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Syntheses of molecularly imprinted polymers and their molecular recognition study for doxazosin mesylate

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Abstract

Molecular imprinted polymers (MIPs) (diameter is about 1.7 μm) were prepared from methacrylic acid as the functional monomer and triallyl isocyanurate as the cross-linker in methanol solution using doxazosin mesylate as the template molecule and 2,2′-azobis-isobutyronitrile as the initiator. The effects of amount of the cross-linker, the ratio of template molecule and functional monomer, amount of solvent and amount of the radical initiator on the polymerization were examined. Release of the template was performed by continuous extraction with methanol containing 10% acetic acid. These MIPs showed specific binding affinity toward doxazosin mesylate and the imprinting mechanism is discussed. The principal advantage of the method is that the MIPs prepared can recognize doxazosin mesylate and the particles size can be controlled by the amount of initiator and the volume of methanol employed.

Keywords: Molecularly imprinted polymer; Microsphere; Doxazosin mesylate; Methacrylic acid; Triallyl isocyanurate; 2,2′-Azobis-isobutyronitrile

1. Introduction

The use of molecularly imprinted polymer (MIPs) is a highly specific detection technology [1,2], which is used extensively in many fields, such as separation of isomers and enantiomers [3], preparation of highly selective solid extraction sorbent by molecular imprinting [4], preparation of chemical sensor [5–7], molecular recognition between enzyme and substrates [8], molecular imprinting for drug bioanalysis [9]. Molecularly imprinted polymers have been prepared in various configurations

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including, polymer beads, monoliths and membranes for different applications. Takeuchi et al. [10] reported a combinatorial approach to the synthesis of MIPs. The most common form of imprinted polymer is, however, still the irregularly shaped particle obtained by grinding the traditional, macroporous polymer monolith. The doxazosin mesylate is a quinazoline compound that is a selective inhibitor of the α1 subtype α of adrenergic acceptor. It is used to treat hypertension (high blood pressure) [11]. When doxazosin mesylate was used as the template molecule, the polymer was synthesized via non-covalent interactions using methacrylic acid (MAA) as the functional monomer, triallyl isocyanurate as a functional cross-linker. A precipitation polymerization technique allows preparation of uniform spherical imprinted beads with diameters down to 200 nm having excellent recognition properties for different target molecules [12–14]. However, whilst the imprinted polymers can be synthesised by heat-initiated or light-initiated polymerization techniques, the procedure suffers from the inconvenience of grinding and seiving. To prepare MIPs by suspended polymerization requires stabilizing agent such as polyglycerol in the process of polymerization [15]. Unfortunately, that stabilizing agent may cause non-selective bonding to template molecule results in decrease of imprinting rate in the bonding process. Molecular imprinting in polymers is achieved by incorporating a template, or imprint molecule into a highly cross-linked polymer matrix. Literature [16] showed non-covalent imprinting was generally occurred between the template and functional monomer. Although covalent interaction between the template and functional monomer was used for preparation of MIPs [17], it was difficult to break the covalent bond between the template and functional monomer by acid hydrolysis. Therefore, we were able to synthesize MIPs that have a strong and specific affinity for doxazosin mesylate, using volumes of reaction medium and non-covalent approach. The average diameter of particles of MIPs is in the range of 1.0–2.0 μm depending on the volume of solvent and amount of initiator in the method proposed. Releasing of the template was performed by continuous extraction with methanol containing 10% acetic acid. Such carefully synthesized MIPs showed specific affinity toward doxazosin mesylate in the bonding process using methanol solvent.

2. Experimental

2.1. Apparatus

All spectrophotometric measurements were made with an ultraviolet visible recording spectrophotometer (UV-265, Shimadzu, Japan) with matched 1-cm quartz cells. In order to compare all spectrophotometric measurements and ensure reproducible experimental conditions, the UV-265 spectrophotometer was checked daily in wavelength accuracy and linearity. Scanning electron microscope (S-570, Hitach, Japan) was used for measuring size of polymer particles. A pHS-3C digital pH meter (Shanghai Lei Ci Device Works, Shanghai, China) was used for pH measurement. Super constant temperature bath (Chongqing experimental Instruments Factory, China) was used for controlling temperature (60 ± 0.01 °C).

2.2. Reagents

Doxazosin mesylate (99.0%), the chemical name is 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-ylcarbonyl)piperazine methanesulfonate, (Shandong medicine industry institute, Shandong, China) was used as the template molecule. Triallyl isocyanurate (TAIC) (purchased from Laiyu Chemical Plant, Shandong, China); methacrylic acid (MAA) (Shanghai Chemical Reagents Co., China) and 2,2′-azobisisobutyronitrile (AIBN) (Shanghai Fourth Chemical Reagents Factory, China) were redistilled to remove the inhibitor before being used. Other reagents were of analytical-reagent grade. Doubly distilled water was used in all experiments.

2.3. Procedure

2.3.1. Procedure I

Preparation of MIPs for doxazosin mesylate. Refer to Table 1, Doxazosin mesylate, MAA,

Table 1
Preparation of MIPs for doxazosin mesylate and binding capacity of MIPs

No.	Doxazosin mesylate (mmol)	MAA (mmol)	TAIC (mmol)	Doxazosin mesylate:MAA:TAIC	Q (μmol/g)
P_1	0	50	200	0:1:4	9.8
P_2	20	0	200	1:0:10	6.4
P_3	20	10	200	1:0.5:10	25.7
P_4	20	40	200	1:2:10	48.3
P_5	20	120	200	1:6:10	76.2
P_6	20	140	200	1:7:10	77.4

and methyl alcohol were accurately weighed into 300 ml ampoule and allowed to stand for 5 h to ensure complete association of the imprint molecule with MAA. The TAIC and AIBN were then added and the solution ultrasonicated for10 min. The ampoule flask was charged repeatedly with pure nitrogen (purity is 99.99%) 15 min to expel air, and sealed immediately with high temperature flame. The ampoule was warmed for 30 h at 60 °C to ensure complete cross-linking and the polymer particles were obtained. The polymer particles were filtrated and put in a Soxhlet extractor to extract template molecule. The releasing of the template was performed by continuous extraction with methanol containing 10% acetic acid in a Soxhlet extractor till the extract phase did not contain doxazosin mesylate. The extracted phase can be detected spectrophotometrically at 341 nm. The MIPs for doxazosin mesylate was washed with water to remove residual methanol and acetic acid. Finally, the MIPs for doxazosin mesylate was dried at 60 °C, stored in desiccator.

2.3.2. Procedure II

Determination of binding capacity for MIPs. Binding capacities of MIPs for doxazosin mesylate were determined spectrophotometrically at 341 nm. The standard solutions of doxazosin mesylate were prepared by methanol and its absorbances were measured against a reagent blank prepared with the same amount of methanol, but no doxazosin mesylate. A calibration graph was found between the absorbance and concentration of doxazosin mesylate. An accurately weighed 0.1000 g portion of the polymer was transferred into a 250 ml conical flask, 10.0 ml of 4.5 mmol l^{-1} doxazosin mesylate–methanol standard solution was added and the flask shaken for 5 h. This solution was centrifuged at 4500 rpm for 5 min. The centrifugate (1.00 ml) was transferred into 5.0 ml volumetric flask and diluted to the mark with methanol. The absorbance of the solution was measured at 341 nm with spectrophotometer. The concentration of doxazosin mesylate was estimated based on calibration graph of doxazosin mesylate.

3. Results and discussion

3.1. Imprinting mechanism

Many imprinted polymers were synthesized using MAA as functional monomer and ethylene dimethacrylate (EDMA) or ethylene glycol dimethacrylate (EGDMA) as cross-linker by heat initiated or light initiated polymerization [18–21]. The template molecule, doxazosin mesylate, is an aquinazoline compound. The doxazosin mesylate molecule has an $-NH_2$ and N atom which link with amine site. This structure ensures to form a three-dimensional molecular imprinting with functional monomer (MAA) [22] and cross-linker (TAIC). The oxygen atom or nitrogen containing functional groups of doxazosin mesylate molecule can form hydrogen bond with functional monomer (MAA) [23–27]. According to the Scatchard model the processing of data showed that there were two bonding sites in the MIPs (see verification of Section 3.8.1). One binding site is located at the point that the amine group and N atom which link with amine site of doxazosin mesylate molecule bond with carboxyl of MAA through hydrogen bond. Binding is also possible at the point that any oxygen atom or nitrogen atom of doxazosin mesylate molecule interact with the

carboxyl of MAA (gave a particular description in Verification of Section 3.8.1). In order to remove the imprint molecule from MIPs after cross-linking, it is necessary to break the non-covalent bond between doxazosin mesylate molecule and carboxyl group of the polymer. This was performed by continuous extraction with methanol containing 10% acetic acid in a Soxhlet extractor. Fig. 1 is a schematic representation of the synthesis of molecularly imprinted polymer for doxazosin mesylate.

3.2. Binding capacity for MIPs

According to procedure II, the binding capacity of MIPs was measured, and calculated according to following equation:

$$Q = \frac{\mu\text{mol(doxazosin mesylate bound)}}{g(\text{MIP})} = \frac{(C_i - C_f)V_s \times 1000}{M_{MIP}},$$

where Q is binding capacity of MIPs (μmol/g), C_i the initial doxazosin mesylate concentration (μmol/ml), C_f the final doxazosin mesylate concentration (μmol/ml), V_s the volume of solution tested (ml), M_{MIP} the mass of dried polymer (mg). The results were showed in Table 1.

3.3. Amount of cross-linker

The cross-linker polymerizes with double bonds of functional monomer to form meshwork polymer in the presence of initiator. As all know, the monomer plays the most important role in the imprinting process; however, the cross-linker is an important factor as well [28]. The amount of cross-linker is a key to forming a suitable imprint in a polymer network. If the density of cross-linker is low in the polymerization, lack of cross-linker cannot fix template molecule to from three-dimensional molecular imprinting. The result showed that the binding capacity of MIPs was satisfactory when the molar ratio (template molecule: functional monomer: cross-linker) equal to 1:6:10 (see Table 1). This ratio was lower than that of ethylene glycol dimethacrylate (EGDMA) used often as functional monomer [29] and may be due to having one less double bond in EGDMA than TAIC.

3.4. Ratio of template molecule and functional monomer

As can be seen (Table 1), the ratio of template molecule and functional monomer was evaluated by comparison of the binding capacity of blank polymer (P_1) and MIPs (P_3–P_6). Obviously, the Q value of P_1 was lower than that of P_3–P_6; this fact showed that the binding of blank polymer was non-selective physical adsorption. P_2 was not an imprinting polymer because of lack of functional monomer in the polymerization. In addition, the ratio of template molecule and functional monomer was a key in the polymerization. If amount of MAA is too low, the template molecules are present in excess and cannot complex with MAA. Clearly, the decrease of Q value was due to decrease of the imprint quantity of the MIPs. So 1:6, the molar ratio of doxazosin mesylate and MAA, was used in preparation of MIPs for doxazosin mesylate.

3.5. Amount of initiator

The amount of initiator is also a key to synthesizing suitable imprint in the MIPs. The amounts of doxazosin mesylate, MAA and TAIC were 4, 24 and 40 mmol, respectively; and the volume of methanol was 120 ml. We changed amount of AIBN to prepared MIPs in above the system. The particle sizes of MIPs were measured with scanning electron microscope, the results showed that average diameter of MIPs increased with a rise in amount of AIBN. When $\frac{\text{AIBN}}{\text{MAA+TAIC}}$ was above 1.0%, the average diameter of MIPs was no longer change. Therefore, a ratio of 1.0% was selected as polymerizing condition.

3.6. Amount of solvent

Mayes and Mosbach reported a suspension polymerization method to prepare the molecular imprinted polymer that was synthesized using MAA as functional monomer and EDMA as cross-linker in presence of perfluoropolymeric surfactant [30]. Pérez-Moral and Mayes [31]

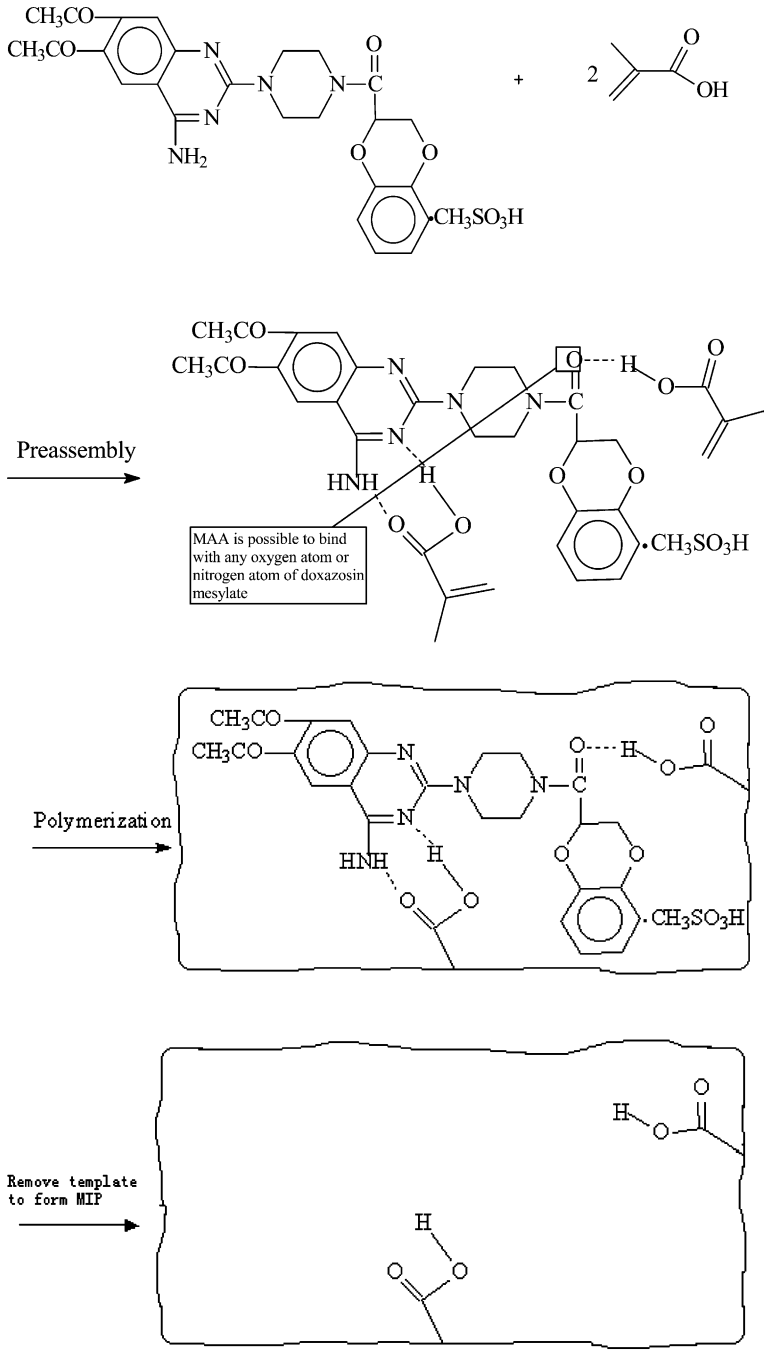

Fig. 1. Schematic representation of the synthesis of MIPs for doxazosin mesylate.

studied the preparation of imprinted polymer particles by different polymerization methods. A monolithic network polymer particles was formed by the bulk polymerization [32], but the polymeric block needs to be crushed and to obtained particles of irregular shape. The advantage of

suspension polymeristion was that the polymerized particles could be obtained directly when the reaction was completed. The beads obtained had a diameter that could vary between 5 and 50 μm depending on the stirring speed and the amount of surfactant [31] In our test the amounts of doxazosin mesylate, MAA, TAIC and AIBN were 4, 24, 40 mmol and 0.1306 g, respectively. To prepared different particle size of MIPs, different volumes of methanol were added to this system. The test found that particles average diameter increased with decrease of volume of methanol. The polymerized particle average diameter was about 1.7 μm and the beads were microspherical when volume of methanol was only 120ml. The result was showed in Fig. 2. Our result showed that the polymerized particles could obtain by the suspension polymerization and the size between 1.0 and 2.0 μm depending on the amount of initiator and the volume of methanol. The polymerized particle shape in Fig. 2 was accorded generally with the result reported by Pérez-Moral and Mayes [31].

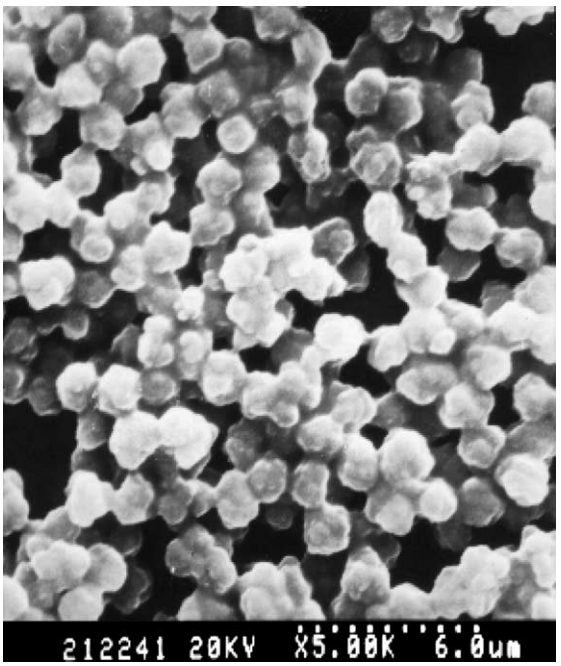

Fig. 2. Micropheral MIPSs Scale: the length of eleven bright dots was 6 μm on the bottom of photo.

3.7. Selectivity

In order to investigate the selectivity of the imprinted polymer, P_1 (corresponding blank polymer) and P_5 (MIPs for doxazosin mesylate) were used to determine binding capacity in doxazosin mesylate and guanine hydrochloride solutions. The guanine is selected to investigate the selectivity of MIPs due to this compound has similar chemical structure as doxazosin mesylate. The guanine molecule has amine group and N atom which link with amine group, and aromatic ring. The binding capacities of P_1 and P_5 were determined according to procedure II. It was note that the determination of binding capacity in guanine solution was carried out spectrophotometrically at 275 nm. The result was showed in Table 2.

As can be seen from Table 2 the binding capacity of P_5 to doxazosin mesylate was stronger than that of P_1, but the binding capacity of P_1 to guanine was similar to P_5. This indicated that MIPs for doxazosin mesylate could recognize doxazosin mesylate molecule, and doxazosin mesylate molecule size to match with the imprinted cavity.

3.8. Characteristic of the recognition of the MIPs for doxazosin mesylate

3.8.1. Verification of imprinting mechanism

MIPs for doxazosin mesylate were prepared based on the synthetical condition.The binding capacities of the MIPs to doxazosin mesylate were measured according to procedure II and the results showed in Fig. 3.

Fig. 3 showed that the change of Q value tend to flat with a rise in the concentration of doxazosin mesylate. This indicated that the cavities of the MIPs were gradually saturated by doxazosin mesylate molecule. In the molecularly imprinted polymer technique, Scatchard et al. [33] model was

Table 2
Selectivity of MIPs for doxazosin mesylate

No.	Q (μmol/g)	
	Doxazosin mesylate	Guanine
P_1	9.6	9.4
P_5	77.4	9.8

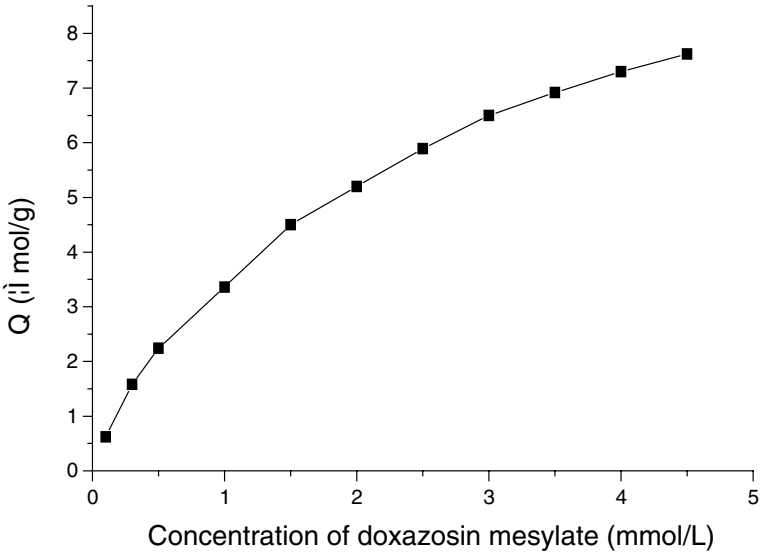

Fig. 3. The logarithmic bonding isotherm of MIPSs for doxazosin mesylate (25 °C).

often used to evaluate the characteristic of the recognition of the MIPs. Scatchard equation [34] is follows: $Q/C = (Q_{max} - Q)/K_d$. In the equation: Q is binding capacity; C concentration of doxazosin mesylate (mmol l^{-1}); Q_{max} maximum apparent binding capacity; K_d disassociation constant at binding site. Test found a relationship between Q/C and Q, namely, Scatchard plot showed in Fig. 4.

The Scatchard plot obtained for the being of doxazosin mesylate to its polymer imprint consists

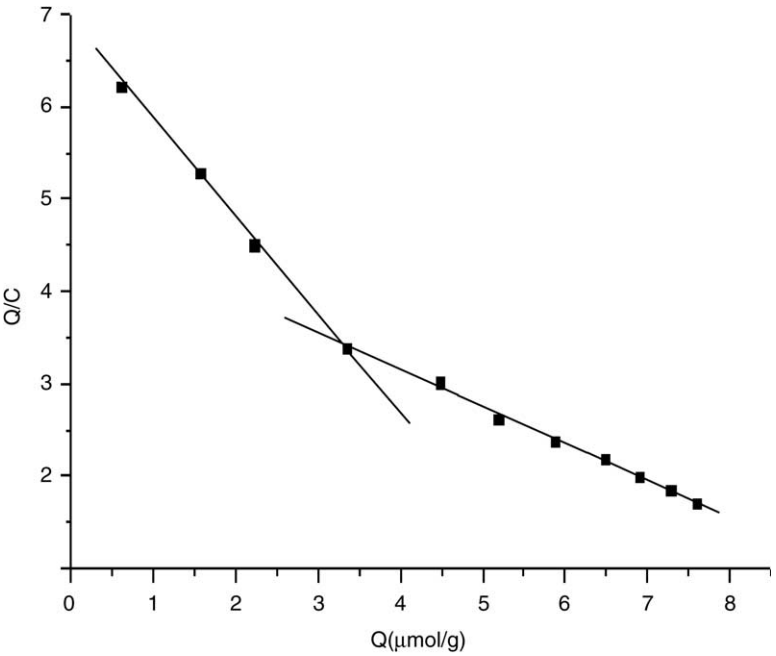

Fig. 4. Scatchard graph of MIPSs for doxazosin mesylate.

of two distinct straight lines with slop which gave $K_{d1} = 1.85$ and $K_{d2} = 0.79$. The Scatchard plot showed two lines with different slopes corresponding to high and low affinity populations of binding sites. i.e., K_{d1} and K_{d2} were disassociation constant at binding site of low affinity and at binding site of high affinity, respectively. Based on the existence of two binding sites in the MIPs we infer that one binding site locates at the point that amine group and nitrogen atom which links with amine group of doxazosin mesylate molecule bond with carboxyl of MAA through non-covalent; other is possible to locate at the point that any oxygen atom or nitrogen atom of doxazosin mesylate molecule interact with carboxyl of MAA. Certainly, there is still much work to be done in efforts to verify which oxygen atom or nitrogen atom of doxazosin mesylate molecule bond with carboxyl of MAA.

4. Conclusion

The novel molecularly imprinted polymer was prepared in the volumes of methanol. The MIPs could recognize doxazosin mesylate and the particles size of MIPs could be controlled by a mount of initiator and volume of methanol. Obviously, this MIPs is potential to prepare stationary phase for separating doxazosin mesylate by chromatography.

References

[1] D. Kriz, O. Ramström, K. Mosbach, Anal. Chem. 69 (1997) A345.
[2] D.A.G. Cormack, A.Z. Elorza, J. Chromatogr. B 804 (2004) 173.
[3] L. Schweitz, L.I. Andersson, S. Nilsson, Anal. Chem. 69 (1997) 1179.
[4] L.I. Andersson, A. Paprica, T. Arvidsson, Chromatographia 46 (1997) 57.
[5] M.F. Lulka, J.P. Chambers, E.R. Valdes, R.G. Thompson, J.J. Valdes, Anal. Lett. 30 (1997) 2301.
[6] F.L. Dickert, O. Hayden, Trends Anal. Chem. 18 (1999) 192.
[7] B. Adhikari, S. Majumdar, Progr. Polym. Sci. 29 (2004) 699.
[8] K. Polborn, K. Severin, Chem. Commun. 24 (1999) 2481.
[9] L.I. Andersson, J. Chromatogr. B 739 (2000) 163.
[10] T. Takeuchi, D. Fukuma, J. Matsui, Anal. Chem. 71 (1999) 285.
[11] A.M. Rey, K. Grauer, J.G. Gums, Postgrad. Med. 89 (1991) 75.
[12] O. Brüggemann, K. Haupt, L. Ye, E. Yilmaz, K. Mosbach, J. Chromatogr. A 889 (2000) 15.
[13] R.J. Ansell, K. Mosbach, J. Chromatogr. A 787 (1997) 55.
[14] F.G. Tamayo, J.L. Casillas, A. Martin-Esteban, Anal. Chem. Acta 482 (2003) 165.
[15] V. Istratov, H. Kautz, Y.-K. Kim, R. Schubert, H. Frey, Tetrahedron 59 (2003) 4017.
[16] A.G. Mayes, L.I. Andersson, K. Mosbach, Anal. Biochem. 222 (1994) 483.
[17] G. Wulff, S. Schauhoff, J. Org. Chem. 56 (1991) 395.
[18] G. Ciardelli, B. Cioni, C. Cristallini, N. Barbani, D. Silvestri, P. Giusti, Biosens. Bioelectron. 20 (2004) 1083.
[19] W. Cai, R.B. Gupta, Separ. Purif. Technol. 35 (2004) 215.
[20] H. Dong, A.J. Tong, L.D. Li, Spectrochim. Acta A 59 (2003) 279.
[21] F.G. Tamayo, J.L. Casillas, A. Martin-Esteban, Anal. Chim. Acta 482 (2003) 165.
[22] W.J. Wizeman, P. Kofinas, Biomaterials 22 (2001) 1485.
[23] M.-J. Syu, J.-H. Deng, Y.-M. Nian, Anal. Chem. Acta 504 (2004) 167.
[24] H.Y. Wang, S.L. Xia, H. Sun, Y.K. Liu, S.K. Cao, T. Kobayashi, J. Chromatogr. B 804 (2004) 127.
[25] F. Chapuis, V. Pichon, F. Lanza, B. Sellergren, M.-C. Hennion, J. Chromatogr. B 804 (2004) 93.
[26] J. Jodlbauer, N.M. Maier, W. Lindner, J. Chromatogr. A 945 (2002) 45.
[27] P.T. Vallano, V.T. Remcho, J. Chromatogr. A 888 (2000) 23.
[28] J.-P. Lai, R. Niessner, D. Knopp, Anal. Chim. Acta 522 (2004) 137.
[29] L.H. Gan, G.R. Deen, X.J. Loh, Y.Y. Gan, Polymer 42 (2001) 65.
[30] A.G. Mayes, K. Mosbach, Anal. Chem. 68 (1996) 3769.
[31] N. Pérez-Moral, A.G. Mayes, Anal. Chim. Acta 504 (2004) 15.
[32] K. Mosbach, O. Ramson, Biotechnology 14 (1996) 163.
[33] G. Scatchard, I.I. Scheiberg, S.H. Armstrong, J. Am. Chem. Soc. 72 (1950) 535.
[34] J. Matsui, Y. Miyoshi, O. Doblhoff-Dier, Anal. Chem. 67 (1995) 4404.