

IDENTIFICATION OF DRIVING FORCES FOR THE RECOGNITION PROCESSES ON MOLECULARLY IMPRINTED POLYMERS

NATALIA DENDERZ¹, JOZEF LEHOTAY^{1,2}

¹*Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinskeho 9, Bratislava, SK-812 37,*

Slovak Republic (natalia.denderz@stuba.sk)

²*Department of Chemistry, Faculty of Natural Sciences, University of Ss. Cyril and Methodius, Nám. J. Herdu 2, Trnava, SK-917 01, Slovak Republic*

Abstract: In this paper the thermodynamic analyses were used to calculate the contributions of entropic and enthalpic terms of the binding processes of selected derivatives of alkoxy-substituted phenylcarbamic acid (MEP) and phenolic acids (PAs) on the series of molecularly imprinted polymers (MIPs) and corresponding non-imprinted polymers (NIPs). All polymers were prepared by a bulk polymerization method with different porogens and functional monomers. The thermodynamic assessments were based on the quantification by HPLC measurements of the analytes tested in different mobile phases and at temperature range from 293 K to 333 K. The thermodynamic parameters were determined from the van't Hoff plots – dependences between logarithms of the retention factors of studied analytes ($\ln k$) and the inverse value of the temperature ($1/T$). Almost all data showed that enthalpic term was the dominating driving force for the investigated analytes.

Key words: molecularly imprinted polymers, thermodynamic analysis, van't Hoff equation

1. Introduction

Molecularly imprinted polymers (MIPs) are highly crosslinked polymers which usually possess outstanding molecular recognition ability. The molecular recognition is the result of the presence in the MIPs tailor-made of the template three-dimensional binding sites which can recognize only one structure or group of structures on which was designed. Those specific properties caused that MIPs have found a wide range of applications as stationary phases (SELLERGREN, 2001; LUO Y. *et al.*, 2011), chemical or biological sensors (LI *et al.*, 2011; CHEN *et al.*, 2012; IRSHAD *et al.*, 2013), biochemical assays (BAGGIANI *et al.*, 2013), catalysts *etc.* (KIRSCH *et al.*, 2009; TONG *et al.*, 2008).

The sorption mechanisms of target analytes on the MIPs is commonly studied by simple approach – the van't Hoff analysis. This method allows determining the retention mechanisms as enthalpically or entropically driven only by examining the temperature dependence of retention.

Retention of analytes on each stationary phase under given conditions is specified by the retention factors, k_i and the distribution of the solute between the mobile and stationary phases can be describe using the standard free energy change, ΔG_i° . Combination of these two quantities over a sufficiently broad temperature range is commonly used to investigate recognition mechanisms in chromatography as the thermodynamic analysis. If the chromatographic retention is specified as a partitioning

process between two phases, the temperature dependence on retention should be then modelled by the van't Hoff plot (HAIDACHER *et al.*, 1996; COYM *et al.*, 2008; CHESTER and COYM, 2003):

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

where k_i is the retention factor, ΔH_i° [kJ/mol] is the standard partial molar enthalpy of transfer, ΔS_i° [J/mol K] is the standard 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 ratio of stationary and mobile phase volumes, V_S and V_M respectively, within the column).

Lot of valuable informations can be drawn from thermodynamic studies with structurally related to the template compounds. Therefore, in order to gain some insight into the sorption mechanisms on the made imprinted polymers, two main groups of compounds were chosen – morpholinoethyl esters of alkoxy-substituted phenylcarbamic acid (MEP) and phenolic acids (PAs) (Fig. 1).

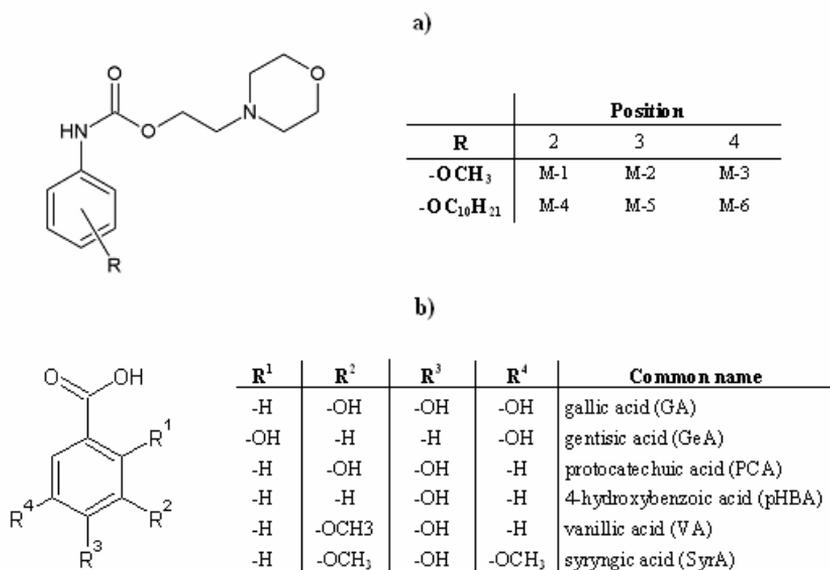


Fig. 1. Structures of analytes under study, (a) derivatives of phenylcarbamic acid (MEP) and (b) phenolic acids (PAs).

2. Material and methods

2.1 Chemicals and columns

The following chemicals and columns were used in experiments: MEP (analytical standards, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius

University in Bratislava, Slovakia), PAs (analytical standards, Sigma-Aldrich, Steinheim, Germany), methanol (MeOH) and acetonitrile (ACN) (gradient grade for HPLC, Sigma-Aldrich, Steinheim, Germany), toluene (TOL) (p.a., ANALYTIKA spol. s.r.o., Prague, Czech Republic), acetic acid (HAc) and acetone (p.a., MIKROCHEM, Pezinok, Slovakia), methacrylic acid (MAA), acrylamide (AA), ethylene glycol dimethacrylate (EGDMA) and azobisisobutyronitrile (AIBN) (for synthesis, MERCK, Darmstadt, Germany), piston columns ECO^{PLUS} (125 × 5mm) (KronLab, Dinslaken, Germany) and GraceSmart RP18 analytical column (5µm, 150 × 4.6mm) (Maryland, USA).

2.2 Preparation of the imprinted and non-imprinted polymers

All MIPs and corresponding non-imprinted polymers (NIPs) were prepared by a bulk polymerization method. Functional monomers (1.8 mmol) and porogens (3.0 ml) in the presence of suitable templates were mixed together in a glass tubes (see Tab. 1.). Then cross-linker monomer (9.0 mmol) and AIBN (0.12 mmol) as an initiator were added. The polymerization of the MIPs was allowed to proceed in a water bath at 60 °C for 24 h. In the next, prepared polymers were grounded and passed through 40 µm sieve. Fine particles were removed by flotation in acetone. The Soxhlet extraction of dried particles (24 h, 100 ml MeOH/HAc (90:10, v/v)) was used in order to purify all MIPs from their print molecules. The NIPs were prepared in the same manner like the MIPs but without the presence of templates in the polymerization mixtures.

Table 1. A combination of templates, porogens and functional monomers used to the preparation of investigated MIPs.

Template	Porogen	Functional monomer
2-(morpholin-4-yl)ethyl (2-methoxyphenyl)carbamate	TOL	MAA
PA	ACN	AA

2.3 Preparation of the piston columns

Piston columns were filled with definite amount of the MIPs or the NIPs (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 in columns. In the same time, sorbents in the columns were pressed by the pistons as long as the resistance was not felt.

2.4 Apparatus and measuring conditions

An Agilent Technologies 1260 Infinity system, consisting of a pump with a degasser, a diode-array detector (DAD), 20 µL injection valve (Rheodyne) and an Agilent Technologies Chemstation was used. Thermodynamic analyses were carried out on piston columns ECO^{PLUS} at temperature range of 293 K – 333 K. Diode array detection was used in the range of 200 – 400 nm.

2.5 Chromatographic experiments

Solvents and corresponding mobile phases were used according to the solubility of each analyte. Analyses in methanol and acetonitrile mobile phases were carried out on the piston columns directly connected with the HPLC system. In the case of toluene-mobile phase, the piston columns were connected only with the pump and the fractions were collected, evaporated to dryness, dissolved in methanol and then analyzed on C18 column. The concentration of all solutions was 5 $\mu\text{g/mL}$ or 200 $\mu\text{g/mL}$ for the MEP and PAs, respectively. 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 procedures were used in the case of NIPs.

3. Results and discussion

The thermodynamic study was applied to investigate the influence of temperature on the binding performance of tested analytes (MEP and PAs) by using the prepared polymers (M1-MIP and PAs-MIP, respectively).

In the first step the temperature influence on the retention behaviour was investigated in the case of M1-MIP in three different mobile phases – porogen (TOL), MeOH and ACN. As it can be seen in the graph (Fig. 2.), in all cases the sorption of analytes was favoured by the enthalpic, not by the entropic term. In the case of the ACN-mobile phase calculated values were significantly higher for the MIP. As to the NIP lower enthalpic contribution was compensated by the higher entropy contribution. The similar results were achieved in the case of toluene-mobile phase. The binding mechanisms of tested analytes in ACN and TOL are probably very similar. Thermodynamic data indicates that prepared imprinted polymer does not possess “lock to key” mechanism of the recognition processes and the sorption of analytes used in the investigation did not proceed according to the mechanisms expected for the imprinted polymers. The results strongly suggested that the affinity of analytes tested for the MIP-binding sites was controlled not by the steric hindrances of substituents in the space but probably by the strength of interactions between functional groups of analytes and complementary functional groups of polymers. Obtained observations did not confirm a good molecular imprinting effect for this imprinted polymer.

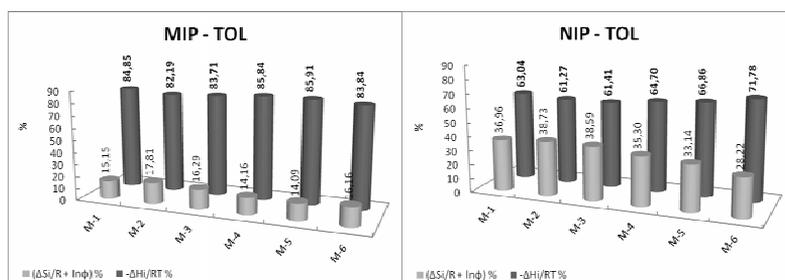


Fig. 2. cont. on the next page

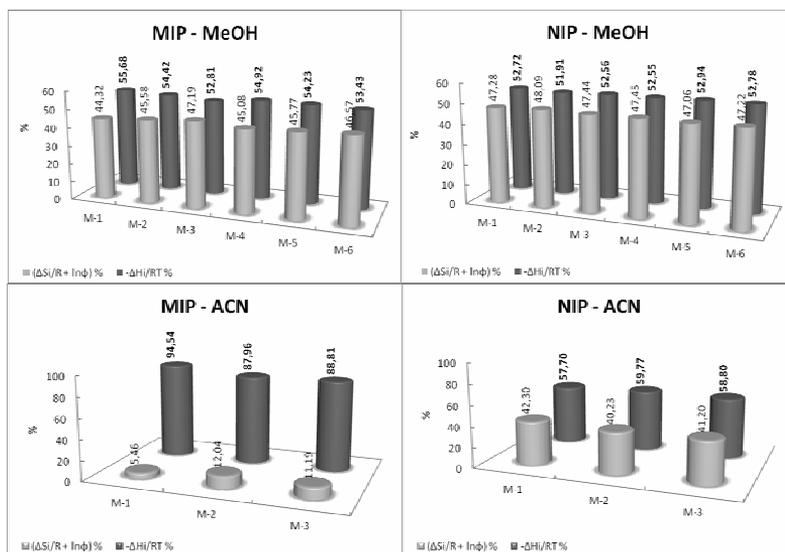


Fig. 2. Graphical representation of the thermodynamic terms values of linear regression ($\ln k = f(1/T)$) in % for MEP in different mobile phases, calculated at $T = 293$ K.

An influence of temperature on the binding performance of PAS-MIP against PAS in two different mobile phases – ACN (porogen) and MeOH was investigated in the second experiment. The affinity of tested analytes for the prepared imprinted polymers was again explained by considering the thermodynamic functions derived from the van't Hoff equation. The affinity of tested analytes for the polymers in methanolic mobile phase in most cases was very low.

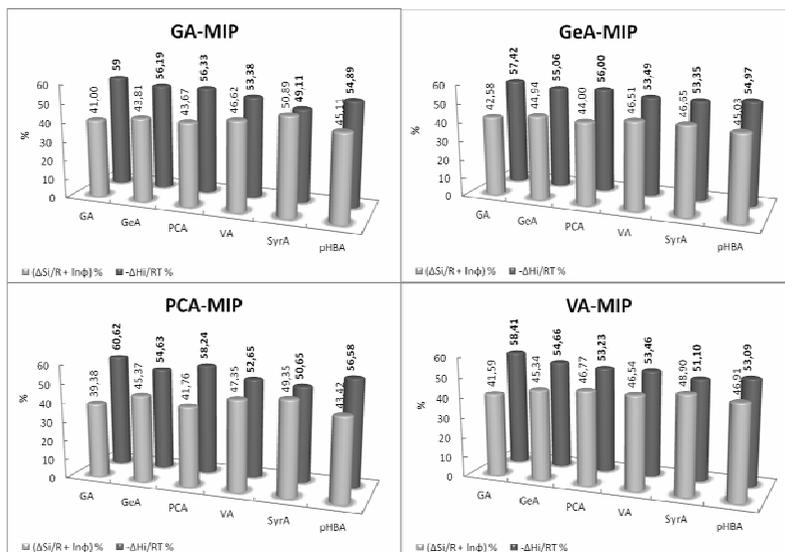


Fig. 3. cont. on the next page

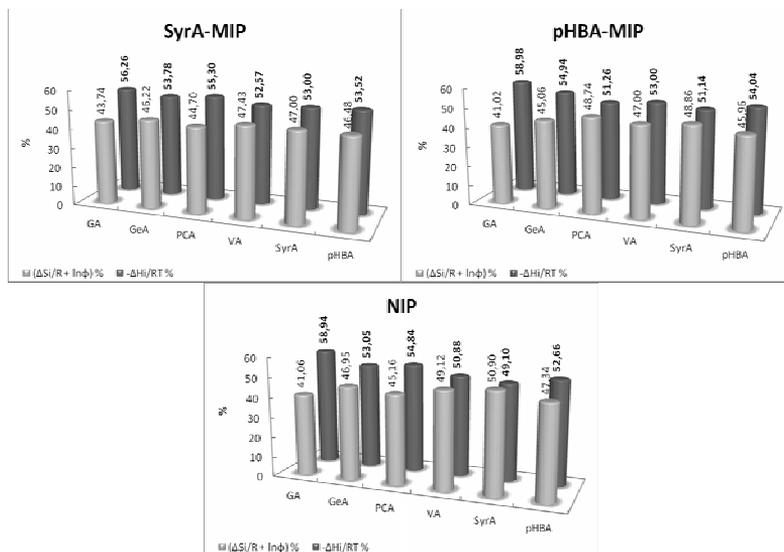


Fig. 3. Graphical representation of the thermodynamic terms values of linear regression ($\ln k = f(1/T)$) in % for PAs in ACN-mobile phase (porogen), calculated at $T = 293$ K.

Figure 3 illustrates the percentage contributions of determined thermodynamic parameters for the PAs-MIPs in ACN-mobile phase. Similar situation like those for the M1-MIP occurred during the specific sorption of PAs on prepared polymers. Values of the enthalpic and entropic terms derived from the van't Hoff dependency were negative for all systems studied. Almost in all cases there were small or very small differences between the calculated values of enthalpic and entropic terms contributions for PAs-MIPs and the corresponding NIP. In most cases the transfer of analytes tested from the mobile to the surface of the polymeric stationary phases (to the PAs-MIPs and the NIP) was enthalpically favoured. However, there were some exceptions. In the case of SyrA, its affinity to the NIP and to the GA-MIP was slightly entropically driven, while its affinity to the other MIPs was energy favourable. These phenomena indicate very complicated interactions between analytes tested and MIP-surface during the sorption processes. Nevertheless, experimental data set the recognition mechanisms on the PAs-MIPs also are not consistent with the MIP-theory.

4. Conclusions

Presented work showed that there were differences between the calculated thermodynamic parameters responsible for the recognition properties of analytes on the tested imprinted polymers in comparison to the corresponding non-imprinted polymers. The results indicated that sorption processes of the target analytes on the MIPs were mostly energy favourable. Presented experiments proved to be very helpful in the identification and specification of which term of the van't Hoff relationship was less responsible for the processes occurring on the MIP-stationary phases.

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