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Preparation and Characterization of Imprinted Polymers Based on Cobalt(II)-Enrofloxacin Coordination

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Molecularly imprinted polymers of enrofloxacin were investigated firstly with 4-vinylpyridine as functional monomer which based on cobalt(II)-enrofloxacin coordination. The results of equilibrium binding experiments show that the molecularly imprinted polymers had higher binding capacity for enrofloxacin than the control imprinted polymer prepared using the same method but in the absence of both cobalt(II) and enrofloxacin or cobalt(II) ion. The influences of metal ions and pH of solution on the recognition performance of the molecularly imprinted polymers were investigated in protic solvent. Scatchard analysis show that two classes of binding sites existed in the metal complex imprinted polymers, with their maximum apparent binding capacity estimated to be 102.76 μmol g^{-1} and 65.23 μmol g^{-1}, respectively. The results on substrate selectivity of imprinted polymer revealed that the molecularly imprinted polymers had better binding affinity for template among the tested compounds. This type of molecularly imprinted polymers could be prepared in protic solvent because of the relative strong interaction between the template complex and the functional monomer.

Key Words: Molecularly imprinted polymers, Complex, Enrofloxacin.

INTRODUCTION

Molecular imprinting is a process for synthesizing organic polymers that contain recognition sites for special molecules. Molecularly imprinted polymer (MIP) is synthesized through a self-assembling process of functional monomers around template molecules. These monomers are further connected *via* cross-linking to form a three-dimensional (3D) polymer network. Due to the synthesis method, the MIP has its connatural advantages in the recognition of molecules. It can form microcavities and binding sites for the target molecules after removed the template molecules[1]. There is considerable interest in molecular imprinting because of its importance in a broad range of applications[2,3].

The preparation of MIP has been limited to traditional imprinting formulations which rely on hydrogen-bonding interactions between template and functional monomers[4,5]. Although protic solvent such as methanol and water are compatible with free radical polymerization, these have been largely excluded from use in imprinting due to the reason that they can weaken the hydrogen-bond. However, as targets with more biological relevance such as fluoroquinolone and oligonucleotides are identified, these traditional organic formulations are no longer effective or suitable because of their dissolubility.

Metal ions have the ability to bind to functional groups through the donation of electrons to the unfilled orbitals of the outer coordination sphere[6]. The strength of interaction can vary enormously from weak, readily exchangeable bonds to strong bonds which behave like covalent links, depending on the nature of metal ion, its oxidation state and ligand characteristics.

Fujii *et al.*[7] firstly prepared metal complex imprinted polymers. They succeeded in forming a polymer complex gel which discriminates the chirality of amino acid in high optical yield (maximum optical purity = 74 %). Dhal and Arnold[8] reported a novel variation of this polymerization technique to synthesize rigid macroporous polymers containing strategically distributed Cu(II)-iminodiacetate (Cu^{II}IDA) complexes. The resulted polymers exhibit selectivity for bisimidazole "protein analogues". Hart and Shea[9] synthesize MIP for the recognition of peptides using peptide-metal interactions in aqueous solutions. The results indicate that the using water in the polymerization and recognition steps has obvious advantages over organic systems. Wu and Li[10] synthesized simple acids-imprinted polymer based on copper ion coordination, which can be looked upon as a first step towards the solution to the problem of imprinting of those compounds having intramolecular hydrogen bond. Recently, many studies are

focused on the recognition of target molecule using metal coordination interaction[11-14].

To the best of our knowledge, there is no report on the using of coordination interaction in the preparation of fluoroquinolone-imprinted polymers. It is well-known that metal ions coordinate to quinolones and several complexes have been isolated and characterized[15,16]. Earlier studies have pointed out that the metal ions play an intermediary role in the interaction between fluoroquinolone antibiotics and DNA[17], but the mechanism of reaction is not precisely known at this time. And now the metal-complex MIP can be used as the imprinted receptor mimics to understand the basic mechanism of interaction of fluoroquinolone antibiotics with their targets in a biological system[18].

In this paper, we report the synthesis of enrofloxacin-imprinted polymer by using the coordination interaction for recognition of enrofloxacin (ENR) in protic solvent. The MIP was developed by copolymerization of Co(II)-ENR complex with 4-vinylpyridine (4-Vpy) and ethyleneglycoldimethacrylate (EDMA) in the presence of 2,2'-azo-*bis*-isobutylonitrile (ABIN). The characterization of the synthesized material and its recognition mechanism are described and discussed.

EXPERIMENTAL

Spectrophotometry was performed using the T6 UV-visible spectrophotometer (Beijing purkinje general instrument Co., Ltd.). 4-Vinylpridine (4-Vpy) and ethylene glycol dimethacrylate (EDMA) were purchased from Sigma-Aldrich (Shanghai), Trading Co., Ltd. (Shanghai, China), respectively and were freshly distilled to removing inhibitors prior to use. Enrofloxacin (ENR) and ciprofloxacin (CIP) were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 2,2-Azo*bis*isobutyronitrile (AIBN) from Beijing Chemical Reagent Company (Beijing, China) recrystallized prior to use. All the other chemicals were of the analytical or the HPLC grade and used without further disposal.

Preparation of metal complex imprinted polymer: A mixture of cobalt(II) acetate and enrofloxacin was dissolved in 7 mL of methanol and 1.5 mL of toluene for 2 h, then added 1.6 mmol of 4-vinylpyridine (0.2 mL). The formation of the complex was confirmed by spectrophotometric studies. The imprinted polymers were produced as follow. Ethylene glycol dimethacrylate (3 mL) and 2,2-azo*bis*isobutyronitrile (30 mg) were added into the prepolymerization solution. The polymerization mixture were purged with nitrogen gas for 10 min and then sealed and heated in a water bath at 60°C for 24 h. The resultant bulk polymers (MIP1) were ground and sieved and the particles between 45 and 73 μm were collected and washed with methanol-acetic acid (volume ratio, 8:2) over-night to remove the template and then with EDTA solution [5 % in methanol/water (50 %, v/v)]. The removal of enrofloxacin was ensured by the absence of the characteristic absorbance at 278.8 nm. Two control polymer systems were similarly prepared except for the exclusion of cobalt(II) acetate (MIP2) or both enrofloxacin and cobalt(II) acetate (NIP) during the polymerization. The preparation of MIP1 is schematically shown as in Fig. 1.

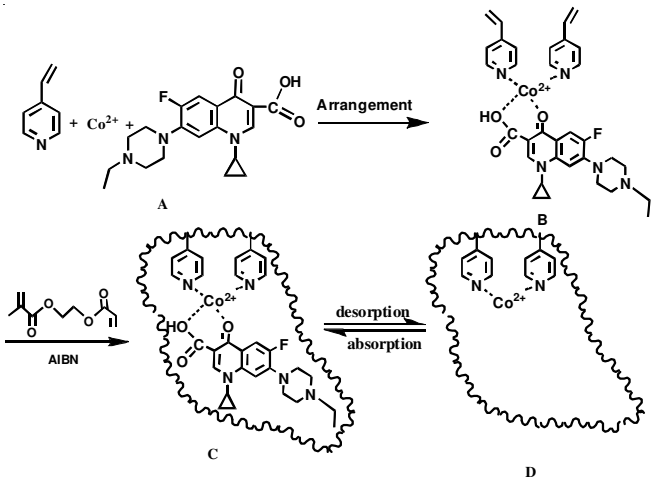

Fig. 1. Schematic representation of the Co(II)-ENR molecule imprinting process. (A) ENR, Co^{2+} and 4-vinylpyridine were allowed to form solution complexes; (B) the complex was fixed using a crosslinking agent (EDMA); (C) locking the complexes in position in the resulting material; (D) recognition sites which are selective for the template structure

Binding experiments of enrofloxacin on molecularly imprinted polymer: 5 mL of ENR solution (2 μmol mL^{-1}) with equivalent metal ion solution was added to 50 mg of polymer in screw-cap vials and then shaked for 10 h and centrifuged for UV measurements at 278.8 nm. The binding capacity Q (μmol g^{-1}) of ENR on MIP was calculated as follows: $Q = V(C_0-C)/m$ where Q represents the binding capacity, V is the volume of initial solution, C_0 and C are the initial and equilibrium concentrations of ligand and m is the mass of polymer sample.

RESULTS AND DISCUSSION

UV characteristics of the prepolymerization solution: A series of solutions was prepared with a fixed concentration of equivalent ENR and metal ion (0.1 μmol mL^{-1}) and various amounts of 4-vinylpyridine (the concentration range 0-0.25 μmol mL^{-1}) in methanol. The UV absorbance of these solutions shown a blue shift near the 278.8 nm absorption band (curve e) which is typical for coordinate bonding effects as the electron clouds density decreased with the formation of the ENR-Co(II)-4-vinylpyridine coordination system (Fig. 2). This indicates that 4-vinylpyridine binds effectively to Co^{2+}-ENR in methanol solution. Formation of a complex coinciding with a 1:2 stoichiometry between Co(II)-ENR complex and 4-vinylpyridine was observed in Fig. 2.

IR spectra: The obtained particles were characterized by IR spectroscopy. Fig. 3 shows the IR spectra of NIP, extracted MIP1 (remove the template) and un-extracted MIP1 (without remove the template). It can be seen that the shape and position of all peaks in IR spectra are exhibited similarly. The peaks of 2956 and 1735 cm^{-1} correspond to absorption bands of $-CH_2-$ or $-CH_3$ and -C=O stretching vibration, respectively. The symmetric and dissymmetry stretching vibration of -C-O-C of ester appeared near 1252 and 1150 cm^{-1}. The band 1636 cm^{-1} in Fig. 2 corresponds to absorption of pyridine ring which is clearly observed in a-c and these peaks are somewhat shifted, indicating the binding of template. The peaks of 1046 and 874 cm^{-1} in Fig. 2(c) which was not find in (a) and (b) also prove

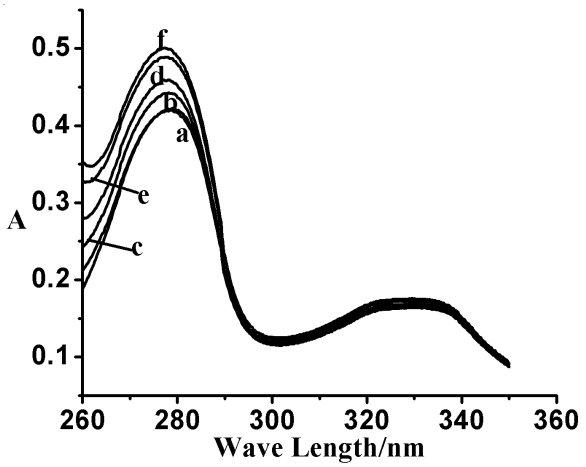

Fig. 2. UV spectrum of Co(II)-ENR-4-vinylpyridine complex in methanol. [Co(II)-ENR] = 0.1 μmol mL^{-1}, with different concentration of 4-vinylpyridine (μmol mL^{-1}), (a) 0; (b) 0.05; (c) 0.1; (d) 0.15; (e) 0.2; (f) 0.25

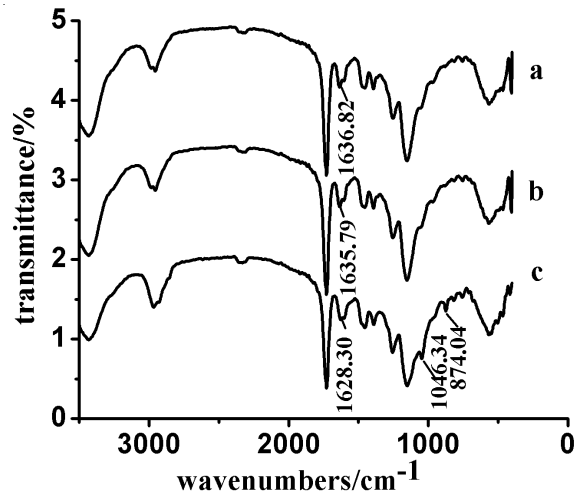

Fig. 3. IR spectra of imprinted and nonimprinted polymers: (a) NIP; (b) extracted MIP1 (remove the template); (c) un-extracted MIP1 (without remove the template)

Co(II)-ENR complex can bind to the MIP1. No obvious bands were present in the region 1648-1638 cm^{-1} indicating the absence of vinyl groups in surface layer of the MIP1, which suggests that both 4-vinylpyridine and EDMA are polymerized.

Influence of concentration of Co(II) acetate on the binding capacity: The influence of concentration of Co(II) acetate on the binding capacity of the polymer was studied. With concentration of ENR fixed at 2 μmol mL^{-1}, the concentration of Co(II) acetate was changed from 0.4 to 2.8 μmol mL^{-1}.

As shown in Fig. 4, the observed binding capacity of ENR was higher for MIP1 than those for the control polymer systems. Lower cobalt ion mediation of ligand binding was observed in the control polymer systems which indicated that the Co(II) plays an bridge-like role in the recognition process and the binding target should be $[Co\text{-}ENR]^{2+}$ with coordination interaction. Optimal binding capacity of MIP1 for ENR was achieved at cobalt ion concentration of 1.8 μmol mL^{-1}. The decrease in capacity above this point indicate that the excess Co^{2+} in the solution competes more successfully on MIP1 than the analyte $[Co\text{-}ENR]^{2+}$.

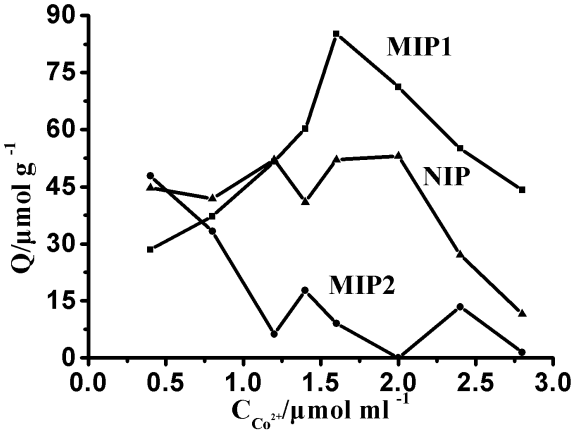

Fig. 4. Influence of Co^{2+} concentration on the binding capacity of MIP1, MIP2 and NIP

Influence of cations and anions on the binding capacity: The binding capacities of ENR on MIP1 were 85.2, 54.42 and 76.76 μmol g^{-1} using Co^{2+}, Zn^{2+} and Cu^{2+} acetate salts as coordinate metal ions, respectively. These ions posses similar ionic radii, $r_{Co(II)}$ = 0.072 nm, $r_{Zn(II)}$ = 0.070 nm, $r_{Cu(II)}$ = 0.071 nm, suggesting that amongst this group ionic size should not be a controlling factor. The effect of different metal ions reveals that this recognition process is not only targeted on the metal-ENR complex but also controlled by the special structure and spatially arranged functional groups of MIP1, which is the result of molecular imprinting.

The influence of anion on the binding capacity of MIP1 was examined by substitution of cobalt chloride and cobalt sulfate for cobalt acetate. The binding capacities are 32.2 and 54.98 μmol g^{-1} with cobalt chloride and cobalt sulfate, respectively. It is observed that the cobalt, acetate, expressed stronger imprinting effect than the others. This demonstrated the acetate ion, had participated in the molecular recognition. So the template recognized by MIP1 was composed of ENR, cobalt ion and acetate. Based on the nature of cobalt(II), it was likely to have six ligands and form an octahedral coordination, so one possible complex with no charge in prepolymerization was composed of two 4-vinylpyridine, one ENR, one cobalt ion and two acetate ions.

Because the anion had participated in recognition process, when anion had been changed, the size and even the shape would be changed due to the differences of the anions and their binding to Co(II). Because Cl^- and SO_4^{2-} were much different from CH_3COO^-, the complex was not suitable for the cavity formed by the cobalt acetate-ENR in the MIP1 and the molecular-imprinting effect was lost. A similar possible complex model recognized by MIP1 was shown in Fig. 5.

Influence of pH on the binding capacity: Since the interaction between the MIP1 and the analyte depends on metal coordination association, the influence of pH of the feed solution on binding performance of polymers was investigated. Cobalt hydroxide precipitation occurs above pH = 6, which also depends on the concentration of cobalt in the medium. However, considering the hydrolysis, pH above 6 was not tested. In this work, a total Co(II) concentration of 2 μmol mL^{-1} with equivalent ENR was employed. As shown in Fig. 6, the binding capacities of MIP1, MIP2 and NIP were influenced by the pH of the

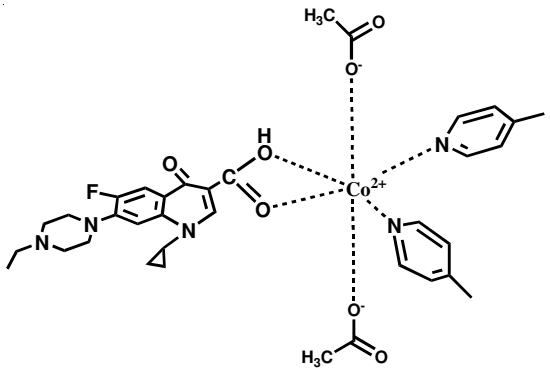

Fig. 5. Proposed octahedral complex formed among Co(II), 4-vinyl-pyridine, enrofloxacin and acetate

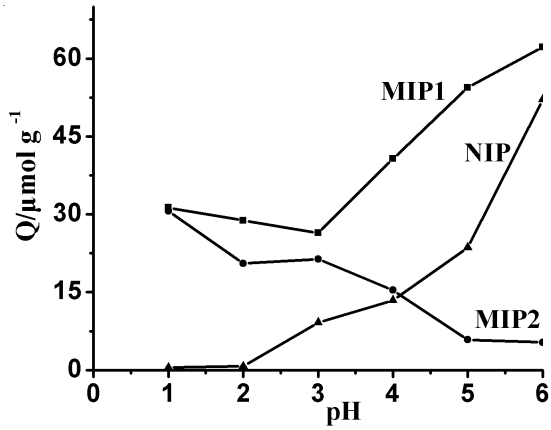

Fig. 6. Influence of pH on the binding capacity of MIP1, MIP2 and NIP

feed solution. With the pH increasing, the binding capacities increased for both MIP1 and NIP, but decreased for MIP2. This result can be attributed to the imprinting effect: the Co (II)-ENR complex can not access to the selective cavity or binding sites created in the MIP2 effectively. It is very important to note that the selectivity could be adjusted by varying the pH of feed solutions, because it is a dynamic transferring process of binding, rebinding and transport.

Binding specificity of Co(II)-ENR imprinted polymers: Binding studies were performed to evaluate the MIP1 uptake of the template. Methanol solutions of Co(II)-ENR complex (the concentration range 0.4-3.2 μmol mL^{-1}) were added to vials containing 50 mg of MIP1. After equilibration, the concentration of unbound ENR was measured by spectrophotometry. The obtained data were plotted with the Scatchard analysis to estimate the binding parameters of the MIP1: $Q/C_f = (Q_{max}-Q)/K_d$ (1) where Q_{max} is the maximum apparent binding capacity and K_d is the equilibrium dissociation constant. As shown in Fig. 7, the Scatchard plot is not linear, indicating that the binding sites in MIP1 are heterogeneous in respect to the affinity for $[Co\text{-}ENR]^{2+}$. Clearly within the plot, there are two distinct sections and two straight lines can be obtained from the linear regression. This indicates that the binding sites in the MIP1 could be classified into two distinct groups with specific binding properties. K_{dl} and Q_{maxl} of higher affinity binding sites can be calculated to be 0.770 μmol mL^{-1} and 102.762 μmol g^{-1} for dry polymer, from the slope and the intercept of the Scatchard plot. K_{d2} and Q_{max2} of lower affinity binding sites were 0.295 μmol mL^{-1} and 65.225 μmol g^{-1}, respectively.

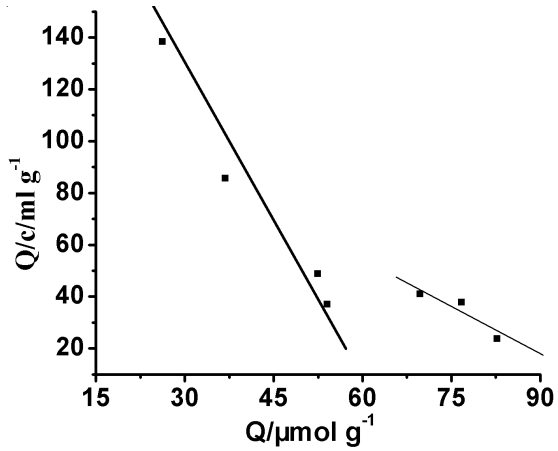

Fig. 7. Scatchard plots of data obtained by rebinding Co(II)-ENR complex to the MIP1

Comparison of the binding behaviour of enrofloxacin and ciprofloxacin: To study the selectivity of the MIP, ciprofloxacin was selected and their binding behaviours were investigated. Table-1 summarizes the data for the binding capacity, distribution coefficient (K_d), selectivity coefficient (k) and the relative selectivity coefficient (k').

Comparison of the k values for the imprinted polymer with the control polymer reveals a significant increase in k for ENR through imprinting. During the preparation of the imprinted polymer, the presence of Co (II)-ENR complex made the ligands arrange orderly. After the removal of Co (II)-ENR complex, the imprinted cavity and specific binding sites of functional groups in a predetermined orientation was formed, whereas steric factors limit accessibility to the sites generated by the smaller ligands, ENR, in the control polymer.

Conclusion

The metal ion-complex imprinted polymer was synthesized through a simple and convenient method. It is interesting to

TABLE-1
COMPETITIVE ADSORPTION OF ENROFLOXACIN (ENR) AND CIPROFLOXACIN (CIP) BY MIP1, MIP2 AND NIP

Polymers	C_0 (μmol mL^{-1})		C_f (μmol mL^{-1})		Capacity (μmol g^{-1})		K_d (mL g^{-1})		k	k'
	ENR	CIP	ENR	CIP	ENR	CIP	ENR	CIP		
MIP1	2.00	2.00	1.43	1.50	57.34	49.74	40.10	33.16	1.21	1.70
MIP2	2.00	2.00	1.73	1.49	27.53	51.19	15.91	34.36	0.46	0.65
NIP	2.00	2.00	1.59	1.47	40.69	52.92	25.59	36.00	0.71	

K_d, distribution coefficient, $K_d = \{(C_0-C_f)/C_f\} \times \{\text{volume of solution (mL)}\}/\{\text{mass of polymers}\}$, where C_0 and C_f represent the initial and free solution concentrations, respectively. k, selectivity coefficient, $K_d(ENR)/K_d(CIP)$. k', relative selectivity coefficient, $k' = k_{imprinted}/k_{non\text{-}imprinted}$. MIP1: molecular imprinting polymer prepared with Co(II)-ENR as template; MIP2: molecular imprinting polymer prepared with ENR as template; NIP: molecular imprinting polymer prepared without template.

note that despite of the use of a protic solvent, methanol, it seems that the obtained imprinted polymer present slightly better rebinding characteristics than those prepared in non protonic solvent. It is also found that acetate ions had participated in recognition process which may improve the spatial selectivity of the imprinted polymer. The high selectivity and affinity available through the use of metal ion mediated ligand binding in MIP1 suggests that their use in application, such as in biosensor and artificial enzyme systems and for separation systems operating in aqueous environments where the use of conventional hydrogen bonding interactions are less competitive.

ACKNOWLEDGEMENTS

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REFERENCES

1. Z.J. Zhao, Q. Wang, L. Zhang and T. Wu, *J. Phys. Chem. B*, **112**, 7515 (2008).
2. K. Lettau, A. Warsinke, M. Katterle, B. Danielsson and F.W. Scheller, *Angew. Chem. Int. Ed.*, **45**, 6986 (2006).
3. T. Takeuchi, D. Goto and H. Shinmori, *Analyst*, **132**, 101 (2007).
4. H.Y. Yan, M.L. Tian and K.H. Row, *J. Sep. Sci.*, **31**, 3015 (2008).
5. C. Zheng, Z.S. Liu, R.Y. Gao and Y.K. Zhang, *Chin. J. Chem.*, **26**, 1857 (2008).
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. Y. Fujii, K. Kikuchi, K. Matsutani, K. Ota, M. Adachi, M. Syoji, I. Haneishi and Y. Kuwana, *Chem. Lett.*, **113**, 1487 (1984).
8. P.K. Dhal and F.H. Arnold, *J. Am. Chem. Soc.*, **113**, 7417 (1991).
9. B.R. Hart and K.J. Shea, *J. Am. Chem. Soc.*, **123**, 2072 (2001).
10. L.Q. Wu and Y.Z. Li, *Anal. Chim. Acta*, **517**, 145 (2004).
11. X.J. Wang, Z.L. Xu, N.C. Bing and Z.G. Yang, *Chin. J. Chem. Eng.*, **15**, 595 (2007).
12. E.H. Papaioannou, M.L. Kyriakides, R.M. Papi and D.A. Kyriakidis, *Mater. Sci. Eng. B*, **152**, 28 (2008).
13. Y.H. Zhai, Y.W. Liu, X.J. Chang, X.F. Ruan and J.L. Liu, *React. Funct. Polym.*, **68**, 284 (2008).
14. M.X. Zheng, S.J. Li and X.J. Luo, *Macromol. Sci. Pure Appl. Chem.*, **44**, 1187 (2007).
15. B. Macias, M.V. Villa, M. Sastre, A. Castiñeiras and J. Borras, *J. Pharm. Sci.*, **91**, 2416 (2002).
16. S.A. Sadeek, *J. Mol. Struct.*, **753**, 1 (2005).
17. L.L. Shen and A.G. Pernet, *Proc. Nati. Acad. Sci. USA*, **82**, 307 (1985).
18. A.J.H. Marshall and L.J.V. Piddock, *J. Antimicrob. Chemother.*, **34**, 465 (1994).