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키랄(S)-이부푸로펜 함유 고분자의 합성과 제조된 고분자의 분자 인식 메카니즘

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Synthesis of Molecularly Imprinted Polymers for Chiral (S)-Ibuprofen and Their Molecular Recognition Mechanism

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Abstract: A group of molecularly imprinted polymers (MIPs) with specific recognition for chiral (S)-ibuprofen were successfully prepared based on hydrogen bonds, utilizing α-methacrylic acid as a functional monomer. The IR analysis of MIPs showed that the blue- and red-shifted hydrogen bonds were formed between templates and functional monomers in the process of self-assembly imprinting and re-recognition, respectively. According to UV-Vis analysis, we found that the ratio of host-guest complexes between template molecule and functional monomer was 1:1. The effect of cross-linker's quantity on the polymerization was studied by transmission electron microscope (TEM). The adsorption selectivity experiments indicated that MIPs exhibited higher selectivity to (S)-ibuprofen than those to ketoprofen and (R)-ibuprofen, (S)-ibuprofen's structural analogs.

Keywords: (S)-ibuprofen, molecularly imprinted polymer, chiral separation, hydrogen bond, separation factor.

Introduction

Molecular imprinting technology (MIT) is a promising technology that confers specific molecular recognition capabilities.[1] The molecular imprinting process involves the formation of a complex between template molecules with appropriate functional monomers that can be cross-linked into a macromolecular matrix. Subsequent removal of the template reveals binding sites that are complementary in size and shape to the original imprinted template molecule.[2,3] Molecularly imprinted polymers (MIPs) have been prominently used as separation media to achieve enantiomers' separation of racemic compound.[4,5] Due to their promising applications, the study on the interaction between template and functional monomer in the pre-assembled and re-recognition process has been especially important. In principle, any kind of non-covalent interaction should be effective and employable for the molecular imprinting,[6,7] whereas hydrogen bond has been one of the most appropriate interactions for precise molecular recognition, since it highly depends on both distance and direction between the monomer and the template.[8,9]

In this study, we prepared (S)-ibuprofen MIPs according to hydrogen bond imprinting which exhibited significant adsorption selectivity for (S)-ibuprofen. (S)-ibuprofen,[10] an indole derivative (Scheme 1(a)), is a well-known nonsteroidal anti-inflammatory drug (NSAID) broadly used for release of acute joint and backbone pain and treatment of degenerative diseases of joints and ligaments.[11] As acrylic acid derivatives, (S)-ibuprofen contains one disubstituted benzene ring and one carboxyl group with three-dimensional structure, which avoids competition of many functional groups in process of self-assembly and re-recognition. It is very useful to explore the role of molecularly imprinted polymers in chiral separation by

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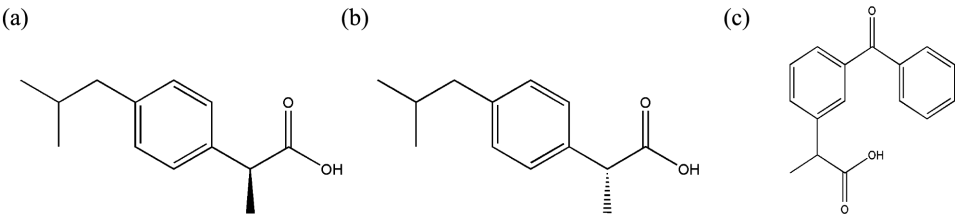


Scheme 1. Chemical structures of (S)-ibuprofen and its structural analogues: (a) (S)-ibuprofen; (b) (R)-ibuprofen; (c) ketoprofen.

the study of hydrogen bond mechanism in molecular recognition.

Experimental

Reagents and Materials. (S)-Ibuprofen(S-IBF), (R)-buprofen(R-IBF) and Ketoprofen(99%) were obtained from Shandong Xinhua Co. and used as received. α-Methacrylic acid (α-MAA) [functional monomer] was purchased from Tianjin Chemical Reagent Research Institute and purified by vacuum distillation before use. Ethylene glycol dimethacrylate (EGDMA) [cross-linker] was supplied by Shanghai Haiqu Chemical Co. and used as received. 2,2-Azo-bis-isobutyronitrile (AIBN) [initiator] from Shanghai Hewei Co. was of analytical grade and recrystallized from ethanol. Dichloromethane (porogen) was obtained from Tianjin Damao chemical reagent Co. All the other reagents and solvents were of analytical grade and used as received without any further purification.

Instruments. UV-2100 uv-vis spectrophotometer was from Shimazu Corp., Japan. FTIR analyses were taken on a TENSOR37-Fourier transform infrared spectrometer (Bruker Corporation, Germany). H-7650 transmission electron microscope (Hitachi, Ltd., Japan) was used to observe the morphology of imprinted polymers.

Ultraviolet Spectrograph Analysis. The absorbance of a series of solution with different ratios of S-IBF: α-MAA (1:6, 1:8, 1:10, 1:16, 1:20) were measured at UV (283 nm) using S-IBF solution of 5 mmol/L as reference.

Preparation of Molecularly Imprinted Polymers. S-IBF (0.206 g, 1 mmol) with different amounts of α-MAA (0.340 mmol) were weighted in conical Erlenmeyer flasks and dissolved in 15 mL dichloromethane. After ultrasonic oscillation for 2 h, EGDMA and AIBN (20 mg) were added, followed by vibration for 1 h and purge with nitrogen gas for 15 min. The polymerization was achieved under UV (365 nm lamp) irradiation at 25 °C for 72 h. Table 1 shows the composition of synthesized polymers in this study. The resultant bulk polymers were grounded and sieved. The particles with particle size between 37 and 74 μm were collected and washed with acetic acid/methanol mixed solution (volume ratio of 2:8) overnight to remove the template until the template could no longer be detected in the elution under UV (283 nm).

Table 1. Chemical Compositions for Making Imprinted Polymers

MIP	*n* (template molecule): *n* (functional monomer): *n* (cross-linker)	Porogen amount (mL)
P_1	1:4:20	20
P_2	1:4:30	20
P_3	1:4:40	20
P_4	0:4:30	20

Adsorption Dynamics Experiment of MIPs. 10 samples of MIPs with equivalent mass of 100 mg were put into a group of 10 mL S-IBF solutions with constant concentration of 5 mmol/L. The mixtures were oscillated in constant temperature bath at 25 °C for different time (15, 30, 45, 60, 75, 90, 120, 150, 180, 210 min), and the concentration of S-IBF in the upper clear liquid was determined using UV absorption spectroscopy (λ = 283 nm), The binding amount Q (μmol/g) was calculated based on the difference of S-IBF concentration before and after adsorption, the volume of solution and the weight of polymers according to the eq. (1).

$$Q = V(C_0 - C_t)/m \quad (1)$$

where, C_0 and C_t are the initial substrate concentration and the equilibrium concentration of substrate after adsorption (mmol/L), V and m are the volume of the substrate solution (mL) and the mass of polymer particles (g), respectively.

Selective Adsorption of MIPs to Different Substrates. In order to investigate the adsorption of MIPs to different substrates, the same method as above was adapted to calculate dissociation coefficient (K) and separation factor (α). The MIPs (100 mg) were mixed with 10 mL of a known concentration of S-IBF, R-IBF and Ketoprofen solution (5 mmol/L), respectively. Dissociation coefficient (K) and separation factor (α) were calculated based on the following eq. (2) and

(3), respectively.

$$K = \frac{C_p}{C_s} \quad (2)$$

$$\alpha = \frac{K_i}{K_j} \quad (3)$$

where, C_p is the polymer-bound substrate concentration (μmol/g) and C_s is the substrate concentration (mmol/L) at equilibrium. And K_i is the dissociation coefficient of template molecule; K_j is the dissociation coefficient of substrate. Furthermore, the value of α is 1.0 when $i = j$.

Results and Discussion

UV Analysis for the Interrelation between Template Molecule and Functional Monomer. The stability of the host-guest complex between template molecule and functional monomer was one of essential influence factors for the selectivity of molecularly imprinted polymers.[12,13] Thus, we measured the absorbance of MIPs under UV 283 nm, the characteristic adsorption peak of S-IBF's conjugated benzene ring, to study the interaction between template and functional monomer, and calculated the host-guest complex binding constants and the complex ratio.

In the preparation process of MIPs, the concentration of the α-MAA (host, b_0) was larger than one of the S-IBF (guest, a_0). The interaction between guest and host of the formed complex can be expressed using the following equations:

$$\text{S-IBF} + n\alpha\text{-MAA} \rightarrow \text{S-IBF}[\alpha\text{-MAA}]_n \quad (4)$$

$$K = [\text{S-IBF}[\alpha\text{-MAA}]_n]/[\text{S-IBF}][\alpha\text{-MAA}]_n \quad (5)$$

where, K is the combination constant. On the basis of mass balance, eq. (6) can be obtained:

$$[\text{S-IBF}] + [\text{S-IBF}[\alpha\text{-MAA}]_n] = a_0$$
$$[\alpha\text{-MAA}] + [\text{S-IBF[MAA}]_n] = b_0 \quad (6)$$

Because b_0 is much larger than a_0, [S-IBF[MAA]$_n$] among the formula (3) can be neglected. Namely:

$$[\alpha\text{-MAA}] \approx b_0 \quad (7)$$

Take eqs. (6) and (7) into eq. (5):

$$[\text{S-IBF[MAA}]_n] = a_0 \; b_0^n K/(1 + b_0^n K) \quad (8)$$

According to Lamber-Beer's law, if S-IBF and S-IBF [MAA]$_n$ have absorption under the measuring wavelength, the absorption of the solution can be expressed as eq. (9):

$$A = \varepsilon_1 l\,[\text{S-IBF}] + \varepsilon_2 l\,[\text{S-IBF[MAA}]_n] = \varepsilon_1 l(a_0 - [\text{S-IBF[MAA}]_n]) + \varepsilon_2 l\,[\text{S-IBF[MAA}]_n] = (\varepsilon_1 - \varepsilon_2) l\,[\text{S-IBF[MAA}]_n] + \varepsilon_1 l\, a_0 \quad (9)$$

where, ε_1 and ε_2 are the mole absorption factors of the S-IBF and S-IBF[MAA]$_n$, respectively.

$$A_0 = \varepsilon_1 l\, a_0$$
$$\Delta A = A - A_0 = (\varepsilon_2 - \varepsilon_1)\; l\,[\text{S-IBF[MAA}]_n] = \Delta\varepsilon l\,[\text{S-IBF[MAA}]_n] \quad (10)$$

Take eq. (10) into eq. (8):

$$\Delta A/b_0^n = -K\Delta A + K\Delta\varepsilon l\, a_0 \quad (11)$$

where, n is the composition of complex. When $n = 1$, a linear curve can be drawn between $\Delta A/b_0^n$ and ΔA (Figure 1). The results show that the complex with template-to-monomer ratio of 1:1 were formed mainly under this condition. The host-guest equation was $\Delta A/b_0^n = -0.0471\Delta A + 0.0109$, and the combination constant ($k = 4.71*10^4 L^2*\text{mol}^{-2}$) was obtained from the slope.

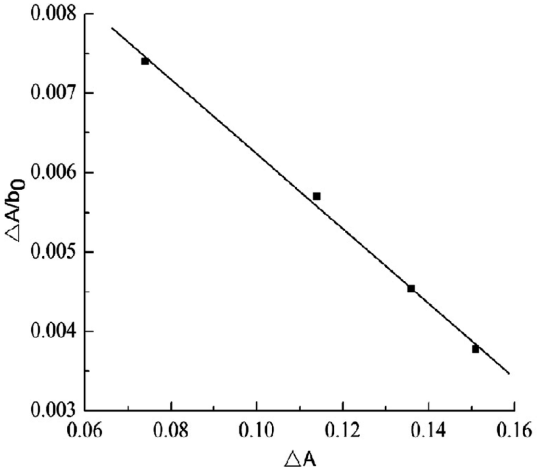


Figure 1. Plot of host-guest equation between (S)-Ibuprofen and α-MAA.

Morphology of Molecularly Imprinted Polymers. The properties of a material are commonly determined by its microstructure and surface, which are impacted by the material's composition, synthesis environment and post-treatment course.[14,15] Transmission electron microscope (TEM) has successfully revealed the correlation between macro-properties and micro-structure of a material. As cross-linker is one of important factors in the process of producing MIPs, the effect of the cross-linker's dosage on the morphology and network

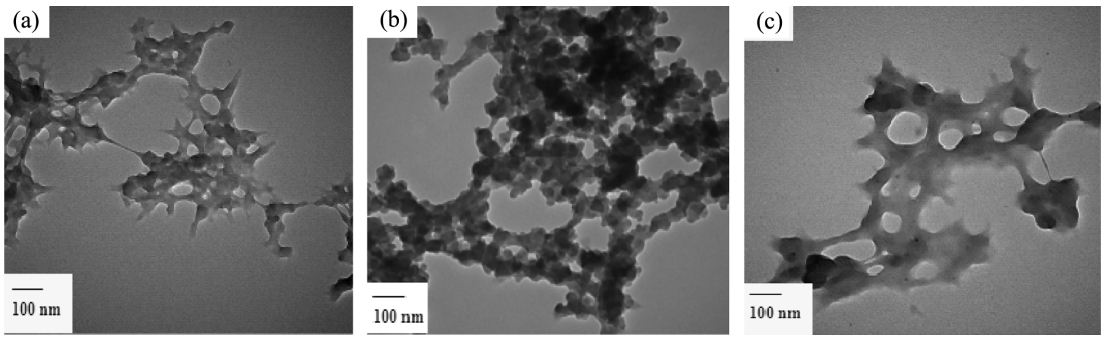


Figure 2. TEM images of (S)-Ibuprofen MIPs: (a) P_1n (template molecule): n (cross-linker)=1:20; (b) P_2n (template molecule): n (cross-linker)=1:30; (c) P_3n (template molecule): n (cross-linker)=1:40.

structure of MIPs was studied by TEM. By comparing three TEM images in Figure 2, we can see that the polymers have significantly different morphologies with different cross-linker's dosage. Less cross-linkers slowed free-radically initiated polymerization rate, and further decreased polymerization degree of products, which causes MIPs' loose structure as shown in Figure 2(a). However, excessive cross-linkers resulted in higher viscosity during polymerization, and made porogen hard to diffuse into the matrix completely to form large pore structures. And excessive cross-linkers also affected the wrap degree of network to pre-polymerization complex, as shown in Figure 2(c). In Figure 2(b), the polymer presented irregular three-dimensional mesh pattern. The pore channels running through the material greatly increased the surface area of the material and reduced the number of embedded imprinting points in MIPs, so as to enhance the accessibility of binding sites. Moreover, the pore channels provided a passing medium for in-house molecules. This was conducive to the achievement of combination and absorption. Based on the above analysis, the template-to-cross-linker ratio of P_2 was the best choice.

Adsorption Dynamics of MIPs to S-IBF. Adsorption kinetic study is one of the important methods to illustrate the efficiency of an adsorption process and to estimate the necessary residual time for the whole process.[16,17] Figure 3 shows that, at a given initial concentration, the adsorption capacity of MIPs towards S-IBF increased with time to attain a maximum value and finally reached equilibrium. The curves also show that the adsorption process was quite rapid over the first 120 min and then slow down to reach the equilibrium stage. This phenomenon indicates that there were specific binding sites complementary to the template molecule inside of MIPs. Although the adsorption of S-IBF was initially fast, after the MIPs reached the saturated adsorption stage, the penetration of

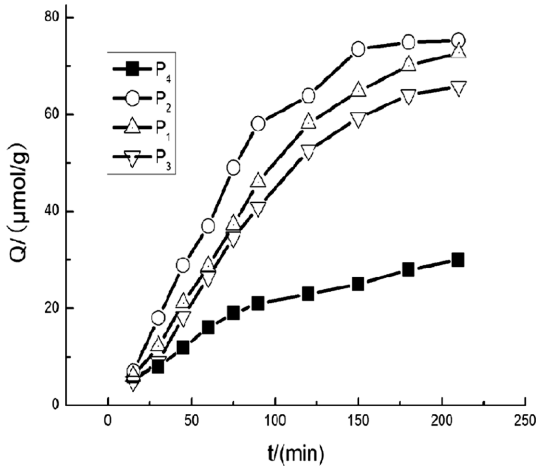


Figure 3. Adsorption kinetics curves of MIPs.

the S-IBF molecule through MIPs became much more difficult. This can be explained that stereo cavities of MIPs weren't well distributed due to copolymerization with cross-linkers, and the depth of cavities were different from each other. The shallow cavities were benefit for the adsorption of MIPs to S-IBF, and presented the rapid adsorption at the initial experiment. This trend indicated that there were some channels in MIPs for the template molecules. The curve of non-selective combined volume to the initial concentration should increase linearly, and it is difficult to reach the saturation stage. From the curves in Figure 3, we might infer that the adsorption of P_4 on S-IBF was non-selective.

Structural Analysis of MIPs. FTIR analysis was conducted to characterize the synthetic polymers' structure and certify the functional groups existing in the molecules. The formation of hydrogen bond effects the stretching vibration of the functional groups and causes the frequency shifted. The identification properties of hydrogen bond can be investigated according to the displacement.[18,19] As shown in Figure 4(b), the observed

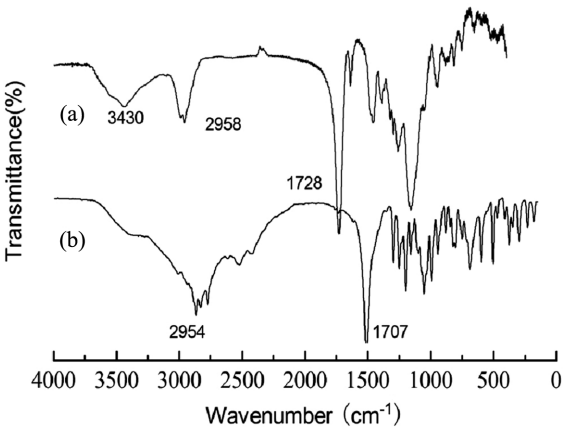


Figure 4. Infrared spectra of (S)-Ibuprofen and polymer: (a) MIP before elution; (b) (S)-Ibuprofen.

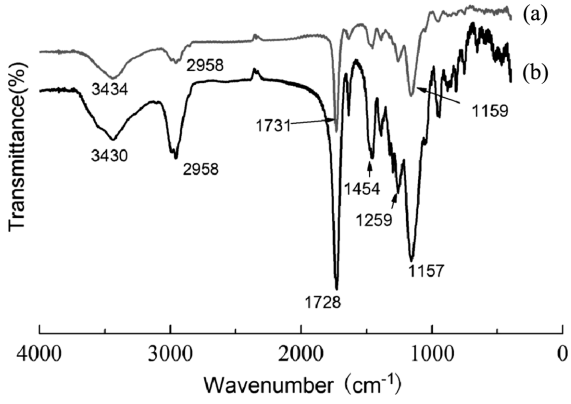
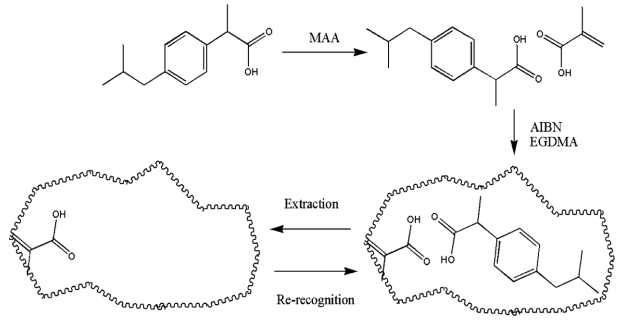


Figure 5. Infrared spectra of polymer: (a) MIPs after elution; (b) MIPs after adsorption.

features around 3000 and 1707 cm^{-1} indicated υ_{O-H} and $\upsilon_{C=O}$, the characteristic peaks of S-IBF. The peaks appeared in fingerprint region represented the vibration peaks of benzene's ring. By comparing (a) with (b) in Figure 4, we can see that the stretching vibration bands were moving to higher frequency, and the absorption intensity and width has increased to some extent. The $\upsilon_{C=O}$, which was blue-shifted to 1728 cm^{-1}, indicate that -C=O of S-IBF formed hydrogen bond with O-H of α-MAA and fixed by EGDMA in copolymerization. While Figure 5 presents the infrared spectra of MIPs after elution and after adsorption. As observed in Figure 5(b), the vibration peaks of S-IBF's and benzene ring in fingerprint region appeared. The $\upsilon_{C=O}$ (1731 cm^{-1}) in MIPs was red-shifted to 1728 cm^{-1} after elution, which indicates that the template S-IBF was successfully imprinted in the polymer and the MIPs successfully re-recognized the template molecule.

In addition, according to the Figure 4 and Figure 5, blue-



Scheme 2. Schematic diagram of MIPs synthesis processing and recognition mechanism.

shifted[20] and red-shifted[21] hydrogen bonds were formed between the templates and functional monomers in the process of self-assembly imprinting and re-recognition, respectively. Based on the principle of "X-H bond lengthen" due to n(Y)-δ(H-X) hyperconjugative and "X-H bond shorten" due to orbital rehybridization, the template molecule and functional monomers were completely pre-assembled. The first step of electron transfer and bond elongation was completed easily by stabilization energy between n(O)→δ(O-H). The second step of O-H bond orbital re-hybrid caused orbital contraction. Therefore, the blue-shifted hydrogen bond was formed between the molecule and functional monomer. However, in the re-recognition process, the template molecule was needed to enter the polymer to combine with the functional monomer. And the spatial structure with orientation and irregular channel left in the polymer limited the interaction between the molecule and functional monomer. So the first step of electron transfer and bond elongation caused by stabilization energy between n(O)→δ(O-H) was completed, and the second step of O-H bond orbital re-hybrid that caused the orbital contraction was hard to reach, therefore, the red-shifted hydrogen bond was formed between the S-IBF and α-MAA. Simultaneously, the template also contained -C=O, the degree of red-shifting was small. Based upon the analysis result of UV and IR, the mechanism of template molecule and functional monomer is shown in Scheme 2.

Selectivity Evaluation of MIPs to Different Substrates. The selective adsorption of MIPs to S-IBF and the other two structurally related compounds (Scheme 1) was evaluated using in this study. The adsorption capacities of P_2 and P_4 to S-IBF, R-IBF and ketoprofen are shown in Table 2. Obviously, P_2 exhibits the highest selectivity to S-IBF compared to the other two compounds, which indicates that P_2 presented specific adsorption to S-IBF due to its matrix matching to S-IBF

Table 2. Values of *K* and α of Different Substrates on MIP, NIP

Substrate	P_2		P_4	
	K	α	*K*	α
(S)-Ibuprofen	29.18	1.00	3.46	1.00
(R)-Ibuprofen	22.62	1.29	3.24	1.06
Ketoprofen	10.27	2.84	3.45	1.12

in shape and reactive groups. But multi-dimensionality of spatial configuration and binding sites of R-IBF and ketoprofen can not match the matrix of MIPs, which caused difficulty for MIPs to diffuse into the matrix. The separation factors (α) of P_4 to R-IBF and ketoprofen were all close to 1.00, which showed P_4's non-specific adsorption to the substrates. And the separation factors (α) of P_2 to R-IBF and ketoprofen were 1.29 and 2.84, respectively.

Conclusions

A series of molecularly imprinted polymers for chiral (S)-ibuprofen were synthesized, and displayed the high specific and selective binding ability toward the template molecules. The influence of cross-linker on the microstructure demonstrated that the pore channels running through the polymer were very useful. According to the UV analysis, the ratio of the host-guest matrix between the template and the functional monomer was 1:1. And IR analysis showed that blue-shifted and red-shifted hydrogen bonds were formed between the template and the functional monomer in the process of self-assembly imprinting and re-recognition, respectively. All of these studies provide evidence of the possible use of (S)-ibuprofen as a template in the field of molecular imprinting technology, and support the promising applications in the field of chiral separation.

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References

1. L. X. Chen, S. F. Xu, and J. H. Li, *Chemical Society Reviews*, **40**, 2922 (2011).
2. Y. H. Jung, J. W. Lee, and Y. T. Yoo, *Polymer(Korea)*, **36**, 434 (2012).
3. H. Q. Shi, W. B. Tsai, M. D. Garrison, S. Ferrari, and B. D. Ratner, *Nature*, **398**, 593 (1999).
4. L. I. Andersson, *J. Chromatogr. B,* **745**, 3 (2000).
5. D. C. Apodaca, R. B. Pernites, R. Ponnapati, F. R. Del Mundo, and R. C. Advincula, *Macromolecules*, **44**, 6669 (2011).
6. C. Alexander, H. S. Andersson, L. I. Andersson, R. J. Ansell, N. Kirsch, I. A. Nicholls, J. O'Mahony, and M. J. Whitcombe, *J. Mol. Recognit.*, **19**, 106 (2006).
7. A. Ameli and N. Alizadeh, *Anal. Biochem.*, **428**, 99 (2012).
8. L. Q. Lin, Y. C. Li, Q. Fu, L. C. He, J. Zhang, and Q. Q. Zhang, *Polymer*, **47**, 3792 (2006).
9. W. R. Bowen and R. R. Nigmatullin, *Sep. Sci. Technol.*, **37**, 3227 (2002).
10. B. U. Ebeshi, K. E. Oseni, A. A. Ahmadu, and J. O. Oluwadiya, *Afr. J. Pharm. Pharmacol.*, **3**, 426 (2009).
11. T. Ezzat, M. A. J. van den Broek, N. Davies, C. H. C. Dejong, A. Bast, M. Malago, D. K. Dhar, and S. Damink, *J. Surg. Oncol.*, **106**, 72 (2012).
12. S. Aitipamula, G. R. Desiraju, M. Jaskolski, A. Nangia, and R. Thaimattam, *Cryst. Eng. Comm.*, **5**, 447 (2003).
13. Y. Kim and J. H. Chang, *Polymer(Korea)*, **36**, 478 (2012).
14. D. K. Kim, S. D. Park, J. W. Oh, and J. B. Kyoung, *Polymer(Korea)*, **36**, 413 (2012).
15. N. Holland, J. Frisby, E. Owens, H. Hughes, P. Duggan, and P. McLoughlin, *Polymer*, **51**, 1578 (2010).
16. K. Y. Zhao, G. X. Cheng, J. J. Huang, and X. G. Ying, *React. Funct. Polym.*, **68**, 732 (2008).
17. S. G. Del Blanco, L. Donato, and E. Drioli, *Sep. Purif. Technol.*, **87**, 40 (2012).
18. R. Gupta and A. Kumar, *J. Sol-Gel Sci. Techn.*, **58**, 182 (2011).
19. I. V. Alabugin, M. Manoharan, S. Peabody, and F. Weinhold, *J. Am. Chem. Soc.*, **125**, 5973 (2003).
20. K. Hermansson, *J. Phys. Chem. A*, **106**, 4695 (2002).
21. S. Scheiner and T. Kar, *J. Phys. Chem. A*, **106**, 1784 (2002).