Molecularly Imprinted Polymers and Their Use in Biomimetic Sensors

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I. Introduction

Chemical sensors and biosensors are of increasing interest within the field of modern analytical chemistry, as can be seen both from the number of papers published and from the diversity of approaches and techniques applied. This is essentially due to new demands and opportunities that are appearing particularly in clinical diagnostics, environmental analysis, food analysis and production monitoring, as well as the detection of illicit drugs, genotoxicity, and chemical warfare agents. Another application area that has been opening up during the past few years is drug screening.

The central part of a chemical or biosensor is the recognition element, which is in close contact with an interrogative transducer. The recognition element is responsible for specifically recognizing and binding the target analyte in an often complex sample. The transducer then translates the chemical signal generated upon analyte binding or conversion into an easily quantifiable output signal. Biosensors rely on biological entities such as antibodies, enzymes, receptors, or whole cells as the recognition elements. With the advent of recombinant antibodies[1] and phage display antibody libraries,[2] a suitable recognition element can now be found even for analytes for which a natural receptor does not exist. There have also been numerous attempts to replace natural receptors

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Karsten Haupt was born in Annaberg-Buchholz, Germany, in 1964. He received his diploma (M.S.) in Biochemistry from the University of Leipzig, Germany, in 1991. He then worked for three years as a graduate student at the University of Technology of Compiègne, France, where he received his Ph.D. degree in Bioengineering in 1994. After a one-year lecturership at Compiègne University, a three-year postdoctoral position at Lund University, Sweden (with Klaus Mosbach), and a one-year research contract at INSERM, Paris, France, he is currently holding a position as a research lecturer at the University of Paris 12, as well as a position as a guest lecturer at Lund University. His present research interests include affinity technology, bioseparation, chemical and biosensors, molecular imprinting, and biomimetic polymers.

Klaus Mosbach began his university studies at Lund University, Sweden, in 1953. After receiving his Ph.D. degree in Biochemistry, he has been a postdoctoral fellow and visiting professor in several laboratories. In 1970 he was appointed Professor in Biochemistry at Lund University, where he founded the Department of Pure and Applied Biochemistry at the Center for Chemistry and Chemical Engineering. He was until recently the Head of the department and is now Director of the Center for Molecular Imprinting and Recognition at the same university. Klaus Mosbach has also held a professorship in biotechnology at the Swiss Federal Institute of Technology, Zurich. He received numerous international prices for his achievements in the areas of Biotechnology and Biochemistry, for example, the prize in Enzyme Technology from the Engineering Foundation, New York, and the first award by the International Organization of Affinity Chromatography and Biorecognition. He has published about 450 original articles and filed approximately 60 patents. His major research interests presently lie in the areas of nanosensing, molecular imprinting, and biomimetic polymers.

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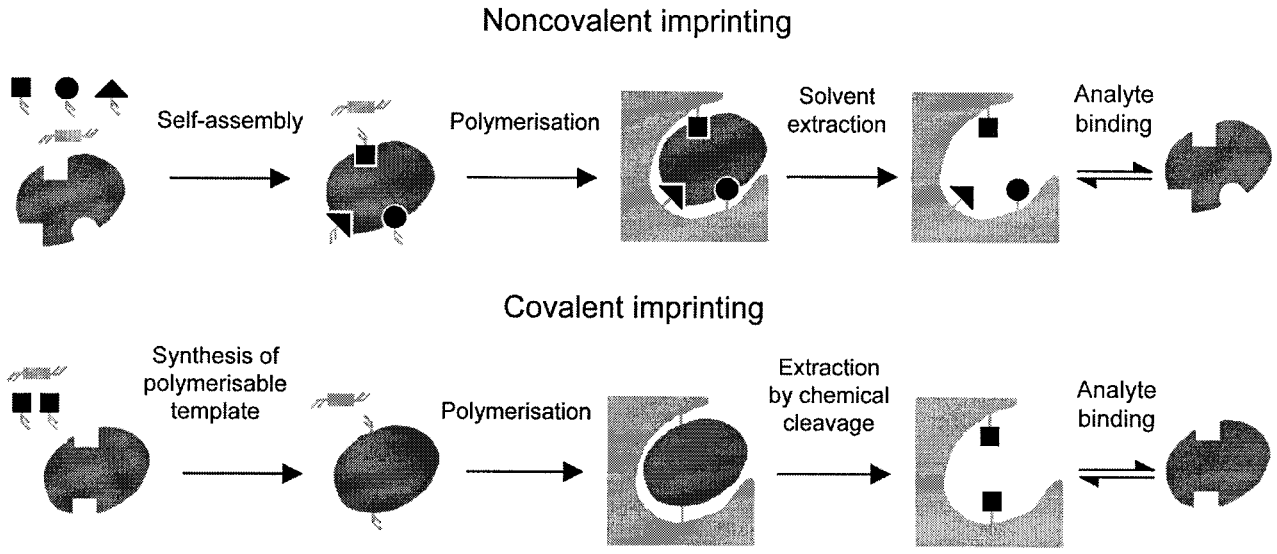
Scheme 1. Schematic Representation of the Covalent and Noncovalent Molecular Imprinting Procedures



with smaller, more stable counterparts. This has led, for instance, to the development of bioengineered antibody fragments such as single-chain variable fragments (ScFv), which have already been used in biosensors.[3] Other small protein domains, such as an α-helical domain derived from staphylococcal protein A ('affibodies'),[4] have also been used for specific binding of target proteins, after selection from phage display libraries. Another new type of semisynthetic receptor for biosensors are nucleic acids and peptide nucleic acids.[5,6] Unfortunately, the, in general, poor chemical and physical stability of biomolecules sometimes prevents their use in harsh environments, although in principle they are very attractive for the design of biosensors for continuous process or environmental monitoring.

An alternative approach involves the use of biomimetic receptor systems capable of binding target molecules with affinities and specificities on a par with natural receptors. Whereas for small target molecules, such as inorganic ions, artificial receptors can often be obtained through rational design and chemical synthesis,[7] this may prove difficult if the analyte is a larger and somewhat more complex molecule. In this case, other techniques might be preferred, for example, the design of biomimetic ligands for proteins using combinatorial chemistry[8] or the creation of tailor-made receptors by templating with the target analyte. One technique that is being increasingly adopted is molecular imprinting in synthetic polymers. The binding sites that are generated during the imprinting process often have affinities and selectivities approaching those of antibody−antigen systems, and molecularly imprinted materials have therefore been dubbed 'antibody mimics'.[9] These mimics display some clear advantages over real antibodies for sensor technology: due to their highly cross-linked polymeric nature, they are intrinsically stable and robust, facilitating their application in extreme environments, such as in the presence of acids or bases, in organic solvents, or at high temperatures and pressures. Moreover, these materials are cheap to produce and can be stored in the dry state at room temperature for long periods of time. Even though the great potential of this technology has been recognized only recently, in particular after the introduction of synthetic organic polymers as imprinting matrices, there is now a strong development toward the use of molecularly imprinted polymers (MIPs) as recognition elements in sensors.

This review focuses on recent advances and developments in the molecular imprinting area, with special emphasis on the application of molecularly imprinted polymers in sensors. For a more detailed presentation of the technology itself, the reader is directed to some general reviews on molecular imprinting that have appeared over the past few years.[10−16]

II. Molecular Imprinting Technology

A. General Principle

Molecular imprinting of synthetic polymers is a process where functional and cross-linking monomers are co-polymerized in the presence of the target analyte (the imprint molecule), which acts as a molecular template. The functional monomers initially form a complex with the imprint molecule, and following polymerization, their functional groups are held in position by the highly cross-linked polymeric structure. Subsequent removal of the imprint molecule reveals binding sites that are complementary in size and shape to the analyte. In that way, a molecular memory is introduced into the polymer, which is now capable of rebinding the analyte with a very high specificity.

There are two distinct approaches to molecular imprinting which are depicted in Scheme 1. A prepolymerization complex between imprint molecule and functional monomers can be formed via noncovalent interactions (self-assembly). Alternatively, monomers can be covalently coupled to the imprint molecule, that is, a polymerizable derivative of the imprint molecule is synthesized. Owing to the greater stability of covalent bonds, covalent imprinting protocols should conceivably yield a more homogeneous population of binding sites, and indeed, there have

been reports suggesting that this might be the case.[17] Moreover, the yield in binding sites relative to the amount of imprint molecule used (imprinting efficiency) should be higher than with noncovalent protocols. This approach has been developed primarily by Wulff and co-workers.[11] On the other hand, the noncovalent imprinting approach, which has been pioneered by Mosbach and co-workers,[14] is more flexible concerning the choice of functional monomers, possible target molecules, and the use of the imprinted materials. Moreover, it is more similar to natural processes in the sense that most biomolecular interactions are noncovalent in nature. Hybrid protocols have also been suggested that try to combine the advantages of both covalent and noncovalent imprinting. For example, a tripeptide (Lys-Trp-Asp) has been imprinted using both covalent and noncovalent interactions. During rebinding, the peptide interacts with the polymer only via noncovalent interactions (Figure 1).[18]

B. Physical Forms of Imprinted Polymers

Molecularly imprinted polymers can be prepared in a variety of physical forms to suit the final application desired. The majority of these are based on organic polymers synthesized by radical polymerization from functional and cross-linking monomers having vinyl or acrylic groups, although other organic polymers and silica have also been used. One reason for the "popularity" of vinyl and acrylic polymers for imprinting is the vast choice of functional monomers available. These can be positively or negatively charged, hydrogen bonding, hydrophobic, metal coordinating, etc. More sophisticated monomers have recently started to appear with specialized functions related to analyte detection, as discussed below. In most cases, the polymerization mixture contains an inert solvent, which is required not only to dissolve all ingredients and, in particular, the imprint molecule but also to generate a highly porous structure that allows for the elution of the imprint molecule and for the analyte to access of the imprinted sites. However, it has been demonstrated that if the imprint molecule is immobilized onto a solid support prior to polymerization, a solvent is not needed.[19]

A common method for preparing MIPs is via solution polymerization followed by mechanical grinding of the monolithic block generated to give small particles with diameters usually in the micrometer range. As an alternative, particles can be prepared directly in the form of spherical beads of controlled diameter.[20−22] In two-phase systems, the use of liquid perfluorocarbons instead of water as the continuous phase might be preferred since water may have a detrimental effect on the noncovalent complex between monomers and imprint molecule.[22] Beads synthesized in this way can be rendered magnetic through inclusion of iron oxide particles.[23] A rather simple method for the preparation of imprinted supports not requiring mechanical grinding is dispersion polymerization, which yields aggregates of spherical particles[24] or, if the system is sufficiently dilute, uniformly sized microspheres.[25] For some applications, the polymerization can be performed in situ,

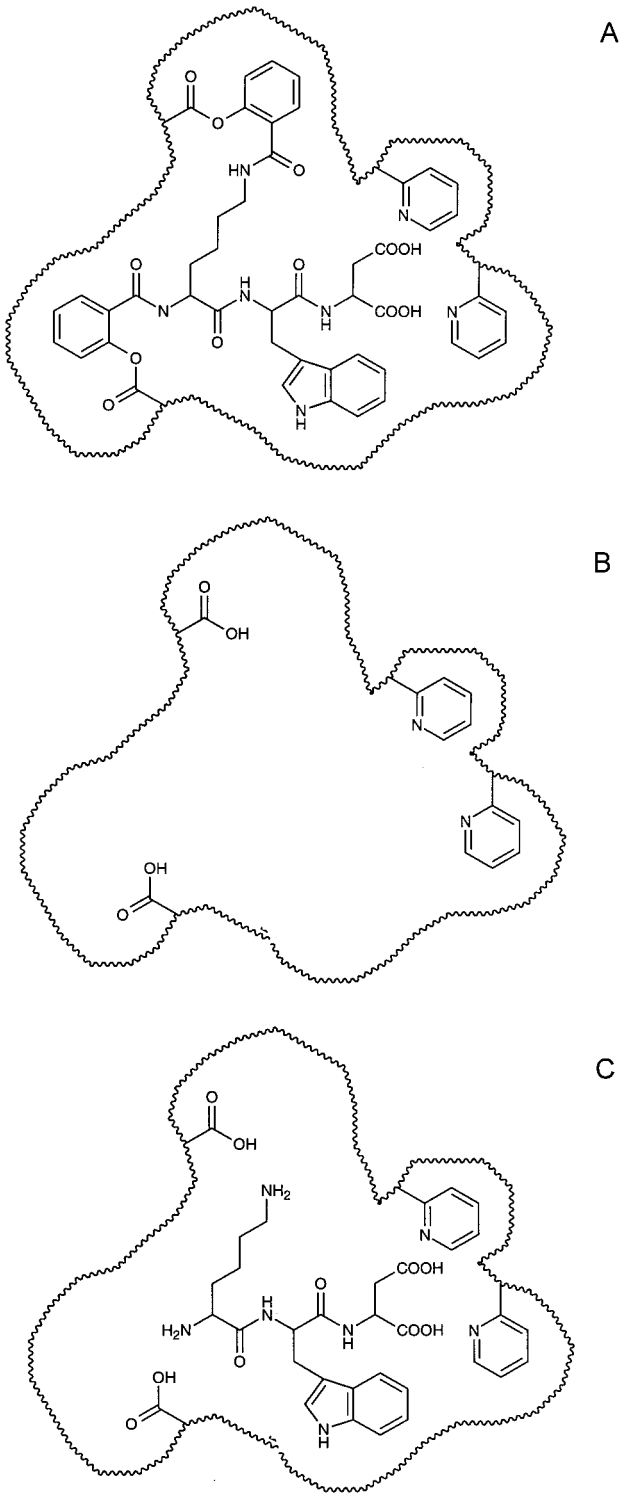


Figure 1. Molecular imprinting of the tripeptide Lys-Trp-Asp using both covalent and noncovalent interactions: (a) binding site with covalently bound imprint molecule; (b) binding site after chemical cleavage and extraction of the imprint molecule; (c) rebinding of the imprint molecule via only noncovalent interactions. (Adapted with permission from ref 18. Copyright 1999 Wiley-VCH.)

for example directly inside a chromatography column[24,26] or in a capillary.[27] One final format, which is particularly suitable for sensor applications, involves imprinted membranes. These can be prepared as thin cross-linked imprinted polymer films,[28,29] by precipitation from solutions of linear polymers in the

presence of the analyte[30] or by casting an imprinted polymer in the pores of an inert support membrane.[31,32]

C. Target Molecules and Applications

One of the many attractive features of the molecular imprinting method is that it can be applied to a diverse range of analytes. The imprinting of small, organic molecules (e.g., pharmaceuticals, pesticides, amino acids and peptides, nucleotide bases, steroids, and sugars) is now well established and considered almost routine. Somewhat larger organic compounds (e.g., peptides) can also be imprinted via similar approaches, whereas the imprinting of much larger structures is still a challenge, although specially adapted protocols have been proposed for, e.g., proteins,[33−35] cells,[36] and even mineral crystals.[37] It has also been shown that the porogenic solvent itself can introduce some kind of molecular memory into the polymer,[38,39] which can be regarded as a molecular imprinting process in a wider sense. The same is true for metal and other ions, which have been used as templates to induce the specific arrangement of functional groups in organic polymers[40−42] and silica.[43,44]

To obtain an optimized polymer for a given target analyte, combinatorial approaches to MIP synthesis have been developed[45,46] where the ingredients of the imprinting recipe, in particular the kind and molar ratio of the functional monomers, are varied using automated procedures.

Originally, MIPs were employed as stationary phases in HPLC, notably for chiral separation.[47−49] Subsequently, their use has been extended to other analytical techniques including thin-layer chromatography,[50] capillary electrochromatography,[27] solid-phase extraction,[51] immunoassay-type binding assays,[9] and chemical sensors. The use of molecularly imprinted artificial receptors for the screening of combinatorial libraries has also been suggested.[52]

D. Related Techniques

There are of course close relations and overlaps between the molecular imprinting technique and other techniques operating at or close to the molecular level. Apart from micro- and nanofabrication methods[53] and template-assisted polymer synthesis,[54] the imprinting of biomolecules should be mentioned here. It has been shown that the substrate specificity of enzymes can be altered by precipitating or freeze-drying the enzyme in the presence of something other than its natural substrate.[55] In a similar way, specific binding sites for certain small molecules[56] or even catalytic activity[57,58] has been introduced into proteins.

Also noteworthy and of relevance for sensor technology is the templating of self-assembled monolayers (SAMs),[59−63] which can be regarded as two-dimensional molecular imprinting. For example, when a SAM of alkane thiols is formed on a gold surface, the presence of a foreign molecule results in a hole in the SAM, which is complementary in size with the guest molecule, thus forming a binding site (Figure 2).

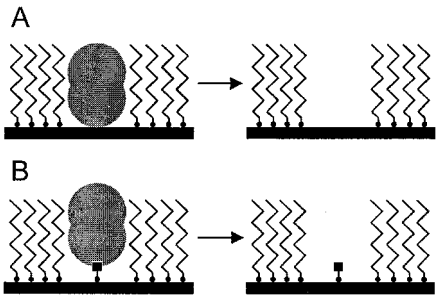


Figure 2. Schematic representation of the two-dimensional imprinting of a self-assembled monolayer without (A) and with (B) creation of a specific attachment point for the analyte in the binding site.

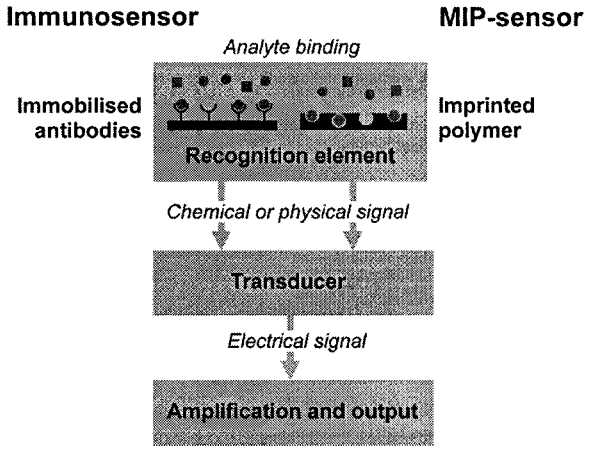


Figure 3. Schematic representation of a MIP-based biomimetic sensor compared to an immunosensor.

III. Molecularly Imprinted Polymers in Sensors

A. General Considerations

In biosensors, a signal is generated upon the binding of the analyte to the recognition element. The transducer then translates this signal into a quantifiable (in most cases electrical) output signal. The same general principle applies if a MIP is used as the recognition element instead of a biomolecule (Figure 3). Certain general properties of the analyte, or changes in one or more physicochemical parameters of the system upon analyte binding, are used for detection. This principle is widely applicable and more or less independent of the nature of the analyte. Alternatively, reporter groups may be incorporated into the polymer to generate or enhance the sensor response. In other cases, the analyte may possess a specific property that can be used for the design of a MIP-based sensor. Table 1 summarizes different transducer types that have been or that could conceivably be used with MIPs and are described in more detail in the following section.

B. Transducers

1. General Detection Principles

Early attempts to utilize the recognition properties of MIPs for chemical sensing were, for example, ellipsometric measurements on thin Vitamin K_1-imprinted polymer layers,[64] the measurement of changes in the electrical streaming potential over an

Table 1. Examples of Transducers Employed in Imprinted Polymer-Based Sensors

transducer	analyte (example)	useful range (μM)	ref
General Formats			
ellipsometry	vitamin K_1	qualitative	64
surface plasmon resonance	theophylline	5000−33000	77
capacitance	phenylalanine anilide	qualitative	67
	phenylalanine	6000	68
conductometry	atrazine	0.005−0.05	80
surface acoustic wave	solvent vapors	(0.1 μL/L)	39
quartz crystal microbalance	solvent vapors	(4 μL/L)	39
	glucose	1000−20000	73
	S-propranolol	50−1300	72
love-wave	2-methoxy 3-methylpyrazine	n.c.	70
infrared evanescent wave	2,4-D	4.5−1000	97
Analyte Generates Signal			
fluorescence	dansyl phenylalanine	25−250	82
	PAH (pyrene)	0.00015−0.2	69
amperometry	morphine	3.5−35	81
Competitive Binding Formats			
colorimetry	chloramphenicol	10−3000	87
voltammetry	2,4-D	0.1−100	88
Polymer Generates Signal			
pH	glucose	1000−25000	90
fluorescence	cAMP	0.1−100	92

HPLC column packed with a MIP,[65] or permeability studies of imprinted polymer membranes.[66] The first reported *integrated* sensor based on a MIP[67] was a capacitance sensor. The device consisted of a field-effect capacitor containing a thin phenylalanine anilide-imprinted polymer membrane. Binding of this model analyte resulted in a change in capacitance of the device, thus allowing for the detection of the analyte in a qualitative manner. More recently, capacitive detection was employed by others in conjunction with imprinted electropolymerized polyphenol layers on gold electrodes.[68]

During the last 3 years, mass-sensitive acoustic transducers, such as the surface-acoustic wave (SAW) oscillator,[39,69] the Love-wave oscillator,[70] or the quartz crystal microbalance (QCM),[39,69,71−75] have become increasingly popular for the design of MIP-based sensors. For example, polymers of the polyurethane type have been synthesized at the surface of SAW and QCM oscillators in the presence of a certain organic solvent.[39] The polymer films subsequently showed a preferential uptake of the "imprinting" solvent over other solvents. This uptake could be quantified by piezoelectric microgravimetry, that is, via the change in oscillation frequency resulting from the mass change at the oscillator surface. A QCM has been used by another group to construct an imprinted polymer-based sensor for glucose.[73] The polymer, poly(*o*-phenylenediamine), was electrosynthesized directly at the sensor surface in the presence of 20 mM glucose. In that way, a very thin (10 nm) polymer layer was obtained that could rebind glucose with certain selectivity over other compounds such as ascorbic acid, paracetamol, cysteine, and to some extent fructose. However, only millimolar concentrations of the analyte could be measured. Others have relied on common acrylic polymers for the design of MIP-based QCM sensors.[72,74−76] With such polymers, it has been demonstrated that the sensor selectivities are similar to those obtained in other applications of acrylic MIPs. For example, a QCM sensor coated with an *S*-propranolol-imprinted polymer was able to discriminate between the *R*- and *S*-enantiomers of the drug with a selectivity coefficient of 5.[72] Measurements with acoustic sensors have been performed both in solution[69,72,73,75] and in the gas phase.[39,70,74]

An alternative way of detecting mass accumulation at a surface is by optical means, such as ellipsometry or surface plasmon resonance. Indeed, the use of these detection principles in combination with MIPs has been reported.[64,77]

Other sensors have been designed based on conductometric transducers.[29,78,79] Here, two electrodes are separated by an imprinted polymer, often in the form of a membrane. Binding of the analyte to the polymer changes its conductivity, which is translated into an electrical signal. A sensing device for the herbicide atrazine which is based on conductometric measurements on a free-standing atrazine-imprinted acrylic polymer membrane has recently been constructed.[80] The authors carefully optimized the polymer recipe, in particular with respect to the kind and molar ratio of cross-linking monomers used, and the relative amount of porogenic solvent in the imprinting mixture. This turned out to be an important factor not only in obtaining flexible and stable membranes, but also because the conductometric response seemed to depend on the ability of the MIP to change its conformation upon analyte binding. Attractive features of this sensor were the comparatively short time required for one measurement (6−10 min), its rather low detection limit of 5 nM, and its high selectivity for atrazine over structurally related triazine herbicides.

2. Analyte Generates the Signal

If the target analyte exhibits a special property such as fluorescence or electrochemical activity, this can be exploited for the design of MIP-based sensors. For example, an amperometric morphine sensor was developed where the analyte morphine was selec-

tively enriched on a MIP and subsequently quantified by electrooxidation.[81]

Optical sensors for the detection of fluorescent analytes belong to the same group. As a model system, a fluorescence sensor for dansyl−phenylalanine has been constructed.[82] The fluorescence of the MIP after analyte binding was measured using fiber optics, and the signal was found to be a function of the analyte concentration. Moreover, the sensor showed a certain degree of stereoselectivity for the L-form of the analyte, which was the original imprint molecule. Others have used polyurethanes imprinted with different polycyclic aromatic hydrocarbons (PAHs) in conjunction with fluorescence measurements in a flow system.[69] Optimum binding could be directed to a specific PAH of interest, which was not necessarily the template PAH. The sensitivity of the system for PAH detection in water, obtained using a fluorescence spectrometer, was rather high (ppt range) owing to a large enrichment factor of the polymer (10^7).

A potential problem that can arise when a special property of the analyte, such as fluorescence is used for detection, is that traces of the imprint molecule can remain entrapped in the polymer, which may cause a high background signal resulting in decreased sensitivity. A remedy could be to imprint the polymer with a structurally closely related but nonfluorescent analyte analogue. This has also been suggested for MIP-based solid-phase extraction matrices, where residual template leakage can be a major problem.[83]

3. Competitive Measurements

If the analyte itself does not exhibit a suitable property that can be used for detection, a competitive or displacement sensor format may be used. A labeled analyte derivative is allowed to compete with the analyte for the binding sites in the MIP, or the labeled analyte is allowed to bind first and is subsequently displaced upon binding of the analyte. Since many analytes cannot be labeled easily, it may be preferable to use nonrelated probes for detection.[84−86] These can be conceived as compounds that can bind to some extent to the imprinted sites in the polymer, without being functionally related to the target analyte. Such compounds may need to have at least some degree of structural similarity with the analyte. The selectivity of sensors based on such competitive formats is not jeopardized since selectivity is determined by the specificity of analyte (the original imprint molecule) binding to the imprinted sites.

For example, a chromatography-based sensing device for chloramphenicol has been designed[87] by circulating a chloramphenicol−methyl red conjugate through a chloramphenicol-imprinted polymer column which was mounted in an HPLC system. The conjugate adsorbed to the imprinted sites (and probably to nonspecific sites too), and upon injection of the analyte, some of the conjugate was displaced, thus generating a peak in the UV monitor. The peak area was related to the analyte concentration, and it was possible to quantify chloramphenicol in blood serum after solvent extraction, thereby covering the therapeutically relevant range (10−20 μg/mL). In another application, a voltammetric sensor for the herbicide 2,4-D was reported[88] where the electroactive compound 2,5-dihydroxyphenylacetic acid was used as a probe instead of the labeled analyte. MIP particles were coated as a thin layer onto a screen-printed carbon electrode and incubated with the sample to which the probe was added. In the presence of the analyte, some of the probe was competed out of the imprinted sites whereas the remaining probe was directly quantified by differential pulse voltammetric measurements (Figure 4).

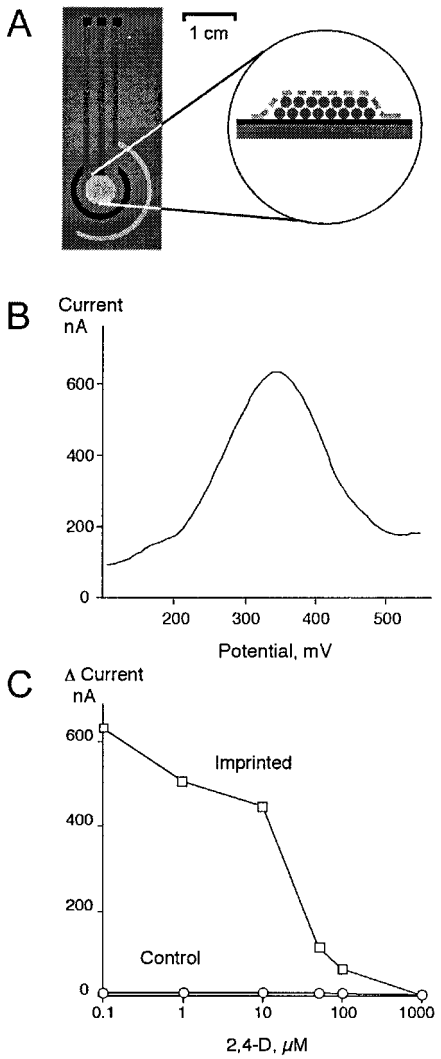


Figure 4. (a) Disposable sensor element based on a screen-printed carbon electrode. The MIP is coated onto the carbon working electrode (middle) which is surrounded by a carbon counter electrode (small arc) and a Ag/AgCl reference electrode (large arc). (b) Differential pulse voltammetric scan of homogentisic acid which is used as an electroactive probe. (c) Calibration curve for 2,4-D, with the imprinted polymer (□) and the control polymer (○). (Adapted with permission from ref 88. Copyright 1999 American Chemical Society.)

4. Polymer Generates the Signal

An attractive design of the recognition element/transducer couple is to have the signal generated by the polymer itself. This is somewhat analogous with a recent trend in biosensor development which involves the incorporation of the recognition element

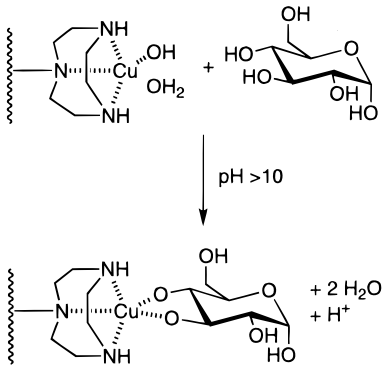


Figure 5. Analyte binding site in a glucose-sensing polymer. Upon coordination of the metal chelate by glucose, a proton is released.[90]

into the bulk of a composite matrix.[89] One example for such a format is a glucose-sensing polymer that works in ligand exchange mode.[90] A complex of a polymerizable copper chelate and methylglucoside was used during preparation of the polymer. Extraction of copper and methylglucoside from the polymer and subsequent reloading with copper yielded the active form of the polymer. Addition of the analyte glucose resulted in its coordination to the metal accompanied by proton release (Figure 5), which was a function of analyte concentration and could be quantified by simple pH measurements. Since a polymer prepared in the presence of ethylene glycol instead of methylglucoside released only one-half as many protons upon analyte binding, the authors suggested that the templating with methylglucoside might have increased the specificity of the polymer for glucose. They also demonstrated that the polymer could be used to measure glucose in blood plasma, although with a slightly reduced response compared to measurements in a pure saline solution.

Optical sensing systems have recently been described where fluorescent reporter groups are incorporated into the MIP, the properties of which are altered upon analyte binding.[91−93] For example, a fluorescent functional monomer, *trans*-4-[*p*-(*N*,*N*-dimethylamino)styryl]-*N*-vinylbenzylpyridinium chloride, has been used together with a conventional functional monomer to prepare a polymer imprinted with cyclic adenosine monophosphate (Figure 6).[92] Upon binding to the imprinted sites, the analyte interacts with the fluorescent groups and their fluorescence is quenched, thus allowing the analyte to be quantified. Others have used a similar system with a metalloporphyrin as the reporter group, of which a polymerizable derivative was used as the functional monomer.[94] Binding of the analyte 9-ethyladenine then resulted in a shift in the visible absorption spectrum of the polymer. Although the above detection methods have not yet been applied to construct integrated sensors, they appear promising since they do not depend on a special property of the analyte and, moreover, should facilitate the integration and production of the sensing device.

A very sensitive sensor for a hydrolysis product of the chemical warfare agent Soman has been described based on a polymer-coated fiber optic probe and a luminescent europium complex for detection.[95]

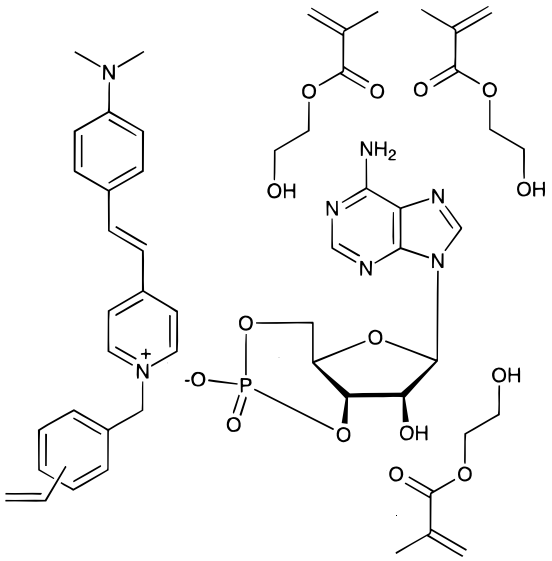


Figure 6. Molecular imprinting using a fluorescent reporter group. Schematic representation of the hypothetical prepolymerization complex formed by the imprint molecule cAMP, the signaling monomer *trans*-4-[*p*-(*N*,*N*-dimethylamino)styryl]-*N*-vinylbenzylpyridinium chloride, and the functional monomer 2-hydroxyethyl methacrylate.[92]

The complex of europium ligated by divinylmethyl benzoate (ligating monomer) and by the analyte pinacoyl methylphosphonate was co-polymerized with styrene, whereafter the analyte molecule was removed by washing. Rebinding of the analyte was quantified from laser-excited luminescence spectra. Although it is not clear whether imprinting has contributed to the selectivity of the sensor, this detection principle appears very promising, taking into account the very low detection limits that can be obtained (7 ppt in this particular case).

C. Interfacing the MIP with the Transducer

An important aspect in the design of a MIP-based sensor is to find an appropriate way of interfacing the polymer with the transducer. In most cases, the MIP has to be brought into close contact with the transducer surface. An obvious advantage would be to integrate this step in an automated production process. Thereby, the polymer can either be synthesized in situ at the transducer surface or the surface can be coated with a preformed polymer.

In situ synthesis of a polymer can be done by electropolymerization on conducting surfaces such as gold.[68,73] This is convenient but requires specialized polymer recipes that are, at least to date, less thoroughly studied than the common acrylic and vinyl polymers with respect to the possibility of being molecularly imprinted. More generally applicable are standard surface coating techniques such as spin coating and spray coating, which have both been used to apply a thin film of monomer solution to acoustic transducer surfaces.[39,70] With these two techniques, thin and even polymer layers can be produced, although if radically polymerized vinyl or acrylic systems are used, the coating has to be done under oxygen-free conditions, due to the radical scavenging effect of oxygen which would inhibit polymerization. A rather simple way to synthesize a polymer layer

on a flat surface is to use a sandwich technique. The imprinting solution is cast between the transducer surface and another flat surface such as a glass or quartz disk, whereafter the polymerization is initiated.[72,76]

Preformed polymers, for example, in the form of nanometer- or micrometer-sized particles, can be interfaced with the transducer in different ways. It has been suggested to entrap MIP-particles into gels[81] or behind a membrane[82,88] for use with electrochemical transducers. Others have spin-coated a suspension of MIP particles in a solution of an inert, soluble polymer (PVC), which served as glue, onto an acoustic transducer surface.[75] Among the potential problems that can arise when using these approaches are diffusion limitations resulting in long response times of the sensor, nonspecific analyte binding, or a decrease in binding capacity.

Imprinted polymers exhibiting, at the same time, electrical conductivity facilitate their assembly with an electrochemical transducer in an integrated device. In this context, the preparation of composite particles consisting of an electrically conducting polymer (polypyrrole) and an acrylic MIP should be mentioned.[96] The polypyrrole which was grown into the preformed porous MIP did not alter its recognition properties; however, in this way MIP particles could be mechanically and electrically connected to a gold-covered silica substrate.

D. Outlook

The signals generated by most of the above-mentioned transducer types are two-dimensional and provide only limited information about the composition of the sample. Although this is normally compensated by the high selectivity of MIPs, a different strategy could conceivably be the use of "intelligent" transducer mechanisms, which generate signals with a higher inherent information content. One way to achieve that is to exploit the high molecular specificity of absorption spectra in the mid-infrared spectral region (3500−500 cm^{-1}). The combination of MIPs and FTIR spectrometry might allow analytical problems to be addressed where the selectivity of the MIP alone is not sufficient, e.g., when samples with complex matrices are to be investigated or when structurally very similar analytes are present in the sample. A recent report described an approach toward a chemical sensor based on an imprinted polymer and infrared evanescent-wave spectroscopy.[97] A polymer molecularly imprinted with the herbicide 2,4-D was coated in the form of a thin film onto a ZnSe attenuated total reflection element, which was mounted in a flow cell. Accumulation of 2,4-D in the MIP layer could be followed on-line and in real time by FTIR spectrophotometric measurements. Analyte binding was concentration dependent and could be quantified by integrating characteristic analyte bands.

In some biosensors, enzymes are involved as the recognition element and/or for the generation or amplification of the signal. Such sensors are often superior to sensors in which the signal is only due to the binding event itself. Analyte conversion and turnover result in an increased sensitivity and lower interference by nonspecific binding. There are some obvious parallels to MIP-based sensors, that is, with catalytic MIPs, a similar approach could conceivably be used. In an early attempt to create MIPs with catalytic activity, imprints of a substrate analogue were made and the resulting polymer was able to hydrolyze the *p*-nitrophenyl ester of an amino acid.[98] Since then, several reports on catalytic MIPs using substrate, transition state, or product analogues as the imprint molecule have followed.[99−101] Unfortunately, the rate enhancements which have been achieved so far are still modest compared to enzymes or even catalytic antibodies and the application of catalytic MIPs in sensor technology will depend on further improvement of their performance.

An important aspect in the development of sensor technology is the need for mass-produced and low-cost disposable transducers.[102] This is especially relevant for environmental and biomedical analysis. As an example, for electrochemical sensors, screen-printed electrodes fulfill this need. The ease of preparation and low cost of MIPs make them attractive as recognition elements for such devices. A first report on this topic recently demonstrated that an imprinted polymer could be coated onto screen-printed carbon electrodes, and the resulting devices could be used to measure an analyte in a competitive format (Figure 4).[88]

A significant trend in the biosensor field goes toward miniaturization[103] and the development of multisensor arrays.[104] Certain transducers based on, for example, electrochemical, capacitance, or optical detection would allow for the preparation of array structures containing several MIPs with different specificities. In consequence, the appearance of microprocessor-controlled multisensing devices that detect multiple analytes simultaneously and that allow for pattern recognition can be expected soon.

IV. Conclusions

In terms of sensitivity, MIP-based biomimetic sensors are, with some honorable exceptions (see Table 1), still somewhat inferior to biosensors. This situation will certainly improve through further optimization of the MIPs and the transducers. In particular, what one hopes to achieve is the development of MIPs that contain a more homogeneous binding site population, have a higher affinity for the target analyte, and can be used in aqueous solvents. A considerable part of the current research efforts on MIPs already deals with these problems. In fact, some of the above-mentioned detection methods are, apart from their use in sensors, equally well suited for investigating the recognition of analytes by MIPs at the molecular level. On the other hand, the outstanding stability of MIPs, their low price, as well as the fact that they can be tailor-made for analytes for which a biological receptor cannot be found are among the properties that make them especially suitable for sensor applications. It appears that the development of imprinted polymer-based sensors is just about to leave the proof-of-principle stage, and researchers are starting to address specific analytical problems and to measure real samples. Fortunately,

national and international funding agencies such as the European Commission have recognized the potential of MIPs for analytical chemistry, and several large research projects aimed at demonstrating the validity and practical usefulness of MIP-based analytical methods and devices are currently under way. So, where will the development go—MIP sensors or biosensors? For the time being this is perhaps not the right question to ask. MIP sensors or enzyme-based sensors or immunosensors or receptor-based sensors, or DNA-sensors... It is this decision which will have to be made to resolve a specific detection or monitoring problem, and MIPs will thereby undoubtedly find their niches of application.

V. References

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