Molecularly Imprinted Electrochemical Sensors

Vembu Suryanarayanan,[a]* *Cheng-Tar Wu*,[b] *Kuo-Chuan Ho*[b,c]*

[a] ElectroOrganic Division, Central Electro Chemical Research Institute, Karaikudi 630006, India
tel: +91-4565-227550; fax: +91-4565-227779
[b] Department of Chemical Engineering, National Taiwan University, Taipei 10617, Taiwan
[c] Institute of Polymer Science and Engineering, National Taiwan University, Taipei 10617, Taiwan
tel: +886-2-2366-0739; fax: +886-2-2362-3040
*e-mails: manasvini1@rediffmail.com; kcho@ntu.edu.tw

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Abstract
In this review, the applications of molecularly imprinted polymer (MIP) materials in the area of electrochemical sensors have been explored. The designs of the MIPs containing different polymers, their preparation and their immobilization on the transducer surface have been discussed. Further, the employment of various transducers containing the MIPs based on different electrochemical techniques for determining analytes has been assessed. In addition, the general protocols for getting the electrochemical signal based on the binding ability of analyte with the MIPs have been given. The review ends with describing scope and limitations of the above electrochemical based MIP sensors.

Keywords: Electrochemical sensors, Molecularly imprinted polymers (MIPs), Biosensors

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1. Introduction

Biosensors and chemical sensors have attracted considerable attention in the field of modern analytical chemistry because of their extreme sensitivities and specificities. They can be successfully employed for the analysis of materials in different fields such as clinical diagnostics, environmental control, food analysis and drug screening. The central part of such sensors is the chemical or biological molecular recognition element, also named as, receptors, which can recognize and bind the target analyte specifically. For biosensors, these recognition elements include antibodies, enzymes, DNA probes and cells. The receptors are in close contact with transducers that converts the recognition between biological element and the target in to quantifiable signal. One such example is the immunosensor that results from the antigen-antibody binding where the structurally related molecules are rejected from the binding sites.

These immunosensors have only limited applications in chemical assays as they involve expensive extraction and need highly controlled environment process for the production of antigen. In order to replace these natural systems, artificial receptors are gaining particular importance in recent years and even they mimic the recognition properties of biological materials (biomimetics). One such artificial receptor follows the group namely Molecularly Imprinted Polymers (MIPs). The MIPs have high selectivity and affinity constant when compared to naturally occurring systems for sensor applications. They are very stable and robust, and are resistant to degradation in extreme environments such as in acids and bases or at high temperatures and pressures. Moreover, these materials are much cheaper than their counterpart and can be stored in a dry state at room temperature for long periods of time. The first report on this field was published by Wulf et al., from Düsseldorf University, who described the experiments on the preparation of synthetic polymers with receptor properties for sugar derivatives [1]. Thereafter, different kind of substances such as sugars, amino acids, peptides, drugs, steroids, metals ions, dyes etc., have been imprinted and studied successfully.

As reflected from several reviews, though the main application of the MIPs continues to be in the field of separation technology [2–3], the employment of this imprinting method in the area of chemical sensors is also progressing very well [4–9]. Chemical sensors may be classified into three general categories such as optical, piezoelectric and electrochemical, on the basis of their transduction. Of this, electrochemical transducers become very popular due to ease of measuring and the availability of instrumentation. Moreover, they are found to be effective in offering good limits of detection (LOD), at low cost with the possibility of easy miniaturization and automation. Hence, this type of transduction is very attractive in making small devices for recognition based on the MIP technology in the field of clinical and pharmaceutical industries. Based on this, in recent years, more studies on this field have been explored and this has been reflected in three reviews, published already [10–12].

The aim of this article is to explore the application of the MIPs in electrochemically based sensors. The present

review focuses on recent advances in the literature on the preparation of both noncovalent and covalent MIP-based electrochemical sensors with special emphasis on the achievements to date in analytical chemistry.

2. Molecular Imprinting Technique

Molecular imprinting technique involves bulk polymerization of monomer in presence of target analyte (imprint molecule) containing a porogenic solvent. The functional monomer initially forms a complex with the target molecule and after polymerization, their functional groups are held in position by the highly cross-linked polymeric structure. Upon removal of the molecular template, the material retains binding sites that are complementary in size and shape to the analyte. This process is very similar to the "lock and key" theory of enzymes and as result of this, a molecular memory is introduced into the polymer, in which the analyte is now able to rebind on the same imprinted material with a very high specificity. The porogenic solvent helps not only to dissolve all the ingredients, but also to create pores or tiny holes in the finished polymer through which the binding sites will be accessible to the analyte. The polymerization must be carried out in the absence of air as the oxygen molecules may interfere with intermediates formed during the course of the reaction. The force of attraction between the monomer and the imprint molecule may be of either covalent or noncovalent and sometimes, a combination of these two. Typical two-dimensional representation of the process of an imprinting technique for the both covalent and noncovalent interaction is shown in Figures 1a and b respectively. In general, it is noted that imprinting efficiency (yield of binding sites relative to amount of the imprint molecule) is higher for covalent imprinting than its counter part and former protocols yields more homogeneous population of the binding sites [13]. However, the noncovalent imprinting is more flexible for the choice of monomer, target molecules and the use of the imprinted materials. Moreover, it is very similar to a natural process in a sense that most of the biomolecular bindings are noncovalent in nature [14].

2.1. Different Recognition Elements

Following the preassembly step, the polymerization is started thermally or photochemically with an initiator and a cross linker dissolved in an inert solvent via free radical pathway. Ultra violet (UV) light was used as a source for polymerization of the monomer in case of the noncovalent imprinting and after the polymerization is complete, the template is removed either by extracting with solvents/hydrolysis for noncovalent imprinting or by chemical cleaving from the polymer for the covalent imprinting method and this process leaves sites complementary in size and shape to the template molecule. The block of the polymer is then grounded, sieved and finally, they are coated on the surface of transducer as monolith or film.

2.1.1. Organic Polymers

The polymer materials can be prepared directly in the form of spherical beads of specified diameter by different routes such as precipitation or emulsion polymerization and the physical configuration of the MIPs varied with the different methods of their preparation. In the case of the precipitation polymerization, the preparative method is very similar to regular polymerization process except the relative amount of the solvent taken is very high. In a recent work, the morphologies of microspherical particles of methacrylate polymers (from methacrylic acid as monomer and trimethylpropane trimethaacrylate as cross-linker) prepared by both precipitation polymerization and traditional bulk polymerization route were compared using Scanning Electron Microscopy (SEM) [15]. The morphologies of the MIPs formed by the precipitation polymerization and the traditional bulk polymerization are shown in Figures 2a and b respectively. It can be seen from the SEM micrographs that the precipitation polymerization gives a uniform, microspherical particle. Owing to the control of the separation point during precipitation polymerization that begins from a dilute monomer solution, uniform microspheres were obtained which possessed higher surface areas and more complementary sites than those produced by bulk polymer-

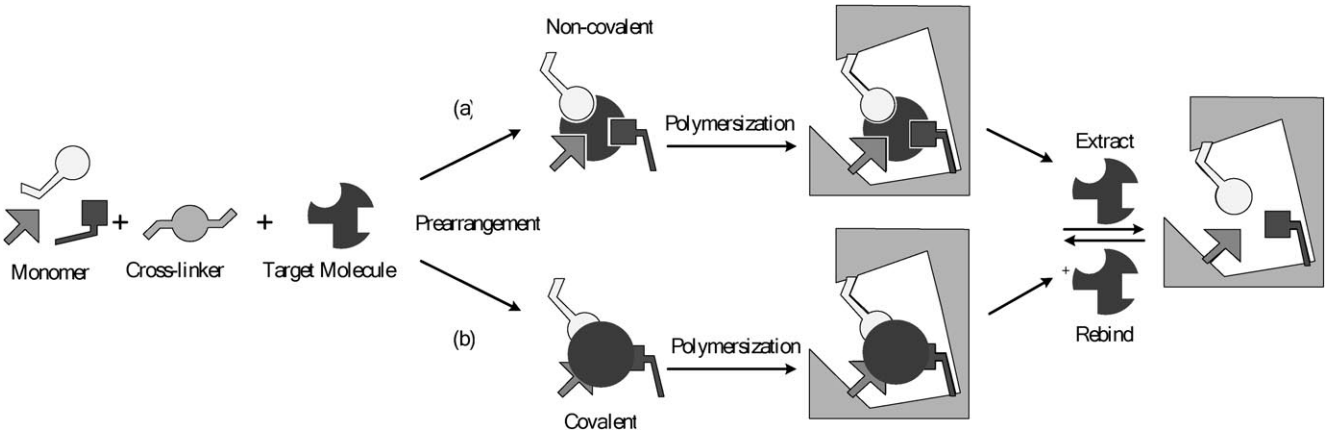

Fig. 1. Schematic overview of molecular imprinting technique; (a) and (b) shows the pathway for noncovalent and covalent imprinting.

ization. Table 1 summarizes the preparation conditions and characteristics for traditional and precipitation polymerizations. The mean sizes of particles are about 500 nm and the surface area is 265.6 m^2/g [15]. In case of emulsion polymerization, small beads are created in an oil-water biphasic system stabilized by surfactant. As an example, cholesterol was imprinted using pyridinium 12-(cholesteryloxycarbonyloxy)dodecane sulfate [16].

The selection of functional monomers and cross linkers is very important to the imprinting process and they must have accessible binding sites for the interaction of the analyte. Better results could potentially be achieved using combinatorial approaches, where tens, hundred and even thousands of polymers can be synthesized and tested in order to select the best one [17–18]. Further, the kind of molar ratio of the monomers is varied by using automated procedures. Turner et al proposed a method which included computational screening of a virtual library of functional monomers against a target molecule followed by section of those able to form the strongest complex with the template [19, 20].

The most common functional monomer poly(methacrylate) (PM), is prepared from the bulk polymerization of methacrylic acid (MAA) [21–40]. For this polymerization reaction, ethylene glycol dimethacrylate (EDMA) [21–24, 27–32, 36, 37, 40–45], tri(ethylene glycol) dimethacrylate (TEDMA) [25, 26], trimethylpropane trimethacrylate (TRIM) [15, 33–35], divinylbenzene (DVB) [37, 38] and dipentaerythrithol hexaacrylate (DPHA) [35] were employed as cross linkers. Other molecularly imprinted electrochemical transducers synthesized from polymeric materials such as poly(vinylimidazole) (poly(VID)) [46, 47], and poly(vinylpyridine) (poly(VPD)) [38, 39, 48–50] and poly(diethylamino)ethylmethacrylate) ((poly(DEAEM)) [51] in combination with EDMA have also been employed. Further, studies on the MIPs composed of poly(pyrrole) (PPy) [52], poly(stryne)/(DVB) [53] and poly(melamine-*co*-chloranil) (poly(mel-*co*-chl)) [54–56], polyphenylboronic acid (p-APBA) [57] and polyethylamine (p-EA) [58] were reported.

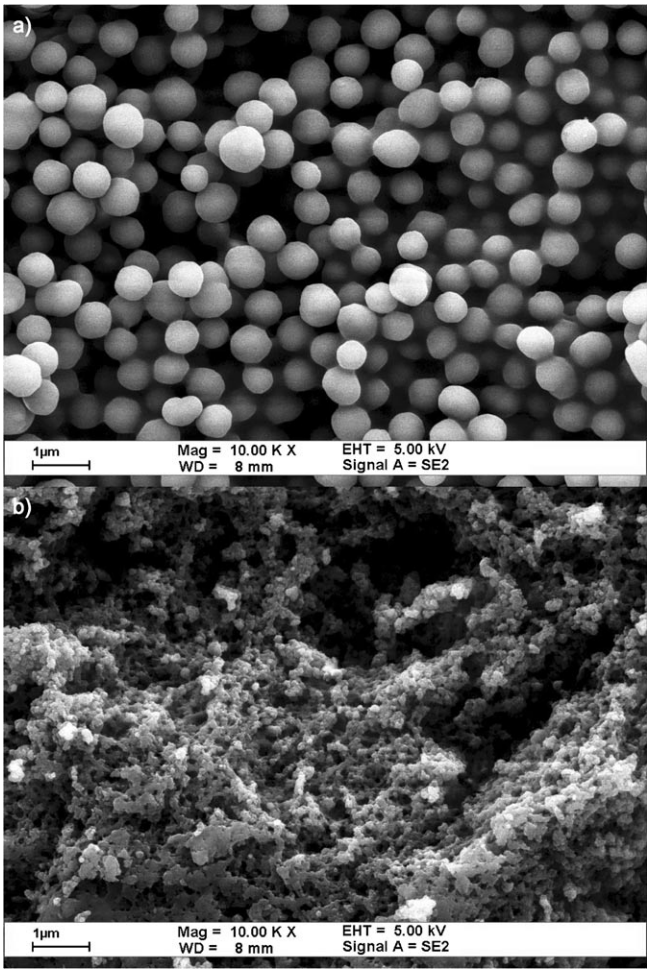

Fig. 2. The morphologies of MIP particles prepared by (a) precipitation polymerization, (b) traditional bulk polymerization [15].

Thermal polymerization was initiated with 2,2′-azobis 2-isobutyronitrile (AIBN) whereas photochemical polymerization was undertaken with acetophenone, benzophenone and AIBN. Example of porogenic solvent is dimethylformamide (DMF) and in aqueous solution, poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (poly(AMPS)) was prepared using a cross linker namely, *N,N′*-methylenebisacrylamide (MBA) [59–61].

In addition to the importance of selecting the functional monomers and cross linkers, their relative ratio plays an important role in determining the performance of the sensors. The presence of higher level of cross linkers makes the MIPs extremely hard and fragile. The optimal ratio of the cross linker and functional monomer was found to be 85:15 (w/w) with excellent specificity and flexibility of the imprinted membrane [26]. Polymers can be made more flexible without loosing their specificity by adding a plasticizer such as oligourethane acrylates to the composition [25–26]. Sometimes, as low as 20% addition of cross linker does work well and this has been observed with batch binding assays [18]. The other conditions that should be monitored during the course of polymerization are temperature rate (thermal) and light flux (photo).

2.1.2. Self-Assembled Monolayers (SAMs)

The imprinting method in the polymer materials has been focused on the three dimensional networks. In contrast, a two dimensional imprinting of self-assembled monolayers (SAMs) of mercaptan and template molecules, on any metal, has been the object of many studies in sensor technology. The technique involves the simultaneous sorption of the template and the mercaptan molecules on gold electrode, followed by the extraction of template. An imprinting of SAMs by alkyl-siloxanes formed on glass surface in the presence of dye was first studied by Sagic [62]. In a later work, Pilestsky et al. used hexadecyl mercaptan for the sensing of cholesterol [63].

The investigation on the kinetic and the thermodynamic features of the imprinted recognition sites with quinone as a function of the chain length of supporting alkane thiols such

Table 1. Preparation conditions and characteristics for traditional and precipitation polymerization [15].

Precursors	Traditional polymerization conditions		Precipitation polymerization conditions	
MAA: TRIM* ratio	1:4		1:1	
Solvent fraction (MeCN)	60%		98%	
Reaction	Thermal		Thermal	
	Traditional polymerization characteristics		Precipitation polymerization characteristics	
Particles characteristics	MIP Monolith	NMIP Monolith	MIP Microsphere	NMIP Microsphere
Particle size (μM)	1–100	1–100	−0.5	−0.5
Surface area (m^2/g)	199.2	160.6	265.6	246.4

as 1-decanethiol, 1-tetradecanethiol and 1-octadecanethiol reveals the dependence of their chain length on the imprinting selectivity [64]. Geometry dependent permeability of probes is also found out on the matrix formed by binary monolayers comprised of mercaptopyrimidine on gold electrode [65]. This kind of permselectivity to probe quinines offers the prospect of developing highly sensitive electrochemical sensors for organic samples [65]. Although these types of sensors function very fast, they suffer from lack of stability of the imprinted monolayers. For example, during the template removal, the destruction of the receptor structure by lateral diffusion of bounded molecules was found out. However, this difficulty was overcome by developing a stable monolayer where the template (thiol derivative of barbituric acid) and functional monomer (hexadecyl mercaptan) were co-immobilized on the electrode by spreader-bar approach. Depressions in the monomer layer, due to template, were able to accommodate barbituric acid and the analyte was recognized without removing the template [66]. Furthermore, in another example, poly(2-mercaptobenzimidazole) (2-MBI) was electrochemically deposited on gold electrodes in the presence or absence of the template pyrene and the pyrene derivative, *N*-(1-pyrenyl)maleimide was covalently bound to 1,3-propane thiol that had been previously self-assembled on a cleaned gold surface. Resorcinol was then electrochemically polymerized onto the electrode followed by electrochemical stripping of the thiolated pyrene from the polymer-coated electrode [67].

Similar types of affinity sensors were also prepared using silanes. Kurihara et al. reported a guest selective electrode covered with an octadecylsilyl monolayer [68]. The electrode was modified with a mixture of octadecylsilyl monomer and template guest molecules. After modification, the templates were extracted to leave host-binding sites that excluded all but the object molecules. For e.g., *n*-hexadecane as an inert template host was chemisorbed on silica or titanium dioxide and the above two dimensional imprinted material was used for the detection of vitamins K_1, K_2 and E [69]. Later, Crookes et al. developed a similar electrode by self-assembling nanoporous monolayers and examined the selective detection of ruthenium hexamine [70]. The imprinted monolayer, specific for phenylalanine, was prepared by adsorption of the template on indium oxide electrode followed by the treatment of the electrode with the adsorbed template using trimethyl chlorosilane from the gas phase [71].

SAMs of aminopropyl-derivatized organosilane, aminopropyltriethoxysilane, (APTES) was prepared on an ITO electrode and the dopamine (DA) imprinted sol was then spin coated on the modified surface. APTES which can interact with dopamine through hydrogen bonding brought more binding sites and thus made the sensor more sensitive for DA [72]. Immobilization of *trans*-resveratrol imprinted film on the surface of functionalized ITO electrode was carried out followed by further modifcation with γ-methacyloxypropyl triethoxysilane (γ-MPS) [73]. In a recent work, SAMs of gold nanoparticles were prepared on ITO electrode via sol-gel technology for imprinting imipramine [74]. Though there are more studies related to sensors containing silanes as the recognition element, their practical applications are very few as the stability of the alkyl chlorosilanes SAMs is found to be smaller than that of the alkanethiols SAMs.

2.1.3. Other Imprinting Matrices

Silica has been used as the imprinting matrix for the detection of dopamine. Here, the bulk material can be imprinted by sol-gel method, thus creating microporous materials with specifically arranged functional groups [75]. Core shell structural MIP has been successfully prepared by polymerization of MIP on the silica nanospheres surface and the vinyl groups, modified on their surface, have directed the copolymerization of functional monomers and cross linkers in the presence of template, hydroquinone [76] Another material that has been imprinted using the sol-gel technique is titanium dioxide [77–79].

2.2. Immobilizing the MIP on the Transducer

The function of the transducer is of paramount importance in the MIP sensing technology as it translates the signal, which was generated upon the binding of the analyte with the recognition element, into a quantifiable electrical

output. It is important to mention here that the MIP must be in a close proximity to the transducer surface and this can be achieved by two ways: (i) the polymer itself may be generated by in situ method such as electropolymerization of the monomer or (ii) the preformed polymers will be coated on the transducer surface

2.2.1. Electrochemical Polymerization

In situ polymerization helps to synthesize polymer electrically on the surface of the conductive substrate without any processing requirement. Moreover, there is no need for removal of the template by the extraction process. The big advantage of this method is that the thickness of the polymer can be controlled by various parameters such as current density and applied voltage resulting in a uniform coating of the polymer on the electrode surface. Moreover, the deposition of the polymer can be done at a precise spot of the detector surface.

Previously, few attempts were made by Vinokurov et al. [80] and Boyle et al. [81] to prepare polyaniline and PPy imprinted electrode respectively with molecular recognition properties by electrochemical methods and later, the similar result was demonstrated with electropolymerized polyphenol for sensing various amino acids [82]. However, the first successful application of electropolymerization method was established by Malitesta et al., who used poly(*o*-phenylenediamine) (poly(*o*-PD)) as the polymer matrix for sensing glucose [83]. Several non-cross-linked electrogenerated polymers such as PPy [84 – 106], poly(protopothyrin IX) [107], polyphenol [29, 108], poly(*o*-PD) [109 – 120], poly(mercaptobenzimidazole) [121], poly(methylene green) [122], poly(*m*-aminophenol) (poly(*m*-AP)) [123], poly(*o*-aminophenol) (poly(oAP)) [124], poly(3,4-ethylenedioxythiophene) (PEDOT) [15, 125 – 127] and polyaniline [128] have been prepared on the surface of the electrode. The medium for the preparation of above polymers is water except for poly(protopothyrin IX) [107] as well as poly(EDOT) [15, 125] where a nonaqueous solvent system was used.

It is important to mention here that among the other polymer species, overoxidised polypyrrole is most frequently applied as the MIP sensor material. During the oxidation process at high positive potentials, complementary nanopores and nanocavities to removed dopant can be easily created by extracting an anionic template molecule [94, 95]. This leads to partial degradation of polypyrrole polymeric backbone and introduction of oxygen containing carbonyl and carboxyl groups in to the MIP moiety that determines semi-permeability as well as ability to recognize the imprinted molecule [94]. Sensors based on imprinted PPy for analytes such as amino acids [52, 96 – 98], 1-naphthlalenesulfonate [100], caffeine [102, 104, 106] and bile acids [103] were analyzed using Quartz Crystal Microbalance (QCM) technique. Other molecular species such as glycoproteins [85], serotonin [99], DNA [101] and adenosine triphosphate [105] (amperometry) as well as ascorbic acid [89] and sulfamethoxazole [91] (Differential Pulse Voltammetry) were also traced using imprinted PPy.

Further, during electrochemical deposition of poly(*o*-PD), amino acids [116], atropine [117], saccharide [118], 4,6-dinitro-*o*-cresol [119] and dimethoate [120] were also detected. The defect and the insulating property of poly(*o*-PD) during imprinting were further improved by adding 1-dodecanethiol (1-DDT) [109 – 110]. However, in one study involving electropolymerization of poly(*m*-AP), a smaller potential was applied to improve the insulating property of the imprinted polymer instead of adding 1-DDT [123]. Further, a mixture of electropolymerized *o*-PD and resorcinol was also employed for the detection of 2,4-dichlorophenoxy acetic acid (2,4-D) [111, 112]. In this study, the experiment protocol did not require extensive extraction of the dopant molecule from the 2,4-D doped polymer coated electrode for the current response to the target analyte (i.e 2,4-D), added to the solution [111, 112]. The capacitive current response may be correlated with the association of free 2,4-D with bound 2,4-D within the membrane as the selective surface association with the 2,4-D modified polymer may not need to occupy an empty cavity [111, 112]. In a similar manner, a novel capacitive sensor was prepared by electrochemical copolymerization of *o*-PD and dopamine (DA) for the enantioselective recognition of glutamic acid (Glu) [113]. It was observed that the L-Glu imprinted copolymer, poly(*o*-PD-*co*-DA), showed relatively higher capacitance changes when compared to L-Glu imprinted poly(*o*-PD) and L-Glu imprinted poly(DA). The formation of imprint cavities was affected extremely by the polymer structures and functional groups, where the L-Glu imprinted copolymer and D-Glu imprinted copolymer showed good affinity towards L-Glu and D-Glu, respectively [113].

Nonconducting imprinted polymers can also be integrated with an electrochemical transducer by in situ electropolymerization of monomers. For example, traditional polymers such as EDMA and MAA can be made conductive by electropolymerization of aniline [30] and EDOT [15] and by this way, the MIP particles could be mechanically and electrically connected to the electrode surface.

2.2.2. Other Methods of Immobilization

The most general methods of integration of imprinted polymers on the surface of the transducer as a thin film involves either spin coating or spray coating [28]. In order to synthesize a polymer layer on a flat surface is to use a sandwich technique where, the polymerization solution is cast between the transducer surface and quartz disc and thereafter, the polymerization is initiated [24 – 26, 48]. Few studies applied the technique of silanization for immobilizing the polymers. In this, the transducer substrate was first dipped in 3-methacryloxypropyl-trimethoxy silane, followed by the monomer and the *co*-polymer was removed by simple washing after polymerization [23, 27]. Other nanoporous silanes such as bis(trimethoxysilylethyl)benzene and 1,4bis(triethoxy)silylbenzene had also been used

as inorganic and organic polymers [129]. In some cases, excess of benzophenone (initiator) and the monomer was made to adsorb on the sensing surface and after the photo polymerization, the excess initiator was removed simultaneously [53, 59].

Preformed polymers in the form of particles can be immobilized on the transducer in different ways [130]. The MIP particles were entrapped into gels [7] or behind the membranes [47]. However, problems may arise in the performance of the sensor, when using these approaches, resulting in nonspecific analyte binding or decrease in binding capacity. Other forms of particle immobilization include casting a composite ink [52], incorporating carbon paste electrode [46] and composite electrode involving MIP, graphite and solid binding matrix such as *n*-eicosane [22]. The ideal size and thickness of the immobilized particles as well as layers are from to 25 to 50 μm [22, 46, 52] and 10 – 100 nm [53, 59] respectively.

3. Transducers

Next to the recognition element, the other important part of the sensor is the transducer. The selection of a transducer depends on various factors such as temperature range, atmospheric nature, the size or volume of the constraints, presence of corrosive materials and the desired sensitivity. The electrochemical transducers can be divided into four categories according to the origin of signal detection: (i) signal due to change in properties of the system as a result of binding of the analyte with the MIP (ii) signal by analyte itself (iii) signal by competitive measurements with displacement and (iv) by electrochemical probe. Any electrochemical transducers that have been used for the preparation of the MIP based electrochemical sensors will be in any one of these four categories. The nature and properties of the above transducers will be discussed broadly in the following section.

3.1. Transducers Based on Signal Produced by Analyte Binding

Electrochemical transduction such as capacitance, conductometry, field effect transistors and potentiometry belong to this category. Here, the direct detection of an inert analyte can be realized by the change in conformation or surface potential as a result of analyte binding with the MIPs. Among this, the sensors based on the first three transduction method proceed without any electrodic reaction and the potentiometry is based on electrochemical reaction on the surface of the electrode.

The first reported integrated sensor based on an MIP was reported based on the capacitance sensor [22]. Capacitive or impedance detection is based on the principle of plate-capacitor with double layer phenomenon. To the donator-receptor combination, the sensor capacitance will be changed by the analyte concentration. In order to enable impedometric binding detection, the receptor layer should posses perfect insulating properties and this can be achieved by electropolymerization on the surface of gold electrode with an additional anchor layer of mercaptan.

The schematic imprinting modification of *o*-PD on the gold surface by 1-DDT is shown in Figure 3. After the polymerization in the presence of the template, surface uncovered areas were plugged with 1-DDT to make the layer dense and insulating and this will further decrease the capacitance. The Differential Pulse Voltammetry (DPV) depicting the insulating property of (a) the bare gold (b) gold electrode electropolymerized with *o*-PD and (c) self-assembled gold electrode by 2-mercaptoethanesulfonate (MES) before polymerization using $K_3[Fe(CN)_6]$ as the probe for detecting glutathione is shown in Figure 4 [110]. Here, the peak current still could be seen in the DPV for MES anchored electrode. For additional insulating property, long chain 1-DDT was employed and after this treatment, the peak current disappeared completely (Figure 4d) [110]. Using this technique, glutathione was detected in the concentration ranges of 0.025 to 0.30 mmol L^{-1} (Table 2) [110]. Other examples of capacitive measurement

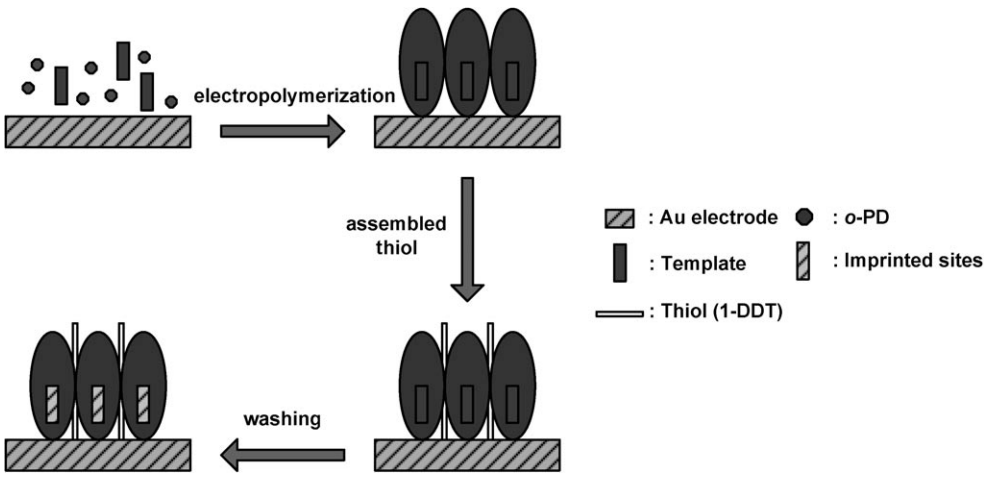

Fig. 3. Schematic representation of the imprinting modification of *o*-PD on the gold surface by 1-DDT.

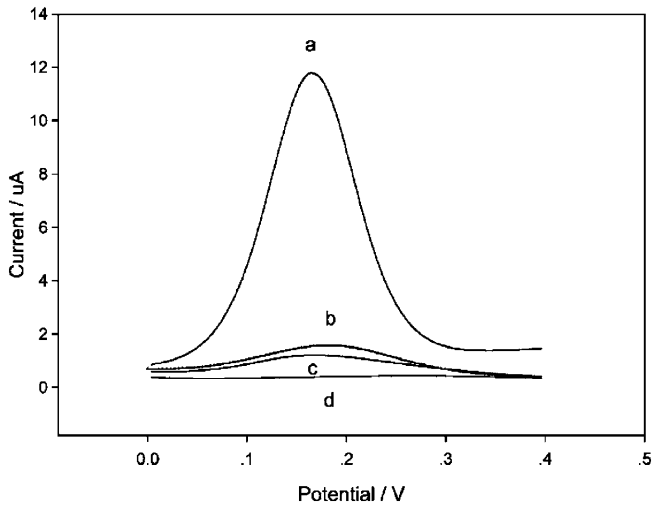

Fig. 4. Differential pulse voltammograms of four kinds of gold electrodes in 1 mmol L^{-1} $K_3[Fe(CN)_6]$ solution containing 0.1 M KCl. a) bare gold electrode, b) gold electrode electropolymerized with *o*-PD, c) self-assembling MES monolayer on gold surface before electropolymerization, d) by additional modification with 1-dodecanethiol besides treatment as (c). Amplitude: 0.05 V; pulse width: 0.05 s; pulse period: 0.2 s (reproduced from [110]).

using different imprinted polymers for various templates are shown in Table 2. Sometimes photografting the imprinted polymer also hold good for detecting the template molecules [59]. Barbituric acid was traced in the thiol substrate by spreader bar approach [66]. Analytes such as L-nicotine [42], desmetryn [60, 61], phenylalanine [93, 108], glucose [109], teagafur [123], pyrene [67], Glu [113] *O*,*O*-dimethyl-α-hydroxylphenyl phosphonate (DHP) [114], theophylline [122] and human serum albumin [146] were also detected using capacitance sensors.

In the conductometric transducers, the two electrodes are separated by an imprinted polymer membrane. The interaction between the template molecules and the polymers leads to conformational organization of polymeric structure, affecting the diffusion of counter ions and as result of this, an increase in the template concentration causes alterations in the membrane electroconductivity. The double layer phenomenon is totally absent here. Stable and flexible polymers such as poly(DEAEM) and PMAA [51] were used as membranes on glass support and quartz tube respectively for the detection of atrazine with a detection limit of 5 nM. The response time interval of the sensor in the quartz plate, varied from 6 – 10 minutes for 60 μm membrane and 12 – 15 minutes for 120 μm membrane [25 – 26]. Similarly, 1-phenylalaninesialicic acid [24], morphine [30] and trichloroacetic acid [39] were also detected by conductometry on PMAA membrane.

The other nonelectrodic transducer is the sensing based on Ion Selective Field Effect Transistors (ISFETs) or Chemical Field Effect Transistors (CHEMFETs). These are semiconductor devices where a chemical reaction on the surface of silicon chip affects both the surface potential and current on the gate of field effect transducers and the rate of the reaction can be easily monitored. For example, in one study, MIP film containing 4-chlorophenoxy acetic acid (CPAA), as the template was prepared by imprinting in TiO_2 films on the surface of SiO_2 chips individually [78]. The schematic representation of the steps involved in the process is shown in Figure 5a.

Hydrolytic polymerization of the Ti(IV) carboxylate or tributoxide complex on SiO_2 surface of the ISFET followed by the removal of carboxylate in the presence of ammonia yields the imprinted sites in the resulting TiO_2 film (Fig. 5a). Here, the equilibrium step with the addition of 1% NH_3

Table 2. The MIP sensors based capacitance.

Template [a]	Recognition elements (Functional monomers) [b]	Method of polymerization	Form/electrode [c]	Detection range	Ref.
Glutathione	*o*-PD/MES/1-DDT	Electrochemical	Film/Au	0.025 – 0.30 mM $LOD = 1.25$ μM	[110]
Creatinine	AMPS/thiol	Photochemical	Photo grafted film/Au	10 – 600 mM	[59]
Barbituric acid	Thiols	SAMS	Film/Au	N.A.	[66]
Desmetryn	AMPS/MBA/thiol	Photochemical	Film/Au	1 – 7 mM	[60, 61]
Phenylalanine	Phenol/thiol	Electrochemical	Film/Au	0.5 – 8 mg/ml	[108]
Glucose	*o*-PD/1-DDT	Electrochemical	Film/Au	0.1 – 20 mM $LOD = 0.05$ mM	[109]
Tegafur	*m*-AP	Electrochemical	Film/Au	N.A.	[123]
Pyrene	MBI or thiol/RSC	SAMS/Electrochemical	Film/Au	N.A.	[67]
L or D-Glu	*o*-PD/DA/1-DDT	Electrochemical	Film/Au	16.7 – 250 μM $LOD = 4.7$ μM	[113]
DHP	*o*-PD	Electrochemical	Film/GC	0.05 – 0.050 μM $LOD = 0.01$ μM	[114]
THO	MG	Electrochemical	Film/GC	0.003 – 0.075 mM	[122]
Phenylalanine	Py	Electrochemical	Film/Pt	N.A.	[93]
L-Nicotine	MAA/EGDM/AIBN	Photochemical	Film/Pt	2 – 5 nM LOD = N.A.	[42]
HSA	TGA/PTMOS	Chemical	Film/Au	N.A.	[146]

[a], [b], [c] see Table 7.

controls the charge and potential of the gate. Figure 5b depicts the operational principle of the MIP ISEFET device. During binding and rebinding of the analytes, the potential between gate and source (V_{gs}) changes, the potential between source and drain (V_{ds}) and drain current (I_d) being constant. The plot of ΔV_{gs}, difference between V_{gs1} (before binding) and V_{gs2} (after binding) vs. log (concentration of the analyte) reveals that the analysis of CPAA is specific and other acids such as cinnamic acid, 2,4-D and benzoic acids are not being sensed. In the similar manner, other organic acids such as methylferrocene carboxylic acids [79], maleic acid as well as fumaric acids [78] were detected on the surface of Al_2O_3 ISFET with the same imprinted site. There is also one example in which silicon wafer ISFET was used for detecting L-phenylalanine anilide on the imprinted poly(MAA-*co*-EDMA) [22].

The next class of electrochemical transducer is based on electrode reaction where the potential difference across a membrane between two solutions with charged species of different activity is measured under conditions of zero current flow (potentiomety). The well-known example for this type of sensor is ion-selective electrodes. The advantages of this type of sensor are that there is no need to remove the template from polymer membrane and there are no size restrictions of the template species. Conducting polymers offer versatile molecular recognition properties and the first study on this sensor was reported in 1992 for various polymeric materials such as PPy, polyaniline etc.,

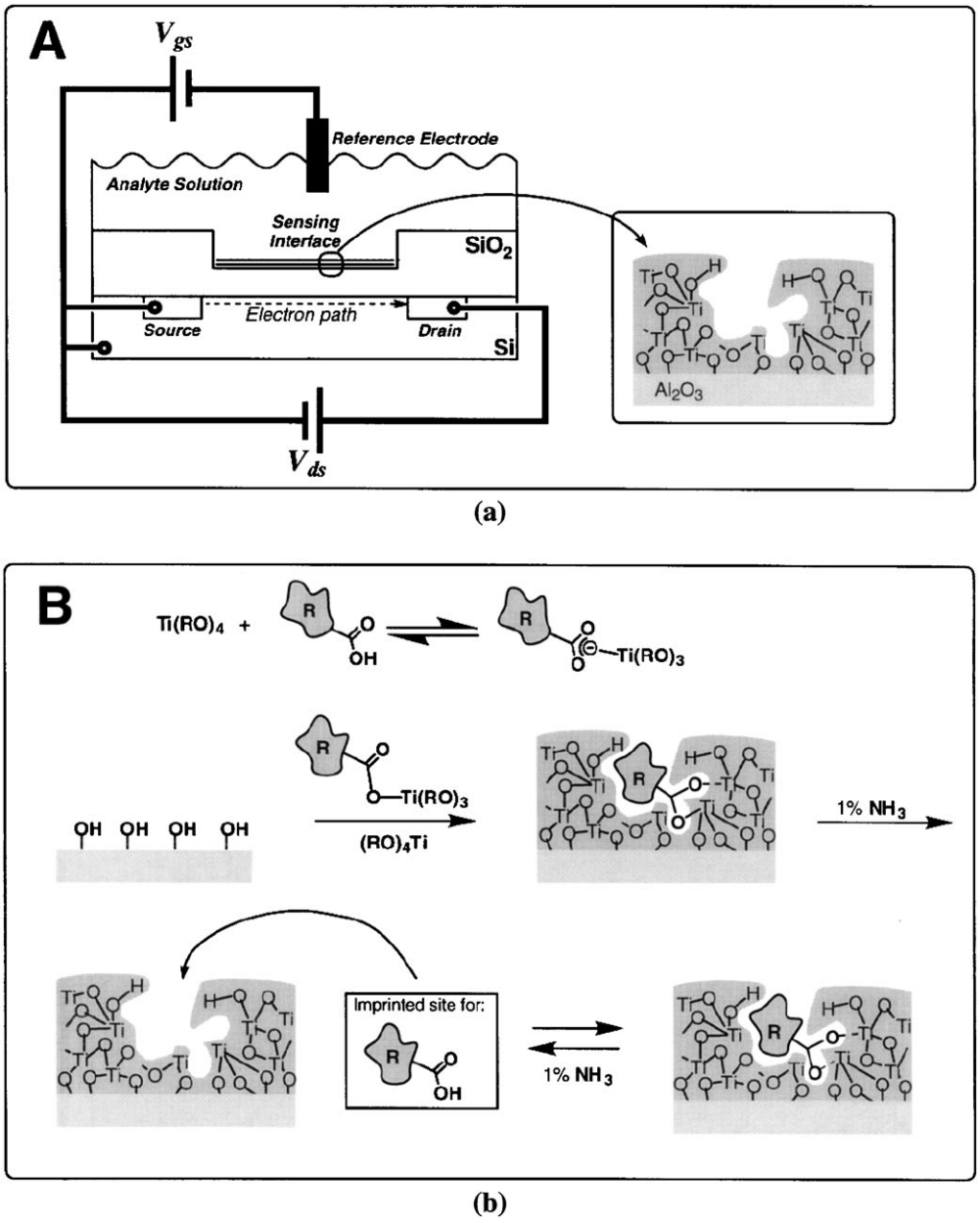

Fig. 5. (a) Schematic configuration of molecular imprinted ISFET device. (b) Preparation of the MIP on TiO_2 thin film acting as a sensing interface on the ISFET gate (reproduced from [78]).

exhibiting memory effect for the anion of the electrolyte [80]. These polymeric materials have anion exchange properties and the selectivity pattern can be easily altered by the exchange of counter ions [131]. For example, dithiocarbamaters can be exchanged during the polymerization of materials [132] and different kinds of metal can be also detected by this potentiometric technique [133]. Kunitake et al. detected sugars by this technique using SnO_2 electrode covered with monolayers of resorcinol-dodecanal cyclotetramer [134]. In another report, glucose concentration in the range of 0 to 25 mM in clinical assays was detected based on measuring the concentrations of proton released during interaction of meta-complexing MIP with glucose [135]. Further, analytes such as melamine [31] and cetirizine [32] were also detected using this technique.

3.2. Generation of Signal by the Analyte Itself

If the analyte undergoes electron transfer reaction, other electroanalytical techniques such as amperometry and voltammetry may be employed for the sensors based on imprinted polymer. This electron transfer step leads to products, which may get fouled on the electrode surface. As a result of this adsorbed products, the reversibility of binding is affected on the transducer surface. Hence, the property of diffusion of analyte species and their products must be required in both directions and this will be provided through the pores or channels present in the MIP layers. Other alternatives such as the use of disposable electrodes [47, 52 – 53] or the surface renovation either by simple mechanical polishing [23] or by washing with solvents [28] were also tried.

In the amperometric determinations, a linear relationship is obtained between the concentration of the analyte species and the current density at constant potential. Before obtaining this plot, a polarization curve under different potentials is recorded and from this curve, a suitable operating potential was determined. Table 3 shows the direct measurements of different analyte by amperometric method. Morphine was detected in the concentration range of 0.1 to 5.0 mM, 0.1 to 2.0 mM, and 0.01 to 0.2 mM by imprinted PEDOT [125], MIP particles immobilized by PEDOT electrode [15], and by imprinted PEDOT microelectrode integrated on the microfluidics system [126], respectively (Table 3). Similarly, the amperometric sensing of methyl valine (m-val) against fructosyl valine (Fru-val) and methyllysine (Z) (m-ε-lys) [47] as well as various glycoproteins [85] was also carried out. One report reveals the development poly(vinylimidazole) MIP with enzyme-like catalytic activity for the sensing of paraxon [46]. Recently, micro-electro-mechanical-systems (MEMS) technique with microfluidic system was fabricated in our laboratory to sense morphine amperometrically and using

Table 3. The MIP sensors based on amperometry.

Template [a]	Recognition elements (Functional monomers) [b]	Method of polymerization	Form/electrode [c]	Detection range	Ref.
	MAA/EDOT	Thermal/electrochemical	Film/ITO	0.1 – 2.0 mM $LOD = 0.3$ mM	[15]
MO	EDOT	Electrochemical	Film/ITO	0.1 – 5.0 mM $LOD = 0.2$ mM	[125]
	EDOT	Electrochemical	Film/Pt	0.01 – 0.2 mM $LOD = 0.3$ μM	[126]
Fru-val, m-val, m-ε-lys	EDMA/1-VID/4-VPB	Thermal	Powder/CP	N.A.	[47]
Paraxon	vinylimidazole	Chemical	Film/CP	$LOD = 100$ μM	[46]
Glycoproteins	Py	Electrochemical	Film/Pt black	N.A.	[85]
Chloroguaiacol	vinylimidazole/EDMA	Thermal	Preconcentrated column	0.05 – 5.0 μM $LOD = 27$ nM	[136]
D4NP	MAA/Zn^{2+}/DVB	Photochemical	Powder/CP	$LOD = 0.1$ mM	[153]
2,4-D	RSC/*o*-PD	Electrochemical	Film/Au	N.A.	[111]
(+)-C	Aniline	Electrochemical	Film/ITO	0 – 150 μM $LOD = 5$ μM	[155]
NIC	TiO_2	Thermal	Film/ITO	0 – 5mM $LOD = 11.1$ μM	[155]
HQ	MAA/TRIM	Thermal	Powder/agarose gel/GC	2 – 100 μM $LOD = 1$ μM	[34]
OTA	Py/CNT	Electrochemical	Preconcentrated column	0 – 15 ppb $LOD = 12$ ppt	[88]
DA	TGA/Q/MMA	SAMS/Photochemical	Film/Au/Glass	0 – 116 μM $LOD = 5.4$ μM	[156]
SR	MAA/EDMA	Thermal	Membrane	1 – 1000 μM $LOD = 0.3$ μM	[44]
Bilirubin	MAA/EDMA	Photochemical	Film/Au	to 1 mg/dL $LOD = 0.01$ mg/dL	[41]

[a], [b], [c] see Table 7.

Table 4. The MIP sensors based on differential pulse voltammetry (DPV).

Template [a]	Recognition elements (Functional monomers) [b]	Method of polymerization	Form/electrode [c]	Detection range	Ref.
VMA	MAA/EDMA	Photochemical	Film/GC	0.1 – 1.5 mM	[28, 29]
Rifamycin SV	Polyphosphazene	Preformed polymer	Film/GC	0.25 – 6.3 μM	[130]
HVA	4-VPD, MAA/EDMA	Photochemical	Film/GC	0.05 – 10 μM $LOD = 7$ nM	[137]
1-OHP	VBTMAC/EDMA, DVB	Thermal	Powder/SPC	N.A.	[142]
Cholesterol	MBI	Electrochemical	Film/Au	20 – 100 μM $LOD = 0.7$ μM	[121]
Albuterol	BzMA/MAA/HEMA/ TRIM/DPHA	Thermal/Photochemical	Film/Pt	1 – 50 and 100 – 200 μM	[35]
UA	mel-*co*-chl /silica gel	Thermal	Preconcentrated column	0.0025 – 0.4600 and 3.92 – 75.00 mg/L $LOD = 0.0008$ mg/L	[54]
PC	Py	Electrochemical	Film/PG	5 – 500 μM and 1.25 – 4.5 mM $LOD = 0.79$ μM	[138]
p-Nitrophenol	MAA/EDMA	Photochemical	CP	5 μM to 8 nm $LOD = 3$ nM	[43]
Creatinine	mel-*co*-chl	Thermal	Film/graphite	1.23 to 100 μM $LOD = 0.37$ μM	[55]
UA	mel-*co*-chl	Thermal	Film/graphite	14.56 to 177.42 μM $LOD = 3.71$ μM	[56]
t-Resveratrol	γ-(MPS)/acrylamide	SAMS	Film/ITO	2.0 to 20 μM $LOD = 0.8$ μM	[73]
imipramine	Au-NPS	SAMS	Film/ITO	5.0 to 1 mM $LOD = 1$ nM	[74]
AA	Py	Electrochemical	Film/Graphite	0.25 to 7 mM $LOD = 0.74$ μM	[89]
Sulfamethoxazole	Py	Electrochemical	Film/Graphite	0.75 mM to 25 mM $LOD = 0.36$ μM	[91]
DA and AA	o-AP	Electrochemical	Film/gold	0.25 μM to 20 μM $LOD = 1.98$ μM	[124]

[a], [b], [c] see Table 7.

such microsystem device, a high sensitivity, low detection limit, and readily response were achieved [126]. A Portable amperometric potentiostat was used for detection of bilirubin based on an SOC based chip [41]. A hydroquinone (HQ) amperometric sensor utilizing MIP particles immobilized by agarose was also reported [34]. Moreover, sorbent flow preconcentration system coupled to amperometric detection of chloroguaiacol (4-chloro-2-methoxyphenol) at submicromolar levels is described [136]. Similarly, a micro-solid phase preconcentration device was prepared by imprinted PPy, for amperometric sensing of ochratoxin A is also described [88]. A flow injection analysis system coupled to an amperoemtric detector was optimized using multivariate analysis for the detection of serotonin [44].

Voltammetric detection involves the measuring the current density generated by the application of a potential sweep and this includes linear sweep voltammetry (LSV), cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV). All these techniques depend upon the applied potential function on the current generation of the electrochemical system. Among these techniques, DPV and SWV offer high sensitivity. The great advantage of the voltammetric sensor is that it can discriminate between species and identify their adsorption at bare electrode surface or imprinted sites, since it does not relay totally on the MIP element for the recognition.

The application of the DPV for sensing analytes using imprinted method is shown in Table 4. Vanillyl mandelic acid (VMA) and homovanillic acid (HVA) can be easily detected through the effective binding of $-$OMe and $-$COOH groups, present in the organic moiety, with the imprinted sites [28 – 29]. From this, VMA and HVA can be differentiated from closely structural-related compounds, having no such groups [28 – 29]. A methodology based on density functional theory calculations for the design of the MIPs has been used for detecting VMA [137].

Rifamycin SV, an antibiotic, was detected on the imprinted poly(phosphazene), which was preformed on the electrode surface by solidification process in the presence of the template [130]. The metabolite,1-hydroxy pyrene, was traced by single use screen printed carbon composite MIP electrode [142] (Table 4). Cholesterol was detected in the presence of mediator, potassium ferricyanide ($K_3[Fe(CN)_6$ at PMBI imprinted electrode [121]. Batch binding assays were carried out with the recognition elements for 2,4-D in order to avoid the unspecific adsorption of the interferences [48]. A multi-array acrylic MIP sensor showed good

selectivity for the recognization of albuterol from its analogies, terbutaline and clenbuterol [35]. Also, paracetamol imprinted PPy sensor immobilized on a pencil graphite electrode was reported [138] where the response of the imprinted electrode was not affected by the interferents, such as phenol, glucose, phenacetin, dopamine and ascorbic acid. Besides, this study showed two LDR, 5 μM – 0.5 mM and 1.25 – 4.5 mM for paracetamol sensing [138].

Different solvents such as methanol, tetrahydrofuran, acetonitrile, dichloromethane and chloroform were checked as washing solvent, in which chloroform was able to establish the specific interaction between catechol and those sites of the MIPs. Recovery higher than 95% was obtained for extraction of catechol even in the presence of structurally similar phenolic compounds [139]. Further, p-nitro phenol was also detected using DPV [43].

Electrochemically prepared polypyrrole MIP for the detection of ascorbic acid [89] as well as sulfamethoxazole [91] and poly(*o*-aminophenol) film for dopamine [124] was fabricated. Creatinine [55] and uric acid [56] was also analyzed using preanodized sol-gel coated graphite electrode with a MIP brush of poly(melamine-*co*-chloroanil) grafted to exterior surface. In another work, an artificial receptor for UA, silica gel-bonded with the same MIP was used as a sorbent for molecularly imprinted solid-phase extraction (MISPE) in column chromatography [54]. Recently, self assembled modification of gold nanoparticles and γ-methacycloxypropyl trimethoxy silane on ITO electrode was used for imprinting imipramine [74] and *trans*-resveratrol [73] respectively.

Reports are in the literature exhibiting the employment of SWV technique for the detection of analyte species. These analytes include 2,4-D [112] and sulfamethazine [40, 140], glucose [141], salicylic acid [115], trinitrotoluene [129] and fenbendazole [37]. A combination of DPV and SWV was used for the trace determination of 1-hydroxy pyrecene using disposable MIP modified screen-printed electrode [142].

High selectivities for enantiomers of D- and L-tyrosine (Tyr) were obtained (L/D = 9.4/1 and D/L = 27.2/1) with PPy imprinted on Ni electrodes with different charge capacities obtained from LSV measurement [52]. It is interesting to see the mechanism of potential induced rebinding, which is shown in Figure 6. After extraction of the template from the MIP film, the complementary cavity of D-Tyr exists within a PPy scaffold or backbone. By applying a positive potential to induce the adsorption, the rebinding of D-Tyr occurred rapidly to the shape imprinted cavity when a fast sweeping rate was applied, but L-Tyr could not favorably enter into the D-Tyr imprinted cavity during a short period of time, primarily due to shape and functional group mediated diffusional constrains. The result shows that imprinted PPy processes a good capability to recognize enantioselectively D and L-Tyr molecules [52].

Similarly, by employing CV technique, adenosine inosine, [84] dopamine [72, 75], parathion [58] and cholesterol [63]

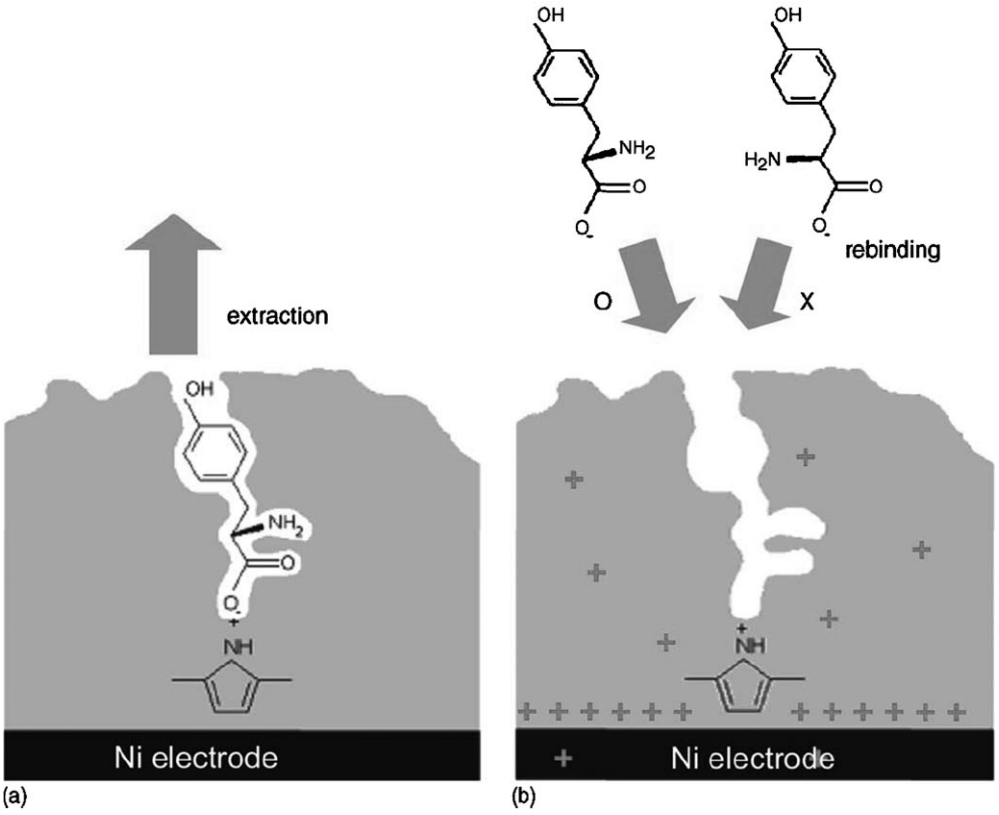

Fig. 6. Schematic diagram of (a) the extraction of template from a D-tyrosine imprinted polypyrrole film and (b) the rebinding of D-tyrosine induced by a positive potential (reproduced from [52]).

Table 5. The MIP sensors based cyclic voltammetry (CV).

Template [a]	Recognition elements (functional monomers) [b]	Method of polymerization	Form/electrode [c]	Detection range	Ref.
1-OHP	St/DVB	Thermal	Powder/SPC	0.1 – 1.0 mM	[53]
Cholesterol	Hexadecyl mercaptan	SAMS	Monolayer/Au	15 – 60 μM	[63]
PQ	Thiols	SAMS	Monolayer/Au	N.A.	[64]
DA	SiO_2-sol-gel	N.A.	GC	N.A.	[75]
Adenosine, Inosine, ATP	Py	Electrochemical	Film/GC	N.A.	[84]
Nitrobenzene	Ni-(PPIX)	Electrochemical	Film/GC	0.01 – 1.0 M	[107]
Theophylline	MAA/EDMA	Photochemical	Membrane/ITO	N.A.	[27]
	MG	Electrochemical	Film/GC	0.01 – 0.08 mM	[122]
	MAA/EDMA	Thermal	Film/ITO, Si	N.A.	[143]
D or L-PAA	MAA/EDMA	Thermal	Film/ITO	N.A.	[45]
Parathion	p-EA	Thermal	Film/SiO_2	0.015 to 15 ppm $LOD = 0.003$ ppm	[58]
DA	APTES	SAMS	Film/ITO	0.8 mM to 2 μM	[72]
(+) Ephedrine	Py*	Electrochemical	Film/GC	0.5 to 3 MM	[90]
Atrazine	EDOT	Electrochemical	Film/Pt	15 mM to 1 nM $LOD = 1$ μM	[127]
Catechol and DA	Aniline/N,N′DDCBEMAA	Electro/Photochemical	Film/Au or ITO	144 μM $LOD = 228$ nM	[128]

[a], [b], [c] see Table 7.

(Table 5) were determined. Theophylline was detected with the MIP composed of poly(MAA-*co*-EDMA), prepared by thermal [143] and photochemical reactions [27] on two platform substrates of ITO/silicon and ITO respectively as well as poly(methylene green), immobilized on GC electrode by electrochemical polymerization [122]. Further, ephedrine [90], atrazine [127], catechol as well as dopamine [128] were detected using electrochemical polymerization of pyrrole, EDOT and aniline respectively. The analysis of nitrobenzene was carried out with 45 – 60 minutes of incubation on protoporphyrin IX MIP, integrated on GC electrode electrochemically [107] (Table 5). Other analytes recognized using this technique are also listed in Table 5.

3.3. Competitive Measurements

In this study, a labeled or structurally similar analyte derivative was employed to compete with the analyte molecules for the binding sites in the MIP. At first, the analyte was allowed to bind selectively with the MIP and in the second step, an electroinactive competitor was added in excess, whereas some of the bound analyte is released. The released template is detected by the amperometric method. In a particular example, morphine was detected in the presence of its electroinactive competitor, codeine [21]. The MIP was MAA cross-linked with EDMA, which was immobilized on Pt electrode by agarose gel.

Clenbuterol was detected in presence of isoxsuprine at MIP modified solid binding matrix composite electrode (SBMCE) using DPV technique [23] in the concentration range of 0.004 – 25 mM, with a detection limit of 20 nM. If the analyte is electroinactive, a competitor with electrochemical activity will be used. In a different approach, a suspension of the electroinactive analyte, 2,4-D, the competitor and the MIP particles were mixed and incubated for few minutes. Finally, the MIP particles were removed and the detection of unbound electroactive competitor was carried out by DPV and LSV respectively [48, 49].

3.4. Electrochemical Probe

This method is very similar to the competitive measurements for electroinactive analyte, except the addition of an electroactive mediator to the imprinted polymer electrolyte system. The cavities present in the MIP serves as charge-transfer channel where the mediator diffuses through the MIP layer, immobilized on the electrode surface and the resultant current measured in presence of template relates to the mass transfer of the mediator inside the pores. Cholesterol was detected in the presence of mediator, potassium ferricyanide $K_3[Fe(CN)_6]$ at PMBI imprinted electrode [121]. One more analyte namely theophylline was detected using this method [27].

The general measurement protocols and the operating procedures for the electrochemical sensing of templates based on MIP technology involve the extraction of templates. However, few reports say that it is still possible to sense the analytes without extracting the templates from the polymers. Examples of applications without initial template extractions include potentiometric determination of NO_3^- [86], capacitive determination of barbituric acid by mercaptans [66] and amperometric detection of 2,4-D at poly(*o*-PD)/resorcinol polymer matrix [111].

As mentioned in previous section (Section 3.2), the development of integrated microarray electrode with protocol for MIP synthesis is also progressing well [126]. The first integrated catechol-imprinted microelectrode as an electrochemical detector for liquid chromatography was

Table 6. A partial list of *IE* obtained for non-EC and *SE*/*PE* for EC MIP sensors from the literatures.

Recognition elements (functional monomers) [b]	Transduction [d]/electrode [c]	Template [a]	*IE*	Ref.
Non-EC MIP sensors/assays				
MAA/EGDMA	HPLC-UV	THO	2.36	[147]
		CAF	1.84	
MAA/DVB	HPLC	THO	2.26	[148]
TEOS/PTMOS/APTES	QCM	PT	1.78	[149]
MAA/EDMA	QC-TSM/Ag	NIC	2.44	[150]
AN/St			1.07	
AN/2-VPD	QCM/Au	CAF	1.30	[151]
AN			1.73	
AN/4-VPD			1.88	
Py	PQC/Pt	SDS	4.33	[87]
MAA/EDMA	QCM/Au	DM	6.0 – 8.4	[36]
Recognition elements (functional monomers) [b]	Transduction [d]/electrode [c]	Template [c]	*SE* or *PE*	Ref.
EC MIP sensors/assays				
MAA/TRIM	Amperometry/ITO	MO	1.11	[15]
MAA/EDMA	CV/ITO	THO	2.97	[27]
MAA/EDMA	DPV/GC	VMA	5.01	[29]
		Fru-val	1.71	[47]
1-VID/4-VPB/EDMA	Amperometry/CP	m-val	1.20	
		m-ε-lys	1.44	
EGDMA/4-VPD	DPV/C	2,4-D	1 – 4.67	[48]
St/DVB	CV/SPC	1-OHP	3.11	[53]
Hexadecyl mercaptan	CV/Au	Cholesterol	3.33	[63]
Py	CV/GC	Adenosine	0.71	[84]
EDOT	Amperometry/ITO	MO	1.32	[125]
Polyphosphazene	DPV/GC	Rifamycin SV	4.00	[130]
4-VPD, MAA/EDMA	DPV/GC	HVA	20.0	[137]
VBTMAC/EDMA, DVB	CV/SPC	1-OHP	0.48	[142]
	DPV/SPC		ca. 2.0	
MAA/EDMA	CV/Au	Atrazine	1.74	[152]
MAA/Zn^{2+}/DVB	Amperometry/CP	D4NP	ca. 1.4	[153]
RSC/*o*-PD	Amperometry/Au	2,4-D	2.65	[111]
Aniline	Amperometry/ITO	(+)-C	1.42	[154]
TiO_2	Amperometry/PEDOT/ITO	NIC	1.24	[155]
BzMA/MAA/HEMA/ TRIM/DPHA	DPV/Pt	Albuterol	10.67	[35]
Py	DPV/PG	PC	2.74	[138]
MAA/EDMA	SWV/GC	Sulfamethazine	1.76	[40]
MAA/EDMA	CV/ITO	L-PAA	7.25	[45]
		D-PAA	2.56	
4-VPD/EDMA	Conuctometry/SPAu	TCAA	3.06	[39]
TGA/Q/MMA	Amperometry/PEDOT/Au	DA	∞	[156]

[a], [b], [c], [d] see Table 7.

fabricated, which has a diminished response against all catecholamines [144]. Imprinted polymer with 20 μm relief structure was prepared in the pores of poly(dimethylsilane) stamp on a silicon wafer and this had been used for the detection of labeled ^{14}C-2,4-D [145].

4. Imprinting Efficiency

Till date, there is no specific definition to distinguish the performance between MIPs and NMIPs. In order to compare the performances between MIPs and NMIPs, an imprinting efficiency (*IE*) is introduced. The *IE* is defined as the bound amount of MIP divided by that of NMIP to quantitatively compare the bindings of MIP and NMIP with respect to the template, as shown in Equation 1:

$$\textit{Imprinting efficiency}\ (IE) = \frac{\textit{Bounded amount of MIP}}{\textit{Bounded amount of NMIP}} \quad (1)$$

Since it is difficult to measure the bound amount of template on MIP-modified electrodes in electrochemical biosensing systems, including amperometry, CV, DPV and SWV, a

Table 7.

[a] Templates			
1-OHP	1-Hydroxypyrene	2,4-D	2,4-Dichlorophenoxyacetic acid
(+)-C	(+)-Catechin	APU	6-Amino-1-propyluracil
CAF	Caffeine	D4NP	Diethyl(4-nitrobenzyl)
DA	Dopamine	DHP	*O*,*O*-Dimethyl-α-hydroxylphenyl phosphonate
DM	Daminozide	Fru-val	Fructosyl valine
Glu	Glutamic acid	HQ	Hydroquinone
HVA	Homovanillic acid	MO	Morphine
m-val	Methylvaline	m-ε-lys	Methyllysine (Z)
NIC	Nicotine	PAA	Phenylalanine
PC	Paracetamol	L-Phe	L-Phenylalanine
PQ	Phenoxynaphthacene quinine	PT	Parathion
SDS	Sodium dodecyl sulfate	TCAA	Trichloroacetic acid
THO	Theophylline	UA	Uric acid
VMA	Vanillyl mandelic acid.	AA	Ascorbic acid
HAS	Human serum albumin		
[b] Recognition elements (functional monomers)			
1-DDT	1-Dodecanethiol	1-VID	1-Vinylimidazole
2-VPD	2-Vinylpyridine	4-VPB	4-Vinylphenylboronate
4-VPD	4-Vinylpyridine	AN	Acrylonitrile
APTES	Aminopropyltriethoxysilane	BzMA	Benzyl methacrylate
DPHA	Dipentaerythrithol hexaacrylate	DVB	Divinylbenzene
EDMA	Ethylene glycol dimethacrylate	EDOT	3,4-Ethylenedioxythiophene
HEMA	2-Hydroxyethyl methacrylate	MAA	Methacrylic acid
MES	2-Mercaptoethanesulfonate	MG	Methylene green
MMA	Methyl methacrylate	Ni-(PPIX)	Ni-(Protoporphyrin IX) dimethyl ester
o-PD	*o*-Phenylenediamine	p-VBBA	p-Vinylbenzeneboronic acid
PTMOS	Phenyltrimethoxysilane	Py	Pyrrole
Q	Quercetin	RSC	Resorcinol
St	Styrene	TEOS	Tetraethyl orthosilicate
TEAMA	*N*,*N*,*N*-Trimethylaminoethyl methacrylate	TGA	Thioglycolic acid
TRIM	Trimethylolpropane trimethacrylate	VBTMAC	(Vinylbenzyl)trimethylammonium chloride
mel-*co*-chl	Melamine-*co*-chloroanil	γ-(MPS)	Methacyloxypropyl trimethoxysilane
APTES	Aminopropyltriethoxysilane	N,N′DDCBE	Diethyldithiocarbamic benzyl ester
BTESB	1,4-Bis(triethoxysilyl) benzene	BTMSEB	Bis(trimethoxysilylethyl) benzene
[c] Electrodes			
Ag	Silver	Au	Gold
CP	Carbon paste	GC	Glassy carbon
ITO	Indium tin oxide	Pt	Platinum
PG	Pencil graphite	SP	Screen printed
[d] Transduction			
CV	Cyclic voltammetry	DPV	Differential pulse voltammetry
FPD	Flame photometric detector	PQC	Piezoelectric quartz crystal
TSM	Thickness-shear-mode	SWV	Square wave voltammetry
QCM	Quartz crystal microbalance		

sensitivity enhancement (*SE*) factor or a peak current enhancement (*PE*) factor is defined as the imprinting efficiency of amperometry assay and CV, DPV or SWV assay in electrochemical (EC)-based MIP sensing system, respectively. *SE* or *PE* factor have been modified below as the ratio of the sensitivity or the peak current of an MIP electrode to that of an NMIP electrode (Eqs. 2 and 3):

$$\textit{Sensitivity enhancement}\ (SE) = \frac{\textit{Sensitivity of MIP electrode}}{\textit{Sensitivity of NMIP electrode}} \quad (2)$$

$$\textit{Peak current enhancement}\ (PE) = \frac{\textit{Peak current of MIP electrode}}{\textit{Peak current of NMIP electrode}} \quad (3)$$

A partial list of *IE* values for non-EC MIP sensors and *SE* or *PE* values for EC MIP sensors reported in the published work has been documented in Table 6 [147–156]. From this table, it is noted that comparison of the *SE* or *PE* values with *IE* values, one can explain that why the EC-MIP sensor are widely expanded in these years.

5. Limitations and Scope

An increase of number of research papers in the field of molecularly imprinted electrochemical sensors indicates that interest in this field is expanding with continuous opportunities for research. Despite this, the potential level of their applications in the field of biosensors and in real analysis is limited. In order to overcome this problem, the research must be focused on the development of the MIPs containing more homogeneously binding site population with high affinity for the target analyte. Moreover, fabrication of the MIPs for use in water and other polar-based solvents must be developed. Finally, improvement in the effective protocols for the MIPs specificity, their immobilization on the electrode surface, selectivity and sensitivity for their commercial success is also necessary. A considerable part of the current research efforts on the MIPs have already dealt this problem. On the other hand, the excellent stability of the MIPs system unlike the antibodies, which require controlled environment and their low price, keeps them as an effective candidate in the field of analytical chemistry. Though the often seen *SE* or *PE* of molecularly imprinted electrochemical sensors is less than 2 (Table 6), there is much room for improvement in the sensing property, i.e. choosing a suitable recognition element and a satisfactory transducer for a specific template to enhance the imprinting efficiency and the electrochemical signal between MIP and NMIP, of the proposed MIP based electrochemical sensors. It is important to note that the application of electrochemically synthesized conducting polymer based MIPs, will find proper place in future molecular technology since they are complex and versatile. We hope that in the near future, the MIP based electrochemical sensors will have the potential to find clear niches in the sensor market.

Acknowledgement

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