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Specific binding of cholic acid by cross-linked polymers prepared by the hybrid imprinting method

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Abstract

A cholic acid-imprinted polymer has been prepared by a hybrid imprinting method involving the use of a template-containing monomer, 3α-methacryloyl cholic acid methyl ester. The removal of the template can afford the polymer with cavities and carboxylic acid groups well positioned in these cavities for the specific binding of cholic acid via hydrogen bonding. The maximum binding capabilities for the imprinted and non-imprinted polymers were determined to be 344.8 and 43.6 μmol/g, respectively. Scatchard analysis indicates the existence of two kinds of binding sites on the imprinted polymer. The imprinted polymers exhibit high affinity and good recognition selectivity for cholic acid in comparison to other compounds with similar molecular structures. The size-specific binding and hydrogen bonding may be both important for the binding of cholic acid by the imprinted polymers.
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Keywords: Molecular imprinting; Molecular recognition; Cholic acid

1. Introduction

The development of synthetic receptors that recognize steroids is an important area of research because of the significance of steroids to living organisms. Bile acids are important steroidal compounds which are synthesized from cholesterol in the liver, stored in the gall bladder, and released in the small intestine for the digestion of fats and lipids. The concentration of bile acids in body is related with hepatitis, gallstone and other diseases in liver. Bile acids such as ursodeoxycholic acid have clinical significance in the treatment of primary biliary cirrhosis [1] and non-alcohol induced steatohepatitis [2]. The qualitative and quantitative analysis of bile acids has both clinical and pharmaceutical significance. Medically, it is feasible to reduce cholesterol content in body by removing bile acids, especially for the treatment of hyperlipidemia [3,4]. It is thus important to prepare bile acid sorbents with high selectivity for analysis and potential medical applications.

Molecular imprinting has been used for the preparation of polymers with a high affinity for a specific target molecule [5–9]. Covalent or non-covalent molecular imprinting method has been used to prepare imprinted polymers, for instance imprinted polymers with cholesterol [10,11], testosterone [12], corticosteroid [13,14], β-estradiol [15] and cholic acid [16,17]. Hybrid imprinting method [18], where a covalent template structure is used in the polymerization step but binding is entirely non-covalent in nature, combines some of the advantages of both covalent and non-covalent methodologies. This approach has been used here to prepare cholic acid-imprinted polymers, which are useful in the analysis and separation of these compounds of biological significance.

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2. Experimental

2.1. Materials

Cholic acid (CA) was purchased from Tianjin Chemical Reagent Company (Tianjin, China) and purified by re-crystallization in ethyl acetate prior to use. Ethylene glycol dimethacrylate (EGDMA) (technical grade) was purchased from Suzhou Anli Chemical Factory (Suzhou, China). It was extracted three times with sodium hydroxide solution to remove inhibitors, dried over $MgSO_4$ and stored at 4 °C. Azo-bis(isobutyronitrile) (AIBN) was obtained from Nankai University Special Reagent Factory (Tianjin, China) and was recrystallized from methanol before use. Cholesterol was purchased from Biosino Biotechnology Company Ltd. (Beijing, China). All solvents were of analytical grade and used as received.

2.2. Synthesis of polymer sorbents

3α-Methacryloyl cholic acid methyl ester (MECAME) was synthesized from cholic acid methyl ester and methacryloyl chloride according to a method reported previously [19,20].

For the preparation of the molecularly imprinted polymers (MIPs), 0.245 g MECAME (0.5 mmol), 1.98 g EGDMA (10 mmol) and 82 mg AIBN (0.5 mmol) were dissolved in 10 ml of chloroform. The mixture was transferred to a test tube, sealed and kept in a water bath at 60 °C for 24 h. The resultant rigid polymer was ground to particles ranging from 75 to 97 μm in diameter, then suspended in a solution of 2 M NaOH and THF (volume ratio of 1:2) and heated to reflux for 12 h to cleave the templates by hydrolysis. Subsequently the concentration of cholic acid in the solution was determined to calculate the degree of hydrolysis. Thereafter the particles were dried in a vacuum oven.

A reference polymer without imprinting templates was prepared with a similar procedure, in which methacrylic acid (MAA) was used as the monomer instead of MECAME and no hydrolysis was performed.

Polymers cross-linked with DVB were synthesized using the same procedure as for polymers cross-linked with EGDMA.

2.3. Batch binding experiments

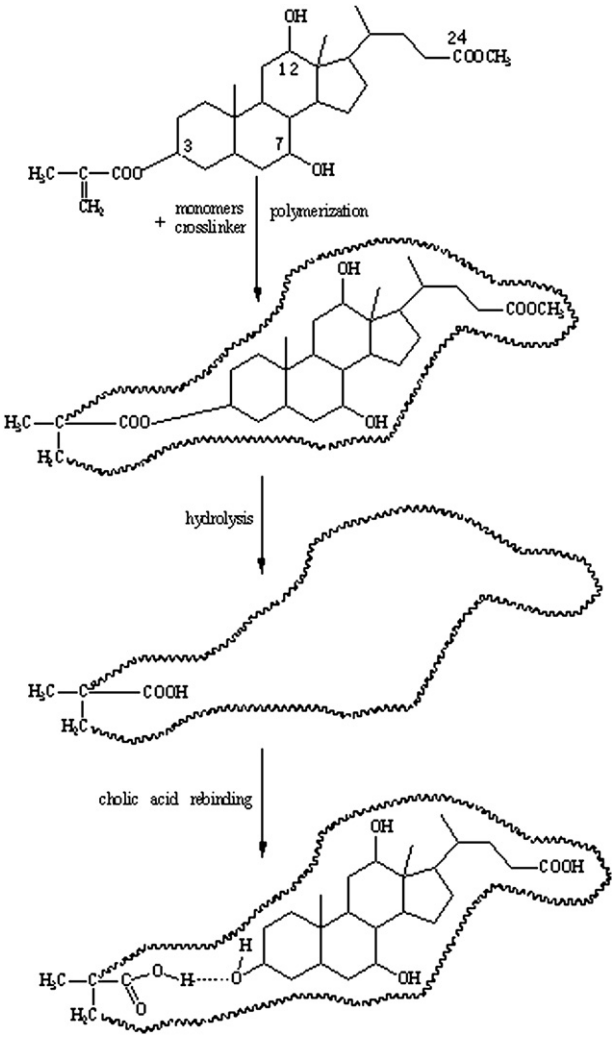
To evaluate the binding ability of the MIPs, a specific amount (20 mg) of a dry polymer was mixed with 3 ml solution of cholic acid with the concentration ranging from 0 to 2.5 mM (in phosphate buffer or ethyl acetate), shaken for a predetermined time at 25 °C to allow the binding equilibrium to be established. The sample was then centrifuged and the cholic acid concentration in the supernatant was determined quantitatively by spectrophotometry after cholic acid reacted with sulphuric acid [21]. A standard calibration curve with the same method was established. This method provides the same results as liquid chromatography in simple solutions but takes less time. The amount of cholic acid adsorbed by unit weight of the polymer (Q) was calculated from the difference between initial and final concentrations of cholic acid.

Equilibrium binding experiments were performed in the same manner with a series of structural analogues of cholic acid (2.5 mM in ethyl acetate) to determine their binding to polymers. Cholesterol was detected by an enzyme kit. Hydroxybenzoic acid and 3,5-dihydroxybenzoic acid were analyzed by UV absorbance on a UV-2100 spectrophotometer (UNICO, China).

3. Results and discussion

3.1. Polymer synthesis

Cholic acid was used since it is one of the major bile acids in the body. The cholic acid-imprinted polymer was prepared by co-polymerization of a mixture of a template-containing monomer (MECAME) and a cross-linker followed by hydrolysis as shown in Scheme 1. The template molecule (cholic acid) linked with the methacrylate monomer covalently. Prior to



Scheme 1. Preparation of the cholic acid-imprinted polymer (MIP) based on poly(3α-methacryloyl cholic acid methyl ester) cross-linked by EGDMA.

polymerization, the monomers bearing the template and the cross-linkers are assembled in an orderly manner with respect to each other. Upon polymerization, the structure was frozen in a three-dimensional network of polymers. The cleavage of the template from the imprinted polymer matrix was performed using a mixture of aqueous NaOH solution and THF. This left specific cavities in the polymer which are topographically complementary to the template and a carboxylic group arranged at the desired site in the cavities which could subsequently interact with the hydroxyl group of cholic acid through hydrogen bonding. Multiple functional groups may help further in the specific recognition process, but even a single functional group in the cavity as in this case has shown to be quite effective in the specific binding process. It is the combined effect of the size and shape of the cavities and the functional group(s) in the cavities that determined the binding specificity.

The complete hydrolysis of covalent bonds in highly cross-linked polymers is difficult. By monitoring the concentration of cholic acid in the hydrolyzed solution, the degree of the hydrolysis of the imprinted polymer was calculated to be about 44.7%, which is consistent with results reported in literature [22]. Prolonged hydrolysis did not improve the result further, likely due to the steric hindrance in the polymer matrix, which is typical in cross-linked polymers.

Imprinting solvent would also affect the properties of imprinted polymer. Several solvents (chloroform, ethyl acetate, toluene, DMSO and MeCN) were used in this work. Imprinted polymers prepared in non-polar solvents had high binding capacities. We can speculate that it may be related to the solvation of the templates by the solvents during the imprinting process and the self-associating property of cholic acid in the different solvents.

3.2. Effect of the cross-linker

Ethylene glycol dimethacrylate (EGDMA) and divinylbenzene (DVB) were used as cross-linkers in the preparation of imprinted polymers. The binding isotherms of the two polymers are shown in Fig. 1. Fitting by the Langmuir equation showed that the EGDMA-based polymer had a higher binding capability (344.8 μmol/g) than the DVB-based material (140.0 μmol/g). Their imprinting factors (IF), defined as the ratio of the maximum binding capacity of the MIP to that of the non-imprinted reference polymer, $IF = Q_{max}(MIP)/Q_{max}(reference)$, were determined to be 7.9 and 6.8, respectively. This result is in agreement with the reports which showed that the EGDMA-based polymers were better than the DVB-based materials in terms of the binding capacity and separation factors [23]. Therefore, EGDMA was selected as the cross-linker for all of the polymers used in the following experiments.

3.3. Kinetic binding studies

The study of binding kinetics can provide information on the time required to reach equilibrium. Kinetic experiments were performed by adding a solution of cholic acid to the MIP and monitoring the concentration of unbound CA at intervals. The amount of CA bound increased over time until it reached a plateau (Fig. 2). This value depends on the concentration of cholic acid and differs from the maximum binding capacity, which is the amount of binding extrapolated to a very high concentration of the substrate. The results of the kinetic binding studies showed that the binding equilibrium could be reached within 3 h for these systems.

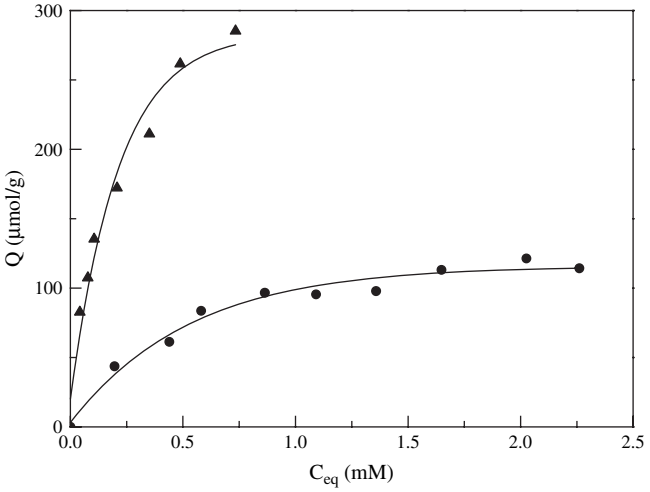


Fig. 1. Binding isotherms for cholic acid by imprinted polymers cross-linked with EGDMA and DVB in ethyl acetate at 25 °C (triangles, EGDMA, circles, DVB). Binding time: 4 h, polymer: 20 mg, $V = 3.0$ ml, initial concentration: 0–2.5 mM.

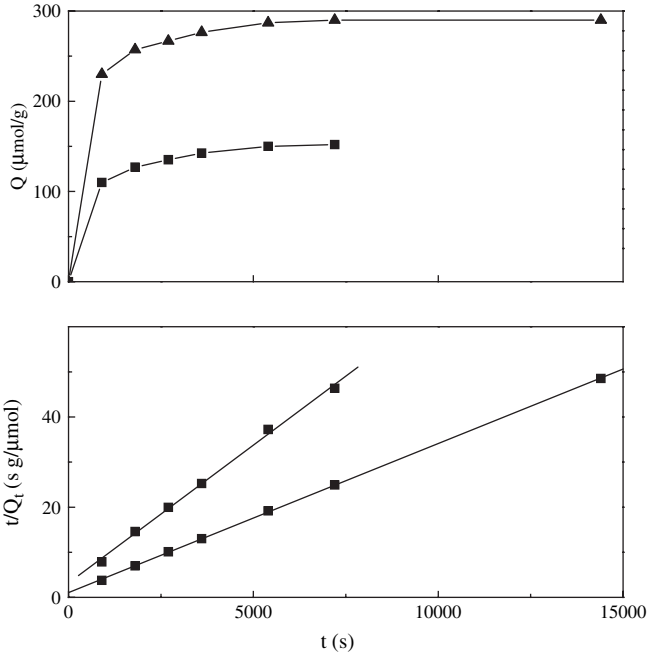


Fig. 2. Binding kinetics for cholic acid by MIP in ethyl acetate and phosphate buffer at 25 °C (triangles, in ethyl acetate; squares, in phosphate buffer). Polymer: 20 mg, $V = 3.0$ ml, initial cholic acid concentration: 2.5 mM.

Two models are often used to describe the adsorption process, the pseudo-first order kinetic model (1) and the pseudo-second order kinetic model (2).

$$-\ln(1-F) = k_1 t \tag{1}$$

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{t}{Q_e} \tag{2}$$

where F is the percentage of the substrate bound, k_1 the rate constant of first order sorption (s^{-1}), t (s) the time, Q_t the adsorption capacity at any time t (μmol g^{-1}), Q_e the adsorption capacity at equilibrium (μmol g^{-1}), k_2 the rate constant of second order sorption (μmol g^{-1} s^{-1}).

The data here provide a good linear relationship for Eq. (1), but the intercepts are not zero. Therefore, the sorption does not really fit well to a pseudo-first order kinetic model. Although this model has been often used, its numerical linear regression may yield tangent and abscissa values in some cases, which will depend on both the initial concentration and on the time intervals [24]. When the pseudo-second order kinetic model was applied to the experimental data, the correlation coefficients reached 0.9999 and 0.9974, respectively, for binding kinetics in phosphate buffer and in ethyl acetate.

3.4. Characteristics of binding for cholic acid by the polymers

Batch binding experiments were carried out to evaluate quantitatively the binding characteristics of the polymers. As shown in Fig. 3, MIP showed a much higher binding capacity than the MAA-based reference polymer. The binding data at equilibrium for the two polymers can fit well to a Langmuir isotherm (Eq. (3))

$$\frac{C_{eq}}{Q} = \frac{C_{eq}}{Q_{max}} + \frac{1}{b} \tag{3}$$

where C_{eq} is the free CA concentration (mM), Q the amount bound at C_{eq} (μmol/g), Q_{max} the maximum binding capacity (μmol/g) and b a constant. The maximum binding capabilities for imprinted and reference polymers are calculated to be 344.8 and 43.6 μmol/g, respectively. The results provide a relative comparison of the different polymers. The imprinting factor of this system is as high as 7.9. The good fit of data to the Langmuir isotherm suggests that a unimodal heterogeneous distribution of binding sites was present in the polymers.

The available binding sites are estimated to be 100 μmol/g from the degree of hydrolysis of the imprinted polymer. The binding capacity of the imprinted polymer calculated from the binding isotherm is higher than this number. Such a phenomenon is not unusual due to the cooperative effect in the binding process of molecules that tend to self-associate. Huval and coworkers [16] prepared bile acid sequestrants by molecular imprinting techniques and showed that the binding capacity of the imprinted polymer was higher than the amount of imprinted cavities. But imprinting created high-affinity

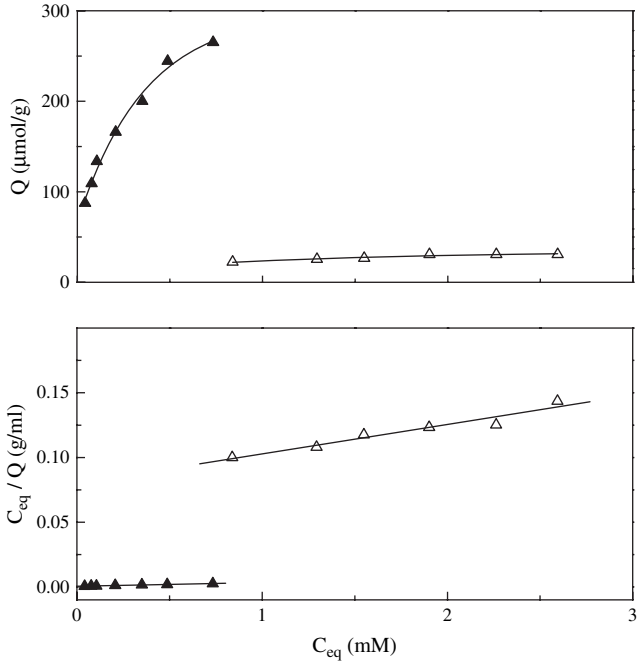


Fig. 3. Binding isotherms for cholic acid by the imprinted (solid symbols) and the reference polymers (open symbols, prepared by co-polymerization of methacrylic acid and EGDMA under the same conditions of MIP) in ethyl acetate at 25 °C. Polymer: 20 mg, $V = 3.0$ ml, initial cholic acid concentration: 0–2.5 mM.

binding sites in the polymers, as demonstrated by *in vivo* studies. It appears that molecular imprinting has imparted an additional binding capacity to the polymers in both covalent imprinting [25] and non-covalent imprinting.

The geometrical location of the binding sites and the relative hydrophilicity/hydrophobicity of the cavities in the polymer render a range of binding affinities of the different sites. The binding may involve both electrostatic and hydrophobic interactions [26]. The increased dielectric constant shifts the balance between electrostatic and hydrophobic interactions. Six solvents with different polarities were tested as the medium (Fig. 4). Ethyl acetate has the similar dielectric constant to that of chloroform which was used to prepare the MIP. It provides a suitable environment for cholic acid to interact with the MIP. When the imprinted polymer was used to recognize cholic acid, in addition to the specific binding defined by the size and shape of the cavities, the carboxyl group provided an extra interaction through hydrogen bonding with the hydroxyl group of cholic acid. This could be impaired by an increase in the polarity of the solvents. Therefore, the amount of CA bound increased with decreasing polarity of the solvents. But in aqueous solutions, the MIP had a high binding capacity for cholic acid, while the binding by MIP is actually lower than that by the non-imprinted reference polymer (Fig. 4), indicating the differences of the binding sites between these polymers.

In addition, the self-associating properties of bile acids also impart a cooperative effect in the binding process. Even this cooperative effect is only induced by the presence

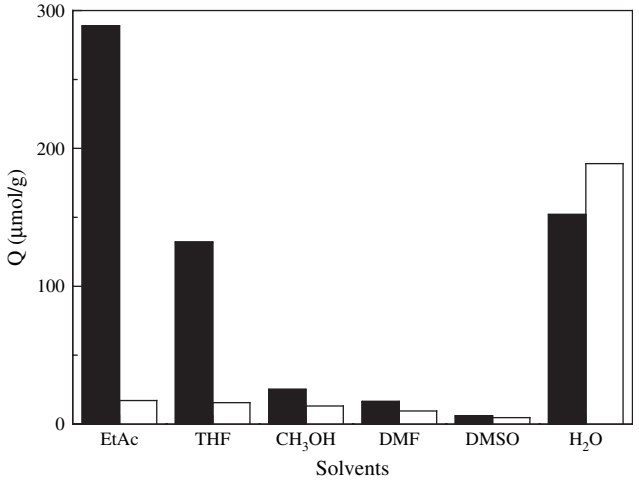


Fig. 4. Comparison of the difference in the binding capacity for cholic acid (including both specific and non-specific bindings) by the imprinted (filled bars) and reference (open bars, prepared by co-polymerization of methacrylic acid and EGDMA under the same conditions of MIP) polymers from different solvents at 25 °C. Polymer: 20 mg, $V = 3.0$ ml, initial cholic acid concentration: 2.5 mM.

of specific binding between the imprinted polymer and bile acids, as evidenced by the significant difference between the imprinted and non-imprinted polymers (Fig. 4). Cholic acid is known to be able to self-aggregate, leading to an apparent higher capacity of the polymer than a simple formation of 1:1 complexes, as one would have expected for an imprinted polymer.

3.5. Determination of binding parameters of the polymer

Scatchard analysis (Eq. (4)) [27,28] is usually used to estimate the binding parameters of polymers:

$$\frac{Q}{C_{eq}} = \frac{Q_{max}}{K_D} - \frac{Q}{K_D} \tag{4}$$

where Q is the amount of bound CA (μmol/g), C_{eq} the concentration of free CA (mM), Q_{max} the apparent maximum number of binding sites (μmol/g) and K_D the equilibrium dissociation constant.

Fig. 5A shows two distinct sections that can be regarded as straight lines in the range of concentrations of CA studied. The coefficients of determination (R^2) are 0.993 and 0.926, respectively. It indicates that there exist two classes of binding sites in the MIP. From the slope and intercept, the equilibrium dissociation constant K_{D1} and the apparent maximum number Q_{max1} of the higher affinity binding sites are calculated to be 7.99×10^{-5} M and 232.4 μmol/g, respectively. K_{D2} and Q_{max2} of the lower affinity binding sites are 6.43×10^{-4} M and 570.7 μmol/g, respectively. From the binding constants, apparent free energy changes (ΔG_0) of these binding sites with high and low affinities were calculated to be −23.4 and −18.2 kJ/mol, respectively. This indicates the existence of two kinds of binding sites: the cavities containing the carboxylic acid group arranged at

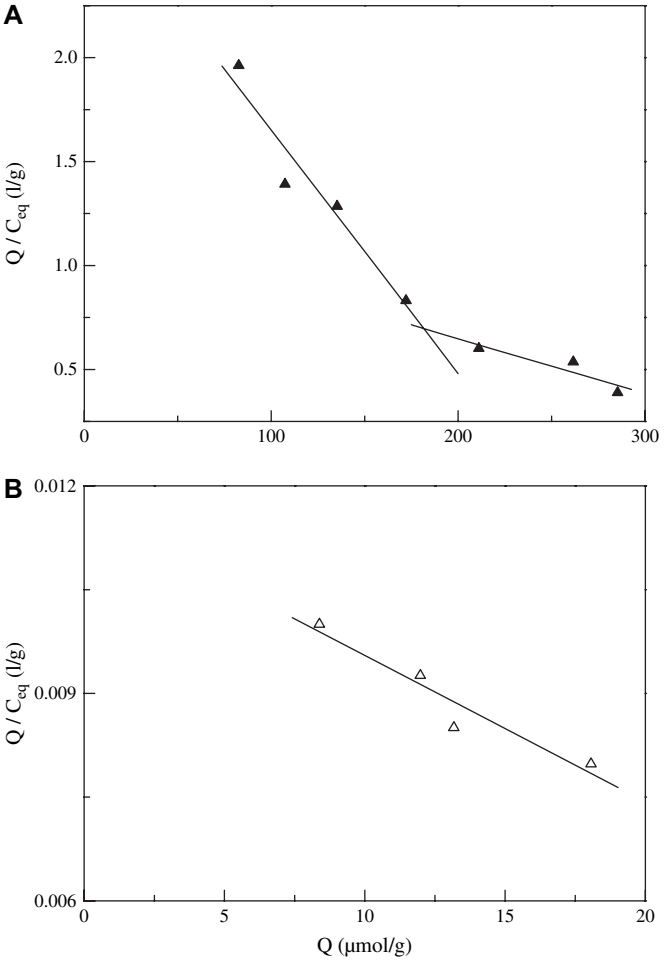


Fig. 5. Scatchard plots for the binding of CA by the imprinted (A) and the reference (B, prepared by co-polymerization of methacrylic acid and EGDMA under the same conditions of MIP) polymers under the same conditions as described in Fig. 3.

the desired sites (high affinity) and binding sites without specific binding (low affinity). According to a report on a MIP imprinted with cholesterol prepared by a similar method, the MIP only bound the guest at 114 μmol/g as analyzed by Scatchard method [29] or 38–50 μmol/g as analyzed by HPLC [30]. In comparison, the cholic acid-imprinted polymer has a higher capacity for cholic acid due to its higher binding affinity for the ligand.

It should be noted that under the same experimental condition there is only one straight line (R^2 is 0.978) for the reference polymer (shown in Fig. 5B) analyzed by Scatchard analysis. The K_D and Q_{max} values for the reference polymer are 3.11×10^{-3} M and 39.8 μmol/g, respectively. The apparent free energy change ΔG_0 is −14.3 kJ/mol. The reference polymer has a lower Q_{max} and a lower binding affinity than the binding sites in the MIP.

3.6. Selectivity of the polymers

A control polymer, prepared without the template molecule, is usually used to verify the imprinting effect. In covalent

imprinting, it is difficult to prepare the control polymers that have the same number of functional groups located in the polymer matrix as in the MIP [22]. In this work a copolymer of methacrylic acid and EGDMA was used as the reference polymer. A set of competing adsorption experiments were carried out with substrates having different chemical structures including deoxycholic acid (DCA), cholesterol (CHOL), hydroxybenzoic acid (HBA) and 3,5-dihydroxybenzoic acid (DHBA) (Scheme 2). Deoxycholic acid has only two hydroxyl groups instead of three on the steroid backbone. Cholesterol is a structurally related molecule and has only one hydroxyl group. HBA and DHBA also have hydroxyl and carboxyl groups but they are smaller in size than cholic acid.

The amounts bound to the imprinted and reference polymers were determined by the equilibrium binding method. IF is used as a reference parameter to describe the selectivity of the polymers. The data in Table 1 show that the imprinted polymer exhibits higher selectivity for cholic acid than for the other analytes, which gives a high IF value of 7.9. Although the reference polymer binds DCA and CHOL in considerable amounts, only the binding of CA is greatly enhanced by molecular imprinting. The MIP also binds HBA and DHBA molecules, but their binding is limited because of the mismatch of spatial arrangement in the cavities. It indicates that the imprinting creates a microenvironment based on the selection of molecular shape and functional groups that resemble those of the template molecule. Both hydrogen bonding and the size of the substrates are important in the binding process. Although the reference polymer has a similar chemical composition as the imprinted polymer, it does not contain any proper cavities and recognition sites. It can only bind the test substrates by non-specific adsorption.

DCA could also be bound in significant amounts, but CA is bound more efficiently than DCA. The MIP can discriminate between the bile acids with different numbers of hydroxyl groups, showing the molecular selectivity of the imprinted polymer. These selectivity tests were accomplished by a batch operation. The guest selectivity is expected to be much higher if the MIP is used for column separation, which is being studied.

Table 1
Binding selectivity of imprinted and reference polymers

Substrate	MIP	Reference polymer[a]	IF
	Q (μmol/g)	Q (μmol/g)	($Q_{MIP}/Q_{reference}$)
CA	285.5	36.0	7.9
DCA	219.6	46.2	4.7
CHOL	19.4	25.6	0.9
HBA	138.4	Not measurable	–
DHBA	101.3	Not measurable	–

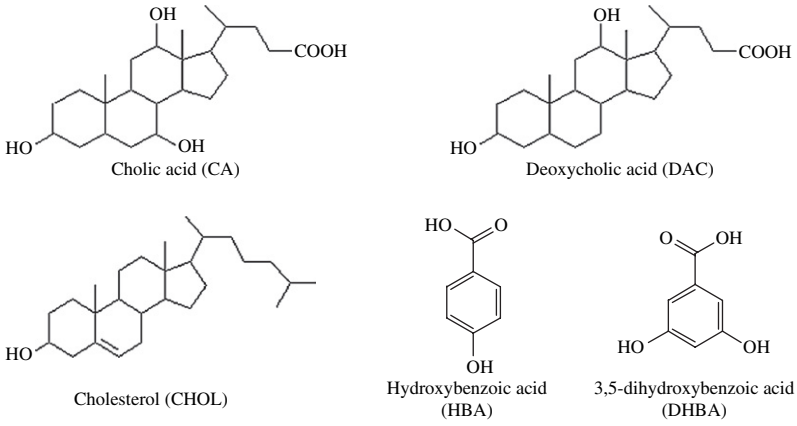
Polymer: 20 mg, $V = 3.0$ ml, initial concentration: 2.5 mM in ethyl acetate, binding time: 4 h.

[a] Reference polymer: prepared by co-polymerization of methacrylic acid and EGDMA under the same conditions of MIP.

The preparation method used here combines the advantages of both covalent and non-covalent imprinting. In covalent imprinting, the imprinted polymers show good specificity in binding, while the non-covalently imprinted polymers have the advantage of fast guest binding. The covalent imprinting in this method helps to avoid the use of the monomer in excess, which is typical in non-covalent imprinting. The cholic acid-imprinted polymer prepared in this work exhibited high affinity for cholic acid.

4. Conclusion

The imprinted polymer prepared by hybrid method exhibited relatively high binding capability and good selectivity for the guest molecule. The selectivity originates from imprinting with templates of a certain shape and certain spatial arrangement of functional groups. This method is a valid way to synthesize imprinted polymers that can selectively recognize the target molecules. The study of binding capacity shows that the cooperative effect in the binding process may be helpful for improving the properties of imprinted polymer. The preparation of MIP with a predetermined selectivity may be useful in the fields of analysis, separation, catalysis and bioassay of related biocompounds and the method may be particularly useful in the preparation of artificial receptors for molecular screening.



Scheme 2. Chemical structures of compounds used in the binding study.

Acknowledgments

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References

[1] Corpechot C, Carrat F, Bonnand AM, Poupon RE, Poupon P. Hepatology 2000;32:1196–9.
[2] Laurin J, Lindor RD, Crippin JS. Hepatology 1996;23:1464–7.
[3] Mandeville WH, Goldberg DI. Curr Pharm Des 1997;3:15–28.
[4] Mandeville WH, Braulin W, Dhal P, Guo A, Huval C, Miller K, et al. Mater Res Soc Symp Proc 1999;550:3.
[5] Sellergren B, Lepisto M, Mosbach K. J Am Chem Soc 1988;110: 5853–60.
[6] Vlatakis G, Andersson LI, Müller R, Mosbach K. Nature 1993;361:645–6.
[7] Wulff G, Sartran A. Angew Chem Int Ed Engl 1995;34:1812–32.
[8] Nishino H, Huang CS, Shea KJ. Angew Chem Int Ed Engl 2006;45: 2392–6.
[9] Alexander C, Andersson HS, Andersson LI, Ansell RJ, Kirsch N, Nicholls IA, et al. J Mol Recognit 2006;19:106–80.
[10] Hwang CC, Lee WC. J Chromatogr A 2002;962:69–78.
[11] Kugimiya A, Kuwada Y, Takeuchi T. J Chromatogr A 2001;938:131–5.
[12] Sreenivasan KJ. J Appl Polym Sci 1998;70:15–8.
[13] Ramstrom O. Chem Biol 1996;3:471–7.
[14] Baggiani C, Giraudi G, Trotta F, Giovannoli C, Vanni A. Talanta 2000;51:71–5.
[15] Wei ST, Molinelli A, Mizaikoff B. Biosens Bioelectron 2006;21: 1943–51.
[16] Huval CC, Bailey MJ, Braunlin WH, Holmes-Farley SR, Mandeville WH, Petersen JS, et al. Macromolecules 2001;34:1548–50.
[17] Idziak I, Gravel D, Zhu XX. Tetrahedron Lett 1999;40:9167–70.
[18] Komiyama M, Takeuchi T, Mukawa T, Asanuma H. Molecular imprinting: from fundamentals to applications. Wiley-VCH Verlag GmbH & Co. KgaA; 2003. p. 19.
[19] Zhang YH, Zhu XX. Macromol Chem Phys 1996;197:3473–82.
[20] Zhu XX, Nichifor M. Acc Chem Res 2002;35:539–46.
[21] Fini A, Zaman P. Collect Czech Chem Commun 1993;58:53.
[22] Ikegami T, Mukawa T, Nariai H, Takeuchi T. Anal Chim Acta 2004;504:131–5.
[23] Wulff G, Vietmeier J, Poll HG. Makromol Chem 1987;188:731–40.
[24] Rudzinski W, Plazinski WJ. Phys Chem B 2006;110:16514–25.
[25] Boonpangrak S, Prachayasittikul V, Bulow L, Ye L. J Appl Polym Sci 2006;99:1390–8.
[26] Rosengren AM, Karlsson JG, Andersson PO, Nicholls IA. Anal Chem 2005;77:5700–5.
[27] Quaglia M, Chenon K, Hall AJ, De Lorenzi E, Sellergren B. J Am Chem Soc 2001;123:2146–54.
[28] Zhang TL, Liu F, Chen W, Wang J, Li K. Anal Chim Acta 2001;450: 53–61.
[29] Whitcombe MJ, Rodriguez ME, Villar P, Vulfson EN. J Am Chem Soc 1995;117:7105–11.
[30] Zhong N, Byun HS, Bittman R. Tetrahedron Lett 2001;42:1839–41.