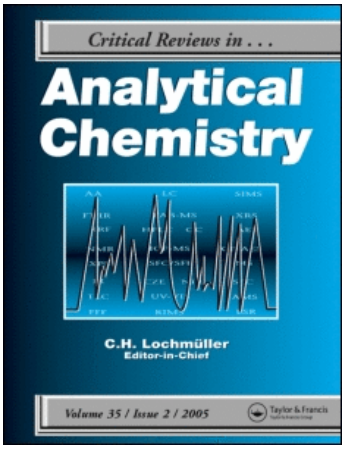


Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Molecularly Imprinted Polymers and Optical Sensing Applications

Salma Al-Kindy[a]; Rosana Badía[b]; Jorge Luis Suárez-Rodríguez[b]; Marta Elena Díaz-García[b]
[a] Qaboos Sultan University, Oman [b] Department of Physical and Analytical Chemistry, University of Oviedo, Oviedo, Spain

To cite this Article Al-Kindy, Salma , Badía, Rosana , Suárez-Rodríguez, Jorge Luis and Díaz-García, Marta Elena(2000) 'Molecularly Imprinted Polymers and Optical Sensing Applications', Critical Reviews in Analytical Chemistry, 30: 4, 291 — 309
To link to this Article: DOI: 10.1080/10408340008984162
URL: http://dx.doi.org/10.1080/10408340008984162

Critical Reviews in Analytical Chemistry, 30(4):291–309 (2000)

Molecularly Imprinted Polymers and Optical Sensing Applications

Salma Al-Kindy,[1] Rosana Badía,[2] Jorge Luis Suárez-Rodríguez,[2] and Marta Elena Díaz-García[2]*

[1]Qaboos Sultan University, Oman; [2]Department of Physical and Analytical Chemistry, University of Oviedo; C/ Julian Clavería, 8, Oviedo, 33006, Spain; e-mail: medg@sauron.quimica.uniovi.es

ABSTRACT: The development of optical sensing approaches for analytes of biological, industrial, or environmental concern has reached an enormous significance. Different natural systems such as enzymes or antibodies have been employed in the development of these kind of sensors, but they often lack of stability. Molecular imprinting has become a universal tool for preparation of artificial and robust recognition materials mimicking natural systems. Molecular imprinting polymers (MIPs) are easily obtained by copolymerization of suitable functional monomers and crosslinkers in the presence of the target molecule. These polymers exhibit a tremendous thermal, chemical, and mechanical stability besides having selectivity similar to that of natural systems. Here we report an overview of molecular imprinting technique focussed to its sensing applications, emphasizing those systems based on an optical transduction.

KEY WORDS: molecular imprinting, imprinted polymers, molecular recognition, sensor, optosensing.

I. INTRODUCTION

A problem of paramount importance in analytical chemistry is selectivity, particularly at low analyte concentrations in the presence of interfering substances. The sensitive and selective determination of a large number of trace compounds is of great relevance in many fields, such as the food industry, biotechnology, environment, and health care for the diagnosis of disease.

Living beings are capable of recognizing chemical changes in their metabolic states and in their environment with high selectivity and sensitivity by using receptors. These interactions rely on selective binding between the receptors and their ligands and are based on the exquisite recognition properties such as those exhibited by antibodies, which can differentiate between their specific antigens and other structurally related compounds.

The outstanding specificity and sensitivity of these biological receptors make them highly attractive for the development of sensors, as a result of which biosensors attracted lot of attention. However, devices that rely on biological molecular recognition elements often lack storage and operational stability and are accompanied by difficulties in generating the affinity sensing phase and the specificity toward the desired target molecule. Due to these limitations, biosensors have not become quite the commercial source expected in the early euphoric development phase.

The studies of the complex interactions between molecular species, the molecular recognition process, and the ability to mimic natural binding phenomena have intrigued scientists over a long period. These studies have led to the establishment of biomimetic chemistry in which

1040-8347/00/$.50

imitations of natural binding entities, such as enzymes, antibodies and receptors, are being studied. As the structure and mechanisms of biochemical systems become known, scientists are attempting to transfer this knowledge to synthetic strategy. The aim in the design of these artificial receptors is the development of systems capable of mimicking the molecular level selectivities observed in nature by constructing host systems possessing steric and electronic configurations complementary to those of the ligands. Extremely high selectivity is obtained if, as in nature, a cavity exits with both an adequate shape to match that of the ligand and with binding sites in a spatial arrangement, so that the ligand is bound inside the cavity. Small molecule receptor surrogates such as cyclodextrines, cyclophanes, crown-ethers, and calixarenes[1-3] have been studied actively in connection with molecular recognition.

Another approach to create receptor-like binding sites is molecular imprinting. The concept of molecular imprinting emerged by Pauling's antibody formation theory in which an antigen is used as a template to mold the antibody polypeptide chains, so that it gets a configuration that complements the antigen molecule.[4] Although Pauling's speculation was abandoned in favor of the more appropriate "clonal selective" theory of antibody formation, chemists found useful the idea of forming a three-dimensional structure around a template for preparing synthetic analogues of antibodies that can recognize target molecules with selectivities similar to their biological counterparts. Hence, these models laid the foundations for molecular imprinting.

In this review the preparations of molecular imprinted polymers (MIPs) and their potential in optical sensing approaches are presented. Emphasis is given to the recent developments in this field and the challenges that lie ahead.

II. MOLECULAR IMPRINTING PROCESS

Molecular imprinting is a technique for constructing tailor-made receptor binding sites in a three-dimensional, cross-linked polymer matrix. Distinct interactions points are created in the presence of a template by the interactive monomer incorporated into the network. The process involve three main key steps (Figure 1):

1. Complex formation of the template (print) molecule with the functional monomers (prearrangement step).
2. Co-polymerization of the resulting printing assembly with an excess of a cross-linking agent in an inert solvent to form a rigid polymer.
3. Removal of the template molecule by hydrolysis or extraction.

The analyte creates a specifically imprinted groove in the polymer in the form of well-defined cavities that are complementary to the imprint molecule. Thus, MIPs posses a permanent memory of the original template in terms of complementary size, geometry, and orientation. These artificial interactive sites mimic their biological counterparts and can recognize the target molecule, even in a complex solution, and are able to differentiate the analyte and its optical or structural isomer.

Two distinct imprinting strategies have been employed to introduce specific recognition sites into highly cross-linked porous polymers (Figure 1): pre-organized (covalent) imprinting approach and the self-assembly (noncovalent) imprinting approach.

A. Preorganized Molecular Imprinting

In covalent imprinting, as suggested by Wulff and co-workers,[5,6] strong reversible

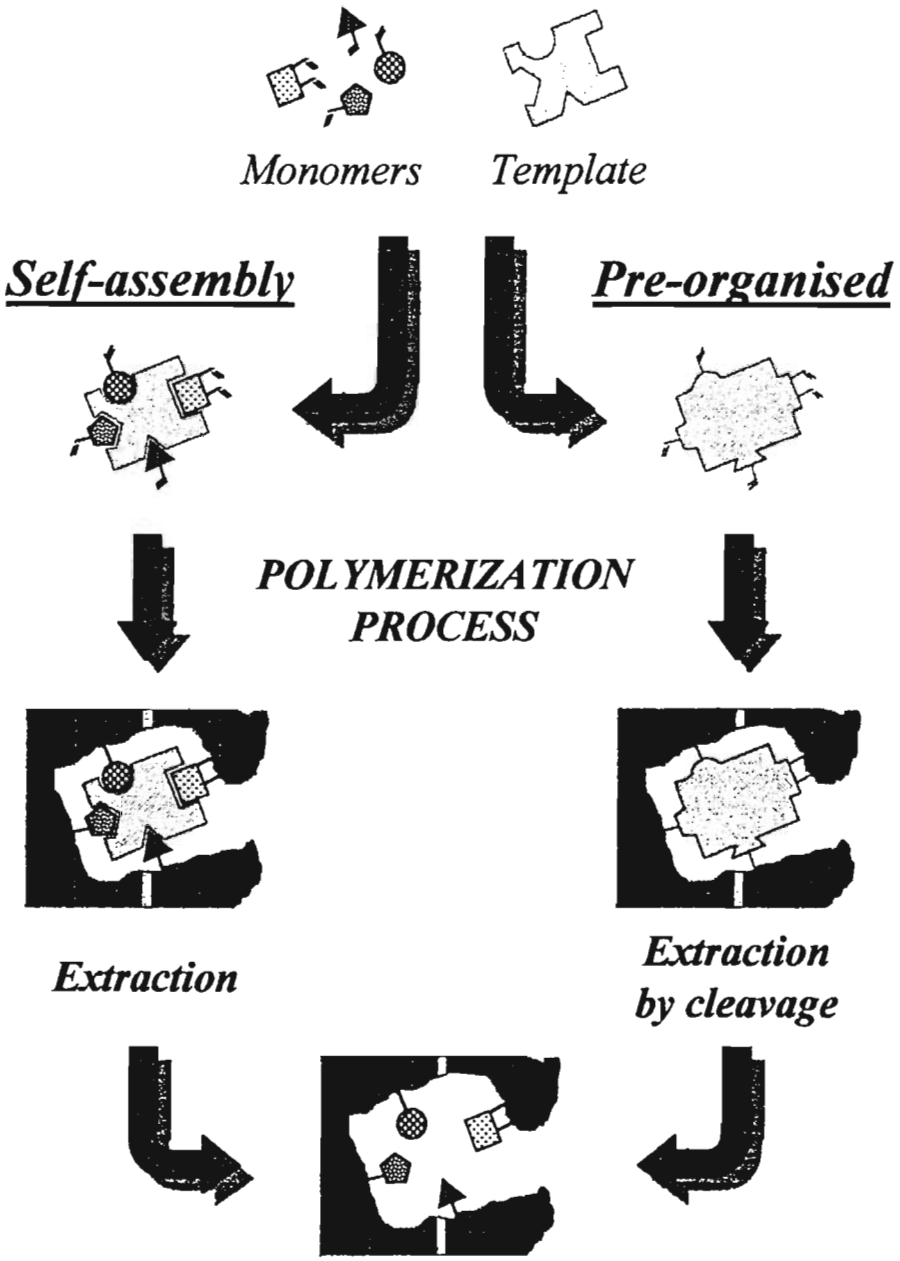


FIGURE 1. Schematic diagram of the molecular imprinting principle.

covalent bonds are formed between the monomer and the template. These labile covalent arrangements involve bonds such as boronate esters,[7] Schiff bases,[8] or ketal.[9] Covalent imprinting has been highly successful in the cases of molecules bearing 1,2 and 1,3-diol groups, for example, various sugar derivatives. As the maintenance of a stable template monomer complex is the cornerstone of an imprinting process, in order to yield high specific and homogenous binding sites, this approach would be advantageous. However, covalent imprinting is accompanied by several disadvantages such as slow binding kinetics and lack of suitable reactions that can be used for this approach. Metal coordination, however, would be a good candidate for further development of covalent systems because they involve fairly stable bonds with fast ligand-exchange ki-

netics compared with other covalent bonds. Furthermore, metal coordination interactions can be tailored for a specific application by replacing the metal ion used during imprint with other metals better suited for the recognition.

B. Self-Assembly Molecular Imprinting

The simplest method of producing selective polymers uses noncovalent interactions in the recognition of the imprinting species. This relies on the self-assembly of monomeric species around the template. The interaction between the analyte and the monomers can be single or multiple point in nature. It involves host-guest complexes such as electrostatic, hydrophobic, dipole-dipole, π-π and hydrogen bonding.

The most important interaction is electrostatic followed by hydrogen-bond formation. Electrostatic interaction alone lead to low selectivity.[10] While highly selective separation and excellent ligand recognition are obtained by polymers that mimic biological interactions by forming multiple bonds between the template and the monomers.[11] Furthermore, a change in selectivity of MIPs in comparison to nonimprinted polymers is the resultant of multiple interaction or contact between the analyte and the polymer (Figure 2).[12]

Noncovalent imprinting is more flexible in terms of choice of functional monomers and template and easier to implement than covalent imprinting. Therefore, it has been predominantly used to produce biomimetic materials. The principal means of exerting specificity have been attributed to ionic interaction and hydrogen bond formation between the analyte and the polymer functional groups, much akin to natural systems as well as to the overall three-dimensional geometry of the sites. However, polymers prepared using self-assembly imprinting protocol are accompanied by nonuniform binding sites, as a result of which part of the imprinted sites may react favorably with the template, whereas other imprinted sites may interact in a less favorable or even nonspecific manner.[13] Polymers with high-affinity binding sites and selectivity can be produced using noncovalent protocol by careful optimizing the polymerization conditions that will stabilize the monomer—template complexes. The reaction variables include the type and relative amount of print molecule, functional monomer, and cross-linking agent, type of porogen solvent used for imprinting and rebinding of the analyte, polymerization temperature, as well as pressure.

III. POLYMER COMPOSITION

A. Templates

The binding strength of the polymer as well as the fidelity in the recognition depends on the number and type of interaction sites, the template shape, and the monomer template rigidity.[14] Templates offering multiple sites of interactions for the functional monomer are likely to yield binding sites of higher specificity and affinity for the template. A quantitative structure binding relationship was demonstrated by imprinting a number of structurally related basic *N*-heterocycles by co-polymerization of methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA).[15,16] High affinity and selectivity were reported when 9-ethyladenine was used as the template.[17,18] This was thought to be due to a combination of Watson and Crick- and Hoogsten-type hydrogen bonds between the base and carboxylic acids. Sellergren and Dauwe observed similar effects[19] in their studies on imprinting a series of similar structurally triazine templates. A notable increase in affinity and selectivity was obtained with an increase in the basicity of the templates.

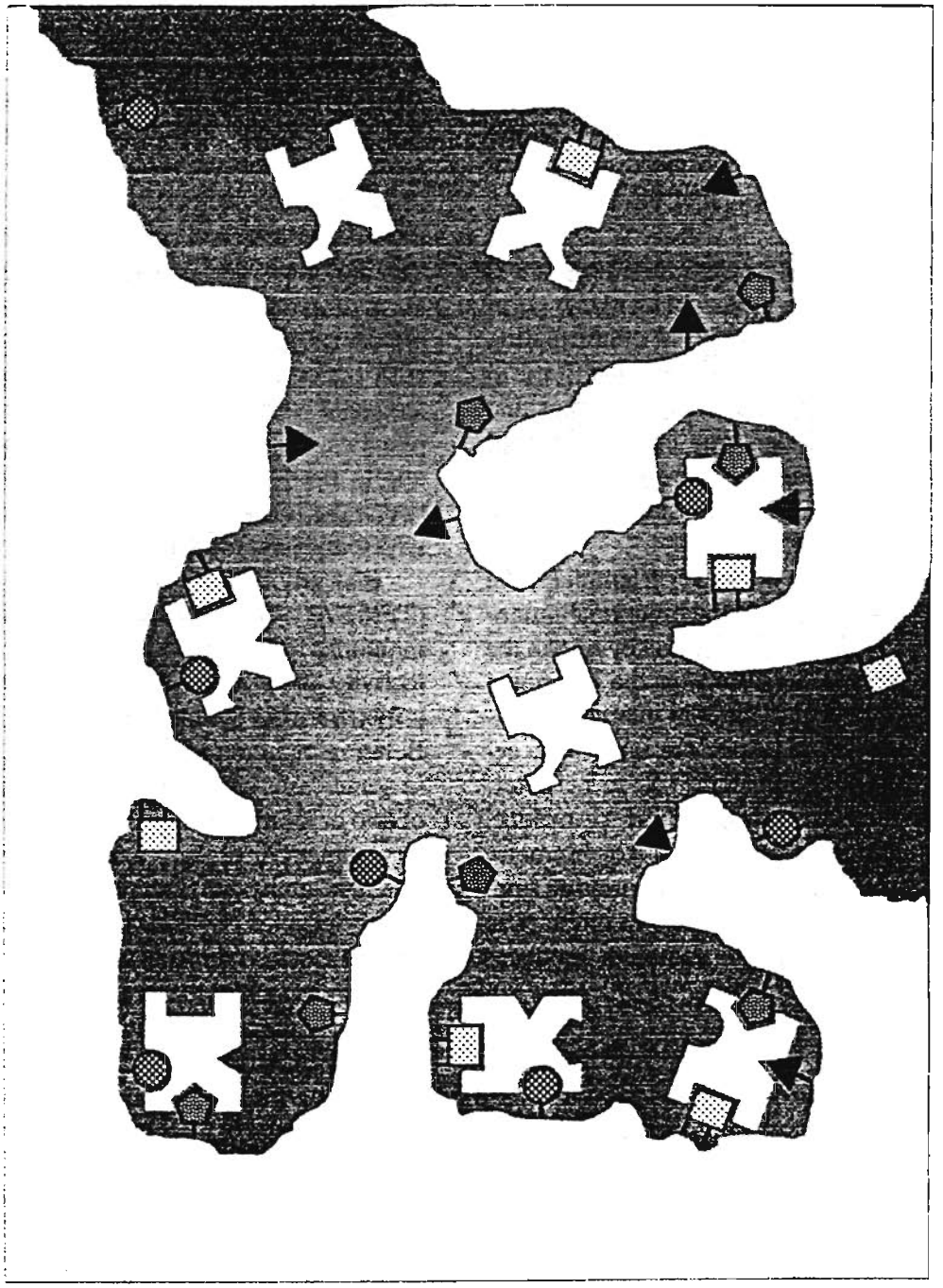


FIGURE 2. Specific recognition in an imprinted polymer with multiple binding sites.

These improvements were attributed to the formation of a 2:1 complex between the monomers and the template. Furthermore, MIPs prepared with a template that forms a two-point interaction with MAA, such as ametryn exhibited higher affinity and selectivity for the template than for a structurally similar compound such as prometryn.[19] Prometryn is less prone to form a 2:1 complex with MAA due to steric hindrances.

The shape and size of the template may in some cases be sufficient to create steric complementarity for efficient discrimination between two molecules. An example for the influence of template structure on the recognition properties of MIPs was observed in

the imprinting of amino acids with N-protecting groups. Selectivity of amide MIPs was adscribed to the hydrogen bonds formed between the sample molecule and amide groups at the recognition sites of the MIP and to the size and shape of the amino acid molecule.[18]

Templates that possess conformational rigidity that can fit in the cavity of the polymer with minimal change in conformation will increase the affinity and selectivity in the recognition. This is due to the fact that templates that fit perfectly into the site will involve minimal loss in entropy due to conformational changes in the site as well as in the template after binding.[14]

In addition to the type of template, the ratio of the template to the functional monomer has been known to play a key role in the selectivity and sensitivity in the imprinted polymers when the possible interactions involved inside the matrix are taken into account. The optimum ratio has to be determined for each individual template. Investigations on the effect of stoichiometry have been reported in several imprinting studies.[11,20-22] In most cases an excess molar ratio of the monomer to the template produces more favorable results.

B. Type of Monomer and Cross-Linker

The type of functional monomer used for producing a useful MIP is very important, as it is the component that is principally involved in forming an effective chemical bond with the print molecule. The functional monomer must strongly interact with the template to achieve a high yield of imprinted binding sites and allow the maximum number of complementary interactions to be developed in the polymeric matrix. In general, analytes containing basic functional groups are best imprinted with monomers containing acidic functional groups and vice versa.

Methacrylic acid (Figure 3) is one of the most widely used monomers. It interacts ionically with the amine functional group and via hydrogen bonding with a variety of polar functionalities such as carboxylic acids, carbamates, and carboxylic esters.[23] The former interaction is stronger than the later. The introduction of 4-vinylpyridine (4-Vpy) as a functional monomer in noncovalent molecular imprinting[24,25] produces polymers with better selectivity for carboxylic acid templates when compared with polymers prepared with MAA.[26] This fact is explained as due to the formation of ionic bonding between the recognition sites of the polymers with templates containing carboxylic groups.

Recently, 2-(trifluoromethyl) acrylic acid (TFMAA) was introduced as a functional monomer for basic templates, and better selectivity and affinity were obtained for such polymers when compared with MAA.[21,27,28] Better recognition ability has been achieved in some cases with polymeric combinations of two or more functional monomers (giving terpolymers or higher) compared with recognition observed with the corresponding copolymers.[24,27,29-31] The success of this process depends on the kind of template and the relative strength of the template and the complexes of functional monomer as compared with their interaction with one another.[32]

Lately, a combination of covalent imprinting approach has been used for imprinting of cholesterol.[33] Covalent binding between the template (cholesterol) and the functional monomer (4-vynilphenol) was used during imprinting process. After hydrolytic cleavage, complementary binding sites capable of hydrogen bonding with cholesterol were generated.[33] This is an excellent way of producing more precise homogeneous binding sites with specificity characteristics similar to a true biological receptor.

In the imprinting process, a very high degree of cross-linking is used. This is necessary in order to achieve a high specificity

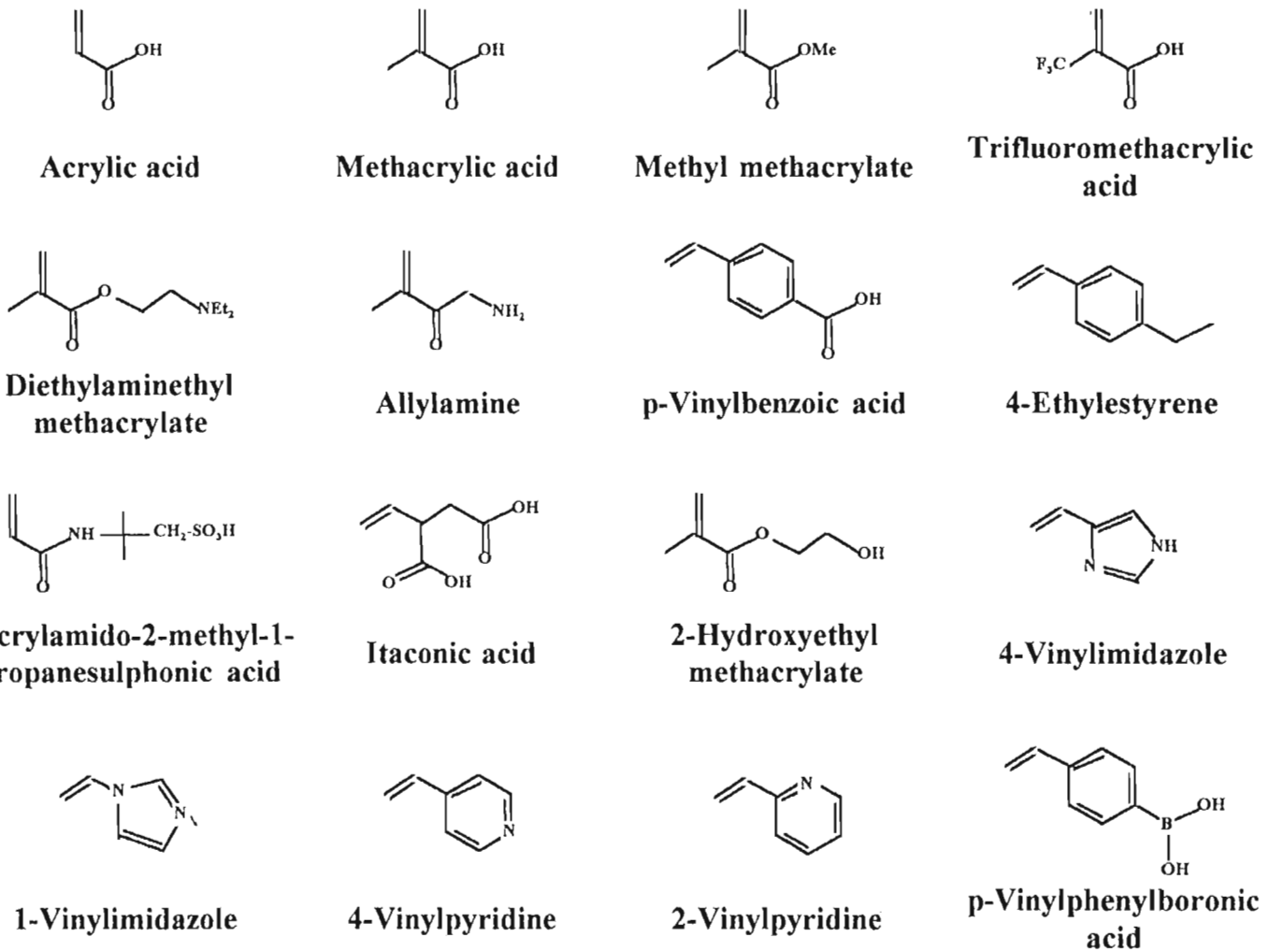


FIGURE 3. Common monomers for MIP production.

and selectivity for the polymer. The enhancement in selectivity is mainly due to the rigidity that the imprint cavity bring about by the preservation of monomer-template assemblies during polymerization, which allows functional groups to be fixed in a stable arrangement complementary to the template.

Ethylene glycol dimethacrylate (Figure 4) has been used extensively as a cross-linker in noncovalent molecular imprinting. New cross-linkers, which are tri- or tetrafunctional, have been studied in some detail.[34] These include trimethyl propane trimethyl acrylate (TRIM), pentaerythritol tetracrylate (PETEA), and pentaerythritol tri acrylate (PETRA). Molecular imprinted polymers with high load capacity and excellent resolving capability were obtained using TRIM when compared with EDMA polymers.[34]

Despite these recent successes, there is still considerable scope to explore new functional monomers, cross-linkers, new polymer materials, as well as completely different polymer systems. Matrix combinatorial techniques, allowing a wide range of possible combinations of monomers, cross-linkers, solvents, etc. to be screened with a template of interest would be fruitful. Although some efforts have been done,[35,36] this approach is still limited by lack of a simple and rapid screening method to evaluate the large number of polymers that would be created.[32]

C. Type of Porogen

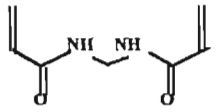
The choice of the porogenic solvent is critical in most molecular imprinting procedures. Porogens govern the strength of noncovalent interactions and influence the polymer morphology such as inner surface area and average pore size. The solvent used in the polymer formation should be as nonpolar as possible in order to maximize the strength of hydrogen and ionic interactions between the print molecule and the monomer while allowing rapid dissolution of the print molecule.

The recognition ability of the MIP depends on the type of solvent used in the rebinding step. In general, better recognition ability is obtained with nonpolar solvents. The morphology is also affected by swelling when exposed to different kinds of porogens. It is generally observed that the choice of recognition solvent should be more or less identical to the imprinting solvent in order to avoid any swelling problems, which will affect the recognition of the polymer.[37]

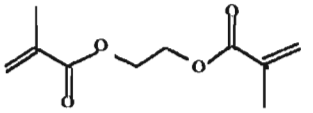
The effects of porogens have been investigated by several researchers.[23,33,38,39] Whitcombe et al.[33] have reported that the addition of as little as 3% of hydrogen bonding solvents to hexane completely suppress the binding of cholesterol to an imprinted polymer. The influence of porogen on rebinding of 9-ethyladenine was studied in detail.[17] It was reported that superior selectivity for the template was attained when similar solvents are use during imprinting and rebinding. One possible explanation of this effect is due to the adjustment of the microenvironment of the binding sites to the porogen during imprinting. Furthermore, the affinity and selectivity in the binding step decrease with an increase in hydrogen bonding capacity of the porogen. This fact is explained by the destabilization of the monomer template assemblies due to the higher polarity of the solvent as well as to the stronger solvation of the free monomer and template.[17]

D. The Effect of Temperature and Pressure

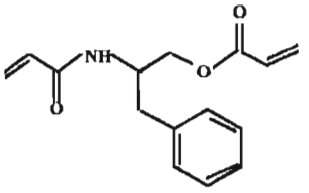
MIPs prepared at low temperatures using a photo-initiator exhibited higher enantiomer separation capabilities.[37,40] This fact was attributed to the stability of the monomer/imprint complexes due to the more favorable entropy, leading to well-defined imprints in the resultant polymer.



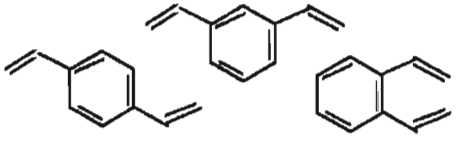
N,N'-Methylene-bisacrylamine



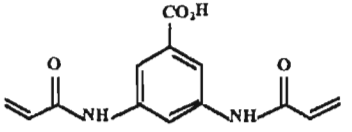
Ethylene Glycol Dimethacrylate



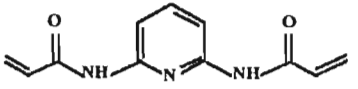
N,O-Bisacryloyl-L-phenylalaninol



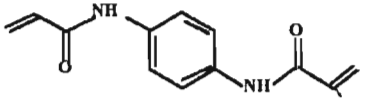
Divinylbencene



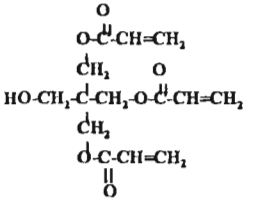
3,5-Bisacryloamidobenzoic Acid



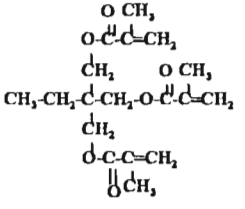
2,6-Bisacryloamidopyridine



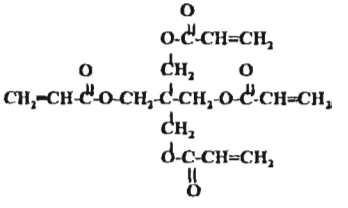
N,N'-Phenylendiacrilamide



Pentaerythritol Triacrylate



Pentaerythritol Tetraacrylate



Trimethylolpropane Trimethacrylate

FIGURE 4. Cross-linkers for MIP production.

Shea et al.[17] reported that the rebinding of 9-ethyladenine was superior to the polymer prepared at low temperatures using photo-initiator when compared with polymers prepared by thermal polymerization. This agrees with earlier reports that polymerization under conditions of low temperature gives optimal rebinding results.[37,40]

The effect of pressure on molecular imprinting polymers was demonstrated by imprinting of triazine templates using MAA and EDMA at a pressure of 1 bar and 100 bar using 2-propanol as porogen solvent.[40] The affinity and selectivity of ametryn was 30% higher in a polymer prepared at 100 bar compared with the one at normal pressure. This effect was, however, not observed for the atrazine-imprinted material. It was believed that pressure induced stabilization of the monomer-template assemblies contributes to the pressure effect observed.[41]

IV. APPLICATIONS OF MIPS IN OPTICAL SENSING

Interest in chemical sensing has blossomed during the last decade.[42-45] Progress in material science, advances in electronic devices and microelectronics, have focussed attention on developing devices incorporating a recognition component connected to a transducer that can output an electronic signal that is proportional to the species being recognized. These devices are analytical chemical sensors.

The basic principle of a chemical (or biochemical) sensor is described in terms of two basic components. One of the components is the recognition layer, where a specific interaction to the target analyte enables it to be recognized. The function of the second component (the transducer) is to transform that interaction into an electrical signal proportional to the interaction energy. A range of combinations of recognition layer/transducers have been proposed and studied. Spectrophotometry, fluorimetry, and luminescence measurements are well established and proven techniques in chemical sensors. These optical sensors make use of changes in absorption, fluorescence, light scattering, refractive index, reflection, etc., which, at present, are mainly detected using conventional instrumentation.

Selectivity is clearly one of the essential characteristics of a chemical sensor, as it determines whether a reliable measurement in the sample is feasible. In this sense, the recognition phase is the most important component and the development of new recognition principles (or the improvement of the known ones) is a task for chemists.

The ability to make chemical sensors with the typical high selectivity of biomolecules while having the robustness to operate in harsh environments such as high temperature is the long-term goal in sensor research. MIPs have unique properties that make them suitable for sensor technology. They have the affinity and selectivity required in a sensing device. They exhibit good specificity for various analytes of medical, environmental, and industrial interest. They are also highly robust and have excellent operational stability. MIPs are an attractive alternative if natural recognition elements are not available.

There have been developed some electrochemical sensing devices based on MIPs,[46-57] but in the field of optical sensing there is a long way to walk. There are many possibilities for the design of optical sensors based on MIPs. We present a concise critical review of current technology. The coverage is not intended to be all-inclusive but assessing the relative merits of the most recent and significant advances in these optical sensing approaches.

A. Analytes with Native Optical Properties

The function of the imprinted polymer as recognition phase is to interact with the

analyte so as to render it detectable. In this situation, the MIP acts as an adsorbent/extractant material to preconcentrate an optically detectable analyte in the field of view of the detection system.

Besides increasing sensitivity by concentrating the analyte, sensing based on this concept is straightforward, but the analytical potential is limited to those species amenable of being detected directly. Sensor devices based on MIP for analytes with native optical properties are reviewed in Table 1.

Sensing approaches based on this principle have been reported recently for polycyclic aromatic hidrocarbons (PAHs) in aqueous solution[58,59] using fluorescence detection. MIPs have been prepared using polyurethanes in order to get a hydrophilic polymer to guarantee enough wetting when working in aqueous media. Also, aromatic monomeric components were used in the polymer synthesis, which allowed efficient interaction via π-π bonds for the PAHs. In these systems, enrichment factors of up to approximately 10^7 have been reported and detection limits down to the parts per trillion were obtained.

The combination of MIP recognition phases and flow injection systems offers the adventages of high sample throughput, the reuse of the same polymeric material for multiple measurements without loss of sensitivity and/or selectivity, and the possibility of real-time monitoring using conventional materials and instrumentation (Figure 5). As an example, a polymer imprinted with flavonol using MAA as the functional monomer, EDMA as the cross-linker, and AIBN as the radical initiator in chloroform as the porogenic solvent were studied[62] (Figure 6).

TABLE 1
Sensor Devices Based on MIPs for Analytes with Native Optical Properties

Analyte	Detection mode	Detection Range	Reference
Pyrene	Fluorescence	0.030–40 mg/mL	58
PAHs[b]	Fluorescence	----	59
NATA[a] and fluorescein	Fluorescence	----	60
Dansyl.L-phenylalanine	Fluorescence	0-30 μg/mL	61
Flavonol	Fluorescence	0.12-2.40 μg/mL	62
Sialic acid	Spectrophotometry	----	63
Testosterone	Spectrophotometry	30-350 μg/mL	64
Salicylic acid	Spectrophotometry	0-300 μg/mL	65

[a]PAHs: Polycyclic aromatic hydrocarbons.
[b]NATA: N-acetyltryptophananide.

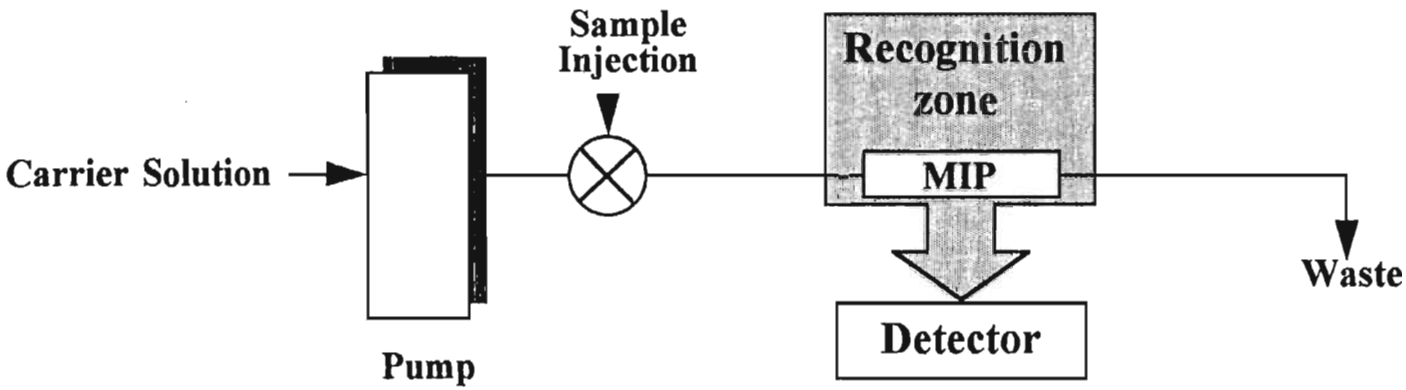


FIGURE 5. Principle of the molecular imprint-based optosensing approach.

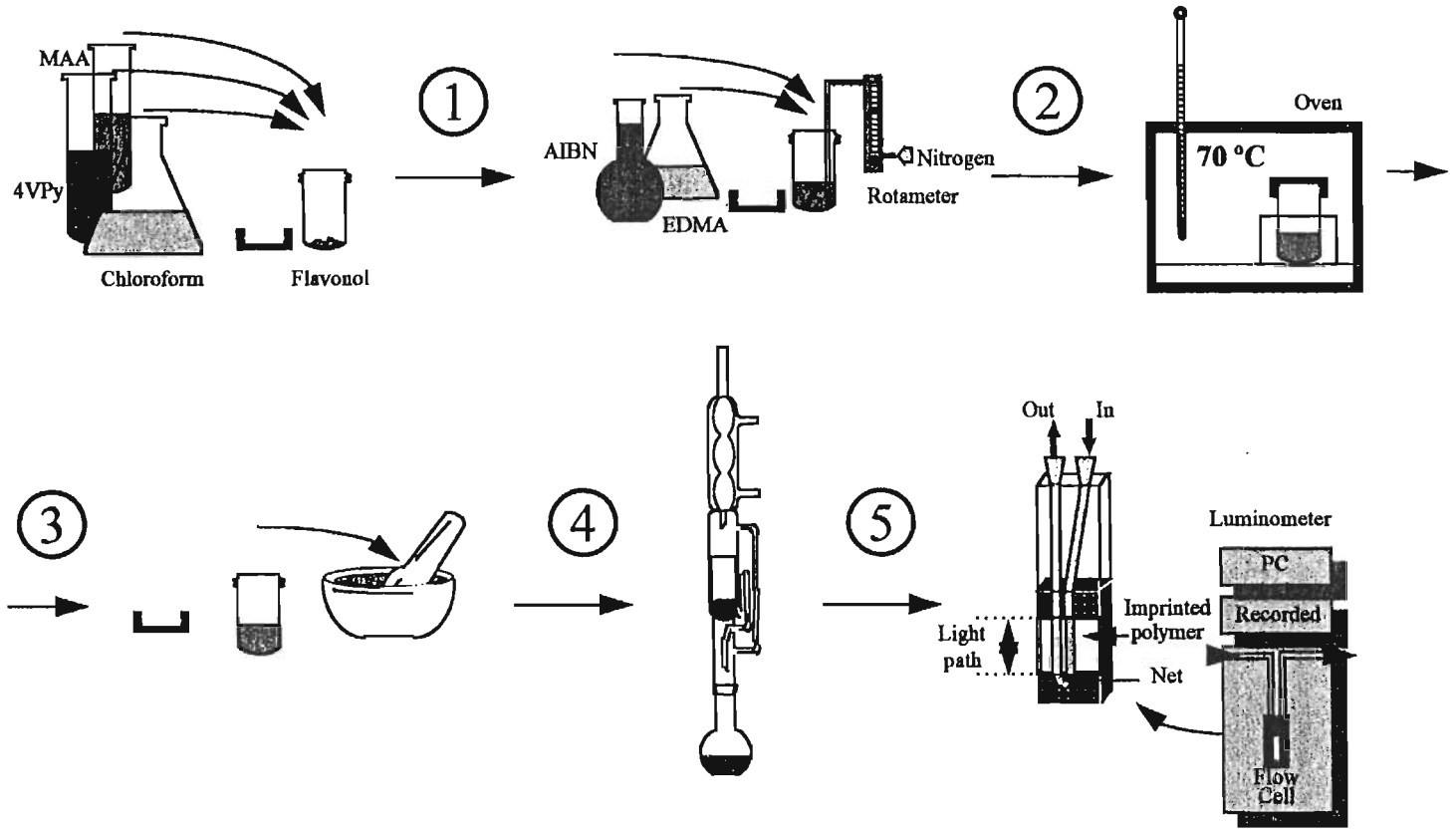


FIGURE 6. (1) Dissolution of flavonol and functional monomers in a poorly hydrogen-bonding solvent of low polarity. (2) Free radical polymerization initiated with an azo initiator and a crosslinking agent, termochemically at 70ºC. (3) Crosslinking the block polymer (150 to 250 µm). (4) Removal of flavonol from the polymer matrix using Soxhlet apparatus or washed. (5) Packing the flow-cell with the MIP for its evaluation as sensing material.

The application of the polymer in a fluorescent flow-through sensing approach for the determination of flavonol was reported. The recognition properties of the polymer were evaluated by injecting flavonol and structurally similar compounds such as morin and quercetin. High selectivity was obtained for the template. This was attributed to be due to inadequate functional groups with correct orientation inside the cavity to interact with flavonol analogues. The influence of the solvent on the sensitivity and recovery time for the sensor was investigated. A mixture of hexane:chloroform 70:30 was reported to be the optimum. The sensor was applicable for the analysis of flavonoids in hydrophobic samples such as food samples without the use of a separation step. The sensor was found to be mechanically stable, being the loss in performance with continuous use for 2 months only 2%.

The direct fluorimetric sensing of *N*-acetyltryptophanide and fluorescein using imprinted polymers has also been reported,[60] and the analytical potential of using MIPs as sensing materials for different analytes is described in recent research papers. The possibility to analyze impure samples containing small amounts of analyte that requires no labeling should make the use of MIP the first option in developing optical sensing approaches for tackling such kind of samples.

B. Analytes with No Optical Properties

Sensor devices based on MIP for analytes with nonnative optical properties are reviewed in Table 2.

1. Indirect Sensing

This situation arises when an MIP with a signaling element is used to selectively recognize and sense the analyte. Most MIP sensor layers developed for applications involving optical detection have the property that change color or fluorescence after interacting with the analyte. Dickert et al.[67] have prepared polyurethane polymers imprinted with several kinds of volatile organic solvents. This kind of volatile analytes creates cavities by evaporation. Furthermore, intercalation of chromogenic or fluorescent dyes into the polymers results in sensitive materials for detection of solvent vapors in air via changes in optical properties. According to Dickert et al.,[67] two principles of selectivity are combined in these materials, "concave chemistry" due to molecular cavities and "convex chemistry" realized by donor-acceptor (solvent-dye) interactions.

Powell et al.[31] have developed a fluorescent chemosensor for adenosine 3′,5′-cyclic monophosphate (cAMP) based on molecular imprints that contain a fluorescent dye, *trans*-4-[*p*-(*N*,*N*-dimethylamin)styryl]-*N*-vinylbenzylpyridinium chloride, as an integral part of the recognition cavity. This MIP displays a quenching of fluorescence in presence of aqueous cAMP, and according to Powell et al. it represents a starting point in the development of highly synthetic fluorescent sensors for cAMP.

Most conventional optical sensors based on fluorescence quenching usually lack selectivity, and, normally, the sensing phase is separated from samples by adequate analyte-permeable membranes in order to eliminate interferents from sample components. Furthermore, the presence of the membrane results in unacceptable slow sensor responses. The use of MIPs in fluorescence quenching-based sensors appears to be viable strategy for effectively remove interfering species (through selective recognition sites) and improve sensor response characteristics as analyte transport into the recognition layer is not impeded by diffusion through a membrane.

TABLE 2
Sensor Devices Based on MIPs for Analytes without Optical Properties

Analyte	Detection mode	Detection limit	Reference
Indirect sensing			
CAMP[a]	Fluorescent quenching	0.035-35 μg/mL	31
9-ethyladenine	Absorbance shift	0.016-1630 μg/mL	66
Polar solvents	Absorbance intensity	----	67
Theophyline	Surface plasmon resonance	0.4-6 mg/mL	68
Vitamin K	Ellipsometry	0–4 μg/mL	69
Direct sensing			
Beryllium	Fluorescence	10-500 ng/mL	70
Aluminium	Fluorescence	----	71
PMP[b]	Fluorescence	0.125-150000 ng/mL	72
PMP	Fluorescence	0.010-10000 ng/mL	73
Competitive assays			
Sialic acid	Fluorescence	0.15-30.9 μg/mL	74
Triazine	Fluorescence	0.81-8100 μg/mL	75
Chloramphenicol	Spectrophotometry	3-1000 μg/mL	76
Chloramphenicol	Spectrophotometry	3-30 μg/mL	77

[a]cAMP: Adenine-3,5-cyclicmonophosphate.
[b]PMP: Pinacolylmethyl phosphate.

2. Direct Sensing

It is possible to design MIP to optically sense species such as metal ions using ligands that change color and/on fluorescence after complex formation with the MIP. The approach is based on the use of a nonfluorescent ligand that forms a fluorescent complex with the metal ion. This fluorescent complex is the template used during polymer synthesis. After removal of the metal ion from sites for the metal ion are constructed. The functional groups of the ligand inside the cavity are arranged at the suitable position for selective interaction with the metal ion.

Al(III)[71] and Be(II)[70] can be sensed based on the formation of fluorescent complexes using polymers imprinted with the corresponding morin-metal chelate. It was demonstrated that the MIPs were highly selective and sensitive for the target metal ion, indicating that the ionic radius and the metal complex stoichiometry are the factors governing the arrangement of morin molecules into polymer cavities and the size of recognition sites. This imprinting strategy provides signaling active sites that match a given ion (see scheme in Figure 7). Although examples have yet to be reported in the literature, many potential applications for metal ion sensing can be envisaged as the methodology offers a simple, selective, sensitive, and fast mean for metal trace analysis in aqueous solutions.

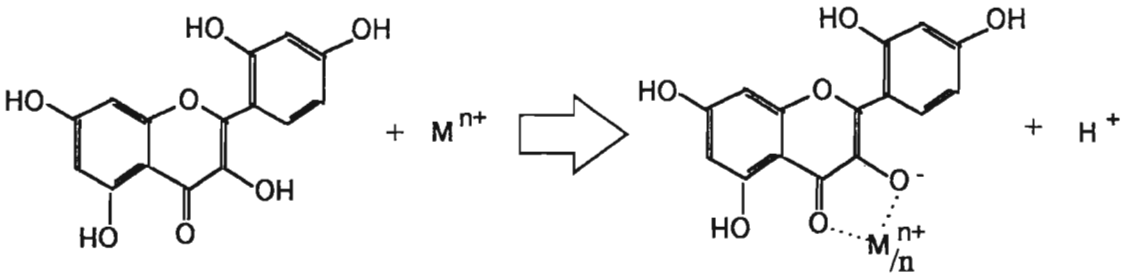
3. Competitive Sensing Assays

It is possible to design competitive binding sensors using MIPs as antibody mimics and basing detection on displacement of a labeled analyte by the unlabeled one. These systems can use either a fluorescent[75] or a colored mark.[76,77] This idea was first proposed by Kriz et al. in 1995.[61] Using a fiber-optic device and an MIP phase against dansyl-phenylalanine, they described the preparation of this MIP and their intention to use it in a competitive assay. Piletsky et al.[74,75] also developed competitive fluorescent sensing assays for the pesticide triazine. The feasibility of competitive sensing assays has been also demonstrated for the antibiotic chloramphenicol[76,77] using methyl-red as colorimetric mark. We are currently developing a competitive flow-through fluorimetric sensing approach for chloramphenicol using a MIP as recognition phase and dansyl-labeled chloramphenicol.[78]

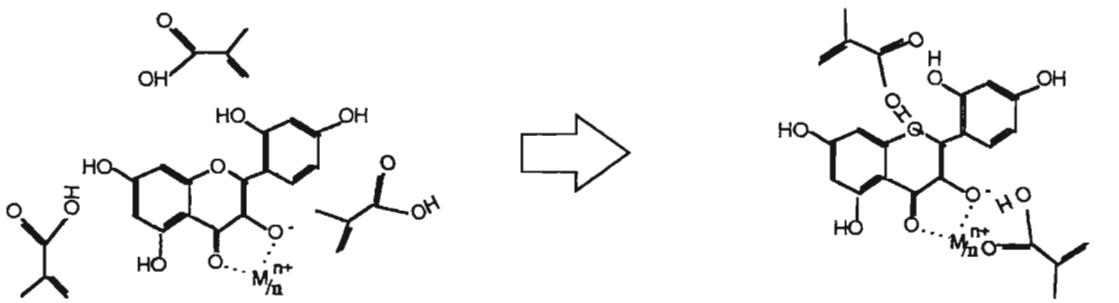
Improvements in sensitivity and detection limits of MIP competitive sensing assays are still required for them to compete with affinity sensors. Progress in this area could be achieved by the use of chemiluminescent or long-lived luminescent labels to increase the signal-to-noise ratio and eliminate background signal problems.

V. CONCLUSIONS AND PROSPECTS

The research effort spent on the development of molecular imprinted polymers has brought many rewards in some fields such as chromatographic separations and stereoselective catalysis. The examples given in this article illustrate that the use of MIPs in optical sensing is still in its infancy and is currently receiving a significant amount of attention. Different optical MIP sensing concepts have been demonstrated in the laboratory, and their reduction to practice may be slow. Besides the many advantages of the technique, a number of problems remains open. The development of new approaches for MIP synthesis, improvement of the MIP specificity, lowering nonspecific binding, or use of different optical transduction schemes (e.g., luminescence with time discrimination, evanescent wave spectroscopy) are some guidelines for future effort to develop novel biomimetic sensors or ligand-binding assays, applicable to a wide range of analytes.

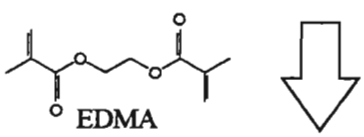


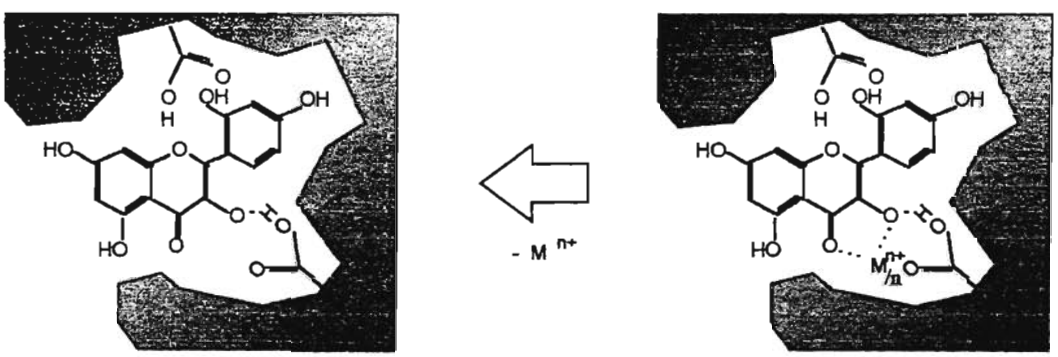
1. Complex formation



2. Mixture of reactives

3. Monomer rearrangement





5. Removed metal

4. Polymerization

FIGURE 7. Molecular imprinting using a metal-chelate as the template.

REFERENCES

1. Lehn, J.M.; *Angew. Chem. Int. Ed. Eng.* **1988**, 27, 89.
2. Rebk, J. Jr, *Angew. Chem. Int. Ed. Eng.* **1990**, 29, 245.
3. Cram, D.J. *Nature*. **1992**, 356, 29.
4. Pauling, L.J. *J. Am. Chem. Soc.* **1940**, 62, 2643.
5. Wulff, G.; Vesper, W. *J. Chromatogr.* **1978**, 167, 171.
6. Wulff, G.; Schauhoff, S. *J. Org. Chem.* **1991**, 56, 395.
7. Wulff, G. *Angew. Chem. Int. Ed. Eng.* **1995**, 34, 1812.
8. Wulff, G.; Vietmeir, J. *Makromol. Chem.* **1989**, 1901, 1727.
9. Shea, K.J.; Sasaki, D.Y. *J. Am. Chem. Soc.* **1991**, 113, 4109.
10. Kriz, D.; Ramström, O.; Mosbach, K. *Anal. Chem.* **1997**, 69, 345A.
11. Yano, K.; Tanabe, K.; Takeuchi, T.; Matsui, J.; Ikebukuro, K.; Karube, I. *Anal. Chim. Acta.* **1998**, 363, 111.
12. Ensing, K.; DeBoer, T. *Trends in Anal. Chem.* **1999**, 18(3), 138.
13. Vincent, T.; Rhemcho, Z.; Tan, J. *Anal. Chem.* **1999**, 71, 248A.
14. Sellergren, B. *Trends in Anal. Chem.* **1999**, 18(3), 164.
15. Vlatakis, G.; Andersson, L.I.; Muller, R.; Mosbach, K. *Nature*. **1993**, 361, 645.
16. Shea, K.J.; Spivak, D.A.; Sellergren, B. *J. Am. Chem. Soc.* **1993**, 115, 3368.
17. Spivak, D.A.; Gilmore, M.A.; Shea, K.J. *J. Am. Chem. Soc.* **1997**, 119 ,4388.
18. Yu, C.; Mosbach, K. *J. Org. Chem.* **1997**, 62, 4057.
19. Dauwe, C.; Sellergren, B. *J. Chromatogr. A.* **1996**, 735, 191.
20. Yano, K.; Nakagiri, T.; Takeuchi, T.; Matsui, J.; Ikebukuro, K.; Karube, I. *Anal. Chim. Acta.* **1997**, 357, 91.
21. Matsui, J.; Doblhoffdie, O.; Takeuchi, T. *Anal. Chim. Acta.* **1997**, 343, 1.
22. Jie, Z.; Xiwen, H. *Anal. Chim. Acta.* **1999**, 381, 85.
23. Kempe, M.; Mosbach, K. *J. Chromatogr. A.* **1995**, 694, 3.
24. Kempe, M.; Fischer, L.; Mosbach, K. *J. Mol. Recog.* **1993**, 6, 25.
25. Kempe, M.; Mosbach, K. *J. Chromatogr. A.* **1994**, 664, 276.
26. Andersson, L.I.; Mosbach, K. *J. Chromatogr.* **1990**, 516, 313.
27. Matsui, J.; Miyoshi, Y.; Takeuchi, T. *Chem Lett.* **1995**, 1007.
28. Matsui, J.; Nicholls, I.A.; Takeuchi, T. *Anal. Chim. Acta.* **1998**, 365, 89.
29. Ramström, O.; Andersson, L.J.; Mosbach, K. *J. Org. Chem.* **1993**, 58, 7562.
30. Hosaya, K.; Shirasu, Y.; Kimata, K.; Tanaka, N. *Anal. Chem.* **1998**, 70, 943.
31. Tukerkewitsch, P.; Wandelt, B.; Darling, G.D.; Powell, W.S. *Anal. Chem.* **1998**, 70, 2025.
32. Mayes, A.G.; Mosbach, K. *Trends in Anal. Chem.* **1997**, 16(6), 321.
33. Whitcombe, M.J.; Rodríguez, M.E.; Villar, P.; Vulfson, E.N. *J. Am. Chem. Soc.* **1995**, 117, 7105.
34. Kempe, M. *Anal. Chem.* **1996**, 68, 1948.
35. Takeuchi, T.; Fukuma, D.; Matsui, J. *Anal. Chem.* **1999**, 71, 285.
36. Lanza, F.; Sellergren, B. *Anal. Chem.* **1999**, 71, 2092.
37. Sellergren, B.; Shea, K.J. *J. Chromatogr.* **1993**, 635, 31.
38. Matsui, J.; Miyoshi, Y.; Doblhoffdie, O.; Takeuchi, T. *Anal. Chem.* **1995**, 67, 4404.
39. Muldorn, M.J.; Stanker, L.H.. *J. Agr. Food Chem.* **1995**, 43, 1424.
40. O'Shannessy, D.J.; Ekberg, B.; Mosbach, K. *Anal. Biochem.* **1989**, 177, 147.
41. Sellergren, B.; Dauwe, C.; Schneider, T. *Macromolecules*. **1997**, 30, 2454.

42. Cammon, K.; Lemke, U.; Rohen, A.; Sander, J.; Wilken, H.; Winter, B. *Angew. Chem.* **1991**, 103, 512.

43. Takeuchi, T.; Haginaka, J, *J. Chromatogr. B.* **1999**, 728, 1.

44. Yano, K.; Karube, I. *Trends in Anal. Chem.* **1999**, 18(3), 199.

45. Dickert, F.L.; Hayden, O. *Trends in Anal. Chem.* **1999**, 18(3), 192.

46. Hedberg, E.; Winquist, F.; Lundstrom, I.; Andersson, L.J.; Mosbach, K. *Sens. Act. A.* **1993**, 37/38, 796.

47. Hong-Seok, J.; McNiven, S.; Ikebukuro, K.; Karube, I. *Anal. Chim. Acta.* **1999**, 390, 93.

48. Andersson, L.I.; Miyabayashi, A.; O'Shannessy, D.J.; Mosbach, K. *J. Chromatogr.* **1990**, 516, 323.

49. Kriz, D.; Mosbach, K. *Anal. Chim. Acta.* **1995**, 300, 71.

50. Kriz, D.; Kempe, M.; Mosbach, K. *Sens. Actuators B.* **1996**, B33, 178.

51. Kriz, D.; Andersson, L.I.; Khayyami, M.; Danielsson, B.; Larsson, P.O.; Mosbach, K. *Biomimetics.* **1995**, 2, 81.

52. Piletsky, S.A.; Piletskaya, E.V.; Elgersma, A.V.; Yano, K.; Karube, I.; Parhometz, Y.P.; El'skaya, A.V. *Biosens. Bioelectron.* **1995**, 10, 959.

53. Sergeyeva, T.A.; Piletsky, S.A.; Brovko, A.A.; Slinchenko, E.A.; Sergeeva, L.M.; Panasyuk, T.L.; El'skaya, A.V. *Analyst.* **1999**, 124, 331.

54. Piletsky, S.A.; Piletskaya, E.V.; Panasyuk, T.L.; El'skaya, A.V.; Levi, R.; Karube, I.; Wulff, G. *Macromolecules.* **1998**, 31, 2137.

55. Kröger, S.; Turner, A.P.F.; Mosbach, K.; Haupt, K. *Anal. Chem.* **1999**, 71, 3698.

56. Panasyuk, T.L.; Mirsky, V.M.; Piletsky, S.A.; Wolfbeis, O.S.; *Anal. Chem.* **1999**, 71, 4069.

57. Chen, C.T.; Chen, G.; Guan, Z.; Lee, D.; Arnold, F.H. *Polym. Prepr.* **1996**, 37, 216.

58. Dickert, F.L.; Besenbock, H.; Tortschanoff, M.. *Adv. Mater.* **1998**, 10, 149.

59. Dickert, F.L.; Tortschanoff, M.; Bulst, W.E.; Fischerauer, G. *Anal. Chem.* **1999**, 71, 4559.

60. Lulka, M.F.; Chambers, J.P.; Valdés, E.R.; Thomson, R.G.; Valdés, J.J. *Anal. Lett.* **1997**, 30, 230.

61. Kriz, D.; Ramström, O.; Svensson, A.; Mosbach, K. *Anal. Chem.* **1995**, 67, 2142.

62. Suárez-Rodríguez, J.L.; Díaz-García, M.E. *Anal. Chim. Acta*, **2000**, 405, 67.

63. Kugimiya, A.; Takeuchi, T.; Matsui, J.;. Ikebukuro, K.; Yano, K.; Karube, I. *Anal. Lett.* **1996**, 29(7), 1099.

64. Cheong, S.H.; Rachkov, A.; Park, J.K.; Yano, K.,; Karube, I. *J. Polym. Chem.* **1998**, 36, 1725.

65. Sreenivasan K. *Talanta.* **1997**, 44, 1137.

66. Matsui, J.; Tachibana, Y.; Takeuchi, T. *Anal. Commun.* **1998**, 35, 225.

67. Dickert, F.L.; Thierer, S. *Adv. Mater.* **1996**, 8, 987.

68. Lai, E.P.C.; Fafara, A.; VanderNoot, V.A.; Kono, M.; Polsky, B. *Can. J. Chem.* **1998**, 76, 265.

69. Andersson, L.J.; Mandenius, C.; Mosbach, K. *Tetrahed. Lett.* **1988**, 29, 5437.

70. Carvalho-Torres, A.L.; Díaz-García, M.E. Unpublished results.

71. Al-Kindy, S.; Badia, R.; Díaz-García, M.E. *Chem. Comm.* Submitted.

72. Jenkins, A.L.; Uy, O.M.; Murray, G.M. *Anal. Commun.* **1997**, 34, 221.

73. Jenkins, A.L.; Uy, O.M.; Murray, G.M. *Anal. Chem.* **1999**, 71, 373.

74. Piletsky, S.A.; Piletskaya, E.V.; Yano, K.; Kugimiya, A.; Elgersma, A.V.; Levi, R.; Kahlow, U.; Takeuchi, T.; Karube, I. *Anal. Lett.* **1996**, 29(2), 157.

75. Piletsky, S.A.; Piletskaya, E.V.; El'skaya, A.V.; Levi, R.; Yano, K.; Karube, I. *Anal. Lett.* **1997**, 30(3), 445.

76. Levi, R.; McNiven, S.; Piletsky, S.A.; Cheong, S.H.; Yano, K.; Karube, I. *Anal. Chem.* **1997**, 69, 2017.

77. McNiven, S.; Kato, M.; Levi, R.; Yano, K.; Karube, I. *Anal. Chim. Acta.* **1998**, 365, 69.

78. Suárez-Rodríguez, J.L.; Díaz-García, M.E. Unpublished results.