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Recognition of hydroxybenzoic acids and their esters by molecularly imprinted polymers

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The sorption properties of molecularly imprinted polymers with imprints of hydroxybenzoic acids and their derivatives were found to depend on the number and location of hydroxyl and carboxyl groups.

In the past decade, great interest in a new class of materials, molecularly imprinted polymers (MIPs), has been expressed by chemists.[1–3] The areas of application of MIPs in chemical analysis are diverse. Among them are the solid-phase extraction of organic compounds from solutions and biological fluids;[4–10] the selective separation of structurally similar organic compounds, including enantiomers, by HPLC,[11–13] capillary electrochromatography and capillary electrophoresis;[12,14,15] the selective binding and determination of substances with chemical and biochemical sensors;[12,16] and immunoassay.[17] In theory, MIPs can be prepared for analyte species such as drugs,[7,11,18,19] amino acid derivatives,[11,12] sugars,[12,20] nucleic acids and hormones.[12] However, only a limited number of compounds have been used for molecular imprinting. For the better understanding of the imprinting process, it is necessary to take into account the structure and nature of imprint – analyte and polymer surface – analyte interactions.

Here, we report on the synthesis of MIPs with several templates of various hydroxybenzoic acids (HBAs) and their esters. The main reason for the choice of such compounds was the possibility to investigate the influence of the nature and structure of template molecules and the number and location of hydroxyl and carboxyl groups on the process of molecular recognition.

The process of polymerization was based on the method reported previously.[21] Acrylamide (AA) and ethylene glycol dimethacrylate (EGDMA) were used as a functional monomer and a cross-linking agent, respectively, and 2,2′-azobisisobutyronitrile (AIBN), as a radical polymerization initiator. The polymers were prepared by a self-assembly imprinting protocol using only noncovalent interactions between the print species and the functional monomers. The print molecules structures, synthesized polymers and their specific surface areas are represented in Table 1.

The template molecules were extracted from the polymers by washing with methanol–acetic acid (9:1) and then methanol–water (3:1) mixtures and sorption properties of the synthesized polymers were investigated. A weighed portion of the sorbent (20 mg) was placed in a 25 ml bottle with a ground-glass stopper containing the test solution, the concentration of solution

Table 1 Template molecules used in the synthesis of molecularly imprinted polymers, specific surface areas (S) of the polymers and recovery (R), distribution coefficients (D) and imprinting factors (IF) of template molecules on polymers with their imprints. $C_{HBA} = 5\times10^{-5}$ mol dm^{-3}, $C_{HCl} = 1\times10^{-3}$ mol dm^{-3}, $V = 5$ ml, $m_S = 0.020\pm0.001$ g, $t = 60$ min, $n = 3$, $P = 0.95$.

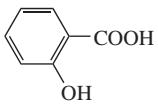
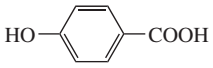
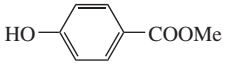
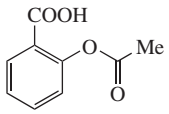
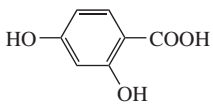
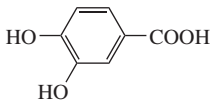
Polymer	Template	Structure	S/m^2 g^{-1}	R (%)	D	IF
						2.5
MIP_1	2-Hydroxybenzoic acid (2-HBA)		49.8	43±2	219	—
BP_1	—		2.3	25±1	86	
MIP_2	4-Hydroxybenzoic acid (4-HBA)		8.2	64±1	444	7.1
BP_2	—		1.8	20±1	63	—
MIP_3	Methyl 4-hydroxybenzoate (methyl 4-HB)		21.6	41±1	177	2.4
BP_3	—		2.3	22±1	73	—
MIP_4	2-Acetoxybenzoic acid (2-AcOBA)		3.4	49±2	259	2.1
BP_4	—		2.2	30±2	122	—
MIP_5	2,4-Dihydroxybenzoic acid (2,4-DHBA)		17.9	30±1	105	3.3
BP_5	—		2.3	11±1	32	—
MIP_6	3,4-Dihydroxybenzoic acid (3,4-DHBA)		7.1	48±1	221	7.9
BP_6	—		2.5	10±1	28	—

Table 2 Hydrophobicity (log P_{ow}), logarithms of distribution coefficients (log D), and imprinting factors (IF) of organic compounds on imprinted polymers (MIP_1, MIP_2, MIP_5 and MIP_6) and non-imprinted blank polymers (BP_1, BP_2, BP_5 and BP_6). $C_{HBA} = 5\times10^{-5}$ mol dm^{-3}, $C_{HCl} = 1\times10^{-3}$ mol dm^{-3}, $V = 5$ ml, $m_S = 0.020\pm0.001$ g, $t = 60$ min, $n = 3$, $P = 0.95$.

Substance	log P_{ow}	lg D		IF	lg D		IF	lg D		IF	lg D		IF
		MIP_1	BP_1		MIP_2	BP_2		MIP_5	BP_5		MIP_6	BP_6	
3,4,5-THBA	0.9±0.3	1.48	1.20	1.9	1.77	1.32	2.8	1.64	1.20	2.8	1.28	1.20	1.2
3,4-DHBA	1.2±0.3	1.45	1.45	1.0	1.96	1.53	2.7	1.48	1.45	1.1	2.34	1.45	7.9
4-HBA	1.4±0.2	1.85	1.79	1.2	2.65	1.80	7.1	1.85	1.79	1.2	1.85	1.79	1.1
Ph	1.5±0.3	1.70	1.43	1.9	1.85	1.49	2.3	1.59	1.43	1.4	1.62	1.43	1.6
2,4-DHBA	1.6±0.3	1.65	1.65	1.0	1.68	1.68	1.0	2.02	1.50	3.3	1.80	1.65	1.4
4-NPh	1.9±0.2	1.66	1.58	1.2	2.12	1.60	3.3	1.70	1.62	1.2	2.07	1.58	3.1
BA	1.9±0.2	1.70	1.67	1.1	2.36	1.71	4.5	2.07	1.68	2.4	1.88	1.68	1.6
2-HBA	2.1±0.3	2.34	1.93	2.5	2.45	1.92	3.4	2.07	1.86	1.6	1.96	1.86	1.2

varied from 0 to 0.2 mmol dm^{-3}, and the bottle was shaken until the sorption equilibrium was attained. The sorption isotherms were plotted and the imprinting factors (IF) were calculated for hydroxybenzoic acids in order to compare their binding properties using the equation $IF = D_{MIP}/D_{BP}$, where D_{MIP} is the distribution coefficient of a substrate on the MIP and D_{BP} represents that on the blank polymer (BP).

Hydroxybenzoic acids contain both hydroxyl and carboxyl groups and a hydrophobic aromatic nucleus; thus, they are convenient models for studying the molecular recognition properties of MIPs. The structural variety of these compounds makes it possible to systematically investigate the influence of the number and location of functional groups on the sorption properties of synthesized MIPs. In this study, for a better understanding of the imprinting process, hydroxybenzoic acids (2-HBA, 4-HBA), their esters (methyl 4-HB, 2-AcOBA) and dihydroxybenzoic acids (2,4-DHBA and 3,4-DHBA) were selected as model template molecules. The binding characteristics of the imprinted polymers compared to the binding characteristics of the corresponding non-imprinted blank polymers are summarized in Table 1.

Compared of two pairs of polymers with imprints of 2-HBA and 4-HBA (MIP_1, MIP_2) and polymers with imprints of 2,4-DHBA and 3,4-DHBA (MIP_5, MIP_6), the only difference between them is the position of the hydroxyl groups, we observed the influence of positional relationship of functional groups on the properties of MIPs. Sorption isotherms of the isomeric hydroxybenzoic acids are shown in Figure 1. As we see (Table 1), the IF values of 4-HBA (MIP_2) and 3,4-DHBA (MIP_6) are much bigger than those of 2-HBA (MIP_1) and 2,4-DHBA (MIP_5). This indicates that the molecular recognition abilities of the MIP_2 and MIP_6 are higher. The phenomenon revealed may be explained by formation of intermolecular hydrogen bonds in 2-HBA and 2,4-DHBA molecules that results in weakening bonding interaction with AA. In contrast to this 4-HBA and 3,4-DHBA molecules yield stable complexes with AA that leads to the formation of specific binding sites in MIP_2 and MIP_6. It was noted[22] that intermolecular hydrogen bonds make the major contribution to the formation of the prepolymerization monomer-template complex; our experimental results proved these data.

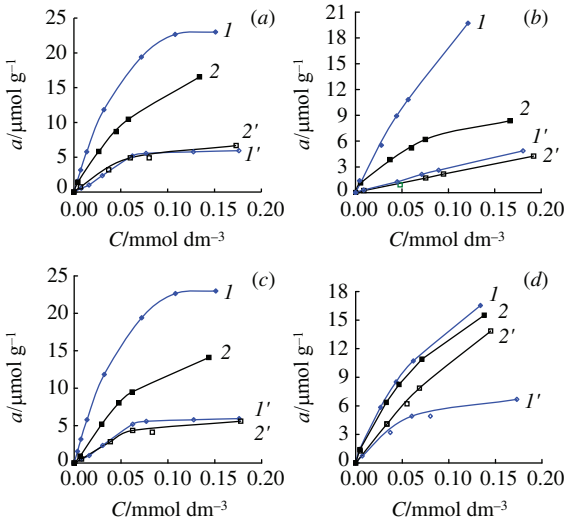


Figure 1 Adsorption isotherms of hydroxybenzoic acids and their esters on the imprinted polymers with their imprints (*1*), (*2*) and their reference blank polymers (*1′*, *2′*): (*a*) 4-hydroxy- (*1*), (*1′*) and 2-hydroxybenzoic acids (2), (2′); (*b*) 3,4-dihydroxy- (*1*), (*1′*) and 2,4-dihydroxybenzoic acids (*2*), (*2′*); (*c*) 4-hydroxybenzoic acid (*1*), (*1′*) and methyl 4-hydroxybenzoate (2), (2′); (*d*) 2-hydroxybenzoic (*1*), (*1′*) and 4-acetoxybenzoic acids (*2*), (*2′*). $V = 5$ ml, $C_{HCl} = 1\times10^{-3}$ mol dm^{-3}, $m_S = 0.020\pm0.001$ g, $t = 60$ min.

The influence of the number of hydroxyl groups was studied by comparing two pairs of polymers with imprints of 4-HBA and 3,4-DHBA (MIP_2, MIP_6) and polymers with imprints of 2-HBA and 2,4-DHBA (MIP_1, MIP_5). As we see (Table 1), the IF values have a minor difference between each other. From these experimental data, we made a conclusion that the presence of additional hydroxyl groups in molecules of 2,4-DHBA and 3,4-DHBA does not influence the molecularly recognition ability of MIPs. But sorption for dihydroxybenzoic acids was worse than for hydroxybenzoic acids [Figure 1(*a*),(*b*)]. It may be concerned with decreasing a hydrophobicity of these acids.

To clarify the role of the carboxyl group in imprinting process, we compared two pairs of polymers with imprints of 4-HBA and methyl 4-HB (MIP_2, MIP_3) and polymers with imprints of 2-HBA and 2-AcOBA (MIP_1, MIP_4) [Figure 1(*c*),(*d*)]. It can be pointed out (Table 1) that the IF value of 4-HBA (7,1) is much bigger than that of methyl 4-HB (2,4) but the IF value of 2-HBA (2,5) is near to equal of 2-AcOBA (2,1). The absence of the carboxyl group in the molecule of methyl 4-HB leads to worse imprints in the MIP_3, it is seen from adsorption isotherms [Figure 1(*c*)]. On the contrary, the presence of the carboxyl group in the molecule of 2-AcOBA leads to the imprints in the MIP_4, that are comparable by the effectivity with MIP_1; it is seen from adsorption isotherms [Figure 1(*d*)]. The differences between adsorption isotherms of BP_1 and BP_4 may be concerned with different hydrophobicity of 2-HBA and 2-AcOBA, which is bigger for 2-HBA. The experimental results indicate that the carboxyl group plays the important role in the formation of intermolecular hydrogen bond between template molecule and amide group of functional monomer.

To estimate the selectivity of the synthesized imprinted polymers, we studied the sorption of structurally related compounds on these materials. The four pairs of polymers based on acrylamide (MIP_1, MIP_2, MIP_5 and MIP_6) and corresponding non-imprinted polymers (BP_1, BP_2, BP_5 and BP_6) were compared. We studied the sorption of benzoic acid (BA), 2-HBA, 4-HBA, methyl 4-HB, 2,4-DHBA, 3,4-DHBA, 3,4,5-trihydroxybenzoic acid (3,4,5-THBA), 2-AcOBA, phenol (Ph) and 4-nitrophenol (4-NPh). The distribution coefficients (log D) and imprinting factors (IF) of studied compounds are listed in Table 2. One can see that the hydrophobicity of compounds substantially affects

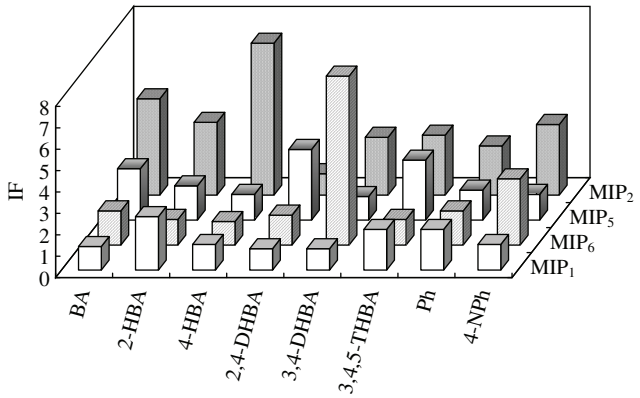


Figure 2 Selectivity and imprinting factors of molecularly imprinted polymers (MIP_1, MIP_2, MIP_5 and MIP_6) when 5 ml of a standard solution of each analyte (5×10^{-3} mol dm^{-3}) was sorbed on MIPs.

their sorption on non-imprinted blank polymers. In the most cases, the distribution coefficients of the studied compounds on these polymers increase with their hydrophobicity. On the contrary, imprinted polymers better adsorb the template molecule and some its structural analogues (Figure 2).

The binding parameters of MIPs were also estimated by the Scatchard analysis.[23] It reveals that two classes of binding sites were produced in MIPs. Association constants and amounts of binding sites were calculated (Table 3). It was established that the total number of binding sites in MIPs is larger than that in corresponding blank polymers and the number of specific binding sites in MIPs is 2–4 times lower than that of non-specific. It was shown that the values of association constants for specific binding sites are higher than those for nonspecific. This testifies to the stronger binding of these substances with MIPs in comparison with BPs.

Table 3 Association constants (K_{ass}) and total amount of binding sites (Q_{max}) for hydroxybenzoic acids and their esters.

Polymer	K_{ass1}/ dm^3 mol^{-1}	K_{ass2}/ dm^3 mol^{-1}	Q_{max1}/ μmol g^{-1}	Q_{max1}/ μmol g^{-2}
MIP_1	2.6×10^4	7.8×10^3	14.4	33.6
BP_1	—	1.1×10^4	—	10.4
MIP_2	2.1×10^4	6.2×10^3	29.8	72.2
BP_2	—	1.4×10^4	—	8.7
MIP_3	—	1.1×10^4	—	22.9
BP_3	—	1.4×10^4	—	7.8
MIP_4	3.1×10^4	8.6×10^3	11.5	28.6
BP_4	—	2.8×10^3	—	47.8
MIP_5	5.0×10^4	6.5×10^3	4.6	16.5
BP_5	—	4.3×10^3	—	4.5
MIP_6	2.3×10^4	3.6×10^3	15.2	64.8
BP_6	—	2.8×10^3	—	12.9

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References

1 B. Sellergren, *Molecularly Imprinted Polymers. Man-made Mimics of Antibodies and Their Application in Analytical Chemistry*, Elsevier, Amsterdam, 2001, p. 582.
2 S. G. Dmitrienko, V. V. Irkha, A. Yu. Kuznetsova and Yu. A. Zolotov, *Zh. Anal. Khim.*, 2004, **59**, 902 [*J. Anal. Chem.* (*Engl. Transl.*), 2004, **59**, 808].
3 K. Haupt, *Analyst*, 2001, **126**, 747.
4 J. Olsen, P. Martin and I. D. Wilson, *Anal. Commun.*, 1998, **35**, 13H.
5 B. Sellergren, *Trends Anal. Chem.*, 1999, **18**, 164.
6 I. Ferrer and D. Barcelo, *Trends Anal. Chem.*, 1999, **18**, 180.
7 L. I. Andersson, *J. Chromatogr. B*, 2000, **739**, 163.
8 N. Masque, R. M. Marce and F. Borrul, *Trends Anal. Chem.*, 2001, **20**, 477.
9 A. Martin-Esteban, *Fresenius J. Anal. Chem.*, 2001, **370**, 795.
10 L. I. Andersson, *Bioseparation*, 2002, **10**, 353.
11 M. Kempe and K. Mosbach, *J. Chromatogr. A*, 1995, **694**, 3.
12 T. Takeuchi and J. Haginaka, *J. Chromatogr. B*, 1999, **728**, 1.
13 B. Sellergren, *J. Chromatogr. A*, 2001, **906**, 227.
14 L. Schweitz, L. I. Andersson and S. Nilsson, *J. Chromatogr. A*, 1998, **817**, 5.
15 V. T. Remcho and Z. J. Tan, *Anal. Chem.*, 1999, **71**, 248A.
16 K. Haupt and K. Mosbach, *Chem. Rev.*, 2000, **100**, 2495.
17 J. Stevenson, *J. Chromatogr.*, 2000, **745**, 39.
18 L. Fischer, R. Muller, B. Erberg and K. Mosbach, *J. Am. Chem. Soc.*, 1991, **113**, 9358.
19 X. Xu, L. Zhu and L. Chen, *J. Chromatogr. B*, 2004, **804**, 61.
20 G. Wulff, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1812.
21 S. G. Dmitrienko, V. V. Irkha, T. V. Duisebaeva, Yu. V. Mikhailik and Yu. A. Zolotov, *Zh. Anal. Khim.*, 2006, **61**, 18 [*J. Anal. Chem.* (*Engl. Transl.*), 2006, **61**, 14].
22 T. Zhang, F. Liu, W. Chen, J. Wang and K. Li, *Anal. Chim. Acta*, 2001, **450**, 53.
23 J. Matsui, Y. Miyoshi, O. Doblhoff-Dier and T. Takeuchi, *Anal. Chem.*, 1995, **67**, 4404.

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