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Thermodynamic study of molecularly imprinted polymer used as the stationary phase in high performance liquid chromatography

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ABSTRACT

Molecularly imprinted polymer (MIP) and non-imprinted polymer (NIP) on the base of methacrylic acid prepared by a bulk polymerization were used as stationary phases for the HPLC analysis. The thermodynamic processes were carried out to investigate the temperature effects during sorption processes of potential local anaesthetics – morpholinoethyl esters of alkoxy-substituted phenylcarbamic acid (MEsP), local anaesthetic – diperodon, flavonoid – quercetin in methanol, acetonitrile and toluene (porogen) as mobile phases. Mobile phases and corresponding solvents were selected according to the solubility of each analyte. The template was chosen from the set of homologous of MEsP – 2-(morpholin-4-yl)ethyl (2-methoxyphenyl)carbamate. Values of retention factors were measured over the temperature range of 20–60 °C. There were determined van't Hoff curves – dependences between logarithms of the retention factors ($\ln k$) and the inverse value of the temperature ($1/T$). Observed graphs were linear directly indicating that there were no changes of interaction mechanisms in the studied range of temperature. Selectivities (evaluated by the separation factors, α) and sorption selectivities (evaluated by the imprinting factors, IFs) of the MIP and the NIP toward template, related and not-related structures with the template were evaluated chromatographically. The highest separation factors and the imprinting factors (IF = 4.73 ± 0.35 for the template) were observed in methanol, not in porogen. Only in the case of quercetin the highest IF was observed in ACN (1.88 ± 0.13). Contrary to expectations, the driving force for the affinity of the target molecules for both of polymers was enthalpic term (with an average of 54%, 82% and 84% contribution of enthalpic term for MeOH, ACN and toluene, respectively on the MIP and 53%, 57% and 65% for MeOH, ACN and toluene, respectively on the NIP). The MIP and NIP were also characterized by attenuated total reflectance analysis Fourier transform infrared spectroscopy (ATR-FTIR) and thermogravimetric analysis (TGA).

1. Introduction

The temperature is a parameter, which together with the mobile phase composition, has a great influence on the effect of chromatographic separation. This crucial factor plays very important role in solute transfer from the mobile phase to the stationary phase in separation processes and it is used for separation factor, resolution and column efficiency controlling [1,2]. A transfer of molecules between phases is related with a reduction of the Gibbs function in the mobile phase and an increase it in the stationary phase. The change of Gibbs function equals the chemical potential of the solute in the concrete phase times the quantity of solute decreased or increased for transfers of infinitesimally small amounts of solute at constant pressure and temperature [3,4].

Generally, there are two main temperature effects governing performance of stationary phase in chromatography. A first effect changes viscosity and the diffusion coefficients of the analyte in both phases. At higher temperatures viscosity decreases, which results in increasing of the diffusion of solute from the mobile phase to the stationary phase and so it can get a better mass transfer at lower pressures. Inversely, if the temperature is decreasing, the viscosity of the mobile phase is higher and molecules cannot transfer between the two phases so fast as at the higher temperatures. Second effect, is the influence of temperature on separation factors (α). The α-values usually decrease as the temperature is increased. This effect is controversial, depends much more on the nature of the chromatographic system and there are no universal rules [5,6].

In order to understand the sorption processes occurring during the chromatographic separation a determination of molar enthalpy and molar entropy changes together with the phase ratio is needed. Thermodynamic analysis can be carried out using van't Hoff approach for different chromatographic systems. In each

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system we can change the mobile phase, stationary phase or we can use a mobile phase modifier [7]. The van't Hoff analysis is convenient to determine the thermodynamic values and construct a plot $\ln k_i = f(1/T)$ according to the equation:

$$\ln k_i = \frac{-\Delta H_i}{RT} + \frac{\Delta S_i}{R} + \ln \varphi \quad (1)$$

where k_i is the retention factor, ΔH_i [kJ/mol] is the partial molar enthalpy of transfer, ΔS_i [J/(mol K)] is the partial molar entropy of transfer, R [J/(mol K)] is the gas constant, T [K] is the absolute temperature and $\ln \varphi$ is the phase ratio (the volume of the stationary phase, V_S, divided by the volume of the mobile phase, V_M).

Eq. (1) shows that a plot of $\ln k_i$ versus $1/T$ should be linear with a slope of $-\Delta H_i/R$ and intercept of $\Delta S_i/R + \ln \varphi$. However, a non-linear van't Hoff behaviour is also possible. This may happen when the separation processes governed by the mechanisms of retention on the non-homogenous stationary phase are different, for example when there are no single sorption mechanisms [1,6,8]. Nevertheless, a linear behaviour of van't Hoff function indicates that there are no changes in the retention mechanisms over the temperature range studied [6] or that the changes in enthalpy for two or more mechanism are similar.

Continuing, Eq. (1) provides a way to determine of the thermodynamic parameters, ΔH_i and ΔS_i, for a chromatographic system if the phase ratio is known, does not vary with the temperature or can be calculated. Determination of the phase ratio is always difficult, because the information about the volume of the active stationary phase, V_S, is mostly approximate and only for pure liquid–liquid chromatography usually is relatively easy to calculate. In the case of commercially available stationary phases for RP-HPLC, when there is no accurately information about bonding density or surface coverage the determination of its value also is quite complex [2,6,8–11].

Kim and Guiochon [12] determined the Langmuir isotherms for two enantiomers of Fmoc-tryptophan using the MIP (molecularly imprinted polymer). Within the measured concentration ranges, they selected best models of these isotherms for the MIP and the NIP. The resulting isotherm parameters showed there are no significant changes of the enantiomeric selectivity with the temperature and the affinity of the analytes tested for both of polymers significantly decrease with increasing temperature. They proved that enthalpy was the driving force for the affinity of the template (Fmoc-L-tryptophan) onto the prepared MIP. Liu and co-workers [13] synthesized various MIPs-stationary phases with cinchonidine (CD) as a template. The temperature effect on the thermodynamic distribution of CD and its stereoisomer – cinchonine (CN) onto the prepared MIPs were explained using van't Hoff plots generated from the chromatographic parameters. The thermodynamic analysis showed that the stereoseparation process depends on the pH mobile phase, is entropy driven for the mobile phase of pH 4.2 and the energy driven for the mobile phase of pH 11.7. Zakaria et al. [14] described the sorption of 2,4-dinitrophenol from the aqueous solutions at different pH using acrylamide-MIP. The adsorption processes were found effective at pH 6.0 and fitted well to the Langmuir adsorption equation. Xia et al. [15] prepared haemoglobin-imprinted polymer beads which were dynamically and thermodynamically tested. The adsorption processes were well explained by the Freundlich isotherm. The results showed that the adsorption was endothermic and had a low potential barrier. Liu et al. [16], in their another work, depicted the sorption process of Pb(II) from the aqueous solutions by Pb(II) ion-imprinted polymer (IIP). The studies indicated that the selective adsorption obeys a Langmuir adsorption isotherm. Additionally, calculated thermodynamic data showed that the adsorption of the template onto the IIP was exothermic and spontaneous. Chen and co-workers [17] prepared the 4-vinylpirydyne-MIP and studied the temperature effect on the behaviour of 2,4-dichlorophenoxyacetic (template) at different pH values. The nature of the interactions, contributions of the enthalpy and the entropy to the binding process were determined by equilibrium binding isotherm analysis and isothermal titration microcalorimetry (ITC). Thermodynamic data proved that the entropy was the driving force at pH < 6, while the enthalpy was the driving force at pH > 6.

In this work an examination of the thermodynamics of the molecular recognition was done in order to gain some insight into the sorption mechanisms on the MIP. As a template was used 2-(morpholin-4-yl)ethyl (2-methoxyphenyl)carbamate and as a functional monomer methacrylic acid was used. We focused on the evaluation of the effect of temperature on the behaviour of homologues of the potential local anaesthetics – MEP (morpholinoethyl esters of alkoxy-substituted phenylcarbamic acid) with different lengths and positions of alkoxy-substituents, local anaesthetic – diperodon and flavonoid – quercetin during specific sorption on the imprinted and non-imprinted stationary phases. By examining the chromatographic retention as a function of temperature, differences in the retention thermodynamics between the MIP and the NIP could be compared. The effect of varying mobile phases – toluene (porogen), acetonitrile and methanol on the enthalpic and the entropic terms was examined as well.

The relationship between the template and the imprinted cavity is based on a "lock-to-key" relationship. The MIPs theory is established on the recognizing processes of specific substrate–target molecule by carefully shaped centres. Thermodynamic kinetics during the sorption processes between the template and the active centre should be the major difference between the MIP and the NIP.

To achieve our goals, we measured the retention factors of all analytes, in three mobile phases and at different temperatures. We calculated sorption selectivities, evaluated by the imprinting factors and defined as $IF = k_{MIP}/k_{NIP}$, where k_{MIP} and k_{NIP} are the retention factors of MEP, quercetin and diperodon on the MIP and the NIP, respectively. The thermodynamic terms ($-\Delta H_i/RT$ and $\Delta S_i/R + \ln \varphi$) were determined and discussed in order to estimate which kind of distribution is more or less responsible for the sorption processes on the MIP.

The thermodynamic parameters estimated from the thermodynamic data for each system were used to understand the temperature effect on the MIP and the NIP.

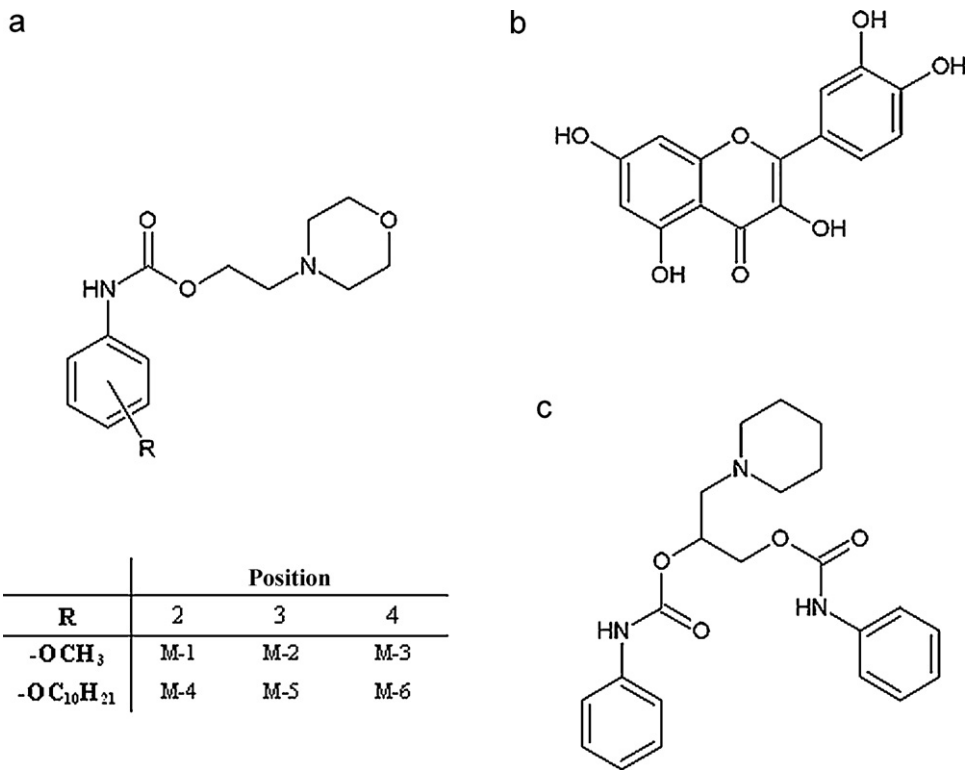
2. Experimental

2.1. Chemicals and columns

MEsPs were prepared at Department of Pharmaceutical Chemistry (Faculty of Pharmacy, Comenius University in Bratislava, Slovakia), diperodon (analytical standard) and quercetin (HPLC grade) were purchased from Sigma–Aldrich (Steinheim, Germany). Structures of all analytes used in this study are shown in Fig. 1. Methanol and acetonitrile (gradient grade) were obtained from J.T. Baker (Deventer, Netherlands), toluene (p.a.) was ordered in ANALYTIKA spol. s.r.o. (Prague, Czech Republic), acetic acid (p.a.) (HAc) and acetone (p.a.) were purchased from MIKROCHEM (Pezinok, Slovakia), methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA) and azobisisobutyronitrile (AIBN) for synthesis were obtained from MERCK (Darmstadt, Germany), piston columns ECO^{PLUS}, 125 mm × 5 mm were delivered from KronLab (Dinslaken, Germany), GraceSmart RP18 column, 5 μm, 150 mm × 4.6 mm (MD, USA).

2.2. Polymers preparation

The MIP was prepared by a bulk polymerization method according to the Zhang et al. method [18]. MAA as a functional



	Position		
R	2	3	4
$-OCH_3$	M-1	M-2	M-3
$-OC_{10}H_{21}$	M-4	M-5	M-6

Fig. 1. Structures of analytes used in the study: (a) morpholinoethyl esters of alkoxysubstituted phenylcarbamic acid, (b) quercetin, and (c) diperodon.

monomer (1.8 mmol), toluene as a porogen (3.0 mL) in presence of 2-(morpholin-4-yl)ethyl (2-methoxyphenyl)carbamate (M-1) (0.3 mmol) as a template were mixed together in a glass tube. Then EDMA as a cross-linker monomer (9.0 mmol) and AIBN (20 mg) as an initiator were added. The polymerization of the MIP was allowed to proceed in a water bath at 60 °C for 24 h. In the next, the prepared polymer was grounded and passed through 40 μm sieve, to receive smaller particles than 40 μm. Fine particles were removed by flotation in acetone. The Soxhlet extraction of the dried particles (24 h, 100 mL MeOH/HAc (9:1), v/v) was used in order to purify the MIP from the template. The NIP was prepared in the same manner like the MIP but without presence of the template in the polymerization mixture. Because of keeping the same conditions of the polymerization process, the identical steps in the preparation of the control polymer (NIP) were used.

2.3. Columns preparation

Piston columns were filled with definite amount of the MIP or the NIP (200 mg). They were washed with methanol for 24 h to elute all remaining residues. The flow rate of the mobile phase was gradually growing up to precise pack of particles of the polymers. The sorbent in the column was pressed by the pistons as long as the resistance was felt.

2.4. Apparatus

An Agilent Technologies 1260 Infinity system (Waldbronn, Germany), consisting of a pump with a degasser, a diode-array detector (DAD), a 20 μL injector and an Agilent Technologies Chemstation were used. In the case of toluene the fractions were collected. Analyses were carried out on piston columns ECO^{PLUS} in temperature range of 20–60 °C. The mobile phases were methanol (for methanolic solutions of compounds under study), acetonitrile (for acetonitrile solutions) and toluene (for toluene solutions) at a flow rate of 0.2 mL/min. For the determination of the compounds under study in toluene fractions, C18 column at flow rate 0.5 mL/min with methanol as a mobile phase was used. Diode array detection was used in the range of 200–400 nm and the chromatograms were acquired at wavelengths of 235, 254 and 360 nm.

IR spectra of grounded polymers and the template were measured by ATR technique on FTIR Spectrometer Nicolet 5700 in the 4000–400 cm^{-1} region.

Simultaneous DTA/TG analyzer SHIMADZU DTG60 was employed to study the thermal properties of the MIP and the NIP. 2–4 mg of sorbent was placed into standard aluminium pans and heated from 50 to 400 °C at the heating rate 10 °C/min under a nitrogen atmosphere with the flow rate 50 mL/min and the corresponding TGA curves were obtained.

2.5. Chromatographic experiments

Solutions of MEP, quercetin and diperodon in methanol, M-1, M-2, M-3, quercetin and diperodon in acetonitrile (M-4, M-5 and M-6 were not soluble in acetonitrile) and MEP in toluene (quercetin and diperodon were not soluble) were prepared. Analytes in methanol and acetonitrile were directly detected by DAD. If toluene was used as a mobile phase, the fractions from the piston columns were collected, evaporated to dryness, dissolved in methanol and analyzed using C18 column. The concentration of all solutions was 5 μg/mL. Prior to the each analysis, the piston columns were conditioned with solution of methanol with addition of HAc (9:1) (v/v) (6 mL), methanol (6 mL) and then the mobile phase (6 mL). The same procedure was used for the NIP.

3. Results an discussion

3.1. Attenuated total reflectance analysis

An ATR analysis provides information about the state of the polymerization both of polymers and an extraction process of the

Table 1
Retention factors at different temperatures and imprinting factors (IFs) of analytes under study for MIP and NIP.

	MIP					NIP					IF
T [K]	293.15	303.15	313.15	323.15	333.15	293.15	303.15	313.15	323.15	333.15	293.15
$1/T$	0.0034	0.0033	0.0032	0.0031	0.0030	0.0034	0.0033	0.0032	0.0031	0.0030	
	k										
Analyte	**MeOH**[a]										
M-1 (the template)	9.60 ± 0.41	6.76 ± 0.20	4.27 ± 0.41	3.65 ± 0.20	2.61 ± 0.19	2.03 ± 0.04	1.82 ± 0.15	138 ± 0.01	1.15 ± 0.02	0.86 ± 0.00	4.73 ± 0.35
M-2	6.11 ± 0.72	3.19 ± 0.17	2.73 ± 0.14	2.57 ± 0.12	2.18 ± 0.54	1.82 ± 0.11	1.58 ± 0.16	1.26 ± 0.02	1.02 ± 0.07	0.81 ± 0.04	3.36 ± 0.29
M-3	8.96 ± 1.02	5.36 ± 0.42	3.36 ± 0.12	2.64 ± 0.91	1.97 ± 0.14	2.24 ± 0.18	1.77 ± 0.11	1.43 ± 0.01	1.18 ± 0.00	0.83 ± 0.06	4.00 ± 0.43
M-4	5.87 ± 0.79	4.09 ± 0.22	2.51 ± 0.63	1.91 ± 0.03	1.57 ± 0.02	2.27 ± 0.03	1.80 ± 0.19	1.30 ± 0.02	1.02 ± 0.02	0.75 ± 0.01	2.59 ± 0.26
M-5	6.30 ± 0.51	5.01 ± 0.67	2.70 ± 0.05	2.58 ± 0.26	1.92 ± 0.22	2.30 ± 0.17	1.96 ± 0.23	1.45 ± 0.01	1.12 ± 0.00	0.91 ± 0.05	2.74 ± 0.22
M-6	8.53 ± 0.57	5.58 ± 0.55	2.81 ± 0.59	2.51 ± 0.17	1.74 ± 0.13	2.28 ± 0.05	1.87 ± 0.20	1.44 ± 0.00	1.19 ± 0.15	0.93 ± 0.04	3.74 ± 0.29
Diperodon	11.40 ± 1.03	9.22 ± 1.01	5.54 ± 0.33	3.78 ± 0.33	2.36 ± 0.01	6.98 ± 0.51	5.26 ± 0.23	3.75 ± 0.25	2.63 ± 0.12	2.17 ± 0.25	1.63 ± 0.17
Quercetin	1.23 ± 0.01	1.18 ± 0.04	1.13 ± 0.08	0.62 ± 0.05	0.55 ± 0.01	2.46 ± 0.14	1.81 ± 0.03	1.35 ± 0.03	1.06 ± 0.01	0.88 ± 0.03	0.50 ± 0.01
	ACN[a]										
M-1	33.59 ± 0.36	30.41 ± 0.46	27.13 ± 0.29	24.37 ± 0.26	21.66 ± 0.38	21.35 ± 0.35	15.45 ± 1.10	10.81 ± 0.27	8.05 ± 0.23	5.34 ± 0.37	1.57 ± 0.11
M-2	32.36 ± 0.51	29.19 ± 0.18	25.98 ± 0.34	23.11 ± 0.30	20.06 ± 0.36	19.28 ± 0.18	16.05 ± 0.38	12.82 ± 0.51	8.99 ± 0.30	6.50 ± 0.19	1.68 ± 0.13
M-3	32.73 ± 0.35	29.53 ± 0.07	26.42 ± 0.11	23.24 ± 0.21	20.49 ± 0.18	18.55 ± 1.47	14.74 ± 0.18	11.78 ± 0.20	8.30 ± 0.12	5.49 ± 0.39	1.76 ± 0.18
Diperodon	32.52 ± 0.14	28.55 ± 0.07	25.05 ± 0.19	22.18 ± 0.99	19.47 ± 0.00	20.74 ± 1.34	16.85 ± 0.34	13.78 ± 0.49	9.67 ± 0.37	7.04 ± 0.38	1.57 ± 0.11
Quercetin	20.79 ± 0.05	15.21 ± 0.14	10.59 ± 0.09	6.74 ± 0.03	3.53 ± 0.15	11.07 ± 0.33	8.01 ± 0.25	5.50 ± 0.60	3.39 ± 0.21	2.14 ± 0.11	1.88 ± 0.13
	Toluene (the porogen)[a]										
M-1	44.83 ± 3.82	37.64 ± 1.77	33.85 ± 2.82	27.79 ± 2.79	23.24 ± 2.83	30.75 ± 2.96	23.21 ± 2.96	18.84 ± 2.96	13.29 ± 0.00	8.13 ± 2.96	1.46 ± 0.11
M-2	43.32 ± 3.01	38.02 ± 2.37	32.71 ± 2.81	27.03 ± 2.82	22.11 ± 2.65	31.14 ± 0.00	22.41 ± 2.96	18.05 ± 0.00	12.49 ± 2.96	7.73 ± 2.96	1.39 ± 0.12
M-3	43.32 ± 0.00	35.88 ± 2.82	32.09 ± 0.00	26.79 ± 2.82	21.11 ± 2.82	31.14 ± 0.00	23.60 ± 2.96	18.44 ± 2.96	13.29 ± 0.00	7.73 ± 2.96	1.39 ± 0.13
M-4	45.97 ± 3.01	37.64 ± 0.00	34.23 ± 0.00	28.55 ± 0.00	22.62 ± 2.82	31.94 ± 2.96	24.79 ± 2.96	20.03 ± 2.96	14.87 ± 2.96	9.32 ± 2.96	1.44 ± 0.15
M-5	44.83 ± 5.65	38.02 ± 2.82	33.09 ± 0.00	27.92 ± 2.82	22.86 ± 0.00	32.33 ± 0.00	25.19 ± 0.00	19.63 ± 2.96	14.87 ± 2.96	9.71 ± 0.00	1.39 ± 0.14
M-6	46.35 ± 2.82	39.91 ± 0.00	33.85 ± 2.82	29.68 ± 0.00	23.62 ± 2.82	32.73 ± 2.96	25.98 ± 2.96	21.62 ± 0.00	16.46 ± 2.96	11.30 ± 2.96	1.42 ± 0.13

[a] Solvent.

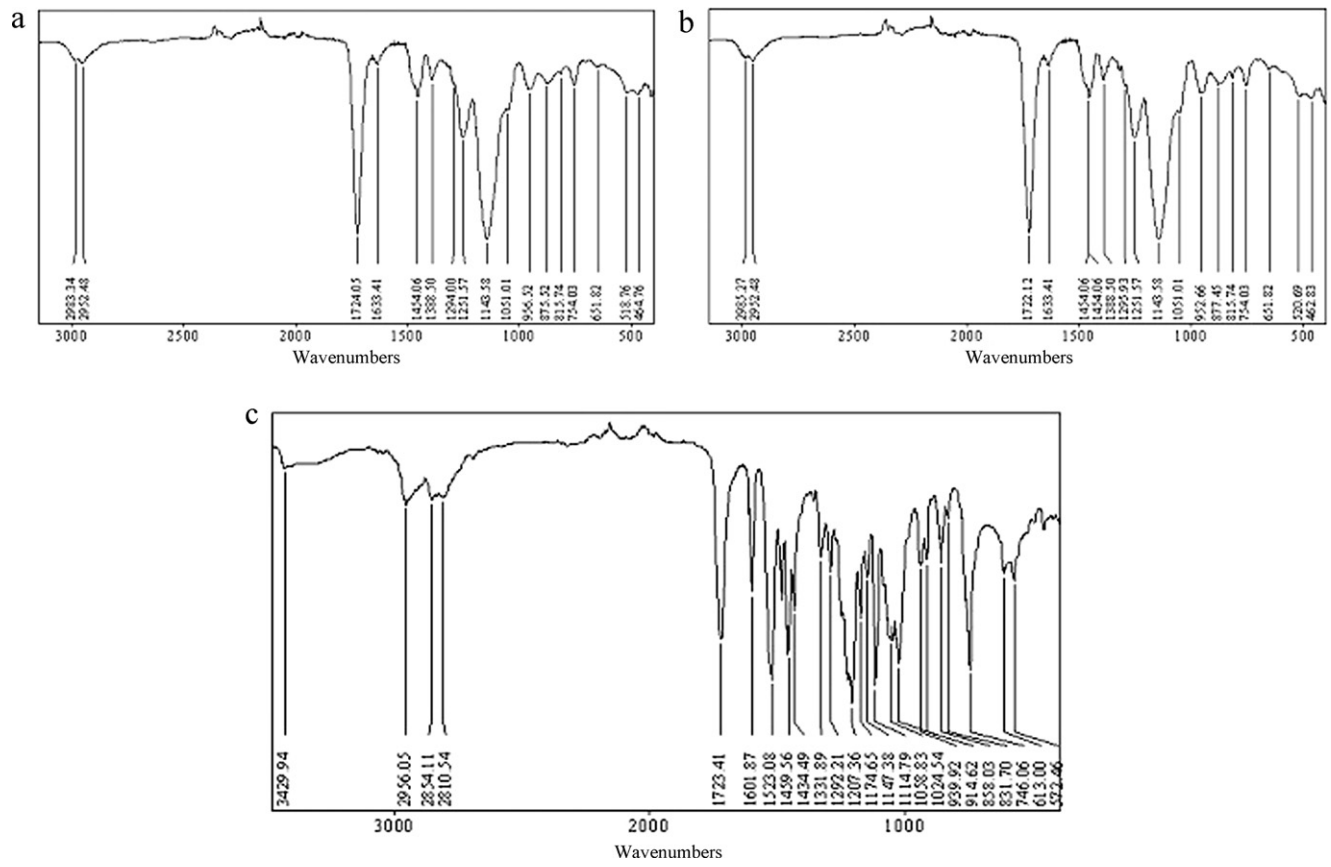


Fig. 2. ATR spectra of MIP (a), NIP (b) and the template (c).

MIP. By controlling of the content of the remaining carbon–carbon double bonds in prepared polymers we are able to check the polymerization extent.

No bands are present in the region 1680–1640 cm^{-1} indicating the absence of vinyl groups in the polymers, what confirms total polymerization of vinyl groups (see Fig. 2). The absence of bands characteristic for the template also indicates a successful extraction process.

3.2. Thermogravimetric analysis

In order to compare mechanical properties of the MIP to the NIP the TGA analysis was performed (see Fig. 3). The decomposition of the imprinted polymer particles starts at 250 °C and the non-imprinted polymer at 235 °C. This proves that presence of the template in the polymerization mixture affects the formation of the imprinting cavities resulting in higher mechanical strength of the MIP in comparison with the NIP.

3.3. Thermodynamic analysis

The thermodynamic study, used in this work, was done in order to explore the retention of analytes tested on the imprinted polymer. Retention thermodynamics were assessed for eight solutes: M-1–M-6 analytes, quercetin and diperodon, in three mobile

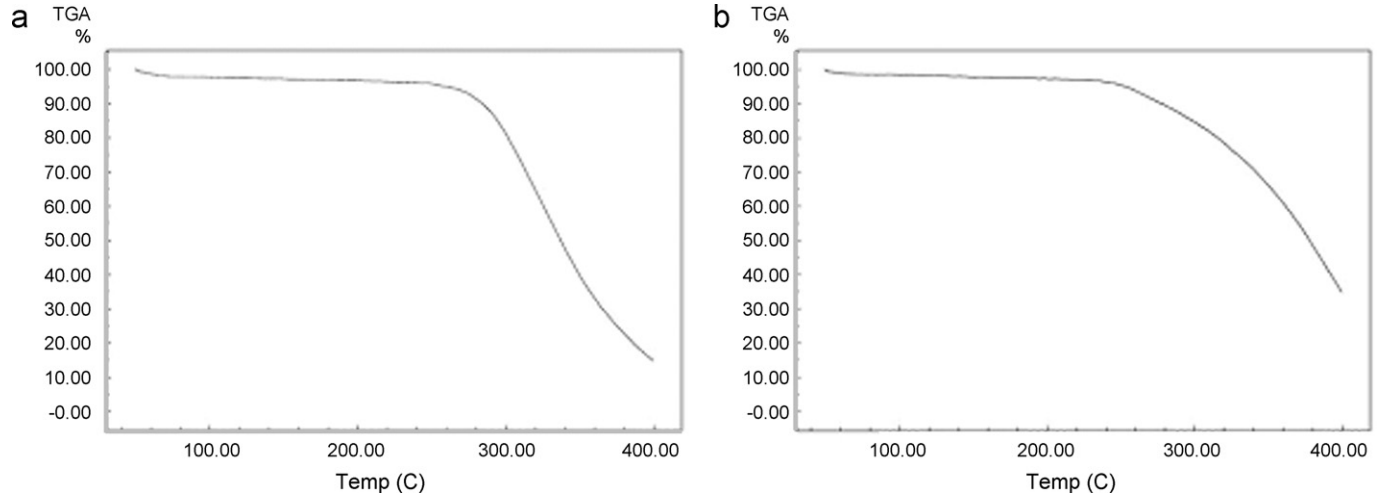


Fig. 3. TGA plots of MIP (a) and NIP (b).

Table 2
The values of thermodynamic terms of linear regression ($\ln k_i = f(1/T)$), the values of thermodynamic terms in % and correlation coefficients (r).

Analyte	MIP					NIP				
	$-\Delta H_i/RT$ [a]	$-\Delta H_i/RT$ (%)	$\Delta S_i/R + \ln \varphi$	$\Delta S_i/R + \ln \varphi$ (%)	r	$-\Delta H_i/RT$ [a]	$-\Delta H_i/RT$ (%)	$\Delta S_i/R + \ln \varphi$	$\Delta S_i/R + \ln \varphi$ (%)	r
	MeOH[b]									
M-1	10.99 ± 0.19	55.68	−8.73 ± 0.14	44.32	0.990	7.45 ± 0.11	52.72	−6.66 ± 0.13	47.28	0.991
M-2	7.66 ± 1.42	54.42	−6.04 ± 1.11	45.58	0.937	6.97 ± 0.87	51.91	−6.26 ± 0.69	48.09	0.996
M-3	12.15 ± 2.34	52.81	−10.06 ± 0.98	47.19	0.989	8.20 ± 0.25	52.56	−7.35 ± 0.24	47.44	0.993
M-4	11.38 ± 1.09	54.92	−9.63 ± 1.46	45.08	0.988	9.49 ± 0.19	52.55	−8.62 ± 0.16	47.45	0.998
M-5	10.36 ± 1.07	54.23	−8.50 ± 0.98	45.77	0.968	8.28 ± 0.17	52.94	−7.39 ± 0.17	47.06	0.998
M-6	13.58 ± 1.63	53.43	−11.46 ± 1.28	46.57	0.981	7.61 ± 0.62	52.78	−6.75 ± 0.58	47.22	0.996
Diperodon	13.79 ± 0.17	55.18	−11.22 ± 0.17	44.82	0.993	10.34 ± 0.61	54.90	−8.36 ± 0.65	45.10	0.995
Quercetin	7.86 ± 0.38	51.18	−7.49 ± 0.33	48.82	0.914	8.86 ± 0.39	52.90	−7.96 ± 0.36	47.10	0.995
	ACN[b]									
M-1	3.75 ± 0.05	94.54	−0.22 ± 0.04	5.46	0.998	11.68 ± 0.40	57.70	−8.56 ± 0.38	42.30	0.999
M-2	4.06 ± 0.25	87.96	−0.56 ± 0.23	12.04	0.998	9.40 ± 0.08	59.77	−6.34 ± 0.07	40.23	0.991
M-3	4.01 ± 0.13	88.81	−0.50 ± 0.10	11.19	0.998	10.27 ± 1.00	58.80	−7.25 ± 1.23	41.20	0.990
Diperodon	4.36 ± 0.14	83.47	−0.87 ± 0.11	16.53	0.999	9.27 ± 0.65	52.92	−6.16 ± 0.60	40.08	0.992
Quercetin	14.88 ± 0.31	55.86	−11.70 ± 0.30	44.14	0.989	14.16 ± 0.87	55.23	−11.64 ± 0.84	44.77	0.996
	Toluene[b]									
M-1	5.60 ± 0.38	84.85	−0.99 ± 0.18	15.15	0.994	11.37 ± 1.58	63.04	−7.11 ± 0.15	36.96	0.985
M-2	5.85 ± 0.38	82.19	−1.25 ± 0.15	17.81	0.992	11.92 ± 2.08	61.27	−7.66 ± 1.38	38.73	0.988
M-3	5.64 ± 0.79	83.71	−1.05 ± 0.09	16.29	0.990	11.88 ± 2.32	61.41	−7.62 ± 1.53	38.59	0.983
M-4	5.57 ± 0.70	85.84	−0.95 ± 0.12	14.16	0.991	10.47 ± 2.01	64.70	−6.18 ± 1.16	35.30	0.985
M-5	5.85 ± 0.90	85.91	−0.95 ± 0.15	14.09	0.993	11.92 ± 2.46	66.86	−6.01 ± 0.68	33.14	0.991
M-6	5.64 ± 0.89	83.84	−1.04 ± 0.20	16.16	0.991	11.88 ± 2.22	71.78	−4.77 ± 0.98	28.22	0.989

[a] Calculated at T = 293 K, ΔH_i [kJ/mol], ΔS_i [J/(mol K)], R [J/(mol K)], T [K].
[b] Solvent.

phases: toluene (porogen), acetonitrile and methanol and with each of the two stationary phases – MIP and NIP.

The influence of temperature (from 20 to 60 °C) on the retention factors (k_i) and the imprinting factors (IFs) is shown in Table 1. Since a linear relationship $\ln k_i = f(1/T)$ was observed for all studied compounds, the van't Hoff equation could be used to calculate corresponding thermodynamic terms calculated at 293 K, listed in Table 2. The calculated terms at other temperatures were changed only within 3% and this value can be neglected in the first approximation. The linearity of relationships proved that there are no changes in retention mechanisms of studied compounds at the temperature range under study. All significant differences were tested by statistical analysis at $\alpha = 0.05$.

Calculated retention factors can be used as approximation on the sorption capacity of given compound in various mobile phases. The MIP and also the NIP strongly retained analytes in the case of toluene (porogen) and acetonitrile. The big affinity of the MEP to the polymers surface is manifesting especially in porogen. Increased retention/separation factors on the NIP surface indicate that steric interactions of all target molecules probably have very small influence on the molecular recognition by the imprinted cavities on the MIP. The strong binding of analytes with the NIP surface and low differences in the retention factors between the polymers reflected low values of IFs. These values indicate small recognition selectivity of prepared MIP, if porogen and acetonitrile as eluents were used. Studying the dependence of IFs on MEP structure, we have found that the position and length of alkoxy chain for the tested analytes have no significant influence (similar values of the IFs).

As methanol replaces acetonitrile and toluene in the mobile phase, a different behaviour is observed. The retention of the target molecules on both, the MIP and the NIP, becomes rapidly smaller. Although the retention factors are lower, the differences between the MIP and the NIP are more significant and values of IFs have increased. Studying the dependencies of IFs on MEP structure, we have found that the position and length of alkoxy chain: (a) in the case of the template has a significant influence (the highest value of the IF), and (b) for another MEP have no significant influence (practically similar values of the IFs).

Inversely, quercetin shows greater retention behaviour onto the MIP if acetonitrile was used. There can be observed a higher value of the IF than in methanol. Diperodon does not show significant difference of IFs in methanol and acetonitrile. It seems that its molecular interactions should be similar in both mentioned mobile phases.

Though analytes show better retention behaviour on the MIP in all used mobile phases, nevertheless, a high retention on the NIP does not confirm a good imprinting effect. Probably the template had very small influence on the polymerization process.

In order to calculate thermodynamic parameters and to understand the specific sorption and molecular recognition on the MIP, the van't Hoff plots were constructed. Determined data show that the transfer of the analytes from the mobile phase to the surface of the MIP and the NIP is enthalpically favoured. The predominance of the contribution of the energy term (in %, Table 2) was greater in toluene and acetonitrile on the MIP. The smaller contribution of enthalpic term in methanol was compensated by the higher contribution of the entropic term. However, in all cases the entropic term was less favourable. In the case of the NIP, the values of both terms were similar in acetonitrile and methanol mobile phases.

The dominance of enthalpic term implies more significant energetic interactions between the target molecules and the surface of the MIP and the NIP than steric interactions based on the designed cavities (entropic term). Our results on the temperature dependence of the retention of studied compounds and predominance of the energy driven distribution do not support the MIP-theory.

4. Conclusions

This article presents a work aiming at thermodynamically interpreting the specific sorption and molecular recognition by the MIP and the NIP. Investigated polymers based on methacrylic acid were

synthesized by a bulk polymerization. The effect of temperature on the imprinting factors and the thermodynamic distribution of solute molecules for a series of structurally related and not-related compounds were interpreted using van't Hoff plots generated from the chromatographic data. MEP, quercetin and diperodon were used to examine the selectivity (k_j/k_i) regarding to the studied compounds and the sorption selectivity – defined by the imprinting factors, IFs. Observed values of IFs indicate that the best selectivity shows the MIP when methanol as the mobile phase is used (higher values of IFs). Probably, methanol supports hydrogen bonds created during the sorption processes. Contrary to expectations, the entropic term was not a driving force for the sorption of the investigated analytes on the MIP. If methanol was used as a mobile phase the entropic term was more important than in toluene and ACN mobile phases, reaching 46% and 47% contribution for the MIP and the NIP, respectively. Data provides by the ATR and the TGA analyses were found to be very helpful to characterize of the polymeric materials.

Our study may provide useful information to the knowledge of the mechanisms of the sorption processes on the MIP and the NIP. Thanks to thermodynamic studies, we were able to specify which term of the van't Hoff equation was responsible for the processes occurring on their surface. The study of the temperature effect helps to estimate the interaction behaviour on the MIP and the NIP, and is the key to understanding the mechanisms governing the chromatographic processes.

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