, **2**, 799 (1931).
- M. J. Whitcombe and E. N. Vulfson, *Adv. Mater.*, **13**, 467 (2001).
- J. X. He, S. Wang, G. Z. Fang, H. P. Zhu, and Y. Zhang, *J. Agric. Food Chem.*, **56**, 2919 (2008).
- S. F. Su, M. Zhang, B. L. Li, H. Y. Zhang, and X. C. Dong, *Talanta*, **76**, 1141 (2008).
- H. S. Wei, Y. L. Tsai, J. Y. Wu, and H. Chen, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, **836**, 57 (2006).
- M. M. Collinson, *Crit. Rev. Anal. Chem.*, **29**, 289 (1999).
- O. Cauli and M. Morelli, *Behav. Pharmacol.*, **16**, 63 (2005).
- E. C. European, *Eur. Commun. Council Regul.*, **No 2377/90**, 224/1 (1990).
- N. A. Littlefield, W. G. Sheldon, R. Allen, and D. W. Gaylor, *Food Chem. Toxicol.*, **28**, 157 (1990).
- A. E. Cribb, M. Miller, A. Tesoro, and S. P. Spielberg, *Mol. Pharmacol.*, **38**, 744 (1990).
- H. C. Neu, *Science*, **257**, 1064 (1992).
- M. P. Davies, V. D. Biasi, and D. Perrett, *Anal. Chim. Acta*, **504**, 7 (2004).
- A. V. Trask and W. Jones, *Top. Curr. Chem.*, **254**, 41 (2005).
- N. Zheng, Y. Z. Li, W. B. Chang, Z. M. Wang, and T. J. Li, *Anal. Chim. Acta*, **452**, 277 (2002).
- A. M. Siouffi, *J. Chromatogr., A*, **1000**, 801 (2003).
- Z. Y. Chen, R. Zhao, D. Shangguan, and G. Q. Liu, *Biomed. Chromatogr.*, **19**, 533 (2005).
- T. C. Gilmer and D. J. Pietrzyk, *Anal. Chem.*, **43**, 1585 (1971).
- M. Kahle and C. Stamm, *Chemosphere*, **68**, 1224 (2007).
- W. Tappe, C. Zarfl, S. Kummer, P. Burauel, H. Vereecken, and J. Groeneweg, *Chemosphere*, **72**, 836 (2008).
- X. J. Liu, C. B. Ouyan, R. Zhao, D. H. Shangouan, Y. Chen, and G. Q. Liu, *Anal. Chim. Acta*, **571**, 235 (2006).
- Z. Zhang, Y. Tanigami, R. Terai, and H. Wakabayashi, *J. Non-Cryst. Solids*, **189**, 212 (1995).