Analytical characterization and application of molecularly imprinted polymers

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thesis

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Introduction

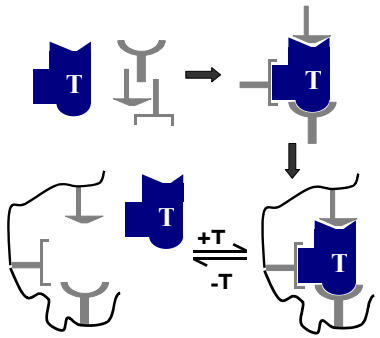
According to the objectives of an EU project during my doctoral work I set myself the task to characterize molecularly imprinted polymers and based on the results to develop analytical methods using the molecular recognition phenomenon.

Molecularly imprinted polymers (MIP)

Molecular imprinting means formation of selective binding sites in a polymer matrix for a predetermined molecule (template). This needs the addition of such monomers which are able to form interactions with the functional groups of the template. The structure of preformed monomer(s)-template complex can be fixed with polymerization by adding a crosslinker. After removing the template from the polymer binding sites (cavities) complementary in shape and functionality are left behind, which are able to rebind the template (see Figure).

Using tebutylazine imprinted polymer I have studied different solvents and conditions and developed a coupled solid

phase extraction procedure for the determination of terbutylazine from large volume surface water samples.



I have investigated a phenytoin imprinted polymer in elution HPLC mode by injecting molecules wich are similar in structure, in biological action or in hydrophobicity to the template. From the results I deduced the structure and the formation of the selective binding site.

Based on the adsorption isotherm of phenytoin on the phenytoin imprinted polymer and the peak shapes measured by elution chromatography I drew the conclusion that the reason of the tailing observed in case of MIPs is mostly the nonlinear isotherm.

Studying the simultaneous adsorption of phenytoin and atrazine I demonstrated positive (cooperative) interference effect on MIPs with two different molecules for the first time.

THESIS

1. I have developed a sample preparation method optimized for selective enrichment of terbutylazine. In this method I firstly employed coupling of C18 disk and molecularly imprinted polymer sorbent filled SPE cartridge for the preparation of large volume surface water samples.
2. Studying the tebutylazine imprinted polymer in solid phase extraction mode I demonstrated that small water content in acetonitrile can dramatically decrease the H-bond based selective interaction, but higher water contents lead to hydrophobic selectivity. I have observed the same phenomenon in HPLC mode with phenytoin imprinted polymer too.
3. Comparing the capacity factors of molecules which are chemically related to phenytoin or have similar hydrophobicities to phenytoin in acetonitrile eluent I concluded that the diphenyl group of phenytoin does not interact significantly with the surface. The phenytoin molecule binds to the binding sites with the functional groups of the hydantoin ring. In the rebinding process it is essential that the diphenyl group is not hindered sterically in the access of the site. This interpretation of the imprinted

sites differs from the usual MIP model and eliminates its contradictions.

4. I have proved that the calibration curve of a MIP based ligand binding assay can be calculated from the adsorption isotherm and the amount of used reagents. Based on these results one can optimize his assay with calculations (according to any function) after measuring the isotherm without the need of using any kind of model for the isotherm.
5. I have modified the batch adsorption method for the measurement of adsorption equilibrium. With this modified method one needs fewer measurements for similar results, especially in case of simultaneous adsorption of two compounds.
6. I proved with simultaneous adsorption measurements of two compounds that on MIPs and NIPs not only competition (proved by others) but also cooperation can be observed. I firstly demonstrated this cooperation for different compounds.

Publications:

1. **Tímea Pap, Viola Horváth, Antal Tolokán, George Horvai, Börje Sellergren**
   Effect of solvents on the selectivity of terbutylazine imprinted polymer sorbents used in solid-phase extraction
   Journal of Chromatography A, 973 (2002) 1-12

2. **Tímea Pap, George Horvai**
   Characterization of the selectivity of a phenytoin imprinted polymer
   Journal of Chromatography A, 1034 (2004) 99-107

3. **Tímea Pap, George Horvai**
   Binding assays with molecularly imprinted polymers – why do they work?
   Journal of Chromatography B, 804/1 (2004) 167-172