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RESEARCH ARTICLE

ELECTROGENERATED MOLECULARLY IMPRINTED POLYMERS IN ELECTROCHEMICAL SENSING

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ABSTRACT

This review points out the importance of coupling electrochemical techniques with molecularly imprinted polymers, in order advanced sensing devices to be developed. Recently, the growing interest in molecularly imprinted polymers (MIPs) as recognition elements, which mimic natural receptors, has made the researchers to design novel formats for improvement of MIP electrochemical sensors. Among other possible approaches used in the literature, this review will discuss the electrogeneration of MIPs and on less common hybrid technology (e.g. based on electrochemistry and classical MIPs, or nanotechnology).

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INTRODUCTION

Conducting polymers (CPs) attracted attention not only for their numerous applications in batteries, electronics devices or ion-selective membranes, but also as sensitive layers in electrochemical sensors (Nishizawa, 1992 and Barlett, 1987). CPs are characterized by a high electrical conductivity and a good electrochemical reversibility, justifying their use as transducers in electrochemical sensors. Moreover, they can be chemically functionalized with various chemical groups, which can be chosen as tags for their ability to recognize biological or chemical target molecules. These latter can thus be quantitatively analyzed. For instance, the functionalization of polypyrrole with oligonucleotides, ODNs, allowed the quantitative recognition of complementary ODNs (Garnier, 1989). In addition, electropolymerization process leads to the simple and reproducible formation of films with precise spatial resolution over surfaces, whatever their size and geometry (Cosnier, 2003) Also, electropolymerization allows easy control over the properties of the polymeric coating such as morphology and thickness (Cosnier, 2003). Moreover, the electrogeneration of polymer films, known to be compatible with bulk manufacturing procedures, leads to polymeric coatings that are stable in organic and aqueous solvents (Cosnier, 2003). Thanks to the synthetic access to a wide variety of monomers, such films have become a powerful platform for the development of electrochemical sensors or electrochemical biosensors. The physicochemical properties of these films strongly depend on the electropolymerization conditions, i.e., solvent, supporting electrolyte, electrode material, polymerization potential, and electropolymerization method (Emr, 1995). In CPs, e.g., polypyrrole (Cosnier, 2003) polyaniline (Lu, 1994), polythiophene (Hiller, 1992), the delocalization of charges along the polymer chains induces the formation of states in the gap, polarons and bipolarons, which are involved in charge transport. The formation of charge carriers on their conjugated backbone realized by oxidation (p-doping) or by reduction (n-doping) allows the appearance of a metal-like intrinsic conductivity. In the case of p-doping of polymers such as polypyrrole or polythiophene, the cationic charges carried by the polymer backbones, are counter balanced by negative charges carried by anions. Concerning the morphology of CPs, which is an important feature when considering sensing applications, it has been suggested that their porosity is dependent on the doping anion used, which controls the polymer network spacing, as proposed for ion-selective electrodes (Wang, 2006). However, even if the use of

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CPs as transducers has largely been described, their associated sensing devices have been mainly limited to the detection of large bioactive species (such as ODNs or peptides). On the other hand, the detection of small (bio) active molecules still remains an open challenge, as their simple association to the sensing groups on the CP matrix is not sufficient to create the needed variation of the electrochemical signal. The solution could be to build highly specific recognition sites in the CP matrix, which will both increase the selectivity and improve the sensitivity of the recognition process. As specificity is concerned, molecularly imprinted polymers (MIPs) can be applied to the manufacture of polymers with pre-determined molecular recognition properties. MIPs are obtained through polymerization in the presence of a template molecule (target) (Owens, 1999 and Haupt, 2000). Hence, highly specific cavities are created into the polymeric matrix. After target removal by extraction, MIPs demonstrate interesting binding sites complementary to the template with recognition properties towards the template (analyte), originating from shape and chemical functionality considerations in the cavities present in the polymer matrix matrices that allow the rebinding of the template with high specificity. The force of attraction between the monomer and the imprint molecule may be of either covalent or noncovalent and sometimes, a combination of these two (Qi, 2010). The promising advantages of MIPs include desirable selectivity and affinity constant compared to naturally occurring systems for sensor applications, physical robustness and stability, resistant to degradation in extreme means such as acids and bases or high temperature and pressure, low cost, storage in a dry state at room temperature for long periods, and excellent reusability (Haupt, 2001). As shown from the large amount of reviews, the main application of the MIPs still is in the field of separation technology (Piletsky, 2001; Mayes, 1997 and Sharma, 2012), but also MIPs are used in the area of chemical sensors as well (Haupt, 2000). Chemical sensors are usually classified into three general categories such as optical, piezoelectric and electrochemical, on the basis of their transduction. Of this, electrochemical transducers become very popular, because measuring is very simple and the instrumentation is easily available. Furthermore, they offer low limits of detection (LOD), at low cost, while they can easily miniaturized and automated. Thus, electrochemical transduction is often attractive in making small devices for recognition based on MIPs in the field of clinical, food, environmental and pharmaceutical industries. Imprinted polymers can be prepared for virtually any kind of substance. The examples of successful imprinting include inorganic ions, drugs, nucleic acids, proteins and even cells. On this perspective, more studies on this field have been conducted, reflected in two already published reviews (Suryanarayanan, 2010 and Malitesta, 2012). This review is focused on MIP-based materials prepared using electrochemical techniques. Under this topic it will be included the electrosynthesis of MIPs and its combination with up-to-date technologies (e.g. nanotechnology) to integrate electrochemically prepared MIPs and electrochemical sensors and their analytical chemistry application.

MATERIALS AND METHODS

Briefly, an MIP is generally prepared from the co-polymerization of functional and cross-linking monomers in the presence of a target molecule (the imprint), acting as a molecular template. Firstly, the functional monomer is forming a complex with the target molecule and after polymerization their functional groups are held in position by the highly cross-linked polymeric structure. With the removal of the template, the material gains binding sites which are complementary in size and shape to the analyte. This procedure is similar to the "lock and key" theory of enzymes, introducing a molecular memory into the polymer matrix, where the analyte is able to specifically rebind on the same imprinted material. The attraction force between the monomer and the imprint molecule could be of either non-covalent or covalent (Fig. 1 a and b, respectively) and sometimes, a combination of these two. Generally, imprinting efficiency (yield of binding sites relative to amount of the imprint molecule) is higher for covalent imprinting than its non-covalent (Whitecombe, 1995). Nevertheless, noncovalent imprinting is more flexible regarding the choice of monomer, target molecules and the use of the imprinted materials. Furthermore, it is similar to natural processes because most of the biomolecular bindings are noncovalent in nature (Mosbach, 1996).

Electrochemical molecular imprinting methodology

Usually, MIPs are synthesized using bulk polymerization. Bulk polymerization methods, though, suffer from some intrinsic limitations, such as the poor site accessibility, time-consuming, high diffusion barrier, and complicated preparation process (Luo, 2013). The synthesis of molecularly imprinted thin polymer films directly on the transducer surface could be effectively done by using electrochemical polymerization is (Lakshmi, 2009), without any processing requirement (Da Silva, 2014). The electrochemical deposition methods provide a simple, repeatable, and green method for the formation of thin and uniform polymer film on the electrode surface in order to overcome the bulk polymerization limitation (Kan, 2012). Imprinting electropolymerization is a very attractive in developing electrochemical sensors (Chang, 2010). This method is very easy and provides deposition of a sensitive layer with high precision on electrode surface, which is extremely important for micro-and multi- sensor production.

Usually, MIPs are synthesized using bulk polymerization. Bulk polymerization methods, though, suffer from some intrinsic limitations, such as the poor site accessibility, time-consuming, high diffusion barrier, and complicated preparation process (Luo, 2013), MIPs could also synthesize in situ on the electrode surface by electropolymerization without any processing requirement (Da Silva, 2014). The electrochemical deposition methods provide a simple, repeatable, and green method for the formation of thin and uniform polymer film on the electrode surface in order to overcome the bulk polymerization limitation (Kan, 2012). Imprinting electropolymerization is a very attractive in developing electrochemical sensors (Chang, 2010). This method is very easy and provides deposition of a sensitive layer with high precision on electrode surface, which is extremely important for micro-and multi- sensor production. Generally, electropolymerized MIPs have gained great attractions as recognition elements due to the ease of preparation (by cycling potential sweeps from solutions of their monomers and templates) and the possibility of obtaining very thin films with good reproducibility on many conductive substrates (Suryanarayanan, 2010). The most significant advantage of electrogenerated MIPs is that the thickness of the polymer can be controlled by various parameters such as number of cycling scans, current density and applied voltage resulting in a uniform coating of the polymer on the electrode surface

(Suryanarayanan, 2010). In addition, the deposition of the polymer can be done at a precise spot of the detector surface. Furthermore, these polymers are especially attractive in developing applications for analytes of large molecular size, which would otherwise be significantly impeded in diffusing through the polymeric layer (Blanco-Lopez, 2004). In this procedure, neither a polymerization initiator nor UV light or heat is needed. Furthermore, the MIP film is deposited from a functional monomer solution, sometimes with the use of a cross-linking monomer (Pietrzyk, 2009), directly onto an electrode surface in the presence of a template. This film adheres well to a (roughened) electrode surface. The film thickness is governed by the amount of charge transferred during electropolymerization. Surface morphology is controlled by the selection of a suitable solvent and supporting electrolyte. Solvent swelling and inclusion of ions of a supporting electrolyte tunes the rigidity and porosity of this film.

Electropolymerized MIPs

Thus, the possibility of inducing selectivity by the presence of the template during polymerization has been tested with several polymers, such as polypyrrole (PPy), poly(o phenylenediamine) (o-PPD), polyphenol, and poly(thiophene) and is well described in reference (Malitesta, 2012). These polymers (polyphenol, PPy, o-PPD and poly(thiophene)) can be prepared easily by electropolymerization from aqueous solutions of their monomers. On this basis, a more recent example, described by Kan et al. (Kan, 2012), demonstrates the application of electrogenerated MIPs in electrochemical sensing. In this study a simple and efficient MIPs based electrochemical sensor was developed. The sensor was based on the electropolymerization of pyrrole in the presence of bovine hemoglobin (BHb) in an aqueous solution. The fabrication process of the sensor was characterized by differential pulse voltammetry and electrochemical impedance spectroscopy, in which $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ was used as an electrochemical active probe. The measurements carried out by using different electrochemical techniques, including cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) was used. The influence factors including electropolymerization cycles, scan rate, the concentration of pyrrole, and the extraction conditions were investigated in detailed. Under the optimized conditions, the experimental results showed that the MIPs based sensor possessed a fast rebinding dynamics and an excellent recognition capacity to BHb, compared to the other non-template proteins. Moreover, the prepared sensor also exhibited a dependent relationship between the concentration of template protein and peak current of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$.

Mazouz et al (Mazouz, 2017), have also used electrogenarated PPy in an MIP based electrochemical sensor to detect glyphosate (Gly), a global herbicide. In this study, PPy was electrodeposited on the surface of a gold electrode, using chronamperometry (CA). Thus, a thin polypyrrole blocking layer (BL) on the gold electrode was electrodeposited; which was necessary to prevent the formation of complexes with gold. They prepared the BL by CA at a constant potential of +1.05 V vs. SCE during 2 s and MIPs were then prepared by CA at a fixed potential of +1.05 V vs. SCE at pH 5.0. Square wave voltammetry (SWV) measurements were performed to determine the optimal incubation duration of the template in the pyrrole solution. Thin films of non-imprinted polypyrrole polymer (NIP) have also been prepared under identical electropolymerization conditions but without Gly, in order to evaluate nonspecific adsorptions. Two transduction techniques were investigated in this study: gravimetry and electrochemistry. This study was the first one, concerning the detection of Gly with a surface acoustic wave sensor functionalized with a molecular imprinted polymer, and the first work that permited the estimation of dissociation constant values between the polypyrrole matrix and Gly from two different transductions techniques. The limits of detection of the proposed methodology were on the order of 1 pM, which were among the lowest values ever reported in literature. This work also concluded that gravimetric and electrochemical results were indicative that both MIP-based sensors are perfectly able to detect and distinguish glyphosate without any ambiguity.

An electrochemical sensor (Afkhami, 2015), based on molecularly imprinted polypyrrole membrane is reported for the determination of dapsone (DDS). The sensor was prepared by electropolymerization of pyrrole at a glassy carbon electrode in the presence of DDS as a template. The electropolymerization was performed at a glassy carbon electrode using cyclic voltammetry. Some of the parameters, important to controlling the performance of the polypyrrole, were investigated and optimized, including the effects of pH, monomer and template concentrations, electropolymerization cycles. The molecularly imprinted film exhibited a high selectivity and sensitivity toward dapsone. The differential pulse voltammetry (DPV) peak current showed a linear dependence on the DDS concentration and a linear calibration curve was obtained in the range 1.0 μM to 110 μM with a detection limit of 3.0×10^{-7} M. The electrode was successfully applied to the determine dapsone in spiked human serum and urine. A novel and selective PPy molecularly imprinted electrochemical sensor (PPy-MIP) for halofuginone (HFG) detection was developed (Radi, 2015). The imprinted sensor was fabricated by electropolymerization of pyrrole (Py) in the presence of halofuginone (HFG) on a glassy carbon electrode (GCE) surface. The electrochemical sensor exhibited high sensitivity. Different techniques such as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were employed to characterize the proposed sensor. Different parameters like the effects of pH, the monomer concentration, the number of cycles for the electropolymerization, and the scan rate for the sensor preparation were optimized. Under the selected conditions, the DPV peak current was linear to the HFG concentration in the range from 7.5×10^{-9} to 1.0×10^{-5} M, with a detection limit of 2.5×10^{-9} M. The prepared sensor also had good storage stability, reproducibility and regeneration ability. The electrochemical sensor was applied to determine HFG real samples (chicken meat samples).

A novel strategy (Li, 2012) to improve the sensitivity of molecularly imprinted polymer (MIP) sensors was proposed. An electrocatalytic Prussian blue (PB) film was electrochemically polymerized on an electrode surface to fabricate an MIP electrochemical sensor using oxytetracycline (OTC) as a template. The OTC determination relied on a competition reaction between OTC and glucose-oxidase-labeled OTC and the catalytic reduction of hydrogen peroxide by the modified PB film. Experimental results show that double amplification, which is based on the catalysis of inorganic PB films and the enzymatic effect of glucose oxidase, can remarkably increase the assay sensitivity. The main experimental conditions (including electrocatalysis of the PB film, pH effects, incubation and competition times, and anti-interference) were optimized. This novel MIP sensor can offer an femtomole detection limit for OTC. In addition, the feasibility of its practical applications has been demonstrated in the analysis of a series of real milk samples. Lakshmi et al. (Lakshmi, 2009), proposed a simple approach, combining a hybrid an molecularly imprinted – conducting polymer recognition element. Using this procedure they tried to create closer integration between a MIP and transducer. In this paper the development of a sensor based on a novel hybrid material, prepared from N-phenylethylene diamine methacrylamide (NPEDMA), was reported. The monomer, NPEDMA, combines orthogonal polymerizable functionalities, comprising an aniline group and a methacrylamide function, each of which can be independently polymerized. Electropolymerization of NPEDMA resulted in the formation of a layer of poly(aniline) onto the electrode surface with pendant double bonds capable of participating in an addition polymerization. In this way MIPs can be grafted in intimate contact with the substituted poly(aniline) layer, which therefore can act as a network of "molecular wires", facilitating the conduction of electrons from the recognition sites in the MIP to the electrode surface. In the first demonstration of this approach, conducting films were prepared on the surface of gold electrodes by electropolymerization, followed by photochemical grafting of the MIP.

This was achieved by N,N′-diethyldithiocarbamic acid benzyl ester (iniferter) activation of the methacrylamide groups on the electropolymerized layer before addition of the MIP components. The MIP chosen for this study had previously been developed within our group as a tyrosinase-mimicking polymer, imprinted with catechol.32 Catalytic MIP conducting polymer hybrid electrodes were prepared and characterized, and the results are presented below. We also report the sensor characteristics, reproducibility, sensitivity, linearity, and selectivity of the sensor with respect to catechol, dopamine, and potential interfering substances. Finally, the catalytic activity for catechol was assessed using data obtained by chronoamperometry. A novel molecularly imprinted electrochemical sensor was developed by Zhao group (Zhao, 2017), for the determination of bisphenol A (BPA). Au nanoparticles (AuNPs) were modified onto the surface of a glassy carbon electrode (GCE) by electrodeposition method. BPA was used as the template molecule, while 4-aminothiophenol (4-ATP) and chitosan (CS) were used as the binary functional monomers. After 4-ATP was modified on the AuNPs by self-assembly, electropolymerization was carried out by cyclic voltammetry (CV) in the presence of CS and BPA to produce molecularly imprinted polymers (MIPs). The prepared sensor was denoted as MIPs/AuNPs/GCE. The morphologies of the electrodes were characterized by scanning electron microscopy (SEM).

The MIPs were characterized by Fourier transform infrared spectroscopy (FT-IR). The electrochemical performance of the imprinted electrochemical sensor was investigated by cyclic voltammetry and electrochemical impedance analysis (EIS) techniques in detail. The experimental results showed that the gold nanoparticles could increase the conductivity of the MIPs significantly. The imprinted electrochemical sensor was successfully applied to determine BPA by linear scan voltammetry (LSV). Under the optimal condition, the oxidation peak currents of BPA exhibit a well linear relationship toward the BPA concentrations in the range from 1.5×10^{-8} mol L^{-1} to 5.5×10^{-5} mol L^{-1}, with a detection limit of 1.1×10^{-9} mol L^{-1} and a correlation coefficient of 0.9996. The sensor possesses good stability, selectivity and high sensitivity to BPA, which was successfully applied to detect BPA in actual samples such as plastic and milk. The recoveries were in the range of 97.6–106.5 %. An electrochemical sensor based on molecularly imprinted polymer (MIP) film for dodecyl gallate detection at the surface of a glassy carbon electrode (GCE) was proposed by Pedroso et al. (Pedroso, 2017). The GCE was modified with f-MWCNT and the MIP synthesis was performed in situ by means of electropolymerization using ortho-phenylenediamine as the monomer. The stepwise preparation of the MIP and NIP (non-imprinted polymer) was characterized electrochemically by means of cyclic and square wave voltammetry employing ferrocyanide/ferricyanide as a redox probe. The selective capacity performance of the MIP and its imprinted effect to the template molecule (analyte) was compared to the NIP. They were also characterized by scanning electron microscopy technique (SEM). The analytical performance of the MIP sensor performed using square wave voltammetry showed linear range from 0.50 to 8.0×10^{-9} mol L^{-1}, with a correlation coefficient of 0.9921. The sensor presented detection and quantification limit of 0.22×10^{-9} and 0.67×10^{-9} mol L^{-1}, respectively. The apparent dissociation constant (KD) calculated was of 1.26×10^{-4} mol L^{-1} and 5.27×10^{-1} mol L^{-1} for the MIP and NIP respectively.

Akyıldırım et al. (Akyıldırım, 2017) developed an imprinted electrochemical surface based on glassy carbon electrode (GCE) modified with palladium nanoparticles (PdNPs) involved in 5-(4-Hydroxybenzylidenamino)-2-mercaptobenzimidazole (BZ) functionalized graphene quantum dots (GQDs) was formed for citrin (CIT) analysis. The formation of the surfaces was characterized by scanning electron microscope (SEM), transmission electron microscope (TEM), electrochemical impedance spectroscopy (EIS) and X-ray photoelectron spectroscopy (XPS). CIT imprinted electrochemical surface was formed in the presence of 80.0 mM pyrrole as monomer and 20.0 mM CIT as template. The linearity range and the detection limit (LOD) of the developed nano sensor were calculated as 1.0×10^{-9} – 5.0×10^{-9} M and 2.0×10^{-10} M, respectively. Highly selective determination of enantiomers by electrochemical sensors with simple recognition elements is of great interest in pharmaceutics, biochemistry and medical fields. Thus, an innovative electrochemical sensor was developed by Wang et al. (Wang, 2017), which was based on boron-embedded molecularly imprinted hybrid film (B-MIHF) modified carbon paste electrode (CPE), for enantioselective determination of *S*-ornidazole (*S*-ONZ). A boron-embedded conductive copolymer was firstly electro polymerized on carbon paste electrode (CPE), and then molecularly imprinted polysiloxanes (MIP) were hydrolyzed and condensed over the conductive copolymer layer via a sol-gel process. The B-MIHF, comprised the advantages of boron-embedded conductive copolymer and molecularly imprinted polysiloxanes, could not only implement direct signal transformation between imprinted cavities and sensing elements, but show amazing selectivity to template *S*-ornidazole. Above all, the introduction of coordinate interaction formed between trisubstituted boron atoms embedded in B-MIHF matrix and nitrogen atoms of template endow the sensor with intensive enantioselectivity.

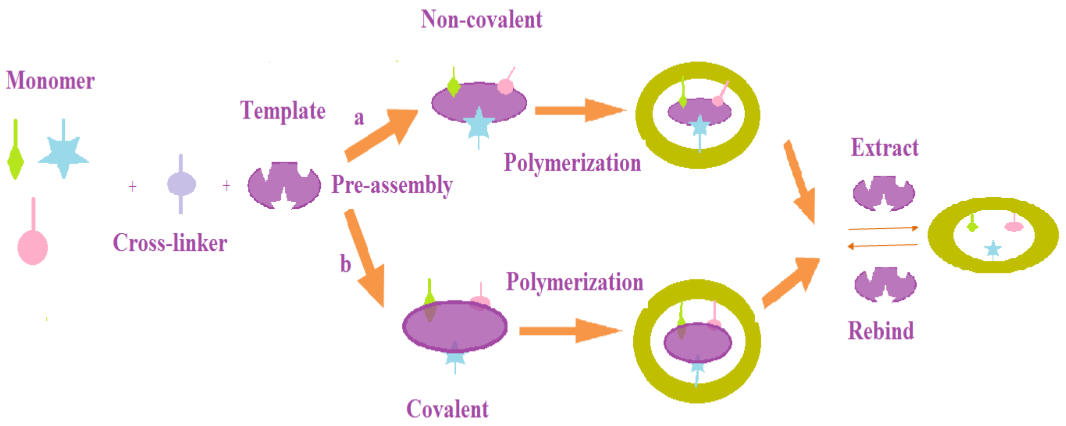

Fig. 1. Typical preparation of MIP: (a) non-covalent and (b) covalent imprinting

Under optimum experiments conditions, the reduction peak currents of *S*-ONZ by differential pulse voltammetry were linear to *S*-ONZ concentrations in the range from 6.0 $\times10^{-8}$ to 1.0 $\times10^{-6}$ mol L^{-1} and 1.0 $\times10^{-6}$ to 3.5 $\times10^{-4}$ mol L^{-1} with a detection limit of 1.1 $\times10^{-8}$ mol L^{-1}. The B-MIHF-based sensor has been successfully applied for the determination of *S*-ONZ in pharmaceutical and biological samples with good precision (RSD 2.25–4.11 %) and acceptable recoveries (96.00–108.00 %). A novel and simple imprinting route based on graphene (GN) was proposed by Znang et al. (Zhang, 2017), to fabricate an electrochemical sensor for sensitive and selective determination of imidacloprid (IDP) residue. In this route, *p*-vinylbenzoic acid (VBA) was utilized as functional monomer to immobilize onto GN in *N*, *N*-dimethylformamide (DMF) solvent via π-π interaction, directing the polymerization of uniform molecularly imprinted polymers (MIP) layers on the surface of GN sheets. Besides, quantitative control of the synthesis of MIP/GN and the preparation of MIP/GN modified glassy carbon electrode (GCE) were carried out, which were rarely found in other papers. Thus, the proposed sensor possesses highly improved stability and specific recognition ability. The adsorption isotherm study indicated that the MIP layer on GN surface is monolayer and the structure is homogeneous. The peak current of IDP on MIP/GN modified GCE is linear with IDP concentration in the range of 0.5–15 μM with a detection limit of 0.10μM. In addition, the novel method was further applied to detect IDP in brown rice samples with satisfactory results. A novel nanosized substrate imprinted polymer (MIPs-GO-Fe_3O_4) was developed on a magnetic graphene oxide (GO-Fe_3O_4) surface for selective recognition and fast removal of 17β-estradiol (17β-E_2) [37].

The characteristics of MIPs-GO-Fe_3O_4 were evaluated by transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy and vibrating sample magnetometer (VSM). Results suggested that GO had a thin single-layer structure anchoring Fe3O4 nanoparticles and that the imprinted film was coated on the GO-Fe_3O_4 surface. MIPs-GO-Fe_3O_4 was sensitive to the magnetic field and could be easily separated using an external magnet. The adsorption results indicated that the kinetic value and binding capacity of MIPs-GO-Fe_3O_4 were 0.0062 g $(mg\cdot min)^{-1}$ and 4.378 μmol g^{-1}, respectively. MIPs-GO-Fe_3O_4 was also used to analyze 17β-E_2 in real food samples, and satisfactory recoveries such as 84.20 % with relative standard deviation (RSD) of 4.67 % at a spike of 0.5 μmol L^{-1} were obtained. Thus, the MIPs-GO-Fe_3O_4-based method provided a convenient and practical platform for the separation, enrichment, and removal of 17β-E_2 in food samples. The fabrication of a molecularly imprinted, polymer-based disposable electrochemical sensor for paraoxon (PO) determination was proposed by Li group (Li, 2017). The sensor was based on a screen-printed carbon electrode (SPCE) modified with a surface molecularly imprinted poly (p-aminothiophenol) (PATP)/gold nanoparticles (AuNPs) composite film, which consisted of a PATP outer layer and an AuNPs inner layer. We report a novel strategy, combining surface molecularly imprinting and self-assembly directed electro-polymerization with high densely imprinting PO molecules in the PATP/AuNPs film. Firstly, AuNPs were in situ electrodeposited at the electrode surface, and then assembled with electropolmerizable functional monomer p-aminothiophenol (ATP).

Subsequently, PO molecules were assembled onto the ATP monolayer-modified AuNPs, forming a basis of surface molecular imprinting. After that, replenished PO molecules were embedded in the PATP/AuNPs film by PO and the ATP molecular self-assembly directed electro-polymerization in the polymerization precursor mixture. The resulting imprinted PATP/AuNPs/SPCE possesses high sensitivity, affinity, and selectivity toward PO, with a low detection limit of 1 $\times10^{-9}$ M. The proposed sensor was successfully applied for the determination of PO in fruit and vegetables, giving satisfactory recoveries. The strategy reported herein can be further expected to fabricate various molecular imprinted sensors for the determination of other pesticide residuals. A novel strategy to improve the sensitivity of molecularly imprinted polymer sensors based on a double-layered molecularly imprinted polymer hybrid film-modified gold electrode was investigated for the determination of testosterone (Fourou, 2018). A conductive poly (anilinomethyltriethoxysilane) film was electrodeposited on the surface of the electrode, and a molecularly imprinted polysiloxane thin film was formed on the conductive layer by a sol-gel process to enhance the electrochemical response. The performance of the sensor for testosterone was investigated by square-wave voltammetry in the presence of a hexacyanoferrate/ferrite as a redox probe. The prepared sensor provided high recognition for testosterone with a broad linear range from 10 to 100 fM and a low detection limit of 10 fM. Good sensitivity, selectivity, and stability were observed with relative standard deviations less than 5%. Good recovery of testosterone was obtained when the sensor was used for the determination of testosterone in fortified urine. A novel electrochemical sensor based on the molecularly imprinted polymer (MIP) was fabricated by electrochemical polymerization of β-cyclodextrins (β-CD) and epigallocatechin-gallate (EGCG) on the graphene oxide (GO) modified glassy carbon (GO/GC) electrode (Liu, 2017). The MIP/GO/GC electrode exhibits an excellent ability of specific binding of EGCG and a rapid electrochemical response, high sensitivity and selectivity for determination of EGCG. This prepared MIP sensor presents distinct advantages over conventional electrochemical methods for EGCG determination because it is a one-step preparation and the template molecule can be easily removed by cyclic voltammetry scans, and no elution reagent is required. Under the optimal experimental conditions, the linear response range for EGCG concentrations by the sensor was 3 x10^{-8} mol L^{-1} to 1 x10^{-5} mol L^{-1} and the detection limit was 8.78 x10^{-9} mol L^{-1}. The results demonstrate that the proposed MIP sensor can be a potential alternative for the determination of EGCG in tea samples.

Conclusion

MIPs prepared by electropolymerization of electroactive functional monomers reveal several advantages over MIPs prepared by free-radical polymerization combined with drop-coating or spin-coating of a solution of a monomer or preformed polymer. Particularly, the film thickness can be conveniently controlled during electropolymerization by the electrochemical parameters of the polymerization and deposition. Furthermore, application of suitable solvents and supporting electrolytes can tune the viscoelastic properties and porosity of the MIP films. MIP films using CP matrices facilitate the charge transport between the electrode substrate and the analyte occupying molecular cavities of the MIP. In addition, there is a possibility of increasing the conductivity of these films by integrating them with conducting NPs such as AgNPs or carbon nanotubes to form composite MIP matrices. Moreover, synthesis and use of derivatized electroactive functional monomers helped to fabricate more selective CP-based MIPs. These derivatized electroactive functional monomers contain additional functional groups, which form stable complexes with templates during a preorganization step. Stabilization of these complexes is further enhanced by π-delocalized bonds in polymer chains.

Therefore, these electroactive functional monomers have successfully been used in imprinting of different target analytes ranging from amino acids to pesticides, drugs, and proteins. Conclusively, the use of electroactive functional monomers for preparation of MIP films as recognition units of sensors is of great importance. These monomers have shown their suitability for fabrication of electrochemical sensors using different transduction platforms. They effectively transduce an event of analyte binding, improving the detectability. For higher selectivity of the electrosynthesized MIP, these electroactive functional monomers should be derivatized with additional functional groups.

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