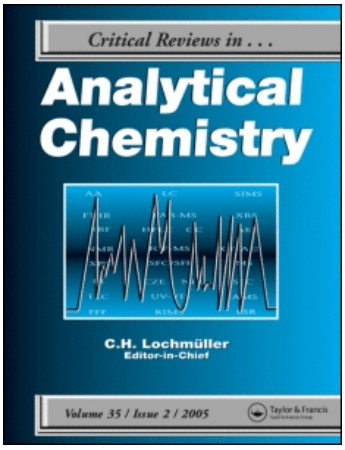

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Biomimetic Sensors for Toxic Pesticides and Inorganics based on Optoelectronic/Electrochemical Transducers—An Overview

T. Prasada Rao[a]; K. Prasad[a]; R. Kala[a]; J. Mary Gladis[a]
[a] Regional Research Laboratory (CSIR), Trivandrum, India

To cite this Article Rao, T. Prasada , Prasad, K. , Kala, R. and Gladis, J. Mary(2007) 'Biomimetic Sensors for Toxic Pesticides and Inorganics based on Optoelectronic/Electrochemical Transducers—An Overview', Critical Reviews in Analytical Chemistry, 37: 3, 191 — 210
To link to this Article: DOI: 10.1080/10408340701244664
URL: http://dx.doi.org/10.1080/10408340701244664

Critical Reviews in Analytical Chemistry, 37:191–210, 2007

ISSN: 1040-8347 print / 1547-6510 online
DOI: 10.1080/10408340701244664

Biomimetic Sensors for Toxic Pesticides and Inorganics based on Optoelectronic/Electrochemical Transducers—An Overview

T. Prasada Rao, K. Prasad, R. Kala, and J. Mary Gladis
Regional Research Laboratory (CSIR), Trivandrum, India

Toxic pesticides and heavy metals constitute an important class of pollutants that degrade the environment due to their persistent nature and their unavoidable use in increasing the agricultural output and industrial importance respectively. The design and development of portable devices such as sensors rather than laboratory based instruments in monitoring the above species at trace levels in real samples is prime challenge to analytical chemists at this juncture. Because of the poor physical and chemical stability of biosensors despite their specificity and sensitivity preclude their use in environmental analysis. On the other hand, in conventional chemical sensors are beset with problems of selectivity. Molecularly imprinted polymers (MIPs) are being increasingly used as recognition elements in mimicking molecular/ionic recognition by natural receptors. A brief survey of synthetic strategies and characterization of MIPs, transducers that convert binding event into a detectable signal, integration strategies of recognition element with a suitable transducer and finally the reported sensors for toxic pesticides and inorganics is discussed. Future outlook of such biomimetic sensors in environmental analysis has been highlighted.

Keywords molecular imprinting, pesticides, inorganics, sensors

INTRODUCTION

Man and technology are inseparable parts of modern civilization. In order that technological advances make minimum impact on biosphere, it has become necessary to take adequate steps to cleanse the environment and check its further pollution through various control measures. In executing these control measures, it has become necessary to identify and assess the extent of pollution in order to determine the type and degree of treatment required to render the waste harmless.

The classification of trace metals as nutrients/toxic is in a state of change. The toxic and essential elements are no longer separated into two rigid categories. It is now recognized that all metals will exert toxic effects when present in excess and certain toxic metals, so classed because of adverse effects at relatively small doses, may be fulfilling some essential function at more minute concentrations (1).

As a result of changing and extending the use patterns of pesticides and ongoing product development, several trends can be observed in pesticide science. For example, there has been a clear shift from the use of "long-life" persistent insecticides such as organochlorine compounds to more polar and readily degradable "short-life" pesticides such as N-methyl carbamate pesticides. Other major trends are the extensive use of "traditional" herbicides eg. triazines, chlorophenoxy acids and polyureas and so called "modern" herbicides eg. sulphonyl ureas and imidazolinones, with favourable properties such as a low dose rate of application and a high degree of (bio)degradation (2). Long-life pesticides, though banned in few countries are still in use in other countries in the world.

Table 1 lists the maximum permissible concentrations, potential health effects and common sources of contamination for selected toxic inorganics and pesticides. It is hard to imagine a more topical subject than toxic pesticides/inorganics in view of almost daily references to the dangers of one or other of them in environment.

Advances in analytical instrumentation and their subsequent application in developing refined, sensitive, selective and accurate techniques for trace toxic species have made yesterday's esoteric investigations today's routine analyses. As a consequence, the normal concentrations in environmental samples and what may be toxic are becoming more clearly delineated. Thus, the requirements of analytical technologies for monitoring environment ranging from threats such as occupational health and safety

Address correspondence to T. Prasada Rao, Regional Research Laboratory (CSIR), Trivandrum, India. E-mail: tprasadarao@rediffmail.com

TABLE 1
Maximum permissible level, potential health effects and common sources of contamination for selected toxic inorganics and pesticides

Contaminant	Maximum permissible level (MPL) in drinking water (mg/l)	Potential health effects	Common sources of contamination
Inorganics			
Arsenic	0.01	Skin damage or problems with circulatory systems	Erosion of natural deposits, run-off from orchards, run-off from glass and electronics production wastes
Cadmium	0.005	Kidney damage	Corrosion of galvanized pipes, erosion of natural deposits, discharges from metal refineries, run-off from waste batteries and paints
Chromium (total)	0.1	Allergic dermatitis	Discharge from steel and pulp mills, erosion of natural deposits
Lead	0.015	Infants and children: Delays in physical or mental development Adults: Kidney problems, high blood pressure	Corrosion of household plumbing systems, erosion of natural deposits
Mercury (inorganic)	0.002	Kidney damage	Erosion of natural deposits, discharge from refineries and factories, run-off from land fills and croplands
Uranium	0.03	Increased risk of cancer, kidney toxicity	Erosion of natural deposits
Pesticides			
Atrazine	0.003	Cardiovascular system or reproductive problems	Run-off from herbicide used on crops
Chlordane	0.002	Liver or nervous system problems, increased risk of cancer	Residue of banned temiticide
Dioxin	0.00000003	Reproductive difficulties, increased risk of cancer	Emissions from waste incineration and other combustion, discharge from chemical factories
Diquat	0.02	Cataracts	Run-off from herbicide use
2,4-D	0.07	Kidney, liver or adrenal gland problems	Run-off from herbicide used on crops
Endrin	0.002	Liver problems	Residues of banned insecticide
Ethylene dibromide	0.00005	Problems with liver, stomach, reproductive system or kidneys, increased risk of cancer	Discharge from petroleum refineries
Glyphosate	0.7	Kidney problems, reproductive difficulties	Run-off from herbicide use
Heptachlor	0.004	Liver damage, increased risk of cancer	Residue of banned termiticide
Simazine	0.004	Problems with blood	Herbicide run-off
Toxaphene	0.003	Kidney, liver or thyroid problems, increased risk of cancer	Run-off/leaching from insecticide used on cotton and cattle
2,4,5-T	0.05	Liver problems	Residue of banned herbicide

risks, to chemical warfare prompted the researchers to design a host of miniaturized analytical instruments for field studies rather than elaborate laboratory based instruments. Sensor devices fit very well in this changing scenario. The poor physical and chemical stability of biosensors, despite their specificity and sensitivity, preclude their use in environmental analysis. On the other hand, chemical sensors are beset with problems of selectivity. Molecular imprinted polymers (MIPs) are being increasingly used as recognition elements in mimicking molecular/ionic recognition by natural receptors.

PREVIOUS REVIEWS

We direct the reader to the articles (3–14) listed in Table 2 for reviews on MIP based sensors. As a starting point to understand the basic concepts of "Molecular Imprinting", one can refer to a tutorial lecture of Haupt (15).

THE REVIEW: GOALS AND CONTENT

As seen from Table 2, the reviews by Vidyasankar and Arnold (3), Dickert and Hayden (4, 5) Ensing and de Boer (6) and Haupt and Mosbach (7) dwells on the early developments of MIP based sensors during the period of 1995–2000. Other review articles mentioned in Table 2 concern with electrochemical (8–11)/optical (12–14) sensors based on Molecular Imprinting. However, there is no concerted effort to review the developments in design and development of sensor devices for the quantification of toxic pesticides and heavy metals and uranium which assume distinct importance as mentioned above. It

TABLE 2
Reviews on molecularly imprinting polymer based sensors complimentary to this review

S. No.	Title	Comments
	General	
1	Molecular imprinting: selective materials for separations, sensors and catalysis (3)	First of this kind published in 1995. But is a general review on applications of molecular imprinting wherein a section on sensors is also included
2	Molecular imprinting in chemical sensing (4)	Dedicated to mass-sensitive MIP sensors for PAHs, isomer VOCs as well as complex oil mixtures
3	Imprinting with sensor development—on the way to synthetic antibodies (5)	Describes optical and mass sensitive MIP sensors for PAHs, isomer VOCs as well as complex oil mixtures
4	Tailor made materials for tailor made applications: application of molecular imprints in chemical analysis (6)	General review on application of molecular imprints in chemical analysis with emphasis on sample pretreatment, separations, ligand binding assays and sensors
5	Molecularly imprinted polymers and their use in biomimetic sensors (7)	A comprehensive and highly informative review and first of its kind dedicated fully to MIP based sensors as on 2000
	Electrochemical sensors	
6	Electrochemical sensors based on molecularly imprinted polymers (8)	Discusses the problems associated with MIP based sensor development and indicate possible solutions
7	New materials for electrochemical sensing IV. Molecular imprinted polymers (9)	An overview of the use of MIPs for the design of electrochemical sensors based on different signal-transduction schemes is presented
8	Electrochemical sensors based on molecularly imprinted polymers (10)	Examines the literature on non-covalent MIP-based electrochemical sensors over the period 1993–2003
9	Electrochemical and piezoelectric enantioselective sensors and biosensors (11)	Reports on the developments in electrochemical and piezoelectric enantioselective sensors during 1992–2004
	Optical sensors	
10	Ion-selective optodes: current developments and future prospects (12)	Characteristics of various optical sensing devices were explained and discussed with experimental data
11	Molecularly imprinted polymers and optical sensing applications (13)	An overview of sensing applications of MIPs based on optical transduction upto 2000 was presented
12	Optical interrogation of molecularly imprinted polymers and development of MIP sensors: a review (14)	Reviews the progress and developments achieved in the past 5 years (2000–2005) in the application of optical analytical techniques to the evaluation of MIP characteristics with a brief coverage on optical sensor based MIPs

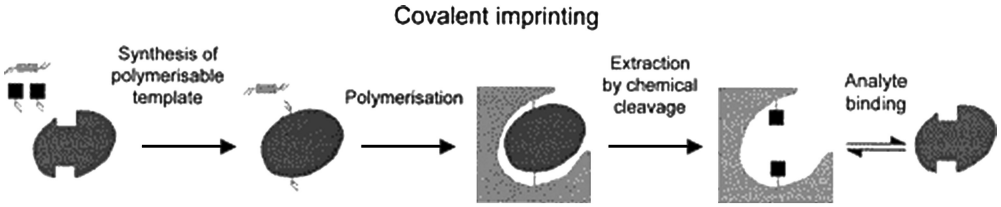

FIG. 1. Schematic representation of the covalent imprinting procedures (7).

is pertinent to mention here the two review articles by Zolotov (16) and by our group (17) on ion imprinted polymer–solid phase extraction for preconcentrative separation of organic/ inorganic pollutants. Hence, this review deals with the analytical methodologies developed for the determination of traces of toxic pesticides/inorganics using biomimetic sensors as of today. We earnestly hope this review and two articles Zolotov et al. and Prasada Rao et al. (16, 17) enthuse analytical chemists to gauze the potential of "Molecular Imprinting Technology" in undertaking this exciting area which has immense scope in environmental science i.e. either in detection/removal of toxic pesticides/inorganics. Hence, we tried to give bird's eye view of various strategies for synthesis and characterization of molecularly imprinted polymer recognition elements, transducers, employed to convert binding event into a detectable signal and integration of recognition element with transducer in addition to describing various biomimetic sensors developed for toxic pesticides and inorganics.

SYNTHETIC STRATEGIES FOR MIPs

Polymers with high affinity binding sites and selectivity can be produced by carefully optimizing the polymerization conditions that will stabilize the template-monomer complexes. The experimental variables include the type and relative amount of imprint molecule/ion, functional monomer and crosslinking agent, porogen used for imprinting, initiator and rebinding of the analyte, polymerization temperature and pressure. Various commonly employed imprinting strategies to introduce size and shape specific recognition sites into cross-linked polymers are described below.

Covalent or Stoichiometric Non-Covalent Imprinting

The covalent or pre-organized approach was primarily developed by Wulff and his coworkers (18) which employ strong, reversible covalent bonds usually involving a prior chemical synthesis step to link the monomers to the template (See Fig. 1) After polymerization, the imprinting molecule can be removed only by chemical cleavage. When covalent interactions are used, the binding site monomers can be employed in the exact stoichiometric ratio to the template molecule resulting in homogenous and higher binding constants resulting in most efficient catalytic systems. It is important to remark that the functional groups in the imprinted cavities are located on different polymer chain segments, which are held in definite mutual orientation simply by cross-links (reminiscent of the structure of active centres of enzymes).

Noncovalent or Self Assembly Imprinting

Noncovalent or self assembly approach was pioneered by Mosbach and coworkers (19). This involves host-guest complexes produced from weak intermolecular interactions (such as ionic or hydrophobic interactions, hydrogen bonding, van der Waals forces, $\pi - \pi$ bonds and metal coordinations) between analyte and monomer precursors. These self-assembled complexes are spontaneously established in the liquid phase and are then sterically fixed by free radical polymerization with a crosslinking monomer. After template extraction, various recognition sites that are specific to print molecule are established (See Fig. 2). The range of templates that can be used are wide and easier but results in heterogeneity.

Semicovalent or Sacrificial Spacer Imprinting

This is a hybrid imprinting strategy proposed by Whitcombe et al. (20, 21) that comprise a covalent imprinting step and subsequent rebinding by noncovalent interactions. This strategy was used for molecules with few functional groups and for one capable of creating recognition sites for templates carrying single or multiple spatially separated groups.

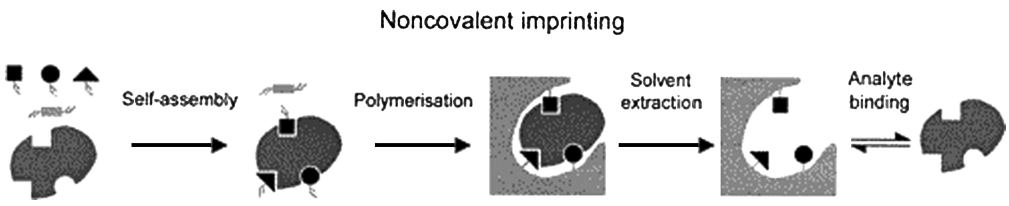

FIG. 2. Schematic representation of the non-covalent imprinting procedures (7).

Surface Imprinting

In 1992, Takagi's group (22) proposed a new imprinting technique based on oil-in-water (o/w) emulsion named as "Surface Imprinting." In 1994, Goto's group (23) have developed a similar technique but with water-in-oil (w/o) emulsion. Schematic diagram of latter technique of surface template polymerization with w/o emulsions is shown in Fig. 3. In both the techniques, the MIPs are prepared by emulsion using a functional host molecule, an emulsion stabilizer, a polymer matrix forming a monomer and a print molecule.

The functional host molecule, which is amphiphilic in nature, forms a complex with a print molecule during emulsion. Thus, the formed complex remains at the reaction surface. The organic phase containing the cross-linking agent is polymerized so that target selective cavities are created on the polymer surfaces, not inside the polymer matrix unlike covalent/non-covalent imprinting techniques. Thus, surface imprinting techniques offer faster rebinding kinetics and can handle water soluble templates such as metal ions and biological components.

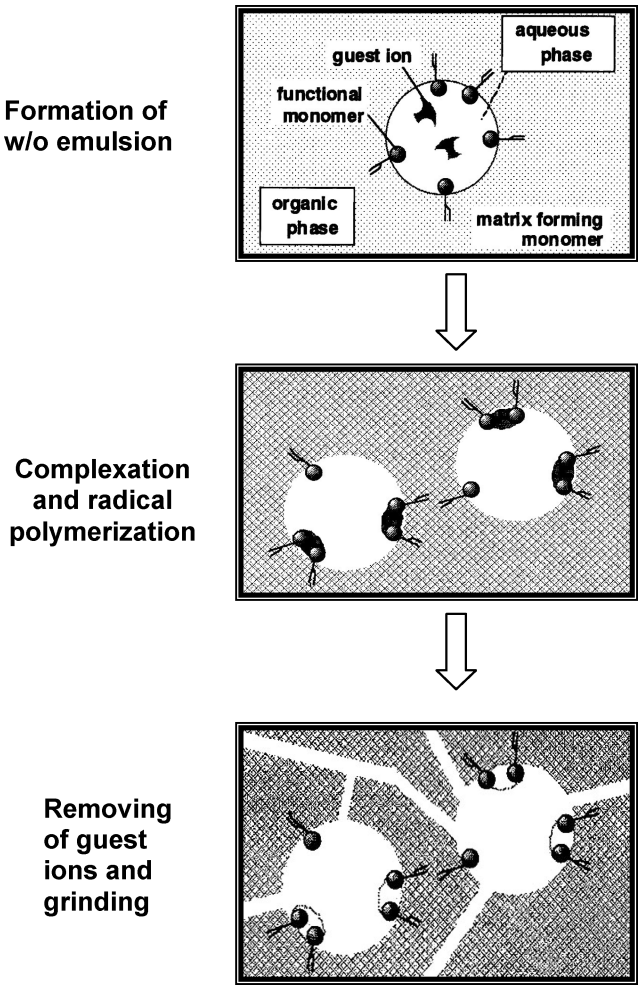

FIG. 3. Schematic diagram of surface template polymerization with w/o emulsions (23).

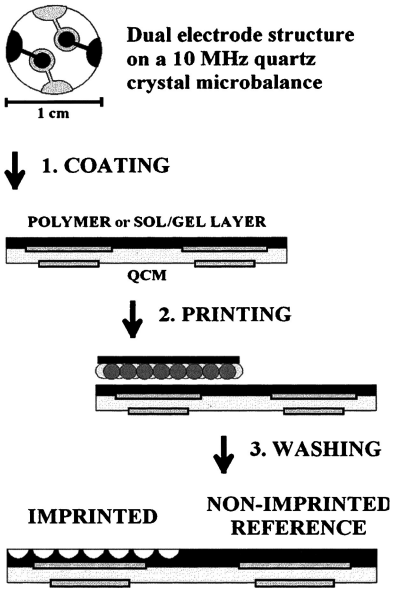

FIG. 4. Surface-imprinting process of precoated quartz crystal microbalances with dual-electrode structures (25).

Surface Imprinting on Inorganic Supports

In this, the print molecule is allowed to form adducts with functional monomers in solution and the formed complexes are subsequently allowed to bind to active inorganic supports such as silica wafers (24) quartz crystal (25) or titanium dioxide (26). Fig. 4 shows a typical surface imprinting process of precoated quartz crystal microbalances with dual electrode structures.

Imprinting by Sol-gel Technique

Templated sol-gel glasses has been prepared using organoalkoxysilanes. In one approach, organoalkoxysilanes, chosen for their affinity towards a template molecule, are combined with tetramethoxysilane to form a hybrid composite (27). In another approach, the template can be covalently attached to inorganic framework for e.g., a silicon alkoxide derivatized template can be coupled with tetramethoxysilane to form the cross-linked network (28).

Hierarchial Imprinting

The print molecule is first immobilized on the surface of a porous silica mould prior to polymerization. Subsequent dissolution of silica results in a "mirror image" pore system containing binding sites uniquely residing at the surface. The above imprinting process is known as Hierarchial templated synthesis (29, 30) (See Fig. 5).

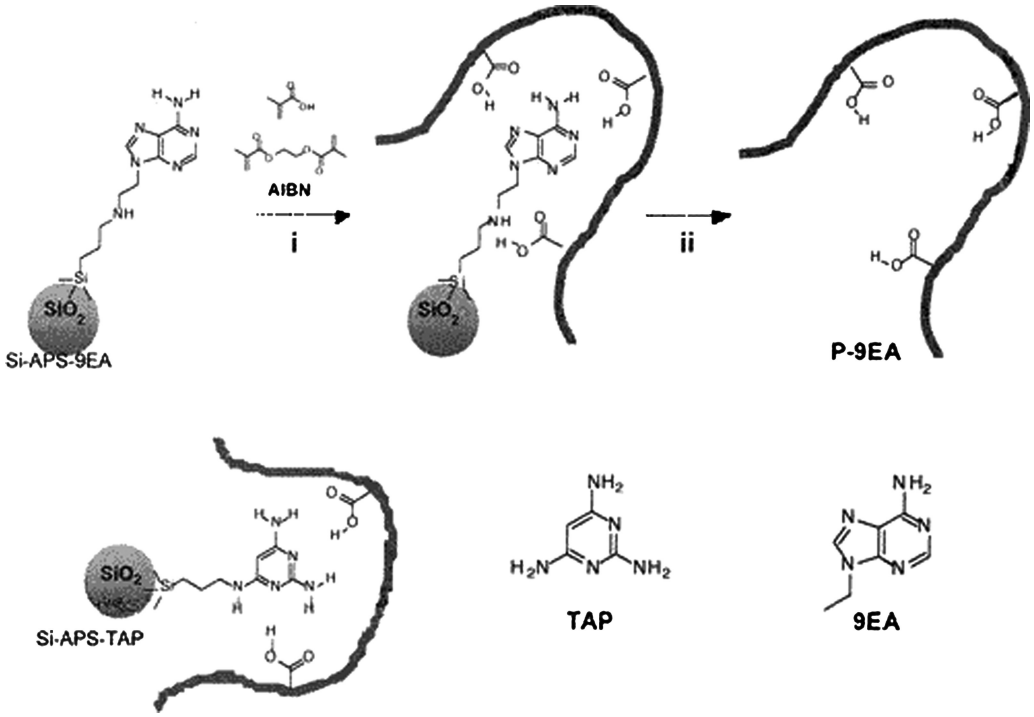

FIG. 5. Approach used to prepare surface-confined templated sites for structures containing the adenine or triaminopyrimidine functionality (29).

Heirachial Double Imprinting

Dai's group (31, 32) has synthesized novel materials with heirarchial structures based on double imprinting methodology (See Fig. 6). On the microporous level (1–3 Å), metal ions served as template. On the mesoporous level (diameters of 25–40 Å) micellar structures produced by self assembly of surfactant molecules were used as templates. Removal of both metal ions and surfactant micelles resulted in the formation of imprints with different sizes within the silica matrix, each with a specific function.

Hydrogels

Molecular imprinted polymers generally prepared by extensive crosslinking polymerization in the presence of template molecules, are obtained as rigid, insoluble, solid materials with frozen binding sites complementary to the template molecules. Understanding the physical basis of enzymes viz. solubility in aqueous media, preserving defined globular conformations in solution, and preparing the synthetic materials is of great interest to synthetic chemists. In recent years, a class of novel aqueous swellable polymers (hydrogels) has been synthesized which can respond to environmental changes and amplify them in the form of phase transitions (33). These gels are called "responsive" or "smart" gels and have been used to prepare artificial muscles, actuators, controlled release systems, sensors, optical shutters etc.

Ion Imprinting by Template Embedding

The first example of this method of imprinting was reported by Nishide and coworkers (34), who polymerized metal complex of 1-vinyl imidazole with 1-vinyl-2-pyrollidone and divinyl benzene. More recently, Lemaire and coworkers (35), Murray's group (36) and Say's group (37) have adopted this approach for selective separation of lanthanides, actinides and transition metal ions, respectively.

Ion Imprinting by Trapping

Prasada Rao and his coworkers have prepared lanthanide/actinide/noble metal IIP particles by single pot polymer synthesis by copolymerizing mixed ligand ternary complex (metal-chelating ligand-vinylated ligand) with styrene/HEMA/MAA (monomer), divinyl benzene/EGDMA (cross-linking monomer) in presence of 2,2′-azobis-isobutyronitrile (initiator). The imprint ion is selectively leached with mineral acid, while the chelate ligand is trapped in the polymer matrix. This allows selective rebinding of template ion from dilute aqueous solutions resulting in preconcentrative separation (38, 39).

CHARACTERISATION OF MIPs

The direct evidence for the formation of noncovalent monomer-template interactions can be studied by traditional spectroscopic techniques like NMR, CHN-analysis, FT-IR, UV-Visible or fluorimetric techniques. On the other hand, the study of MIP-template interactions is rather difficult on account of the

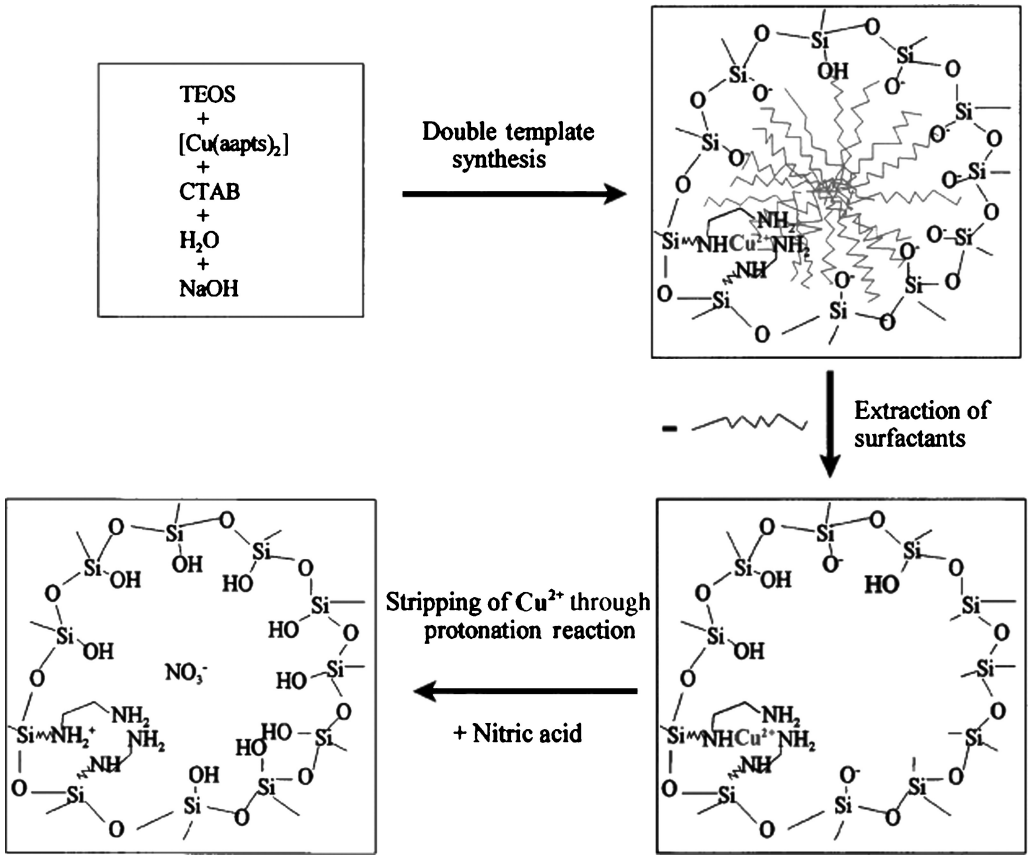

FIG. 6. Schematic diagram showing the synthesis of a hierarchically imprinted sorbent using Cu^{2+} and CTAB simultaneously as templates (32)

fact that MIPs are insoluble and intractable. Hence, these MIPs cannot be characterized by more commonly employed polymerization characterization methods that would require polymer solutions e.g., gel permeation chromatography, solution NMR techniques and UV measurements. Furthermore, since MIPs are amorphous, crystallographic or microscopic methods cannot be used to determine the structure of the MIP binding sites, although microscopy has aided the macroscopic understanding of MIP morphology (40, 41). Therefore, there are only a limited number of direct physical characterization methods for imprinted polymers. Cormack and Elorza (42) have briefly listed various morphological and chemical characterization methods that can possibly find use in MIP characterization. We now present a brief outline of various characterization techniques employed by MIP researchers with appropriate recent citations.

Morphological Characterization

The morphology of MIPs can be probed in the same way as that of the porous solids. Depending on the characterization technique, useful information can be deduced regarding pore volumes, pore diameter, pore size distribution and BET surface areas of the materials.

Water Uptake. Macroporous polymers are predominantly porous even in the dry state and uptake experiments can estimate the specific pore volume. Even though water uptake of PVC with various plasticizers is available, the corresponding data for MIPs are not available.

Swelling Ratio. Yoshida et al. (43) and Kala et al. (44) have evaluated the rigidity of the surface imprinted and erbium(III) IIPs synthesized by γ-irradiation by swelling ratio experiments with toluene by soaking for 30 minutes. These researchers have successfully correlated the swelling ratio values of IIPs formed by using different functional and crosslinking monomers with imprinting effect, percent extraction, equilibrium loading and selectivity coefficients.

Surface Area and Porosity. N_2 sorption porosimetry has been used for obtaining BET surface area, specific pore volume, average pore diameter and pore size distribution by measuring the amount of gas sorbed as a function of pressure, constructing sorption isotherms and application of BET theory and mathematical models. Karrison et al. (45), Biju et al. (46) and Daniel et al. (47) have employed this technique to distinguish the MIPs

based on pore size as microporous, mesoporous or macroporous. Sellergren and Shea (48) have studied the effect of porogens on surface area, pore volume and size of MIPs.

Relative Dielectric Constant. The relative dielectric constant of the plasticizer added to PVC while the forming membrane has significant influence on subsequent membrane sensor performance using potentiometry. Prasad et al. (49) have shown that the use of plasticizer with high dielectric constant gave Nernstian response over a wider range in case of dysprosium(III) IIP potentioselectrode as observed in the case of lanthanum(III) conventional ion selective electrode (50).

Microscopy (SEM/TEM/AFM). Optical microscopy can be used to verify the structural integrity of MIP beads and SEM/TEM/AFM can often image the surface morphology and correlate to the selective rebinding of the template. Gonzalez et al. (51) have employed SEM as a tool for studying the surface morphology of MIPs by altering the functional monomer, porogen or the volume of the latter during MIP synthesis. Ye et al. (52) and Yoshida et al. (53) have characterized molecularly imprinted microspheres and surface imprinted polymer materials using SEM. Perez-Moral and Mayes (54) and Daniel et al. (55) have characterized propanolol- and palladium(II)-imprinted polymer particles prepared by different polymerization methods using SEM. Same technique is used for characterization of polymer membranes prepared by embedding of UO_2^{2+}-vinyl benzoate in an ionically permeable membrane (56) and zinc(II) surface imprinted membrane (57). Koenig and Chechick (58) have employed TEM to characterize polymerizable gold nanoparticles embedded into macroporous cross-linked polymers. AFM has been employed to characterize the molecularly imprinted composites for the first time by Hilal et al. (59).

Chemical Characterization

The chemical characterization methods that can be used for solid samples can also find application in characterizing solid MIPs.

Elemental Microanalysis. Elemental microanalysis has been used to calculate the comonomer composition of the polymer and has been advantageously utilized by Lemaire's group (60) and our group (61) while characterizing gadolinium(III) and palladium(II) IIP resins/particles, respectively.

FTIR. The investigations of MIPs by FTIR spectroscopy are simpler with solid polymers than with a mixture of monomers in solution. Indeed solid polymers can be used directly, without other constituents, which do not interfere with the determination of polymer functionalities. Kobayashi et al. (62), Shea et al. (63), Lu et al. (64), Oral and Peppas (65) and our group (47, 61) have characterized imprinted polymers synthesized by different strategies using FT-IR spectra.

Solid-State NMR. Solid-state NMR was used to determine the polymerization yield and also to verify if the template molecule was still bound to the polymer after MIP synthesis (66). However, it was not possible to determine the presence of selective cavities with this experiment. Sasaki and Alam (67) examined the binding sites in an imprinted silica xerogels by using ^{31}P MAS NMR. MIPs prepared by phase inversion (which are not cross-linked) are not completely insoluble in some solvents and facilitate their characterization by proton NMR (68).

Energy Dispersive X-ray Spectroscopy (EDS). Gladis and Rao (69) have characterized uranyl IIP particles by EDS in ascertaining the complete removal of uranyl imprint ion and trapping of non-vinylated ligand viz. 5,7-dichloroquinoline-8-ol during leaching with 6M HCl.(See Fig. 7)

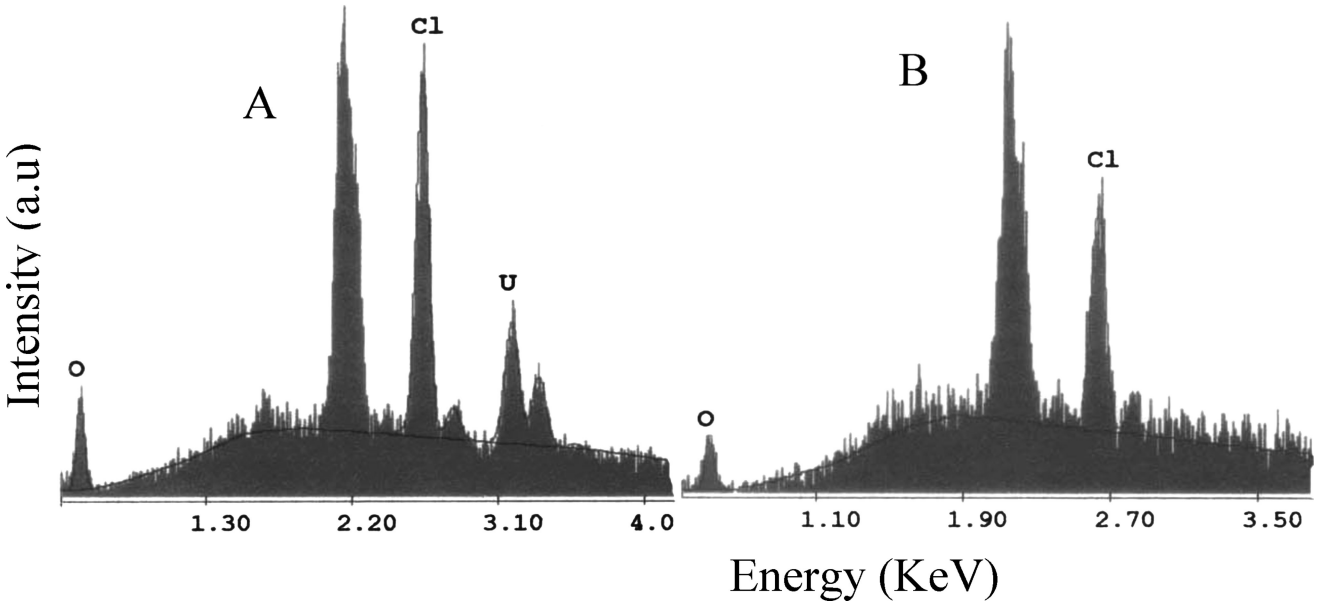

FIG. 7. EDS spectrum of (A) unleached and (B) leached uranium IIP particles.

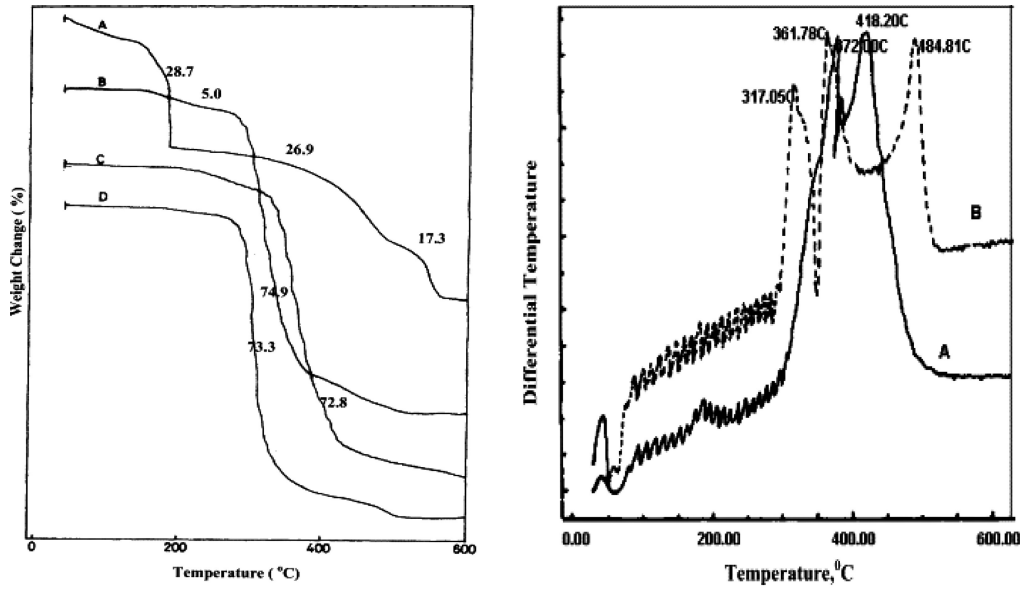

FIG. 8. (a) TGA plots of Er+DCQ+VP ternary complex (curve A), unleached IIP (curve B), CP (curve C) and leached IIP (curve D) particles. 8(b) DTA plots of unleached (curve A) and leached (curve B) erbium IIP particles.

Thermogravimetric (TGA) and Differential Thermal (DTA) Analysis. TGA/DTA studies were conducted by our group to characterize the unleached and leached imprinted polymer particles to prove that non-vinylated ligand viz. 5,7-dichloroquinoline-8-ol is intact even after leaching with mineral acid (70). (See Fig. 8)

X-ray Diffraction (XRD). The complete leaching of lanthanide/actinide/noble metal imprint ion on treatment with mineral acid was established by our group by comparing X-ray diffractograms of unleached and leached IIP particles with ternary complex of imprint ion (61,71,72). (See Fig. 9 for palladium IIP particles(61)). However, this method is not that sensitive to establish quantitative leaching of imprint ion.

UV-Visible Spectroscopy. As mentioned earlier, UV-visible spectra of solid IIP is not that sensitive unlike solution studies. However, the analysis of leachant solutions and from the blank values obtained during enrichment experiments using UV-Visible spectroscopy offer conclusive proof for complete leaching of particular inorganic imprint ion (47, 71, 72).

TRANDUCERS FOR MIP SENSORS

Various optoelectronic and electrochemical transducers that can be employed to convert a binding event into a detectable signal are shown in Chart 1. Blanco-Lopez et al. (10) in 2004 have reviewed the electrochemical transduction techniques while designing MIP-based sensors. We now review the optoelectronic transducers, viz. fluorimetric, UV-Visible, FT-IR, surface plasmon resonance and chemiluminescence.

Fluorescence

Fluorimetry is perhaps the most sensitive optically based measurement technique and is capable of yielding very low detection limits ($<10^{-7}$ M). Consequently, many groups have tried to exploit the development of fluorescence based sensors. The first real use of optical transducer in MIP based sensors was that of Kriz et al. (73) based on fibre optic detection of fluorescent templates. Piletsky et al. (74) have studied the effect of template on formation of fluorophores within the MIP. In subsequent work, Piletsky et al. (75) devised a somewhat simpler system for triazine herbicide based upon the competition of the template and a fluorescent analogue (reporter) for the binding sites within the MIP. Lulka and Chambers (76) describes the imprinting of silicas with fluorophores.

Cooper et al. (77) report the utilization of two novel fluorescent functional monomers in EGDMA based MIPs. Murray et al. (78) have further extended the scope of MIP based sensors by developing a fluorescent transducer based lead(III) sensor based on formation of fluorescent complex with binding of imprint ion. In a similar vein, Murray and coworkers (79, 80) have developed fluorescent transducer based optical sensors for chemical warfare agents or rather their hydrolysis products based on interaction of ancillary ligand with fluorescent metal complexes within the MIP. Subsequently, Dickert et al. (81) have created urethane-based MIPs for the detection of polycyclic aromatic hydrocarbons. Turkewitsch et al (82) have prepared fluorescent transducer based MIP sensor based on the quenching of functional monomer fluorescence upon binding of analyte. Haupt et al. (83) have devised a fluoroimmunoassay procedure for the herbicide, 2,4-D based on MIPs.

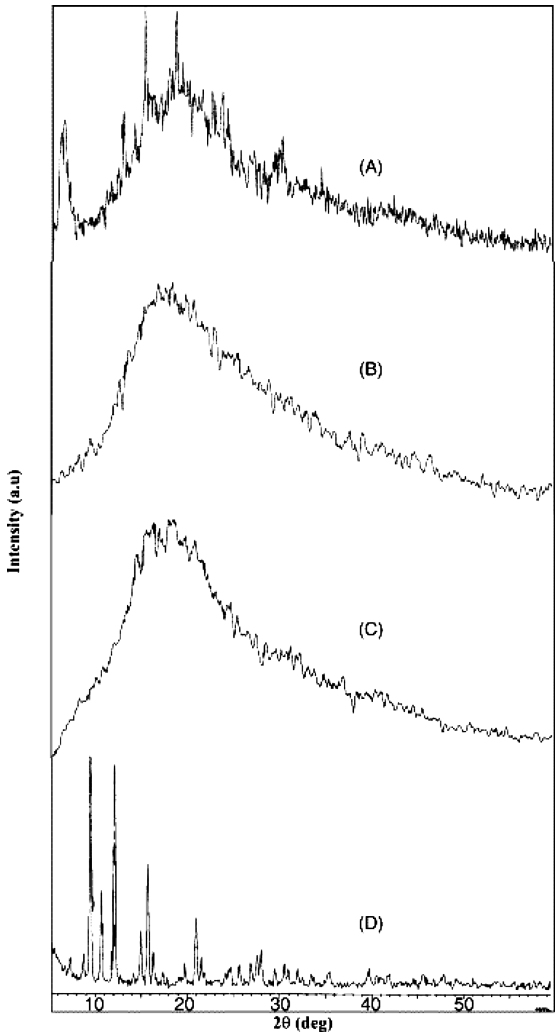

FIG. 9. XRD patterns of unleached (curve A); leached (curve B) palladium(II) IIP particles; CP (curve C); and Pd–AQ–VP ternary complex (curve D).

Absorptiometry

Kunitake and coworkers (84) have developed devices that allow the confirmation of optical observation, i.e., by colorimetric detection of solvent vapours using MIPs deposited on quartz crystals, using extremely sensitive independent mass-sensitive measurements.

Ellipsometry

The first demonstration of the use of MIPs in optical transducer based sensors was the work of Andersson et al. (85) using ellipsometry to quantify the amount of vitamin K bound in a monolayer of octadecylsilane supported on a silicon wafer.

Surface Plasmon Resonance (SPR)

SPR is a technique somewhat akin to ellipsometry, whereby the binding of organic molecules to a surface results in a change in the angle of incident light. Lai et al. (86) prepared MIP against theophylline, caffeine and xanthine and deposited these on silver-coated glass substrates. This is the first report that uses SPR to detect the binding of substrates to MIPs.

Chemiluminescence

The detection of light emitted from the biochemical degradation of a compound has the advantage that it requires neither an excitation source, as in fluorescence, nor a monochromator or an optical filter for the detection of resulting signal. Because the intensity of the emitted light is directly proportional to the concentration of analyte present, chemiluminescence is a very attractive detection technique for the study of MIP materials during rebinding. Haupt made an attempt to develop a chemiluminescent adsorbent assay for the detection of 2,4-D using non-related probes for competitive binding detection (87). More recently, Zhou et al. (88) prepared a flow-through sensor for the determination of chenbuterol in urine based on chemiluminescence transducer(See Fig. 10). Lin and Yamada (89) investigated the

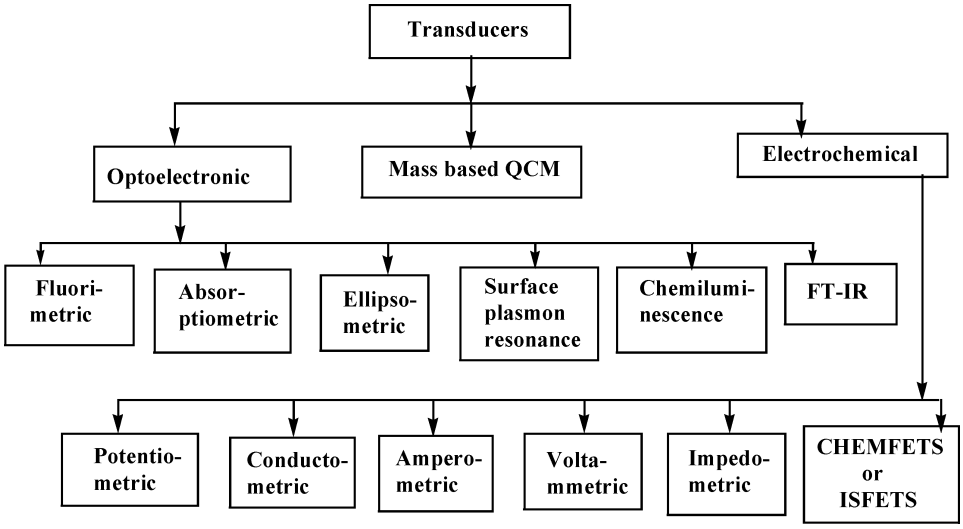

CHART 1. Types of transducers.

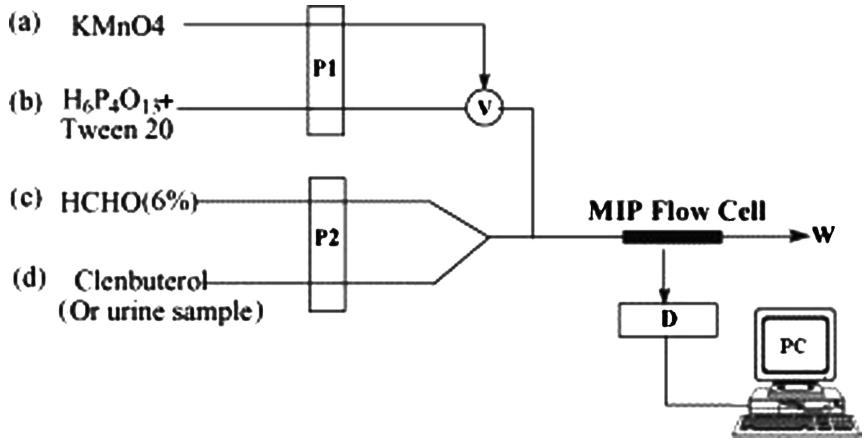

FIG. 10. Schematic diagram of the MIP-CL sensor flow system: (a) potassium permanganate solution; (b) polyphosphate + Tween-20 carrier; (c) formaldehyde; (d) clenbuterol solution; (P1, P2) peristaltic pump; (v) six-way injection valve; (W) waste; and (D) detector (88).

potential to prepare MIP sensor for 1,10-phenanthroline based on the decomposition of H_2O_2 with a ternary complex catalyst viz. Cu(II)-1,10-phenanthroline-4-vinylpyridine employing chemiluminescence transducer.

FT-IR

Jakush et al. (91) have developed a MIP sensor with IR transducer for 2,4-D by immobilizing MIP onto zinc selenide attenuated total refraction elements. Thick films (~5 mm) were immobilized onto the surface of transducers and upon exposure to test solutions, selective enrichment of the analyte in MIP layer was measured by observing mid-IR bands at 1595 and 1410 cm^{-1}, assigned to the anionic form of the carboxyl groups. Consequently, the approach could be very attractive for the continuous monitoring of pollution in water.

Quartz Crystal Microbalance (QCM)

Zhang et al. (92) developed MIP sensor with QCM transducer (see Fig. 11 for schematic diagram) by modifying piezoelectric quartz crystal with MIP membrane. For formation of MIP membrane, the MIP particles were suspended in 10 ml of tetrahydrofuran dissolving 5 mg of polyvinylchloride powder in it. About 10 μl of the suspension was spread onto the centre of the quartz crystal surface resulting in formation of thin membrane at room temperature. The sensor was coated with homogenous membrane of polymer by repeating the coating for 3 times.

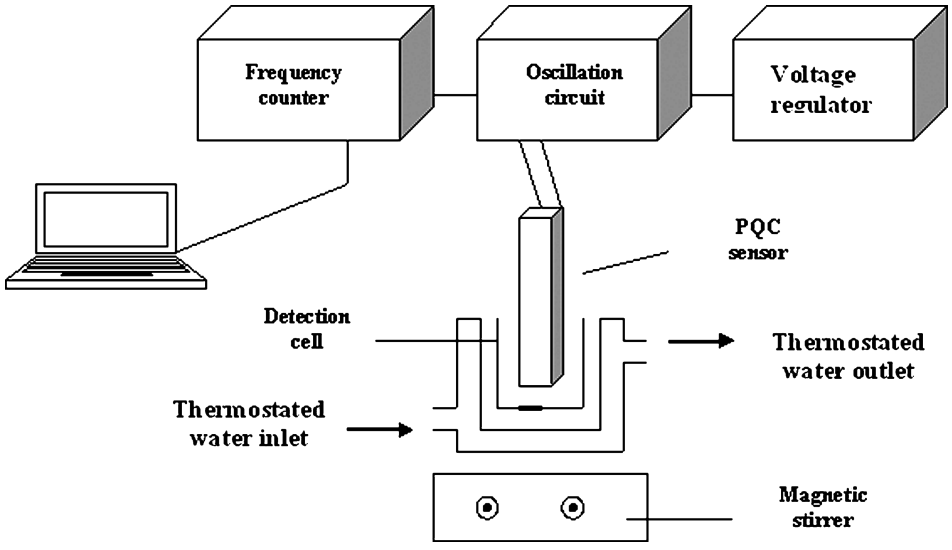

FIG. 11. Schematic diagram of the QCM-sensor device.

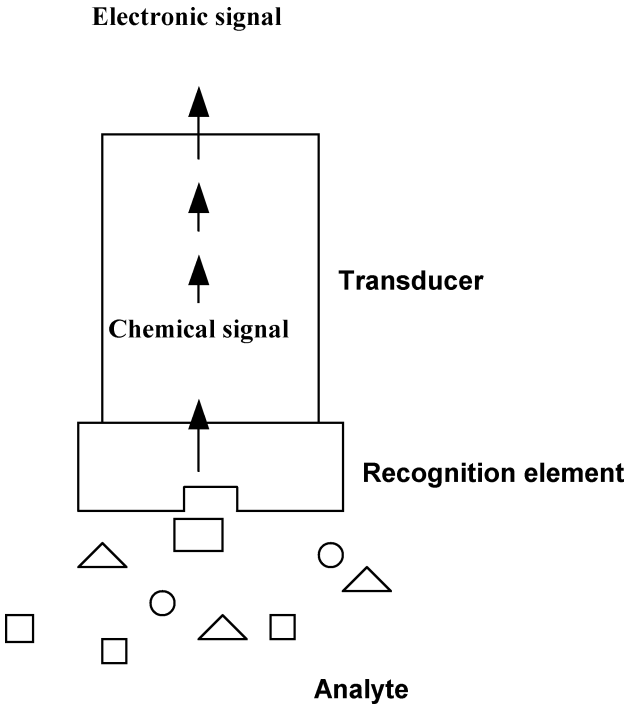

FIG. 12. Principle of MIP sensor.

IMMOBILIZATION/INTEGRATION OF MIPS WITH TRANSDUCERS

There is no doubt that polymers that display higher selectivity for a given analyte can be synthesized via MIPs. For use in sensors, however, one of the fundamental difficulties is combining the recognition element with a suitable transducer, in order to convert the binding event into a detectable signal. The pictorial representation of MIP sensor is given in Figure 12.

In-situ Polymerization

In-situ polymerization is the best immobilization procedure and consists of in situ synthesis of MIPs at the transducer surface and has the advantage of integrating the immobilization step into an automatic mass-production process.

Surface Coating

Spin or spray coating is another method of immobilizing MIPs as film onto a transducer surface. This strategy is more often employed todate. In any case, the control of layer thickness is necessary to adapt sensor response time and sensitivity. In general, three dimensional networks are preferred to two dimensional because they are more stable and more favourable for anchoring the molecule at several points. The affinities for the polymer are higher, although at the cost of a slower response, so thickness—response time must be in compromise. Manual deposition of a MIP layer is also used on various occasions.

Entrapment/Dispersion

Entrapment/dispersion of MIP particles into gels (93) membranes (94) is another approach of integrating MIPs with transducers. These prepared materials are applied onto the transducer surface. An inert soluble polymer such as PVC entraps the MIP particles. For sensing layers using particulate MIP, the sensor response time is closely related to the particle size. Studies hitherto reported used particles of fraction upto 25 or 50 μm, which generally led to slow kinetics for intraparticle diffusion and consequently long response times.

MIP-Based Composites

Another approach of integrating MIPs with transducer component is mixing of graphite or carbon paste, in the MIP matrix (95). In this way, the binding sites and the conducting particles are in close contact. A simple mechanical polishing can renew the sensor surface, hence offering the advantages in the mass production of biomimetic sensors.

MIP-BASED PESTICIDE SENSORS

Pesticides are classified based on their target group as insecticides, fungicides, herbicides and others. Again, based on chemical composition and structure of pesticide, compounds can be categorized into (i) organophosphate, (ii) carbamate, (iii) phenyl urea, (iv) triazine (v) chlorophenoxy acid and (vi) chlorinated hydrocarbon pesticides.

Organophosphate Pesticides

Marx et al. (96) developed thin films of molecularly imprinted sol-gel polymer with specific binding sites. The films were cast on glass substrate and on glassy carbon electrodes and were used to detect parathion in aqueous solutions by GC-FPD and cyclic voltammetry. Gas-phase binding measurements were performed on quartz crystal microbalance resonators. The binding was shown to be very sensitive to the type of functional monomer used for imprinting. The imprinted films showed high selectivity towards parathion in comparison to similar organophosphates. Furthermore, the authors discuss difference between molecular recognition in gas- and liquid-phase imprinted polymers. Li et al. (97) fabricated a novel sensor by sol-gel method using p-tert-butyl calix[6]–1,4-crown-4 as functional monomer. A fast response of parathion can be obtained in the range 5×10^{-9} to 1×10^{-4} M with a detection limit of 1×10^{-9} M after incubation in 0.1 M phosphonate buffer solution for 20 minutes. The extremely low detection limit allows the reliable monitoring of parathion in natural waters. The results of the analysis of real samples, such as rice, using the developed sensor compare well with HPLC.

Carbamate Pesticides

Flow through optosensors developed for carbaryl and warfarin make use of β-cyclodextrin bound to synthetic polymer (98). The detection limits were reported to be 6.3×10^{-8} M for

warfarin and 6.5×10^{-8} (aqueous) and 2.5×10^{-8} M (organic) for carbaryl, respectively. The R.S.Ds are 0.58 (at 1×10^{-6} M level) and 5% (at 9.45×10^{-7} M level) for warfarin and carbaryl, respectively. The low detection limits offered by optosensors allow a rapid reliable and precise determination of warfarin and carbaryl in natural waters.

Triazine Herbicides. Electrochemical and optical transducer based biomimetic sensors developed for triazine herbicides (especially atrazine) are summarized in Table 3 (90, 99–109). As seen from Table 3, TSM accoustic (104) and ISFET-QCM-based (105) sensors offer detection limits of 4 ppm (2000 nM) for atrazine which is much higher than maximum permissible limit in drinking water i.e., 3 ppb. On same vein, cyclic voltammetric (106) and potentiometric (108) based sensors offer detection limits of 10 ppb for atrazine are lower than above sensors but still higher than maximum permissible limits. Hence, all the above mentioned sensors are useful for monitoring contaminated natural waters. Of these, the potentiometric-based sensor (108) is ideal for field studies. On the other hand, conductometric transducer based atrazine sensor (90, 102–103) allows the monitoring of atrazine both in contaminated and uncontaminated natural waters as the detection limit is 1 ppb. The only draw back is that it required laboratory based instrumentation and is not suitable for field studies. The simplification in instrumentation while designing conductometric sensors will go a long way to solve this problem.

Chlorophenoxy Acid Herbicides. MIP-based recognition materials synthesized by non-covalent imprinting and then coupling with various signal transduction strategies for the detection of 2,4-D are summarized in Table 4 (83, 110–114). Of the few sensors designed for 2,4-D, it is seen from Table 4 that fluorescence (83) and bulk acoustic wave-based (113) sensors alone offer detection limits of 20 ppb (100 nM), which are lower than maximum permissible limit in drinking water, viz. 70 ppb. Hence, these two sensors should find wide application in monitoring contaminated and uncontaminated natural waters. On the other hand, on-line FTIR (112) and electrochemical (111) transducer-based sensors can only be useful in monitoring contaminated natural waters.

MIP-BASED IONOMER SENSORS

Until recently, most of the published accounts for "sensors" have described the phenomena that may lead to a sensing device, not actual devices. Unlike gas sensors, ionic sensors must be used in solution. Mass-sensitive devices such as quartz crystal microbalance or surface wave acoustic sensors cannot usually be employed. The two major methods used for signal transduction in ionic sensors or selectrodes are based on electrochemical and spectroscopic properties (115). The synthesis of polymers exhibiting selective binding of a specific cation involves the formation of cavities equipped with complexing groups or "ligands" so arranged as to match the charge, coordination number, coordination geometry and size of the target cation. The combination of molecular imprinting and transduction selectivities can result in sensors that exclusively recognize target analytes and not interfering species. The selectrodes so far designed for toxic inorganic ions are of two types: (1) Potentioselectrodes (potentiometry transducer based and (2) Optrodes (optical transducer based).

Potentioselectrodes

The first imprinted polymer potentioselectrode was described for calcium and magnesium ions by Mosbach's group (116). The monomer used in the fabrication of electrode was a neutral ionophore N,N′-dimethyl-N,N′-bis(4-vinylphenyl)-3-oxapentadiamide. The imprinting process enhanced the selectivity for calcium by factors of 6.0 and 1.7 on calcium binding using Ca^{2+}- and Mg^{2+}-imprinted polymers respectively, over an unimprinted blank, as measured by back extraction. Murray's group (117) has prepared lead(II) potentioselectrode by employing lead (vinyl benzoate)$_2$ complex for preparing imprinted polymer material. This selectrode results in Nernstian response from 10^{-6}–10^{-2} M of lead(II). In view of this, the developed lead(II) potentioselectrode is not useful for the monitoring of lead(II) in uncontaminated natural water samples as the maximum permissible limit in drinking water is 30 ppb or 1.5×10^{-7}M. The same group (118) has subsequently developed uranyl ion potentioselectrode by employing uranyl-vinyl benzoate/vinyl salicylaldoxime complexes for the preparation of imprinted polymer particles. Our group was successful in fabrication of uranyl (119) and dysprosium ion (49) sensing potentioselectrodes by a single pot synthesis of imprinted polymer particles (sensing materials) with a mixed ligand complex–UO_2^{2+} /Dy^{3+}- 5,7-dichloroquinoline-8-ol-4-vinyl pyridine. In the above-mentioned devices, the IIP particles after leaching imprint ion were dispersed homogenously in a polyvinylchloride matrix via sonication. The dysprosium ion potentioselectrode senses dysprosium(III) ion in the concentration ranges 8×10^{-6}–10^{-1} M (Nernstian response) with a detection limit of 2×10^{-6} M while the blank membrane electrode does not respond below 10^{-4} M. The selectivity coefficients for dysprosium(III) over alkali, alkaline earth and transition metal ions are in the range of $\sim 10^{-4}$. The dysprosium ion potentioselectrode enables the determination of fluoride in mouthwash solution by an indirect potentiometric titration with ethylenediaminetetraacetic acid. On the other hand, the uranyl ion potentioselectrode (schematically shown in Fig. 13) senses uranyl ion in the concentration range 2×10^{-8} to 1×10^{-2} M with a detection limit of 2×10^{-8} or 4.8 ppb. Hence, the developed potentiometric sensor has the capability of monitoring even uncontaminated natural or sea water samples as the maximum permissible limit for uranium in drinking water is 30 ppb. In addition, the sensor showed a good selectivity for uranyl ion over alkali, alkaline earth, transition and heavy metal ions. The analysis results of sea water samples by uranyl ion sensor is comparable with neutron activation analysis values.

TABLE 3
Biomimetic sensors for triazine herbicides

Template/Analyte	Monomers	Initiator	Imprinting strategy	Transducer	Working range (mM)	Detection limit (nM)	Ref.
Atrazine	MAA- (or) Diethylamino-ethylmethacrylate (DEAEM)/EGDMA	AIBN	Non-covalent	Conductometry	0.001–0.004	—	(99)
Atrazine	MAA-DEAEM/EGDMA	AIBN	Non-covalent	Fluorescence	0.01–100	—	(100)
Atrazine	DEAEM/MAA/allylamine/4-vinylphenyl boromic acid—EGDMA	AIBN	Covalent/non-covalent	Conductometry	0.001–0.05	—	(101)
Atrazine	MAA-tri(ethyleneglycol) dimethacrylate MAA-tri(TEGDMA)	AIBN	Non-covalent	Conductometry		5	(90)
Atrazine	MAA-TEGDMA	AIBN	Non-covalent	Conductometry	—	5	(102)
Atrazine	MAA-TEGDMA	AIBN	Non-covalent	Conductometry	—	5	(103)
Trialkylmelanain/ atrazine	MAA-EGDMA	AIBN	Non-covalent	Conductometry	—	5	(103)
Atrazine	MAA-EGDMA	AIBN	Non-covalent	TSM acoustic	—	2000	(104)
Triazine herbicides	Acrylamide-MAA	AIBN	Non-covalent	ISFET/QCM	0.001–0.08	2000	(105)
Atrazine	MAA-EGDMA	AIBN	Surface	Cyclic voltammetry	0.001–0.01	50	(106)
Atrazine	MAA-EGDMA	AIBN	Surface	Cyclic voltammetry	0.001–0.01	—	(107)
Atrazine	Styrene-DVB	AIBN	Non-covalent	Potentiometry	0.001–10	50	(108)
Triazine herbicides	—	—	—	—	46–2200	—	(109)

TABLE 4
Biomimetic sensors for chlorophenoxy acid herbicides

Template/ Analyte	Monomers	Initiator	Imprinting strategy	Transducer	Working range (mM)	Detection limit (nM)	Ref.
2,4-D	—	—	Non-covalent	Fluorescence		100	(83)
2,4-D	MMA, EGDMA	—	—	—	—	—	(110)
2,4-D	4-Vinylpyridine—EGDMA	AIBN	Non-covalent	Electrochemical	1–1000	—	(111)
2,4-D	4-Vinyl functional pyridine/ / EGDMA MAA filling inert monomers	2,2′-Azobis (2,4-dimethylaeronitrile)	Non-covalent	Online FT-IR	4.5–4500	—	(112)
2,4-D	MAA, EGDMA	AIBN		Bulk acoustic wave	2×10^{-7} to 5×10^{-4}	100	(113)
2,4-D	3-[N,N′-bis(9-anthrylmethyl amino]propyl triethoxysilane	—	Sol-gel	Luminescence quenching	—	—	(114)

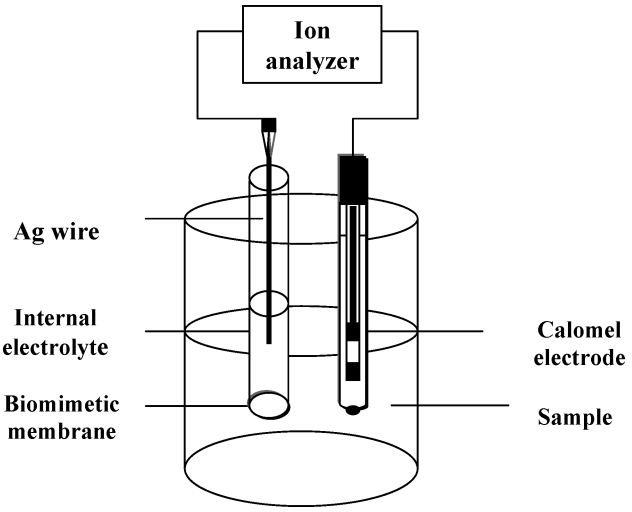

FIG. 13. Schematic diagram of IIP-based dysprosium(III) ion selective electrode.

Optrodes

Murray's group (118) has described a fluoro-optrode for the first time by employing lead(II)–methyl-3,5-divinylbenzoate (DVMB) complex during IIP preparation. This optrode was prepared by binding 3% lead(II)–DVMB complex (2% divinylbenzene) by in situ copolymerization on a vinylated 400 μm optical fibre surface. The lead(II) imprint ion was removed from the polymer by first swelling in a mixture of methanol and water and then soaking in stirred solution of EDTA ($\sim$1 hour each). The calibration from 70–7 $\times$ 10^5 ppb showed a detection limit of 50 ppb. Unlike the potentioselectrode developed by the same group, the lead(II) optrode can be used for monitoring lead in uncontaminated natural waters as the detection limit is in the same range as that of maximum permissible limit in drinking water, i.e., 30 ppb.

Al-Kindy et al. (120) prepared aluminium sensing materials via non-covalent imprinting technique using the aluminium(III)–morin complex. Based on the fluorescent properties of the chelate, a selective optical flow-through sensor was developed for aluminium. The affinity of the polymer binding sites was higher for aluminium than for other di- and trivalent ions (e.g., Be(II), Ca(II), Mg(II), Eu(III), Zn(II), Fe(III)) suggesting that the nature of metal ion, its ionic radius and the metal-morin stoichiometry play important roles in the ionic recognition.

CONCLUSIONS AND OUTLOOK

We have outlined various synthetic strategies to prepare molecularly imprinted polymer recognition elements and their characterization and transducers employed for converting binding event into a detectable signal. However, one of the fundamental difficulties in the design and development of biomimetic sensors is coupling of chemical recognition element with an appropriate transducer. In spite of this, both electrochemical and opto-electronic transducer-based MIP sensors have been developed for toxic pesticides and inorganics by various researchers. A brief outline of such biomimetic sensors is given.

As mentioned earlier, the low-key attitude in developing electrochemical biomimetic sensors lies in integrating artificial recognition elements with electrochemical transducers. With the developments in MIP technology viz. formation of thin films, composite materials conducting polymers, catalytic polymers etc it is very likely that a new generation of MIP based electrochemical sensors will be established in the next decade. To circumvent the limitations of macroporous cross-linked methacrylate, acrylamide and styrene polymers as materials, other materials and morphologies based on self-assembled monolayers, sol-gels, surface and hierarchial imprinting and several other approaches will be investigated in future. A development worth mentioning for future work is the expanding research on electronic noses and tongues.

The development of optical MIP-based sensors essentially rely on chromogenic (either inherent or labeled) or non-chromogenic analytes and majority of them rely upon fluorescence measurements to achieve optimal sensitivity. Thus, it seems that the fluorimetric technique will continue to receive increasing attention. Direct measurements using a variety of sensing devices such as flow analytical devices, wave guide devices, sensing plate devices and fibre optic devices should prove extremely useful.

Historically, regarding the discipline of MIPs and MIP sensor areas as well, most researchers are skilled in polymer chemistry and are interested in polymers rather than sensing. Recently, analytical chemists have realized the potential of MIPs as analytical tool, be it separation or detection and few groups have initiated programmes in this direction. With this changed scenario, one can envisage the fabrication of biomimetic sensors which enable validation and analysis of various environmental, biological, geological and metallurgical samples.

ACKNOWLEDGMENT

The corresponding author is thankful to Kerala State Committee on Science, Technology and Environment Department (STED), Govt. of Kerala, India for sponsoring a project on "MIP-based sensors for pesticides."

REFERENCES

1. E. Berman, *Toxic Metals and their Analysis* (Heyden & Son Ltd., London, 1980).
2. E. Hogendoorn and P. Van Zoonen, Recent and future developments of liquid chromatography in pesticide trace analysis. *J. Chromatogr. A* 892 (2000):435–453.
3. S. Vidyasankar and F. H. Arnold, Molecular imprinting: Selective materials for separations, sensors and catalysis. *Curr. Opin. in Biotech.* 6 (1995):218–224.
4. F. L. Dickert and O. Hayden, Molecular imprinting in chemical sensing. *Trends Anal. Chem.* 18 (1999): 192–199.

5. F. L. Dickert and O. Hayden, Imprinting with sensor development—On the way to synthetic antibodies. *Fresenius J. Anal. Chem.* 364 (1999):506–511.
6. K. Ensing and T. deBoer, Tailor-made materials for tailor-made applications: Application of molecular imprints in chemical analysis. *Trends Anal. Chem.* 18 (1999):138–145.
7. K. Haupt and K. Mosbach, Molecularly Imprinted Polymers and Their use in Biomimetic Sensors. *Chem. Rev.* 100 (2000):2495–2504.
8. S. A. Piletsky and A. P. F. Turner, Electrochemical sensors based on molecularly imprinted polymers. *Electroanalysis* 14 (2002):317–323.
9. A. Markoci and S. Alegret, New materials for electrochemical sensing IV. Molecular imprinted polymers. *Trends Anal. Chem.* 21 (2002):717–725.
10. M. C. Blanco-Lopez, M. J. Lobo-castanon, A. J. Miranda-Ordieres, and P. Tunon-Blanco, Electrochemical sensors based on molecularly imprinted polymers. *Trends Anal. Chem.* 23 (2004):36–48.
11. M. Trojanowicz and M. Weislo, Electrochemical and piezoelectric enantioselective sensors and biosensors. *Anal. Lett.* 38 (2005):523–547.
12. H. Hisamoto and K. Suzuki, Ion-selective optodes:Current developments and future prospects. *Trends Anal. Chem.* 18 (1999):513–524.
13. S. Al-Kindy, R. Badia, J. Luis Swarez-Rodriguez, and M. Elana Diaz-Garcia, Molecularly imprinted polymers and optical sensing applications. *Crit. Rev. Anal. Chem.* 30 (2000):291–309.
14. O. Y. F. Henry, D. C. Cullen, and S. A. Piletsky, Optical interrogation of molecularly imprinted polymers and development of MIP sensors: A review. *Anal. Bioanal. Chem.* 382 (2005):947–956.
15. K. Haupt, Molecularly imprinted polymers in analytical chemistry. *Analyst* 126 (2001):747–756.
16. S. G. Dmitrienko, V. V. Irkha, A. Yu. Kuznetsova, and Yu. A. Zolotov, Use of molecular imprinted polymers for the seperation and preconcentration of organic compounds. *J. Anal. Chem.* 59 (2004):808–817.
17. T. Prasada Rao, S. Daniel, and J. Mary Gladis, Tailored materials for preconcentration or seperation of metals by ion-imprinted polymers for solid phase extraction (IIP-SPE). *Trends Anal. Chem.* 23 (2004): 28–35.
18. G. Wulff and A. Sarhan, The use of Polymers with enzyme-analogous structures for the resolution of racemates. *Angew Chem. Int. Ed. Engl.* 11 (1972):341–343.
19. R. Arshady and K. Mosbach, Synthesis of substrate-selective polymers by host-guest polymerization. *Makromol. Chem.* 182 (1981):687–692.
20. M. J. Whitcombe, M. E. Rodriguez, P. Villar, and E. N. Vulfson, A new method for the introduction of recognition site functionality into polymers prepared by molecular imprinting: Synthesis and characterization of polymeric receptors for cholesterol. *J. Am. Chem. Soc.* 117 (1995):7105–7111.
21. N. Kirsch, C. Alexander, M. Lubke, M. J. Whitcombe, and E. N. Vulfson, Enhancement of Selectivity of imprinted polymer Via post-imprinting modification of recognition sites. *Polymer* 41 (2000):5583–5590.
22. K. Y. Yu, K. Tsukaghoshi, M. Maeda, and M. Takagi, Metal ion-imprinted Microspheres prepared by reorganization of the coordinating groups on the surface. *Anal. Sci.* 8 (1992):701–703.
23. M. Goto, Imprinted metalselective ion exchanger, in *Ion exchange and Solvent Extraction: Series of Advances*, Eds. A. K. Sengupta, Y. Marcus and J. A. Mariusky (Marcel Dekker Inc., New York, 2001), 259–293.
24. D. C. Tahmassebi and T. Sasaki, Synthesis of a new trialdehyde template for molecular imprinting. *J. Org. Chem.* 59 (1994):679–681.
25. F. L. Dickert and O. Hayden, Bioimprinting of polymers and sol-gel phases. Selective detection of yeasts with imprinted polymers. *Anal. Chem.* 74 (2002):1302–1306.
26. M. Lahav, A. B. Kharitonov, O. Katz, T. Kunitake, and I. Wilner, Tailored chemosensors for Chloroaromatic acids using molecular imprinted TiO_2 thin films on ion-sensitive field-effect transistors. *Anal. Chem.* 73(2001):720–723.
27. R. Makote and M. M. Collinson, Template recognition in inorganic-organic hybrid films prepared by the sol-gel process. *Chem. Mater.* 10 (1998):2440–2445.
28. M. Hunnius, A. Rufinska, and W. F. Maier, Selective surface adsorption versus imprinting in amorphous microporous silica. *Microporous Mesoporous Mater*. 29 (1999):389–403.
29. M. M. Titrici, A. J. Hall,and B. Sellergreen, Hielrarchially imprinted stationary phases: Mesoporous polymer beads containing surface-confined binding sites for adenine. *Chem. Mater.* 14 (2002):21–23.
30. E. Yilmaz, K. Haupt, and K. Mosbach, The use of immobilized templates—A new approach in molecular imprinting. *Angew Chem. Int. Ed.* 39 (2000):2115.
31. S. Dai, M. C. Burleigh, Y. H. Ju, H. J. Gao, J. S. Lin, S. J. Pennycook, C. E. Barnes, and Z. L. xue, Hierarchically imprinted sorbents for the seperation of metal ions. *J. Am. Chem. Soc.* 122 (2000): 992–993.
32. S. Dai, Hierarchically imprinted sorbents. *Chem. Eur. J.* 7 (2001):763–768.
33. A. R. Khare, and N. A. Peppas, The structure and transport properties of environmental sensitive hydrogels. *Polymer News* 16 (1991):230–236.
34. M. Kato, H. Nishide, E. Tsuchida, and T. Sasaki, Complexation of metal ion with poly(1-vinylimidazole) resin prepared by radiation-induced polymerization with template metal ion. *J. Polym. Sci. Polym. Chem. Ed.* 19 (1981):1803–1809.
35. R. Garcia, C. Pinel, C. Madic, and M. Lemaire, Ionic imprinting effect in gadolinum/lanthanum Separation. *Tetrahedron Lett.* 39 (1998):8651–8654.
36. S. Y. Bae, G. L. Southard, and G. M. Murray, Molecularly imprinted ion exchange resin for purification preconcentration and determination of UO_2^{2+} by spectrophotometry and plasma spectrometry. *Anal. Chim. Acta* 397 (1999):173–181.
37. R. Say, E. Birlik, A. Ersoz, F. Yilmaz, T. Gedibey, and A. Denizli, Preconcentration of copper on ion-selective imprinted polymer microbeads. *Anal. Chim. Acta* 480 (2003):251–258.
38. T. Prasada Rao, S. Daniel, and J. M. Gladis, Tailored materials for preconcentration or separation of metals by ion-imprinted polymers for solid-phase extraction (IIP-SPE). *Trends Anal. Chem.* 23 (2004):28–35.
39. V. M. Biju, J. M. Gladis, and T. Prasada Rao, Ion imprinted polymer particles: Synthesis, characterization and dysprosium ion uptake properties suitable for analytical applications. *Anal. Chim. Acta* 478 (2003):43–51.

40. K. Karim, F. Breton, R. Rouillon, E. V. Piletska, A. Gurreiro, I. Chianella, and S. A. Piletsky, How to find effective functional monomers for effective Molecular imprinted polymer? *Adv. Drug Delivery Rev.* 57 (2005):1795–1808.
41. A. Spivak, Optimization, evaluation, and characterization of molecularly imprinted polymers. *Adv. Drug Delivery Rev.* 57 (2005):1779–1794.
42. P. A. G. Cormack, and A. Z. Elorza, Molecularly imprinted polymers: Synthesis and characterisation. *J. Chromatogr. B* 804 (2004):173–182.
43. M. Yoshida, K. Uezu, M. Goto, and S. Furusaki, Required properties for functional monomers to produce a metal template effect by a surface molecular imprinting technique. *Macromolecules* 32 (1999): 1237–1243.
44. R. Kala, V. M. Biju, and T. Prasada Rao, Synthesis, characterization, and analytical applications of erbium(III) ion imprinted polymer particles prepared via γ-irradiation with different functional and crosslinking monomers. *Anal. Chim. Acta* 549 (2005):51–58.
45. J. G. Karlsson, B. Karlsson, L. I. Andersson, and I. A. Nicholls,The roles of Template Complexation and ligand binding conditions on recognition in bupivacaine Molecularly imprinted polymers. *Analyst* 129 (2004):456–462.
46. V. M. Biju, J. M. Gladis, and T. Prasada Rao, Effect of gamma-irridation of ion imprinted polymer (IIP) particles for the preconcentrative separation of dysprosium from other selected Lanthanides. *Talanta* 60 (2003):747–754.
47. S. Daniel, J. M. Gladis, and T. Prasada Rao, Synthesis of imprinted polymer material with palladium ion nanopores and its analytical application. *Anal. Chim. Acta* 488 (2003):173–182.
48. B. Sellergren and K. J. Shea, Influence of polymer morphology on the ability of imprinted network polymers to resolve enantiomers *J. Chromatogr.* 635 (1993):31–49.
49. K. Prasad, R. Kala, T. Prasada Rao, and G. R. K. Naidu, Ion imprinted polymer based ion-selective electrode for the trace determination of dysprosium (III) ions. *Anal. Chim. Acta* 566 (2006):69–74.
50. M. Shamsipur, M. Yousefi, M. Hosseini, and M. Reja Ganjali, Lanthanum (III) PVC membrane electrodes based on 1,3,5-trithiacyclohexane. *Anal. Chem.* 74 (2002):5538–5543.
51. G. P. Gonzalez, P. F. Hernando, and J. S. D. Alegria, A morphological study of molecularly imprinted polymers using the scanning electron microscope. *Anal. Chim. Acta* 557 (2006):179–183.
52. L. Ye, R. Weiss, and K. Mosbach, Synthesis and characterization of molecularly imprinted microspheres. *Macromolecules* 33 (2000):8239–8245.
53. M. Yoshida, Y. Hatate, K. Uezu, M. Goto, and S. Furusaki, *Coll. Surf. A Physicochem. Eng. Aspects* 169 (2000):259–269.
54. N. Perez-Moral and A.G. Mayes, Comparative study of imprinted polymer particles prepared by different polymerisation methods. *Anal. Chim. Acta.* 504 (2003):15–21.
55. S. Daniel, P. Prabhakara Rao, and T. Prasada Rao, Investigation of different polymerization methods on the analytical performance of palladium (II) ion imprinted polymer materials. *Anal. Chim. Acta* 536 (2005):197–206.
56. A. Kimaro, L.A. Kelly, and G.M. Murray, Molecularly imprinted ionically permeable membrane for uranyl ion. *Chem. Commun.* 14 (2001):1282–1283.
57. K. Araki, T. Maruyama, N. Kamiya, and M. Goto, Metal ion-selective membrane prepared by surface molecular imprinting. *J. Chromatogr. B* 818 (2005):141–145.
58. S. Koenig and V. Chechik, Au nanoparticle-imprinted polymers. *Chem. Commun.* (2005):4110–4112.
59. N. Hilal, V. Kochkodan, L. Al-Khatib, and G. Busca, Characterization of molecularly imprinted composite membranes using an atomic force microscope. *Surf. Interface Anal.* 33 (2002):672–675.
60. O. Vigneau, C. Pinel, and M. Lemaire, Ionic imprinted resins based on EDTA and DTPA derivatives for lanthanides(III) separation. *Anal. Chim. Acta* 435 (2001):75–82.
61. S. Daniel, P. E. J. Babu, and T. Prasada Rao, Preconcentrative separation of palladium (II) using palladium(II) ion-imprinted polymer particles formed with different quinoline derivatives and evaluation of binding parameters based on adsorption isotherm models. *Talanta* 65 (2005):441–452.
62. P. S. Reddy, T. Kobayashi, M. Abe, and N. Fujii, Molecular imprinted Nylon-6 as a recognition material of amino acids. *Eur. Polym. J.* 38 (2002):521–529.
63. K. J. Shea and D. Y. Sasaki, An analysis of small molecule Binding to functionalised synthetic polymers by 13C CP/MAS NMR and FT-IR Spectroscopy. *J. Am. Chem. Soc.* 113 (1991):4109–4120.
64. Y. Lu, Ch. Li, x. Wang, P. Sun, and X. Xing, Influence of polymerization temperature on the molecular recognition of imprinted polymers. *J. Chromatogr. B* 804 (2004):53–59.
65. E. Oral and N. A. Peppas, Dynamic studies of molecular imprinting polymerisations. *Polymer* 45 (2004): 6163–6173.
66. D. Valihinger, K. Landfester, I. Krauster, H. Brunner, and G. E. M. Tovar, Molecularly imprinted polymer nanospheres as synthetic affinity receoptors obtained by miniemulsion polymerisation. *Macromol. Chem. Phys.* 203 (2002):1965–1973.
67. D. Y. Sasaki and T. M. Alam, Solid-state P-31 NMR study of phosphonate binding sites in guanidine-functionalized, molecular imprinted silica xerogels *Chem. Mater.* 12 (2000):1400–1407.
68. T. Kobayashi, T. Fukuya, M. Abe, and N. Fujii, Phase inversion molecular imprinting by using template copolymers for high substrate recognition. *Langmuir* 18 (2002):2866–2872.
69. J. M. Gladis and T. Prasada Rao, Effect of porogen type on the synthesis of uranium ion imprinted polymer materials for the preconcentration/separation of traces of uranium. *Microchim. Acta* 146 (2004): 251–258.
70. R. Kala, J. M. Gladis, and T. Prasada Rao, Preconcentrative separation of erbium from Y, Dy, Ho, Tb and Tm by using ion imprinted polymer particles via solid phase extraction. *Anal. Chim. Acta* 518 (2004): 143–150.
71. P. Gopi Krishna , J. M. Gladis, T. Prasada Rao, and G. R. K. Naidu, Selective recognition of neodymium (III) using ion imprinted polymer particles. *J. Mol. Recognit.* 18 (2005):109–116.
72. P. Metilda, J. Mary Gladis and T. Prasada Rao, Influence of binary/ternary complex of imprint ion on the analytical applications of uranyl ion imprinted polymer materials. *Ana. Chim. Acta* 512(2004):63–73.
73. D. Kriz, O. Ramstrom, A. Svensson, and K. Mosbach, A biomimetic sensor based on a molecularly imprinted polymer as a recognition element combined with fiber-optic detection. *Anal. Chem.* 67 (1995):2142–2144.

74. S. A. Piletsky, E. V. Piletskaya, K. Yano, A. Kugimiya, A. V. Elgersma, R. Levi, U. Kahlow, T. Takeuchi, I. Karube, T. I. Panasyuk, and A. V. Elskaya, A biomimetic receptor system for sialic acid based on molecular imprinting. *Anal. Lett.* 29 (1996):157–170.
75. S. A. Piletsky, E. V. Piletskaya, A. V. Elskaya, R. Levi, K. Yano, and I. Karube,Optical detection system for triazine based on molecularly imprinted polymers. *Anal. Lett.* 30 (1997):445–455.
76. M. F. Lulka, J. P. Chambers, E. R. Valdes, R. G. Thompson, and J. J. Valdes, Molecular imprinting of small molecules with organic silanes: Fluorescence detection. *Anal. Lett.* 30 (1997):2301–2313.
77. M. E. Cooper, B. P. Hogg, and D. L. Gin, Design and synthesis of novel fluorescent chemosensors for biologically active molecules. *Polym. Prepr.* 38 (1997):209–210.
78. G. M. Murray, A. L. Jenkins, A. Bzhelyansky, and O. M. Uy, Molecularly imprinted polymers for the selective sequestering and sensing of ions. *Johns Hopkins APL Technical Digest* 18 (1997):464–472.
79. A. L. Jenkins, O. M. Uy, and G. M. Murray, Polymer based lanthanide luminescent sensors for the detection of nerve agents. *Anal. Commun.* 34 (1997):221–224.
80. A. L. Jenkins, O. M. Uy, and G. M. Murray, Polymer-based lanthanide luminescent sensor for detection of the hydrolysis product of the nerve agents. *Anal. Chem.* 71 (1999):373–378.
81. F. L. Dickert, H. Besenbock, and M. Tortschanoff, Molecular Imprinting through van der Waals Interactions: Fluorescence Detection of PAHs in Water. *Adv. Mater.* 10 (1998):149.
82. P. Turkewitsch, B. Wandelt, G. D. Darling, and W. S. Powell, Fluorescent functional recognition sites through molecular imprinting. A Polymer-based fluorescent chemosensor for aqueous cAMP. *Anal. Chem.* 70 (1998): 2025–2030.
83. K. Haupt, A. G. Mayes, and K. Mosbach, Herbicide assay using an imprinted polymer-based system analogous to competitive fluoroimmunoassays. *Anal. Chem.* 70 (1998):3936–3939.
84. S. W. Lee, I. Ichinose, and T. Kunitake, Molecular imprinting of azobenzene carboxylic acid on a TiO_2 ultrathin film by the surface sol-gel process. *Langmuir* 14 (1998):2857–2863.
85. L. I. Andersson, C. F. Mandenius, and K. Mosbach, Studies on guest selective molecular recognition on an octadecyl silylated silicon surface using ellipsometry. *Tetrahedron Lett.* 29 (1988):5437–5440.
86. E. P. C. Lai, A. Fafara, V. A. Vandernoot, M. Kono, and B. Polsky, Surface plasmon resonance sensors using molecularly imprinted polymers for sorbent assay of theophylline, caffeine, and xanthine. *Can. J. Chem.* 76 (1998):265–273.
87. K. Haupt, Molecularly imprinted sorbent assays and the use of non-related probes. *React. Funct. Polym.* 41 (1999):125–131.
88. H. J. Zhou, Z. J. Zhang, D. Y. He, Y. F. Hu, Y. Huang, and D. L. Chen, Flow chemiluminescence sensor for determination of clenbuterol based on molecularly imprinted polymer. *Anal. Chim. Acta* 523 (2004): 237–242.
89. J. M. Lin, and M. Yamada, Chemiluminescent flow-through sensor for 1,10-phenanthroline based on the combination of molecular imprinting and chemiluminescence. *Analyst* 126 (2001):810–815.
90. T. A. Sergeyeva, S. A. Piletsky, A. A. Brovko, E. A. Slinchenko, L. M. Sergeeva, and A. V. Elskaya, Selective recognition of atrazine by molecularly imprinted polymer membranes. Development of conductometric sensor for herbicide detection. *Anal. Chim. Acta* 392(1999):105–111.
91. M. Jakush, M. Janotta, and B. Mizaikoff, Molecularly imprinted polymers and infrared evanescent wave spectrocopy. A chemical sensors approach. *Anal. Chem.* 71 (1999):4786–4791.
92. Z. Zhang, H. Li, H. Liao, L. Nie, and S. Yao, Infleunce of cross-linkers amount on the performance of the piezoelectric sensor modified with molecularly imprinted polymers. *Sensors Actuators B* 105(2005):176–182.
93. D. Kriz and K. Mosbach, Competitive amperometric morphine sensor based on an agarose immobilised molecularly imprinted polymer. *Anal. Chim. Acta* 300 (1995):71–75.
94. S. Kroger, A. P. F. Turner, K. Mosbach, and K. Haupt, Imprinted polymer-based sensor system for herbicides using differential-pulse voltammetry on screen-printed electrodes. *Anal. Chem.* 71 (1999):3698–3702.
95. A. Pizzariello, S. Miroslav, S. Stredanska, and M. Stanislav, A solid binding matrix/molecularly imprinted polymer-based sensor system for the determination of clenbuterol in bovine liver using differential-pulse voltammetry. *Sensors Actuators B* 76 (2001):286–294.
96. S. Marx, A. Zaltsman, I. Turyan, and D. Mandler, Parathion sensor based on molecularly imprinted sol-gel films. *Anal. Chem.* 76 (2004):120–126.
97. Ch. Li, Ch. Wang, B. Guan, Y. Zhang, and S. Hu, Electrochemical sensor for the determination of parathion based on p-tert-butylcalix[6]arene-1,4-crown-4 sol-gel film and its characterization by electrochemical methods. *Sensors Actuators B: Chem.* 107 (2005):411–417.
98. R. Badía and M. E. Díaz-García, Cyclodextrin-based optosensor for the determination of warfarin in waters. *J. Agri. and Food Chem.* 47 (1999):4256–4260.
99. S. A. Piletsky, E. V. Piletskaya, A. V. Elgersma, K. Yano, I. Karube, Yu. P. Parhometz, and A. V. El'skaya, Atrazine sensing by molecularly imprinted membranes. *Biosensors Bioelectronics* 10 (1995):959–964.
100. S. A. Piletsky, E. V. Piletskaya, A. V. El'skaya, R. Levi, K. Yano, and I. Karube, Optical detection system for triazine based on molecularly-imprinted polymers. *Anal. Lett.* 30 (1997):445–455.
101. S. A. Piletsky, E. V. Piletskaya, T. L. Panasyuk, A. V. El'skaya, R. Levi, I. Karube, and G. Wulff, Imprinted membranes for sensor technology: Opposite behavior of covalently and noncovalently imprinted membranes. *Macromolecules* 31 (1998):2137–2140.
102. T. A. Sergeyeva, S. A. Piletsky, A. A. Brovko, E. A. Slinchenko, L. M. Sergeeva, T. L. Panasyuk, and A. V. El'skaya, Conductometric sensor for atrazine detection based on molecularly imprinted polymer membrane. *Analyst* 124 (1999):331–334.
103. J. Matsui, K. Fujiwara, and T. Takeuchi, Atrazine-selective polymers prepared by molecular imprinting of trialkylmelamines as dummy template species of atrazine. *Anal. Chem.* 72 (2000):1810–8113.
104. C. Luo, M. Liu, Y. Mo, J. Qu, and Y. Feng, Thickness-shear mode acoustic sensor for atrazine using molecularly imprinted polymer as recognition element. *Anal. Chim. Acta* 428 (2001):143–148.
105. S. V. Pogorelova, T. Bourenko, A. B. Kharitonov, and I. Willner, Selective sensing of triazine herbicides in imprinted membranes using ion-sensitive field-effect transistors and microgravimetric quartz crystal microbalance measurements. *Analyst* 127 (2002):1484–1491.

106. R. Shoji, T. Takeuchi, H. Suzuki, and I. Kubo, Atrazine sensor based on a nano chemical receptor modified electrode. *Bunseki Kagaku* 52 (2003):1141–1146.
107. R. Shoji, T. Takeuchi, and I. Kubo, Atrazine sensor based on molecularly imprinted polymer-modified gold electrode. *Anal. Chem.* 75 (2003):4882–4886.
108. K. Prasad, K. P. Prathish, J. M. Gladis, and T. Prasada Rao, Molecularly imprinted polymer (biomimetic) based potentiometric sensor for atrazine (Communicated to Sensors and Actuators B 123 (2007):65–70.
109. Y. Andreu, F. Baldini, A. Giannetti, and A. Mencaglia, Mathematical model for the analytical signal of an herbicide sensor based on the reaction centre of *Rhodocbacter sphaeroides*. *Talanta* 65 (2005): 586–592.
110. Y. Nomura, H. Mugurama, K. Yano, A. Kugimiya, S. McNiven, and K.Ikebukuro, Selective recognition of 2,4-dichlorophanoxyacetic acide using a molecularly imprinted polymer. *Anal. Lett.* 31 (1998):973–980.
111. S. Kroger, A. P. F. Turner, K. Mosbach, and K. Haupt, Imprinted polymer-based sensor system for herbicides using differential pulse voltammetry on screen-printed electrodes. *Anal. Chem.* 71 (1999):3698–3702.
112. M. Jakusch, M. Janotta, B. Mizaikoff, K. Mosbach, and K. Haupt, Molecularly imprinted polymers and infrared evanescent wave spectroscopy. A chemical sensors approach. *Anal. Chem.* 71(1999):4786–4791.
113. Ch. Liang, H. Peng, L. H. Nie, and S. Z. Yao, Bulk acoustic wave sensor for herbicide assay based on molecularly imprinted polymer. *Fresenius J. Anal. Chem.* 367 (2000):551–555.
114. M. K. P. Leung, Ch-F. Chow, and M. H. W. Lam, A sol-gel derived molecular imprinted luminescent PET sensing material for 2,4-dichlorophenoxyacetic acid. *J. Mater. Chem.* 11 (2001):2985–2991.
115. G. M. Murray, and O. M. Uy in *Molecularly Imprinted Polymers*, Ed. B. Sellergren (Elsevier, Amsterdam, 2001), 441.
116. T. Rosatzin, L. I. Andersson, W. Simon, and K. Mosbach, Preparation of Ca^{2+} selective sorbents by molecular imprinting using polymerisable ionophores. *J. Chem. Soc. Perkin Trans.* 2 (1991):1261–1265.
117. X. Zeng, A. C. Bzhelyansky, and G. M. Murray, *Proc. of 1996 ERDEC Conf. on Chem. & Bio. Def. Res.* Aberdeen Proving Ground, Bethesda, Maryland (1997):545.
118. G. M. Murray, A. L. Jenkins, A. C. Bzhelyansky, and O. M. Vy, Molecularly imprinted polymers for the selective sequestering and sensing of ions. *John Hopkins APL Tech.* 18 (1997):464–472.
119. P. Metilda, K. Prasad, R. Kala, J. M. Gladis, T. P. Rao, and G. R. K. Naidu, Ion imprinted polymer based sensor for monitoring toxic uranium in environmental samples. *Anal. Chim. Acta* 582 (2007):147–153.
120. S. Al-Kindy, R. Badia, and M. E. Diaz-Garcia, Fluorimetric monitoring of molecular imprinted polymer recognition events for aluminium. *Anal. Lett.* 35 (2002):1763–1774.