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Molecularly Imprinted Polymers (MIPs) in Biomedical Applications

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1. Introduction

Generations of scientists have been intrigued by the binding phenomena involved in interactions that occur between natural molecular species, and over the years, numerous approaches have been used to mimic these interactions. Complex formation between a host molecule and the guest involves recognition, which is the additive result of a number of binding forces (Figure 1).

Fig. 1. Schematic representation of molecular recognition process. Adapted from Hillberg & Tabrizian, 2008.

Within biological systems, these are usually dynamic and are the result of a mass of non-covalent interactions, which act collectively to form a very stable system. Molecular imprinting is a relatively new and rapidly evolving technique used to create synthetic receptors, having recognition properties comparable to the biological systems and it also possesses great potential in a number of applications in the life Sciences. Primarily, molecular imprinting aims to create artificial recognition cavities within synthetic polymers (Alvarez-Lorenzo & Concheiro, 2004; Ramström & Ansell, 1998; Mosbach & Ramström, 1996). It is a relatively simple concept, which involves the construction of sites of specific

recognition, in synthetic polymers (Owens et al., 1999; Wulff, 1995; Caro et al., 2002; Joshi et al., 1998). The template of choice is entrapped in a pre-polymerization complex, consisting of functional monomers with good functionality, which chemically interact with the template. Polymerization in the presence of crosslinker serves to freeze these template-monomer interactions and subsequent removal of the template results in the formation of a molecularly imprinted polymer matrix (Figure 2).

Enormous interest has also been shown in imprinted materials as they mime biological receptors for the screening of new substances with potential pharmacological activity or to specifically detect drugs in biological fluids in screening assays for drugs of abuse. Such specificity is comparable with monoclonal antibodies used in immunoassay techniques (Pap et al., 2002; Chapuis et al., 2003; Caro et al., 2003; Vandeveld et al., 2007). Molecular imprinting is a well-developed tool in the analytical field, mainly for separating and quantifying very different substances, including drugs and bio-active molecules contained in relatively complex matrices. Moreover, the information generated about polymer synthesis procedures and the properties outlined for optimum performance in separation-based technologies may be a good starting point to create imprinted polymers useful in biomedical applications such as drug delivery systems, polymeric traps for toxic metabolites, etc. (Cunliffe et al., 2005). The chapter will focus on the most representative applications of MIPs in the biomedical field.

Fig. 2. Schematic representation of MIP synthesis.

2. Synthesis of MIP

Molecular imprinting is a very useful technique to incorporate specific substrate recognition sites into polymers. Molecular recognition characteristics of these polymers are attributed to complementary size, shape, and binding sites imparted to the polymers by the template molecules. The specific binding properties of MIP must be attributed to specific interactions

between the template and the functional groups in the polymeric network, thus the choice of the functional monomers is of primary importance to obtain performing imprinted materials (Puoci et al., 2005; Curcio et al., 2009).

MIPs can be synthesized following three different imprinting approaches (Caro et al., 2002), as follows:

1. The non-covalent procedure is the most widely used because it is relatively simple experimentally and the complexation step during the synthesis is achieved by mixing the template with an appropriate functional monomer, or monomers, in a suitable porogen (solvent) (Joshi et al., 1998). After synthesis, the template is removed from the resultant polymer simply by washing it with a solvent or a mixture of solvents. Then, the rebinding step of the template by the MIP exploits non-covalent interactions.
2. The covalent protocol, which requires the formation of covalent bonds between the template and the functional monomer prior to polymerization. To remove the template from the polymer matrix after synthesis, it is necessary to cleave the covalent bonds. To this end, the polymer is then refluxed in a Soxhlet extraction or treated with reagents in solution (Ikegami et al., 2004).
3. The semi-covalent approach is a hybrid of the two previous methods. Thus, covalent bonds are established between the template and the functional monomers before polymerization, while, once the template has been removed from the polymer matrix, the subsequent re-binding of the analyte to the MIP exploits non-covalent interactions, as the non-covalent imprinting protocol.

The binding sites obtained by molecular imprinting show different characteristics, depending on the interactions established during polymerization. The average affinity of binding site prepared using bonding by non-covalent forces is generally weaker than those prepared using covalent methods because electrostatic, hydrogen bonding, π - π and hydrophobic interactions, between the template and the functional monomers, are used exclusively in forming the molecular assemblies (Hwang & Lee, 2002). Moreover, an excess of functional monomer relative to the template is usually required to favor template-functional monomer complex formation and to maintain its integrity during polymerization. As a result, a fraction of the functional monomers is randomly incorporated into the polymer matrix to form non-selective binding sites.

However, when covalent bonds are established between the template and the functional monomer prior to polymerization, this gives rise to better defined and more homogeneous binding sites than the non-covalent approach, since the template-functional monomer interactions are far more stable and defined during the imprinting process.

Nevertheless, non covalent imprinting protocol is still the most widely used method to prepare MIP because of the advantages that it offers over the covalent approach from the point of view of synthesis.

In some polymers prepared by the non-covalent procedure, it has been observed that the binding of the template to the polymer can sometimes be so strong that it is difficult to remove the last traces of template, even after washing the polymer several times (Martin et al., 2003; Andersson et al., 1997).

When the MIP is used, small amounts of residual template can be eluted. This bleeding is a problem mainly when the MIP has to be applied to extract trace levels of the target analyte. To overcome this drawback, some authors have synthesized MIP using an analogue of the target molecule as a template (the template-analogue approach) (Dirion et al., 2002). In this

way, if the MIP bleeds template, then the elution of the template does not interfere in the quantification of the target analyte. Andersson was the first author to synthesize a MIP using a template analogue. In this case, a MIP selective for sameridine was prepared using as a template a close structural analogue of sameridine. However, it should be pointed out that the use of template analogues is not always the solution, because sometimes it is not possible to identify and to source a suitable analogue. For this reason, other methods, such as thermal annihilation, microwave-assisted extraction (MAE) and desorption of the template with supercritical fluids have also been developed to remove the template from the MIP (Ellwanger et al., 2001).

It should also be mentioned that, as a control in each polymerization, a non-imprinted polymer (NIP) is also synthesised in the same way as the MIP but in absence of the template. To evaluate the imprinting effect, the selectivities of the NIP and MIP are then compared.

It is important to state that MIP can be obtained in different formats, depending on the preparation method followed. To date, the most common polymerizations for preparing MIPs involve conventional solution, suspension, precipitation, multi-step swelling and emulsion core-shell. There are also other methods, such as aerosol or surface rearrangement of latex particles, but they are not used routinely.

When a MIP is obtained by conventional solution polymerization, the resultant polymer is a monolith, which has to be crushed before use, except when the MIP is prepared in situ. However, suspension polymerization (in fluorocarbons or water) and precipitation polymerization allow MIPs to be prepared in the form of spherical polymer particulates.

Conventional solution polymerization is the most common method because of its simplicity and universality. It does have some drawbacks as the processes of grinding and sieving not only are wasteful and time consuming, but also may produce irregularly sized particles.

Another important parameter to be considered in the synthesis of MIP is the type of initiator system.

The widespread use of traditional free radical polymerization methods for the preparation of molecularly imprinted polymers can be attributed to a tolerance for a wide range of functional groups and template structures. In essence, the free radicals generated during the addition polymerization do not interfere with the intermolecular interactions critical for the non-covalent imprinting system.

Generally, in the synthesis of MIP, the free radicals are generated by decomposition of azo-compounds, peroxides and thermal iniferters which require relatively high polymerization temperature to ensure their rapid decomposition.

The polymerization temperature is also an important parameter to be considered in order to obtain performing MIP. A high temperature, indeed, is expected to drive the equilibrium away from the template-functional monomer complex toward the unassociated species, resulting in a decrease in the number of imprinted cavities. Thus, several strategies have been planned to create a stable pre-polymerization complex by decreasing the kinetic energy of the system, a parameter that strongly depends on the polymerization temperature. For example, UV induced polymerization processes were successfully employed in the synthesis of MIP selective for different kinds of template (Puoci et al., 2008a; Puoci et al., 2007a). Moreover, even if conventional initiator systems have been applied in polymerization and copolymerization with the convenience of working at a lower temperature, they show the disadvantage of the possible introduction of harmful and toxic chemical side products.

In a recent work, (Cirillo et al., 2010a) $\text{FeCl}_2/\text{H}_2\text{O}_2$ redox initiator system was employed to synthesize a theophylline imprinted polymer. Hydroxyl radical is the active species that is generated from the reduction of hydrogen peroxide at the expense of Fe^{2+} ions.

A great number of studies have investigated the use of Fenton reactions for water remediation through pollutant degradation. Fenton reagents have been used as radical initiator in vinylic polymerization or grafting for more than 50 years. However, almost no reference has been made to its use to initiate molecularly imprinted polymerization.

The advantages of this kind of initiator system consist of the low working temperature, the absence of any kind of toxic reaction products, that is desirable for materials to be employed in biomedical field, and the possibility to decrease the polymerization time (2h for the synthesis of Redox MIP *vs* 24 h for the synthesis of conventional MIP synthesized by azo-initiators). The whole of these aspects contributes to preserve the stability of the pre-polymerization complex, thus improving the imprinting efficiency of the obtained materials.

3. Applications of MIP

Molecular imprinting has now become an established method and has also been applied in the areas of biomedical and analytical chemistry. MIP have been used as chromatographic stationary phases (Turiel & Martin-Esteban, 2004) for enantiomeric separations (Bruggemann et al., 2004), solid-phase extraction (Haupt et al., 1998), catalysis (Ye & Mosbach, 2001a) and sensor design (Mosbach, 2001), as well as for protein separation (Hansen, 2007), as receptor (Haupt, 2003), antibody (Ye & Mosbach, 2008) and enzyme mimics (Yu et al., 2002), and most recently as drug delivery systems (DDS) (Alvarez-Lorenzo & Concheiro, 2008).

3.1 MIP as basis of Drug Delivery Systems

In the last few years, a number of significant advances have been made in the development of new technologies for optimizing drug delivery (Schmaljohann, 2006). To maximize the efficacy and safety of medicines, drug delivery systems (DDS) must be capable of regulating the rate of release (delayed- or extended-release systems) and/or targeting the drug to a specific site. Efficient DDS should provide a desired rate of delivery of the therapeutic dose, at the most appropriate place in the body, in order to prolong the duration of pharmacological action and reduce the adverse effects, minimize the dosing frequency and enhance patient compliance. To control the moment at which delivery should begin and the drug release rate, the three following approaches have been developed (Chien & Lin, 2002):

- a. **rate-programmed drug delivery:** drug diffusion from the system has to follow a specific rate profile;
- b. **activation-modulated drug delivery:** the release is activated by some physical, chemical or biochemical processes;
- c. **feedback-regulated drug delivery:** the rate of drug release is regulated by the concentration of a triggering agent, such as a biochemical substance, concentration of which is itself dependent on the drug concentration in the body.

When the triggering agent is above a certain level, the release is activated. This induces a decrease in the level of the triggering agent and, finally, the drug release is stopped. The sensor embedded in the DDS tries to imitate the recognition role of enzymes, membrane receptors and antibodies in living organisms for regulation of chemical reactions and for maintenance of the homeostatic equilibrium.

Molecular imprinting technology can provide efficient polymer systems with the ability to recognize specific bioactive molecules and a sorption capacity dependent on the properties and template concentration of the surrounding medium; therefore, although imprinted DDS have not reached clinical application yet, this technology has an enormous potential for creating satisfactory dosage forms.

The following aspects should be taken into account:

a. Compromise between rigidity and flexibility.

The structure of the imprinted cavities should be stable enough to maintain the conformation in the absence of the template, but somehow flexible enough to facilitate the attainment of a fast equilibrium between the release and re-uptake of the template in the cavity. This will be particularly important if the device is used as a diagnostic sensor or as a trap of toxic substances. In this sense, non-covalent imprinting usually provides faster equilibrium kinetics than the covalent imprinting approach (Allender et al., 2005). The mechanical properties of the polymer and the conformation of the imprinted cavities depend to a great extent on the proportion of the cross-linker. Mostly imprinted systems for analytical applications require around 25-90% of cross-linker agent. These cross-linking levels increase the hydrophobicity of the network and prevent the polymer network from changing the conformation obtained during synthesis. As a consequence, the affinity for the template is not dependent on external variables and it is not foreseen that the device will have regulatory or switching capabilities. The lack of response capability to the alterations of the physico-chemical properties of the medium or to the presence of a specific substance limits their potential uses as *activation-* or *feedback-modulated* DDS. A high cross-linker proportion also considerably increases the stiffness of the network making it difficult to adapt the shape of the administration site and causing mechanical friction with the surrounding tissues (especially when administered topically, ocularly or as implants).

b. High chemical stability.

MIP for drug delivery should be stable enough to resist enzymatic and chemical attack and mechanical stress. The device will enter into contact with biological fluids of complex composition and different pH, in which the enzymatic activity is intense. Ethylene glycol dimethacrylate (EGDMA) and related cross-linkers, which are the most usual ones, have been proved to provide stable networks in a wide range of pHs and temperatures under *in vitro* conditions (Svenson & Nicholls, 2001). However, additional research should be carried out to obtain information about its behaviour in vivo environments, where esterases and extreme pHs seem to be able to catalyse its hydrolysis (Yourtee et al., 2001). Additionally, it has to be taken into account that the adaptability of molecular imprinting technology for drug delivery also requires the consideration of *safety* and *toxicological* concerns. The device is going to enter into contact with sensitive tissues; therefore, it should not be toxic, neither should its components, residual monomers, impurities or possible products of degradation (Aydin et al., 2002). Therefore, to ensure biocompatibility it might be more appropriate to try to adapt the imprinting technique to already tested materials instead of creating a completely new polymeric system. On the other hand, most classical MIP are created in organic solvents to be used in these media, taking advantage of electrostatic and hydrogen bonding interactions. The presence of residual organic solvents may cause cellular damage and should be the object of a precise control. In consequence, hydrophilic polymer networks that can be synthesised and purified in water are preferable to those that require organic solvents. A hydrophilic surface also enhances biocompatibility and avoids adsorption of

proteins and microorganisms (Anderson, 1994). Additionally, many drugs, peptides, oligonucleotides and sugars are also incompatible with organic media.

A wide range of cross-linked hydrogels have been proved to be useful as drug delivery platforms (Davis & Anseth, 2002). Molecular imprinting in water is still under development and difficulties arise due to the considerable weakness of electrostatic and hydrogen-bonding interactions in this polar medium, which decrease the affinity and selectivity of MIP for the ligand (Komiyama et al., 2003). Nevertheless, hydrophobic and metal co-ordination interactions are proving to be very promising to enhance template and functional monomer association in water (Piletsky et al., 1999).

It is clear that the polymer composition and solvent are key parameters in the achievement of a good imprinting and that, in consequence, a compromise between functionality and biocompatibility is needed.

To date, several MIP based drug delivery devices were prepared for the sustained/controlled release of anticancer, antibiotic and anti-inflammatory drugs, obtaining a great efficiency in the release modulation.

One of the most relevant challenges in this field is intelligent drug delivery combined with molecular recognition. Intelligent drug release refers to the release, in a predictable way, of a therapeutic agent in response to specific stimuli such as the presence of another specific molecule or small changes in temperature, pH, solvent composition, ionic strength, electric field, or incident light (Gil & Hudson, 2004; Peppas & Leobandung, 2004). The ability of polymers to reversibly respond to small environmental changes mainly depends on different interactions between functional segments of the polymer network (Puoci et al., 2008b).

3.1.1 pH responsive MIP

pH-responsive polymers are characterized by swelling/shrinking structural changes in response to environmental pH changes (Morikawa et al., 2008; Oh & Lee, 2008; Pérez-Añvarez et al., 2008). Such a polymeric network, containing ionizable groups, is able to accept or donate protons at a specific pH, thereby undergoing a volume phase transition from a collapsed state to an expanded state. Weak polyacids and weak polybases represent two types of pH-sensitive polyelectrolyte. To date, there have been a number of papers in the literature describing the synthesis and applications of pH-sensitive polymer hydrogels based on molecular imprinting technology to be applied as base excipients for drug delivery formulations. (Gil & Hudson, 2004).

As reported, in the synthesis of an efficient imprinted polymers, the first parameter to be considered is the choice of the suitable functional monomer, and for this scope, a screening of different functional monomers should be made. In a recent work (Cirillo et al., 2010b), three different MIP for the selective release of glycyrrhizic acid were synthesized employing methacrylic acid (MAA) as acidic, 2-(dimethylamino)ethyl methacrylate (DMAEMA) as basic, and 2-hydroxyethylmetacrylate (HEMA) as neutral functional monomer, in order to evaluate the effect of the different monomer to the recognition properties of the resulting materials. The most promising matrix to be applied as glycyrrhizic acid controlled delivery device in gastrointestinal was found to be the MAA-containing MIP, while the DMAEMA-MIP was not effective in this direction because of the high non-specific hydrophobically driven interaction between polymeric matrices and template. The HEMA-containing MIP was found to be less effective as a result of the lower capacity of HEMA to form hydrogen bond comparing to MAA.

Another work (Puoci et al., 2007b) reports on the synthesis of MIP for the sustained release of this molecule in gastro-intestinal simulating fluids. The imprinted polymers were found to have a better ability to control drug release compared with non-imprinted polymers due to the presence of specific binding sites in the polymeric network that are able to release the drug much more slowly: the drug release from NIP was indeed remarkably faster than that observed from MIP. These remarkable differences depend on the different recognition properties of the two polymeric matrices (Figure 3).

Fig. 3. Gastrointestinal release profile of 5-FU by MIP (—■—) and NIP (-♦-). Adapted from Puoci et al., 2007a.

The non-imprinted polymers, indeed, do not have specific binding cavities for the drug, while the MIP samples, because of their specific structure, strongly bound the drug by non-covalent interactions in the cavities formed during the polymerization procedure in the presence of the analyte. This observation supports a model of retention mechanism, which assumes that the acid groups of the selective sites have stronger interaction with the drug than the non-selective sites. At low pH (1.0) values, the carboxylic groups are not ionized and there is a good interaction with the template. These results might help us to understand the behavior of these matrices when the pH increases. Under these conditions, that simulate the intestinal fluid, in the non-imprinted polymers the antioxidant is bound with non-covalent interactions on the surface of the matrices. At pH 6.8, the diffusion rate of the buffer on the polymer surface is fast, the carboxylic groups are ionized, and the drug is rapidly released. Instead, in the MIP case, the diffusion rate of the buffer into specific cavities of imprinted polymers is slower, and the functional groups are ionized more slowly, resulted in well controlled release.

Similar results were obtained for the release of antioxidant molecules such as tocopherol (Puoci et al., 2008c), and phytic acid (Cirillo et al., 2009a) confirming that MIPs represent a very useful polymeric device for the selective and controlled release of a therapeutic agent in gastrointestinal fluids.

However, the reported synthetic approaches (bulk polymerization) yields particles with limited control on particle size and shape. In literature, several attempts have been applied to produce monodispersed molecularly imprinted polymeric particles using methods such

as suspension polymerization in water (Lai et al., 2001), dispersion polymerization (Say et al., 2003), liquid perfluorocarbon (Mayes & Mosbach, 1996), and via aqueous two-step swelling polymerization (Piscopo et al., 2002). However, during the polymerization procedure, these techniques require water or highly polar organic solvents, which frequently decrease specific interactions between functional monomers and template molecules. Precipitation technique not only allows to avoid these disadvantages, but also to obtain monodispersed molecularly imprinted micro- and nanospheres, without the integrity and stability of recognition sites compromised (Wei et al., 2006). Moreover, spherical shape should be advisable in order to avoid swelling anisotropic behavior associated with other geometries (Iemma et al., 2008).

Based on these considerations, micro- and nano-spherical imprinted polymers (Figure 4) were prepared for the sustained release of sulfasaleazine in gastrointestinal simulating fluids (Puoci et al., 2004) and 5-FU in plasma simulating fluids (Cirillo et al., 2009b), respectively. A better control on drug delivery was obtained, the spherical shape, indeed, allows to eliminate the anisotropic swelling normally associated with other geometries.

Fig. 4. SEM image of 5-FU molecularly imprinted nanospheres. Adapted from Cirillo et al., 2009b.

Recently, furthermore, a different approach was used for the synthesis of imprinted microspheres to be applied in the sustained release of paracetamol. Most of the developed imprinting protocols, indeed, can be successfully used to produce MIP for recognition of a large range of guest molecules predominantly in organic solvent-based media, while they often fail to generate MIP for use in pure aqueous environments (Benito-Peña et al., 2009). This depends on the non-specific hydrophobically driven bonds between template and surface of materials. In addition, biological sample components, such as proteins and lipids, are strongly adsorbed to the polymeric surfaces, negatively interfering with their recognition properties (Boos & Fleischer, 2001). Thus, in order to obtain MIP able to work in aqueous media, such as biological fluids or environmental waters, a considerable reduction of these non-specific interactions is required (Bures et al., 2001). For this purpose, different methodologies were developed (Mullet & Pawliszyn, 2003; Sambe et al., 2007). A widely

used approach is the insertion of a hydrophilic monomer such as 2-hydroxyethyl methacrylate (HEMA) in the pre-polymerization mixture. This compound is known to impart water compatibility in a number of unrelated systems, but it is also able to interfere with the formation of the pre-polymerization complex interacting with several analytes by hydrogen bonds formation (Tunc et al., 2006). Another approach, involving a two step polymerization procedure, is the hydrophilic modification of MIP surface using glycerol monomethacrylate (GMMA) and glycerol dimethacrylate (GDMA). These materials avoid the destructive deposition of biomacromolecules on the polymeric surface, allowing an enhanced imprinting effect, especially in SPE protocols (Sanbe & Haginaka, 2003; Haginaka et al., 1999). A more promising approach is to use a monomer that less interferes in the pre-polymerization complex formation, but able, at the same time, after a post-polymerization straight forward modification, to impart water compatibility to the system. Glycidylmethacrylate (GMA) is useful for this purpose because its oxygen atom, bounded to two carbons, has lower capacity to form hydrogen bonds than a free hydroxy group. Furthermore, the epoxide ring opening carried out to the formation of a hydrophilic external layer on the polymeric surface. With this reaction, it is possible to modify hydrophobic matrices in more water compatible ones, more suitable to be employed in biological media because of the reduction of non-specific hydrophobic interactions (Puoci et al., 2009; Parisi et al. 2009).

3.1.2 Thermo-responsive MIP

A great number of synthetic, naturally occurring, and semisynthetic polymers display discrete, rapid, and reversible phase transformations as a result of conformational changes in response to temperature (Curcio et al., 2010). Polymers can exhibit either a lower critical solution temperature (LCST), below which they are soluble in deionized water, or an upper critical solution temperature (UCST), above which they are soluble. A balance of hydrophilic/ hydrophobic groups in the network determines the onset of the response that switches these “smart” materials in a controlled manner by adjusting the temperature. The responsive behavior of polymers with LCST properties is characterized by interactions between the hydrophobic groups, such as methyl, ethyl, and propyl groups, which become stronger than the hydrogen bonds with increasing temperature. On the other hand, in materials with UCST properties, the opposite is true and they swell at high temperature and shrink at low temperature. Poly(*N*-isopropylacrylamide) (PNIPAM) is the polymer most widely studied in this context because of its low critical solution temperature (LCST) in the range of 25-32 °C, i.e. close to the temperature of the human body (Iemma et al., 2009). In recent years, MIPs exhibiting thermoresponsive behavior have also been studied. One of the first reports concerned temperature-sensitive imprinted polymeric gels based on *N*-isopropylacrylamide (NIPAM), acrylic acid, and *N,N'*-methylenebis(acrylamide) (BIS), which were prepared in the presence of a template such as DL-norephedrine hydrochloride or DL-adrenaline hydrochloride (Watanabe et al., 1998). The imprinted and non-imprinted gels prepared in 1,4-dioxane showed a volume change in aqueous solution as a function of temperature. However, when the guest molecule was present in a saturated solution, the polymers exhibited another phase (“molecular recognition phase”), the volume of which was responsive to the concentration of the guest molecule. An interesting study (Alvarez-Lorenzo et al., 2001) reported on temperature-sensitive polymeric gels based on NIPAM, methacrylic monomers, and *N,N'*-methylenebis(acrylamide) as cross-linker, which were

capable of reversibly adsorbing and releasing divalent ions. The effects of various methacrylate salts on the binding of divalent ions were reported. Imprinted gels prepared with calcium methacrylate or lead methacrylate showed higher affinity for target molecules as compared to randomly polymerized gels containing methacrylic acid (MAA) or lithium methacrylate as adsorbing monomers. The affinity decreased in the swollen state but was recovered upon shrinking. This suggests that the imprinted gels possessed a reversible adsorption ability, which was controlled by the folding and unfolding of the polymer, i.e. the volume phase transition. A different procedure was employed to prepare temperature responsive imprinted polymers without using a template (D'Oleo et al., 2001). These polymers were based on NIPAM and *N,N*-cystaminebis(acrylamide) weakly cross-linked with *N,N*-methylenebis(acrylamide). After polymerization, the disulfur bridges in the pendant cystamine groups were cleaved and oxidized to form a pair of sulfonic functions capable of interacting with divalent cations.

3.1.3 Photo-responsive MIP

The interaction between light and a material may be used to modulate drug delivery. This can be accomplished by using a material that absorbs light at a specific wavelength and then uses the energy from the absorbed light to modulate drug delivery (Suzuki & Tanaka, 1990). Since light stimulus can be imposed instantly and delivered in specific amounts with high accuracy, light-sensitive hydrogels may have special advantages over systems that rely on other stimuli. The capacity for instantaneous delivery of the stimulus makes the development of light-sensitive materials important for various applications in both the engineering and biochemical fields (Yui et al., 1993). For example, molecularly imprinted membranes, based on a polymerizable derivative of azobenzene, *p*-phenylazoacrylanilide (PhAAAn), with photoregulated ability to interact reversibly with a predetermined compound such as dansylamide, were synthesized (Minoura et al., 2003). A mixture of ethylene glycol dimethacrylate and tetraethylene glycol diacrylate was used to prepare PhAAAn-containing membranes in the presence of the template. PhAAAn serves not only as a photoresponsive monomer but also as a functional monomer. Upon UV irradiation of these membranes, PhAAAn undergoes *trans*-to-*cis* isomerization and upon visible light irradiation, *cis*-to-*trans* isomerization occurs. Correspondingly, the shape, intensity, and positions of the absorption bands change.

3.3 MIP as chemo/biosensors

A sensor is a device that responds to a physical or chemical stimulus by producing a signal, usually electrical. As highlighted by Hillberg et al., 2005, although this is often the case for physical effectors, such as temperature, light, or weight, this is less commonly the case when a sensor's target is a particular molecule, ion or atom. In these situations an "effect" can either be specific or non-specific, can be informative or misleading. For instance the absorbance or emission spectra of an excited metal atom in a flame can be diagnostic of a particular metal whilst the UV absorption spectra of a mystery solution can be indicative but is seldom specific. And of course an additional, and often overriding complication, is that it is unusual, in "real" samples, for there to be a single species present. More commonly an analyte of interest is accompanied by a number of different species, all present at different concentrations and all adding to the complexity of the analytical problem.

All over the world, billions of dollars are spent annually on chemical/biological detections related to medical diagnosis, environmental monitoring, public security and food safety because lab analysis using expensive equipment is usually cumbersome and time-consuming. Therefore, there has been a pressing societal need for the development of chemo/biosensors for the detection of various analytes in solution and atmosphere, which are both less expensive and simpler to construct and operate. Although considerable progress was made in the past several decades, the chemo/biosensor field remains underdeveloped and at a low level of commercialization because of the lack of alternative strategies and multidisciplinary approaches (Guan et al., 2008).

The standard approach to the analytical analysis of complex matrices is the separation of the different components. Typically, therefore, before a sensor can be used to perceive and quantify one component in a mixed solution, the various components of the complex mixture must be separated, usually by a chromatographic process, so that some form of non-selective sensor, e.g. UV absorbance measurements, can be used to detect and quantify each individual component.

In order to improve the performance of chemical sensors, an improvement of their selectivity is required, so that a particular chemical species can be detected and assayed without the need for a possibly lengthy separation stage. In this direction, a technological approach is the development of the biosensor (Updike & Hicks, 1967). A biosensor is a sensor that uses biological selectivity to limit perception to the specific molecule of interest. A typical biosensor consists of two main components: the chemosensory materials (receptors) that can selectively bind target analytes and the efficient transducer that can transform the binding events into a readable signal output related to the analyte concentration in the Sample (Eggins, 2002). The efficiency of chemosensors is largely dependent on the selectivity and sensitivity of the used sensory materials to a target species. The traditional approaches are to immobilize a biological or biologically derived sensing element acting as receptor on the surface of a physical transducer to provide selective binding of analytes (Figure 5) (Orellana & Moreno-Bondi, 2005; Jiang & Ju, 2007). As sensing element, it is possible to use either biological macromolecules (e.g. antibodies, enzymes, receptors and ion channel proteins, nucleic acids, aptamers and peptide nucleic acids) or biological systems (e.g. ex vivo tissue, microorganisms, isolated whole cells and organelles). However, the small surface area and non-tunable surface properties of transducers greatly limit the efficiency of chemosensors, especially for the detection of ultratrace analytes.

Recently, nanomaterials have found a wide range of applications as a material foundation of chemosensors, and have exhibited various degrees of success in the improvement of detection sensitivity and selectivity (Gao et al., 2007; Xie et al., 2006; Banholzer et al., 2008). Nanomaterials themselves can also form a novel platform of chemical/biological detections due to their unique electrical, optical, catalytic or magnetic properties (Chen et al., 2004). Moreover, the large surface-to-volume ratio and good dispersivity of nanomaterials provide a huge adsorptive surface for enriching target species (Xie et al., 2008). Although biological receptors have specific molecular affinity and have been widely used in diagnostic bioassays and chemo/biosensors, they are often produced via complex protocols with a high cost and require specific handling conditions because of their poor stability, and the natural receptors for many detected analytes don't exist (Whitcombe et al., 2000; Wulff, 2002; Haupt & Mosbach, 2000; Ye & Haupt, 2004). Thus, there has been a strong driving force in synthesizing artificial recognition receptors. Molecular imprinting is one of the most

efficient strategies that offer a synthetic route to artificial recognition systems by a template polymerization technique (Ye & Mosbach, 2001b; Spivak, 2005; Zhang et al., 2006). In this direction a recent review (Hillberg et al., 2005) highlight the importance of the concept of “engineerability” of MIP, defining as “engineerability” the materials ability to be integrated into an electro-mechanical device (Adhikari & Majumdar 2004). To date molecularly imprinted polymers have been successfully used with most types of transduction platforms and a range of methods have been used to bring about close integration of the platform with the polymer.

Fig. 5. Schematic representation of Biosensor. Adapted from Guan et al., 2008.

During the past ten years, the literatures on the development of MIP-based sensors, in particularly electrochemical (Riskin et al., 2008; Kan et al., 2008a) and optical (McDonagh et al., 2008; Basabe-Desmonts et al., 2007; Li et al., 2007; Feng et al., 2008) sensors, have been dramatically growing.

3.3.1 Electrochemical sensors

MIP-based electrochemical sensors were first reported in the early 1990s by Mosbach's group (Andersson et al., 1990). They described the integration of a phenylalanine anilide imprinted polymer into a field effect capacitance sensor and reported a significant reduction in the overall capacitance of the system when the sensor was exposed to the template (Hedborg et al., 1993). It was also observed that no such effect was observed when the sensor was exposed to the potential cross-reactants tyrosine anilide and phenylalaninol. The capacitance sensors based on MIPs were also fabricated and used to detect many other analytes such as amino acid derivatives with a detection limit of 500 ppm (panasyuk et al., 1999), and barbituric acid with a detection limit of 3.5 ppm (Mirsky et al., 1999). During the past decade, remarkable progress in MIP-based electrochemical sensors have been achieved by the use of conductometric/potentiometric measurements and MIP nanomaterials, greatly extending the range of detected targets and improving the sensitivity, selectivity and

simplicity of electrochemical sensors (Zhou et al., 2003). Different MIP sensing device designed with therapeutic application were prepared, by employing amperometric and/or voltammetry measurements and using several different templates, such as morphine, (Kriz & Mosbach, 1995), atrazine (Kim et al., 2007), benzyltriphenylphosphonium chloride (Kriz & Mosbach, 1995), thiophenol (Kröger et al., 1999), glutamic acid (Ouyang et al., 2007). Recently, the electrochemical sensors are fabricated by installing MIP nanomaterials, as recognition elements, onto the surface of electrode. The changes of current and peak voltage at cyclic voltammetry upon the analyte binding can sensitively respond to the concentration and kind of analytes, respectively, because of the oxidation or reduction of analytes at the MIP-modified electrode. In this direction, sensor for the detection of several analytes were developed: (Prasad et al., 2010a; Prasad et al., 2010b), tolazoline (Zhang et al., 2010a), tryptophan (Prasad et al., 2010c; Kong et al., 2010), clindamycin (Zhang et al., 2010b), 2,4-dichlorophenoxy acetic acid (Xie et al., 2010), histamine (Bongaers et al., 2010), caffeine (Alizadeh et al., 2010; Vinjamuri et al., 2008), uracil and 5-fluorouracil (Prasad et al., 2009a), salicylic acid (Kang et al., 2009), uric acid (Patel et al., 2009), resveratrol (Xiang & Li, 2009), hydroquinone (Kan et al., 2009; Kan et al., 2008a), bisphenol (Kolarz & Jakubiak, 2008), dopamine (Kan et al., 2008b).

3.3.2 Optical sensors

Of various signal transducers, optically addressable sensors based on fluorescent “turn-on” or “turn-off” mechanism have been demonstrated to be highly desirable for a variety of small molecular analytes in many challenging environments, due to their high signal output and feasible measurements (Holthoff & Bright, 2007a; Holthoff & Bright, 2007b). One of the first earliest MIP sensors studies described an optical device for sensing l-dansyl phenylalanine (Kriz et al., 1995). In this simple study polymer particles, imprinted with the fluorescent template l-dansyl phenylalanine, were sealed beneath a quartz window and re-exposed to the template. The fluorescence response of the systems was shown to be related to the concentration of template and importantly that this response was stereoselective. recent progress in the covalent linkage of MIPs to optical transducers has allowed for the realisation of highly efficient and robust optical MIP-based molecular recognition sensors (Henry et al., 2005). Most of the strategies involve in the design and use of fluorescent ligands and fluorotag-ligand conjugates in the preparation of the fluorescent sensors. Fluorescent functional monomers are coupled with imprinted sites, exhibiting fluorescence enhancement or quenching upon the analyte binding. In this direction, a 2-acrylamidoquinoline as a fluorescent functional monomer with a polymerizable acrylate moiety and a fluorescent hydrogen-bonding moiety was designed and synthesized (Kubo et al., 2005). The template cyclobarbitol was imprinted into a polymer matrix by using the fluorescent functional monomer, in which the remarkable fluorescent enhancement upon the hydrogen bonding of the target into the imprinted sites was observed. The fluorescent sensor demonstrated the ability to signal the presence and concentration of the analyte with a detection range of 0.1-2.0 mM.

Wang et al. developed a system that responded to the binding event with a significant fluorescence intensity change without the use of an external quencher (Wang et al., 1999; Gao et al., 2001). The key to this was the use of a fluorescent, anthracene containing monomer that was substituted with a boronic acid containing group. When the template, d-fructose, was re-introduced into the system a large change in fluorescence was observed.

This was attributed to the reformation of the boronic ester with the cis-diol of the fructose. In a different approach, Ye et al. incorporated a fluorescent scintillant into polymer microspheres imprinted with (S)-propranolol (Ye et al., 2002). When the MIP was used in scintillation proximity assays the specific binding of the radio-labelled template resulted in a transfer of energy from template to scintillant resulting in the generation of a fluorescence signal. Furthermore, Detection and quantification of Dextromethorphan is a pharmacological important marker drug used to identify the activity of the CYP2D6 class of p450 monooxygenases, is achieved by measuring the refractive index changes of multiple surface plasmons resulting from the binding to template pockets within the thin layer imprinted β -cyclodextrin polymer.

To date, MIP based optical sensors were successfully prepared for the selective recognition of different templates, such as, digoxin (Gonzalez et al., 2009), monoamine naphthalenes (Valero-Navarro et al., 2009), atrazine (Wu et al., 2008), aflatoxin B1 (Mosbach, 2006), dopamine (Kan et al. 2008b). In addition to the abovementioned fluorescence enhancement, photoinduced electron transfer (Leung et al., 2001), quencher-analyte competition adsorption (Liao et al., 1999) and chemiluminescence (Lin & Yamada, 2000) have been extensively explored to signal the analyte binding events. Photoinduced electron transfer has been a very popular mode of sensing in fluorescent molecular recognition in recent years (Basabe-Desmonts et al., 2007). It was demonstrated that the use of electron transfer mechanism as a means of signal transduction is feasible for the fluorescent detection of non-fluorescent analyte. A sol-gel molecularly imprinted luminescent sensor was fabricated by using a tailor-made organosilane as fluorescent functional monomer and 2,4-D as template molecule. Luminescence of the template was greatly enhanced by the formation of acid-base ion pairs with 2,4-D, because of the suppression of photoinduced electron transfer quenching on the anthryl fluorophore emission. Therefore, the imprinted sol-gel materials exhibited a selective fluorescent response to 2,4-D by the significant enhancement of fluorescence. A gradually rising trend in luminescent intensity was observed with increasing 2,4-D concentration from 10 to 166.6 $\mu\text{g mL}^{-1}$, while the control materials showed negligible response in luminescent intensity (Leung et al., 2001).

3.3.3 Mass sensitive devices

In principle, the measurement of mass is the most general method suitable for the detection of any analyte since the mass is a universal property of matter. Piezoelectric devices such as a quartz crystal microbalance (QCM) can provide an extremely sensitive measurement to the mass of the analyte binding at the surface of piezoelectric materials. When the mass of a piezoelectric material (e.g. quartz) changes there is an accompanying change in the resonant frequency and this change can be measured very precisely. A general rule of thumb being that for a system resonating at 10 MHz a change in mass of 1 ng results in a 1 Hz change in resonant frequency. In practice this means that when an analyte binds to the surface of a piezoelectric device, such as a quartz crystal microbalance (QCM), its presence is detected through a change in the resonant frequency of the system. When a molecular imprinted polymer layer is attached to the surface of a QCM the system can be used to measure template specific binding with high degree of sensitivity. MIP-based piezoelectric sensors has increased at a relatively slow rate compared with electrochemical and optical sensors, the synergetic advantages of the selectivity provided by MIP with the sensitivity provided by piezoelectric sensing makes the sensors almost universally applicable with good limits of

detection, low cost and the possibility of easy miniaturization and automation (Haupt et al., 1999; Tanaka, 2007; Ayela et al., 2007). The applications of MIP nanomaterials in piezoelectric sensors extends from small molecules to biomacromolecules and to bulky analytes such as microorganisms and cells. In 1996 Dickert and Thierer coated QCM surfaces with cross-linked polyurethanes molecularly imprinted with different solvents (Dickert & Thierer, 1996). The resulting sensor was shown to be selective for the template solvent. This is particularly interesting since it suggested that polymer selectivity could be achieved for small and poorly functional molecules such as tetrahydrofuran and chloroform. Krozer (Reimhult et al., 2008) reported the QCM sensor with dissipation (QCM-D) by coating the sensor surface with pre-made molecularly imprinted nanoparticles. The nanoparticles were physically entrapped into a thin poly(ethylene terephthalate) (PET) layer spin-coated on the transducer surface. By controlling the deposition conditions, a high nanoparticle loading can be gained in the stable PET layer, allowing the recognition sites in nanoparticles to be easily accessed by the test analytes. The highest uptake of the nanoparticle film to propranolol corresponded to approximately 2 nmol cm⁻² or about 1x10¹⁵ molecules cm⁻². The detection limit of the MIP-QCM sensor was about 10 µM, and the chiral recognition and discrimination between *R*- and *S*-propranolol can also be achieved.

Mass sensitive MIP sensors have also been viewed as good candidates for use in therapeutic monitoring and a number of therapeutically interesting targets have been studied. Liang et al. developed a highly selective and sensitive caffeine sensor which performed well in both serum and urine samples (Liang et al., 1999), the same group also used a similar bulk acoustic wave mass sensitive techniques to prepare MIP sensors for the direct determination of epinephrine (Liang et al., 2000), the antimicrobial agent pyrimethamine (Peng et al., 2000a), Phenobarbital (Peng et al., 2000b), (Yao et al., 2000), atropine (Peng et al., 2000c), and dopamine (Prasad et al., 2009b).

3.4 MIP as artificial receptors and antibodies

The design and synthesis of biomimetic receptor systems capable of binding a target molecule with similar affinities and specificities to their natural counterparts has long been a goal of bioorganic chemistry. Due to their unique binding characteristics (in terms of affinity and specificity), their high chemical and physical stability, ease availability and low cost, molecularly imprinted polymers are sometimes referred to as artificial antibodies and are considered an alternative to antibodies. (Ye & Haupt, 2004)

Molecularly imprinted polymers are certainly very different from antibodies; they are large, rigid and insoluble, whereas antibodies are small, flexible and soluble. However, as before mentioned, MIPs share with antibodies one of their most important features: the ability to selectively bind a target molecule.

To be used as receptor or antibodies and potentially as a drug, a MIP should be water-compatible and be synthesized from biocompatible building blocks.

At the time being, the majority of reports on molecularly imprinted polymers describe organic polymers synthesized from vinyl or acrylic monomers by radical polymerisation, and using non-covalent interactions. This can be attributed to the rather straightforward synthesis of these materials, and to the vast choice of available monomers with different functional groups. These can be basic (*e.g.* vinylpyridine) or acid (*e.g.* methacrylic acid), permanently charged (*e.g.* 3-acrylamidopropyltrimethylammonium chloride), hydrogen bonding (*e.g.* acrylamide), hydrophobic (*e.g.* styrene), metal coordinating, *etc.* These

functional monomers are sometimes considered analogous to the 20 amino acids that constitute the building blocks of proteins. These simple monomers have association constants with the template that are too low for the formation of a stable complex (although in the final polymer, the formation of several simultaneous interactions and a favourable entropy term normally assure tight binding of the target molecule). During non-covalent imprinting, functional monomers have to be used in excess to shift the equilibrium towards complex formation, resulting in some functional groups being randomly distributed throughout the polymer, which in turn is one of the reasons for non-specific binding. Compared to proteins that nature has selected for the required recognition and binding properties through evolution or, in the case of antibodies, clonal selection, this is a considerable drawback. Therefore, somewhat more sophisticated monomers are being designed that form more stable interactions with the template molecule or substructures thereof, and that can be used in a stoichiometric ratio. Other organic polymers are sometimes used for imprinting that are either better suited for a specific application or easier to synthesise in the desired form, for example poly(phenylene diamine), overoxidised polypyrrole, or polyurethanes. Imprinting is also possible in inorganic matrices, in particular sol- gels of silica or titanium dioxide. The molecular imprinting technique can be applied to different kinds of target molecules, ranging from small, organic molecules (*e.g.* pharmaceuticals, pesticides, amino acids and peptides, nucleotide bases, steroids and sugars) to peptides and proteins.

The first paper in this application area being a report by Mosbach's group on the development of a MIP-based immunoassay against theophylline and diazepam (Vlatakis et al., 1993). In this and other examples, MIPs have been used as substitutes for antibodies in radioimmunoassays (RIA) for drugs, showing strong binding to the target analytes and cross-reactivity profiles similar to those of antibodies. The dissociation constants that have been measured by some authors were found to be in the nanomolar to micromolar range (Andersson et al., 1995; Ramstrom et al., 1996). This is in the same range as the average antibody, although antibodies exist that have an affinity for their antigen several orders of magnitude higher. In a competitive radioimmunoassay based on MIP, the radioisotope-labeled target analyte is incubated with increasing amounts of non-labeled target to compete for binding to a limited amount of MIP. After the equilibrium is reached, the amount of label bound to the MIP, which is inversely related to the concentration of nonlabeled analyte, is quantified by radioactivity measurements.

A plot of bound label against the concentration of non-labeled analyte gives a typical sigmoidal calibration curve, which can be used to calculate the MIP's binding affinity and site population. The concentration of non-labeled analyte displacing 50% of the label is defined as the IC_{50} value. The same experiment can be repeated using other related drugs as the competing ligand, which gives displacement curves (and IC_{50}) shifted to a higher concentration range. The MIP's cross-reactivity for the new ligand is defined as the percentage of IC_{50}/IC'_{50} .

By incorporating an appropriate scintillation reporter element, MIPs can be designed to directly generate a specific physicochemical signal upon binding of an analyte. In Figure 6, the principle of using a "universal" scintillation reporter embedded in molecularly imprinted microspheres is reported (Ye & Mosbach, 2001c; Ye et al., 2002).

The MIP containing the scintillation reporter is imprinted against a β -adrenergic antagonist, S-propranolol. When tritium-labeled S-propranolol binds to the MIP, its β -radiation triggers the nearby reporter to emit long wavelength fluorescence that can be directly quantified.

When used in competitive-assay mode, the fluorescence signal decreases due to the non-labeled analyte competing for the limited number of binding sites. This MIP-based scintillation proximity assay (SPA) has the potential to provide a very high sample throughput, since it is a quasi-homogeneous assay that does not require washing steps to separate unbound radioligand from its bound fraction before quantification.

Fig. 6. MIP-based proximity scintillation assay. **a** The S-propranolol-imprinted microspheres contain a scintillation reporter located in proximity to the specific binding site. Binding of [3H]S-propranolol makes the β -electron from the radioisotope decay stimulate the reporter to generate long wavelength fluorescence. **b** Calibration curve. In competitive mode, the non-labeled S-propranolol displaces the [3H]S-propranolol, and so reduces the fluorescence signal. Adapted from Ye et al., 2002.

Imprinted-polymer-based assays are conveniently performed using radiolabels, because the labelled analyte has the same structure as the original template. However, this involves the handling of radioactive materials and produces radioactive waste, which is sometimes undesirable. Interest is therefore increasing in the development of alternative assay formats based on other detection methods that could use, just like immunoassays, an enzyme reaction or fluorescence for detection. Several years ago competitive immunoassays that use a fluorescent probe (Haupt et al., 1998) or an electroactive probe (Kroger et al., 1999) for detection were proposed. These assays were based on a polymer imprinted with the herbicide 2,4-dichlorophenoxyacetic acid, and the probes were not related to the analyte but had some structural similarity with it. It was shown that although binding of the probes to the polymer was only a few percent as compared to the analyte, specificity and selectivity of the assay were on a par with a competitive radioligand binding assay using the same polymer and the radiolabelled analyte. The fluorescent assay could be performed in

aqueous buffer as well as in organic solvents such as, acetonitrile. The real challenge, however, has always been to use enzyme labels. Although most common with immunoassays, enzymes seemed to be less practical in MIPs assays for two reasons: first, they often only work in aqueous buffers, whereas the use of many imprinted polymers used to be restricted to organic solvents. Second, the rather hydrophobic nature and highly cross-linked structure of the polymer limits the access of the imprinted binding sites by the large protein molecules.

However, during the last few years, MIPs that perform well in aqueous solvents have been developed, and Haupt et al. have shown that the problem of binding site accessibility might be circumvented by using, instead of large porous MIP particles, imprinted microspheres that have binding sites at or close to their surface (Andersson, 1996). They have developed ELISA-type assays where the analyte was labelled with the enzyme peroxidase. Thus, colorimetry or chemiluminescence could be used for detection. A colorimetric assay has also been reported by Piletsky and colleagues. They have developed a method where the polymer is *in situ* synthesised in the wells of a polystyrene microtiter plate. Aminophenylboronic acid was polymerised in the presence of epinephrine (the target analyte) using oxidation of the monomer by ammonium persulfate. This process resulted in the grafting of a thin polymer layer onto the polystyrene surface (Piletsky et al., 2000). The polymer was then used in a competitive enzyme-linked assay with a conjugate of horseradish peroxidase and norepinephrine.

4. References

- Adhikari, B. & Majumdar, S. (2004) Polymers in sensor applications. *Progress in Polymer Science*, 29, 699–766.
- Alizadeh, T.; Ganjali, M.R.; Zare, M. & Norouzi, P. (2010). Development of a voltammetric sensor based on a molecularly imprinted polymer (MIP) for caffeine measurement. *Electrochimica Acta*, 55, 1568-1574.
- Allender, C.J.; Brain, K.R. & Heard, C.M. (2005). Molecularly imprinted polymers-preparation, biomedical applications and technical challenges. *Progress in Medicinal Chemistry*, 36, 235-291.
- Alvarez-Lorenzo, C. & Concheiro, A. (2004). Molecularly imprinted polymers for drug delivery. *Journal of Chromatography B*, 804, 231-245.
- Alvarez-Lorenzo, C. & Concheiro, A. (2008). Intelligent drug delivery systems: Polymeric micelles and hydrogels. *Mini-Reviews in Medicinal Chemistry*, 8, 1065-1074.
- Alvarez-Lorenzo, C.; Guney, O.; Oya, T.; Sakai, Y.; Kobayashi, M.; Enoki, T.; Takeoka, Y.; Ishibashi, T.; Kuroda, K.; Tanaka, K.; Wang, G.; Grosberg, A.Y.; Masamune, S. & Tanaka, T. (2001). Reversible adsorption of calcium ions by imprinted temperature sensitive gels. *Journal of Chemical Physics*, 114, 2812-2816.
- Anderson, J.M. (1994). In vivo biocompatibility of implantable delivery systems and biomaterials. *European Journal of Pharmaceutics and Biopharmaceutics*, 40, 1-8.
- Andersson, L.I. (1996) Application of molecular imprinting to the development of aqueous buffer and organic solvent based radioligand binding assays for (S)-propranolol. *Analytical Chemistry*, 68, 111-117.
- Andersson, L.I.; Miyabayashi, A.; O'Shannessy, D.J. & Mosbach, K. (1990). Enantiomeric resolution of amino acid derivatives on molecularly imprinted polymers as monitored by potentiometric measurements. *Journal of Chromatography*, 516, 323-331

- Andersson, L.I.; Muller, R.; Vlatakis, G. & Mosbach, K. (1995). Mimics of the binding sites of opioid receptors obtained by molecular imprinting of enkephalin and morphine. *Proceedings of the National Academy of Sciences of the United States of America*, 92, 4788-4792.
- Andersson, L.I.; Paprica, A. & Arvidsson, T. (1997). A highly selective solid phase extraction sorbent for preconcentration of sameridine made by molecular imprinting. *Chromatographia*, 46, 57-66.
- Aydin, O.; Attila, G.; Dogan, A.; Aydin, M.V.; Canacankatan, N. & Kanik, A. (2002). The effects of methyl methacrylate on nasal cavity, lung, and antioxidant system (An Experimental Inhalation Study). *Toxicologic Pathology*, 30, 350-356.
- Ayela, C.; Vandeveld, F.; Lagrange, D.; Haupt, K. & Nicu, L. (2007). Combining resonant piezoelectric micromembranes with molecularly imprinted polymers. *Angewandte Chemie - International Edition*, 46, 9271-9274.
- Banholzer, M.J.; Millstone, J.E.; Qin, L.D. & Mirkin, C.A. (2008). Rationally designed nanostructures for surface-enhanced raman spectroscopy. *Chemical Society Reviews*, 37, 885-897.
- Basabe-Desmonts, L.; Reinhoudt, D.N. & Crego-Calama, M. (2007). Design of fluorescent materials for chemical sensing. *Chemical Society Reviews*, 36, 993-1017.
- Benito-Peña, E.; Martins, S.; Orellana, G. & Moreno-Bondi, M.C. (2009). Watercompatible molecularly imprinted polymer for the selective recognition of fluoroquinolone antibiotics in biological samples. *Analytical and Bioanalytical Chemistry*, 393, 235-245.
- Bongaers, E.; Alenus, J.; Horemans, F.; Weustenraed, A.; Lutsen, L.; Vanderzande, D.; Cleij, T.J.; Troost, G.J.; Brummer, R.-J. & Wagner, P. (2010). A MIP-based biomimetic sensor for the impedimetric detection of histamine in different pH environments. *Physica Status Solidi (A) Applications and Materials*, 207, 837-843.
- Boos, K.S. & Fleischer, C.T. (2001). Multidimensional on-line solid-phase extraction (SPE) using restricted access materials (RAM) in combination with molecular imprinted polymers (MIP). *Journal of Analytical Chemistry*, 371, 16-20.
- Brüggemann, O.; Visnjeviski, A.; Burch, R. & Patel, P. (2004). Selective extraction of antioxidants with molecularly imprinted polymers. *Analytica Chimica Acta*, 504, 81-88.
- Bures, P.; Huang, Y.; Oral, E. & Peppas, N.A. (2001). Surface modifications and molecular imprinting of polymers in medical and pharmaceutical applications. *Journal of Controlled Release*, 72, 25-33.
- Caro, E.; Marcè, R.M.; Cormack, P.A.G.; Sherrington, D.C. & Borrull, F. (2003). On-line solid-phase extraction with molecularly imprinted polymers to selectively extract substituted 4-chlorophenols and 4-nitrophenol from water. *Journal of Chromatography A*, 995, 233-240.
- Caro, E.; Masquè, N.; Marcè, R.M.; Borrull, F.; Cormack, P.A.G. & Sherrington, D.C. (2002). Non-covalent and semi-covalent molecularly imprinted polymers for selective on-line solid-phase extraction of 4-nitrophenol from water samples. *Journal of Chromatography A*, 963, 169-178.
- Chapuis, F.; Pichon, V.; Lanza, F.; Sellergren, B. & Hennion, M.-C. (2003). Optimization of the class-selective extraction of triazines from aqueous samples using a molecularly imprinted polymer by a comprehensive approach of the retention mechanism. *Journal of Chromatography A*, 999, 23-30.
- Chen, J.R.; Miao, Y.Q.; He, N.Y.; Wu, X.H. & Li, S.J. (2004). Nanotechnology and biosensors. *Biotechnology Advances*, 22, 505-518.

- Chien, Y.W. & Lin, S. (2002). Optimisation of treatment by applying programmable rate-controlled drug delivery technology. *Clinical Pharmacokinetics*, 41, 1267-1299.
- Cirillo, G.; Curcio, M.; Parisi, O.I.; Puoci, F.; Iemma, F.; Spizzirri, U.G. & Picci, N. (2009a). Gastro-intestinal sustained release of phytic acid by molecularly imprinted microparticles. *Pharmaceutical Development and Technology*, DOI: 10.3109/10837450903397602.
- Cirillo, G.; Iemma, F.; Puoci, F.; Parisi, O.I.; Curcio, M.; Spizzirri, U.G. & Picci, N. (2009b). Imprinted hydrophilic nanospheres as drug delivery systems for 5-fluorouracil sustained release. *Journal of Drug Targeting*, 17, 72-77.
- Cirillo, G.; Parisi, O.I.; Curcio, M.; Puoci, F.; Iemma, F.; Spizzirri, U.G. & Picci, N. (2010b). Molecularly Imprinted Polymers as Drug Delivery Systems for the Sustained Release of Glycyrrhizic Acid. *Journal of Pharmacy and Pharmacology*, 62, 577-582.
- Cirillo, G.; Puoci, F.; Curcio, M.; Parisi, O.I.; Iemma, F.; Spizzirri, U.G. & Picci, N. (2010a). Molecular imprinting polymerization by Fenton reaction. *Colloid and Polymer Science*, 288, 689-693.
- Cunliffe, D.; Kirby, A. & Alexander, C. (2005). Molecularly imprinted drug delivery systems. *Advanced Drug Delivery Reviews*, 57, 1836-1853.
- Curcio, M.; Puoci, F.; Spizzirri, U.G.; Iemma, F.; Cirillo, G.; Parisi, O.I. & Picci, N. (2010). Negative Thermo-responsive Microspheres Based on Hydrolyzed Gelatin as Drug Delivery Device. *AAPS PharmSciTech*, DOI:10.1208/s12249-010-9429-5.
- Curcio, M.; Parisi, O.I.; Cirillo, G.; Spizzirri, U.G.; Puoci, F.; Iemma, F. & Picci, N. (2009) Selective Recognition of Methotrexate by Molecularly Imprinted Polymers" *E-polymers*, 78, 1-7.
- D'Oleo, R.; Alvarez-Lorenzo, C. & Sun, G. (2001). A new approach to design imprinted polymer gels without using a template. *Macromolecules*, 34, 4965-4971.
- Davis, K.A. & Anseth, K.S. (2002). Controlled release from crosslinked degradable networks. *Critical Reviews in Therapeutic Drug Carrier Systems*, 19, 385-423.
- Dickert, F.L. & Thierier S. (1996). Molecularly imprinted polymers for optochemical sensor. *Advanced Materials*, 8, 987-990.
- Dirion, B.; Lanza, F.; Sellergren, B.; Chassaing, C.; Venn, R. & Berggren, C. (2002). Selective solid phase extraction of a drug lead compound using molecularly imprinted polymers prepared by the target analogue approach. *Chromatographia*, 56, 237-241.
- Eggins, B.R. (2002). *Chemical sensors and biosensors*. John Wiley, Chichester, UK.
- Ellwanger, A.; Berggren, C.; Bayouth, S.; Crecenzi, C.; Karlsson, L.; Owens, P.K.; Ensing, K.; Cormack, P.A.G.; Sherrington, D.C. & Sellergren, B. (2001). Evaluation of methods aimed at complete removal of template from molecularly imprinted polymers. *Analyst*, 126 784-792.
- Feng, F.D.; He, F.; An, L.L.; Wang, S.; Li, Y.H. & Zhu, D.B. (2008). Fluorescent conjugated polyelectrolytes for biomacromolecule detection. *Advanced Materials*, 20, 2959-2964.
- Gao, D.; Zhang, Z.; Wu, M.; Xie, C.; Guan, G. & Wang, D. (2007). A surface functional monomer-directing strategy for highly dense imprinting of TNT at surface of silica nanoparticles. *Journal of the American Chemical Society*, 129, 7859-7866.
- Gao, S.H.; Wang, W. & Wang, B.H. (2001). Building fluorescent sensors for carbohydrates using template-directed polymerizations. *Bioorganic Chemistry*, 29, 308- 320.
- Gil, E.S. & Hudson, S.M. (2004). Stimuli-reponsive polymers and their bioconjugates. *Progress in Polymer Science (Oxford)*, 29, 1173-1222.
- González, G.P.; Hernando, P.F. & Alegría, J.S.D. (2009). An optical sensor for the determination of digoxin in serum samples based on a molecularly imprinted polymer membrane. *Analytica Chimica Acta*, 638, 209-212.

- Guan, G.; Liu, B.; Wang, Z. & Zhang, Z. (2008). Imprinting of Molecular Recognition Sites on Nanostructures and its Applications in Chemosensors. *Sensors*, 8, 8291-8320.
- Haginaka, J.; Takehira, H.; Hosoya, K. & Tanaka, N. (1999). Uniform-sized molecularly imprinted polymer for (S)-naproxen selectively modified with hydrophilic external layer. *Journal of Chromatography A*, 849, 331-339.
- Hansen, D.E. (2007). Recent developments in the molecular imprinting of proteins. *Biomaterials*, 28, 4178-4191.
- Haupt, K.; Mayes, A.G. & Mosbach, K. (1998). Herbicide assay using an imprinted polymer-based system analogous to competitive fluoroimmunoassays. *Analytical Chemistry*, 70, 3936-3939.
- Haupt, K. & Mosbach, K. (2000). Molecularly imprinted polymers and their use in biomimetic sensors. *Chemical Reviews* 100, 2495-2504.
- Haupt, K. (2003). Imprinted polymers - Tailor-made mimics of antibodies and receptors. *Chemical Communications*, 9, 171-177.
- Haupt, K.; Dzgoev, A. & Mosbach, K. (1998). Assay system for the herbicide 2,4-dichlorophenoxyacetic acid using a molecularly imprinted polymer as an artificial recognition element. *Analytical Chemistry*, 70, 628-631.
- Haupt, K.; Noworyta, K. & Kutner, W. (1999). Imprinted polymer-based enantioselective acoustic sensor using a quartz crystal microbalance. *Analytical Communications*, 36, 391-393.
- Hedborg, E.; Winqvist, F.; Lundström, I.; Andersson, L.I. & Mosbach, K. (1993). Some studies of molecularly-imprinted polymer membranes in combination with field-effect devices. *Sensors and Actuators, A: Physical* 37-8, 796-799.
- Henry, O.Y.F.; Cullen, D.C. & Piletsky, S.A. (2005). Optical interrogation of molecularly imprinted polymers and development of MIP sensors: A review. *Analytical and Bioanalytical Chemistry*, 382, 947-956.
- Hillberg, A.L.; Brain, K.R. & Allender, C.J. (2005). Molecular imprinted polymer sensors: Implications for therapeutics. *Advanced Drug Delivery Reviews*, 57, 1875-1889.
- Hillberg, A.L. & Tabrizian, M. (2008). Biomolecule imprinting: Developments in mimicking dynamic natural recognition systems. *ITBM-RBM* 29, 89-104.
- Holthoff, E.L. & Bright, F.V. (2007a). Molecularly templated materials in chemical sensing. *Analytica Chimica Acta*, 594, 147-161.
- Holthoff, E.L. & Bright, F.V. (2007b). Molecularly imprinted xerogels as platforms for sensing. *Accounts of Chemical Research*, 40, 756-767.
- Hwang, C.C. & Lee, W.C. (2002). Chromatographic characteristics of cholesterol-imprinted polymers prepared by covalent and non-covalent imprinting methods. *Journal of Chromatography A*, 962, 69-78.
- Iemma, F.; Cirillo, G.; Spizzirri, U.G.; Puoci, F.; Parisi, O.I. & Picci, N. (2008). Removal of metal ions from aqueous solution by chelating polymeric microspheres bearing phytic acid derivatives. *European Polymer Journal*, 44, 1183-1190.
- Iemma, F.; Spizzirri, U.G.; Puoci, F.; Cirillo, G.; Curcio, M.; Parisi, O.I. & Picci, N. (2009). Synthesis and release profile analysis of thermo-sensitive albumin hydrogels. *Colloid and Polymer Science*, 287, 779-787.
- Ikegami, T.; Mukawa, T.; Nariai, H. & Takeuchi, T. (2004). Bisphenol A-recognition polymers prepared by covalent molecular imprinting. *Analytica Chimica Acta*, 504, 131-135.
- Jiang, H. & Ju, H.X. (2007). Enzyme-quantum dots architecture for highly sensitive electrochemiluminescence biosensing of oxidase substrates. *Chemical Communications*, 4, 404-406.

- Joshi, V.P.; Karode, S.K.; Kulkarni, M.G. & Mashelkar, R.A. (1998). Novel separation strategies based on molecularly imprinted adsorbents. *Chemical Engineering Science*, 53, 2271-2284.
- Kan, X.; Geng, Z.; Wang, Z. & Zhu, J.-J. (2009). Core-Shell molecularly imprinted polymer nanospheres for the recognition and determination of hydroquinone. *Journal of Nanoscience and Nanotechnology*, 9, 2008-2013.
- Kan, X.; Zhao, Q.; Zhang, Z.; Wang, Z. & Zhu, J.-J. (2008a). Molecularly imprinted polymers microsphere prepared by precipitation polymerization for hydroquinone recognition. *Talanta*, 75, 22-26.
- Kan, X.; Zhao, Y.; Geng, Z.; Wang, Z. & Zhu, J.-J. (2008b). Composites of multiwalled carbon nanotubes and molecularly imprinted polymers for dopamine recognition. *Journal of Physical Chemistry C*, 112, 4849-4854.
- Kang, J.; Zhang, H.; Wang, Z.; Wu, G. & Lu, X. (2009). A novel amperometric sensor for salicylic acid based on molecularly imprinted polymer-modified electrodes. *Polymer - Plastics Technology and Engineering*, 48, 639-645.
- Kim, S.N.; Rusling, J.F. & Papadimitrakopoulos, F. (2007). Carbon nanotubes for electronic and electrochemical detection of biomolecules. *Advanced Materials*, 19, 3214-3228.
- Kolarz, B.N. & Jakubiak, A. (2008). Catalytic activity of molecular imprinted vinylpyridine/acrylonitrile/divinylbenzene terpolymers with guanidyl ligands-Cu(II) inside the active centres. *Polimery/Polymers*, 53, 848-853.
- Komiyama, M.; Takeuchi, T.; Mukawa, T. & Asanuma, H. (2003). *Molecular Imprinting*, Wiley-VCH, Weinheim.
- Kong, Y.; Zhao, W.; Yao, S.; Xu, J.; Wang, W. & Chen, Z. (2010). Molecularly imprinted polypyrrole prepared by electrodeposition for the selective recognition of tryptophan enantiomers. *Journal of Applied Polymer Science*, 115, 1952-1957.
- Kriz, D. & Mosbach, K. (1995). Competitive amperometric morphine sensor-based on an agarose immobilized molecularly imprinted polymer. *Analytica Chimica Acta*, 300, 71-75.
- Kriz, D.; Ramström, O.; Svensson, A. & Mosbach, K. (1995). Introducing biomimetic sensors based on molecularly imprinted polymers as recognition elements. *Analytical Chemistry*, 67, 2142-2144.
- Kröger, S.; Turner, A.P.F.; Mosbach, K. & Haupt, K. (1999). Imprinted polymer based sensor system for herbicides using differential pulse voltammetry on screen printed electrodes. *Analytical Chemistry*, 71, 3698-3702.
- Kubo, H.; Yoshioka, N. & Takeuchi, T. (2005). Fluorescent imprinted polymers prepared with 2-acrylamidoquinoline as a signaling monomer. *Organic Letters*, 7, 359-362.
- Lai, J.P.; Lu, X.Y.; Lu, C.Y.; Ju, H.F. & He, X.W. (2001). Preparation and evaluation of molecularly imprinted polymeric microspheres by aqueous suspension polymerization for use as a high-performance liquid chromatography stationary phase. *Analytica Chimica Acta*, 442, 105-111.
- Leung, M. K-P.; Choe, C-F. & Lam, M. H-W. (2001). A sol-gel derived molecular imprinted luminescent PET sensing material for 2, 4-dichlorophenoxyacetic acid. *Journal of Materials Chemistry*, 11, 2985-2991.
- Li, J.; Kendig, C.E. & Nesterov, E.E. (2007). Chemosensory performance of molecularly imprinted fluorescent conjugated polymer materials. *Journal of the American Chemical Society*, 129, 15911-15918.
- Liang, C.D.; Peng, H.; Bao, X.Y.; Nie, L.H. & Yao, S.Z. (1999). Study of a molecular imprinting polymer coated BAW bio-mimic sensor and its application to the determination of caffeine in human serum and urine. *Analyst*, 124, 1781-1785.

- Liang, C.D.; Peng, H.; Zhou, A.H.; Nie, L.H. & Yao, S.Z. (2000). Molecular imprinting polymer coated BAW bio-mimic sensor for direct determination of epinephrine. *Analytica Chimica Acta*, 415, 135-141.
- Liao, Y.; Wang, W. & Wang, B.H. (1999) Building fluorescent sensors by template polymerization: the preparation of a fluorescent sensor for L-tryptophan. *Bioorganic Chemistry*, 27, 463-476.
- Lin, J.M. & Yamada, M. (2000). Chemiluminescent reaction of fluorescent organic compounds with KHSO₅ using cobalt(II) as catalyst and its first application to molecular imprinting. *Analytical Chemistry*, 72, 1148-1155.
- Martin, P.; Jones, G.R.; Stringer, F. & Wilson, I.D. (2003). Comparison of normal and reversed-phase solid phase extraction methods for extraction of β -blockers from plasma using molecularly imprinted polymers. *Analyst*, 128, 345-350.
- Mayes, A.G. & Mosbach, K. (1996). Molecularly imprinted polymer beads: Suspension polymerization using a liquid perfluorocarbon as the dispersing phase. *Analytical Chemistry*, 68, 769-3774.
- McDonagh, C.; Burke, C.S. & MacCraith, B.D. (2008). Optical chemical sensors. *Chemical Reviews*, 108, 400-422.
- Minoura, N.; Idei, K.; Rachkov, A.; Uzawa, H. & Matsuda, K. (2003). Molecularly Imprinted Polymer Membranes with Photoregulated Template Binding *Chemistry of Materials*, 15, 4703-4704.
- Mirsky, V.M.; Hirsch, T.; Piletsky, S.A. & Wolfbeis, O.S. (1999). A spreader-bar approach to architecture: formation of stable artificial chemoreceptors. *Angewandte Chemie - International Edition*, 38, 1108-1110.
- Morikawa, H.; Koike, S.; Saiki, M. & Saegusa, Y. (2008). Synthesis and characterization of the PEG-based nonionic surfactants endowed with carboxylic acid moiety at the hydrophobic terminal. *Journal of Polymer Science, Part A: Polymer Chemistry*, 46, 8206-8212.
- Mosbach, K. & Ramström, O. (1996). The emerging technique of molecular imprinting and its future impact on biotechnology. *Bio/Technology*, 14, 163-170.
- Mosbach, K. (2001). Towards the development of artificial recognition elements in sensor technology with emphasis on molecular imprinting (E), *Electrochemistry*, 69, 919-.
- Mosbach, K. (2006). The promise of molecular Imprinting. *Scientific American*, 295, 86-91.
- Mullett, W.M. & Pawliszyn, J. (2003). The development of selective and biocompatible coatings for solid phase microextraction. *Journal of Separation Science*, 26, 251-260.
- Oh, K.T. & Lee, E.S. (2008). Cancer-associated pH-responsive tetracopolymeric micelles composed of poly(ethylene glycol)-b-poly(L-histidine)-b-poly(L-lactic acid)-b-poly(ethylene glycol). *Polymers for Advanced Technologies*, 19, 1907-1913.
- Orellana, G. & Moreno-Bondi, M.C. *Frontiers in chemical sensors: novel principles and techniques*. Springer-Verlag, Berlin, Heidelberg, New York, NY, 2005.
- Ouyang, R.; Lei, J.; Ju, H. & Xue, Y. (2007). A molecularly imprinted copolymer designed for enantioselective recognition of glutamic acid. *Advanced Functional Materials* 17, 3223-3230.
- Owens, P.K.; Karlsson, L.; Lutz, E.S.M. & Andersson, L.I. (1999). Molecular imprinting for bio- and pharmaceutical analysis. *Trends in Analytical Chemistry*, 18, 146-155.
- Panasyuk, T.L.; Mirsky, V.M.; Piletsky, S.A. & Wolfbeis, O.S. (1999). Electropolymerized molecularly imprinted polymers as receptor layers in a capacitive chemical sensors. *Analytical Chemistry*, 71, 4609-4613.

- Pap, T.; Horvath, V.; Tolokàn, A.; Horvai, G. & Sellergren, B. (2002). Effect of solvents on the selectivity of terbutylazine imprinted polymer sorbents used in solid-phase extraction. *Journal of Chromatography A*, 973, 1-8.
- Parisi, O.I.; Cirillo, G.; Curcio, M.; Puoci, F.; Iemma, F.; Spizzirri, U.G. & Picci, N. (2009) Surface modifications of molecularly imprinted polymers for improved template recognition in water media. *Journal of Polymer Research*, DOI 10.1007/s10965-009-9322-7.
- Patel, A.K.; Sharma, P.S. & Prasad, B.B. (2009). Electrochemical sensor for uric acid based on a molecularly imprinted polymer brush grafted to tetraethoxysilane derived sol-gel thin film graphite electrode. *Materials Science and Engineering C*, 29,1545-1553.
- Peng, H.; Liang, C.D.; He, D.L.; Nie, L.H. & Yao, S.Z. (2000a). Bulk acoustic wave sensor using molecularly imprinted polymers as recognition elements for the determination of pyrimethamine. *Talanta*, 52, 441– 448.
- Peng, H.; Liang, C.D.; He, D.L.; Nie, L.H. & Yao, S.Z. (2000b). Nonaqueous assay system for phenobarbital using biomimetic bulk acoustic wave sensor based on a molecularly imprinted polymers. *Analytical Letters*, 33, 793–808.
- Peng, H.; Liang, C.D.; Zhou, A.H.; Zhang, Y.Y.; Xie, Q.J. & Yao, S.Z. (2000c). Development of a new atropine sulfate bulk acoustic wave sensor based on a molecularly imprinted electrosynthesized copolymer of aniline with o-phenylenediamine. *Analytica Chimica Acta*, 423, 221–228.
- Peppas, N.A. & Leobandung, W. (2004). Stimuli-sensitive hydrogels: Ideal carriers for chronobiology and chronotherapy. *Journal of Biomaterials Science, Polymer Edition*, 15, 125-144.
- Pérez-Álvarez, L.; Sáez-Martínez, V.; Hernáez, E. & Katime, I. (2008). Synthesis and characterization of pH-sensitive microgels by derivatization of npa-based reactive copolymers. *Materials Chemistry and Physics*, 112), 516-524.
- Piletsky, S.A.; Andersson, H.S. & Nicholls. I.A. (1999). Combined Hydrophobic and Electrostatic Interaction-Based Recognition in Molecularly Imprinted Polymers. *Macromolecules*, 32, 633-636.
- Piletsky, S.A.; Piletska, E.V.; Chen, B.; Karim, K.; Weston, D.; Barrett, G.; Lowe, P. & Turner A.P.F. (2000). Chemical grafting of molecularly imprinted homopolymers to the surface of microplates. Application of artificial adrenergic receptor in enzyme-linked assay for β -agonists determination. *Analytical Chemistry*, 72, 4381-4385.
- Piscopo, L.; Prandi, C.; Coppa, M.; Sparnacci, K.; Laus, M.; Lagana, A.; Curini, R. & D'Ascenzo, G. (2002). Uniformly sized molecularly imprinted polymers (MIPs) for 17 β -estradiol. *Macromolecular Chemistry and Physics*, 203:532–1538.
- Prasad, B.B.; Madhuri, R.; Tiwari, M.P. & Sharma, P.S. (2010a). Imprinted polymer-carbon consolidated composite fiber sensor for substrate-selective electrochemical sensing of folic acid. *Biosensors and Bioelectronics*, 25, 2140-2148.
- Prasad, B.B.; Madhuri, R.; Tiwari, M.P. & Sharma, P.S. (2010b). Electrochemical sensor for folic acid based on a hyperbranched molecularly imprinted polymer-immobilized sol-gel-modified pencil graphite electrode. *Sensors and Actuators, B: Chemical*, 146, 321-330.
- Prasad, B.B.; Madhuri, R.; Tiwari, M.P. & Sharma, P.S. (2010c). Enantioselective recognition of d- and l-tryptophan by imprinted polymer-carbon composite fiber sensor. *Talanta*, 81, 187-196.
- Prasad, B.B.; Srivastava, S.; Tiwari, K. & Sharma, P.S. (2009a). Development of uracil and 5-fluorouracil sensors based on molecularly imprinted polymer-modified hanging mercury drop electrode. *Sensors and Materials*, 21, 291-306.

- Prasad, B.B.; Srivastava, S.; Tiwari, K. & Sharma, P.S. (2009b). Trace-level sensing of dopamine in real samples using molecularly imprinted polymer-sensor. *Biochemical Engineering Journal*, 44, 232-239.
- Puoci, F.; Garreffa, C.; Iemma, F.; Muzzalupo, R.; Spizzirri, U.G. & Picci, N. (2005). Molecularly imprinted solid phase extraction for detection of sudan I in food matrices. *Food Chemistry*, 93, 349-353.
- Puoci, F.; Cirillo, G.; Curcio, M.; Iemma, F.; Parisi, O.I.; Castiglione, M. & Picci, N. (2008c). Molecularly imprinted polymers for α -tocopherol delivery. *Drug Delivery*, 15, 253-258.
- Puoci, F.; Cirillo, G.; Curcio, M.; Iemma, F.; Spizzirri, U.G. & Picci, N. (2007a). Molecularly Imprinted Solid Phase Extraction for the selective HPLC determination of α -tocopherol in Bay Leaves. *Analytica Chimica Acta*, 593, 164-170.
- Puoci, F.; Curcio, M.; Cirillo, G.; Iemma, F.; Spizzirri, U.G. & Picci, N. (2008a). Molecularly imprinted solid-phase extraction for cholesterol determination in cheese products. *Food Chemistry*, 106, 836-842.
- Puoci, F.; Iemma, F.; Cirillo, G.; Curcio, M.; Parisi, O.I.; Spizzirri, U.G. & Picci, N. (2009). New Restricted Access Materials combined to Molecularly Imprinted Polymers for Selective Recognition/Release in Water Media. *European Polymer Journal*. 45, 1634-1640.
- Puoci, F.; Iemma, F. & Picci, N. (2008b). Stimuli-responsive molecularly imprinted polymers for drug delivery: A review. *Current Drug Delivery*, 5, 85-96.
- Puoci, F.; Iemma, F.; Cirillo, G.; Picci, N.; Matricardi, P. & Alhaique, F. (2007b). Molecularly imprinted polymers for 5-fluorouracil release in biological fluids. *Molecules*, 12, 805-814.
- Puoci, F.; Iemma, F.; Muzzalupo, R.; Spizzirri, U.G.; Trombino, S.; Cassano, R. & Picci, N. (2004). Spherical Molecularly Imprinted Polymers (SMIPs) via a Novel Precipitation Polymerization in the Controlled Delivery of Sulfasalazine. *Macromolecular Bioscience*, 4, 22-26.
- Ramström, O. & Ansell, R.J. (1998). Molecular imprinting technology: Challenges and prospects for the future. *Chirality*, 10, 195-209.
- Ramström, O.; Ye, L. & Mosbach, K. (1996). Artificial antibodies to corticosteroids prepared by molecular imprinting. *Chemistry and Biology*, 3, 471-477.
- Reimhult, K.; Yoshimatsu, K.; Risveden, K.; Chen, S.; Ye, L. & Krozer, A. (2008). Characterization of QCM sensor surface coated with molecularly imprinted nanoparticles. *Biosensors and Bioelectronics*, 23, 1908-1914.
- Riskin, M.; Tel-Vered, R.; Bourenko, T.; Granot, E. & Willner, I. (2008). Imprinting of molecular recognition sites through electropolymerization of functionalized Au nanoparticles: development of an electrochemical TNT sensor based on π -donor-acceptor interactions. *Journal of the American Chemical Society*, 130, 15911-15918.
- Sambe, H.; Hoshina, K. & Haginaka, J. (2007). Molecularly imprinted polymers for triazine herbicides prepared by multi-step swelling and polymerization method. Their application to the determination of methylthiotriazine herbicides in river water. *Journal of Chromatography A*, 1152, 130-137.
- Sanbe, H. & Haginaka J. (2003). Restricted access media-molecularly imprinted polymer for propranolol and its application to direct injection analysis of β -blockers in biological fluids. *Analyst*, 128, 593-597.
- Say, R.; Birlık, E.; Ersoz, A.; Yilmaz, F.; Gedikbey, T. & Denizli, A. (2003). Preconcentration of copper on ion-selective imprinted polymer microbeads. *Analytica Chimica Acta*, 480, 251-258.
- Schmaljohann, D. (2006). Thermo- and pH-responsive polymers in drug delivery. *Advanced Drug Delivery Reviews*, 58, 1655-1670.

- Spivak, D.A. (2005). Optimization, evaluation, and characterization of molecularly imprinted polymers. *Advanced Drug Delivery Reviews*, 57, 1779-1794.
- Suzuki, A. & Tanaka, T. (1990). Phase transition in polymer gels induced by visible light. *Nature*, 346, 345-347.
- Svenson, J. & Nicholls, I.A. (2001). On the thermal and chemical stability of molecularly imprinted polymers. *Analytica Chimica Acta*, 435, 19-24.
- Tanaka, M. (2007). An industrial and applied review of new MEMS devices features. *Microelectronic Engineering*, 84, 1341-1344.
- Tunc, Y.; Hasirci, N.; Yesilada, A. & Ulubayram, K. (2006). Comonomer effects on binding performances and morphology of acrylate-based imprinted polymers. *Polymer*, 47, 6931-6940.
- Turiel, E. & Martín-Esteban, A. (2004). Molecularly imprinted polymers: Towards highly selective stationary phases in liquid chromatography and capillary electrophoresis. *Analytical and Bioanalytical Chemistry*, 378, 1876-1886.
- Updike, S.J & Hicks, G.P. (1967). The enzyme electrode. *Nature*, 214, 986-988.
- Valero-Navarro, A.; Salinas-Castillo, A.; Fernández-Sánchez, J.F.; Segura-Carretero, A.; Mallavia, R. & Fernández-Gutiérrez, A. (2009). The development of a MIP-optosensor for the detection of monoamine naphthalenes in drinking water. *Biosensors and Bioelectronics*, 24, 2305-2311.
- Vandeveldel, F.; Leichlé, T.; Ayela, C.; Bergaud, C.; Nicu, L. & Haupt, K. (2007). Direct patterning of molecularly imprinted microdot arrays for sensors and biochips. *Langmuir* 23, 6490-6493.
- Vinjamuri, A.K.; Burns, S.C. & Dahl, D.B. (2008). Caffeine and Theobromine Selectivity Using Molecularly Imprinted Polypyrrole Modified Electrodes. *ECS Transactions*, 13, 9-20.
- Vlatakis, G. ; Andersson L.I.; Muller, R. & Mosbach K. (1993). Drug assay using antibody mimics made by molecular imprinting. *Nature*, 36, 645-647.
- Wang, W.; Gao, S.H. & Wang, B.H. (1999). Building fluorescent sensors by template polymerization: the preparation of a fluorescent sensor for d-fructose, *Organic Letters*, 1, 1209- 1212.
- Watanabe, M.; Akahoshi, T.; Tabata, Y. & Nakayama, D. (1998). Molecular specific swelling change of hydrogels in accordance with the concentration of guest molecules. *Journal of the American Chemical Society*, 120, 5577-5578.
- Wei, S.; Molinelli, A. & Mizaikoff, B. (2006). Molecularly imprinted micro and nanospheres for the selective recognition of 17 β -estradiol. *Biosensors and Bioelectronics*, 21, 1943-1951.
- Whitcombe, M.J.; Alexander, C. & Vulfson, E.N. (2000). Imprinted polymers: Versatile new tools in synthesis. *Synlett*, 911-923.
- Wu, Z.; Tao, C.-A.; Lin, C.; Shen, D. & Li, G. (2008). Label-free colorimetric detection of trace atrazine in aqueous solution by using molecularly imprinted photonic polymers. *Chemistry - A European Journal*, 14, pp. 11358-11368.
- Wulff, G. (1995). Molecular imprinting in cross-linked materials with the aid of molecular templates - A way towards artificial antibodies. *Angewandte Chemie - International Edition*, 34, 1812-1832.
- Wulff, G. (2002). Enzyme-like catalysis by molecularly imprinted polymers. *Chemical Reviews*, 102, 1-27.
- Xiang, H.Y. & Li, W.G. (2009). Electrochemical sensor for trans-resveratrol determination based on indium tin oxide electrode modified with molecularly imprinted self-assembled films. *Electroanalysis*, 21, 1207-1210.

- Xie, C.; Gao, S.; Guo, Q. & Xu, K. (2010) Electrochemical sensor for 2,4-dichlorophenoxy acetic acid using molecularly imprinted polypyrrole membrane as recognition element. *Microchimica Acta*, 169, 145-152.
- Xie, C.; Liu, B.; Wang, Z.; Gao, D.; Guan, G. & Zhang, Z. (2008). Molecular imprinting at walls of silica nanotubes for TNT recognition. *Analytical Chemistry*, 80, 437-443.
- Xie, C.; Zhang, Z.; Wang, D.; Guan, G.; Gao, D. & Liu, J. (2006). Surface molecular self-assembly strategy for TNT imprinting of polymer nanowire/nanotube arrays. *Analytical Chemistry*, 78, 8339-8346.
- Yao, S.Z.; Peng, H.; Liang, C.D.; Wu, Y. & Nie, L.H. (2000). Biomimetic bulk acoustic wave sensor for determination of trimethoprim in the organic phase based on a molecular imprinting polymer. *Analytical Sciences*, 16, 211 - 215.
- Ye, L. & Haupt, K. (2004) Molecularly imprinted polymers as antibody and receptor mimics for assays, sensors and drug discovery. *Analytical and Bioanalytical Chemistry*, 378, 1887-1897.
- Ye, L. & Mosbach, K. (2001b). Molecularly imprinted microspheres as antibody binding mimics. *Reactive and Functional Polymers*, 48, 149-157.
- Ye, L. & Mosbach, K. (2001c) Generation of new enzyme inhibitors using imprinted binding sites: The anti-idiotypic approach, a step toward the next generation of molecular imprinting. *Journal of American Chemical Society*, 123, 2901-2902.
- Ye, L. & Mosbach, K. (2001a). The technique of molecular imprinting - Principle, state of the art, and future aspects. *Journal of Inclusion Phenomena*, 41, 107-113.
- Ye, L. & Mosbach, K. (2008). Molecular imprinting: Synthetic materials as substitutes for biological antibodies and receptors. *Chemistry of Materials*, 20, 859-868.
- Ye, L.; Surugia, I. & Haupt, K. (2002). Scintillation proximity assay using molecularly imprinted polymers. *Analytical Chemistry*, 74, 959- 964.
- Yourtee, D.M.; Smith, R.E.; Russo, K.A.; Burmaster, S.; Cannon, J.M.; Eick, J.D. & Kostoryz, E.L. (2001). The stability of methacrylate biomaterials when enzyme challenged: Kinetic and systematic evaluations. *Journal of Biomedical Materials Research*, 57, 522-531.
- Yu, Y.; Ye, L.; Haupt, K. & Mosbach, K. (2002). Formation of a class of enzyme inhibitors (drugs), including a chiral compound, by using imprinted polymers or biomolecules as molecular-scale reaction vessels. *Angewandte Chemie - International Edition*, 41, 4459-4463.
- Yui, N.; Okano, T. & Skurai, Y. (1993). Photo-responsive degradation of heterogeneous hydrogels comprising crosslinked hyaluronic acid and lipid microspheres for temporal drug delivery. *Journal of Controlled Release*, 26, 141-145.
- Zhang, H.Q.; Ye, L. & Mosbach, K. (2006). Non-covalent molecular imprinting with emphasis on its application in separation and drug development. *Journal of Molecular Recognition*, 19, 248-259.
- Zhang, J.; Wang, Y.; Lv, R. & Xu, L. (2010a). Electrochemical tolazoline sensor based on gold nanoparticles and imprinted poly-o-aminothiophenol film. *Electrochimica Acta*, 55, 4039-4044.
- Zhang, Z.; Hu, Y.; Zhang, H. & Yao, S. (2010b). Novel layer-by-layer assembly molecularly imprinted sol-gel sensor for selective recognition of clindamycin based on Au electrode decorated by multi-wall carbon nano tube. *Journal of Colloid and Interface Science*, 344, 158-164.
- Zhou, Y.X.; Yu, B. & Levon, K. (2003). Potentiometric sensing of chiral amino acids. *Chemistry of Materials*, 15, 2774-2779.

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Biopolymers are polymers produced by living organisms. Cellulose, starch, chitin, proteins, peptides, DNA and RNA are all examples of biopolymers. This book comprehensively reviews and compiles information on biopolymers in 30 chapters. The book covers occurrence, synthesis, isolation and production, properties and applications, modification, and the relevant analysis methods to reveal the structures and properties of some biopolymers. This book will hopefully be of help to many scientists, physicians, pharmacists, engineers and other experts in a variety of disciplines, both academic and industrial. It may not only support research and development, but be suitable for teaching as well.

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