Preparation and Recognition Mechanism of Gallic Acid Imprinted Polymers

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A series of gallic acid (= 3,4,5-trihydroxybenzoic acid; GA) imprinted polymers were prepared in MeOH by noncovalent imprint technology. Their recognition properties and selectivity were characterized by equilibrium binding experiments. The recognition mechanism of the optimum imprinted polymer MIP_3 which was prepared with the molar ratio 4-VP (= 4-vinylpyridine = 4-ethenylpyridine)/GA 4:1, was explored by UV spectroscopy, *Scatchard*-binding-model analysis, as well as structure-relevant binding. The obtained data consistently indicated that the recognition by MIP_3 was mainly based on the ionic interaction between the COOH group of GA and the pyridine moiety fixed on the matrix of the polymer. MIP_3 showed the maximum specific affinity to GA in H_2O/MeOH 1:1 (*v*/*v*) at an apparent pH of 4.7 (imprinting factor 18.6). Due to the high imprinting efficacy, the optimum polymer MIP_3 can effectively recognize GA in a complex mixture and possesses certainly an important application potential.

Introduction. – Molecular imprinting is a technique of creating recognition sites for a targeted molecule (= template molecule) in a synthetic polymer [1]. The preparation principle of a molecularly imprinted polymer (MIP) is to fix a template–functional-monomer complex into the matrix of the polymer in the presence of a cross-linker. After removal of the template molecule, imprinted sites complementary to the template both in shape and functionality remain in the polymer. Due to their favorable molecular-recognition capability and stability, MIPs have been extensively used as stationary phases for affinity chromatography [2], as artificial antibodies, as sensor components [3], for membrane separation [4], and as adsorbents for solid-phase extraction [5].

Although the molecular-imprinting technique has widely been used for polymer synthesis, few investigations have been developed with the aim to understand the mechanisms and interactions occurring between the template molecule and the functional monomer. However, a thorough understanding of recognition mechanisms is very important to improve the selective recognition of synthesized MIPs.

Gallic acid (= 3,4,5-trihydroxybenzoic acid; GA), a natural plant phenolic acid, is widely used in foods, drugs, and cosmetics because of its versatile biological activity such as its antioxidant, antivirus, antiallergic, and anticancer effects [6]. From the structural viewpoint, GA is a suitable template molecule on account of its rigid structure and multiple functional groups appropriate for typical supermolecular interactions with MIPs such as H-bond and electrostatic interactions. Therefore, the

preparation of GA-imprinted polymers is propitious to the exploration of the recognition mechanisms and the nature of the binding sites in MIPs.

In this work, as a part of our continuous studies on MIPs [7], a series of GA-imprinted polymers were prepared in MeOH with different kinds and amounts of functional monomers. Their recognition properties and selectivity were characterized by equilibrium binding experiments. The recognition mechanism of the optimum MIP, *i.e.*, MIP_3, which was prepared with the molar ratio 4-VP (=4-vinylpyridine=4-ethenylpyridine)/GA 4:1, was explored by UV spectroscopy, by *Scatchard*-binding-model analysis, as well as by structure-relevant binding experiments with H_2O at different pHs as probe. To the best of our knowledge, this is the first report of on MIPs of GA, and the study on its recognition mechanism might be of instructive significance and provide a comprehensive conception for the preparation of highly selective MIPs.

Results and Discussion. – 1. *Optimization of the Imprinted System*. 1.1. *Choice of Functional Monomers*. The choice of functional monomers is essential to maintain stable monomer–template complexes during the imprinting process. As a phenolic acid compound, GA can form an ionic complex with the basic functional monomer 4-VP and exert H-bond interactions on acidic MAA (=methacrylic acid=2-methylprop-2-enoic acid) or neutral AM (=acrylamide=prop-2-enamide). Four imprinted polymers, MIP_1–MIP_4, were synthesized in MeOH with MAA, AM, 4-VP, and the co-monomer composed of AM/4-VP 1:1 as functional monomers, respectively. Their binding amounts for GA compared with the corresponding nonimprinted polymers, $NMIP_1$–$NMIP_4$, were analyzed in equilibrium binding experiments. The results (*Table 1*) showed that (4-VP)-containing polymer MIP_3 gave the best binding affinity for the template. The other MIPs obviously exhibited relatively low binding for the template, because of the absence of significant interaction between the functional monomers and the template molecules.

Table 1. *Binding of GA on MIP_1–MIP_8 Prepared by Different Protocols*[a])

	Template ([mmol])	Functional monomer ([mmol])	Molar ratio	Q_{MIP} [mg/g]	Q_{NMIP} [mg/g]	Q_{MIP}/Q_{NMIP}[b])
MIP_1	GA (1)	MAA (4)	1:4	58.24	34.63	1.68
MIP_2	GA (1)	AM (4)	1:4	61.32	35.10	1.75
MIP_3	GA (1)	4-VP (4)	1:4	73.13	33.38	2.19
MIP_4	GA (1)	AM (2)+4-VP (2)	1:4	59.42	36.83	1.61
MIP_5	GA (1)	4-VP (0.5)	1:0.5	27.74	23.51	1.18
MIP_6	GA (1)	4-VP (1)	1:1	32.56	25.03	1.30
MIP_7	GA (1)	4-VP (2)	1:2	52.78	32.47	1.63
MIP_8	GA (1)	4-VP (6)	1:6	78.07	49.05	1.59

[a]) The binding properties were determined by adding 0.3402 mg of GA in 10 ml of MeOH to 20.0 mg of polymer and equilibration for 6 h. [b]) The imprinting factor (Q_{MIP}/Q_{NMIP}) is the ratio of the binding activity of MIP with respect to that of the NMIP.

In general, MAA or AM interact with a template *via* H-bonds. The 4-VP contains an electrophilic group used as the functional monomer that initiates an ionic interaction between the recognition sites of the polymer and the template. The results of *Table 1*

suggest that the ionic interaction between GA and 4-VP is stronger than the H-bond interaction between GA and MAA (or AM) because of the high acidity of GA. The results also support that templates containing acidic groups are usually best imprinted by using basic, *i.e.*, vinylpyridine, functional monomers [8].

1.2. *Amounts of Functional Monomer*. The molar ratio of the functional monomer to template is important with respect to the number and quality of MIP recognition sites [9]. Too much functional monomer may contribute to high nonspecific binding, while too little will result in a poor yield of imprinted sites due to inadequate stoichiometric complexation of the template. Thus, the adsorption amounts (Q) of GA-imprinted polymers prepared with different amounts of 4-VP, *i.e.*, of MIP_5 – MIP_8, were determined (*Table 1*). The results revealed that the adsorption amounts of the MIPs increased with increasing amounts of functional monomer, but the nonspecific recognition exhibited by the corresponding NMIPs ($NMIP_5$ – $NMIP_8$) also increased gradually. The MIP_3 prepared with the molar ratio 4-VP/GA 4 : 1 exhibited the best specific recognition as compared to other polymers, its imprinting factor (Q_{MIP}/Q_{NMIP}) being 2.19. Therefore, the optimum MIP, MIP_3, was used in the following investigation.

2. *Binding Studies of the Polymer*. The binding isotherm can yield important information concerning binding energies, modes of binding, and site distributions in the interaction of small-molecule ligands with receptors [10]. The binding isotherms for the uptake of GA by MIP_3 and its blank polymer $NMIP_3$ were determined in the range of 0 – 0.6804 mg ml^{-1} GA (initial concentration, c_0). The results in *Fig. 1* showed that the amount of GA (Q) bound to MIP_3 was higher than that to $NMIP_3$, especially in the high-concentration range, indicating an obvious imprinting effect. The isotherm inclined to reach a stable value, showing the characteristics of *Langmuir* isotherms. Thus the obtained data of MIP_3 were fitted to the *Scatchard* equation [6][11] (*Eqn. 1*), where Q (mg/g) is the amount of GA bound to the polymer, Q_{max} is the apparent maximum number of binding sites, K_D is the equilibrium dissociation constant, and [GA] represents the equilibrium concentration of GA. As shown in *Fig. 2*, there are two distinct linear sections within the *Scatchard* plot, indicating the presence of two classes of heterogeneous binding sites in the polymer. From the slope and intercept of the straight line, the K_{D1} and Q_{max1} of the higher-affinity binding sites could be

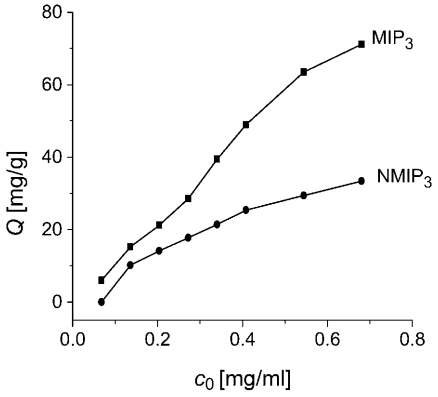

Fig. 1. *Binding isotherm curve for the uptake of GA by MIP_3 and $NMIP_3$.* $M_{polymer} = 20.0$ mg, $V = 10$ ml, 25°, adsorption time 6 h.

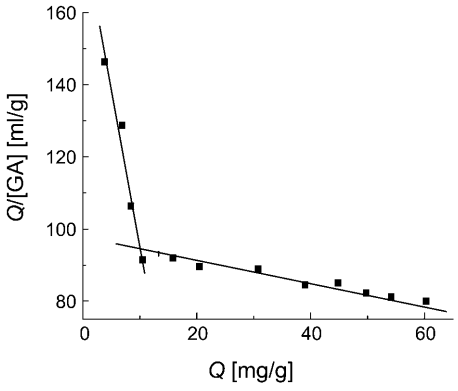

Fig. 2. Scatchard *plot of MIP*$_3$

calculated to be 23.72 μmol l^{-1} and 33.01 μmol g^{-1}dry polymer, respectively. Similarly, the K_{D2} and Q_{max2} of the lower-affinity binding sites were 0.49 mmol l^{-1} and 137.59 μmol g^{-1}, respectively. Compared to other known imprinted polymers [6], the MIP_3 showed higher absorption capacity and a relatively higher percentage of low-affinity binding sites, which may be ascribed to the multiple polar functional groups in the GA molecule and its relatively small molecule volume.

$$Q/[\mathrm{GA}] = Q_{max}/K_D - Q/K_D \quad (1)$$

3. *Spectroscopy Analysis.* To gain more insight into the origin of binding sites in the optimum polymer, the UV absorption spectrum of GA in the presence of 4-VP was measured in MeOH and compared with that of a MeOH solution of pure 4-VP. As a control, the spectrum of GA in the presence of MAA was also measured. On addition of MAA (*Fig. 3,a*), the first absorption peak of GA (B band, at 214 nm) was obviously red shifted with concomitant gradually decreased intensity. Also the second peak (E band, at 272 nm) was slightly red shifted while the absorbance increased. These changes may be ascribed to H-bonding interactions between the phenolic OH groups of GA and the COOH group of MAA. As a consequence of the conjugation effect of the phenolic O-atoms, the bands B and E of GA were shifted to the red with different emission probability [12]. Interestingly, GA showed a very different UV spectrum in the presence of 4-VP (*Fig. 3,b*). Its B absorption band experienced almost no shift on addition of 4-VP but its intensity considerably decreased, indicating another, different, and stronger interaction in the GA/4-VP system. The most plausible explanation for this is the electrostatic interaction between the COOH group of GA and the pyridine moiety of 4-VP. The isolated COOH group is a strong electron-withdrawing group, which reduces the electron density in the benzene ring greatly, resulting in an E-band shift to the violet as well as in a decreased absorption intensity of the B band [12]. This implies the involvement of electrostatics in the GA–(4-VP) complex.

To characterize the GA–(4-VP) complex, a theoretic analysis according to [6][13] was carried out. For a reaction of template A with a functional monomer B to a complex C, *Eqn. 2* holds. If the concentration of A (a_0) is much smaller than that of B (b_0), the equilibrium concentration of B should be approximated as b_0, so the complex

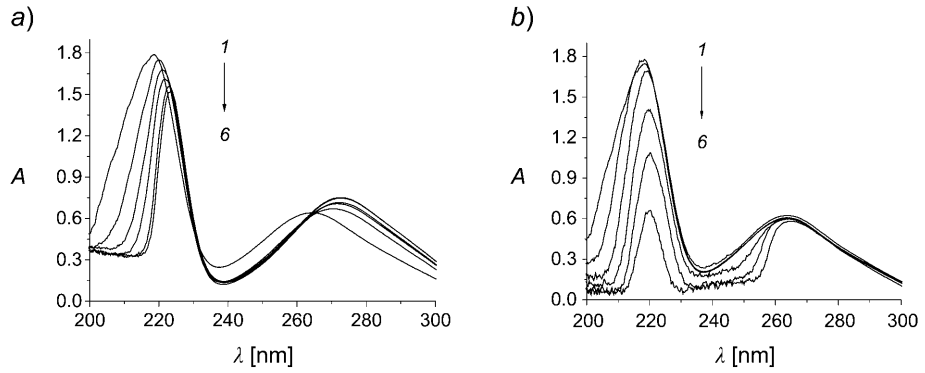

Fig. 3. *UV Difference spectra* (MeOH) *of gallic acid* (GA) *in the presence of* a) *MAA and* b) *4-VP*. [GA] = 0.08 mM, [MAA] or [4-VP] = 0, 0.08, 0.16, 0.24, 0.32, and 0.40 mM (for *1* to *6*); corresponding MeOH solutions of pure MAA or 4-VP were used as blanks.

concentration (c) can be calculated according to *Eqn. 3*, where K is the association constant, with $n = 1, 2, 3\ldots$ The absorbance difference ΔA of the mixture measured against B can be expressed by *Eqn. 4*. The absorption A_0 of pure A is given by *Eqn. 5*, and the difference between ΔA and A_0 by *Eqn. 6*, where $\Delta\varepsilon = \varepsilon_C - \varepsilon_A$. Substituting *Eqn. 6* into *Eqn. 3* yields *Eqn. 7*.

$$A + nB = C \tag{2}$$

$$c = \frac{a_0 b_0^n K}{1 + b_0^n K} \tag{3}$$

$$\Delta A = A_A + A_C = [(a_0 - c)\varepsilon_A + c\varepsilon_C]l \tag{4}$$

$$A_0 = a_0 \varepsilon_A l \tag{5}$$

$$\Delta\Delta A = \Delta A - A_0 = (a_0 - c)\varepsilon_A l + c\varepsilon_C l - a_0\varepsilon_A l = c\Delta\varepsilon l \tag{6}$$

$$\frac{\Delta A}{b_0^n} = -K\Delta A + K\Delta\varepsilon a_0 l \tag{7}$$

$\Delta A/b_0^n$ was plotted *vs.* ΔA of the respective solution measured at 214 nm, and a good linear relationship at $n = 1$ with regression equation was $\Delta A/b_0 = 488.9 - 1968.6 \cdot \Delta A$ ($R = 0.9917$) in the 4-VP concentration range 0.08 – 0.40 mmol/l. The result implied that a 1 : 1 complex was produced between GA and 4-VP. It is reasonable that only the COOH group in GA can form an ionic interaction with the pyridine moiety of 4-VP. Although the phenolic OH groups of GA might form H-bonds with the pyridine moiety of 4-VP, its intensity was much lower than that of the electrostatic interaction between GA and 4-VP. The possible structure of the GA – (4-VP) complex is shown in *Fig. 4*.

4. *Binding Experiments in H_2O-Containing Solution at Different pH*. The effect of the solvent on the recognition ability of MIPs in the adsorption solutions is different

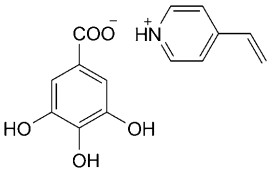

Fig. 4. *Possible structure of the GA – (4-VP) complex*

according to their recognition mechanism. Commonly, a H-bonding interaction is favored by low-polarity solvents, whereas ion-pair and other strong dipolar interactions are favored by polar solvents. To establish the ionic-interaction mechanism of MIP_3, further binding studies were carried out in the presence of the competitive agent H_2O at different pHs as molecular probe.

The effect of the H_2O content in the adsorption solution on the binding capacity of MIP_3 towards GA is shown in *Fig. 5*. With the increase of H_2O content, of the GA taken up by amount both MIP_3 and $NMIP_3$ decreased, but in the case of $NMIP_3$, the decrease was more important, resulting in a relative increase in the recognition ability of MIP_3. When the H_2O content was 50% (*v*/*v*), the difference between MIP_3 and $NMIP_3$ reached a maximum, showing the maximum imprinting effect (imprinting factor 18.6). This result indicated that the imprinted polymer, *via* an ionic interaction mechanism, may recognize quite well a targeted molecule in aqueous solution.

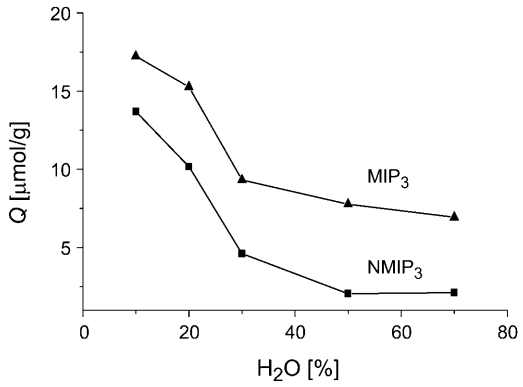

Fig. 5. *The amount* Q *of GA adsorbed on MIP_3 and $NMIP_3$ from H_2O-containing solutions.* $M_{polymer}$ = 20.0 mg, C_0 = 2.0 mmol l^{-1}, V = 10 ml, 25°, adsorption time 6 h

The amounts of GA taken up by MIP_3 were measured at the apparent pH values 2.0, 4.7, and 8.0 of the adsorption solution containing 50% H_2O in MeOH. The results revealed a greater adsorption in acidic solution (pH 2.0) with an adsorbed amount of 119.0 mg/g and a decreased affinity at pH 8.0 with 46.0 mg/g. The maximum affinity was achieved at pH 4.7 with an adsorbed amount of 129.0 mg/g. This might be related to the presence of different forms of the template and functional groups at different pH values. At pH 2.0, the pyridine moiety in the polymer is protonated. As an electron-deficient group, it can bind the electron-rich GA molecule *via* $\pi-\pi$ dusting effects, but not *via* an electrostatic interaction. While in basic solution (pH 8.0), the GA molecule is present in its anion form, it is impossible to form an ionic bond between GA and the

polymer because of the electrostatic repulsion between them which greatly reduces the binding capacity of the polymer for GA; thus the lowest affinity was obtained at pH 8.0. On the contrary, at pH 4.7, GA and 4-VP are present mainly in their neutral form ($pK_a = 4.7$ and 5.6, resp.), allowing an electrostatic interaction of sufficient strength between GA and 4-VP; thus the maximum affinity was achieved. These results are consistent with those reported previously [14]. This implied that ionic interaction was the predominant force for the recognition of GA by MIP_3, and an improved recognition and separation may be obtained by changing the pH of the sample solution.

5. *Recognition Selectivity Studied by HPLC*. The competitive selectivity of the optimum MIP, *i.e.*, of MIP_3, was evaluated with a series of template substrates with similar structure, namely GA and its six analogues 2,5-dihydroxybenzoic acid, 2,4-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, salicylic acid (=2-hydroxybenzoic acid), benzoic acid, and benzene-1,3-diol. Standard mixture solutions of 0.1 mg of each substrate in 1 ml of MeOH were subjected to adsorption by MIP_3 and $NMIP_3$ for 6 h. After filtration, the solutions were analyzed by HPLC under the established optimum conditions (see *Exper. Part.*, *Sect. 5*). The equations of the working curves with good linear relation for the seven substrates (*Table 2*) were obtained by using the HPLC-peak area as dependent variable.

Table 2. *Equations of the Working Curves for the Substrates Gallic Acid and Six Analogues* ($n = 5$)

	Regression equation[a])	Correlation coefficient r	R.s.d.[b]) [%]
Gallic acid	$A = -471146.55 + 668382.17C$	0.9990	0.73
Benzene-1,3-diol	$A = -111959.77 + 252012.742C$	0.9996	0.45
2,5-Dihydroxybenzoic acid	$A = -708058.7 + 451887.98C$	0.9991	1.28
2,4-Dihydroxybenzoic acid	$A = -168670.84 + 447199.292C$	0.9997	1.36
3,4-Dihydroxybenzoic acid	$A = -212094.61 + 489668.658C$	0.9997	1.11
Benzoic acid	$A = -158373.59 + 343433.786C$	0.9996	0.84
Salicylic acid	$A = -378419.2 + 626343.96C$	0.9995	1.69

[a]) C and A in the regression equation represent the concentration of substrate in μmol/ml and the area of the HPLC peak, resp. [b]) R.s.d. = relative standard deviation.

The adsorption capacities of MIP_3 towards various substrates are summarized in *Fig. 6*. As expected, MIP_3 showed the maximum adsorption for GA. The adsorbed amounts of the other six analogues decreased in the order 3,4-dihydroxybenzoic acid > 2,4-dihydroxybenzoic acid > 2,5-dihydroxybenzoic acid > salicylic acid > benzoic acid > benzene-1,3-diol, consistent with the similarities in the structures. This order suggested that the steric effect may be a recognition drive force for MIP_3, but the ionic interaction between the substrates and 4-VP is more important than the steric effect for the binding ability of MIP_3. Compared with benzene-1,3-diol, benzoic acid exhibited a stronger specific affinity to MIP_3, indicating that the COOH group in the substrate contributed more to the recognition of MIP_3 than the OH groups. These results furthermore revealed that the ionic interaction between COOH of GA and the pyridine moiety fixed on the polymer is the predominant recognition drive force of MIP_3.

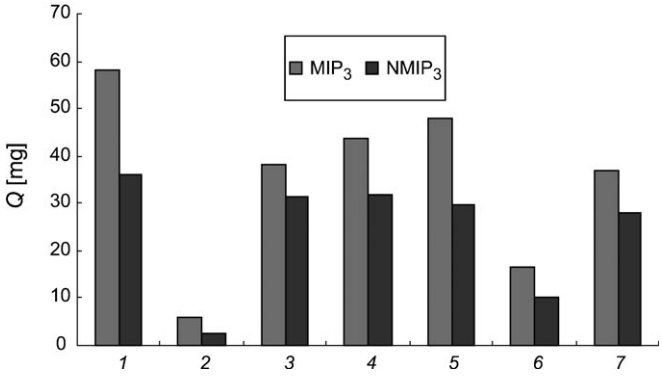

Fig. 6. *Adsorbed amounts* Q *of different substrates by MIP$_3$ and NMIP$_3$. 1* gallic acid, *2* benzene-1,3-diol, *3* 2,5-dihydroxybenzoic acid, *4* 2,4-dihydroxybenzoic acid, *5* 3,4-dihydroxybenzoic acid, *6* benzoic acid, and *7* salicylic acid. Standard mixture solutions of each analogue with 0.1 mg/ml were adsorbed by MIP$_3$ and NMIP$_3$ for 6 h, $V = 10$ ml.

By comparison, the NMIP$_3$ showed a similar adsorption capacity to most substrates, except for benzene-1,3-diol and benzoic acid, indicating a lower recognition selectivity as compared to MIP$_3$ (*Fig. 6*). These observations established the application potential of MIP$_3$ for a complex-mixture system.

6. *Application to Complex Samples.* To explore the application potential of the polymer in the case of a real complex mixture, MIP$_3$ was used as an adsorbent to enrich GA directly from the MeOH extract of the traditional Chinese medicine *Cornus officinalis*. The concentration of GA was analyzed by the HPLC method established in the *Exper. Part* (*Sect. 6*). As a control, the HPLC of the initial extract solution before adsorption was used. The graphs shown in *Fig. 7* revealed that MIP$_3$ could specifically recognize GA with a moderate cross-reactivity effect. The concentration of GA in the initial solution was 39.2 ± 0.67 μg ml^{-1}. After adsorption by MIP$_3$, the concentration of GA in the residual solution decreased to 23.3 ± 0.51 μg ml^{-1}. The ratio of GA adsorption by MIP$_3$ was calculated as 40.5% (r.s.d. 2.6%, $n = 3$). These preliminary

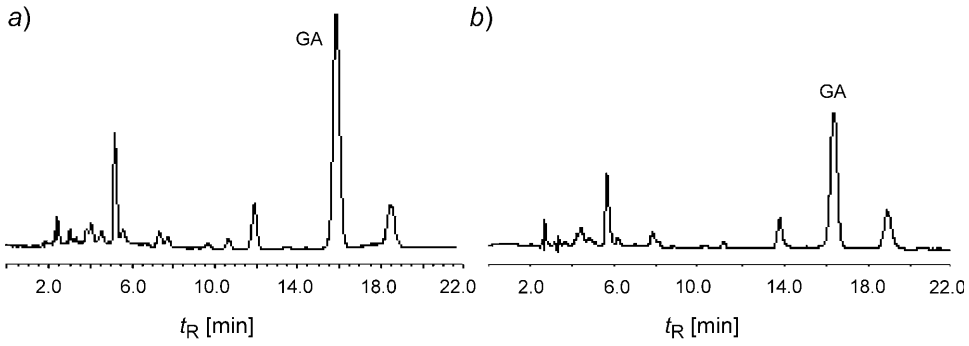

Fig. 7. *HPLC of the MeOH extract from* Cornus officinalis: a) *the initial extract solution and* b) *the residual solution after adsorption by MIP$_3$.*

results established the application potential of MIP_3 as a selective adsorption material to enrich and determine traces of GA in complex samples.

Conclusions. – A series of GA-imprinted polymers were prepared in MeOH with different kinds and amounts of functional monomers. The polymer MIP_3 prepared with 4-VP in a ratio 4-VP/GA 4:1 exhibited the optimum recognition properties. The mechanistic studies revealed that an ionic interaction between the COOH group of GA and the pyridine moiety of 4-VP may be the predominant recognition drive force in the recognition process of the GA-imprinted polymer. MIP_3 showed the maximum specific affinity to GA in 50% H_2O-containing MeOH solution at an apparent pH 4.7 (imprinting factor 18.6). MIP_3 can effectively discriminate GA in a complex sample system, possessing the potential of a selective adsorbent to enrich and determine trace amounts of GA in complex samples. These results may help to understand the recognition process of MIPs and the design of reliable and predictable high-quality MIPs.

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Experimental Part

1. *General*. Gallic acid (= 3,4,5-trihydroxybenzoic acid; GA) was purchased from *Tokyo Kasei Kogyo Co. Ltd.* (Japan); 2,4-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, and 2,5-dihydroxybenzoic acid were purchased from *Sigma Co.* (Germany); 4-vinylpyridine (=4-ethenylpyridine; 4-VP) was purchased from *Acros Organics Co.* (USA); benzoic acid, benzene-1,3-diol, and acrylamide (= prop-2-enamide; AM) were purchased from the *Shanghai Chemical Reagents Plant* (China). Ethylene glycol dimethacrylate (=2-methylprop-2-enoic acid 1,1′-(ethane-1,2-diyl) ester; EDMA; *Shanghai Coral Chemical Plant*, China) and methacrylic acid (=2-methylprop-2-enoic acid; MAA; *Suzhou Anli Chemical Plant*, China) were purified by distillation before use. Azobis[isobutyronitrile] (=2,2′-azobis[2-methylpropanenitrile]; AIBN; *Shanghai Fourth Reagent Plant*, China) was recrystallized from EtOH before use. All other reagents were used without further purification. Solvents were of anal. grade. HPLC: *Waters* system (USA) equipped with a *Waters-1525* pump, *Waters-2996* UV/VIS detector, and *Waters-717* automatic injector; *Empower* workstation (*Waters*, USA); reversed-phase *C18* column (250 mm × 4.6 mm, 5 μm, *SymmetryPrep*™). UV Spectra: *Shimadzu-UV-2401* double-beam spectrophotometer (Japan); *HZ* constant-temp.-bath oscillator (China); λ_{max} in nm.

2. *Ultraviolet Spectrum Characteristics.* A series of solns. containing various amounts of MAA (or 4-VP) and a fixed amount of GA in MeOH were prepared, and their difference UV absorption spectra were determined against a corresponding functional-monomer solution.

3. *Preparation of Polymers.* The required amounts of functional monomers were dissolved in MeOH (10 ml) with a certain amount of template GA (1 mmol, 0.1701 g). After shaking for 3 h, cross-linker EDMA (5.7 ml, 30 mmol) and initiator AIBN (30 mg, 0.18 mmol) were added. The mixture was sparged with N_2 for 10 min, and then sealed under vacuum. The polymerization was initiated in a water bath at 60° for 24 h. The resulting polymer was grounded and sieved to collect 75 μm particles. The material was then placed in a *Soxhlet* apparatus, and washed with 10% (*v*/*v*) AcOH/MeOH until GA could no longer be detected at 272 nm by spectrophotometry in the eluate. The particles were then washed with MeOH to remove residual AcOH and dried to constant weight under vacuum. The compositions of the prepared polymeric matrices $MIP_{1–8}$ are given in *Table 1*.

As a control, the nonimprinted blank polymers $NMIP_{1–8}$ were also prepared and treated in the same manner, except that the template was not added.

4. *Equilibrium Binding Studies by UV Spectrophotometry.* Polymer particles (20.0 mg) were placed in a conical flask and mixed with 10 ml of the GA soln. of different concentration. The mixture was oscillated in a constant-temp.-bath oscillator at 25° for 6 h. The adsorption solns. were filtrated over dry filter paper, and the absorbance A of free GA in the soln. was determined by UV spectrometry at 272 nm. The concentration of GA (c, mg/ml) was obtained from its linear equation in MeOH ($A = 3.675C + 1.925$, $R = 0.9999$, r.s.d. = 0.67). The binding capacity (Q), defined as milligram of substrate bound per 1 g of polymer, was calculated by subtracting the concentration of free substrate from the initial concentration. The average values of triplicate independent results were obtained.

The adsorbed amounts of GA by MIP_3 in solns. of different pH and soln. composition were evaluated by the same procedure. To eliminate the error due to the isolation of GA in the presence of a polar additive, the equilibrium concentration of GA was calculated by the single-spot calibration method comparing with the corresponding initial standard soln.

5. *Competitive Selectivity by HPLC Analysis.* MIP_3 or $NMIP_3$ (20.0 mg) was mixed with 10.0 ml of a standard mixture constituted of gallic acid, 2,5-dihydroxybenzoic acid, 2,4-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, salicylic acid, benzoic acid, and benzene-1,3-diol (0.1 mg/ml, each) under vibrating at 25° for 6 h. Then, the soln. was filtered through a 4.5 μm membrane. The filtrate was analyzed by HPLC (*C18* column, column temp. 30°, MeCN (0.1% CF_3COOH)/H_2O 40 : 60 (*v*/*v*), flow rate 1.0 ml/min, monitoring at 210 nm). An efficient separation of GA and other components in the standard-mixture soln. and in the real-sample soln. was achieved under these conditions.

6. *Complex-Sample Analysis.* A 10.000 g crushed *Cornus officinalis* SIEB. et ZUCC (purchased from the *Juehuachun Chinese Herb Market*, Kunming, China) was extracted twice with MeOH (50 ml) at 85° for 2 h. The combined extract was concentrated to 50 ml. The polymer and crude-extract soln. were mixed in a 20 ml beaker and shaked for 5 h. The filtrate was examined by HPLC (see *Sect. 5*, except for eluent; MeCN (eluent *A*) and 0.4 % (*v*/*v*) phosphoric acid in H_2O (eluent *B*) mixtures, *i.e.*, first 100% *A* → 80% *A* within 5 min, then 80% *A* from 5 to10 min, and then 80% *A* → 0% *A* from 10 to 50 min). The system was equilibrated for 10 min before the next injection. Under these condition, GA could be isolated from the mixture with other disturbing components with the calibration curve $A = -264252.7 + 3610785.9\,C$ ($R = 0.9992$, r.s.d. = 0.86) in the concentration range 0.05 – 0.500 μg/ml.

REFERENCES

[1] K. Haupt, K. Mosbach, *Chem. Rev.* **2000**, *100*, 2495; E. Piletska, S. Piletsky, K. Karim, E. Terpetschnig, *Anal. Chim. Acta* **2004**, *504*, 179.
[2] P. T. Vallano, V. T. Remcho, *J. Chromatogr., A* **2000**, *887*, 125.
[3] T. A. Sergeyevaa, S. A. Piletskya, A. A. Brovkob, *Anal. Chim. Acta* **1999**, *392*, 105.
[4] A. Kamal, B. A. Kumar, M. Arifuddin, S. G. Dastidar, *Bioorg. Med. Chem.* **2003**, *11*, 5135.
[5] F. Puoci, M. Curcio, G. Cirillo, F. Iemma, U. G. Spizzirri, N. Picci, *Food Chem.* **2008**, *106*, 836.
[6] G. Lancelot, *J. Am. Chem. Soc.* **1977**, *99*, 7038; A. Pietrzyk, R. Wiley, D. McDaniel, *J. Org. Chem.* **1957**, *22*, 83.
[7] G.-S. Wang, Q.-E Cao, Z.-T. Ding, Y.-G. Wang, M.-H. Yang, *Helv. Chim. Acta* **2007**, *90*, 1179; G.-S. Wang, Q.-E Cao, J. Xiong, X.-F. Zhu, N.-B. Hou, Z.-T. Ding, *Helv. Chim. Acta* **2006**, *89*, 3032; X.-F. Zhu, Q.-E Cao, N.-B. Hou, G.-S. Wang, Z.-T. Ding, *Anal. Chim. Acta* **2006**, *561*, 171.
[8] C. Y. Hung, Y. T. Huang, H. H. Huang, C. C. Hwang, *J. Appl. Polym. Sci.* **2006**, *102*, 2972; B. W. Sun, L. Q. Wu, Y. Z. Li, *Acta Chim. Sin.* **2005**, *62*, 598.
[9] H. S. Andersson, J. G. Karlsson, S. A. Piletsky, K. Mosbach, *J. Chromatogr., A* **1999**, *848*, 39.
[10] B. Sellergren, *J. Chromatogr., A* **2001**, *906*, 227.
[11] D. A. Spivak, *Adv. Drug Delivery Rev.* **2005**, *57*, 1779
[12] T. Nowicka-Jankowska, K. Gorczyńska, A. Michalik, E. Wieteska, 'Elsevier Science Publishers B. V.', Amsterdam, Oxford, New York, Tokyo, 1986.
[13] J. Zhou, X. W. He, *Anal. Chim. Acta* **1999**, *381*, 85.
[14] T. Pap, V. Horvath, A. Toloka, G. Horva, B. Sellergren, *J. Chromatogr., A* **2002**, *973*, 1.

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