Modern and Dedicated Methods for Producing Molecularly Imprinted Polymer Layers in Sensing Applications

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Abstract: Molecular imprinting (MI) is the most available and known method to produce artificial recognition sites, similar to antibodies, inside or at the surface of a polymeric material. For this reason, scholars all over the world have found MI appealing, thus developing, in this past period, various types of molecularly imprinted polymers (MIPs) that can be applied to a wide range of applications, including catalysis, separation sciences and monitoring/diagnostic devices for chemicals, biochemicals and pharmaceuticals. For instance, the advantages brought by the use of MIPs in the sensing and analytics field refer to higher selectivity, sensitivity and low detection limits, but also to higher chemical and thermal stability as well as reusability. In light of recent literature findings, this review presents both modern and dedicated methods applied to produce MIP layers that can be integrated with existent detection systems. In this respect, the following MI methods to produce sensing layers are presented and discussed: surface polymerization, electropolymerization, sol–gel derived techniques, phase inversion and deposition of electroactive pastes/inks that include MIP particles.

Keywords: molecularly imprinted layers; surface polymerization; electropolymerization; sol–gel derived techniques; phase inversion; electroactive pastes and inks

1. Introduction

Chemical and biochemical sensors are modern devices in analytical chemistry that simplify and miniaturize analytical determinations. Analytical methods based on modern sensor technology have solved many difficult analytical issues in research and society. Many scientific groups are working at the global level in the field and are reporting interesting results [1]. In general, conventional analytical methods have been extensively used due to their accuracy; nevertheless, most of these methods are expensive and require complex equipment, laboratory facilities, a high reagent consumption and well-trained personnel [2]. These drawbacks have prompted the research community to build more performant sensors for specific analysis of samples with complex matrices and very low concentrations, all at a lower reagent cost with inexpensive and easily-handled equipment, in order to perform in situ and on-site determinations [3].

Generally, a sensor is composed of three integrated parts (Figure 1), as follows: (i) a receptor for detecting the target analyte in a selective and sensitive manner, (ii) a physical transducer that converts the information obtained from the sensitive receptor into a measurable signal (usually an electric signal), and (iii) a suitable analytical device to process and show the significant signals formed by transducers and to calculate the results [4].
Biosensors are defined as analytical devices incorporating as a receptor biological material, such as enzymes, antibodies, nucleic acids, whole cells and tissues, a biologically derived material (i.e., engineered proteins, aptamers or recombinant antibodies) or a biomimetic material (i.e., molecularly imprinted polymers or combinatorial ligands). Depending on the incorporated sensitive material, the biosensors are classified as enzymatic, immune affinity recognition, DNA or whole-cell sensors; while considering the type of transducer, sensors can be classified as electrochemical, optical, acoustical, piezoelectrical, gravimetrical or thermal [1]. Each type of sensor class has other subclasses. For instance, the electrochemical sensors maybe amperometric (the majority), potentiometric, voltametric, etc. [5]. However, due to high production costs and the restricted operating conditions of these natural receptors, the development of artificial receptors with molecular recognition capacity, so-called synthetic receptors, have attracted a great interest as appropriate alternatives for the biological elements. Hence, among the existing techniques for the development of artificial receptors, high expectations are set out in the design of molecularly imprinted polymers (MIPs).

MIPs are polymeric materials that are designed and produced with built-in molecular recognition properties. As a result of this fundamental attribute, a growing interest has been observed in their development as inexpensive and robust materials with sensitive and selective molecular recognition. Some of the top current applications that include the use of MIPs are associated with catalysis, separation sciences and monitoring/diagnostic devices for chemicals, biochemicals and pharmaceuticals [6,7]. MIPs have proven to possess important advantages as an alternative sensing material for biosensors, including the ease of preparation, storage stability, low cost, repeated uses without loss of activity, high mechanical strength and resistance to heat and pressure as well as to harsh chemical environments [8,9]. In a typical approach, the MI process (Figure 2) allows the creation of specific molecular recognition sites by the polymerization of a functional monomer in the presence of target molecules (called template) and of a high concentration of crosslinker. Following the template removal, by specific extraction procedures, the specific recognition cavities are revealed [10–12]. This approach endows MIPs with tremendous specific binding properties, as they possess cavities with complementary size, shape and electronic environment with the target molecule [13].
In the classical imprinting process, a complex is formed between the template and functional monomer(s), by covalent, semi-covalent or non-covalent bonds, the non-covalent approach being preferred because of the simplicity in complex forming and the ease of the template extraction. In this case, polymerization takes place in the presence of an initiator and a porogen solvent, the latter having the role to create pores in the polymer matrix that facilitate the access of target molecules near the imprinted sites, during the rebinding assays [15]. A large amount of crosslinker is needed, as well, in order to stabilize the structure. Finally, the template is extracted from the crosslinked polymer and, thus, the imprinted cavities are created. This approach is also called the bulk method [16] because a solid block is first obtained, which is later on crushed for obtaining irregular-shaped particles. This method has little productivity because many of the recognition sites are destroyed during the crushing. This is the main reason why other methods were developed for enhancing the specificity and selectivity of MIPs, such as suspension [17], emulsion [18] or precipitation polymerization [19].

Molecular imprinting (MI) was applied initially for organic small molecule templates and ions [20], due to the molecular size, complexity, conformational flexibility and diffusion difficulties of large molecules [8]. Nevertheless, in the last 15 years, large molecules, such as proteins, were also successfully imprinted. For instance, by using the so-called epitope approach [21], only a characteristic part of the biomacromolecule is imprinted, and the following rebinding process is based on recognizing this part alone. Another clever approach developed from necessity allows the preparing of MIPs for labile, dangerous or very expensive targets, by performing the imprinting using a “dummy” template, meaning a safer or more available compound with a similar structure to that of the target analyte [22].

MIPs can also be used to design enzymatic and immunoaffinity sensors. In agreement with the classification of biosensors, MIP-based devices can work according to two different detection schemes: (1) affinity sensors (“plastic antibodies”) and (2) catalytic sensors (“plas-ticenzymes”) [2,23]. In this respect, MIP layers are usually deposited directly on the transducer surface of a sensor chip to produce the recognition unit [24,25]. As a result of this rather simple procedure, many researchers have found various methodologies for producing MIP layers as receptors for producing sensing devices, such as spin coating of a precursor solution to form a thin film, followed by insitu chemical polymerization [26]; electropolymerization of a pre-assembled complex of an electroactive functional monomer with a template [27,28]; dropcasting of a precursor polymer from solution [25,29,30]; dripping a composite solution containing a conducting material (e.g., graphene), MIP particles, and a binder (e.g., PVC) [31]; and, self-assembly of monolayers [32]. Thereby, the target of this review is to present both modern and dedicated methods for the preparation of MIP-sensitive layers, which are presented in detail in the next sections. In all cases, the authors describe the synthesis procedure and properties of prepared MIPs, giving quantifiable results relative to the control samples, non-imprinted polymers (NIP).

2. Molecular Imprinting by Surface Polymerization

In the surface imprinting process, the recognition sites are formed at the surface of a substrate [33]. Due to this fact, the recognition sites are more accessible and promote faster binding kinetics, compared to monolith MIPs for example. This means that template–polymer interactions are not governed by diffusion processes to the same extent as usually encountered in bulk imprinting [34]. Therefore, the technique is applicable especially for the imprinting of large biomolecules such as proteins [35,36], microorganisms and cells. Moreover, surface imprinting requires lower amounts of template molecules compared to the amounts used for other imprinting techniques because imprinting occurs right at the surface of the films [37].

In principle, the method consists of the preparation of a polymerization mixture, containing the functional monomer, the template, the initiator, the porogen solvent and the crosslinker, similar to the bulk method (see Figure 3). An amount of this mixture is cast on a solid surface, as for instance the sensor surface, and the formed thin film is
polymerized thermically or by UV light curing; the latter being largely preferred. At the end, the template is extracted, thus generating the surface imprinted polymer [16].

![Diagram of MIP layer preparation by surface polymerization](image)

**Figure 3.** The principle of MIP layer preparation by surface polymerization.

Nonetheless, there are many variants of surface imprinting such as soft lithography, template immobilization, surface imprinting via grafting (pre-grafting polymerization and post-crosslinking/imprinting and grafting polymerization synchronized with crosslinking/imprinting [38]), emulsion and precipitation polymerization or epitope imprinting [16].

For example, Cennamo et al. [39], developed a biomimetic optical sensor based on MIP and surface plasmon resonance (SPR) transduction, in connection with tapered plastic optical fiber (POF) to detect selectively low molecular weight substances. The prepared SPR sensor was tested for l-nicotine \((\text{-(−)-1-methyl-2-(3-pyridyl) pyrrolidine, MW} = 162.24)\). In order to realize this SPR sensor, the plastic optical fiber, without protective jacket, was heated and stretched to yield a thinner part with a length of 10 mm, after which it was embedded in a resin block and a thin gold film was sputtered on its surface. According to the MIP classical procedure, the pre-polymerization mixture was prepared using l-nicotine as template, methacrylic acid (MAA) as functional monomer, divinylbenzene (DVB) as crosslinker, in a molar ratio of l-nicotine:MAA:DVB = 1:4:24. The developed device was able to be detected and discriminate between l- and d-nicotine using a small volume of sample, but with sensitivity strongly depending on the characteristics of the optical fiber [39]. Some examples of newsworthy MIP layers via the grafting approach are listed in Table 1.

**Table 1.** MIP-based sensors obtained by surface polymerization.

| Synthesis Method | Receptor | Support | Analyte | Characterization Method(s) | LOD | Refs. |
|------------------|----------|---------|---------|---------------------------|-----|-------|
| Spincoating      | MIP film | Glass   | Atrazine| RIfS \(^1\) measurements.  | >1.7 ppm | [40] |
| Precipitation polymerization/polymer casting | MIP layer | SERS substrate \(^2\) | Enrofloxacin hydrochloride | Raman       | \(10^{-7}\) mol·L\(^{-1}\) | [41] |
| Casting          | MIP membrane | Screen-printed gold electrode | Myoglobin | EIS \(^3\), SWV \(^4\) | 2.25 µg·mL\(^{-1}\) | [42] |
| Grafting polymerization synchronized with crosslinking/imprinting | MIP film | GCE \(^5\) | Olaquindox | CV \(^6\), DPV \(^7\), EIS | 7.5 nmol·L\(^{-1}\) | [43] |
| Covalent imprinting/drop casting | MIP film | Au-TFME \(^8\) | SARS-CoV-2 spike protein subunit S1 | CV, SWV | 4.8 pg·mL\(^{-1}\) | [44] |
| Dropcasting      | MIP membrane | QCM \(^9\) crystal chip | Human serum albumin | Langmuir, Freundlich, Langmuir–Freundlich isotherm | 0.026 µg·mL\(^{-1}\) | [45] |

\(^1\) RIfS: reflectometric interference spectroscopy; \(^2\) SERS: surface-enhanced raman scattering; silver nanoparticles modified by 3-methacryloxypropyltrimethoxysilane; \(^3\) EIS: electrochemical Impedance spectroscopy; \(^4\) SWV: square wave voltammetry; \(^5\) glassy carbon electrode; \(^6\) cyclic voltammetry; \(^7\) differential pulse voltammetry; \(^8\) MicruX™ gold-based thin-film metal electrodes; \(^9\) QCM: quartz crystal microbalance.
Another optical sensor is described by Belmont et al. [40], in which case reflectometric interference spectroscopy (RIfS) was employed as a detection method, while the MIP films were prepared with the pesticide atrazine as the template molecule. In their study, the MIP films were deposited on glass transducers by two different methods: (i) spin-coating of pre-polymerization mixture containing polyvinyl acetate as a sacrificial polymeric porogen, followed by in situ surface polymerization of thin films, and (ii) auto-assembly of MIP nanoparticles with the aid of polyethylene imine (PEI) as an associative linear polymer. The results obtained upon assessment of atrazine solutions in toluene were reproducible for both types of films. However, the film prepared with auto-assembled MIP nanoparticles was more sensitive, tracking atrazine down to 1.7 ppm.

An example of the template immobilization approach is given in reference [42], where Moreira et al. developed a reusable sensor for Myo based on an MIP, prepared by assembling a polymer layer of carboxylated poly(vinyl chloride) (PVC COOH). This polymer was cast on the gold working area of a screen-printed electrode (Au-SPE), creating in this manner a novel disposable device relying on plastic antibodies. The MIP/Au-SPE sensor displayed a linear behavior by electrochemical impedance spectroscopy (EIS) and a limit of detection set-out at 2.25 \( \mu \)g/mL. The MIP/Au-SPE sensor also displayed good results in terms of selectivity. The error found for each interfering species were 11% for BSA, 7% for troponin T and 2% for creatine kinase MB, respectively. Overall, the MIP modification over the Au-SPE was found suitable for producing an electrochemical sensor for screening Myo in biological fluids [43].

One epitope approach by surface polymerization for MIP selective layers is also illustrated by Ma and co-workers [44]. In the first step, the MIP was synthesized, using the epitope of human serum albumin as a template and afterwards, a coating method was applied to produce the quartz crystal microbalance sensor (EMIP-QCM). The MIP solution was prepared using zinc acrylateas a functional monomer, EGDMA as crosslinker and dimethylformamide (DMF) as porogen solvent. The gel precipitate was separated, washed and lyophilized. In order to obtain the sensor, a quartz crystal microbalance (QCM) crystal chip was used to drop-cast the MIP solution. The final MIP-QCM sensor displayed good selectivity and sensibility for human serum albumin, with a detection limit of 0.026 \( \mu \)g/mL. Furthermore, the sensor has also proven good accuracy and reproducibility when real samples were tested [45]. Further on, the study presented by Boysen and co-workers [46] is a typical example of a soft lithography approach and describes the design and synthesis of layer-by-layer MIPs via surface polymerization. The double-layered patterned MIP1/MIP2 was prepared on a silicon wafer. To enable chemical binding of MIP1 layer on the silicon, the surface of the wafer was first activated by sonication and exposed to UV-light, after which the surface was silanized with 3-(trimethoxysilyl)propyl methacrylate. The MIP pre-polymerization mixture was prepared by dissolving the template(\( N \)-dansyl-L-phenylalanine) and MAA with a crosslinker and a photo-initiator in a porogen solvent. After template extraction, a 4-vinylpyridine-MIP thin film layer was deposited by photolithographic etching onto the first MIP film of PMAA, resulting in a grid-patterned surface in which two different MIPs, with pre-determined selectivity for \( N \)-dansyl-L-phenylalanine, alternated at a micron scale. Selectivity differences towards fluorescent template analogues were inspected using fluorescence microscopy [46].

MIP films can be prepared by surface polymerization also on organic support, such as multiwall carbon nanotubes (MWCNT) [47]. In this report, the used template was lysozyme (Lys) from egg white. The functional monomer was acrylamide (AAm), the crosslinker, methylene bis acrylamide (MBA) and the solvent, phosphate buffer (PBS). Besides using PBS for the protein protection, a redox initiation was employed consisting of ammonium persulfate (APS) and N,N,N',N'-tetramethylethlenediamine (TEMED), allowing to perform the surface polymerization at room temperature. The selectivity assays showed that the Lys–MIP film registered higher capacity and affinity for Lys than for the other competitive proteins, such as cytochrome C (Cyt C), myoglobin (Myo), hemoglobin.
(Hb) and bovine serum albumin (BSA). The relative selectivity coefficients for Lys/Cyt C, Lys/Myo, Lys/Hb, and Lys/BSA were 1.30, 1.30, 3.12 and 2.82, respectively, while the adsorption capacity of the Lys–MIP film was 1.86 times higher than that of the non-imprinted polymer (NIP). Although the material was intended for selective separation of lysozyme, it may also be of interest for sensor application as a result of excellent electric conductivity of MWNT [47].

In recent decades, an extensive interest in the application of controlled radical polymerization methods (CRPs) for the imprinting of biomacromolecules has been observed [48]. Generally, MIPs are prepared by a conventional free radical polymerization mechanism, mainly due the fact that it is not disturbed by a large range of functional groups of the monomers and of the template, but also due to mild reaction conditions that can be employed. However, free radical polymerization has a major drawback due to the fact that chain propagation and termination reactions are hard to control, which makes the synthesis of surface imprinted polymer films with constant and targeted thickness difficult. Furthermore, free radical polymerization normally yields crosslinked polymer networks with heterogeneous structures, which might be responsible for the increased heterogeneity of binding sites, and thus, for the decreased affinity and selectivity [49]. On the other hand, the negligible chain termination in CRPs and their thermodynamically-controlled processes allow for more constant rates of the polymer chain growth, leading to more homogeneous polymer networks with narrower distributions of the chain length. This is the reason why several CRPs were developed so far for surface polymerization, in which case the most preferred methods refer to iniferter-induced radical polymerization [50,51], atom transfer radical polymerization (ATRP) [38,52] and reversible addition-fragmentation chain transfer (RAFT) polymerization [38,53]. Some of the successful procedures are provided next.

A selective surface plasmon resonance (SPR) sensor, based on surface polymerization, for the detection of Ochratoxin A (OTA) contamination in dried fig was developed by Akgönülü et al. [54]. OTA is one of the most common mycotoxins that contaminate a wide range of agricultural products, which is why its assessment is very important for the monitoring of the food quality. The MIP layer was produced onto the SPR sensor chip by light-initiated polymerization of N-methacryloyl-L-phenylalanine (MAPA) and 2-hydroxyethyl methacrylate (HEMA) using OTA as a template. In a first step, the gold surface of the chip sensor was modified with allyl groups, by allyl mercaptan. This pretreatment was performed because thiol-end will bind to the gold SPR chip, and the other end, allyl group, will interact with the polymer, insuring the good adherence of the MIP film on the chip. For the preparation of the MIP film, MAPA and OTA were mixed to obtain a pre-complex with the molar ratio of 1:3 for OTA:MAPA. The pre-polymerization complex was then mixed with HEMA, a crosslinker and a radical initiator in methanol. This reaction mix was dripped onto the SPR gold chip surface, distributed uniformly using a spin coater and, finally, polymerized by UV-light to produce the MIP film. The MIP–SPR chip was able to detect the OTA with high specificity (around 4.24 higher than that of the NIP), while the detection limit was close to 1 ng/mL and the response time was about 8 min [54].

Another interesting study on surface imprinting via the grafting (grafting from) method is provided by Heetal [55], which described the obtaining of a sensor for testosterone starting from porous silica by covalently binding azo-initiators and then photografting. First, glycidoxy groups were immobilized on the surface of silica particles by the reaction of silanol groups with 3-glycidoxy-propyltrimethoxysilane (GPS), after which the glycidoxy groups were modified with an azo-initiator (4,4′-Azobis(4-cyanopentanoic acid)) to yield azo group-introduced silica particles. The following polymer grafting was carried out by photopolymerization of MAA as the functional monomer, ethylene glycol dimethacrylate (EGDMA) as the crosslinker, and testosterone as the template, on the surface of the azo-modified particle, which served as the initiator. The prepared particles with specific recognition ability for testosterone (imprinting factor of 1.52) were used for liquid chromatography [55]. Nevertheless, it is obvious that they can also be used for sensor development by nanoparticle auto-assembly [40].
In reference [56], a “grafting from” method is provided by Tarannum and Singh, who reported water-compatible surface imprinting of “baclofen” on silica surface. As a supporting matrix, a silica gel was used and the synthesis of the MIP for a skeletal muscle relaxant, namely baclofen (4-amino-3-p-chlorophenylbutyric acid) was carried out on the surfaces of silica gel. An imprinting network of sulfobetaine polyelectrolyte was prepared in aqueous medium only. This was grafted onto the silica gel matrix using 3-aminopropyltriethoxysilane (APTES) as a silane coupling agent and Michael addition reaction for further propagating the polymer grafting procedure. The rebinding studies showed that the MIP displayed good recognition for baclofen as compared to NIPs. Meanwhile, selectivity tests proved that MIP had a high affinity to baclofen in the presence of interferants (close structural analogues). Hence, a facile, specific, selective and water-compatible technique to detect baclofen in the presence of various interferants is provided. The prepared materials can be applied in HPLC as well as in capillary electrochromatography (CEC).

An interesting “dummy” imprinting approach by surface polymerization is described by Shahhoseini et al. [57]. In order to prepare sensitive layers for tricyclic antidepressants (TCAs) measurement in blood, the surface polymerization was performed on steel blades. In this respect, the pre-polymerization solution was obtained using a dummy template: benzyl[3-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)propyl](methyl) carbamate, MAA and EGDMA and cast on the steel blades. Subsequently, the photo initiator was added and the layers were covered with a glass cover. The idea of using a dummy template, in this case, was derived from the necessity to prevent template bleeding. For trace analysis, such as the one targeted in this work, the risk of false positive results can also be due to incomplete template removal. Thus, to avoid such issues a pseudo-template was employed. The prepared MIPs were used as sensitive layers for liquid chromatography in which case the adsorption efficiencies for the MIPs were 3 to 5 times better than for the non-imprinted analogous, confirming that the use of the pseudo-template led to improved performance in this case.

The work of Hudson et al. [58] provides some insights regarding the surface molecular imprinting polymerization for obtaining a fluorescence sensor for the thermal and optical detection of nafcillin. The production of MIP films and particles was realized by integrating a fluorescent moiety that serves both as an element for template interaction and signaling. Fluorescein methacrylate (FluMa) was synthesized and introduced during the molecular imprinting process, first as the sole monomer and afterwards in an equimolecular mixture with MAA. The thin MIP films were deposited onto functionalized glass slides (to serve as electrodes) and the following UV-light initiated polymerization was performed in the presence of template species, nafcillin sodium salt. Although the specificity of films was not as high as that registered for the particles, the MIPs with FluMa and MAA were by far more performant than the ones with FluMa alone. Hence, the results are promising for developing a portable sensor for antibiotics [58].

3. Molecular Imprinting by Electropolymerization

Electrochemical polymerization is a method used to synthesize conductive polymers that are widely used for the development of biosensors and chemical sensors. This technique involves the deposition of a polymer layer on an electrode surface [59]. The electropolymerization can be performed by two methods, i.e., oxidation or reduction. Of the two methods, oxidation is the most commonly used for the obtaining conducting polymers. Anodic electropolymerization involves the monomer oxidation, thus obtaining cationic radicals, that lead to the formation of the polymer on the electrode surface [60,61]. Pyrrole, aniline and thiophene are the most important classes of conductive monomers, due to their low oxidation potential. Thus, by electropolymerization, polypyrrole, polyaniline and polythiophene are obtained. These polymers find their use in a wide range of domains because of certain advantages, referring to price, stability and synthesis complexity [62,63].
The electrochemical polymerization can be carried out using a three-electrode system (working electrode, counter electrode and reference electrode) or screen-printed electrodes (“3 in 1 electrode”). In a typical procedure, the following components are required: electrode system, solvent, supporting electrolyte, monomer(s) and template molecule (in case of MIP synthesis) [64]. The polymer layer deposition takes place on the working electrode (WE) surface (given schematically in Figure 4). The most common electrodes are made of gold, carbon and platinum, but other options are also available such as silver or indium tin oxide. Since, the properties of the polymer film are influenced by the electrode material, electrode surface, supporting electrolyte, electropolymerization technique, solvent and monomer, the overall procedure usually requires an optimization [65].

![Figure 4. The principle of MIP deposition by electropolymerization.](image)

The electropolymerization can be performed using different techniques, including potentiodynamic (cyclic voltammetry), potentiostatic (constant potential) and galvanostatic (constant current) [66]. The potentiodynamic technique involves the formation of the polymer via cyclic voltammetry (CV). The polymer is formed upon applying a potential, which is changing between the oxidative and reductive state, resulting in a doped and undoped polymer [67]. On the other hand, potentiostatic polymerization is performed at a constant potential. In case of oxidative polymerization, the applied potential is positive, resulting in a doped polymer. In addition, the potential should be high enough to oxidize the monomer in order to initiate the polymerization process, but at the same time, it should be low enough to avoid secondary reactions [61]. Last but not least, the galvanostatic polymerization is carried out at a constant current, thus resulting in a doped polymer. This method has important advantages, such as simplicity, and most importantly, the thickness of the polymer depends on the electropolymerization time [68].

In recent decades, MIPs synthesized by electropolymerization have been widely used in the development of electrochemical sensors. An electrochemical sensor is a device consisting of a recognition element (in this case, MIP selective layer) and the electrochemical transducer. The principle of an electrochemical sensor relies on the interaction of the receptor with the analyte, which is transformed into an analytical signal (Figure 4). The main types of electrochemical sensors include conductometric, potentiometric, impedimetric and amperometric sensors [69,70]. Due to the improved selectivity, sensitivity and stability, limit of detection and low cost that MIPs provide, such MIP-based sensors are widely reported nowadays [71]. According to the recent literature, MIP-based electrochemical sensors are used in various applications in areas of human health, environmental pollution and pharmaceutical domain. The most significant results with MIP-based electrodes prepared by electropolymerization are provided in Table 2.

For instance, Menon and her co-workers [72] prepared an MIP-based electrochemical sensor for acetaminophen detection. Acetaminophen is an analgesic that can be harmful if used in excess. The synthesis of the film was conducted using a three-electrode system consisting of a gold (Au) electrode (working electrode), Ag/AgCl (reference electrode) and platinum electrode (counter electrode). In the first step, the gold electrode surface was modified with AuNPs. After that, the MIP film was deposited onto the modified electrode surface by electropolymerization. The MIP synthesis was performed by cyclic
voltammetry, at a potential ranging from 0 to 1.3 V, at a scan rate of 100 mV/s for 30 scan cycles. The prepared electrodes were analyzed by CV and EIS. The performance of the MIP sensor was also studied by square wave voltammetry (SWV), in a concentration range between $4.5 \times 10^{-5}$ and $5 \times 10^{-7}$ M, resulting in a limit of detection of $2.3 \times 10^{-9}$ M [72].

Another concerning problem is the side effect related to the use of dimetridazole beyond the permitted limits. Recently, Ali et al. [73] depicted the preparation of a poly-arginine MIP based sensor that can be used for electrochemical detection of dimetridazole. In this study, the MIP film synthesis was carried out by CV, using a three-electrode system (glassy carbon electrode GCE–working electrode, Ag/AgCl electrode–reference electrode and platinum wire electrode–counter electrode) and a solution consisting of L-arginine, Dimetridazole dissolved in PBS. The electropolymerization conditions consisted of a potential range between $-2$ and 2.2 V, a scan rate of 100 mV/s and 12 scan cycles. The recognition experiments were performed by differential pulse voltammetry (DPV), using solutions with different concentration ($10^{-10}$ to $10^{-5}$ M). The limit of detection for such sensors was 0.1 nM, which represents a promising future for dimetridazole detection [73].

In the past few years, both the production and consumption of antibiotics increased, which has led to a rise in environmental pollution. In recent years, sensors for antibiotics have been studied in order to improve the quality of life. For example, Ayankojo and co-workers [74] developed a sensor based on MIP for erythromycin detection, using a screen-printed electrode with Au working electrode. The electropolymerization was performed under potentiostatic condition, meaning a constant potential of 0.63 V, using m-phenylenediamine as monomer. The recognition and the rebinding capacity of the sensors were studied by DPV, pointing to a limit of detection of 0.1 nM and also a good selectivity. The results presented in this study may represent a solution for erythromycin.
Another research group prepared an electrochemical sensor for tetracycline detection [75]. They were able to synthesize an MIP film by electropolymerization on the surface of the gold electrode surface. A three-electrode system consisting of Au electrode (working electrode), saturated calomel electrode (reference electrode) and a platinum (Pt) electrode (counter electrode), was used in the electropolymerization process. A first step involved in synthesizing gold nanoparticles, further used in the development of the sensor. The films were prepared by CV upon applying a potential between 0.35 and 0.8 V, at a scan rate of 100 mV/s, for 10 scan cycles, after which the conditions were changed to a fixed potential (0.8 V). The performance of the sensor was studied by linear sweep voltammetry (LSV) and indicated quite high sensitivity, down to 0.22 fM tetracycline in aqueous solutions [75].

Given the toxic effect of dyes on human health, controlling their presence in the food becomes mandatory. Thus, Arvand et al. [76] proposed the development of an electrochemical sensor based on an MIP layer that can be used for food analysis. In this case, the authors used a three-electrode system consisting of a working electrode (functionalized GCE), reference electrode (saturated Ag/AgCl electrode) and counter electrode (Pt electrode). MWCNTs were used for the functionalization of the GCE. The electropolymerization was carried out by CV, in a potential range between −1.4 V and −0.4 V, for 15 scan cycles, using a solution containing AAm as the functional monomer, N,N-methylene-bis-acrylamide (MBA) as the crosslinker, sodium persulfate as the initiator, sunset yellow as the template molecule and sodium nitrate as the electrolyte dissolved in PBS. Ultimately, the sensor was electrochemically tested using CV and was demonstrated to possess good recognition properties for sunset yellow in the 0.05–100 µM concentration range and a low limit of detection (LOD) of 5 nM [76].

Shen et al. [77] described the preparation of an electrochemical sensor based on MIP for tetra-bromo-bisphenol A (TBBPA) detection. TBBPA is widely used for plastics and electronics manufacturing, and it may have negative effects on human health, including neurological and thyroid dysfunctions. Thus, the MIP films were prepared by electropolymerization onto GCE surface, using CV, in a potential range between −0.5 and 0.5 V, at 70 mV/s for 10 scan cycles. The solution used for electropolymerization consisted of ethanol, dopamine, TBBPA (template molecule) and PBS. MIPs showed a good sensitivity for TBBPA, in the 1–50 nM concentration range. Moreover, the MIP films presented selectivity and an LOD of 0.27 nM. The results are promising and comparable with further HPLC applications [77].

Wang and co-workers [78] prepared an MIP based sensor on indium tin oxide (ITO) bare for the determination of resveratrol. In this respect, prior to electropolymerization, the ITO electrode was modified with Ag nanoparticles using cyclic voltammetry and after that with a HAuCl₄ (chloroauric acid) solution. Following this procedure, the MIP film was deposited onto the modified electrode surface via electropolymerization, by CV, applying a potential between 0 and 0.8 V at a scan rate of 50 mV/s, for 30 scan cycles. The electrochemical behavior of the sensors was studied by CV and EIS, in which case a linear response between $2.0 \times 10^{-11}$ to $9.0 \times 10^{-9}$ M was reported. The detection limit was determined to be $7.1 \times 10^{-12}$ M. Moreover, the sensor was tested using structural analogues and proved good selectivity [78].

Li [79] described the development of an MIP sensor for atrazine detection. The MIP film synthesis was carried out by electropolymerization using a classical electrochemical cell. For producing the MIP film, the electrochemical cell consisted of an Au electrode (working electrode), a saturated calomel electrode (reference electrode), a Pt electrode (counter electrode), o-Phenylenediamine (monomer), electrolyte and atrazine (template molecule). In this case, the employed electropolymerization conditions referred to applying a potential between 0 and 0.8 V at a scan rate of 50 mV/s for 15 scan cycles. CV and DPV assays have proven that the prepared sensor can detect atrazine at different concentration, having a very low LOD of $1 \times 10^{-9}$ M [79].
Motia et al. proposed an electrochemical sensor based on an MIP layer for sodium lauryl sulfate (SLS) detection. In this study, a “3 in 1” electrode with Au electrode as working electrode, Ag electrode as reference electrode and gold strip plate as counter electrode, was used. The MIP film was deposited by electropolymerization of 2-Aminothiophenol (2-ATP), in the presence of SLS (template molecule), by applying a potential between −0.35 and 0.80 V for 10 scan cycles at 100 mV/s. Prior to electropolymerization, the surface of the electrode was modified with a layer of 2-ATP by dripping the solution on the electrode surface. The electrochemical response, by CV, DPV and EIS, indicated that the sensor presented good affinity for the SLS, with a limit of detection of 0.18 pg/mL [80].

Seguro et al. [81] have recently present an electrochemical MIP-based sensor for diclofenac detection. The development of the voltametric sensor involved the synthesis of an MIP film by CV directly on screen printed carbon electrode (SPCE) surface, using a solution containing dopamine as monomer, diclofenac sodium as template molecule and KCl as electrolyte. The electrodeposition was performed in a potential range between −0.5 V and 1 V at a scan rate of 100 mV/s. Ultimately, the sensor was tested by DPV, and, according to the authors, the imprinting factor was 2.5. Furthermore, a LOD of 70 nM and a limit of quantification (LOQ) of 200 nM were obtained [81].

Another sensor based on Au nanoparticles and MIPs was introduced by Liu and her group [82] for epinephrine detection. The authors used a three-electrode system consisting of GCE (working electrode), saturated calomel electrode (reference electrode) and a Pt electrode (counter electrode). The working electrode was modified previously with HAuCl₄, after which the electrodeposition was performed by applying a potential between −0.2 and 1.2 V for 20 scan cycles in the presence of epinephrine and 3-thiophene boronic acid (3-TBA). CV and DPV techniques were used to evaluate the performance of the final sensor, in which case a detection limit of 7.6 × 10⁻⁸ M was recorded [82].

Lopes and his group [83] proposed a sensor for naloxone detection. In this respect, the MIP film was synthesized by electropolymerization on the screen-printed carbon electrode that was firstly modified with MWCNT. The MIP film was prepared by CV when applying a potential in a range between −0.2 and 1 V, for 20 scan cycles at a scan rate of 100 mV/s, using a solution of 4-aminobenzoic acid and naloxone in PBS. The performance of the sensor was further studied using DPV and a detection limit of 0.2 µM was acquired [83]. Three years later, Shaabani and co-workers [84] also studied a sensor for naloxone detection, whereas the MIP film was deposited under similar conditions as described by Lopes et al. [83]. Yet, in this case, the SPCE surface was modified with Au nanoparticles and the CVs were obtained after applying a potential between −0.2 and 1 V at a scan rate of 50 mV/s for 15 scan cycles. By doing so, a lower detection limit (0.16 µM) was achieved.

Methylone is a synthetic drug, which produces psychotropic effects similar to ecstasy. Considering the side effects of this drug that include hypertension, paranoia and tachycardia, Couto et al. [85] presented a method for preparing an MIP-based sensor for methylone detection. The film was deposited on screen-printed gold electrode by electropolymerization of 2-mercaptobenzimidazole (monomer) in presence of the template molecule (methylone). The process was performed by CV when applying a potential in a range between −0.2 and 1.3 V for 15 scan cycles. A good performance of the sensor was obtained by SWV, in which case a detection limit of 1.1 µM was obtained. Moreover, the sensor also showed good stability and selectivity [85].

Melamine is a synthetic compound that can be used in the plastic industry as well as the milk industry. Considering the side effects of the melamine on human health (e.g., kidney problems, kidney stones), the monitoring of food is required. In this respect, Li and colleagues [86] developed an SPR sensor based on MIP films for melamine detection. The three-electrode system consisted of an optical fiber probe coated with Cr/Au film (working electrode), an Ag/AgCl electrode (reference electrode) and Pt electrode (counter electrode), while the MIP film was synthesized by electropolymerization of o-aminophenol (functional monomer) in the presence of melamine, by applying a potential between −0.3 and 1.2 V for 30 scan cycles at 50 mV/s. In order to estimate the performance of the
sensor, several melamine solutions with different concentrations were used, leading to an LOD of $5.1 \times 10^{-12}$ M, which indicated a good sensitivity of the sensor [89]. Two years later, Regasa et al. [87] proposed a sensor for melamine detection that involved the electropolymerization of aniline in order to obtain the MIP film. In this case, a three-electrode system with GCE (working electrode), Pt electrode (counter electrode) and Ag/AgCl electrode (reference electrode) was employed and the electrodeposition of the MIP film was carried out in potentiodynamic conditions, by applying a potential between $-0.2$ and 1 V for 10 scan cycles. This group [87] evaluated the properties of prepared sensors by CV and SWV, in which case they obtained a higher detection limit ($4.47 \times 10^{-10}$ M) compared to the previous work [86].

Roushani and his team [88] developed an MIP sensor for electrochemical detection of asulam herbicide or methyl $N$-(4-aminophenylsulfonyl)carbamate. In the first step, the GCE was modified with g-C$_3$N$_4$ (synthesized powder) and, subsequently, used for the electrodeposition of the MIP. The MIP solution consisted of monomer (dopamine), the template molecule (asulam) and electrolyte (KCl) dissolved in tri-buffered saline (TBS) ($pH = 7$), which was electropolymerized in the $-0.5$ to 0.5 V potential range, for 10 cycles. The electrochemical behavior was analyzed using different methods, including CV, DPV and EIS, which led to the conclusion that the sensors possess high selectivity and affinity towards asulam, with an LOD close to 0.17 pM [88].

Wei and colleagues [89] studied and developed a sensor based on MIP for electrochemical detection of luteolin, using a three-electrode system (ITO as the working electrode, SCE as a reference electrode and Pt electrode as counter electrode). The MIP film synthesis was achieved by electropolymerization of $\beta$-cyclodextrin (monomer) in presence of luteolin, applying a potential between $-0.8$ and 1.1 V for 20 cycles. Rebinding and selectivity experiments were performed by contacting the sensor with luteolin, respectively with quercetin and apigenin solutions and studied by DPV. In this case, the sensor detection limit for luteolin was near $2.4 \times 10^{-8}$ M [89].

Zheng and co-workers [90] proposed a two-step fabrication process of an electrochemical MIP-based sensor for detection of 4-nonylphenol (4-NP) in milk samples. In the first step, a multilayer electrode was obtained using reduced graphene and HAuCl$_4$. In the second step, the MIP film was prepared by electropolymerization of p-aminothiophenol (monomer), in presence of 4-NP as template molecule, upon applying a potential between $-1$ and 1 V. CV and DPV were employed for determining the sensor characteristics, in which case indicated a good selectivity and a LOD of 0.01 ng/mL [90].

The study by Mathieu-Scheers and her group [91] presented an MIP sensor for electrochemical detection of anthracene using GCE. The procedure was performed in a potential range between 0 and 1.4 V or 0 and 1.2 V, at a scanning rate of 10 mV/s, for 5 scan cycles, using pyrrole as a monomer and anthracene as a template molecule. The rebinding of anthracene was detected by SWV when a detection limit of 12 nM was calculated [91].

Recently, Grothe et al. [92] proposed a sensor based on MIP that allowed for detecting and identifying components from cocaine samples. Identification of the components provides important information regarding the origin of the cocaine. Herein, the MIP film was synthesized by electropolymerization of 3-amino-4-hydroxybenzoic in the presence of anesthetic benzocaine as the template molecule, on the carbon electrode of a SPCE. The electropolymerization process was performed in a potential range between 0 and 1.5 V for 10 scan cycles at a scan rate of 100 mV/s. SWV and EIS were used to study the electrochemical behavior of the films after contact with artificial urine (containing benzocaine) or caffeine, aminopyrine and procaine, in which case a limit of detection of 2.9 nM was achieved [92].

Radi and co-workers [93] have also described a sensor developed using MIP films that can be used for entacapone detection. A three-electrode system involving a WE (GCE), CE (platinum wire electrode) and RE (Ag/AgCl/KCl) was employed herein, while the MIP layers were prepared using polyphenylenediamine (monomer) and entacapone (template). Electropolymerization was performed using CV by applying a potential between
0 and 0.8 V at a scan rate of 100 mV/s and electrochemical behavior of the films was studied using EIS and DPV. As a result of the study, the sensor presented a good selectivity for entacapone vs. levodopa and carbidopa, with a limit of detection near $5 \times 10^{-8}$ M.

4. Molecular Imprinting Using MIP Particles Embedded in Pastes or Inks

This section brings to the scientific community newsworthy studies through reviews and original articles concerning strategies for preparing pastes or inks, used for the fabrication of molecularly imprinted polymers (MIPs)-decorated layers or films. Currently, preparation of sensitive layers on electrode surfaces is certainly the most frequently used technique for MIP-modified electrodes. Among the reviewed methods for forming MIP layers, dropcasting of a pre-prepared polymer paste and coating processes such as drop/dip or spin coating and screen printing, or through self-assembled monolayers (SAMs) are shortly discussed hereafter. Hence, this part of the review focuses more on the performances of the resulting MIP-modified layers via their preparation and deposition techniques.

Research on pastes or inks for applicability within areas of electrochemistry and electroanalysis began in the mid-1950s. Since then, preparing and deposition of paste (ink)-based electrodes modified with MIP soon became very attractive for sensing applications and photoelectrochemical devices; carbon pastes (CPs) belonged among the most popular electrode materials for preparation of various sensors, justified by their low price, ease of fabrication, fast response time, renewable surfaces, high sensitivity and low background current. Generally, CP, defined as a heterogeneous mixture of a carbonaceous moiety with a suitable (usually liquid) binder, is a graphite/carbon-black paste aimed at the deposition of metal-free, electrically conductive layers by screen-printing or coating techniques.

Likewise, the composition of the pastes used for deposition of the successive layers may be altered by the addition of nanostructured materials such as cofactors, stabilizers and mediators. More recently, silver nanoparticles (AgNPs), Ag nanowires (AgNWs), CuNWs, CuNPs, carbon nanotubes (CNTs), and reduced graphene oxide (rGO) have also been explored as nano-inks and further incorporated either in these pastes or in a later stage on the working electrode. The most applied and comfortable routes to prepare MIP layers using inks and pastes are illustrated in Figure 5.

![Figure 5. Routes for preparing MIP layers using inks or pastes.](image-url)
materials were published [102,104,105]. Nowadays, taking into account the type of ink and substrate, printing technologies that should be mentioned are screen printing (more than 60%, according to Wahyuni, et al. [98]), gravure printing, stereolithography, inkjet printing and aerosol jet printing [100].

The process of screen printing consists of layer-by-layer depositions of proper ink through a patterned mesh or screen onto a solid substrate, followed by a thermal curing treatment. Hence, successive layers can be deposited by this procedure and repeat patterns can be designed onto the same screen to enhance production speed. SPE usually combine a three-electrode configuration (working, reference and counter electrode) printed on different chemically inert substrates, i.e., flexible plastic (polycarbonate, polyimide, polyvinyl chloride and PEEK), ceramic, paper or glass substrates [106]; the substrates are easily modifiable with a great variety of commercial or self-made inks.

The pastes most commonly reported in SPE production are conductive (containing the polymer base dispersed in a solvent and the conductive material) or dielectric inks (often based on polymers or ceramics and form the encapsulating layer of the sensor) [98,107]. Among the solvents that participate in the ink components [108], there are two categories: organic and water-based. Organic solvents, such as ketone, esters and hydrocarbons, in general, have a low flash point, which promotes faster drying of the ink and low viscosity, which are generally qualities that facilitate the processing of inks. Recently, considering the performance and the environmental strategies, water-based inks have also been employed [109–111]. Gold paste is also employed in SPEs but less than carbon due to its higher cost [112]. The composition of the various inks used for printing on the electrodes determines the selectivity and sensitivity required for each analysis. It is important to point out that proper ink formulation is owned by the manufacturer as proprietary information and it has been shown that differences in ink composition, e.g., type, size or loading of graphite particles and in the printing and curing conditions can strongly affect the electron transfer reactivity and the overall analytical performance of the resulting carbon sensors.

An extensively studied path used in the preparation of modified layers is based on the incorporation of pre-synthesized MIP microparticles/nanoparticles [113,114] (acting as a selective recognition element and a pre-concentrator agent for the determination of different templates) into layers or polymer matrices or containing a conducting material and binder (e.g., PVC) (e.g., carbon nanotubes (CNTs), graphene, graphite, or carbon black) [110,115] (Table 3). Cervini and Cavalheiro [116] revealed the most significant approaches for electrodes modified with MIP (including spin and drop coating and self-assembling of films on metal nanoparticles). Thus, the mentioned authors used a graphite-polyurethane composite matrix to prepare electrodes used in paracetamol (APAP) determination. Interference of phenacetin in the APAP response decreased remarkably when the proposed electrode was used.

Khosrokhavar et al. [117] described the successful preparation of an MIP nanoparticle/graphene suspension drop-coated as a thin layer onto the surface of the SPCE. For this purpose, two inks including silver ink (containing silver and PVC powders with mass percentages of 97 and 3% respectively in 1:1 (v/v) acetone/cyclohexanone solution) and carbon ink (containing 80% graphite, 12% dibutyl phthalate (DBP) and 8% PVC in 1:1 (v/v) acetone-cyclohexanone solution) were prepared. The electrochemical sensor used for the selective detection of an antidepressant drug, sertraline (STR) exhibited good sensitivity (177.25 µA L µmol⁻¹) and recoveries above 98%.

Another significant study was described by Nontawong et al. [118], where the team reported the preparation of dual-imprinted electrodes modified with graphene to simultaneously determine 8-hydroxy-20-deoxyguanosine (8-OHdG) and 3-nitrotyrosine (3-NT) and assess oxidative and nitrative biomarkers in urine and plasma samples. The synthesized composites, mixed separately with graphene ink, were further screened as hydrophobic barrier layers on filter paper. The smart devices revealed selectivity and sensitivity leading to low detection limits of 0.0027 µM for 3-NT and 0.0138 µM for 8-OHdG.
In another screen-printing preparation strategy, a graphite ink made of a mixture of graphite powder and hydroxyethyl cellulose was chosen [119]. The electrochemical MIPs were incorporated in a composite paste SPE for the detection of an organic pollutant, Bisphenol A (BPA). The results proved the reliability of the device with an LOD as low as 0.06 nM and also the selectivity vs. carbamazepine and ketoprofen. BPA was also detected using a simple and ultra-low-cost, disposable paper-based potentiometric sensor, reported by Kamel et al. [120]. The prepared BPA–MIP nanobeads were mixed with the PVC membrane cocktail and incorporated by dropcasting into recyclable and biodegradable paper. The sensor was proven to be selective towards other phenols, exhibiting a detection limit of 0.15 µM.

An entrapment agent such as poly(methyl methacrylate) (PMMA) was suitable to carry out the incorporation of the synthesized MIP particles. For example, a QCM-based sensor for volatile organic compounds was prepared by spin coating a dispersion containing the MIP particles and PMMA on an AT quartz crystal resonator. The resulting devices tested towards toluene or p-xylene vapor showed potential for developing QCM sensors with MIP [121].

An interesting design of a biosensor for detecting lipopolysaccharides (LPS) derived from *Pseudomonas aeruginosa* was proposed by Iordache et al. [122] using MIP-modified SPCE. As described in their research, the strategy was based on doping the mixture with electroactive particles of zinc oxide, while the sol–gel precursor solution was dripped directly onto the SPCE using the dropcasting method. The MIP films prove stability at re-use, recognizing the LPS from *Pseudomonas aeruginosa* to a greater extent than the LPS from *Escherichia coli*, when using 16.7 µg/mL aqueous LPS solutions.

Another successful attempt, accomplished by Blanco-López et al. [30], for preparing electrodes modified with MIP layers, refers to imprinting rifamycin SV (RSV), a macrocyclic antibiotic. Their study involved the drop coating of the electrode surface with a solution of a preformed polymer (polyphosphazenes) as recognition element. The final electrode exhibited selectivity towards dopamine and NADH as well as excellent reusability up to 25 times without signs of film loss or memory effects. The same group investigated the behavior of diclofenac by modifying carbon composite electrodes of different nature (polytetrafluoroethylene-graphite, epoxy graphite and epoxy-carbon black) with MIP particles. The drop coating method was also used for the modification of hanging a mercury drop electrode (HMDE) with MIP–DMF casting solution, which was applied in the determination of ascorbic acid [123].

One interesting approach used in the preparation of electrodes modified with MIP, is based on spin coating. The study carried out by Liuduan and co-workers [124] targeted the preparation of a sensor for phenylephrine detection. In this respect, an electrochemical sensor for phenylephrine (as template), was dropped onto the surface of a GCE and the excess solution was eliminated by spin coating. The modified electrode was used in the determination of phenylephrine. Some years later, Ebarvia et al. [125] developed a biomimetic piezoelectric quartz crystal sensor for the determination of antibiotic chloramphenicol in food products. In their work, an MIP sensing layer was obtained by spin coating using a home-made device. The performance of this sensor refers to a sensitivity of about 73 Hz/log (conc., µg mL$^{-1}$), a detection limit of $7 \times 10^{-8}$ µg mL$^{-1}$ and good repeatability (rSD below 10%). The development of an electrochemical sensor for 1-hydroxypyrene (1-OHP) based on a molecular imprinted TiO$_2$ gel matrix was also described by the group of Yang [126]. The 1-OHP-imprinted films were prepared by spincoating using film-forming stock solutions on quartz plates. The resultant 1-OHP-imprinted sensor had a detection limit of $3.353 \times 10^{-10}$ M with the linear range $1 \times 10^{-9}$ M–$2 \times 10^{-7}$ M.

Another approach for MIP preparation relies on the self-assembly-copolymerization of mixtures containing the template and the monomers [127]. Shin et al. [128] reported the preparation of a thin polymer film on a self-assembled monolayer with 4-mercaptophenol and benzenethiol on a gold plate for the recognition of cholesterol. The poly(methyl
methacrylate) (PMMA) was then spincoated on the monolayer, leading to excellent recognition ability. The authors assumed that the difference of regularity and flatness, around 0.233 nm roughness value, after spincoating has an effect on the cholesterol recognition ability.

Roushani and colleagues [129] investigated an MIP electrochemical sensor for fast and direct determination of trazosine (TR). The large effective surface area and good electrical conductivity were obtained through a layer of AuNPs. MIP/AuNPs/SPCE was developed by directly dropping the synthesized MIP onto the surface of the AuNPs/SPCE, exhibiting great electrochemical signals in the potential range 0.6 mV for detecting trace TR with a good selectivity and a low detection limit (S/N = 3) of 0.3 µM.

Liu et al. [130] reported a composite of reduced graphene oxide/Fe$_3$O$_4$-ionic liquid-based MIP, which was dropcasted onto a GCE. RGO/Fe$_3$O$_4$-IL-MIP layer was used to construct an electrochemical sensor for diphenylamine (DPA). The performances of the sensor revealed a detection limit of 0.05 µM (S/N = 3) with a linear range of 0.1–30 µM proving the recognition in real samples.

Meanwhile, Angelis et al. [131] functionalized carbon black (fCB) by the insertion of oxygenated functional groups upon acid treatment with HNO$_3$ and H$_2$SO$_4$, developing an MIP–fCBPE (functionalized carbon black paste electrode) for imazethapyr (IMT) determination in rice samples. This method was applied after microwave-assisted extraction of IMT, leading to 96.3–105.7% accuracy by recovery assays. The electrochemical properties rely on the incorporation of molecularly imprinted polyvinylimidazole (MIP–VN) in the fCBPE, obtaining a limit of detection of 0.03 µmol L$^{-1}$ and good reproducibility of the measurements (RSD% = 3.6).

Table 3. MIP-based sensors obtained using MIP particles embedded in pastes or inks.

| Synthesis Method | Receptor | Support | Analyte | Characterization Method(s) | LOD | Refs. |
|------------------|----------|---------|---------|-----------------------------|-----|-------|
| Drop coating     | MIP film | SPCE$^1$ | Sertraline | CV$^2$ and DPV$^3$ | 1.99 × 10$^{-9}$ M | [117] |
| Coating          | MIP film | Paper-based device | 8-hydroxy-20-deoxyguanosine and 3-nitrotyrosine | CV and SWV$^4$ | 1.38 × 10$^{-8}$ M and 2.7 × 10$^{-9}$ M | [118] |
| Dropcasting      | MIP film | SPCE | Bisphenol A | CV and HPLC$^5$ | 6.0 × 10$^{-11}$ M | [119] |
| Dropcasting      | Membrane | Chromatography paper used as electrode | Bisphenol A | Potentiometric detection | 1.5 × 10$^{-7}$ M | [120] |
| Spincoating      | MIP Membrane | GCE$^6$ | Phenylephrine | DPV and HPLC | - | [124] |
| Spincoating      | MIP film | Au electrode quartz crystal | Chloramphenicol | Oscillation frequency | 7 × 10$^{-8}$ µg·mL$^{-1}$ | [125] |
| Spincoating      | MIP film | Quartz plates | 1-hydroxypyrene | CV | 3.353 × 10$^{-10}$ M | [126] |
| Dropping         | Film | SPCE | Trazosin | CV, DPV and EIS$^7$ | 3.0 × 10$^{-7}$ M | [129] |
| Dropping         | Film | GCE | Diphenylamine | DPV | 5.0 × 10$^{-8}$ M | [130] |
| Functionalization of carbon black paste electrode | Layer | Functionalized Carbon black | Imazethapyr | DPV | 3.0 × 10$^{-8}$ M | [131] |

$^1$ SPCE: screen-printed carbon electrode; $^2$ CV: cyclic voltammetry; $^3$ DPV: differential pulse voltammetry; $^4$ SWV: square wave voltammetry; $^5$ HPLC: high performance liquid chromatography; $^6$ GCE: glassy carbon electrode; $^7$ EIS: electrochemical impedance spectroscopy.

5. Molecular Imprinting by Sol–Gel Derived Techniques

The interest in materials obtained by sol–gel method has grown considerably in recent years, as evidenced by the multitude of published papers. The materials obtained by this method can be used in different applications, including chemical sensors and biosensors, coatings and catalysts [132].

Overall, the sol–gel process implies several actions such as activation of precursor (usually involves hydrolysis), polycondensation, gelation, aging, washing, drying and stabilization [133,134]. The sol–gel process involves the transition from liquid state to a “sol” and then into a “gel” network structure [135]. In this respect, metal alkoxides undergo hydrolysis in aqueous media to obtain hydroxyl groups. The next step involves
the obtaining of a 3D network by the formation of Si–O–Si bonds resulting from the poly condensation reaction of hydroxyl groups and remaining alkoxyl groups. The reaction can be performed under basic or acidic conditions depending on the morphology of the material that is required.

The porosity and the morphology of the sol–gel materials depend on several parameters, i.e., the solvents, the pH, drying process, reaction conditions and catalysts. These aspects are very important because, given the porosity and morphology, the obtained materials can be used for different applications [133,136–138]. This synthesis method presents multiple advantages, namely mild synthesis conditions (for example, the synthesis can be performed at room temperature), the obtained materials possess good optical properties, high thermal stability and high porosity, which can be correlated with a high surface area [139–141]. The sol–gel materials can be prepared in different forms, including thin films (for sensors and coatings) [14,141–143], composites (such as alumina reinforced materials), particles (for solid-phase extraction or for incorporation in thin films for sensor development) [138].

By combining the sol–gel method with molecular imprinting, performant sensitive layers can be obtained for sensor development (Figure 6). The sol–gel MIP films can be prepared by the drop coating or spin coating method and also by electrodeposition [144,145]. Due to the good optical and semi-conductive properties, sol–gel thin films can be used for the development of optical sensors as well as for electrochemical sensors. Thereby, in this section of the review, sensors based on sol–gel MIP films are presented (according to the two procedures presented in Figure 6). Thus, in the first part of the section, MIP sensor recognition elements based on sol–gel are presented, while the second part presents some examples of hybrid methods based on the combined sol–gel/electropolymerization method. Table 4 presents the use of sol–gel technique for MIP-based sensors and their performances.

Guney and his team [146] proposed the preparation of an MIP film using sol–gel method, in order to develop an electrochemical sensor for theophylline (TP) detection. The film was deposited on a GCE surface, which was modified prior to MIP deposition. The functionalization was made using a functional monomer: CoG–TEICPS (Crocein orange G-triethoxy(3-isocyanatopropyl)silane) and TEOS (tetraethyl orthosilicate) in acidic conditions, after which the MIP film was deposited by spin coating using the same recipe but with the addition of the template, TP. The electrochemical behavior of the films was studied using two methods: CV and DPASV (differential pulse anodic stripping voltammetry), which revealed that the sensor was selective and attained a low limit of detection of \(1.4 \times 10^{-9}\) M [146].

![Figure 6. Sol–gel and combined sol–gel/electropolymerization methods for the preparation of MIP layers.](image-url)
Table 4. MIP-based sensors obtained by sol–gel method.

| Method | Receptor | Support | Analyte | Characterization Method(s) | LOD | Refs. |
|--------|----------|---------|---------|-----------------------------|-----|-------|
| Spin coating | MIP film | Modified GCE | Theophylline | CV and DPASV | 1.4 × 10⁻⁹ M | [146] |
| Immersion | MIP film | Modified Au electrode | Melamine | CV and SWV | 0.4 × 10⁻⁹ M | [147] |
| Spin coating | MIP film | Au surface of SPR device | Amoxicillin | SPR and CV | 7.3 × 10⁻¹¹ M | [148] |
| Spin coating | MIP film | Au modified glass substrate | cis-jasmine | FT-IR, LSPR | 3.5 ppm | [149] |
| Spin coating | MIP film | SPR substrate | Trinitrotoluene | SPR | 0.26 ppb | [150] |
| Immersion | MIP film | Modified GCE | Chlorogenic acid | DPV | 3.2 × 10⁻⁸ M | [151] |
| Dripping | MIP film | SPE | Europium | CV, EIS, DPV | 1 × 10⁻⁷ M | [152] |
| Dripping | MIP film | GCE | Aspartic acid | SWSV | 1.77 × 10⁻⁸ M | [153] |
| Coating | MIP film | MCNTs | Aristolochic acid | Adsorption experiments | 0.034 µg/mg | [154] |
| Immersion | MIP film | GCE | Trichlorfon | EIS and CV | 2.8 × 10⁻⁹ g/mL | [155] |
| Electrochemical | MIP film | Au electrode | Clenbuterol | DPV | 3.1 × 10⁻⁹ M | [156] |
| Electrochemical | MIP film | GCE | Melamine | DPV | 6.8 × 10⁻⁹ M | [157] |
| Electrochemical | MIP film | ITO electrode | Naloxone | CV and DPV | 2 × 10⁻⁸ M | [158] |
| Electrochemical | MIP film | GCE | Mephedrone | SWV | 8 × 10⁻¹⁰ M | [159] |
| Electrochemical | MIP film | Modified GCE | Diethylstilbestrol | DPV | 24.3 fg/mL | [160] |
| Electrochemical | MIP film | Pencil | Ketamine | EIS and SWV | 7 × 10⁻¹⁰ M | [161] |

1 GCE: glassy carbon electrode; 2 CV: cyclic voltammetry; 3 DPASV: differential pulse anodic stripping voltammetry; 4 Au electrode: gold electrode; 5 SWV: square wave voltammetry; 6 SPR: surface plasmon resonance; 7 FT-IR: Fourier transform-infrared spectroscopy; 8 LSPR: localized surface plasmon resonance; 9 DPV: differential pulse voltammetry; 10 SPE: screen-printed electrode; 11 EIS: electrochemical impedance spectroscopy; 12 SWSV: square wave stripping voltammetry; 13 MCNTs: magnetic carbon nanotubes; 14 ITO: indium tin oxide electrode.

Bengamra et al. [147] developed a sensor based on an MIP membrane for electrochemical detection of melamine. The gold electrode was first modified with a mercaptopropytrimethoxysilane (MPTMOS) layer. Subsequently, the MIP layer was obtained in acidic conditions using TEOS, phenyltrimethoxysilane (PTMOS) and methyltrimethoxysilane (MTMOS), after immersing the modified electrode in a solution of melamine. The final sensors were characterized by CV and SWV, resulting in a LOD of 0.4 × 10⁻⁹ M [147].

A selective SPR sensor based on an MIP film was developed by Ayankojo and co-workers [155], for detecting amoxicillin. The sol–gel precursor solution for MIP films consisted of methacrylamide (MAAM, functional monomer), TEOS, vinyltrimethoxysilane (VTES, coupling agent) and the template (amoxicillin), which was further used to coat uniformly the gold surface of an SPR sensor. The obtained sensors, tested using SPR and CV, recorded an LOD of 73 pM and a good selectivity for amoxicillin [148].

Another example of sensor based on MIP film is presented by Shang et al. [149]. In this case, a SPR (surface plasmon resonance) sensor for cis-jasmone detection was initiated with TiCl₄. Prior to MIP film deposition by spin coating, the glass substrate was modified with gold. For such SPR sensors, the registered LOD was 3.5 ppm for cis-jasmone detection in vapor state [149].

In 2016, Giustina and co-workers [150] proposed an MIP-based SPR sensor for trinitrotoluene (TNT) detection. The sol–gel films were prepared by dissolving the silanes (APTES, TEOS and MPTMOS) and template (TNT) in ethanol and gelation took place in basic conditions. The films were deposited by spin coating onto the SPR substrate and the final sensor was analyzed by SPR in the 4.9 ± 2.8 ppb concentration range. In this case, a limit of detection for TNT of 0.26 ppb was estimated [150].

Ribeiro and her team [151] studied a sensor for the detection of chlorogenic acid in food samples. In a first step, the GCE electrode surface was modified with a layer consisting of MWCNTs and VTES. Following this procedure, an MIP sol–gel layer was deposited on
the surface of the modified electrode by immersing the electrode in the MIP solution and rotating at 1500 rpm. In this case, the MIP solution was prepared by mixing TEOS, PTMOS and APTES in 2-ethoxyethanol, with the catalyst solution of HCl containing the template (chlorogenic acid). The detection limit of the prepared sensor was 0.032 µM, which was suitable for chlorogenic acid detection [151].

Considering the toxic effect of europium on human health, Chen and co-workers [152] focused their interest on the development of a sensor for its detection. In this respect, they modified the surface of a SPE by electropolymerization using catechol, resulting in a poly (catechol) layer, after which the MIP layer was deposited. The precursor gel solution for the MIP was obtained by mixing TEOS, PTMOS and MTMOS with a solution of europium, in acidic conditions. The imprinted film was obtained by dripping the precursor solution on the modified electrode surface and dried at room temperature. The electrochemical behavior of the sensors was tested by different methods (CV, EIS, DPV), out of which DPV technique revealed a detection limit of 10⁻⁷ M for europium [152].

Chen and his team [153] developed a sol–gel MIP-based sensor for aspartic acid recognition. The MIP precursor solution was prepared by dissolving PEG (Polyethyleneglycol) in ethanol and, after stirring the template molecule [(L-Asp)Cu²⁺ (NC-L-Asp)] was added. Finally, TEOS was added to this solution, at reflux, and the films were cast in double layer, by dripping the solution on the GCE surface. In this case, a limit of detection of 1.77 µM was achieved by SWSV (square-wave stripping voltammetry) [153].

Studies on the effects on human health for the use of aristolochic acid have led to a link between its presence and liver cancer. Therefore, Li and her co-workers [156] developed a sensor based on an MIP, using the sol–gel technique, for aristolochic acid detection. In this respect, they prepared a precursor solution containing the template and the monomer (PTMOS) and kept it in the refrigerator, after which magnetic carbon nanotubes (MCNTs), previously functionalized with carboxyl groups, crosslinker (TEOS) and the catalyst were added. The modified MIP–MCNTs were extracted from the solution with a magnet and tested in rebinding experiments. The imprinting factor of MIP particles was 3.17, with the detection limit 0.034 µg/mg [154].

Other concerning compounds that may affect human health are insecticides. For example, trichlorfon can cause memory problems, depression, disorientation, etc. Thus, Gao and co-workers [155], proposed an electrochemical sensor for its detection. The MIP film was synthesized by a sol–gel method in acidic condition, which involved the use of TEOS, PTMOS, MTMOS and the template (trichlorfon). The authors used a GCE as support for the MIP film deposition, which was performed by immersing the electrode in the previously prepared precursor solution. The films were analyzed by different electrochemical technique, i.e., EIS, CV and presented good sensitivity and selectivity, with a LOD of 2.8 × 10⁻⁹ g/mL [155].

In the next part of this section hybrid methods for sol–gel MIP films synthesis are presented. In this respect, many studies were found for MIP film synthesis by combining the sol–gel method with electropolymerization (according to the procedure sketched in Figure 6). In this case, the MIP solutions are prepared by sol–gel methods, whereas the deposition on the electrode surface is carried out by electropolymerization.

Recently, Liu and co-workers [156] developed a sensor for clenbuterol detection. Clenbuterol is used as veterinary medication. At the same time, if identified in human samples (athletes), it may be corroborated with doping. Therefore, in this study, a three-electrode system was used for sensor development and the imprinted films were deposited on the gold electrode surface. To this end, a sol–gel solution was prepared by dissolving the monomer (APTES), the crosslinker (TEOS), the template (clenbuterol) and KCl in ethanol, followed by electrochemical deposition at −0.8 V for 30 min. The prepared films were characterized by various techniques, from which DPV revealed a good value for the LOD of 31 nM [156].

The group of Xu [157] proposed an electrochemical sensor based on MIP for melamine detection. The MIP solution was prepared by dissolving TEOS, PTMOS, MTMOS and
melamine in ethanol/water/HCl, in which the GCE of the sensor system was immersed. The film was obtained after electrodeposition, by CV, when applying a potential range between 0 and 1.9 V at 50 mV/s. Ultimately, the films were analyzed by DPV using melamine solutions, in which case an LOD of $6.8 \times 10^{-8}$ M was calculated [157].

In 2021, Shaabani et al. [158] proposed the development of an MIP-based sensor for naloxone detection. For this reason, the authors used a MWCNTs modified ITO electrode for the following sol–gel MIP film deposition. The sol–gel solution was prepared by mixing PTEOS, TEOS, trifluoroacetic acid (TFA) and the template in ethanol/water. After homogenization, pyrrole and LiClO$_4$ were added to this solution as well. The ITO modified electrode was immersed in this precursor solution and the film was obtained by electrodeposition in the $-0.8$ and $0.4$ V potential range, for 10 scan cycles at a scan rate of 50 mV/s. CV and DPV were used to evaluate the performance of the MIP film, resulting in a limit of detection of 0.02 µM. Moreover, the sensor presented a good selectivity when contacting compounds with similar structures [158].

Another interesting approach for MIP-based sensors was presented by Razavipanah and his co-workers [159] for mephedrone detection. Mephedrone is a stimulant drug from the class of amphetamines. In their study, a precursor solution containing PTEOS, TEOS, H$_2$O, ethanol, TFA and mephedrone was prepared, after which another solution containing tyramine, MWCNT@AuNPs nanocomposite and sodium dodecyl sulfate(SDS) was added. The electrode was placed into this final solution, and the MIP films were obtained by electrodeposition (potential between $-0.8$ and $1.2$ V at 50 mV/s). The synthesized films were studied by different techniques. The ability of the films to detect the mephedrone was quantified by SWV, which indicated a detection limit of 0.8 nM [159].

In 2018, Bai and colleagues [160] developed an MIP-based sensor for diethylstilbestrol detection. This compound is used as a growth promoter for animals and produces negative effects on humans. During the experiments, a classical three-electrode system containing a GCE (working electrode), an Ag/AgCl (reference electrode) and a Pt electrode (counter electrode) was used. In this study, the MIP film was deposited on the GCE after its modification with MWCNTs, chitosan and AuNPs. For the synthesis of films, a precursor MIP solution was prepared from APTES, OTOMS (octyltriethoxysilane), TEOS, template, 2-ethoxyethanol/water and hydrochloric acid as catalyst. Subsequently, the electrode was immersed in this solution to obtain the MIP film by electrodeposition (potential range $-0.5$ and $0.5$ V). The electrochemical behavior of the films was studied by DPV, resulting a low LOD of 24.3 fg/mL [162].

Deiminiat and colleagues [161] proposed an electrochemical MIP-based sensor for determining the content of ketamine. In this respect, sol–gel films were deposited on the surface of a pencil graphite electrode. The precursor solution contained TEOS, PTEOS, water and ethanol mixed with a solution containing TFA and ketamine. After 2 h, this solution was mixed with another solution containing tyramine, SDS and MWCNT@AuNPs in DMF, and afterwards used for film electrodeposition, by cyclic voltammetry (potential range $-0.8$ and $1.2$ V for 10 scan cycles). The characteristics of the films were studied using EIS and SWV, in which case a limit of detection of 0.7 nM was registered. Moreover, the sensors presented reproducibility, sensitivity and selectivity [161].

6. Molecular Imprinting by Phase-Inversion

Bio-sensing systems based on molecularly imprinted membranes (or MIMs), have received considerable attention from researchers in various fields. In these types of systems, the recognition component is represented by the imprinted membranes, which are integrated with a transducer component. The possibility of direct and rapid determination of the target analyte by the interaction with the recognition element was a stimulating factor for the application of such systems, which are excellent alternatives to the classical bio-analytical methods. The biomimetic sensor-based membranes are used for food, environmental and clinical determinations, due to their high sensitivity, specificity and stability [162].
The first reports about the MIMs were published about 30 years ago [163–165], in which case free-standing membranes or thin polymer membranes were prepared on the surface of a solid support, following classical imprinting recipes [163,164]. Only one of these studies [164] employed a phase-inversion method starting from linear polymer precursors.

Generally, the phase inversion method consists of the use of an already prepared polymer, which is solubilized in a solvent in the presence of the template, so that a complex is formed between the target analyte and the functional polymer (Figure 7). During phase inversion, the template is included in the membrane. The template is extracted afterwards from the solid membrane leading to the formation of a MIM. Phase inversion can be performed using two methods, as follows: (i) wet phase inversion (WPI), in which case the membrane is obtained by coagulation in a coagulation bath, as long as the template is not soluble in the coagulation bath (the solvent diffuses from the polymer solution in the coagulation bath, while the polymer changes to a solid membrane, Figure 7), and (ii) dry phase inversion (DPI), in which case the membrane is obtained by solvent evaporation, as long as the polymer can withstand the heating process (the solvent is evaporated, while the polymer changes to a solid membrane, as shown in Figure 7).

**Figure 7.** Synthesis of molecular imprinted membranes by phase inversion.

Rarely, after the phase inversion, a chemical crosslinking reaction of membranes follows; this is because in most of the cases, the physical crosslinking is sufficient for endowing the MIM with chemical and mechanical stability. The physical crosslinking means that the polymer chains are “fixed” by hydrogen bonds or other physical interactions in the presence of a template by solvent evaporation [166] or phase-inversion precipitation [167]. This process is very similar to “bioimprinting” in proteins, whereas the structure is “frozen” either by lyophilization or by chemical crosslinking in the presence of a template. A range of structures are initially formed when the template interacts with the linear polymer, the heterogeneity of folded structures formed during phase inversion being very similar with the structure formed when the polymer chains crosslink covalently.

Usually, the interactions between the template and the polymer are non-covalent for the phase inversion method. Nevertheless, some studies have presented the possibility for the chemical functionalization of a monomer with the template molecules, and this latter monomer is further polymerized to serve as a functional polymer for the subsequent phase inversion. In this case, the template extraction from the MIMs is performed by destroying the covalent bonds between the template and the polymer.

The advantage of the phase inversion refers to the fact that it usually implies mild conditions for coagulation without affecting the properties of sensible templates. Thus, phase inversion is very much adequate for preparing MIMs for templates with low chemical, thermal or light stability. Moreover, the functional polymer can be prepared in optimum conditions with no interference from the presence of template molecules, which in some cases can inhibit the polymerization process. Thereby, the phase inversion method is appealing; yet, it is somehow limited by the fact that it usually applies for functional (co)polymer and templates, for which a common solvent can be found, to prepare the precursor solution. Another issue encountered when approaching the WPI is the medium-high solubility of the template in the coagulation bath, which leads to low specificity of the
prepared MIM. In this case, to avoid the diffusion of the template during the imprinting process, the DPI can be applied. On the other hand, if the polymer is susceptible to degradation during DPI and an adequate non-solvent for the polymer and template can be found, the preferred method to prepare MIMs is the WPI.

The copolymers of acrylonitrile (AN) with acrylic acid (AA) or MAA are often used for membrane preparation by phase inversion for sensor application [168–170]. AN is used as a structural monomer, due to cyan polar groups that render stability to the imprinted cavities via strong hydrogen bonding, while AA or MAA are chosen as functional monomers due to their ability to interact with polar compounds. For instance, Kobayashi and his group [171] were the first to develop a quartz-crystal microbalance (QCM) sensor employing a PAN copolymer imprinted membrane for the detection of the stimulant drug caffeine. The authors used the phase inversion precipitation method to prepare two imprinted PAN copolymers membranes.

A typical preparation of an electrochemical sensor by WPI is described by Yang et al. [172]. In this respect, a molecularly imprinted film (MIF) was deposited on the surface of a Ti/TiO₂ electrode and used as recognition element of an electrochemical sensor with high selectivity and sensitivity for bisphenol A (BPA). In a first step, p(AN-co-AA) was synthesized according to solution radical copolymerization method using AN and AA in dimethyl sulfoxide (DMSO) with radical initiator AIBN. Step two referred to preparing the precursor solution of p(AN-co-AA) in DMSO, containing BPA as template molecule, followed by casting it directly onto the pretreated Ti/TiO₂ electrode. The MIF resulted after precipitation in the coagulation bath and the sensor assembly was kept in ultrapure water. Maintaining the membrane in the wet form after template removal is necessary, in order to preserve the imprinted network. If the membrane is dried by conventional methods, the shrinkage affects drastically the shape and size of the imprinted cavities which may collapse during this procedure. The Ti/TiO₂/MIF sensor showed a linearity within the range 4.4 nM–0.13 mM, and a very low detection limit of 1.3 nM. Additionally, the sensor presented excellent recognition selectivity for BPA in competition with analogue compounds. This sensor was applied to detect BPA in paper cup samples and seawater, with high recoveries of 86–110% and with low relative standard deviations of 1.3–3.2%. Table 5 summarizes different methods via phase inversion applied for the preparation of MIP-based layers for sensors.

Another paper describing the use of PAN copolymers in the WPI process is that of Stoica et al. [168], where the preparation of a MIM that may be applied for preparing sensors for ephedrine detection is depicted. In this approach, two copolymers of AN and MAA with different weight ratios (80:20 and 75:25) were prepared by emulsion polymerization and used to obtain two membrane variants, using ephedrine chlorohydrate as template. The membranes were tested by batch adsorption measurements in the attempt of finding the optimum recipe for preparing a film with affinity for ephedrine. In this regard, the MIMs with 20 wt% MAA were found to be more adequate to retain ephedrine with an adsorption capacity of 122 mg ephedrine/g polymer and an imprinting factor of 3.4. Furthermore, this same group [173] also used similar copolymer precursors to prepare sensitive membranes for the detection of explosives, more precisely, trinitrotoluene (TNT). The composition of the p(AN-co-AA) was varied this time using the following ratios AN:AA= 80:20 and 85:15.

Another method for TNT and dinitrotoluene (DNT) retention is described by Turner et al. [174]. Using a phase inversion method with the introduction of the template after the polymer synthesis, a range of molecularly imprinted polymer films were prepared consisting of AN as a matrix monomer and methacrylamide (MAAm) as a functional monomer (96:4). After template removal, the films exhibited no rebinding activity, probably due to a collapse of the films’ porous structure. The addition of a crosslinker (EGDMA) stabilized the macroscopic structure of the films, which provided, though limited, adsorption properties.

Besides PAN copolymers, many other synthetic polymers such as polyamide [175], polystyrene and polysulfonemay be used for preparing MIMs by the phase inversion. For example, an amperometric immunosensor for the determination of antirabbit IgG
was developed using phase inversion to prepare a porous conductor polymer graphite–polysulfone electrode [176]. Poly(ethylene-co-vinyl alcohol) copolymers (EVAL), with an ethylene molar content of 32% and 44% were used to produce MIMs, by phase inversion for the selective recognition of saccharides of biomedical interest [177].

Another polymer with great film-forming properties used for MIMs preparation is polyvinyl alcohol (PVA). However, when using PVA, chemical crosslinking is needed. In the two reports from Sarbu and colleagues [178,179], polyvinyl alcohol with a hydrolysis degree of over 99% and molecular weight of 1500 g/mol was used for MIMs forming and the wet membranes were crosslinked by acetylation with glutaraldehyde (GA). The templates in these two studies were the pesticides triclofon (O,O-dimethyl-1-hydroxy-2,2,2-trichlorethyl phosphanate) [178] and atrazine (1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) [179], respectively. The procedure of crosslinking was optimized using various concentrations of GA in sulfuric acid/sodium sulfate media. The crosslinked MIMs were insoluble in water or any other solvents, thus proving the success of the crosslinking reaction. Further in the study, the authors used the remaining free aldehyde groups on the membrane surface to immobilize enzymes covalently (i.e., Tyrosinase). Thereby, these interesting studies may be useful for preparing future immuno–enzymatic electrochemical sensors.

Another important advantage of the phase inversion technology is that natural (bio)polymers are not excluded, since the whole methodology is based on using previously prepared polymers [180]. An example of a biopolymer MIM prepared by DPI is provided by Ma and co-workers [181], in which case a MIM was prepared in aqueous media using chitosan (CS) as the functional polymer, naringin (NG) as the template and polyethylene glycol (PEG) as the porogen. The best performance of imprinted membranes was recorded for a weight ratio of CS:NG = 15:1, when sulfuric acid was used as crosslinker. The FT-IR spectra proved that non-covalent interactions appear between the functional polymer and the template molecules. The prepared membrane was used to separate NG from neohesperidin/NG mixtures in aqueous media.

For the preparation of a hybrid natural-synthetic MIM for \(\alpha\)-amylase retention, Silvestri et al. [182] used a mixture of dextran (Mw 76,900 Da) and poly (ethylene-co-vinyl alcohol) EVAL (intrinsic viscosity 8.44 dl/g) having an ethylene molar content of 40%. Solutions in DMSO with 15 wt% polymers were prepared with two rations of dextran: EVAL = 30:70 and 40:60, after which \(\alpha\)-amylase was added. In this study, the WPI took place in two steps, as follows: first, the casted solutions were immersed in DMSO:H\(_2\)O = 50:50 solution (first phase-inversion bath) and afterwards in water (second phase-inversion bath). Interestingly, the selectivity results showed that \(\alpha\)-amylase was selectively retained by 1.96-fold compared to albumin.

Lee and his co-workers [183] also described an interesting method for the preparation of hybrid MIM by WPI using a covalently bonded dummy template. A hybrid MIM has the advantage of combining the selectivity of molecular imprinting with the mechanical resistance of the support membrane. It is well known that a membrane presents a great specific surface area and possess a great number of binding sites, at the same time, due to the porous structure that offers a facile access of analyze solutions. In this work, core-shell polymer particles were first produced using 1-naphthol as mimic (dummy) template for CIT (Citrinin). The template was covalently bonded to the matrix, by using \(\alpha\) naphthol methacrylate (NAM) monomer to create a polymeric shell around divinyl benzene (DVB) particles. In the second step, hybrid MIMs were prepared by mixing the resultant core-shell MIP particles with polyether sulphone (PES) in a \(N\)-methyl pyrrolidone (NMP) solution. The latter solution was cast on glass plates and transformed into MIMs by WPI in a water coagulation bath. The assessment of CIT was performed by liquid chromatography with fluorescence detection (HPLC–FD), in which case the LOD and LOQ were found to be 0.5 and 1.7 ng g\(^{-1}\), respectively. The overall results pointed towards sufficient sensitivity of the MIMs for CIT, being recommended for the analysis of CIT in rice [183].
Table 5. MIP-based sensors obtained by phase inversion method.

| Synthesis Method | Receptor | Support | Analyte  | Characterization Method(s) | LOD         | Refs. |
|------------------|----------|---------|----------|----------------------------|-------------|-------|
| Casting          | MIP film | Ti/TiO₂ electrode | Bisphenol A | Amperometric measurements | 1.3 × 10⁻⁹ M | [173] |
| Casting Hybrid MIP membrane | Glass support | Citrinin | Batch binding, HPLC | 0.5 ng·g⁻¹ | [182] |
| Casting MIP Membrane | Conductive graphite | Trimethoprim | Potentiometric measurements | 4.01 × 10⁻⁷ M | [184] |
| Casting MIP Membrane | Conductive graphite | Enrofloxacin | Potentiometric measurements | 0.9 µg·mL⁻¹ | [185] |
| Dropwise MIP Film | Screen-printed gold electrode | Regenerating Protein 1B | CV | 0.1 pg·mL⁻¹ | [186] |
| 3D-Imprinting MIP Membrane | Polyvinylidene fluoride/carbon black membrane | Non-woven (Polyester-17153) and 5000 mesh fabric | Morpho-structural, CV | / | [187] |

1 High performance liquid chromatography; 2 CV: cyclic voltammetry.

It is important to mention that hybrid membranes can also be prepared using alternative methods. Such hybrid membranes for sensor development are described by Rebelo and his group [184], where the MIP particles are synthesized by non-covalent complexation of the template with the functional monomers. Herein, the polymeric sensor was prepared using MIP particles, synthesized from MAA and 2-vinyl pyridine as functional monomers, and trimethoprim (TMP) as a template, while the polymer matrix was polyvinylchloride (PVC). Hence, the MIM membrane was obtained without the use of phase inversion. The sensors prepared in this manner displayed a linear behavior against the TMP logarithmic concentration, in a wide concentration range. A very similar method is described in [185], using enrofloxacin as a template.

The epitope approach can be applied for MIP preparation not only by surface polymerization but also by phase inversion. An example of the epitope dry phase inversion is described by Jurcevic and his group [186]. EVAL copolymers were dissolved in DMSO containing the following template peptides: SCSGFKKWKDESEKK (Peptide 2), KSWDTGPSANAGYCAS (Peptide 4) and KESSTDSDNVWIG (Peptide 6) of REG1B. The preparation of peptide–MIMs was performed by the dropwise addition of this precursor solution on the gold substrate of a screen-printed electrode. The urine samples from patients with pancreatic cancer revealed higher electrochemical response in comparison with samples from healthy persons, which was consistent with their elevated levels of the marker protein.

7. Conclusions

This review provided an overview on the progress of MIP layers synthesized by various modern and dedicated techniques such as surface polymerization, electropolymerization, sol–gel technique, phase-inversion and hybrid methods including MIP-dopped inks/pastes or combined methods of sol–gel and electropolymerization. In this respect, using different systems of monomers, template molecules and solvents, some very sensitive and promising sensing tools were developed. In most of the cases, the sensors showed high sensitivity, selectivity, reproducibility and most importantly, low limit of detection. However, electropolymerization seems to be by far the most promising method to prepare highly sensitive sensors, especially MIP-based electrochemical sensors. From the examples described in Section 3, for detecting different pollutants (dyes, polycyclic aromatic hydrocarbon), drugs (antibiotics), illicit drugs (cocaine), the reported limit of detection for such sensors ranges from 1 µM to 1 fM. On the other hand, the combined sol–gel/electropolymerization and ink-based sensors are somewhat less complex and cheaper to produce, but with higher values of LOD, in the µM range.

It is also important to note that surface modification or the sol–gel technique is usually preferred for preparing optical and spectroscopic sensors, while the phase inversion method is more applicable to electrochemical sensors and chromatographic applications. Although the electrochemical sensors are generally the most sensible, the use of MIP for
other detection techniques led in some cases to significant improvements of sensibility and selectivity relative to the state-of-the-art of detection methods applied for optical, spectroscopic or chromatographic applications.

Future challenges in the field of MIP layer preparation targets further improvements of the sensitivity, selectivity and reproducibility for real sample applications, especially for peptides and protein detection [42,44,45]. Due to the intrinsic properties of the MIP sensors such as robustness, reliability, accuracy, reproducibility, long-term stability and portability, future requirements should be considered in scenarios where MIP layers can potentially be tools for routine applications. For instance, MIP layers for optical biosensors could have a significant impact in targeted applications considering that users are interested in solutions with low maintenance and servicing, such as for environmental monitoring (e.g., screening of estrogen [188] or the sensing of small organic contaminants [189,190]).

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