

Chromatographic properties of molecularly imprinted polymers

Doctoral thesis

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1. Introduction

Molecular imprinting is a novel technology for creating selective binding sites in a polymeric matrix by polymerization in the presence of the target compound (the template) (Figure 1). The template can be leached out from the polymer and the resulting molecularly imprinted polymer (MIP) is capable of selectively rebinding the target compound from a sample matrix.

MIPs mimick natural binding sites in many respects. At the same time, imprinting is a simple and efficient synthetic method and the MIPs have high physical and chemical stability.

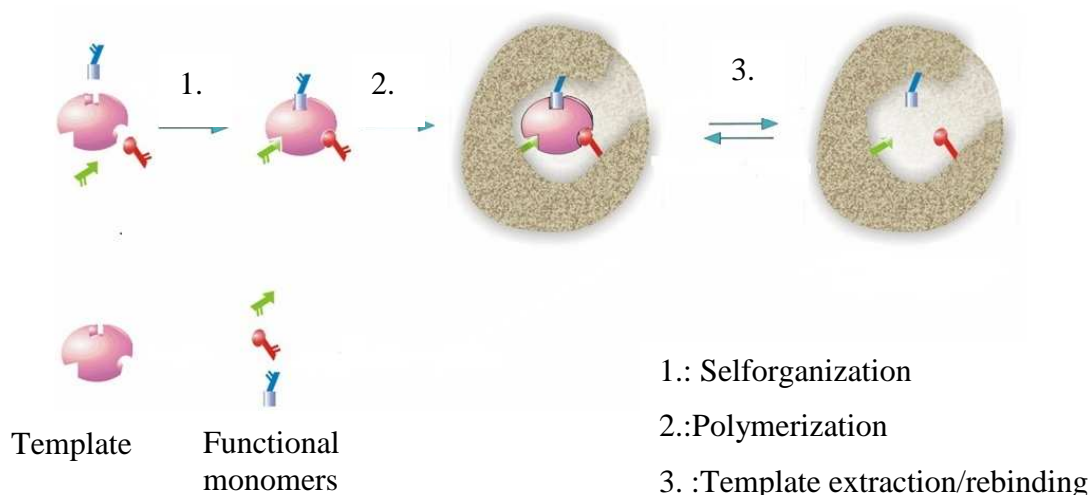


Figure 2. Scheme of molecular imprinting

Selective binding may be utilized in diverse practical applications like environmental technologies, chemical technologies and analytical methods. This explains the strong interest in MIPs.

1.1. MIP preparation methods

MIPs may be produced by covalent or noncovalent methods.

1.1.1. Noncovalent imprinting

Biological recognition is usually based on noncovalent interactions like H-bonding, hydrophobic, ionic or van der Waals interaction. These interactions are weaker than covalent bonds but multiple noncovalent interactions between the target molecule and the polymer may be sufficiently strong.

In the case of noncovalent imprinting the template and a functional monomer interact by noncovalent forces in the prepolymerization mixture.

The noncovalent method has some advantages:

- Removal of the template after polymerization may be carried out under mild conditions due to the noncovalent bondages
- Rebinding of the template is usually a fast process

The method has also some disadvantages:

- The complex between the functional monomer and the template is frequently nonstoichiometric and occasionally quite weak
- The polymerization conditions need to be carefully selected to promote complexation between the monomer and the template
- An excess of the functional monomer is usually required to shift the complexation equilibrium but this can lead also to the creation of nonspecific sites

The variability and simplicity of the noncovalent method have made it much more widespread than the covalent method, where the functional monomer and the template are covalently bound to each other before polymerization.

1.1.2. Bulk polymerization

The most common method for carrying out the polymerization of MIPs is bulk polymerization. The prepolymerization mixture is reacted in a glass container and a solid polymer bulk material is obtained. This is ground and sieved to obtain particles in a given size range.

Bulk polymerization is a simple process. The liquid mixture of monomers, template, porogenic solvent and initiator is deoxygenated and heated to 60 °C for 24 hours or it is thermostated at a lower temperature like 5 °C and irradiated by UV light for several hours.

Disadvantages of this method include the irregularity of the shape and size of the sieved particles, the high workload and the material loss at sieving. A further problem is the uncertain reaction temperature due to the heat of polymerization.

1.1.3. Preparation of uniformly sized spherical MIP beads (silica-MIP composites)

MIPs may also be synthesized in the pores of porous materials, e.g. in surface modified reverse HPLC packing materials.

The polymerization mixture used for bulk polymerization is soaked into the pores of the silica and the polymerization is allowed to occur. The result is a composite consisting of spherical particles of the same size as the original silica (Figure 3). The maximum amount of polymerization mixture to be used is defined by the pore volume of the silica. After polymerization the silica may be leached out to obtain uniformly sized spherical MIP beads but these are mechanically not sufficiently strong. Therefore it is better to use the composite itself. In this case, however, the silica should not itself adsorb any compounds of interest. This can be achieved by using hydrophobically modified silica particles which are commonly used in reversed phase HPLC. It should be noted that the imprinted polymer which is formed in the pores of the silica is porous itself. Naturally the pore size of the polymer cannot exceed the pore size of the silica. Thus a silica with wide pores (e.g., 200 Å) should be used.

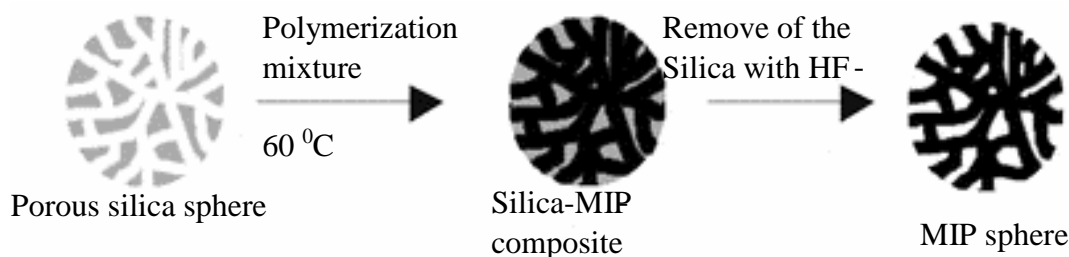


Figure 4. Preparation of MIP in the pores of silica

1.2. Chromatographic behavior of MIPs

Chromatographic experiments with MIPs should be divided in two categories:

- characterization of MIPs,
- separation on MIP columns.

In either case the chromatographic peaks are unusually wide, characterized by extreme tailing. This is most obvious with the template peak but may be also observed with some other compounds. Several explanations have been proposed for this phenomenon:

- the MIP particles are too big
- the MIP particles have irregular shape
- the binding sites are not homogeneous
- mass transfer is slow
- the adsorption isotherm of the analyzed compound is nonlinear on the MIP

All these factors may indeed contribute to the tailing but they have scarcely been separated. Methods have been found to make small, uniformly sized spherical MIP particles (e.g. by precipitation polymerization, by making composites or by multistep swelling) and the peak tailing appeared to be reduced with these but could by far not be eliminated. Therefore some of the remaining other factors may be more important. MIP isotherms are always nonlinear even at low concentrations so this factor needs certainly to be considered.

2. Aims

The subject of my dissertation is the investigation of MIPs, particularly with respect to their analytical applications.

MIPs have been successfully made for many templates and therefore molecular imprinting appears to be a generally useful method. MIPs are stable materials with negligible heat and chemical sensitivity. Thus making MIPs appears to be a more simple method for synthesizing hosts in host-guest chemistry than by synthesis of small host molecules. The MIP binding sites are by their nature part of a solid carrier which makes their use easier than of small molecules.

This was a generally accepted view among MIP researchers when I started my thesis work. It appeared that only a few smaller improvements were left to be done. One of these was the reduction of tailing in MIP chromatography. Many researchers hoped to solve this problem by making smaller (5-10 microns diameter), more uniformly sized and spherical MIP beads instead of the 25-35 micron particles obtained by crushing and sieving bulk MIPs. I have tried to make such a MIP based on a literature method and obtained the good morphologic features mentioned above. In chromatographic testing this material gave somewhat narrower but still badly tailing peaks.

Just around this time Shimizu and coworkers published papers showing that the distribution of binding site strength on MIPs is much wider than previously thought. Instead of one or two kinds of binding sites there is a continuous distribution of sites by their strength. This wide distribution of binding sites began to be held responsible for peak tailing.

There was only one group, that of Guiochon and coworkers, which explained the chromatographic behavior of MIPs by the nonlinear isotherm. They had measured the isotherm of a particular MIP and calculated the expected peak shape from this isotherm. Experimental peak shapes were found to agree quite well with the calculated ones. Their results have not been widely accepted. In fact the MIP researcher who gave them the MIP for testing did not himself use this explanation later.

I felt the results of Guiochon convincing and decided to develop a more simple method for proving that peak tailing on MIP columns was due to isotherm nonlinearity. Guiochon's method required the painstakingly accurate measurement of the isotherm in

a very wide concentration range; moreover, his simulation was based on the fitting of relatively simple isotherm models to the data. The applicability of the simple models was doubtful in view of Shimizu's results. One could also not expect many researchers who synthesize MIPs to do the lengthy isotherm measurements with each new MIP. Therefore I went a different way. I checked the validity of some of the consequences of the nonlinear chromatographic model in such a way that the explicit knowledge about the isotherm was not necessary. My subsequent experimental work had shown that the role of the nonlinear isotherm is indeed more general and not limited to the single case investigated by Guiochon. With this information I thought to be necessary to see how isotherm nonlinearity influences other measurements with MIPs. In view of the difficulties of isotherm measurement and interpretation I tried to find out what general conclusions can be made without using a particular adsorption model and its mathematical form of the isotherm.

When a new MIP is prepared, the effectivity of imprinting and the selectivity of the new MIP are tested by some method. This has often been done by using the MIP as stationary phase in a HPLC column. Different compounds, including the template, are injected one by one on the column and the respective retention times are compared. The retention times have always been determined as the position of the peak maximum. It has been occasionally noted, however, that the peak maximum depends on the concentration of the injected sample. This is an unusual phenomenon in analytical chromatography. From studies in nonlinear chromatography I thought that this concentration dependence is also the result of isotherm nonlinearity. So I decided to check this assumption. It was also natural to check the suitability of any data calculated from retention times and used for the characterization of MIPs, e.g. the imprinting factor and the selectivity.

The nonlinearity of an adsorption isotherm is usually due to the limited amount of binding sites on the sorbent. If a solution contains more than one adsorbable compound these compounds may compete for the limited binding sites. Such competition is well known also at MIPs, but it has been described only in other applications than chromatography, e.g. in binding assays or with sensors. I wanted to understand and explain this apparent contradiction.

3. Methods

I have prepared MIPs by bulk polymerization and as composites in the pores of modified silica. I investigated the physical properties of these materials by electron microscopy, electron beam microanalysis and low temperature gas adsorption.

The adsorption properties of the polymers have been studied by equilibrium batch adsorption and by chromatography in columns packed with the MIP particles.

4. Experimental results

Chromatographic measurements have been made with columns packed with bulk and composite MIP, respectively. The MIPs were imprinted with phenytoin.

At first I compared the bulk and composite MIPs (Figure 3).

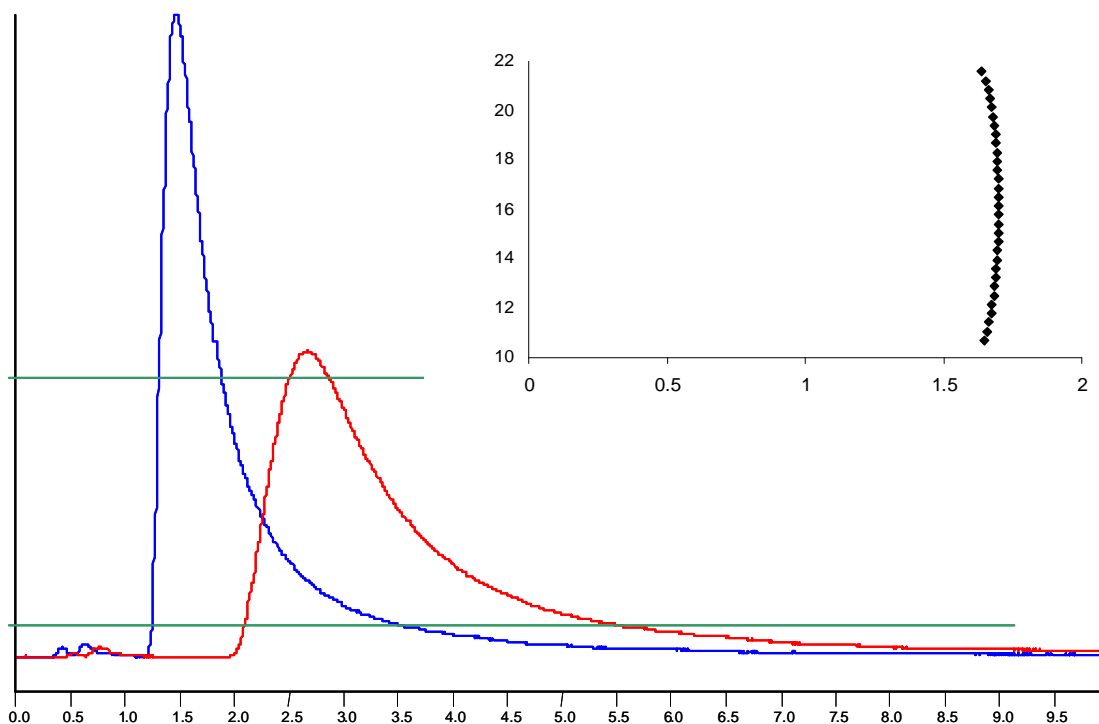


Figure 5. Comparison of HPLC columns packed with bulk and composite MIP, respectively. Inset shows the ratio of the reduced retention times of the two peaks on their descending branches, between the two concentration levels marked by horizontal lines.

Figure 5 shows that the peak on the composite column is narrower and less tailing, but it appears at a lower retention time. The ratio of the reduced retention times of the two descending branches is essentially constant.

The following experiments served to study the general behavior of MIP HPLC columns.

Effect of column length

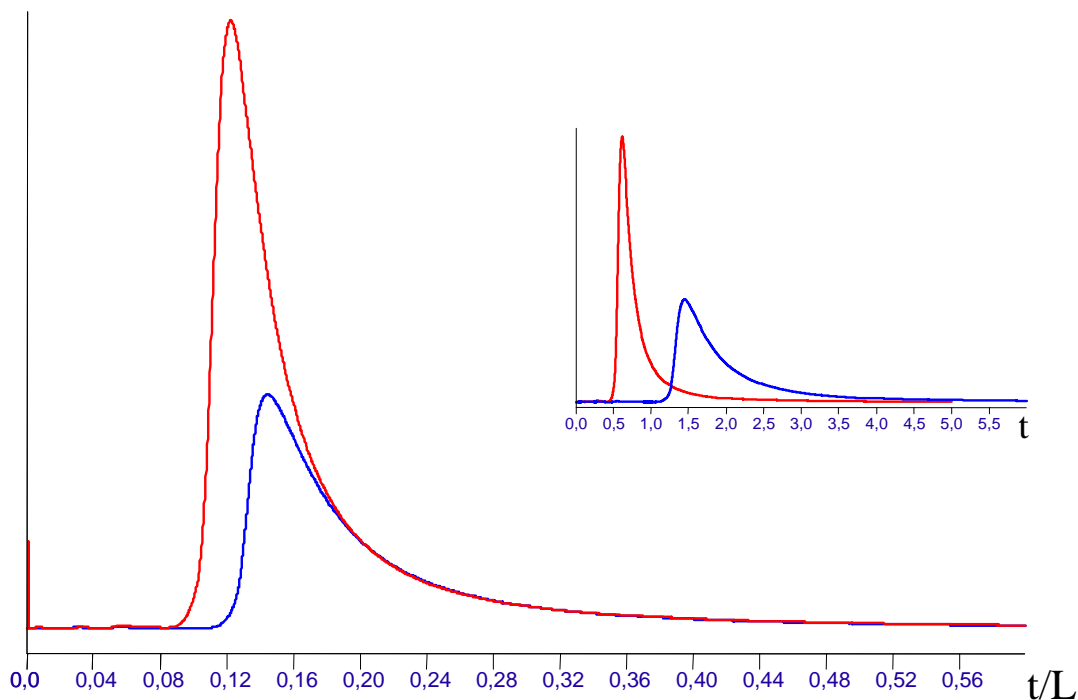


Figure 6. Chromatograms of phenytoin on phenytoin imprinted MIP columns of different lengths. The original chromatograms are shown on the inset. The main figure shows the

chromatograms with $\frac{t}{L}$ plotted on the horizontal scale

Note that the descending branches of the chromatograms in the main part of Figure 6 overlap.

Effect of the flow rate

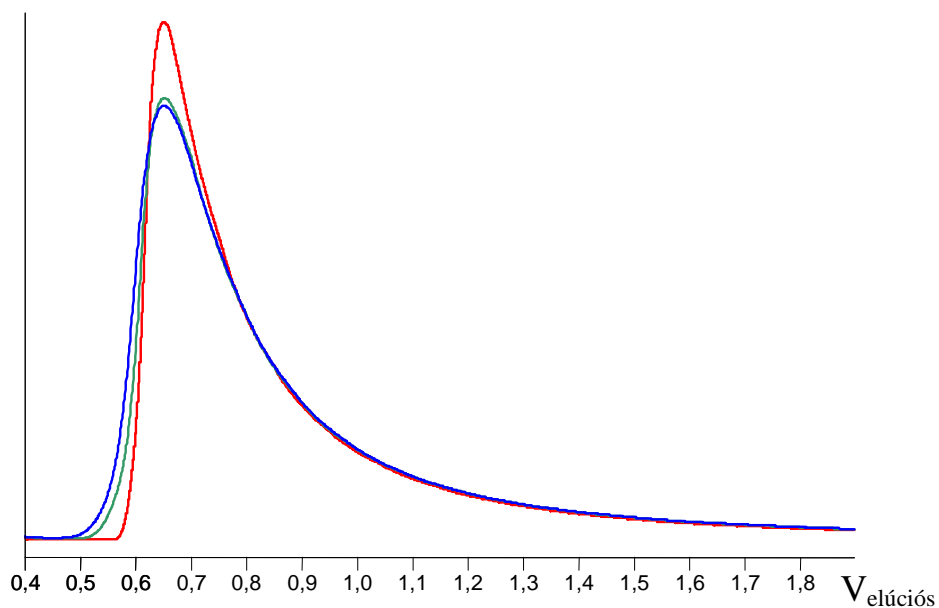


Figure 7. Phenytoin peaks at 0.05; 0.5 and 1 ml/min flow rates. The horizontal axis shows elution volumes in ml

Note the coincidence of the descending branches in Figure 7. The slope of the ascending branches changes with the flow rate.

Effect of particle size

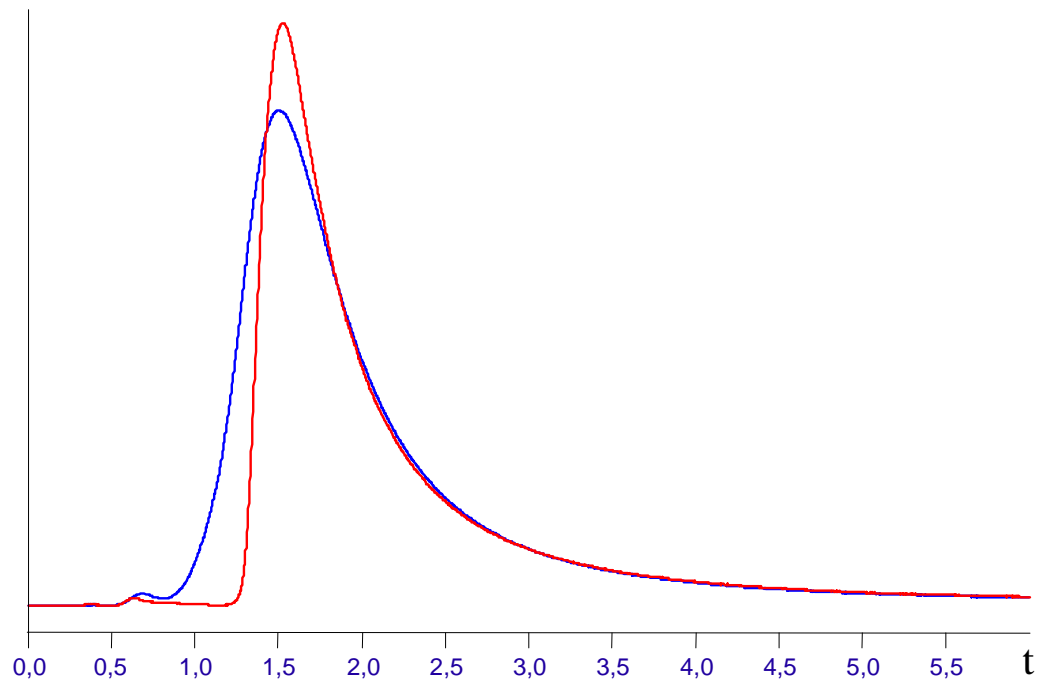


Figure 8. Phenytoin peaks on columns packed with 13 μm and 50 μm -composite MIP particles, respectively.

In Figure 8, the descending parts of the peaks overlap. The ascending branch is much steeper with the smaller particle size.

4.1. Investigation of the quantities used for the characterization of MIPs

4.1.1. Concentration dependence of the retention factor and of the imprinting factor

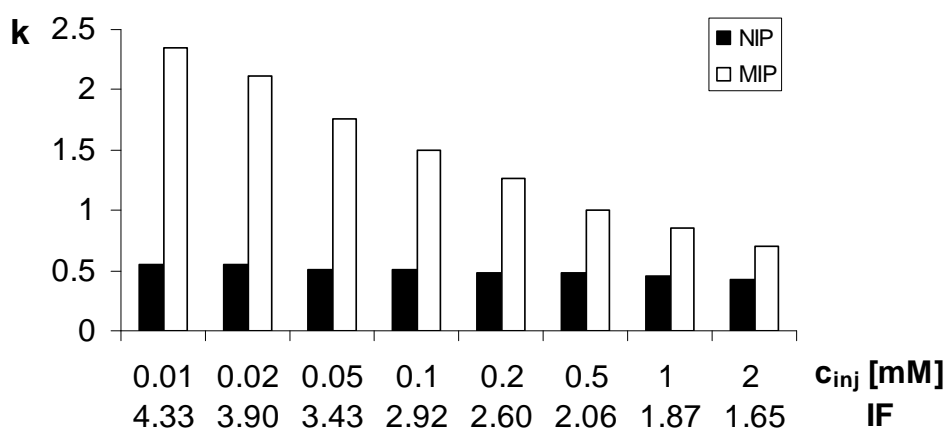


Figure 9. Investigation of the concentration dependence of the retention factor (k) and of the imprinting factor (IF)

Figure 9 shows the marked concentration dependence of both the retention factor and the imprinting factor.

4.1.2. Dependence of the selectivity on the geometrical parameters of the column

L (cm)	d (cm)	α	L (cm)	d (cm)	α
5	0.46	1.92	10	0.2	1.48
10	0.46	2.13	10	0.3	1.82
20	0.46	2.31	10	0.46	2.13

Table 1. Dependence of the chromatographic enantioselectivity (α) on the column length (L), and on the column inner diameter (d)

The results in Table 1 have been calculated from chromatograms obtained by numerical modeling of nonlinear chromatography using the Rouchon algorithm

The data show that the selectivity values obtained from the retention factor ratios depend on the column length and the column diameter, respectively.

4.2. Investigation of the competition effect on MIP chromatographic columns

Competition on MIP HPLC columns has been investigated by experiment and computer modeling. In the experiments the competition between phenytoin and hydroxyphenytoin has been studied on phenytoin imprinted composite MIP column. Computer modeling was carried out with a software written by Krzysztof Kaczmarek. The isotherm data of two enantiomers used in the model calculation were derived from the literature. Competition was studied upon coinjection of two competing compounds and compared to the separate injection of each compound. In experiments not shown here I studies competition by another method, called injection on a plateau.

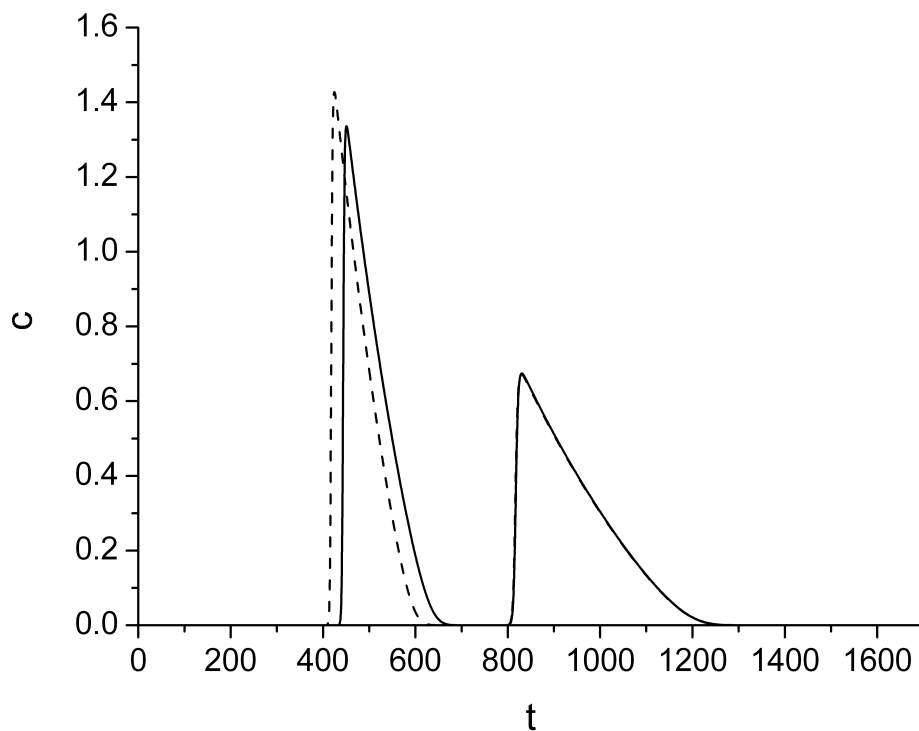


Figure 10: Simulated elution chromatograms of two enantiomers injected separately (continuous line) and simultaneously (dashed line), respectively. (The two lines overlap on the second peak.)

Figure 10 shows that coinjection has only minor influence on the first eluting (less retained) peak and no influence on the second.

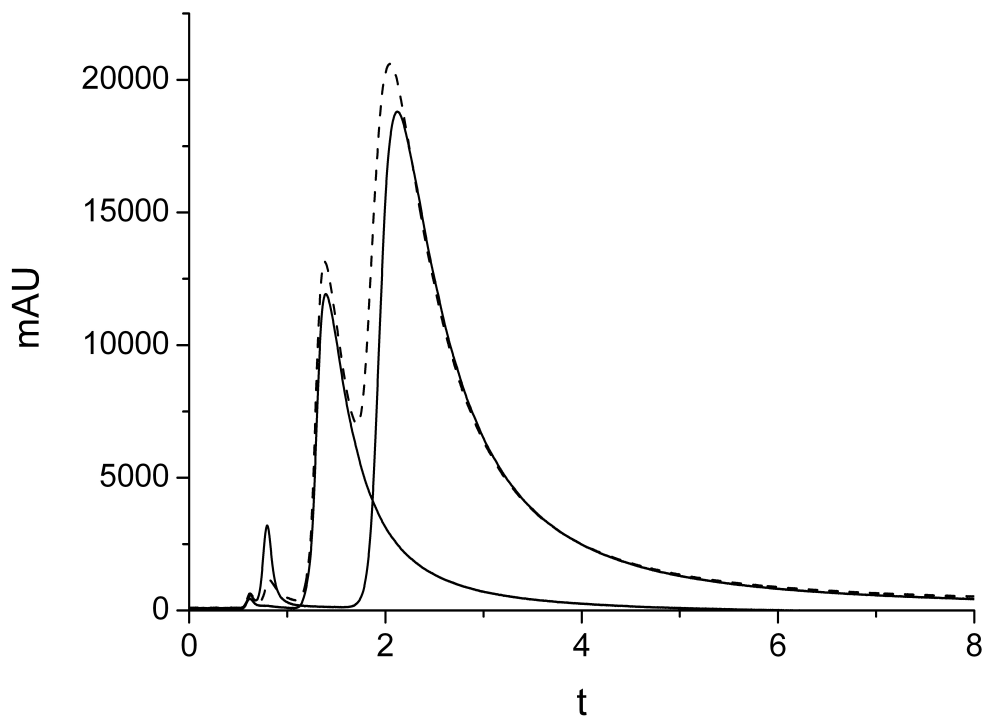


Figure 11. Experimental results for the injection of phenytoin and hydroxyphenytoin, separately (continuous line) and simultaneously (dashed line), respectively

This experiment shows a result similar to that obtained by simulation. The chromatogram obtained by coinjection was only slightly different from the sum of the two peaks obtained by separate injection.

5. Conclusion

1. I have created and verified a novel experimental method for detecting the nonlinear chromatographic behavior of molecularly imprinted polymer (MIP) stationary phases in the concentration range of their intended application.
2. I have proved the nonlinear chromatographic behavior of a phenytoin imprinted MIP made with methacrylic amide functional monomer in the pores of a modified silica stationary phase. This is the second instance that the nonlinear chromatographic behavior of a MIP has been proven and this has been achieved with a MIP substantially different from the one in the precedent work (different template and different functional monomer type).
3. I have proved both by experiments and by computer modeling that the characterization of MIPs by chromatographic experimental parameters like k , α and the imprinting factor (IF) is not appropriate. I have discovered that these parameters, which are all based on measuring the position of the peak maximum, show an unexpected dependence on apparently irrelevant experimental data like the length or inner diameter of the chromatographic column.
4. I have shown that the distribution coefficient D is a useful approximate measure for the characterization of MIPs. Although D is concentration dependent, it is independent from any assumed theoretical model of adsorption. In a not too broad concentration range D is an easily understandable measure. I could extract from published MIP data that the range of D values for different MIPs in their intended applications covers several orders of magnitude.
5. I have shown that the tailing of peaks in MIP chromatography cannot be avoided by merely making a MIP stationary phase with uniform binding sites as had been proposed in the literature. I have also shown that the use of uniformly sized spherical MIP particles does not result in reduced tailing.

6. I have shown experimentally and by computer modeling that competition between coinjected analytes is much less discernible in MIP HPLC than in non-transient techniques like batch competitive binding assays or sensors.
7. I have investigated the competition between the template and other compounds by injection on a concentration plateau. In agreement with my modeling results I have found competition to be more discernible under such conditions than in coinjection. I have shown by modeling and experiment that in special cases (like coinjection of a competitor in large concentration) the template peak may suffer severe distortions.

Publication list of Blanka Tóth

Publications in English, closely related to the dissertation:

B. Tóth, K. László, G. Horvai, Chromatographic behavior of silica–polymer composite molecularly imprinted materials, *J. Chromatogr. A* 1100 (2005) 60–67, IF:3.096

B. Tóth, T. Pap, V. Horvath, G. Horvai, Nonlinear adsorption isotherm as a tool for understanding and characterizing molecularly imprinted polymers *J. Chromatogr. A* 1119 (2006) 29–33, IF:3.554

B. Tóth, T. Pap, V. Horvath, G. Horvai, Which molecularly imprinted polymer is better?, *Anal. Chim. Acta* 591 (2007) 17–21, IF:3.186

Publication in Hungarian, closely related to the dissertation:

Tóth Blanka, Pap Tímea, Horváth Viola, Horvai György, Molekuláris lenyomatú polimerek: kombinatorikus előállítás, nemlineáris kromatográfia és pszeudo-immunanalitika, *Magyar Kémiai Folyóirat* 111 (2005) 110-113

Publications in English, not closely related to the dissertation:

F. Phillips, K. Kaczor, N. Gandhi, B. D. Pendley, R. K. Danish, M. R. Neuman, B. Toth, V. Horvath, E. Lindner, Measurement of sodium ion concentration in undiluted urine with cation-selective polymeric membrane electrodes after the removal of interfering compounds, *Talanta* 74 (2007) 255–264, IF:3.374

V. Horvath, B. Lorantfy, B. Tóth, J. Bogнар, K. Laszlo, G. Horvai, Preparation of terbutylazine imprinted polymer microspheres using viscous polymerization solvents, *Journal of Separation Science* 32 (2009) 3347-3358, IF:2.551