Chapter 5

Summary and Outlook

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Molecular imprinting is now a well established technology in the field of synthetic molecular recognition, offering a generic, robust, and cost-effective alternative to existing techniques such as monoclonal antibodies through either the re-establishment of covalent linkages or via a non-covalent mechanism. MIPs are highly crosslinked polymers that can be tailored with selectivity for a desired chiral guest. When a chirally pure template molecule is used, the generated binding sites are enantioselective. The selectivity observed is a result of the combination of the exact cavity-shape fitting and the exactness of the arrangement of the functional groups.

The aim of this work is to tailor optimized molecular imprinted polymers for the specific and selective recognition of amino acid derivatives and to exploit the specificity and selectivity of the successful system for the selective binding of the enantiomers of amino acid derivatives. The templates selected for this study are Boc-L-tryptophan, Boc-L-phenylalanine and a chiral compound D-mandelic acid. The molecular imprinting protocols employed involve free radical initiated bulk polymerization carried out in apolar, organic solvents to maximize electrostatic interactions such as hydrogen bonding, upon which many MIPs rely for recognition. The binding studies and the selectivity of the polymers were analyzed using UV-vis spectrophotometer. In this study MIPs and non-imprinted polymers were prepared and examined for their ability to bind preferentially the enantiomers of the print molecule. The

imprinted polymer exhibited a higher degree of template binding compared to the non-imprinted polymer revealing that they are inherently different materials and therefore exhibit different degrees of affinity.

It is important to understand the effects of the different variables in the imprinting process in order to improve the binding properties and ultimately the utility of MIPs. Several aspects of polymer preparation were investigated including the effect of percentage of crosslinking, template to functional monomer ratio (T/FM), concentration of the template solution, solvent, mass and time on the binding ability of the polymers to separate the enantiomers of the print molecule. A comparative evaluation and optimization of selectivity and specificity based on the nature and degree of crosslinking agent is also carried out in the present study.

Compounds with functional groups reciprocal to those of the target molecule or template are selected as functional monomer and are used to form a scaffold around the chosen template. In this study, a basic functional monomer 4-vinylpyridine (4-VP), which is an electrophilic monomer, was utilized for the preparation of imprinted polymers for these templates with a carboxyl group in the molecule. The complex formed between 4-VP and the chosen templates is preserved within a matrix to form an imprint that is chemically and sterically complementary to the template. It has been found that D-mandelic acid imprinted polymers made with 4-vinylpyridine as functional monomer yielded better separation than the one made with MAA, which we attribute to the possibility of π stacking interactions with the aromatic rings of the imprint

molecule in addition to the electrostatic interaction with the carboxyl group and H-bonding.

The formation of the pre-polymerisation complex prior to polymerization is crucial, since the structure of the resulting assemblies defines the subsequently formed binding sites, thereby affecting the recognition properties of the materials for the template molecule. The higher the stability of the complex, the higher the fidelity of the resulting recognition sites, and the stronger will be the recognition ability of the MIPs. The interactions between the templates and the used functional monomer 4-vinylpyridine were investigated by FT-IR, ^{1}H-NMR spectroscopic measurements and fluorescent experiments. The obtained results demonstrated the feasibility and utility of using these spectroscopic techniques for determining binding interactions during complex formation based on hydrogen bonding, π-π stacking, hydrophobic, and electrostatic effects in dependence of the applied solvent. IR spectroscopy was applied for the analysis of hydrogen bonded complexes in IR transparent chloroform solution enabling simultaneous studies of the free and complexed species confirming IR spectroscopy as a powerful technique for investigating molecular level interactions. The obtained results demonstrated that the concerted application of IR and NMR spectroscopic techniques and fluorescent titration offers a powerful strategy for pre-screening the interactions between the functional monomer and template molecule.

Presence of sufficient crosslinking is very important for the formation of strong polymer structure that can uphold its conformation in different environments. To decipher the role of these crosslinking agents

on the specificity and selectivity of the imprinted polymers two crosslinkers EGDMA and DVB were employed in this study. In all the three systems the specific binding is higher for the 40% crosslinked system and it decreases with increasing percentage of crosslinking. A comparison was made of the two different crosslinker systems. In fact it has become apparent that moderate degree of crosslinking can display significant affinity toward the print molecule. It was found that DVB-crosslinked Boc-L-tryptophan imprinted polymers prepared under the same conditions showed higher specific binding when compared to the EGDMA-crosslinked polymers. In the Boc-L-phenylalanine and D-mandelic acid imprinted systems EGDMA-crosslinked polymer was superior in specificity and binding than the one crosslinked with DVB. The polymer prepared with DVB seems to be harder as compared with the other polymers. The rigid DVB- and the flexible EGDMA-crosslinked systems would have different mechanical stability which eventually influences the stability of the geometry left by the template molecule. The relatively inflexible highly rigid matrix exhibits kinetic hindrance to reversible binding of the template molecule. The NNMBA system is found to be nonspecific due to the participation of the secondary amide group of NNMBA in the binding of the template. Thus it is clear that the nature of the crosslinking agent influences the specificity of imprinted polymers. The most promising polymer prepared are the one with 4-VP as the functional monomer and EGDMA as the crosslinker for all the three templates and is used to optimize the composition of the polymer for best recognition.

The relative ratios of template and functional monomer are of significant importance for the recognition characteristics of the product polymers. In the DVB-crosslinked Boc-L-tryptophan imprinted polymers the one having 1:4 ratio possesses the highest specific binding. In EGDMA-crosslinked polymers though the binding by the imprinted and non-imprinted polymers of the 1:8 system is higher than the 1:4 system their specificities are comparable. In both Boc-L-phenylalanine and D-mandelic acid imprinted polymers with EGDMA and DVB crosslinking, the specific binding of the 1:4 systems is high compared to the 1:2 and 1:8 T/FM ratios.

The extent of template binding increases with increased initial template concentration C_o and saturation was observed at higher template concentration and then the binding remains constant indicating that the available receptor sites have been saturated with template in all systems. MIP and NIP differ in their time taken for saturation of binding sites. Generally, for imprinted polymers the time taken for saturation of binding sites is higher compared to the non-imprinted polymers. This is because the template molecule has to be penetrated through highly crosslinked network to access the imprinted sites for binding whereas in the non-imprinted system there is no specific arrangement of the binding sites, and nonspecific interactions at the available sites of the polymer leads to a fast random binding of the template. The time for saturation of binding depends on the nature of crosslinking agent. From the studies it is clear that saturation of binding sites occurs within a few hours and overnight equilibration is not necessary.

The influence of the solvent on the extent of template binding on the imprinted polymers was investigated. The studies revealed that the binding was high in the solvent which was used as the porogen. This effect takes place because of the three-dimensional structure of the imprinted sites, those that have a functionality, size, and shape that are perfectly complementary to those of the template is destroyed in the presence of other solvents. A deviation was observed in the case of DVB-crosslinked (40%) Boc-L-Trp-OH imprinted polymer with 1:2 T/FM ratio and DVB-crosslinked (40%) Boc-L-Phe-OH imprinted polymer with 1:4 T/FM ratio which offered maximum specificity in dichloromethane compared to chloroform which is the porogen. This may be attributed to the similar structure and polarity of dichloromethane to chloroform. The 40% EGDMA-crosslinked D-mandelic acid imprinted polymers showed maximum binding in water. An obvious increase upon total binding to the polymers in water arises from increased hydrophobic interaction between the template molecule and polymer matrix.

Molecular imprinted polymers are generally polyclonal in nature, always exhibiting a distribution of affinity for the target compound. Scatchard analysis revealed that two classes of binding sites were formed in the imprinted polymers of the three selected templates. The equilibrium dissociation constant K_D and the maximum number of binding sites S_{max} are determined by the Scatchard-type analysis. The lower value of K_D implies stronger guest binding. The high binding to the imprinted polymers are due to high concentration of template binding sites. As is evident from the studies the specific rebinding increased with increase in the mass of the polymer. But the substrate bound per gram of the polymer

remains almost constant. With increase in the mass of the polymer the number of binding sites is expected to increase with the result that the substrate binding also increased.

The structure of the template molecule governs the efficiency of the binding. The template effects in these cases are confirmed through comparison of the binding of structurally related compounds to the template molecules in different polymers. MIPs of different templates have different degree of molecular recognition to the template and structural analogues. The study was conducted with optimized EGDMA- and DVB-crosslinked polymers. Within the experimental error, the imprinted polymers exhibit selectivity towards the print molecule compared to other structurally related compounds. The MIPs of Boc-L-tryptophan could not separate Boc-L-Phenylalanine effectively and vice versa. The MIPs of Boc-L-tryptophan had the characteristic selective specificity to the imprinted molecule not only due to the complementary functional groups but also due to the matched space structures. The indolyl group in Boc-L-tryptophan is much larger than the phenyl group in Boc-L-phenylalanine. Therefore, the MIPs of Boc-L-phenylalanine also could not bind Boc-L-tryptophan effectively. The lack of recognition of the small molecule like L-Phe by the large molecule imprinted polymers may be due in part to the different conformers of the print molecule resulting in recognition sites for different shapes in combination with different spatial orientation. Also due to the *tert*-butoxycarbonyl group in Boc-L-Phenylalanine the space structure of L-Phe did not match with the MIPs of Boc-L-Phenylalanine. Reduced binding implies the absence of any favorable interaction between the polymer and the structural

analogues. The results confirm that MIP binds the template molecule strongly but that there is partial cross reactivity to structurally closely related compounds.

The ability of the L-enantiomer imprinted polymer to separate the D-enantiomer was investigated. From the studies it is clear that L-enantiomer was more retained than D-enantiomer on the L-specific MIP. This result is anticipated since the stereo structure of D-enantiomer molecule is not complementary to the cavities in the L-enantiomer imprinted polymer which induces the weaker interaction of D-enantiomer. The mechanism of recognition was shown to involve ionic bonding to the carboxyl group H-bonding to the carbamate function of the substrate by the pyridinyl group and π- π interaction between the aromatic ring of the template and the pyridine ring immobilized in a stereo specific manner in the imprinted polymer. All the interactions were necessary for efficient enantiomeric resolution. These results mean that the complementary binding sites such as 'template-fit pockets' in which the position and the alignment of the functional group in the functional host molecule are optimally adjusted for binding the corresponding imprinted isomer are a principal factor to recognize the target molecule. One observation in the case of the Boc-L-tryptophan imprinted EGDMA-crosslinked polymer (40%) with varying T/FM ratio is that though the 1:8 polymer possesses comparable specificity with 1:4 polymer in binding the template molecule, the enantioselectivity is higher for the 1:4 system. In the DVB-crosslinked system though the 1:4 system possesses a high specificity in binding the template molecule, increasing the ratio from 1:2 to 1:4 reveals a decrease in selectivity. The 40% DVB-crosslinked polymer having

template to functional monomer ratio 1:2 is found to be effective in separating the enantiomer of the print molecule.

In the EGDMA-crosslinked Boc-L-phenylalanine imprinted polymers (1:4) the 40% polymer is found to have the highest enantioselectivity. As the percentage of crosslinking increases the enantioselectivity decreases. This is due to the increased rigidity of the imprinted sites with increasing crosslinking which decreases the access of the binding sites by the template molecule. In the DVB-crosslinked 1:4 system the enantioselectivity of the imprinted polymer is not appreciable. Because of the low selectivity of the 40-80 mmol% DVB-crosslinked Boc-L-phenylalanine imprinted polymers, enantioselectivity of the 1:2 polymer was carried out and it has been found that as the percentage of crosslinking increases enantioselectivity also increases. In the D-mandelic acid imprinted polymer system also as in the previous two cases the EGDMA-crosslinked system is proved successful in the specific and selective recognition of the template molecule.

The attempts made in this work led to the successful design of molecular imprinted polymers of the selected templates. With the significant advantages in easy preparation, low cost, predictable specific recognition, and high stability, the synthesized molecular imprinted polymers have the capability of specific adsorption and recognition of the template molecule. The foregoing investigations revealed that molecular imprinting is multidisciplinary in nature and possesses a high potential for applications in particular through their capacity for serving as robust artificial receptors for specific target molecule. In the future there will be a large number of different systems and companies using this exciting

new general platform technology, encompassing the analytical area including solid phase extraction, biosensor mimics, separation, the resolution of racemic mixtures, drug discovery and beyond. It is not clear at the moment to what extent MIPs can replace or more appropriately complement real biological receptors in terms of selectivity, binding strength and homoegeneity. Considering the versatility and high level of specificity and recognition that can be achieved the future of these materials is very promising.