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# Molecularly imprinted polymers for biomedical and biotechnological applications

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This survey covers main advances in the preparation and application of molecularly imprinted polymers which are capable of specific recognition of biologically active compounds. The principles underlying the production of highly efficient and template-specific molecularly imprinted polymers are discussed. The focus is on the imprinting of highly structured macromolecular and supramolecular templates. The existing and potential applications of molecularly imprinted polymers in various fields of chemistry and molecular biology are considered.
The bibliography includes 261 references.

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## I. Introduction

Biopolymers have an intrinsic capability for specific recognition, or binding, of certain molecules to form intermolecular complexes. Large molecular constructs, for example, receptors of protein nature, bind the corresponding biologically active compounds (referred to as ligands) and act as sensing elements that control most biochemical processes in a cell. Intermolecular interactions underlie enzymatic and immune reactions, directed transport of

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biomolecules, their transformations and so on. One of the major prerequisites for the occurrence of specific interactions between molecules is their mutual complementarity, that is, their matching each other as tightly as possible (as key and lock). This is achievable only when the entire ligand molecule, or characteristic moieties in its structure, finds a set of chemical groups in the receptor site that would provide the formation of a network of short-range specific contacts and when the walls of the cavity in this site tightly envelop the ligand to be recognized.

The specific recognition ability underlies many biochemical methods for the separation and assay of biomolecules, starting with affinity chromatography and ending with biosensors and diagnostic test systems. In nature, the specificity of molecular recognition is determined by the spatial and physicochemical complementarity of the receptor and ligand, so in theory there are no bars to the design of synthetic analogues of biological receptors. A technique for preparing such synthetic materials should be facile and available, and the materials themselves be stable. Technology for the preparation of synthetic polymers that contain imprints of test molecules (molecular targets) and can specifically bind to them is referred to as molecular imprinting.[1]

Molecular imprinting consists in forming polymer matrices (materials) based on organic or inorganic compounds capable of recognizing molecules and(or) ions that served as templates to form the polymer framework. In the structure of molecularly imprinted polymers (MIPs), there are regions that can specifically interact with template molecules or with structurally related molecules; recognition can occur with respect to shape or size, or due to interactions between the functionalities of the template and polymer. Documented are both the classic version of MIP synthesis involving the polymerization of functional monomers and cross-linking agents, and a simpler version where the polymer changes its state (transforms from liquid to solid) in the presence of the template to form a molecular imprint.

The preparation of polymers that contain imprints of large biological molecules and their application are now a very topical line of research.[2,3] The main merits of MIPs consist of their stability, moderate costs, and a great diversity of imprinting subjects ranging from low-molecular-weight compounds to biopolymers and even pre-cell species (phages and viruses) and cells.[4,5]

A great many original articles and surveys have been published during the last decade on the design principles of MIPs[6,7] and their usage, *e.g.*, in biochemical assay,[8–15] as artificial antibodies or receptors,[16–19] as catalysts of chemical reactions[20–22] and as recognition elements in biosensors.[23–27] We should mention that these publications mostly highlight specific aspects of molecular imprinting[28,29] or, on the contrary, cite general and common knowledge, leaving aside, in particular, the imprinting specifics of biomolecular templates.[7,30] Many articles, including survey ones, describe the use of MIPs in various research and applied fields, *e.g.*, in analyses of food products and natural objects (including water pools) for toxins[31] and as components of drug delivery systems in the body.[32] The documentation on applications of MIPs is very abundant but not systematized; there are, however, documents that summarize the information given in reviews.[33]

Here, we survey the retrospective, starting with the 1930s, and the state of art in the field of molecular imprinting of polymers. The focus of our survey is on the most important information gained in the last two decades on the principles of formation of molecularly imprinted polymers for biomolecular templates, such as biologically active and high-molecular-weight compounds, viruses and cells, starting with the selection of functional monomers to form the polymer framework and imprinting methods, and ending with the usage of MIPs. We will consider the prospects of their use *in vitro* and *in vivo* in biotechnological and biomedical applications. We systematize molecular imprinting strategies, including nontrivial approaches that make it possible to prepare molecularly imprinted polymers for the recognition and separation of bulk biomolecules having complex three-dimensional organization, for example proteins, and other biological items (viruses, bacteria, and even eukaryotic cells).

## II. Historical background

Materials having an enhanced specificity to molecular templates were prepared by Polyakov[34] in the beginning of 1930s. The silica gels formed in the presence of various alkylbenzenes were shown to have an enhanced (by $\sim$15%) adsorption ability just to the hydrocarbon that was present during the synthesis of polymer framework. The idea to construct artificial antibodies based on molecularly imprinted polymers for molecular biological applications first came to Pauling in 1940.[35,36] In 1949 Dickey,[37] Pauling's disciple, attempted at preparing polymeric matrices that would bind organic dye methyl orange, by mixing an acidic aqueous solution of silicate and methyl orange. The thus-formed silica gel contained dye molecules, and after drying and extraction of the template, the inorganic material adsorbed twice as great amount of methyl orange than of ethyl orange (its structural analogue).

In the 1060s, publications appeared where silica gels having an enhanced affinity to some type of molecule were described, but they did not attract enough attention of researchers. So was until Wulff and Sarhan[38] reported on their success to prepare imprinted organic systems by covalently bonding a polymer to a phenyl-α-D-mannopyranozide molecule. Wulff and Sarhan[38] were the first to call these systems molecularly imprinted polymers (MIPs), and the process to prepare them, molecular imprinting.

The beginning of a next step in the development of technology of organic polymers containing molecular imprints may be dated back to 1981, when Mosbach and Arshady[39] reported a new approach to the preparation of imprinted polymers; the underlying idea of that approach was pre-forming of non-covalent pre-polymerization complexes between a template molecule and framework-forming functional monomers. The approach, having simple methodology, raised the interest in molecular imprinting. Non-covalent imprinting technology was used to prepare polymers containing imprints of complex biopolymer molecules; the first publications about the production of MIPs capable of specifically binding protein molecules appeared in the late 1980s and early 1990s, and this considerably extended the potential application range of molecularly imprinted polymers.

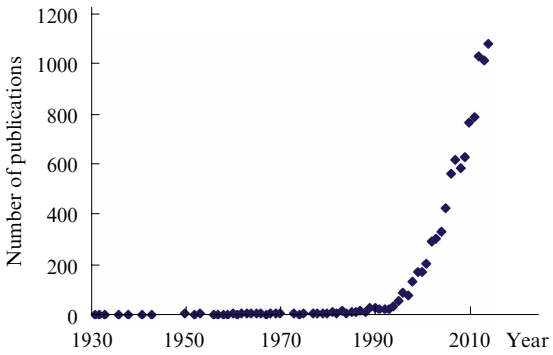



**Figure 1.** Evolution of the yearly number of publications on MIPs since 1930 through 2010 borrowed from www.mipdatabase.com.

According to www.mipdatabase.com, the number of publications devoted to molecular imprinting is rapidly increasing every year (Fig. 1), biomolecular templates becoming most popular.

## III. Principles of production of molecularly imprinted polymers

The preparation of a MIP comprises three stages (Fig. 2). At the first stage, a net of contacts is formed between a template molecule (or its fragment) and functional monomers. These contacts can be formed either *via* covalent bonding (covalent molecular imprinting), or *via* non-covalent intermolecular interactions (hydrogen bonding, ion–ion or hydrophobic interactions, *etc.*) between the functionalities of the monomer and template (non-covalent molecular imprinting). The second stage involves a polymerization (polycondensation) reaction between the complex formed at the first stage and the cross-linking agent. The resulting polymer framework contains regions having the fixed arrangement of functionalities as set by the template molecule. At the third stage, the template molecules are removed to give rise to cavities (imprints) in the polymer, which in an ideal case are complementary to the molecular template in size, shape, and(or) arrangement of functionalities. Such the polymer materials are capable of specific recognition of imprinted molecules, just as native antibodies recognize targets.

A person who intends to develop methods for preparing MIPs is advised to keep in mind several requirements to each component of the reaction. Important factors for imprinting are polymerization parameters (temperature, solvent composition, atmosphere and so on) and the ratio between components in the polymerization mixture (functional monomer, template, and cross-linking agent). The selection of these factors serves to ensure the required stiffness and porosity of the resulting polymer framework and the quality of the imprints to be formed.

The main requirement to a molecular template is its stability upon polymer formation; the template should be free of groups that would be directly involved in chemical transformations during polymerization. For a biomolecular template, the preservation of its three-dimensional structure should be ensured under polymer framework formation conditions, which can frequently be quite severe.[7]

In choosing the functional monomer–template pair, one should keep in mind some limitations. First, the use of a template that reacts with monomer(s) *via* more than one functionalities results in the formation of many specific molecular recognition sites; for example, a template containing acidic groups should be imprinted with the participation of monomers that bear basic functionalities, and *vice versa*.[40,41] Second, the template configuration should have steric and functional uniqueness such that would be sufficient for efficiently binding the test molecule to form an imprint in the presence of molecules of other compounds, specifically its structural analogues.[42] An important criterion for optimal imprinting is the preservation of contacts between the monomer and template during polymerization; a strictly definite spatial fixation of the functionalities that interact with the template upon secondary binding is also required.

Monomers are selected such that their functionalities would provide interaction between batch components (monomer + template) during polymer synthesis; in order to enhance the template-binding efficiency of monomers, functional monomers are frequently taken in excess.

The functional capacity (the accessibility and affinity of binding sites) of a MIP depends on the porosity of the polymer matrix: the larger the pores, the more accessible is the binding site to interaction with the test molecule. Reduced functions of the resulting MIP can arise from the poor quality of imprints (for example, binding sites can merge when the template is taken in excess), or from a large amount of unwashed template.

The main characteristic of a MIP is the binding selectivity toward the test molecule (template). Selectivity (also referred to as a selectivity coefficient, selectivity factor, or imprinting factor) in publications is assessed variously:

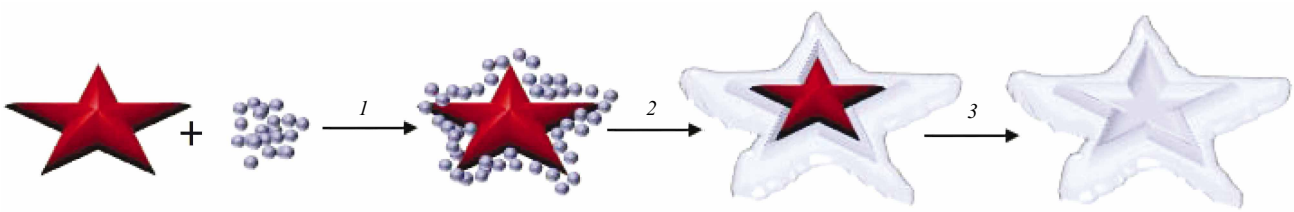



**Figure 2.** MIP preparation scheme.[24]
(*1*) The assembly of the pre-polymerization complex, (*2*) polymerization and (*3*) removal of the template.

— as the ratio of binding efficiencies of the template to the MIP and to the reference non-imprinted polymer (NIP) in isolated systems, derived from the physicochemical parameters that express the amount of template bound to the polymer,

$$S = \frac{C_{MIP}}{C_{NIP}} \quad (1)$$

(this quantity is also referred to as the imprinting factor);[43, 44]

— as the ratio of binding efficiencies of the template to the MIP and to the polymer imprinted in the presence of another template,[45]

$$S = \frac{C_{MIP1}}{C_{MIP2}} \quad (2)$$

— as the ratio of the physicochemical parameters that characterize the sorption, on the MIP, of the template ($C_t$) and the reference molecule ($C_{cm}$),

$$S = \frac{C_t}{C_{cm}} \quad (3)$$

exemplified by the separation factor when the MIP is used as a chromatographic sorbent:

$$S = \frac{k_{MIP}}{k_{NIP}} \quad (4)$$

where $k = (t - t_0)/t_0$, $t$ is the retention time of the template on the column and $t_0$ is the retention time of the unretained substance (the dead time of the column);[46, 47] or

— as the values of association/dissociation equilibrium constants of the template with the polymer.[48]

Each of these parameters can serve as a characteristic of molecular recognition by imprinted polymers, and the choice of a method for assessing selectivity is dictated by the problem to be solved. Naturally, a high selectivity coefficient of the MIP is the goal of researchers engaged in molecular imprinting; high values of selectivity coefficients (100 – 1000) have not yet been achieved for MIPs. Meanwhile, MIPs having selectivity coefficients of 1 – 100 can successfully be used for the isolation and recognition of individual compounds, design of biosensing devices, catalysis, targeted drug delivery in the body, and in other practices.[7, 8, 24, 33]

## IV. Classification of molecular imprinting methods

Molecular imprints in the polymer structure can be created on the surface (2D imprinting) or in the bulk of the polymer framework (3D imprinting). In 3D imprinting, the polymerization reaction is carried out in solution or suspension.[49] In 2D imprinting, the polymer is formed on a solid support (substrate) that bears the immobilized template. The prepared film is removed from the support, and its imprint remains in the film after the template is removed. The support can be glass, gold, plastic or another material in the form of a flat substrate or bulk item.[50] The advantage of supported MIPs is a high accessibility of binding sites to high-molecular-weight templates.

The polymers capable of molecular recognition can be categorized into organic and inorganic depending on the nature of functional monomers used.[2] Organic polymers are most frequently prepared *via* the polymerization of functional monomers and cross-linking agents based on unsaturated hydrocarbons;[7, 41] inorganic polymers are prepared by polycondensation with the use of functionalized silicon compounds, most frequently, substituted silanes,[6, 34, 37, 51, 52] or titanium compounds.[53 – 55] Hybrid organic – inorganic MIPs can also be prepared.[56]

In the character of specific contacts between the template and the functional moieties, non-covalent, covalent, and semi-covalent molecular imprinting modes are distinguished.[57, 58]

### IV.1. Non-covalent Imprinting

The interaction between the template molecule and functionalized monomers to form a pre-polymerization complex involves non-covalent intermolecular contacts, just as in the subsequent recognition of the template by the MIP. This is now a most popular approach to the design of MIPs.[59]

Radical polymerization involving functional monomers and cross-linking agents that contain acryl, vinyl or other groups having C=C bonds is most frequently used to prepare non-covalent MIPs.[60] The functional monomers can be acidic[17] (methacrylic acid, trifluoromethacrylic acid or vinylbenzoic acid) of basic[41] (vinylpyridine), can have a constant charge[43] (*N*,*N*-diethylaminoethyl methacrylate) or can be uncharged[7] (acrylamide or vinylpyrrolidone). Typical non-covalent interactions responsible for the molecular recognition ability of imprinted polymers are hydrogen bonds[61] and ionic contacts,[62] hydrophobic[63] and π–π stacking interactions.[64]

The incorporation and removal of the template upon non-covalent imprinting are, as a rule, performed in non-polar organic solvents, as non-covalent contacts are broken in polar solvents and this hampers the formation of a stable pre-polymerization complex. However, the amount of documentation on non-covalent imprinting in aqueous media is increasing in recent times. Search in this direction is in part dictated by the existing demand for imprinting biomolecular templates, which are very unstable in organic solvents.[54, 59] Non-covalent molecular imprinting does not impose severe limitations on the choice of functional monomers and stable template molecules, and moreover, the resulting polymers more frequently have enhanced template recognition selectivities.

Methacrylic acid (MAA) is the functional monomer most frequently used for MIP preparation, specifically *via* non-covalent imprinting, and ethylene glycol dimethacrylate (EGDMA) is the most frequently used cross-linking agent. Methacrylic acid can complex with various template molecules *via* ionic bonds (for example, with amines) or hydrogen bonds (for example, with amides or alcohols). One embodiment of this approach is the preparation of MIPs for the extraction of theophylline and caffeine from green tea (Fig. 3).[65]

It is noteworthy that the use of a mixture of functional monomers that are capable of reacting with various functionalities of a template molecule to prepare a polymer can enhance the binding selectivity of the template toward the polymer prepared in the presence of only one functional monomer. Ramstrom *et al.*,[41] for example,

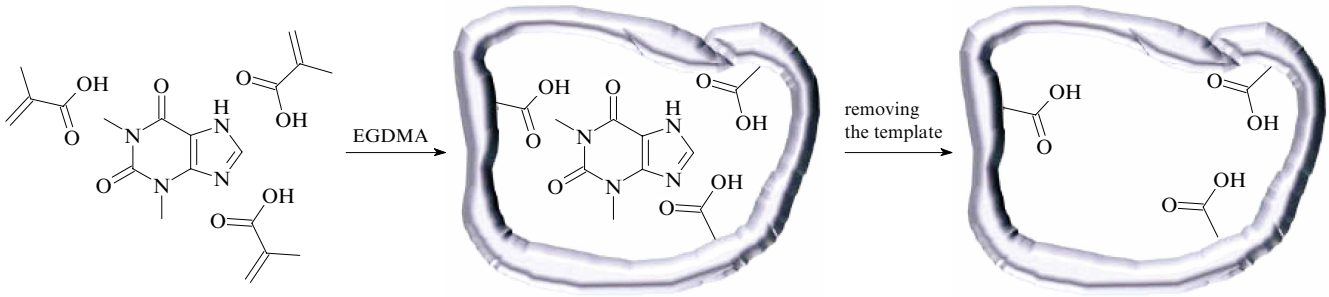



**Figure 3.** Preparation scheme for a MIP intended for theophylline release (adapted from Ref. 65).

compared three types of MIPs for dansyl-L-phenylalanine and Boc-L-tryptophan (Boc stands for *tert*-butoxycarbonyl) that were prepared by the copolymerization of EGDMA (as the cross-linking agent) with MAA, 2-vinylpyridine (2VPy) or a mixture of the two (as the functional monomers); the authors showed that the polymer prepared from the mixture had the highest affinity to the template molecule. The most likely reason behind the high selectivity of this polymer was the higher (relative to one-component polymers) content of the functionalities that were capable of hydrogen bonding and electrostatic interactions with the template molecule, the more so as the carboxy group of the MAA moiety is involved in binding sites for amino groups and the 2VPy moiety is involved in binding to carboxy groups of amino acids.

There are more intricate approaches to MIP production that use some functional monomers and composites, and additional factors to control the polymer structure. Karmalkar *et al.*,[66] for example, used the coordination properties of metals for positioning functional monomer moieties (imidazole rings, carboxy and hydroxy groups) in the microgel structure of the polymer in order to imitate the active centre of α-chymotrypsin, an enzyme of the serine protease family. The polymer produced from a multicomponent mixture of functional monomers in the presence of $Co^{2+}$ ions (which were necessary for coordinating functionalities) and the 2-({[6-(isobutyrylamine)caproyl]-L-phenylalanyl}2-amino)pyridine template, had the catalytic hydrolytic activity like that of α-chymotrypsin, whereas the non-imprinted polymer had only an insignificant activity.

The methodologically simple non-covalent strategy is widely used for imprinting molecules of diverse sizes and structures, including biomolecules, but there are some templates and MIP preparation modes for which covalent imprinting is optimal.

### IV.2. Covalent imprinting

The covalent imprinting technique proposed by Wulff[57] involves preforming of a functional monomer – template system by chemical synthesis in the form of a modified template or a covalent conjugate. The polymerization of such conjugates in the presence of cross-linking agents yields a polymer that contains covalently bonded template molecules. Covalent imprinting can result in the formation of various types of chemical bonds that can easily degrade under certain conditions, such as ester and(or) imine bonds.[67,68] After the polymer mass is produced, the bonds retaining the molecular template are intentionally broken in order to liberate the recognition sites of the MIP. A covalent bond between the polymer and the molecule to be recognized is again formed upon molecular recognition. The distribution of imprints over the polymer and their match to the template molecule is provided by a more strict arrangement of functionalities in the structure of a 'covalent' polymer compared to a 'non-covalent' polymer. However, covalent imprinting yet has some drawbacks, the major being the difficulty to remove the template. For example, relatively severe conditions are required for splitting amides and esters, under which the polymer frameworks can degrade. At the same time, incomplete removal of the imprinted template from the polymer can reduce the capacity and specificity of the MIP during recognition. We should also mention that covalent imprinting imposes some limitations on the choice of template and experimental parameters.

### IV.3. Semi-covalent imprinting

Some researchers (Refs 46, 69 – 72) propose combining the merits of the covalent and non-covalent approaches. This combined approach has been referred to as semi-covalent imprinting.[46,71] According to this technique, imprinting is performed *via* the polymerization of functional monomers that are covalently bonded to the template, and the secondary recognition of test molecules by template imprints involves non-covalent interactions.

One implementation of semi-covalent molecular imprinting was proposed by Zimmerman *et al.*[69] (Fig. 4). The authors prepared a branched (dendrimeric) structure using a porphyrin derivative and a functional monomer. Upon polymerization, the last porphyrin moiety bonded by ester bonds to functional monomers *via* all of the free OH groups, appeared to be a part of the polymer network. The template (porphyrin) was removed in an alkaline medium, which provided the break of the ester bond between the porphyrin and its surrounding. The resulting polymer contained a clear-cut imprint that corresponded to the porphyrin derivative and ensured its selective high-affinity recognition among the structural analogues [the equilibrium association constant was $K_a = (0.5 - 1.4) \times 10^5$ litre $mol^{-1}$].

Some publications on semi-covalent imprinting mention that the efficiency of a MIP for the recognition of reference template analogues varies depending on the structure of the recognized molecule. Using a polymer that can recognize the lysine – tryptophan – aspartate (Lys-Trp-Asp) tripeptide, Klein *et al.*[71] observed a high binding efficiency toward references, which were Lys-Trp

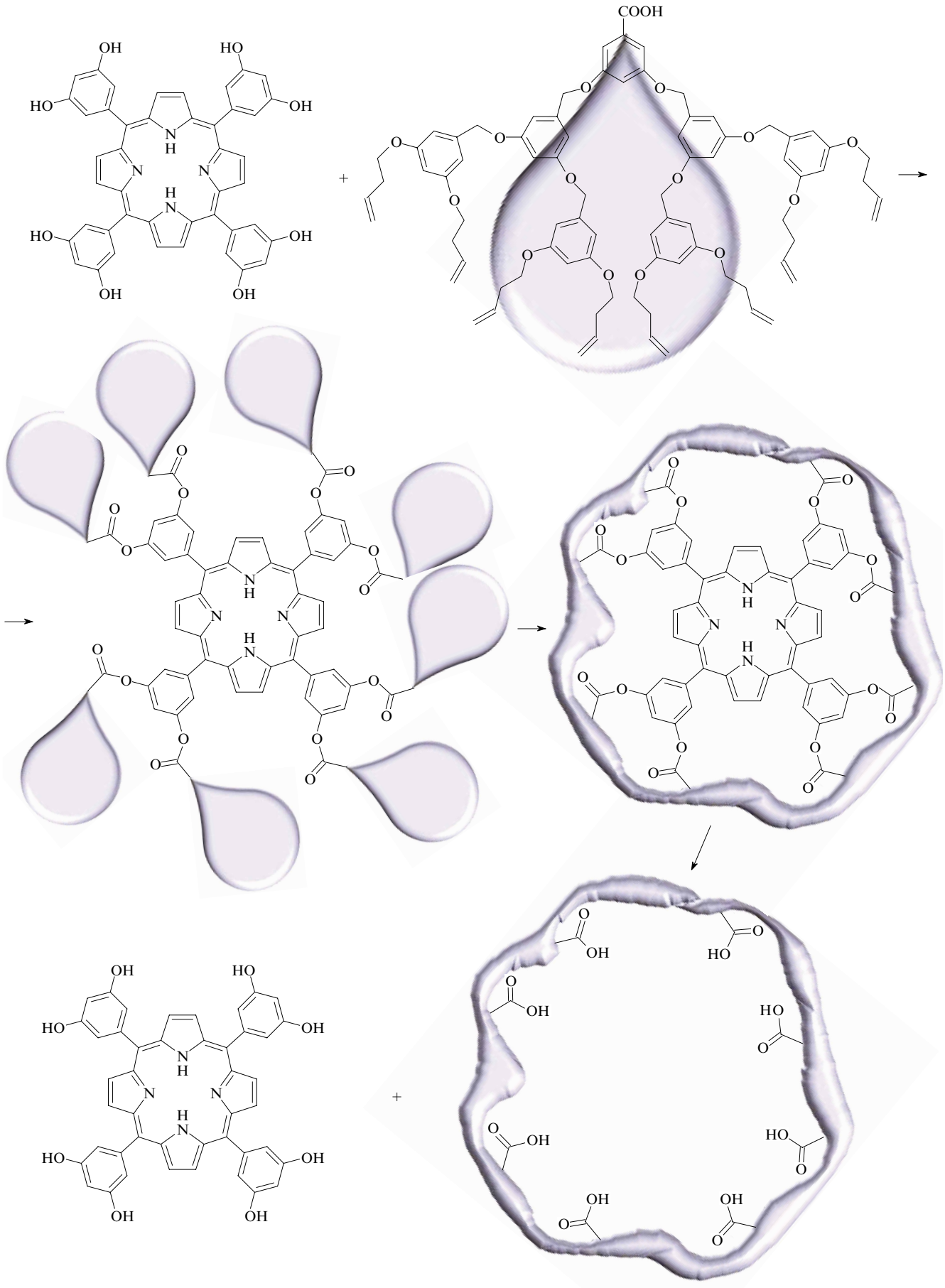



**Figure 4.** Semi-covalent imprinting of porphyrin using dendrimers (adapted from Ref. 69).

dipeptide and Arg-Trp-Asp (Arg is arginine) tripeptide. However, the binding efficiency calculated as the ratio of the peptide amount bound to the MIP to the amount bound to the reference polymer ($C_{MIP}/C_{NIP}$), reached a maximal value of 3.9 only when the tripeptide was used as the imprinting template.

Hwang and Lee[46] performed a comparative study of the cholesterol binding selectivity for polymers prepared by semi-covalent imprinting and non-covalent imprinting. For the semi-covalent imprinting experiment, the polymer was prepared using (4-vinylphenyl)cholesteryl carbonate; the cross-linking agent was ethylene glycol dimethacrylate. Non-covalent imprinting was used to prepare two polymers where the functional monomers were MAA and 4VPy, respectively (Fig. 5). All polymers showed enhanced affinities to cholesterol in the chromatographic separation of a cholesterol – β-estradiol mixture, but the polymer prepared by semi-covalent imprinting had a far greater cholesterol adsorption capacity. The separation factor was 3.2 for semi-covalent imprinting, and 3.0 and 2.9 for non-covalent imprinting with MAA and 4VPy monomers, respectively: [$S = k$(cholesterol)/$k$(β-estradiol), $k = (t - t_0)/t_0$, where $t$ is the retention time of the template on the column and $t_0$ is the dead time of the column]. Therefore, despite the difficulty to form monomer – template covalent binding and then to remove the template, the obvious advantages of the semi-covalent strategy are the clear-cut imprint and the high affinity of the polymer to the template.

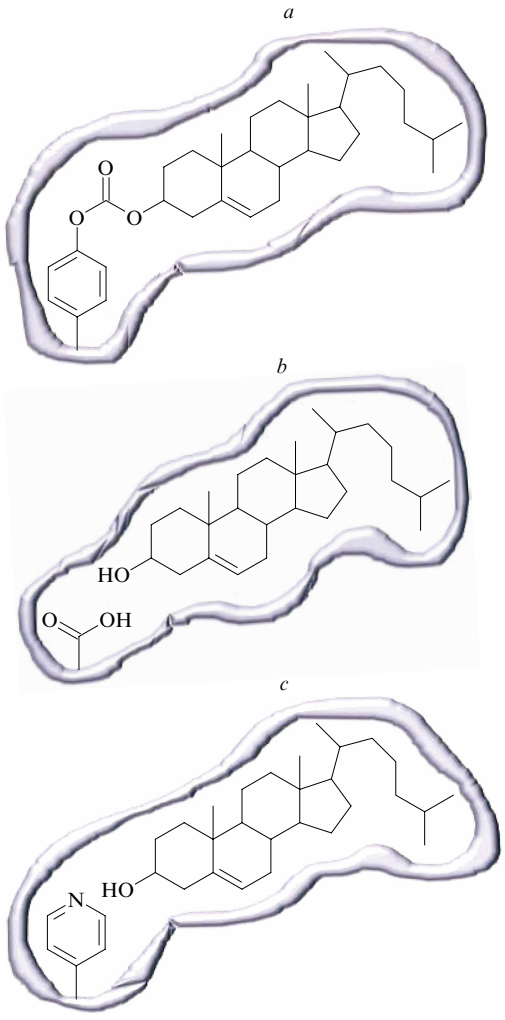



**Figure 5.** Schemes of bond formation between cholesterol and MIPs (adapted from Ref. 46).
(*a*) Semi-covalent imprinting, (*b*) non-covalent imprinting where the functional monomer is MAA and (*c*) non-covalent imprinting where the functional monomer is 4VPy.

Thus, the choice of strategy to prepare molecularly imprinted polymers depends on many factors and on the research or technological task. The preparation of MIPs is a universal procedure that can yield selective polymers for a wide range of compounds, specifically for those having biological activity, by adapting the batch composition, MIP format and polymerization method to each template.[73,74] However, original methods for preparing imprinted polymers are now underway to satisfy one or more of the following criteria:

— facile polymer preparation procedure;

— enhanced polymer capacity to the given template;

— enhanced binding selectivity of the template to polymer; and

— universal MIP preparation technique for various molecules.

We should mention that the design of MIPs for biological macromolecules, specifically proteins and enzymes, remains a challenge.

## V. Molecularly imprinted systems based on off-the-shelf polymers

Polymeric materials are widely used in MIP preparation; polymers, for example polypyrrole or polysaccharides (dextran or chitosan), interact with the template, and MIP formation occurs after polymeric molecules are cross-linked or polymerized with functional monomers.[75–78] The MIPs prepared using preformed polymers *via* the structural reorganization of polymer chains in the presence of the template to form molecular imprints after the template is removed constitute a separate class (Fig. 6). An alternative to classic molecular imprinting consists of the following: a framework-forming polymer is dissolved together with the template molecules to give rise to specific interactions between the polymer chains and template in the solution; after precipitation or drying of the solution and removal of the template, there remains a polymeric framework that is capable of molecular recognition.

Such a MIP is formed upon the transition of the polymer from solute to solid state, *i.e.*, without addition of functional monomers or cross-linking agents[79] and avoiding the polymerization stage. This approach can be exemplified by the preparation of molecularly imprinted polymers from polyamides (specifically, nylon 6)[80–82] and protein fractions.[83]

### V.1. Nylon-based molecularly imprinted systems

Nylon is widely used in scientific research and in biotechnology and medicine. This biocompatible, mechanically and thermally stable material, whose properties have been characterized comprehensively,[84] serves as the basis for diverse bioassay devices, for example DNA chips.[85]

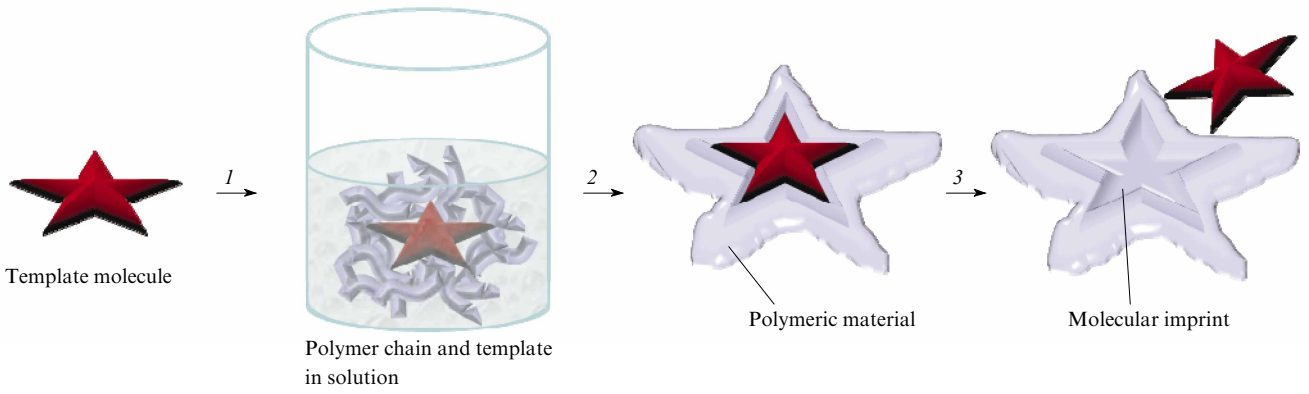



**Figure 6.** MIP preparation scheme based on off-the-shelf polymers (adapted from Ref. 82): (*1*) formation of interactions between polymer chains and the template, (*2*) imprinting and (*3*) removing the template.

The solvents most frequently used for the polyamide are acids (formic acid, acetic acid[86–88] and hydrochloric acid[89]) or 2,2,2-trifluoroethanol.[90–92] Co-solvents, namely water (up to 20%)[84] or chloroform,[91] or a liquefied gas, specifically carbon dioxide,[90] or various microparticles are used to provide the porosity of nylon membranes.[93] Another way to alter the properties of nylon membranes is to use composites.[87,91]

Documents published prior to 2012 describe nylon MIPs that were suitable only for the recognition of low-molecular-weight templates.[80,81,88] MIP preparation from nylon consists in the conversion of the polyamide from solute in an acid solution to solid *via* drying under air or precipitation by addition of a solution that is not a solvent for the polyamide (for example, water or an organic solvent). The functionalities of nylon itself served as functional moieties (Fig. 7).

The thus-prepared MIP was successfully used to separate L- and D-glutamines,[80,81] for which the equilibrium binding constants were $4.9 \times 10^5$ and $1.5 \times 10^5$ litre mol$^{-1}$, respectively. A weakness of this method is the use of strong acids for dissolving the polyamide, which can promote the degradation of biological templates (nucleic acids, peptides or other molecules).

The above-described method was further developed by the authors of Refs 82, 94, 95, who used 2,2,2-trifluoroethanol (which made it possible to preserve the three-dimensional structure of a biomolecule)[96,97] to prepare molecularly imprinted polymers for some low-molecular-weight templates (adenosine triphosphate and riboflavin) and high-molecular-weight templates (haemoglobin, bovine serum albumin and human serum albumin).[82] The selectivity coefficients of these MIPs ranged from 1.4 to 4.6 depending on the polymer formation parameters and the nature of template. The dissociation constants, for example for the haemoglobin template, were $12.3 \times 10^{-6}$ and $27.5 \times 10^{-6}$ mol litre$^{-1}$ for the MIP and the reference polymer, respectively.

The method for preparing molecularly imprinted polymers based on the formation of a polymer network upon transition of nylon from solute to solid state, is distinguished by facility, the stability of MIP and the usefulness of various templates.

### V.2. Biopolymer-based molecularly imprinted systems

Synthetic polyamides are not the only off-the-shelf polymers usable for producing molecular imprints; natural polymers (polypeptides and nucleic acids) can also serve for this purpose.[98–100] Since the active sites of enzymes are based on amino acids, polymers that contain peptide and protein moieties, theoretically, can give rise to a specific binding site for the template-molecule when used for molecular imprinting. Yoshikawa *et al.*[99] attempted to implement this theory; in order to prepare a MIP, they used hybrid polymers based on short peptides consisting of Boc-protected glutamate (Glu) one to eight amino acid residues long, immobilized on an aminomethylpolystyrene support. After the solvent was removed, the template polymeric membranes 80 – 140 μm thick served to separate L- and D-tryptophan optical isomers. The efficiency and selectivity of L-Trp adsorption on the imprinted membranes was shown to depend on the oligopeptide length. The optimal number of amino acid residues in the polymer was 3 – 5; the L-Trp association constant for these polymers was $3.4 \times 10^3 - 1.08 \times 10^4$ litre mol$^{-1}$. The highest selectivity coefficient was observed for the tripeptide-containing polymeric membrane; D-Trp sorption exceeded L-Trp sorption by a factor of 3.8.

Apart from simple linear peptides, 'pincers'-like branched peptide constructs having chain lengths of 1 – 4 amino acids immobilized on a polystyrene support (**1** – **4**) were used to prepare MIPs.[100] The use of branched constructs was shown to increase the affinity constant between the template molecule (Boc-D-Trp) and the imprinted polymer compared to the respective value for linear-oligomer-based polymeric membranes; the highest value of the constant ($1.44 \times 10^4$ litre mol$^{-1}$) was observed for the tetrapeptide-based construct. In the frame of the cited work, it was found that the adsorption selectivity coefficient for Boc-D-Trp increased relative to that for Boc-L-Trp as the oligopeptide chain length increased, and amounted to 1.5, 1.7, 2.1 and 2.5 for chains comprised of one, two, three and four amino acids, respectively.

Later, Yoshikawa *et al.*[83] proposed a new molecular imprinting strategy to prepare MIPs by drying a solution of the total protein fraction of *Geobacillus thermodinitri-*

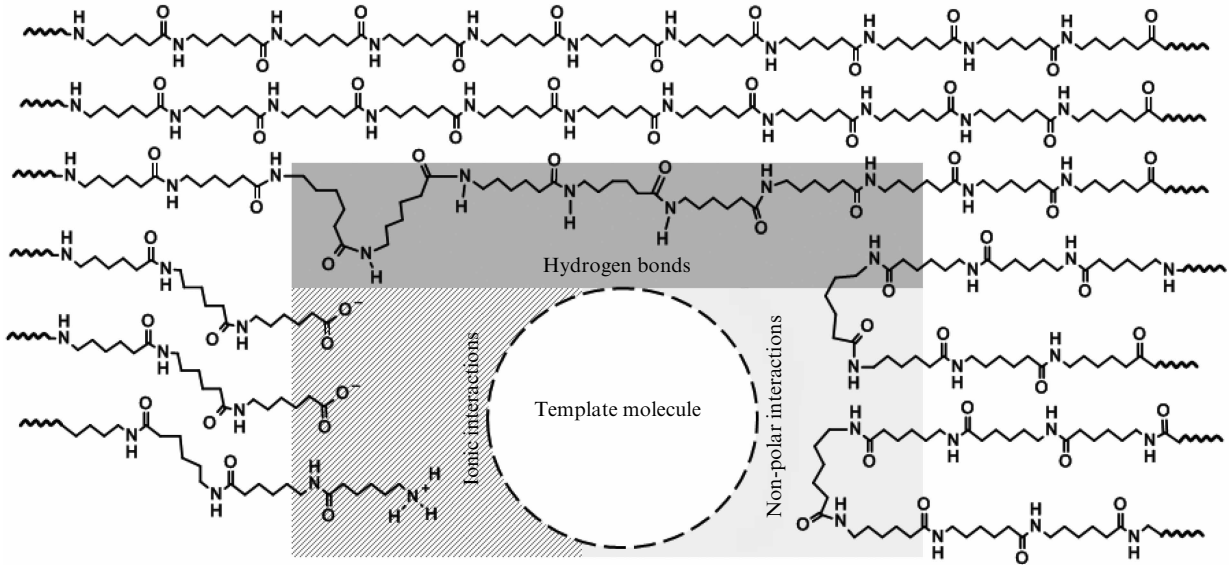
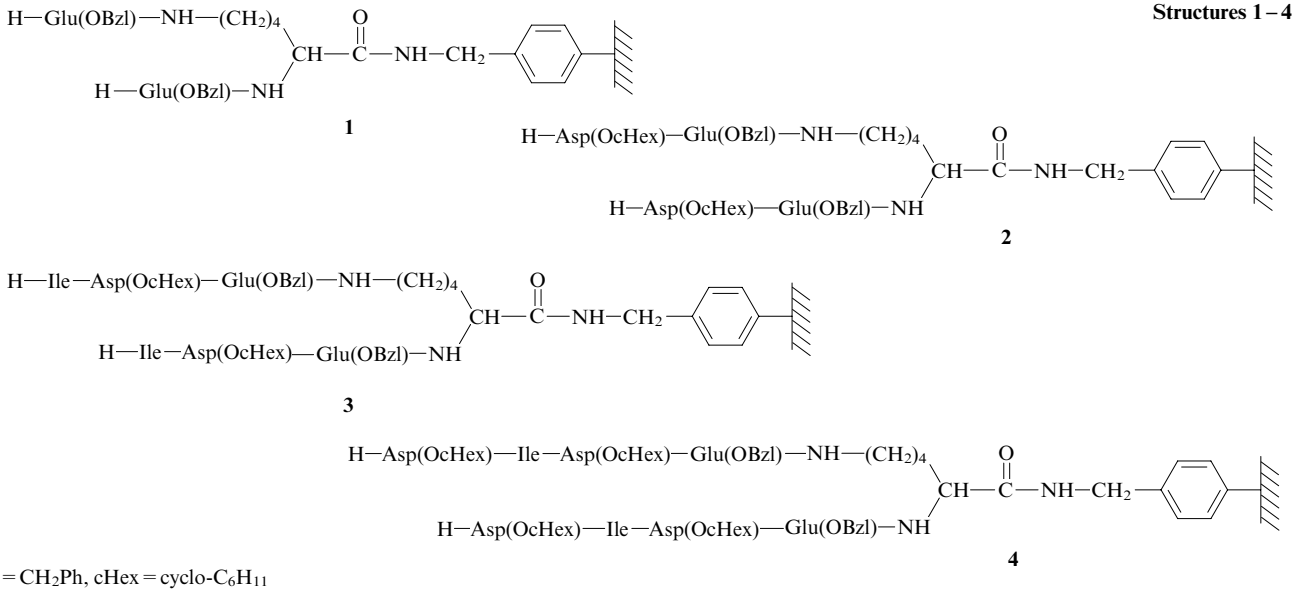



**Figure 7.** Scheme of interactions between molecularly imprinted nylon and the template (adapted from Ref. 82).

**Structures 1 – 4**




Bzl = $CH_2Ph$, cHex = cyclo-$C_6H_{11}$

*ficans* DSM465 in trifluoroethanol in the presence of a template (9-ethyladenine). Molecularly imprinted polymers were formed as protein membranes either without additional cross-linking of biopolymer chains, or with cross-linking by hexamethylene diisocyanate. Expectedly, the membranes prepared in the absence of cross-linking agents had insufficient mechanical strength, so the analysis of the properties of protein MIPs was performed for cross-linked polymeric membranes. Such a MIP preferred to bind to adenine when placed in an aqueous solution of adenine and guanine. The reference was a non-imprinted polymer, namely a protein membrane prepared in the absence of templates. The target binding selectivity factor for the MIP, expressed as

$$S = \frac{A/G}{[A]/[G]} \qquad (5)$$

($A$ and $G$ are, respectively, the adenine and guanine amounts adsorbed on the membrane; and $[A]$ and $[G]$ are, respectively, the residual adenine and guanine concentrations in solution after interaction with the NIP), was 4.2, while for the NIP it was as low as 1.1. The affinity constant between adenine and the molecular recognition site of the membrane was $1.75 \times 10^5$ litre $mol^{-1}$.

An insufficient mechanical strength may be treated as a disadvantage of protein polymeric membranes, against their obvious advantages, which are biocompatibility and biodegradability. The preparation of MIPs that contain

natural building blocks (amino acids and nucleotide bases), can provide for their high specificity, for example, if the intrinsic affinity of some nucleotide sequences to certain proteins is used at the stage of forming the molecular imprint. Umeno *et al.*,[98] for example, took the affinity of restriction endonuclease (EcoR1) to a certain DNA sequence as the basis of MIP specificity. As the polymer base, they chose poly(*N*-isopropylacrylamide), which contains photoactivated terminal groups that can covalently bond to a DNA. As the template, EcoR1 with a DNA was used. The photoinduced reaction of the polymer with the DNA occurred during polymerization to yield a uniform polymeric matrix, where the DNA sequence became part of the polymeric framework. After the template protein was removed, the polymer contained a free specific recognition site for binding the enzyme. This strategy cannot be called universal, as the DNA sequence should be selected for each template.

Thus, the use of off-the-shelf polymers (polyamides or biopolymers) to form molecular imprints offers a suitable means for imprinting structured biomolecules, such as proteins, as the absence of a polymerization reaction mildens the formation conditions for a solid framework.

## VI. Imprinting of biological macromolecules

The manufacture of materials specific to biological objects is of great importance both for research purposes and for medical dianostics and therapy. The first biological subjects of molecular imprinting were low-molecular-weight biologically active compounds: vitamins, hormones, toxins, drugs, nucleotides and their derivatives.[73, 74, 101 – 103] They turned out to be optimal imprinting subjects thanks to their small sizes and stable structures. Going to the imprinting of complexly organized biomolecules (nucleic acids, peptides and proteins) required a considerable alteration of the extant imprinting protocols and the development of new ones. The pioneering studies into molecular imprinting in peptides and proteins were those by Mosbach and co-workers;[104 – 107] these studies concerned the creation of a stationary phase for high-performance liquid chromatography (HPLC). The molecularly imprinted polymers described in these studies were checked for the ability to selectively recognize proper molecules in organic solvents. These experiments laid the basis to methods for preparing MIPs that would have affinity to biomolecular templates.

### VI.1. Problems in imprinting of biomolecules

In recent years various research teams developed MIP preparation strategies for a wide range of molecules, specifically for biologically active ones. Most of the successfully imprinted templates, however, had low molecular weights and were unstable in the organic solvents in which MIPs were synthesized.[108] At the same time, most biological macromolecules are soluble and stable only under physiological conditions; therefore, MIP formation and subsequent recognition of biological macromolecules should be carried out in aqueous or other media that would ensure their preservation. The size and spatial organization of the macromolecular template also impose limitations on the structure of the polymer to be formed. Thus, there are considerable limitations to transfer standard (covalent or non-covalent) imprinting strategies from small molecules to biological macromolecules. For example, the most popular method for preparing methacrylate-based MIPs in its basic version is unsuitable for imprinting biological objects, because the polymerization conditions are too severe and the structure of the resulting polymer fails to provide the accessibility of the binding sites of the MIP to the macromolecular template.

The main distinctions of the templates used in molecular imprinting of macromolecules (proteins, nucleic acids, viruses and even cells) from the templates used in imprinting of low-molecular-weight compounds are the following: the size of the template, its complex three-dimensional structure and conformational flexibility, and solubility.

Suitable natural biopolymers (nucleic acids and proteins) are large molecules; the formation of the MIP polymeric framework in their presence by standard bulk polymerization protocols can give rise to steric hindrances in washing out the template and its secondary binding, and this would appreciably deteriorate the quality of the imprinted polymer. Post-synthetic mechanical destruction of the polymer bulk can help in part to solve this problem, but instead could induce the destruction of template-binding sites. Another strategy is to use porogens (special additives to the reaction batch during polymer formation) to provide the generation of porous structures. It is documented that addition of a porogen to the batch increases the capacity of the resulting material compared to the capacity of the analogue formed without porogens.[109] The choice and amount of the porogen can appreciably influence both the imprinting process and the quality of imprints, and the physical characteristics of the MIP (pore size and shape, the stiffness of the polymer lattice and so on). Porogens can be various compounds: solvents, which alter the strength of various types of interactions between the template molecule and functional monomers;[110, 111] or bulky molecules, which exert the free volume exclusion effect.[112]

The complex organization of protein templates implies the existence of an optimal number of high-efficiency interactions between the template and polymer framework. When there are many weak interactions, strong non-specific binding is observed. Biopolymers, unlike low-molecular-weight organic templates, have many functionalities. Each of these functionalities residing on the surface of template molecules can interact with functional monomers and participate in the recognition of the template by the MIP. The functionality (and the type of interaction, too) can have different physicochemical properties and can change, together with the environment, to create obstacles both to clear imprinting and to the recognition of the template by the polymer.[28]

The flexibility of complex three-dimensional structures of biopolymers also imposes limitations on their molecular imprinting. In accordance with standard molecular imprinting protocols, there are rather severe conditions for polymeric framework formation (organic solvents, high temperatures and highly reactive species). Biopolymers, for example proteins, can change their conformation, can denature, aggregate and be modified under these conditions. As a result, the imprint will not match the initial protein (the protein structure that exists under the physiological conditions). Studies into the conformational specifics of biopolymers under the polymerization condi-

tions (which should be as mild as possible) and the maintenance of these conditions at the stage of molecular template recognition can help to solve this problem.

The above-listed factors limit the bioassay applications of MIPs without pre-separation and(or) pre-treatment of samples. As a rule, special templates modified to be stable under polymerization conditions are used to overcome the difficulties that arise from the conformational alteration of the biomolecular template, and this requires considerable additional expenditures.

The choice of solvent is important to the design of a MIP for a biologically active compound. In most cases, imprinted polymers are prepared in organic solvents in order to increase, to the highest possible extent, the efficiency of electrostatic interactions and hydrogen bonds, which are responsible for the recognition ability of MIPs. However, the low stabilities and solubilities of most proteins under these conditions hamper the preparation of quality molecular imprints, and the use of aqueous buffer solutions, which are optimal for the dissolution and preservation of the structure of a biological polymer molecule, considerably limits the choice of useful functional monomers for MIP preparation. Furthermore, the presence of water during synthesis can cause an adverse effect on the quality of the resulting MIP, as water can compete with the template for hydrogen bonding with the functional monomer and thereby inhibit monomer – template interactions and imprinting during polymerization.[28, 108]

Thus, attempting at protein (biomacromolecular) imprinting, researchers are facing the problems of how to preserve the native template structure upon polymer synthesis, to produce a clear imprint in solvents that do not attack biomolecules, to provide the accessibility of the binding sites of the MIP to biological macromolecules and many other problems. Below we will consider the major strategies implemented hitherto that satisfy the strict requirements for the preparation of MIPs for biological objects, using imprinting of biopolymers as an example.

## VI.2. Bulk imprinting

Bulk polymerization yields a polymeric matrix that contains template molecules. This process is referred to as 3D molecular imprinting.[2] One prerequisite for successful molecular recognition by MIPs consists of the possibility of free removal of a large biological template from the polymer and its re-binding to the specific site upon recognition. Thus, the affinity of a MIP is much determined by the accessibility of binding sites and thereby by the polymer structure and pore size, which is an important characteristic of the polymer synthesized.

Various polymeric matrices can serve as the basis for 3D imprinting of protein molecules.[2, 6] Hydrogel-based composites and solid microparticles are most suitable for this purpose by their chemical and physicomechanical properties, as they make it possible to enhance the stability of the polymer synthesized and to control the processes involved in the formation of polymer framework.

### VI.2.a. Hydrogel-based molecularly imprinted polymers

We mentioned above that acrylic acid and its derivatives are the functional monomers most frequently used for molecular imprinting; imprinting of biomolecules cannot be ruled out in this context.[28] Several research teams[113 – 115] describe examples of successful preparation of molecularly imprinted biogel polymers using acrylamide (AA) and *N*, *N*′-ethylene-bis(acrylamide) (EBA) as cross-linking agents. The concentration of cross-linking agent was chosen so that to provide, on the one hand, a sufficient stiffness of the polymer framework (the framework should swell to a minimal extent and retain binding sites), and on the other, a maximal permeability of the polymeric matrix to the protein template. The accessibility of binding sites was additionally ensured by micronizing the template to the proper particle size. Using the same reactants (AA and EBA) and varying their ratio, Hjerten *et al.*[114] constructed MIPs for a set of proteins (bovine hemoglobin, ribonucleases, cytochrome *c*, transferrin, human growth hormone and equine myoglobin). Ou *et al.*[115] prepared a MIP for lysozyme with a selectivity factor of 1.8 – 3.4 using MAA, AA and DMAEM (dimethylaminoethyl methacrylate) monomers. The authors mentioned that the selectivity factor could have been higher if the initial template had been completely washed out from the polymer. However, this remained unattained: $> 25\%$ of the initial template remained in the polymer after all washing procedures.

Acrylamide and(or) its derivatives were used as functional monomers in more than every second study.[28] In a comparative study of the specificity to template proteins between MIPs prepared from ordinary acrylic acid derivatives, on the one hand, and MIPs prepared from functional monomers that were carefully chosen taking into account the specifics of the imprinted molecule, on the other, however, the latter were shown to give an appreciable enhancement of specificity.[116]

Imprinting of nucleic acids has been addressed far less frequently than imprinting of protein molecules. Ogiso *et al.*[117, 118] are the authors of one of the few studies into the molecular imprinting of nucleic acids in hydrogel; they studied the possibility of imprinting nucleic acids and isolating specific DNA sequences by electrophoresis in a molecularly imprinted gel. The templates used were a 564-bp long fragment of the double-strand DNA of phage λ and a 34-bp long double-strand sequence of a fragment of the verotoxin gene. Prior to polymerization, the DNA was incubated for 15 h at 4 °C with 4,6-diamino-2-vinyl-1,3,5-triazine (VDAT) functional monomer in a buffer solution (50 mmol litre$^{-1}$) to form a pre-polymerization complex. Then, polymerization was triggered in a glass tube with addition of AA and MBA [*N*, *N*′-methylene-bis(acrylamide)] functional monomers and initiators; the template DNA was removed from the gel in an electric field. Non-imprinted gel was prepared in the same manner but without addition of the pre-polymerization complex. The use of the MIP against an ordinary polyacrylamide gel was shown to provide retention of template molecules in the gel due to an additional interaction with the binding sites of the imprinted hydrogel framework.[103, 104] The reference molecules, which were not the template, held a typical of dependence electrophoretic mobility on the molecular size.

In the above-cited studies, the imprinting of biological macromolecules was performed under aqueous conditions using acrylamide gels. However, the efficiency of hydrogel-based MIPs depends considerably on the cross-linkage of the polymer (the mobility of macromolecules in the polymer network) upon gel formation, the efficiency of

removal of the template from the polymer, the stability of polymers and other factors. The drawbacks of polymer gels may be viewed in the loss of steric match to the template molecule, the loss of stability when the medium changes and the need to choose functional monomers.

**VI.2.b. Imprinting using composites**

The use of composites in imprinting offers a means to alter the properties of MIPs.[119] The strengths of this strategy include the possibility to carry out polymerization under mild conditions in aqueous solutions (as for hydrogels) and a greater strength of the polymers, than found for hydrogels, due to the presence of microparticles; further, the support can perform additional functions, for example, carry nonzero residual magnetization due to the incorporation of magnetic particles.[116, 120] Venton *et al.*[119] proposed a method for the selective recognition of protein molecules using a composite based on silica gels and an acrylic gel polymer. The thus-prepared polymers were tested for ability to recognize two proteins [urease and bovine serum albumin (BSA)] upon their secondary binding. The binding efficiency of the test protein to the proper MIP relative to the reference protein was 30% higher for urease and as little as 3% higher for BSA. Another study of the same authors targeted at the synthesis of haemoglobin-specific and myoglobin-specific MIPs did not give desired results. A likely reason for this lies in the lack of access to binding sites, as $>25\%$ of the initial template remained unremoved from the polymer.

We mentioned above that the accessibility of binding sites of the polymer to a protein can be provided by porogens, for example, micronized $CaCO_3$. After the polymer is formed, calcium carbonate is removed by dissolution in acidic medium to generate additional pores for the free transport of the protein through the polymer matrix.[121]

Composites were also used in the preparation of polymers for the specific binding of haemoglobin.[122] The solid matrices used were native chitosan or maleic anhydride modified chitosan beads, on which acrylamide was polymerized in the presence of a template (haemoglobin) with initiators and cross-linking agents. The thus-prepared polymer particles were mechanically stable after the haemoglobin was removed and had a high binding capacity and high selectivity toward the target protein upon competitive binding in the presence of BSA.

Magnetic particles encapsulated into MIPs are frequently used to separate proteins.[123, 124] As a rule, the preparation of magnetic MIPs comprises several stages. First, magnetic nanoparticles are prepared having certain sizes, shapes and magnetic characteristics (saturation magnetization, coercivity and residual magnetization) and rather narrow distributions over these parameters. The second stage involves surface modifying, or functionalizing, of the magnetic particles in order to prepare various MIPs.[125, 126]

Magnetite ($Fe_3O_4$) is one of the most frequently used magnetic components of MIPs. Its widespread use is primarily due to its environmental friendliness and availability and the existence of well-proven methods and technologies for the preparation of disperse iron oxide systems having tailored dimensional and magnetic characteristics.[127, 128] Magnetic particles are frequently coated with $SiO_2$ for functionalization. This coating is then modified using a silane binder, for example 3-(trimethoxysilyl)propyl methacrylate, or surfactants based on ethylene glycol[129] or oleic acid.[130] Chitosan[131] and polychloromethylstyrene[132] are used immediately before polymerization.

Combination materials are frequently used for imprinting nucleic acids. Yin *et al.*,[116] for example, prepared imprinted magnetic nanoparticles for binding a 16-bp long single-strand DNA sequence that bears benzopyren-7,8-diol-9,10-epoxide moieties at various positions (BPDE – DNA). This study is one of the few studies into imprinting of extended oligonucleotides. In most cases of nucleic acid imprinting, the templates were nucleotides, dinucleotides or tetranucleotides.[133, 134] The MIP preparation protocol was as follows: 10-nm $Fe_3O_4$ nanoparticles were silanized, then functionalized by 2-bromoisobutyric acid bromide; around the resulting particles, the emulsion polymerization of functional monomers (allyllated calixarenes and guanosine-based monomers), a cross-linking agent (EGDMA) and detergents was afterwards initiated in the presence of the BPDE – DNA template. Magnetic particles having diameters of $\sim 100$ nm were prepared in this way. Yin *et al.* compared selectivity factors expressed as

$$S = \frac{K_{\mathrm{MIP}}}{K_{\mathrm{NIP}}} \tag{6}$$

$$K = \frac{(C_0 - C_{\mathrm{e}})w_{\mathrm{s}}}{C_{\mathrm{e}}w_{\mathrm{n}}} \tag{7}$$

(where $C_0$ and $C_{\mathrm{e}}$, respectively, are the initial template concentration in solution and after equilibrium was attained; $w_{\mathrm{s}}$ is the template solution weight; and $w_{\mathrm{n}}$ is the weight of nanoparticles), depending on the functional templates used in the synthesis and the polymer layer thickness on magnetic particles. When the functional monomers were allylated calixarenes and guanosine-based monomers, selectivity factors were twice as high as with commonly used monomers, for example MAA and 4VPy. Further, the increase in polymer layer thickness on a magnetic particle up to 20 nm also contributed to the gain in selectivity factor; with greater layer thicknesses, however, the selectivity factor decreased and the magnetic properties of particles deteriorated.

Thus, composites are successfully used for imprinting protein templates, but such the MIPs have a limited applicability because of the dense structure of the polymer framework. Composite-based MIPs are far from always having the required efficiency and template specificity. Therefore, additional strategies are frequently required for forming composite MIPs in order to enhance the template binding selectivity upon recognition.

Thus, MIPs for protein molecules can be prepared by bulk polymerization or using additional factors to provide the affinity and selectivity of the polymer, but these methods suffer from essential drawbacks, namely: unstable polymer structure, steric hindrances to removal of the template and its secondary binding to the polymer, in some cases a limited choice of template molecules and non-adaptability to any template.

### VI.3. Surface imprinting

Surface imprinting [50, 135] has certain advantages over bulk imprinting for macromolecules and biomolecules, as the three-dimensional polymer structure around the binding site appreciably hinders the break (dissociation) of bonds between the polymer and high-molecular-weight template and the secondary binding of the template. In classic understanding of the term, the 2D imprinting strategy consists of the immobilization of a template molecule on the support surface, the treatment of the surface with the monomer and the initiation of polymerization of the latter.[136] Useful supports for 2D imprinting can be gold nanoparticles, glass, mica, porous quartz particles, plastic plates and other solid materials.[135, 137] When the reaction is over, the substrate and template are removed; the thus-formed polymeric structure bears an imprint of the template molecule on the surface. Two-dimensional imprinting offers an additional opportunity to control the MIP structure upon template imprinting through varying the size and porosity of the support. Two-dimensional MIPs are formed by centrifuging, sputtering or soft lithography.[7, 138]

Another 2D imprinting method consists of applying thin polymer layers on planar surfaces or particles that bear immobilized templates so that the template is immersed into the polymer, but is not completely enveloped. A variety of this strategy can be viewed in the preparation of ultrathin polymeric films on the support surface where the polymerization mixture is applied together with the template.[139 – 142]

The pioneering work by Mosbach and co-workers,[143] concerned with 2D protein imprinting, advances a method for preparing a transferrin-imprinted polymer on the surface of silica particles. The polymer framework was formed in the presence of a mixture of functionalized silanes, specifically those intended for interacting with the carbohydrate portion of transferrin in aqueous conditions. The transferrin binding selectivity of the MIP in the presence of BSA was 2.16.

An example of preparation of a polymer bearing the imprint of a protein molecule on a planar surface was demonstrated by Nicholls and Rosengren,[50] who produced thin polymer films on glass surfaces with covalently immobilized BSA *via* the copolymerization of diacryloylpiperazine and methacrylic acid functional monomers. Two-dimensional imprinting was also used to produce a silica-supported MIP for haemoglobin recognition.[139] The covalent attachment of protein molecules to the surface of porous silica particles *via* imine bonds was followed by the polycondensation of 3-aminopropyltrimethoxysilane or trimethoxypropylsilane (Fig. 8). The particles were washed with succinic acid to remove haemoglobin. Comparing the properties of the thus-produced MIP with the properties of pure silica particles and those of the polymer produced in the presence of free haemoglobin, the authors showed that the thus-produced MIP was the only that sorbed haemoglobin with a higher efficiency than other proteins, such as BSA, β-amylase, myoglobin and transferrin.

As mentioned above, a variant where the MIP is formed in thin surface layers can be treated as a variant of 2D imprinting; as a result, a large biomolecule appears immersed but not enveloped in the polymer framework, which appreciably facilitates its removal from the polymer structure and subsequent binding to the MIP upon recognition. This strategy, not complicating the polymer preparation procedure, provides a better accessibility of specific binding sites than in bulk imprinting. This was shown, for example, for imprinting of 34-bp long double-strand sequence of a verotoxin gene fragment.[140] Slinchenko *et al.*[140] obtained an imprint of the nucleic acid on the surface of glass slides pre-treated with 3-(methoxysilyl)propyl methacrylate in ethanol. The polymer was formed on the slide surface by applying one variant of the polymerization blends, namely one-component (V), two-component (V – B) or three-component (V – B – A) mixtures that contained various combinations of VDAT (V), MBA (B) and AA (A) functional monomers. The V – B mixture was free of acrylamide, and composition V had no acrylamide and bisacrylamide. After the template was removed, the specific binding ability of a polymer was assessed by fluorescently labelled DNAs [as the template and reference molecule, which was the 34-bp long self-complementary duplex $d(CG)_{34}$]. The highest template-binding efficiency with the highest specificity was observed for the polymer that contained the three-component mixture of VDAT, MBA and AA functional monomers.

Surface imprinting is the most popular imprinting strategy for large biomolecules. Diverse polymers and diverse imprinting methods can be suitable for this purpose. Examples of most successful relevant studies will be given below.

In one high-tech study into the synthesis of protein MIPs,[45] the mica surface bearing an immobilized protein was coated with a disaccharide (trehalose) layer to generate a network of hydrogen bonds between carbohydrate hydroxy groups and protein functionalities (Fig. 9). The

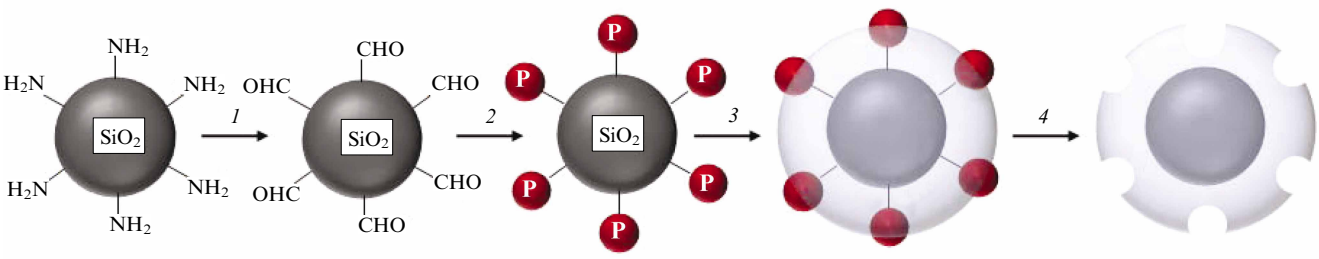



**Figure 8.** MIP preparation scheme (adapted from Ref. 139).
(*1*) Attachment of glutaraldehyde, (*2*) haemoglobin (P) immobilization, (*3*) silane polycondensation and (*4*) removal of the template.

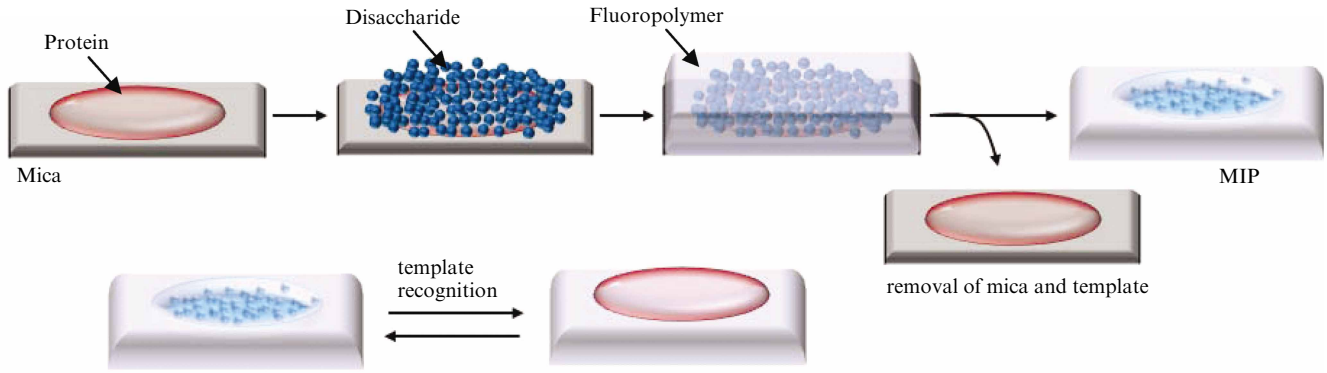



**Figure 9.** Surface imprinting of protein molecules (adapted from Ref. 45).

disaccharide – protein complex was coated with a thin polyhexafluoropropylene film using plasmachemical polymer coating technology. After the mica substrate was dissolved and the protein was removed, the disaccharide hydroxy groups retained a spatial match to the template functionalities; that is, recognition sites were formed (see Fig. 9). This strategy was used to prepare disaccharide-coated imprinted fluoropolymers that were capable of recognizing some protein molecules (BSA, ribonuclease A, immunoglobulin G and lysozyme). The selectivity coefficients for the competitive binding of radioactively labelled proteins in the ratio 1 : 1 varied within the range from 1.5 to 4.7 depending on the template and reference protein.

Ouyang *et al.*[144] used a similar strategy to construct a MIP inside pores of a nanochannel alumina membrane with covalently immobilized protein molecules. The imprinted nanotubes produced in this manner had fairly high specificity to the molecule in the presence of which they had been formed. The ratio of binding efficiencies of the MIP to the template protein (which were bovine haemoglobin and human haemoglobin) and to the reference protein (which were BSA, streptavidin, ribonuclease A or another), was 4.1 and 4.5, respectively.

The method for the production of a gallium-based imprinted surface should be specially mentioned.[145] Bossi *et al.*[145] generated molecular imprints on the gallium surface in the following manner: mica bearing an adsorbate protein was coated with molten gallium and cooled to become solid, and then the mica together with the protein was removed. The horseradish peroxidase dissociation constant from the thus-prepared imprinted surface was $(11 \pm 2) \times 10^{-9}$ mol litre$^{-1}$; the references were the non-imprinted gallium surface and the surface bearing albumin imprints, with the constants $(184 \pm 18) \times 10^{-9}$ and $205 \times 10^{-9}$ mol litre$^{-1}$, respectively.

Two-dimensional MIPs have lower capacities toward template molecules compared to 3D MIPs. However, immobilization makes it possible to attain the strictly definite orientation of the molecular template and to avoid its dissolution before the MIP is formed,[136] and provides the accessibility of binding sites in the polymer framework to structured macromolecules, thereby making it possible to construct high-specificity MIPs. Strategies were also developed to combine the merits of 2D and 3D molecular imprinting modes.[146]

### VI.4. Additional efficiency enhancement factors in imprinting of biomolecules

There are strategies for enhancing the selectivity of MIPs to large biomolecules both in 3D and 2D imprinting modes. One such strategy exploits the coordination properties of metals in imprinting.

The role of metal ions in coordinating the specific binding site of a MIP is documented (see, for example, Refs 51, 66, 147 and 148). The involvement of functionalities of the polymer in a coordination complex makes it possible to ensure the maximal interaction with the template and improves the recognition qualities of the MIP. The research team headed by Mosbach[147] was among the pioneers of the strategy for preparing a composite sorbent that would be capable of selectively interacting with a protein. The underlying idea of this strategy is the ability of copper cations to form coordination complexes with organic ligands (Fig. 10). Mosbach's team developed a method for imprinting ribonuclease A on methacrylate-functionalized silica particles using the *N*-(4-vinyl)benzyliminodiacetic acid (VBIDA) metal-binding functional monomer. The divalent copper cation in the polymer interacted with the protein and VBIDA moieties. The template was removed by a stronger chelating agent (EDTA).

A similar method using the coordination properties of $Ni^{2+}$ was implemented in the 3D imprinting mode to prepare MIPs that were capable of selective recognition of peptides having N-terminal histidine.[148]

Another method extensively used in imprinting of biomolecular templates helpful to partially solve steric and conformation problems, is referred to as 'antigenic determinant' (epitope) approach.[43, 149, 150] Conventionally, an epitope (antigenic determinant) is a set of amino acid residues of a protein antigen or polysaccharide chain fragments, for example in a glycoprotein, that form a site on its surface capable of selectively interacting with a certain site of the proper specific antibody. This approach is suitable for preparing a MIP when a short amino acid fragment of a large protein molecule is used as the imprinting template. The subsequent recognition of the entire protein molecule is due to the recognition of this fragment by the MIP (Fig. 11).

This approach succeeded in the specific binding of oxytocin protein *via* imprinting its terminal tetrapeptide Tyr-Pro-Leu-Gly (Tyr stands for tyrosine, Pro for proline,

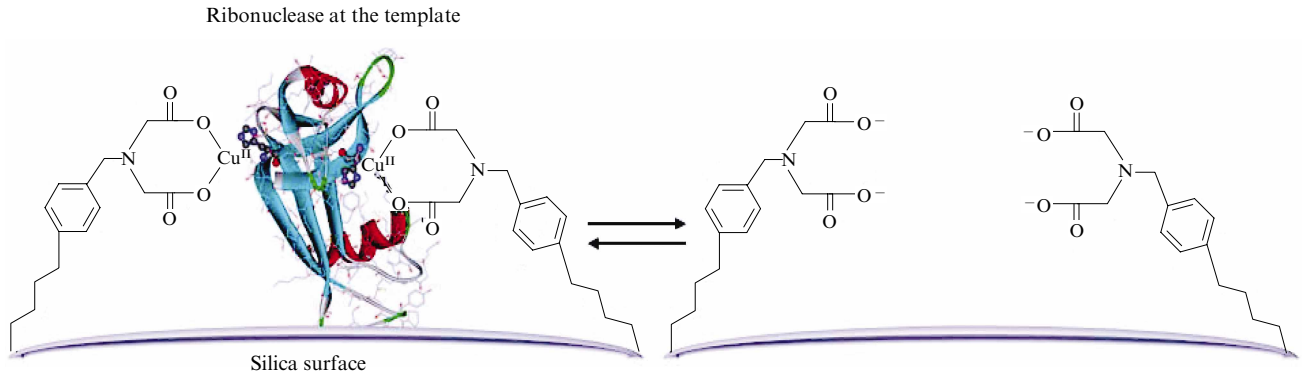



**Figure 10.** Scheme of affinity binding of the MIP to ribonuclease A.[147]

Leu for leucine and Gly for glycine) in a polymer.[151] Polymer binding to the imprinted tetrapeptide and oxytocin was studied by HPLC; the selectivity coefficients (determined as the ratio of capacity factors [see relation (4)] for the given polymer) were 2.6 and 2.4, respectively. However, the binding capacity of the MIP to the tetrapeptide was twice as high as its binding capacity to high-molecular-weight oxytocin.

### VI.5. Imprinting of supramolecular biological objects (viruses, bacteria and cells)

Molecularly imprinted polymers offer new opportunities for diagnosing diseases, and can potentially be used as new therapeutic agents. Articles have been published that describe methods to prepare MIPs for complex biological objects (viruses, phages, bacteria and cells).[152 – 155] Most molecular imprinting strategies for these objects are similar to those for proteins.[156]

The advent of 2D imprinting and soft imprint lithography gave a new impetus to research in the field of imprinting of large biological items. In soft imprint lithography, imprints are formed by bonding two surfaces together by applying pressure, one surface coated with a layer of the preformed polymer and the other coated with a monolayer of the template (Fig. 12).

The binding specificity of viruses to a MIP is most frequently determined by the specifics of shapes of viral capsids. Tobacco mosaic virus (TMV), for example, has a rod-like appearance and is a suitable subject for molecular imprinting. Imprinted polydimethylsiloxane-based polymer layers on glass slides manufactured by soft lithography in the presence of TMV were shown to contain thin extended cavities (imprints) (Fig. 13) and provide for the selective binding of the template to the imprinted polymer.[157] This MIP bound 8.8 mg TMV particles per gram of the imprinted polymer, whereas as little as 4.2 mg of the virus was sorbed per gram of the reference polymer, which was prepared in the presence of tobacco necrosis virus whose virion has a spherical shape.[158]

Bolisay *et al.* also used soft lithography in their study to prepare MIPs that could separate viral strains having similar shapes but differing in the compositions (structures) of proteins exposed on the capsid surface.[159] These MIPs in combination with a quartz crystal microbalance were used to recognize picornaviruses — various types of human rinoviruses (HRV1A, HRV2, HRV14, HRV16). The adsorption efficiency of the 'proper' virus on these MIPs varied depending on the type of virus, but was always two to three times higher than for the other ('improper') viruses (Fig. 14).

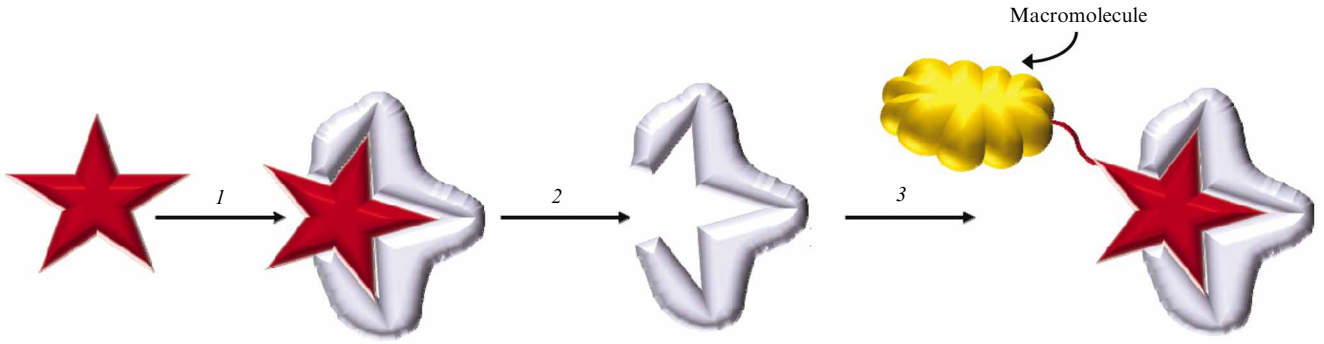



**Figure 11.** MIP preparation scheme *via* antigenic determinant strategy (adapted from Ref. 43).
(*1*) Generation of an imprint from the epitope, (*2*) removal of the template and (*3*) recognition of a molecule.

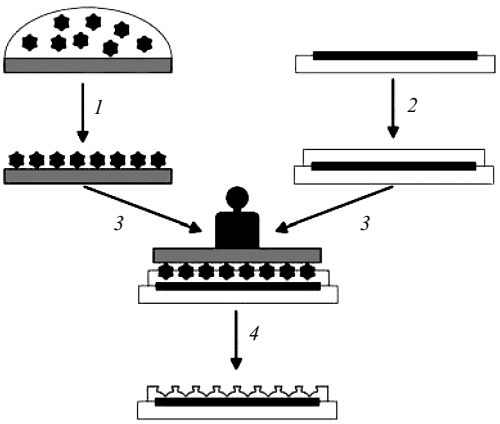



**Figure 12.** Scheme of the soft lithography process for MIP production.[157]
(*1*) Applying the template, (*2*) spreading the polymerization mixture, (*3*) imprinting and (*4*) removing the template.

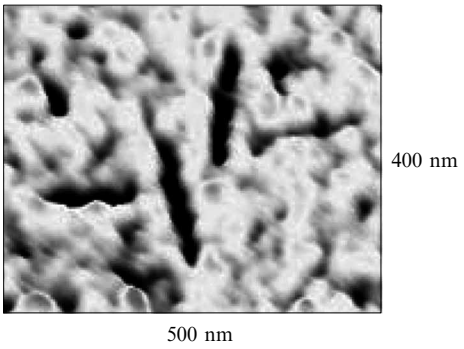



**Figure 13.** Images of the imprints of TMV viral particles in the polymer.[157]

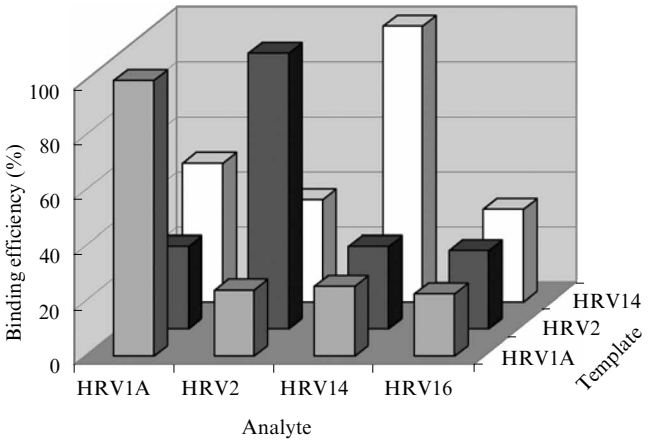



**Figure 14.** Binding efficiency of the tested viral strains to MIPs.[159]

Soft lithography was employed to manufacture MIPs with imprints of yeasts, red blood cells [160, 161] and *Escherichia coli* bacterial particles (Fig. 15).

In red blood cell imprinting,[161] imprints were created in a preformed polyvinylpyrrolidone layer that was prepared by the thermal (70 °C) or photo-induced ($\lambda$ = 254 nm) polymerization of an *N*,*N*′-methylene-bis-(acrylamide) and 1-vinyl-2-pyrrolidone mixture (Fig. 16). Red blood cell imprinting and subsequent removal of the template occurred under mild aqueous conditions. Soft imprint lithography made it possible to shorten the con-

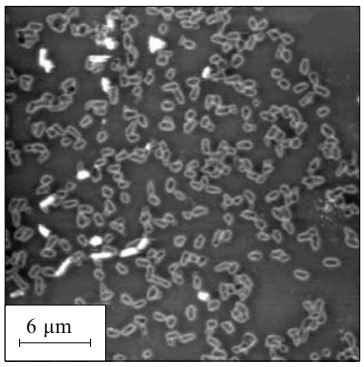



**Figure 15.** Atomic-force electron surface image of a MIP for *Escherichia coli* bacterial particles.[162]

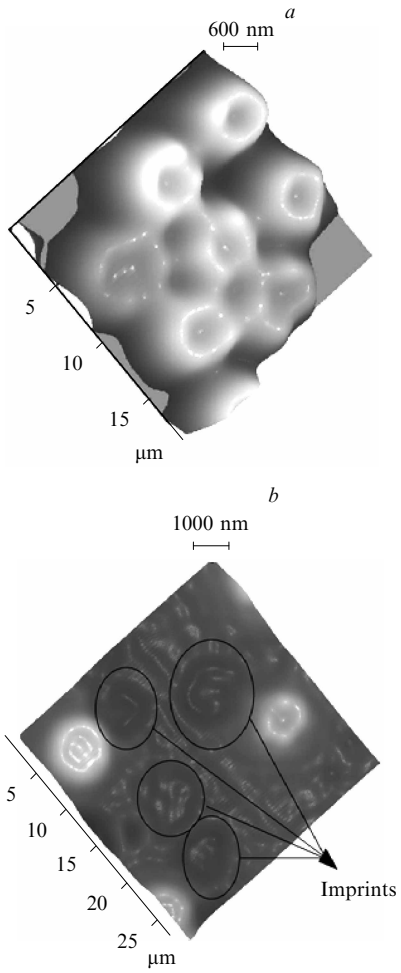



**Figure 16.** Atomic-force electron images of a polymeric layer containing red blood cells (*a*) prior to and (*b*) after removal of the template.[161]

tact time of cells with the preformed polymer and to end the imprinting process before the red cells were destroyed. The thus-prepared polymers specifically bound red blood cells. Aherne *et al.*,[163] relying on the known property of some microorganisms to pile up at the water–organic interface, advanced an approach to produce imprints matching the shape and size of bacteria, on the polymer. First, a polyamide microcapsule was preformed; individual bacterial particles were anchoraged to the capsule surface *via* covalent bonding of polyamine and amino groups of the bacterial wall with adipic acid dichloride. Then, UV-induced polymerization was triggered around preformed microcapsules to obtain a polyacrylate polymer mass. After the template was removed by acid hydrolysis, scanning electron microscopy detected imprints on the polymer surface whose shapes and sizes well matched the microorganisms used in polymerization. Unfortunately, the researchers cited did not determine the binding specificity of microorganisms to these polymers. Harvey *et al.*[164] later employed this approach in imprinting *Bacillus thuringiensis kurstaki* spores to find a 25% enhancement in the cell binding efficiency of the MIP relative to the efficiency of the non-imprinted polymer.

Successful imprinting and recognition strategy for some bacterial cells involving 2D imprinting and soft lithography was described by Ren and Zare.[155] Imprints were formed by bonding glass slides together by applying pressure, one slide containing the template bacteria and the other bearing a polydimethylsiloxane layer.

The antigenic determinant strategy found use not only in the imprinting of characteristic fragments of protein molecules but also in the imprinting of bacterial particles. The bacterial epitope is the bacterial surface protein, for example, *Staphylococcus aureus* protein A.[165] The selectivity coefficient for protein A binding to a gel-based MIP compared to that for egg albumin was 5.17. Adsorption selectivity in the recognition of bacterial particles was slightly lower; however, the resulting polymer particles adsorbed *Staphylococcus aureus* with an efficiency more than twice higher than for *Escherichia coli* or *Streptococcus thermophilus*.

Research in the field of imprinting of viruses, bacteria and cells is still at its early phase: MIPs were prepared only for a limited range of templates, and their efficiency was shown only in model systems. However, we notice a rapid development and a great promise of relevant research and the very wide range of potential applications of imprinted polymers for practical and research purposes.

## VII. Biomedical and biotechnological applications of molecularly imprinted polymers

The most popular application field of molecularly imprinted polymers is the chromatographic separation of individual compounds. Molecularly imprinted polymers are used for HPLC[166–168] and capillary electrochromatography;[169–171] a solid-supported, carefully micronized polymer mixed with a binder can be used in thin-layer chromatography.[172] MIP chromatography appeared to be an adequate alternative to affinity chromatography, for example in the separation of diastereomers of amino acids, peptides, nucleotides, carbohydrate isomers, structurally related β-lactam antibiotics and other compounds,[166–168] as well as proteins.[51,173]

The separation factor, a parameter frequently used to characterize the specificity of an imprinted polymer, is defined as the ratio of the retention times of the template molecule and the molecules to be separated in a column packed with a specific sorbent. According to the related literature, a value of 1.5–2.5 for this factor is sufficient for successful MIP-based chromatographic separation.[166,174,175]

Molecularly imprinted polymers are also used for the pre-purification and pre-concentration of medical samples and for the solid-phase extraction monitoring of composition of products and natural water pools.[176–178] MIP-based systems were developed for the pre-treatment of biological fluid samples (blood plasma and serum,[179–182] urine,[183–185] bile,[179] water[186,187]) and food products.[28,162,188–191] Values ranging from 2 to 30 were reported for the selectivity factors for binding of analyte molecules to MIPs against binding of the same molecules to reference non-imprinted polymers.[185,190] Beltran *et al.*[72] in their analyses of river water for parabens, performed a comparative study of the efficiency of MIPs and commercially available specific sorbents to show the competitiveness of the MIPs.

Catalytically active MIPs were prepared;[192] they can catalyze aldol condensation,[21,193] hydrolysis,[194–199] elimination,[200] oxidation,[201,202] Diels–Alder reaction,[203–205] dehydrofluorination,[206] isomerization[207,208] and other reactions.[209] The difficulty a researcher is facing in creating catalytically active MIPs consists in that the template should be a stable compound whose state should be similar to the transition state of the substrate to be transformed. The principles here are in fact the same as for the production of abzymes (catalytic antibodies).

Design of biosensors is one of the most frequently discussed and promising applications of MIPs.[50,210] The operation idea of a biosensor is the high-specificity recognition of analyte molecules. In ordinary biosensors, a receptor layer (consisting of nucleic acids, antibodies, antigens, enzymes, cells or other) immobilized on the surface of the sensing element interacts with the analyte to alter the physicochemical parameters of the system and thereby generate a signal, which is amplified and enters the recorder and data processing unit.[211–215] MIP sensors have a similar operation principle, but instead of biomolecular probes, they use a polymer coated on the sensing element, which contains molecular recognition sites.[216–220] Many of the MIP production methods described in this survey are directed just to the production of recognition elements of biosensing devices.

MIP-based assays widely use both classic reporter groups (radioisotopes[221–223] or fluorescent labels[224,225]) and advanced detection systems (a quartz crystal microbalance,[152,159] an atomic force microscope[226] and so on). The design of electrochemical MIP sensors is the leader in the abundance of documentation.[227–229]

Thus, assay systems where the recognition element is an imprinted polymer capable of selectively binding low-molecular-weight organic molecules, specifically biologically active ones, are now abundantly documented.[209] There are only few publications focusing on the recognition and isolation of high-molecular-weight items, such as

proteins.[173] Extensive work to develop MIP-based sensors for the determination of drugs, pesticides, and other species in biological and environmental samples is now underway.[230, 231] However, the production of practically applicable MIPs that bear imprints of large biological items (peptides, proteins or cells) yet remains a nontrivial problem.

### VII.1. Application of molecularly imprinted polymers *in vivo*

The *in vivo* use of MIPs as synthetic analogues of antibodies is a very promising and yet poorly documented field. Hoshino *et al.*[232] prepared acrylamide-comprising imprinted polymer nanoparticles for melittin, a 26-amino-acid peptide isolated from bee venom which can be fatal if overdosed. The authors started with selecting the combination and ratio of acrylamide functional monomers to form the polymer framework in the presence of a template molecule that would provide the most efficient binding to melittin *in vitro*.[48] Next, the MIP prepared as nanoparticles was administered to mice immediately after they were injected with a fatal dose of melittin (4.5 mg per kg body weight). The administration of imprinted particles resulted in the survival of more than one-half mice; all control animals died. Some of the mice that received NMP particle survived, too, but Hoshino *et al.* held the opinion that this was not a statistically significant effect. Ultimately, they showed the potential of nanosized MIPs for use as artificial antibodies to function in a living body. Obviously, this type of research stimulates the interest in molecular imprinting.

*In vivo* experiments were also involved in studies of MIPs as components of targeted drug delivery systems in the body.

### VII.2 Molecularly imprinted polymers as delivery systems

Targeted drug delivery systems are promising and demanded subjects of development research; their design can succeed owing to a combination of advanced technologies and intellectual biomaterials, for example MIPs. Molecularly imprinted polymers are able to recognize and bind specific biomolecules; this ability depends on the properties and concentration of the template in the medium, and can also be controlled by changing pH or temperature, addition or change in the concentration of competing molecules. Owing to the affinity to the template molecule, a MIP can maintain a sustained release of the template, thereby extending the residence time of a drug in the body.[233, 234] Thus, molecular imprinting technology has a great potential for medical applications. The advantages of MIPs can be viewed in their stability in most biological and chemical media, which guarantees the steadiness of the drug release rate from the polymer framework.

A MIP suitable for use as a drug delivery system should satisfy the following requirements:

— biocompatibility and nontoxicity;

— suitable sizes and monodispersity of particles and(or) polymer matrix;

— affinity and capacity of the polymer; and

— drug release efficiency and rate.

We should mention that both the functional monomers and the cross-linking agents used to produce a MIP should be biocompatible and nontoxic, which appreciably narrows the range of useful efficient functional monomers. In this context, promising monomers are methacrylic acid, ethylene glycol dimethacrylate and their analogues.[235] The production of MIPs based on off-the-shelf polymers, for example nylon [82, 95] or proteins, can be promising.[83] The lack of polymerization in the last case rules out possible effects of monomer residues, cross-linking agents or reaction initiators on the tissues of a living body.

It should also be kept in mind that polymerization is most frequently carried out in organic solvents, which can have a toxic effect on cells even in trace amounts, and water has a limited applicability as a polymerization medium because of an appreciable reduction in the stability of the pre-polymerization complex and the selectivity of the MIP to be formed. As mentioned above, however, methods recently appeared to successfully produce MIPs in aqueous medium.[226, 236] Residual impurities of other reactants in the polymer matrix, for example surfactants, should also be taken into account. A MIP production scheme comprises the use of a molecular template (or its structural analogue), and the removal of the template should also be complete and controllable. Therefore, careful purification and control of the polymer composition are necessary for providing the safe use of polymers as targeted drug delivery systems.

The homogeneity of the polymer matrix is one more prerequisite for forming MIPs. A non-uniform binding site distribution in the polymer (which is more typical of non-covalent imprinting) can give rise to rather high non-specific sorption and to a non-uniform release of the drug template from the polymer framework. The hydrophilicity of the polymer matrix is also desired; this will enhance the biocompatibility of the material and inhibit the non-specific adsorption of proteins and other components of living systems.

Approaches to the production of MIP-comprising drug delivery systems are differentiated by the degree of control of the drug release level and the delivery onset time.[237] One variant consists in that the drug is released by itself from the system; the drug diffusion rate profile in biological fluids has some specifics. Drug release can also be triggered by some physical, chemical or biochemical process. Lastly, the drug delivery level can be controlled by the concentration of a triggering agent, which depends on the drug concentration in the body. Drug release starts when the concentration of the triggering agent exceeds a certain level; its piling-up in the body causes a reduction in concentration of the triggering agent and drug release stops.

Molecularly imprinted polymers prepared in organic solvents in the presence of large amounts of a cross-linking agent were proposed for use as the base of controlled drug release systems in case where release is due to diffusion. These systems are suitable for the delivery of a drug that has a narrow therapeutic index, that is, a drug that has a small difference between the minimal efficient concentration and the minimal toxic concentration. Drugs such as theophylline, warfarin and other should be delivered by systems that can precisely control their release.[238, 239] In the view of oral administration of theophylline, Norell *et al.*[240] produced imprinted particles for this drug (based on methacrylic acid; particle size: 65 μm) in chloroform. The thus-pre-

pared particles were capable of maintaining the release of theophylline for several hours in pH 7.0 phosphate buffer. At high theophylline loads, high release levels were observed, which was explained by weak interactions with non-specific binding sites. For the same reason, theophylline release rates were higher for non-imprinted particles. Norell *et al.*[240] were among the first to show the ability of MIPs to maintain a constant release rate of molecular templates.

Some approaches to the development of drug delivery systems using MIPs are based on the ability of MIPs to release molecules in response to changes in the environment around them. These changes can influence drug binding to the polymer either directly (due to the competitive binding or hydrolysis of labile bonds), or indirectly (due to changing degree of polymer swelling, pH, ionic strength, *etc.*). An example is the use of competitive binding for the delivery of target molecules.[241] In this approach, the delivery vehicle is the MIP bound to the drug which is structurally related to the molecular template but yet has a weaker affinity to the MIP than the template has. In this manner, hydrocortisone molecularly imprinted particles were obtained to be then bound to testosterone (in that case, the drug). When these particles came in contact with the template (hydrocortisone) that had a higher affinity to the MIP, the dosage form was released.

Frequently, the level of some compound in the body serves as an indirect indicator of the degree of disorder in certain physiological processes. The development of MIPs capable of selectively recognizing these compounds is an important step in the design of drug delivery systems that would tune the release of a drug depending on their concentrations in the body, that is, use them as triggering agents. The release of insulin in response to changing glucose concentration in the body can serve as a simple and illustrative example of such a system. Kataoka *et al.*[242] developed a glucose-dependent gel, embedded into the 3-acrylamidephenylboronic acid polymer matrix, to control the release of insulin. The $pK_a$ value for phenylboronic acid decreases from 8.9 to 7.9 upon reaction with glucose hydroxy groups. As a result, the solubility of polymer increases strongly when glucose concentration at pH 9 increases. This gives rise to a rapid release of insulin, which in fact ceases when the glucose concentration decreases to below the critical value. Although the imprinting effect was not used in implementing this strategy, the usefulness of boronic acid derivatives in molecular imprinting for sugars is possible.[243]

There are no commercial propositions where MIPs is used as a drug delivery device; however, relevant research is now underway.[189 – 200] Most documents in the related literature address the development of MIP devices for the transdermal, ocular or oral drug delivery.

Transdermal administration is widespread for topical and systemic drugs. The transdermal route is an alternative to oral administration. In drugs comprising chiral compounds, only one enantiomer often has an enhanced pharmacological effect; to separate the racemate is not a trivial task. The use of an enantioselective MIP as a component of transdermal drug delivery systems can appreciably enhance the expected therapeutic effect of many agents.[244] A representative example in this context is the use of MIPs for the delivery of (S)-propranolol, which provides an enhanced (more than 100-fold) pharmacological activity compared to (R)-propranolol.[245 – 247]

Most research related to the manufacture of a commercial product is concerned with forming contact lenses from a MIP. The ophthalmic agents present in, for example, eye drops, have low bioavailability because of the protective mechanisms of the body; therefore, the use of therapeutic contact lenses is more promising. However, ordinary lenses have very low drug capacities. The manufacture of contact lenses from MIPs would solve this problem.

The most detailed study into the design of therapeutically efficient contact lenses was performed by a research team headed by Alvarez-Lorenzo.[248 – 254] This team manufactured hydrogel contact lenses based on weakly crosslinked hydroxyethyl methacrylate (HEMA) with or without addition of methacrylic acid or methyl methacrylate, using timolol as the template. The lenses had a transparent, smooth, and nonporous surface, mechanical strength and stability; that is, they completely satisfied the requirements to contact lenses. The kinetics of template release from the polymer was studied under various conditions (0.9% NaCl solution, phosphate buffer, pH 7.4, an artificial tear fluid), and the timolol capacities of the imprinted polymer and of a non-imprinted reference polymer were studied at various pH values. The greatest capacity was observed at pH of 5.5 – 7.5 in a MIP prepared from a HEMA + MAA monomer mixture, equal to 12 mg timolol per gram of dry hydrogel. The timolol diffusion rate from the polymer varied depending both on the solution used (in 0.9% NaCl, for example, it was slower than in phosphate buffer or in tear fluid), and on the polymer composition (HEMA and HEMA + MMA polymers retained timolol for longer times than HEMA + MAA polymers did). The results of that study showed that variations in composition of the polymer offer a way to reach its drug capacity with the maintenance of the tailored drug release characteristics.

The efficiency of similar contact lenses was proven *in vivo*: the drug was detected in the tear fluid of rabbits in therapeutic doses even 180 min after the lenses were mounted, which was unattainable with drops or impregnated lenses (a similar concentration was observed no later than 60 min after the drug was administered).[255] Studying the efficiency of norfloxacin in contact lenses made of a MIP with the proper template, Malakooti *et al.*[256] showed that those lenses were capable of adsorbing larger amounts of the drug than the lenses made of the non-imprinted polymer of the same composition, and were capable of maintaining the therapeutic concentration of the drug for $>24$ h.

Molecularly imprinted polymers can also be used for the oral administration of drugs. Puoci *et al.*[257] showed this for 5-fluorouracil. A permanent use of the free drug causes a severe toxic effect on the body, and moreover, its serum level decreases very rapidly. Molecularly imprinted polymers providing a gradual release of this drug would greatly alleviate the patient's condition and improve the efficiency of treatment. The researchers cited studied the *in vitro* release of 5-fluorouracil from a methacrylic acid polymer in the media that imitated the gastrointestinal tract and blood serum conditions. The drug release time

with the use of MIPs was 30 h. Singh and Chauhan [258] also studied the delivery of 5-fluorouracil by HEMA- and MAA-based MIPs and gained similar results.

MIP-based delivery systems for other oral preparations, for example for tramadol,[259] glycyrrhizic acid [260] and molsidomine,[261] are also documented. The compositions and concentrations of functional monomers and cross-linking agents and the concentration of the template used in imprinting are of importance to prepare a polymer with tailored characteristics.

Thus, MIPs have a great potential for use as components of targeted delivery systems, and although there are no commercial products in the market right now, scientists are exploring MIPs to gain an ever deeper insight into these materials, and in the near future MIPs will possibly be used everywhere.

## VIII. Conclusion

Molecular imprinting technology is now booming; significant results have been achieved, and specific polymers have been prepared for a wide range of low-molecular- and high-molecular-weight compounds. We hold the opinion that the most promising line of molecular imprinting is the imprinting of biological items (proteins, viruses, and bacteria). However, the preparation of MIPs for biological macromolecules and supramolecular entities is a complex task and requires a special approach in each case. The high heterogeneity of recognition sites, the low capacity of polymers, and considerable non-specific binding are the major problems in the imprinting of biomolecules. It is worth noting that the technology for producing polymers having a 'molecular memory' is steadily improved every year. Extensive studies into molecularly imprinted polymers are now underway; new publications appear constantly to expand the knowledge of how to prepare MIPs and where they can be used.

If artificial materials having high specificities in recognition of molecular targets and biological activity in living systems are designed, the applications of these polymers will expand appreciably. Obviously, molecularly imprinted polymers have a potential for wide use, and these 'intellectual' materials are most likely to become artificial agents that would efficiently mimic natural biosensing and protective systems.

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