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Review

Carbon Electrode Modified with Molecularly Imprinted Polymers for the Development of Electrochemical Sensor: Application to Pharmacy, Food Safety, Environmental Monitoring, and Biomedical Analysis

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Abstract: This review aims to elucidate recent developments in electrochemical sensors that use functionalized carbon electrodes with molecularly imprinted polymers (MIPs) for the selective detection of organic compounds in diverse fields including pharmacy, food safety, environmental monitoring of pollutants, and biomedical analysis. The main targets include explosive compounds, dyes, antioxidants, disease biomarkers, pharmaceuticals, antibiotics, allergens, pesticides, and viruses. Following a brief overview of the molecular imprinting principle, the most significant applications are explored. The selection of the functional monomer is subsequently discussed. Notably, various types of carbon electrodes are presented, with a particular emphasis on screen-printed carbon electrodes. The most commonly employed techniques for MIP deposition such as electropolymerization, drop casting, and chemical grafting are introduced and discussed. Electrochemical transduction techniques like cyclic voltammetry, differential pulse voltammetry, square wave voltammetry, and electrochemical impedance spectroscopy are presented. Lastly, the review concludes by examining potential future directions and primary limitations concerning carbon electrodes modified with MIPs.

Keywords: molecularly imprinted polymers; carbon electrode; electrochemical sensor; electrode functionalization; in situ detection; SPCE



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1. Introduction

The need to accurately detect analytes in complex matrices in real time is becoming increasingly significant [1]. This requirement spans a wide range of applications, including food safety [2], quality control for drugs and food [3], monitoring environmental air and water pollution [4], biomedical analysis [5], disease diagnosis through monitoring biomarkers in blood or urine [6], combatting food fraud [7], and enhancing civil security [8]. To address these growing demands, the development of affordable, selective, and sensitive sensors presents an interesting solution. A notable challenge in sensor development involves ensuring selective responses in complex matrices such as food, urine, or blood. Electrochemical sensors offer viable solutions due to their cost-effectiveness, maturity, user-friendliness, ease of miniaturization, and the capacity to analyze small volumes [9]. However, despite these advantages, electrochemical sensors still encounter issues related to selectivity limitations and the potential for electrode fouling [10].

Combining molecularly imprinted polymers (MIPs) with electrochemistry offers a robust strategy to enhance the selectivity of electrochemical sensors [11]. MIPs are synthetic polymers designed to replicate the selectivity found in natural systems like enzyme–substrate, antigen–antibody, or enzyme–substrate interactions. These MIPs can be deposited onto electrode surfaces through various techniques, including physical methods such as spin or drop coating, as well as chemical methods like grafting or electropolymerization.

Numerous electrode materials have been functionalized with MIPs; among them, carbon-based electrodes stand out due to their cost-effectiveness and their compelling

chemical and mechanical resistance properties. They are present in different forms such as glassy carbon electrode, graphene oxide, single-walled carbon nanotubes, or multi-walled carbon nanotube, offering a wide possibility of functionalization [12]. Screen-printed carbon electrodes (SPCEs) enable the miniaturization of the detection system and the analysis of small sample volumes. Furthermore, they are disposable, requiring no maintenance treatment, and carry a minimal risk of fouling [13].

This review aims to develop the recent advancements in electrochemical sensors using carbon electrodes functionalized with MIPs for the specific detection of organic compounds across various domains, including pharmaceuticals, food safety, environmental pollutant monitoring, and biomedical analysis. A special focus has been made on the development of electrochemical sensors based on MIP modified SPCE. The novelty of this review compared to other reviews are the wide applications in several fields and the focus on the development of SPCE.

2. Molecularly Imprinted Polymers

Molecularly imprinted polymers (MIPs) are synthetic polymers that mimic specific interactions occurring in nature. These interactions include those between an antigen and its antibody, an enzyme and its substrate, or a hormone and its receptor. These interactions are generally weak and come in different types: hydrogen bonding, hydrophobic, or ionic interactions [14].

The principle of MIP synthesis is presented in (Figure 1).

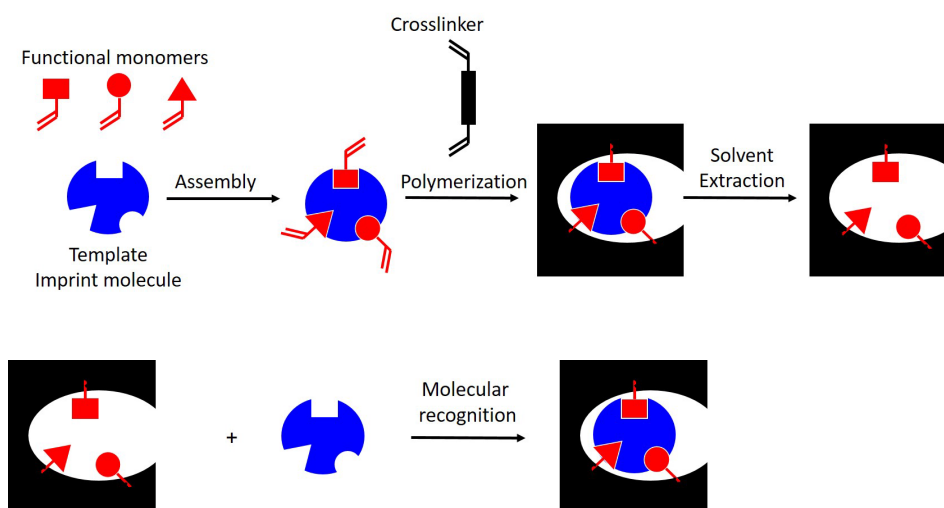


Figure 1. The principle of MIP synthesis and the phenomenon of molecular recognition [15].

Synthesizing an MIP involves selecting several reagents and determining their relative quantities. All of these choices have an impact on the morphological properties and performance of the MIPs. The reagents are the template (T), the functional monomer (FM), the solvent (S), the initiator (I), and the crosslinker (C) [16].

The template is chosen based on the application field. The choice of the functional monomer is a key step; it must demonstrate chemical complementarity to the template. The solvent must solubilize all the reagents and play the role of a porogen, which is responsible for the formation of a porous structure within the polymer [16]. The conditions for eliminating the solvent (time and temperature) play a crucial role in pore formation. The initiator allows the polymerization reaction to take place; it must be present in sufficient quantity to activate all the polymerization functions [17]. The main role of the crosslinker is to create a three-dimensional network structure within the polymer matrix [18].

It is important to make sure that all the reagents are soluble in the reaction medium. The non-solubility of one of the reagents presents a serious obstacle to successful polymerization. A non-imprinted polymer (NIP) is always synthesized in parallel with the MIP

and serves as a blank polymer. The NIP is synthesized in the same way as MIPs but in the absence of the template in the reaction medium.

Molecularly imprinted polymers are mostly acrylate-based. Occasionally, sol-gel imprinted polymers using ethoxysilane or methoxysilane monomers and crosslinkers are employed. In this case, the polymerization takes place in a hydroalcoholic medium. This approach yields more eco-friendly and less toxic polymers [19,20], which are also more hydrophilic, an important criterion for applications in aqueous media. However, the main limitations of silicate-based polymers are the restricted availability of commercial monomers and the low solubility of most organic molecules in the hydroalcoholic medium.

3. Main Targets and Template

A quick search into the application of MIPs in the past 10 years in the field of electrochemical sensing reveals that the primary targets are related to explosive compounds, dyes, antioxidants, disease biomarkers, pharmaceuticals, antibiotics, allergens, pesticides, and viruses. The primary areas of application include medicine, pharmacy, the environment, public safety, and food safety (Table 1).

Examples of explosives sensing include: Triacetone triperoxide (TATP) which is a widely used explosive and is one of the main components of improvised explosive devices (IEDs). Law enforcement agencies and security experts often focus on detecting and preventing the production and use of TATP [21]. RDX is another target example of explosive sensors development: RDX is widely used. It is usually used in mixtures with some other explosives including TNT, PETN, and HMX.

Examples of dyes include synthetic dyes like sunset yellow. Determining their presence in food matrices is crucial, as they pose a potential risk to human health.

Concerning the antioxidants sensing: Catechin is a well-known natural lipid antioxidant [22] and TBHQ and BHA are synthetic antioxidants. Synthetic antioxidants are commonly used in various industries, including food, biodiesel, and cosmetics. Their role in preventing the oxidation of fats and oils make them a valuable additive. However, concerns have been raised about their potential effects on health, prompting their continuous monitoring.

Examples of disease biomarkers include cytokine interleukin-1 β [23], creatinine [24], and cholesterol [25]. Cytokine interleukin-1 β is a biomarker of allergic rhinitis, periodontal disease, and peri-implant disease. Its monitoring in diagnostics is of crucial importance in following up the indication and progress of cancer. Creatinine concentration in urine and blood serves as an indicator of kidney function, muscular functions, and thyroid dysfunctions. Cholesterol monitoring in the blood is very important for the heart health: It is important to maintain a balance of cholesterol levels in the body. High levels of LDL cholesterol and low levels of HDL cholesterol are often linked to an increased risk of cardiovascular diseases.

Pharmaceuticals and drugs are among the primary targets in the development of electrochemical sensors. The following targets have been studied recently in the literature: Atorvastatin belongs to a class of drugs known as statins [26]. Statins are commonly prescribed to lower cholesterol levels in the blood and reduce the risk of cardiovascular diseases, including heart attacks and strokes. Paracetamol is widely used without prescription to treat a variety of symptoms including headache, fever, arthritis, colds and pain [27]. It is considered a mild analgesic (pain reliever) and antipyretic (fever reducer). The most studied drugs are antibiotics. Several electrochemical sensors for antibiotic determination were developed: Chloramphenicol is a wide spectrum antibiotic [28]. It was commonly used to treat a variety of bacterial infections in humans and animals. Metronidazole is an antibiotic and antiprotozoal medication commonly used to treat various types of bacterial and parasitic infections [29]. It belongs to a class of drugs known as nitroimidazoles.

Only one example of allergen marker was reported. It concerns Genistein, a naturally occurring isoflavone compound that is found in various plants, particularly soybeans and soy products. It was used as a marker for the detection of soy allergens in foods [30].

Pesticides were widely studied. Among them, we found Profenofos [31] and Methidathion [32] insecticides and Isoproturon herbicide [33].

The most compelling example is the detection of HIV-1, the most common and widespread type of HIV. It is responsible for the majority of HIV infections worldwide. This detection became achievable through the selective sensing of the HIV-p24 antigen, which is the capsid protein of HIV-1. It appears at an earlier stage of HIV infection than its antibodies. Its early diagnosis helps in the prevention and control of Acquired Immune Deficiency Syndrome (AIDS) [34].

Table 1. Examples of application of MIP in electrochemical sensors using carbon-based electrode.

Analyte	Analyte Family	Matrix *	Electrode	MIP Deposition Details	Deposition Method	Detection Method	Selectivity	LOD	Reference
Cytokine interleukin-1 β	Protein	Human serum solution	SPCE	o-PD under-layer than electropolymerization with C2R	CV electropolymerization	EIS	Other proteins	0.23 ng/L	[23]
Atorvastatin	Drug	Water	SPCE	Electropolymerization with 4-ABA	CV electropolymerization	DPV	Other statins	0.56 μ g/L	[26]
Genistein	Allergen marker	Food products	SPCE	Electropolymerization with o-PD	CV electropolymerization	DPV	Isoflavones and flavones	100 μ g/kg	[30]
Paracetamol	Drug	Plasma	SPCE	Covalent attachment of nanoMIPs after deposition of APTES	Chemical grafting	DPV	Caffeine, procainamide, or ethyl 4-aminobenzoate	7.56 mg/L	[27]
Isoproturon	Herbicide	Groundwater sample	GCE	Electropolymerization with pyrrole	CV electropolymerization	SWV	Carbendazim, diuron, and carbamazepine	2.2 μ g/L	[33]
Tryptophan	AA, precursor of neurotransmitters	Human serum	MWCNTs	Drop-coating of an imprinted chitosan film	Acid polymerization	LSV	Ascorbic acid, dopamine, uric acid, and tyrosine	204 ng/L	[35]
RDX	Explosive	Water	GCE	Drop-coating of MIP/MWCNTs mixture. MIP was prepared MAA as FM	Drop casting	DPV	TNT and HMX	4.4 ng/L	[36]
Profenofos	Insecticide	Vegetable samples	CNTs	Grafting of SiO ₂ and vinyl end groups on the carboxylated CNTs, then thermal polymerization (grafting) of MIP	Thermal polymerization	CV	Carbofuran, chlopyrifos, hydroquinone, caffeine, phenol, MgSO ₄ and NaCl	747 ng/L	[31]

Table 1. Cont.

Analyte	Analyte Family	Matrix *	Electrode	MIP Deposition Details	Deposition Method	Detection Method	Selectivity	LOD	Reference
Sunset Yellow	Synthetic dye	Candy, jelly powder, juice powder, and beverage	MWCNTs	Electropolymerization with acrylamide	CV electropolymerization	DPV	Tartrazine, erythrosine, indigo carmine, glucose, sucrose, and ascorbic acid	2.26 µg/L	[37]
Sulfanilamide	Antibiotic	Buffer aqueous solution	GCE	Synthesis of MIP by precipitation polymerization in the presence of GO. Drop-coating of MIP/GO in a chitosan matrix	Drop casting	SWV	not studied	10 mg/L	[38]
Creatinine	Disease marker	Buffer aqueous solution	GCE	Electropolymerization with aniline and methacrylic acid as bifunctional monomers in the presence of Ni nanoparticles	CV electropolymerization	DPV	Tyrosine, uric acid, dopamine, creatine, and ascorbic acid	22.6 ng/L	[24]
HIV-p24	Virus	Human serum	MWCNTs modified GCE	Grafting of HIV-P24 protein on a drop casted chitosan layer. Polymerization of the MIP using AAM as FM	RT polymerization	DPV	CEA, HCG, AFP, and BSA	83 µg/L	[34]
Bisphenol A	Endocrine disruptor	Mineralized water and fresh milk	GCE	Functionalization of GO with APTES. Template immobilization. Grafting of EGDMA onto the APTES coated GO. MIP thermal polymerization. GO/APTES-MIP was immobilized on a GCE using chitosan	Drop casting	DPV	Estradiol, ethinyl estradiol, and phenol	685 ng/L	[39]

Table 1. Cont.

Analyte	Analyte Family	Matrix *	Electrode	MIP Deposition Details	Deposition Method	Detection Method	Selectivity	LOD	Reference
Paracetamol	Drug	Pharmaceutical formulation	GCE	Nanocomposite: Oxidation of MWCNTs, then functionalization with VTMS, then MIP thermal polymerization using MAA as FM. Drop coating of the nanocomposite	Drop casting	SWV	Acetaminophen, hydroquinone, catechol, 3,4-Dihydroxy-L-phenylalanine, ascorbic acid, and uric acid	166 µg/L	[40]
TBHQ, BHA	Synthetic antioxidant	Soybean oil, margarine, mayonnaise, and biodiesel	GCE	Nanocomposite: Oxidation of MWCNTs, then functionalization with VTMS, then MIP thermal polymerization using MAA as FM. Drop coating of the nanocomposite	Drop casting	DPV	L-ascorbic acid, epinephrine hydrochloride, butylated hydroxyanisole, catechol, dopamine hydrochloride, hydroquinone, acetaminophen, and propyl gallate	90.1 µg/L for BHA and 141.3 µg/L for TBHQ	[41]
Chloramphenicol	Antibiotic	Milk and honey	CKM-3 and P-r-GO modified GCE	Synthesis of a MWCNTs@MIP thermal polymerization using 3-hexadecyl-1-vinylimidazoliumchloride as functional monomer. Coating of the MWCNTs@MIP on the modified GCE	Drop casting	DPV	Glucose, ascorbic acid, uric acid, and glutamic acid	32.3 ng/L	[28]

Table 1. Cont.

Analyte	Analyte Family	Matrix *	Electrode	MIP Deposition Details	Deposition Method	Detection Method	Selectivity	LOD	Reference
Ganciclovir	Antiviral drug	Human serum	MWCNTs modified GCE	Electropolymerization with 2,2'-dithiodianiline as FM in the presence of Au nanoparticles	CV electropolymerization	DPV	Valganciclovir, aciclovir, valaciclovir, guanine, deoxyguanosine, aniline, and cysteine	383 ng/L	[42]
Metronidazole	Antibiotic	Fish meat and pharmaceutical tablets	MWCNTs modified GCE	Electropolymerization with dopamine	CV electropolymerization	CV	Ronidazole, 4-nitroimidazole, 1,2-dimethylimidazole and dimetridazole	49 ng/L	[43]
Methidathion	Insecticide	Waste water	SPCE	Thermal polymerization of bulk MIP using MBAA as FM. Drop casting of MIP@sol-gel/PEG on the surface of SPCE	Drop casting	EIS	Malathion, fenthion, parathion, and chlordfenvinphos	5.14 µg/L	[32]
Metronidazole	Antibiotic, antiprotozoal	Milk and honey	MGCE	Synthesis of a sol-gel and magnetic MIP using APTES as functional monomer (Fe ₃ O ₄ @SiO ₂ -MIP). Coating of Fe ₃ O ₄ @SiO ₂ -MIP on MGCE	Attachment using magnetic force	DPSV	Ronidazole and dimetridazole	2.74 µg/L	[29]
Sucrose	Table sugar	Sugar beet juices	MWCNTs modified GCE	Electropolymerization with o-phenylenediamine	CV electropolymerization	DPV	Raffinose, kestose, glucose, and fructose	1 mg/L	[44]

Table 1. Cont.

Analyte	Analyte Family	Matrix *	Electrode	MIP Deposition Details	Deposition Method	Detection Method	Selectivity	LOD	Reference
Cholesterol	Disease marker	Hydro-alcoholic solution	CCE	Thermal polymerization MWCNT@MIP, graphite powder, and silicon alkoxide	Packing	LSV	Cholic acid and deoxycholic acid	386 ng/L	[25]

* spiked samples.

4. The Rational Selection of the Functional Monomer

The selection of the functional monomer is a crucial step in the synthesis of MIPs. The most commonly used functional monomer is MAA for acrylate-based MIPs. Electropolymerization is the predominant method for depositing MIPs. In this scenario, various functional monomers are employed, including 4-ABA, o-PD, C2R, pyrrole, acrylamide, or dopamine [23,26,30,33,37,43].

An original approach consists of using bifunctional and multifunctional monomers in order to enrich the diversity of recognition sites. Rao et al. used a bifunctional monomer (methacrylic acid and aniline) for the synthesis of an MIP specific for creatinine [24]. Jiang et al. used electropolymerization of o-phenylenediamine and L-lysine for the design of an MIP at the surface of GCE for the selective detection of moxifloxacin antibiotic [45]. Dechtrirat et al. used the co-electropolymerization of 3-aminophenylboronic acid and o-phenylenediamine for the selective detection of salbutamol drug based on a graphene modified SPCE [46].

Often, the selection of the functional monomer relies on a literature search or on the theoretical chemical complementarity of interactions between the template and the functional monomer.

A rational approach to choosing the FM is through molecular modeling. It enables researchers to make informed decisions about the most suitable FMs based on rational design principles, rather than relying on time-consuming experimental trials. By utilizing computational tools, researchers can quickly identify the most appropriate MIP for a given template in terms of favorable binding characteristics [47].

In the synthesis of MIPs, molecular modeling simulates interactions between various FMs and one template, facilitating the choice of the most suitable FM based on the most stable combination (FM-Template), while considering the following factors: (i) Prediction of intermolecular interactions: Molecular modeling enables the prediction of interactions between the FM and the template molecule such as hydrogen bonding, van der Waals forces, electrostatic interactions, etc. Through the analysis of these interactions, researchers can identify which FMs exhibit the strongest and most specific binding sites for the template [48,49]. (ii) Binding site geometry: Molecular modeling provides insights into the spatial arrangement of FMs around the template molecule. This information is crucial for designing MIPs with an optimal binding site geometry. This geometry should align with the shape and size of the template molecule. A well-designed and appropriately shaped binding site enhances the selectivity and affinity of the MIP for the target molecule [50,51]. (iii) Energy calculations: Computational methods are able to calculate the interaction energy between the template and a list of FMs. Lower energy values translate to stronger and more stable binding [47,52]. (iv) Screening different monomers: With the help of molecular modelling, researchers have the possibility to screen a range of potential FMs before conducting costly and time-consuming experimental synthesis [53,54]. This means that less promising candidates can be eliminated, and efforts can be concentrated on the most promising FMs.

Several authors have employed molecular modeling and computational methods to select the most suitable functional monomers. Bakas et al. calculated the binding energy between six different functional monomers and the methidathion template. They compared the binding energy values obtained through computational simulations with the experimental charge transfer resistance of the MIP sensor prepared using the corresponding monomers. The experimental results showed a strong correlation with the computational calculations. MBAA-based MIP sensor demonstrated the largest electron transfer resistance ΔR (22.15 k Ω), which is consistent with highest binding energy between MBAA and methidathion [32]. Alanazi et al. utilized computational screening to choose the most FM for designing a MIP tailored for paracetamol. They demonstrated that the itaconic acid monomer exhibited the highest binding energy, forming six hydrogen interactions and displaying the highest interaction ratio with paracetamol among the 25 FMs tested [27]. Rebelo et al. employed quantum mechanical calculations to determine the functional monomer (FM) that forms the most stable complex with Atorvastatin. Out of ten elec-

tropolymerizable monomers, 4-ABA exhibited the highest binding energy between ABA and Atorvastatin [26].

5. Carbon-Based Electrodes

Glassy carbon electrodes (GCEs) are extensively utilized in conjunction with MIPs for shaping selective sensors. This carbon material is non-graphitizing; it does not transform into crystalline graphite upon heat treatment, resulting in a non-crystalline structure of carbon. GCEs exhibit chemical inertness, high electrical conductivity, facile functionalization, and commendable mechanical robustness. Their chemical inertness ensures stability and response accuracy. Furthermore, GCEs tolerate an extensive range of potentials, maintaining stability while manifesting minimal background current. High electrical conductivity and mechanical strength facilitate prompt electron transfer during redox reactions and durability during physical manipulation. To enhance the selective analyte detection, GCEs can be readily functionalized with an array of materials like polymers, nanoparticles, or biomolecules. GCEs are compatible with a broad spectrum of electrochemical techniques, including cyclic voltammetry, chronoamperometry, and impedance spectroscopy, among numerous others.

GCE are used alone as a base for the immobilization of MIPs, but GCE is often used in combination with GO or MWCNTs. Sadriu et al. have immobilized an MIP on the surface of a GCE using CV electropolymerization of pyrrole for the electrochemical sensing of Isoproturon herbicide in groundwater samples [33]. Rao et al. have immobilized an MIP on the surface of a GCE using CV electropolymerization of aniline and methacrylic acid as bi-functional monomers in the presence of Ni nanoparticles for the electrochemical sensing of creatinine [24].

The integration of GO into electrochemical sensors presents numerous benefits, including enhanced selectivity and simplified functionalization. By applying GO onto electrode surfaces, sensitivity and selectivity are enhanced. The substantial surface area of GO contributes to improved electron transfer kinetics and a more efficient sensor response. Electrodes modified with GO can considerably enhance the sensitivity of electrochemical sensors. The extensive surface area of GO increases interactions with the analyte, thereby lowering the detection limit of the sensor. The functionalization of GO with specific functional groups or MIPs facilitates the selective detection of target analytes. The main challenge remains in the surface electrode regeneration following the removal of MIP.

Wei et al. used a GCE modified with MIP and GO to develop a novel electrochemical sensor for the selective detection of sulfanilamide [38]. Dadkhah et al. modified a GCE with GO and MIP for the electrochemical sensing of Bisphenol A. They have used an elaborated methodology to functionalize GO with APTES. The Bisphenol A was then immobilized onto amino-functionalized GO. The next step was the grafting of EGDMA onto the APTES coated GO then the thermal polymerization of the MIP [39].

MWCNTs possess the same properties as those employed in the development of carbon-based electrochemical sensors, which include excellent electrical conductivity, mechanical stability, and the potential for functionalization. Additionally, their high specific surface area contributes to enhanced sensitivity and performance [55].

MWCNTs represent a type of nanomaterial composed of cylindrical carbon atom tubes. Their structure resembles that of SWCNTs, though with multiple layers of concentrically arranged graphene sheets. Each layer is referred to as a “wall”. The quantity of walls can vary, generally ranging from a few layers to dozens. MWNTs are an allotrope of sp^2 -hybridized carbon, similar to fullerenes and graphite; consequently, they exhibit significant chemical stability. MWCNTs diameters can extend up to 30 nm, in contrast to the typical SWCNT range of 0.7 to 2.0 nm. Nonetheless, SWCNTs are generally 100 to 1000 times more costly than MWCNTs.

These materials have found application as platforms for immobilizing MIPs to facilitate the specific detection of particular analytes. A straightforward and speedy approach to modify an MWCNTs electrode involves the application of a thin layer of MIP through

electropolymerization. Researchers such as Arvand et al. [37], Gholivand et al. [42], Yuan et al. [43], and Shekarchizadeh et al. [44] have utilized CV electropolymerization to deposit MIP onto MWCNTs electrodes, enabling the detection of substances like sunset yellow, ganciclovir, metronidazole, and sucrose, respectively. Wu et al. [35] have drop coated a MIP-chitosan film on the surface of a GCE modified with MWCNTs for the selective sensing of tryptophan in human serum. Ma et al. drop casted a chitosan layer onto MWCNTs modified GCE. Then, they grafted the HIV-p24 protein on the chitosan layer before the polymerization of a MIP. The modified electrode was used for the selective determination of human immunodeficiency virus p24 (HIV-p24) [34].

Screen-printed electrodes (SPEs) are miniaturized, disposable electrodes that fulfill the requirements of in situ monitoring, offering advantages in terms of reproducibility, cost-effectiveness, sensitivity, and portability. Additionally, in alignment with green chemistry principles, they necessitate only small sample volumes for operation, thereby reducing the usage of environmentally unfriendly solvents and minimizing waste production. SPEs represent a novel generation of miniaturized electrodes, and they are increasingly utilized in the development of electrochemical sensors due to their straightforward fabrication, low production cost, and user-friendly nature [56]. A section will be dedicated to the development of SPCEs.

6. Functionalization Methods of the Carbon-Based Electrode with MIPs

Several functionalization methods were used to deposit an MIP onto the surface of the C-based electrodes. The most widely used are electropolymerization and drop casting, and to a lesser extent chemical grafting. Other functionalization techniques that have rarely been used such as packing and magnetic force attachment will be developed.

6.1. Electropolymerization

Electropolymerization is a relatively simple process that can be conducted under mild conditions. This simplicity contributes to the ease of manufacturing sensors and their scalability. The materials employed for electropolymerization and MIP synthesis often demonstrate cost-effectiveness, rendering the production of MIP-coated electrodes economically feasible [57]. Through electropolymerization, a controlled and uniform MIP coating is achieved on the electrode surface using cyclic voltammetry [46]. The number of cycles directly correlates with the thickness of the deposited MIP layer. This uniformity guarantees reproducible results across various sensors by minimizing variations in sensor response. Electropolymerization establishes a stable and adherent MIP layer on the electrode surface, significantly reducing the possibility of detachment or degradation [58]. This layer's durability ensures the sensor's longevity, enabling multiple uses without a notable loss in performance.

It is the most commonly used method for the functionalization of electrodes with MIPs (Table 1). Electropolymerization has been applied directly to SPCEs, GCEs, or MWCNTs. One of the simplest examples is the electropolymerization of 4-ABA onto the surface of an SPCE for the selective sensing of atorvastatin in water samples [26].

6.2. Drop Casting

The drop casting method is relatively simple and does not necessitate complex equipment or specialized expertise. It involves placing a droplet of the MIP suspension onto the electrode surface, making it accessible to non-expert researchers. This technique is versatile and can be applied to a wide range of electrode materials and shapes. By adapting the concentration of the MIP solution and the number of casting steps, researchers can achieve a controlled and adjustable thickness of the MIP layer [59]. Drop casting is a relatively quick process, requiring less time compared to other coating methods. Gentle solvent evaporation during drop casting should be employed, as it can aid in preserving the structure and integrity of the MIP.

The major advantage of drop casting remains the possibility of synthesizing the MIP using conventional methods outside of the electrode, in the form of a monolith or powder, and then depositing the MIP on the electrode surface in a second step. This provides greater freedom in choosing the MIP formulation and the type of polymerization.

On the other hand, drop casting is suitable for small-scale production or research purposes, allowing the creation of a manageable number of sensors but not adapted to large-scale production. However, drop casting does not allow precise control over the orientation and density of binding sites on the MIP layer. While drop casting offers some control over the thickness of the MIP layer, achieving a reproducible thickness across multiple sensors can be challenging. The solvent evaporation step during drop casting can sometimes result in irregular drying, leading to cracks or defects in the MIP layer [59]. The choice of solvent for drop casting must be compatible with both the MIP and the electrode material. Care should be taken to select a solvent that does not damage the electrode or the MIP structure. If the electrode has a non-flat geometry, achieving a uniform layer of MIP through drop casting may be more difficult. PEG and chitosan are often used as immobilization matrices, but they could potentially reduce accessibility to the MIP interaction sites or introduce interferences [60].

In the examples of MIP deposition using drop casting, MIP was deposited alone or in mixture with other polymers or materials such as chitosan [39], MWCNT₅ [28], GO [38], or PEG [32].

In the following example (Figure 2), dos Santos Moretti et al. [40] prepared a nanocomposite by oxidation of MWCNTs, then they functionalized the oxidized material with VTMS before the polymerization of MIP using MAA as FM and TRIM as crosslinker. The prepared nanocomposite was drop casted on the surface of a GCE in the form of a suspension dispersed in DMF followed by addition of Nafion.

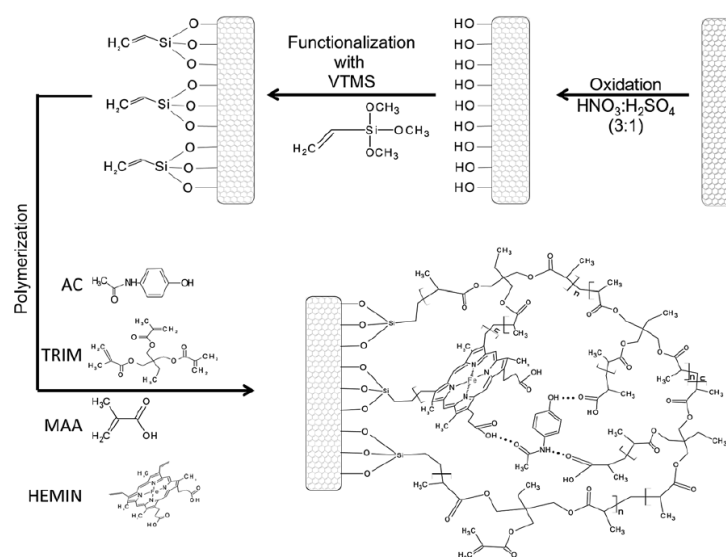


Figure 2. Schematic representation of synthesis of nanocomposite based on multi-walled carbon nanotubes grafted by poly(methacrylic acid-hemin). Reproduced from [40] with the permission of Royal Society of Chemistry.

6.3. Chemical Grafting

Functionalizing electrodes through chemical grafting involves modifying the electrode's surface by covalently attaching MIPs. Prior to functionalization, the electrode surface needs to be cleaned and adequately prepared to ensure the effective attachment of the functional groups of the MIPs. In many cases, the electrode surface requires activation to facilitate the covalent attachment of these functional groups. Activation methods may include chemical treatments, plasma treatments, or electrochemical approaches. The main

advantage of this method is the strong attachment of MIP on the electrode, but this method is often tedious and time consuming.

In the following example (Figure 3), Kan et al. [61] functionalized a MWCNT with a MIP. Firstly, the surface of the MWCNT was activated using HNO_3 . Then, MWCNTs were treated with sulfoxide chloride (SOCl_2) and allyl alcohol in order to transform the surface of the MWCNTs into polymerizable vinyl group (MWCNTs-CH=CH_2). The final step was a selective polymerization of a MIP using dopamine as a template, TRIM as a crosslinker, and AIBN as an initiator. The obtained functionalized material was drop casted at the surface of glassy carbon electrode for the electrochemical sensing of dopamine.

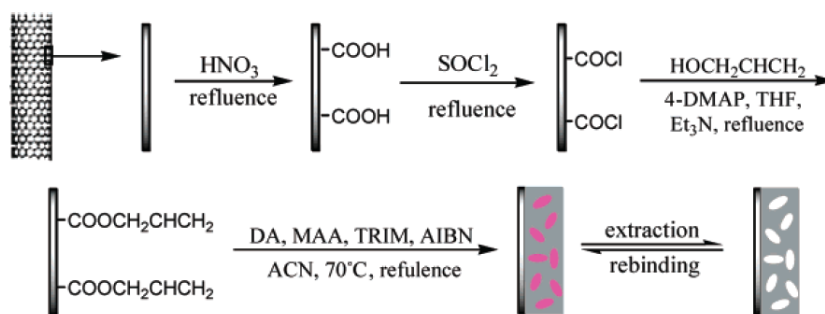


Figure 3. Synthesis route of MWCNTs-MIPs showing the chemical grafting of an MIP at the surface of MWCNTs. 4-DMAP: (4-dimethylamine) pyridine; DA: Dopamine; AIBN: azobisisobutyronitrile. Reproduced from [61] with the permission of ACS Publications.

6.4. Other Functionalization Methods

In this paragraph, we will discuss other infrequently utilized functionalization methods.

Tong et al. [25] employed packing of a mixture of MIP, MWCNTs, graphite powder, and silicon alkoxide to functionalize a ceramic carbon electrode (CCE). The resulting functionalized electrode was utilized for electrochemical cholesterol sensing. A notable advantage of this packing method is the flexibility it offers in synthesizing the MIP externally to the electrode. However, a significant drawback to consider is the potential risk of MIP fragments or monomers leaching into the electrolyte or the environment, leading to system contamination and potential performance effects.

In an innovative approach, Chen et al. [29] used magnetic forces to functionalize the surface of a magnetic glassy carbon electrode with a magnetic MIP. This functionalized electrode demonstrated efficacy in selectively sensing the metronidazole antibiotic in milk and honey samples.

6.5. Comparison of Functionalization Methods

Table 2 summarizes the main advantages and drawbacks of the functionalization methods.

Table 2. Advantages and drawbacks of the functionalization methods.

Functionalization Method	Advantages	Drawbacks
Electropolymerization	Easy, simple Soft polymerization in comparison with UV Control of the layer thickness Covalent attachment of the monomer Suitable for large scale production	Limited to aqueous medium
Drop casting	Fast Does not require specific equipment Freedom in the choice of MIP formulation	Weak interactions between the MIP and the electrode The MIP synthesis is time consuming Not suitable for large scale production Use of immobilization matrix: PEG, chitosan
Chemical grafting	Covalent attachment of MIP	Time consuming Not suitable for large scale production Layer thickness difficult to control

7. Electrochemical Detection Methods

Several electrochemical detection methods were used for the sensing of organic compounds: CV, DPV, SWV, LSV, DPSV, and EIS.

DPV remains the most used transduction method. DPV enhances the sensitivity of electrochemical measurements by applying a minor voltage pulse at each potential step [62]. This leads to an improved signal-to-noise ratio and better detection limits when compared to other voltammetric techniques. DPV reduces the influence of capacitive currents, which are often met in cyclic voltammetry and can complicate data interpretation [63]. The distinctive measurement format of DPV facilitates the differentiation between faradaic and capacitive currents, yielding clearer and more precise quantification [64]. DPV displays reduced susceptibility to interference from non-faradaic processes. It is a suitable technique for complex sample matrices.

The lowest limit of detection obtained with DPV was 4.4 ng/L. It was obtained after drop casting a MIP/MWCNTs mixture onto a GCE for the detection of RDX in water [36]. This value is the second lowest, following the one determined by EIS during the electrochemical detection of the cytokine interleukin-1 β protein after the electropolymerization of an MIP on the surface of an SPCE. In this case, the limit of detection was as low as 0.23 ng/L [23].

Electrochemical impedance spectroscopy (EIS) offers several distinct advantages when applied to electrochemical sensors. EIS is a quantitative technique which allows the determination of analyte concentrations based on impedance changes [65]. Moreover, EIS shows high sensitivity to alterations in the surface properties of electrodes, and it is highly effective at detecting small variations in analyte concentration or surface conditions [66]. This sensitivity is crucial for sensor applications. EIS does not necessitate the detected species to be electroactive, it involves the use of a redox probe in solution [67]. This simplifies the detection process and reduces costs. EIS is a non-invasive technique, it preserves sample integrity [68]. It is a particularly advantageous technique for applications where sample integrity must be maintained. Furthermore, EIS excels in low-frequency analysis, which is highly important for studying biological interactions, redox processes, and reactions involving slow kinetics [69,70].

8. Screen-Printed Carbon Electrode

Screen-printed carbon electrodes (SPCEs) are increasingly being used in electrochemical sensing and analysis. These electrodes are produced using a screen-printing process, which deposits a layer of carbon ink onto a substrate, typically ceramic, plastic, or paper. The screen-printing process employed in their manufacture is relatively straightforward and cost-effective, facilitating mass production while maintaining consistent quality [71]. SPCEs are designed for disposable use, making them well-suited for one-time applications or field usage. Their compact dimensions and lightweight construction contribute to their portability. Generally, the carbon surface of SPCEs exhibits a low tendency to accumulate fouling, ensuring stable and reproducible measurements over extended periods [72].

Moreover, SPCEs have a broad electrochemical potential window, which allows the investigation of various redox processes without experiencing electrode degradation [73]. The limited working area of SPCEs allows for the use of reduced sample volumes, which is highly important when working with scarce or expensive samples [74]. SPCEs are compatible with a range of electrochemical techniques, such as CV, DPV, SWV, chronoamperometry and EIS.

Additionally, SPCEs can be easily functionalized through the incorporation of different MIP materials. Table 3 provides a summary of examples illustrating the functionalization of SPCEs using MIPs.

Most of the applications concern drugs, antibiotics, and disease biomarkers. Applications also include pesticides, food toxins, and allergens. The most developed field of application is biomedical analysis, but fields such as food safety, environmental monitoring, and civil safety are growing rapidly.

Table 3. Examples of functionalization of SPCE with MIP.

Analyte	Matrix	MIP Deposition Method	Detection Method	LOD	Reference
Fenitrothion insecticide	Forest sample (leaves)	EP	SWV	222 µg/L	[75]
C-reactive protein	Blood serum	SC	DPV	0.04 mg/L	[76]
Oxfendazole drug	Milk	EP	DPV, SWV	8.0 µg/kg	[77]
Methidathion insecticide	Waste water	DC	EIS	5.1 µg/L	[32]
Naloxone drug	Urine/human serum	EP	DPV	65 µg/L	[78]
Salbutamol drug	Swine meat feed samples	EP	DPV	23.9 ng/L	[46]
Bisphenol A Plastic component	water/acetonitrile (99/1)	SC	DPV then CV	13.7 ng/L	[79]
Thyroid hormone Thyronamine	PBS buffer	EP	SWV	19 µg/L	[80]
MDMA ecstasy	Human blood serum and urine	EP	SWV	0.15 mg/L	[81]
Cefquinome antibiotic	Phosphate buffer	EP	SWV	26.4 µg/L	[82]
Solatol drug	Tablet and human blood serum	DC	CV	9.53 µg/L	[83]
Sertraline drug	Human serum	DC	DPV	609 ng/L	[84]
Azithromycin antibiotic	Environmental water	EP	DPV	59.9 ng/L	[85]
Cyclocreatine drug	Plasma sample	EP	EIS	55.1 ng/L	[86]
Tau protein, biomarker of Alzheimer's disease	Serum sample	EP	EIS	1.1 ng/L	[87]
Genistein allergen	Food products	EP	DPV	100 µg/kg	[30]
L-hydroxyproline, biomarker of bone disease	Human serum	EP	EIS	0.13 mg/L	[88]
Paracetamol drug	Plasma	CG	DPV	7.56 mg/L	[27]
Atorvastatin drug	Water	EP	DPV	0.56 µg/L	[26]
Cytokine interleukin 1-β protein	Human serum	EP	EIS	0.23 ng/L	[23]
Albumin allergen	PBS buffer	DC	CV, Am	180 mg/L	[89]
Trazodone drug	Tap water samples Human serum	EP	DPV	595 µg/L	[90]
Aflatoxin M1	Milk	CG	Chronoamperometry	0.09 µg/L	[91]
Insulin hormone	Pharmaceutical sample	EP	SWV	11.0 µg/L	[92]

EP: Electropolymerization. CG: Chemical grafting. SC: Spin coating DC: Drop casting. SP: Screen-Printing. Am: Amperometry.

The easiest, fastest, and most cost-effective method for SPCE functionalization is electropolymerization. This is why it was used in the majority of examples found in the literature. Drop coating is the second most used method for the MIP deposition onto the surface of SPCE.

Figure 4 presents an example of the functionalization of SPCE with MIP using electropolymerization of a monomer combination: 3-aminophenylboronic acid and o-phenylenediamine, in the presence of the L-hydroxyproline template. The developed electrochemical sensor was employed for the selective determination of L-hydroxyproline, a biomarker for bone disease, in human serum samples. This was achieved using EIS as the transduction method [88].

The most used electrochemical methods for the detection of analytes are DPV and SWV. EIS is often used when the analyte is not electroactive. The lowest limit of detection recorded was in the range of pM for Tau protein and insulin [87,92].

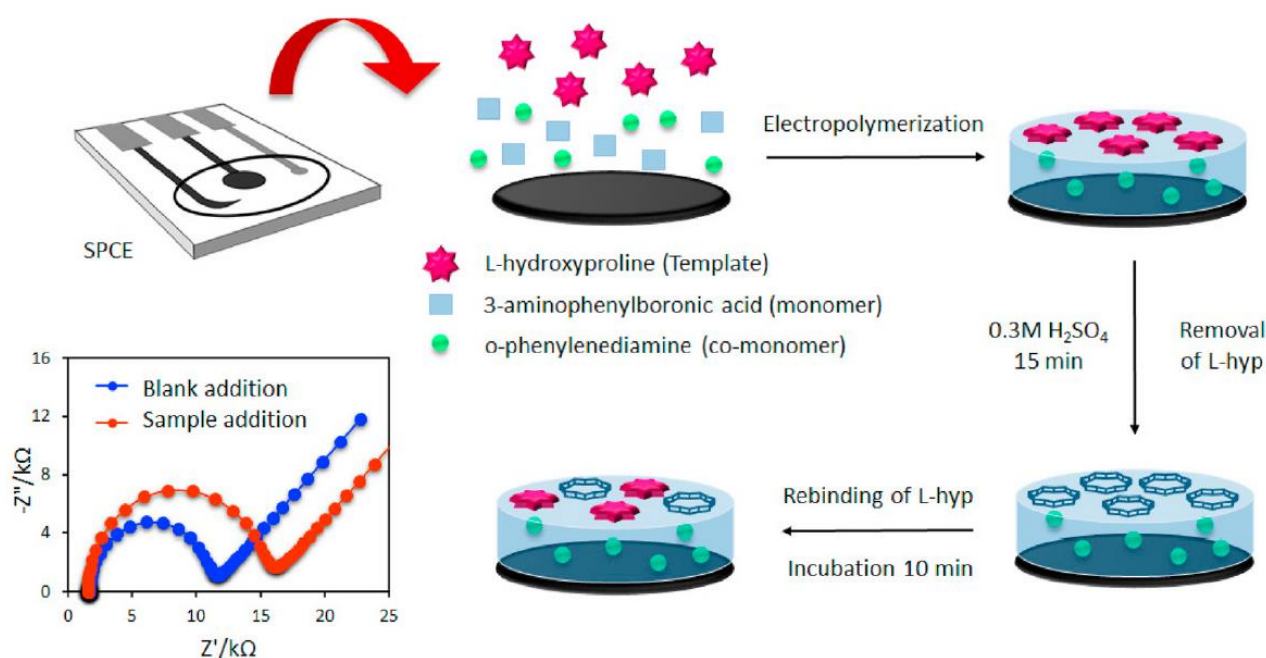


Figure 4. Schematic illustration of the fabrication and operation of MIP/SPCE biosensor. Reproduced from [88] with the permission of Elsevier.

9. Conclusions and Future Directions

In conclusion, electrochemical sensors using carbon electrodes functionalized with MIPs have found applications in various fields. Among these, biomedical analysis has seen the most significant development, including the analysis of drugs, antibiotics, and disease biomarkers. Emerging applications involve the monitoring of environmental and food contaminants. Analytes vary widely, from small molecules like sucrose to 62 kDa proteins such as tau protein. One remarkable application demonstrated the selective detection of HIV viruses using an MWCNTs modified GCE and functionalized with an AAM-based MIP.

Miniature electrodes such as SPCEs are rapidly gaining attractiveness, attributed to their affordability, user-friendly nature, and disposability, which avoids the requirement for frequent maintenance seen in conventional electrodes. The inaugural application of SPCE functionalization with an MIP dates back to 1999; however, it was not until 2010 that the first real-time detection emerged. In the period covering 2010 to 2018, only six applications were documented in literature. It was not until 2019 that the utilization of MIP-modified SPCEs saw significant development, as evidenced by the publication of 4 to 5 articles annually.

Future directions lie in the development of MIP-based electrochemical sensors for the detection of contaminants for food safety and environment monitoring at low cost. The commercialization of sensitive and selective MIP-based electrochemical sensors remains a challenging objective, despite various successful attempts at the laboratory scale. The primary obstacles include relatively high costs associated with extended development periods and the capability to simultaneously detect multiple targets. Researchers often omit the timeframe required for developing new sensors and may not share unsuccessful results, which can be both time-consuming and discouraging. While MIPs have proven effective, they have, thus far, only been applied to the electrochemical detection of individual targets and cannot be utilized for multi-target detection, such as the simultaneous determination of multiple pesticide residues or food and environmental pollutants. One of the main challenges that persists is the creation of MIP-based multi-target sensors.

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Abbreviations

4-ABA	4-aminobenzoic acid
AA	Amino Acid
AAM	Acrylamide
AFP	Alpha fetal protein
APTES	3-Aminopropyltriethoxysilane
BHA	Butylated hydroxyanisole
BSA	Bovine serum albumin
C2R	Chromotrope 2R
CA	Chronoamperometry
CCE	Ceramic carbon electrode
CEA	Carcinoembryonic antigen
CKM-3	Mesoporous carbon
CNTs	Carbon nanotube
CV	Cyclic voltammetry
DMF	N,N-Dimethylformamide
DPSV	Differential pulse stripping voltammetry
EGDMA	Ethylene glycol dimethacrylate
EIS	Electrochemical impedance spectroscopy
FcMMA	Ferrocenyl-methylmethacrylate
FM	Functional monomer
GCE	Glassy carbon electrode
GO	Graphene oxide
HCG	Human chorionic gonadotropin
HIV	Human immune deficiency virus
HMX	1,3,5,7-tetranitro-1,3,5,7-tetrazocane
LSV	Linear sweep voltammetry
MAA	Methacrylic acid
MBAA	N-methylenebisacrylamide
MDMA	3,4-methylenedioxymethamphetamine
MGCE	Magnetic glassy carbon electrode
MWCNT	Multi-walled carbon nanotube
o-PD	O-phenylenediamine
PEG	Poly ethylene glycol
PETN	Pentaerythritol tetranitrate
P-r-GO	3-dimensional porous graphene
RDX	Trinitroperhydro-1,3,5-triazine
RT	Room temperature
SPCE	Screen printed carbon electrode
SWCNT	Single-walled carbon nanotube
SWV	Square wave voltammetry
TBHQ	Tert-butylhydroquinone
TNT	2,4,6-trinitrotoluene
TRIM	Trimethylolpropane trimethacrylate
VTMS	Vinyltrimethoxysilane

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