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A Sensitive Approach on Trace Analysis of Methylparaben in Wastewater and Cosmetic Products Using Molecularly Imprinted Polymer

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***Abstract*—**Parabens are the antimicrobial molecules largely used in cosmetic products as a preservative agent. Among them, the methylparaben (MP) is the most frequently used ingredient in cosmetic preparations. Nevertheless, their potential dangers led to the development of sensible and reliable methods for their determination in environmental samples. Firstly, a sensitive and selective molecular imprinted polymer (MIP) based on screen-printed gold electrode (Au-SPE), assembled on a polymeric layer of carboxylated poly(vinyl-chloride) (PVC-COOH), was developed. After the template removal, the obtained material was able to rebind MP and discriminate it among other interfering species such as glucose, sucrose, and citric acid. The behavior of molecular imprinted sensor was characterized by Cyclic Voltammetry (CV), Differential Pulse Voltammetry (DPV) and Electrochemical Impedance Spectroscopy (EIS) techniques. Then, the biosensor was found to have a linear detection range from 0.1 pg.mL^{-1} to 1 ng.mL^{-1} and a low limit of detection of 0.12 fg.mL^{-1} and 5.18 pg.mL^{-1} by DPV and EIS, respectively. For applications, this biosensor was employed to determine MP content in four wastewaters in Meknes city and two cosmetic products (shower gel and shampoo). The operational reproducibility and stability of this biosensor were also studied. Secondly, another MIP biosensor based on tungsten trioxide (WO_3) functionalized by gold nanoparticles (Au-NPs) assembled on a polymeric layer of PVC-COOH was developed. The main goal was to increase the sensitivity of the biosensor. The developed MIP biosensor was successfully applied for the MP determination in wastewater samples and cosmetic products.

***Keywords*—**Cosmetic products, methylparaben, molecularly imprinted polymer, wastewater.

I. Introduction

ENDOCRINE-DISRUPTORS are naturally occurring compounds or man-made substances that may mimic or interfere with the function of hormones in the body. One antibacterial agent under analysis at this time is MP. As is well known, it is currently used as additive in different common products such as toothpastes, facial cleansers, hand soaps, body washes, cosmetics and numerous other products [1]. Although its effects on human health are controversial and largely unknown, it has been reported to have an effect on the endocrine system [2]. Therefore, in order to reduce the harm of parabens to human health, the use of parabens as preservatives in cosmetic products is permitted in several countries, up to a maximum concentration of 0.4% (w/w) in the finished product for one ester and up to 0.8% (w/w) for their mixtures [3]. Due to their widespread use, the active antibacterial agents in these products have been detected in public water samples.

Although, several analytical methods have been developed for determination of MP in different matrixes, these methods are primarily included gas chromatography (GC) [4], high-performance liquid chromatography (HPLC) [5], liquid chromatography with mass spectrometry (LC-MS) [6], and electroanalytical methods [7]. These techniques need expensive instrumentation and samples extractions and they are time-consuming. The most recent research in monitoring techniques is mainly focused on bioanalytical tools, such as biosensors, which offer advantages over classical analytical techniques in terms of selectivity, sensitivity, short assay times, and reduced cost of analysis. Moreover, recent advances in nanotechnology emerged in a wide range of sensitive biorecognition elements assembled in small platforms, such as molecularly imprinted polymers (MIPs) [8]. Otherwise, deposition of different agents on the surfaces of manufactured electrodes could also be easily achieved to enhance selectivity such as MIP. It is used as an artificial receptor in bioanalytes. MIPs are synthetic materials that are able to specifically recognize and bind target molecules. It allows the creation of artificial recognition sites using the imprint molecule [9], [10]. MIPs have many advantages such us high selectivity, sensitivity, and stability [11]. Moreover, based amplification paths aimed at achieving ultrahigh sensitivity. Au-NPs have potential applications in the construction of electrochemical sensors because of their small dimensional size, good stability, biocompatibility and good conductivity. The integration of NPs in MIP materials has the benefit of enhancing the sensitivity and the fast equilibration with the analyte [12].

The purpose of this study was firstly to develop a sensitive and very selective biosensing device based on MIP using Au-SPE. Secondly, the same MIP procedure using Au-SPE based

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on WO_3 functionalized with Au-NPs was developed. The biosensors were characterized by CV, DPV, and EIS. As application, the first biosensor was successfully used to the MP determination in wastewater and cosmetic products samples.

II. Experimental

A. Reagents and Solutions

MP, PVC-COOH, phosphate buffered saline (PBS 0.01M), 1,4 dioxane, sucrose, citric acid and glucose were from Sigma Aldrich. N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDAC), ammonium persulphate (APS), N, N'-methylenebisacrylamid (NNMBA), acrylamide (AAM), N,N,N',N'-Tetramethylethylenediamine (TEMED), N-hydroxysuccinimide (NHS), potassium ferricyanide $K_3[Fe(CN)_6]$ and potassium ferrocyanide $K_4[Fe(CN)_6]$ were all obtained from Fluka. 2-amino-2-hydroxyméthyl-1,3-propanediol (TRIS 0.5 M) was from Panreac quimica. The buffer solutions used in this work were TRIS and PBS. All solutions of the adopted range were stocked at 4 °C.

B. Preparation of MIPs and NIPs (Non Imprinted Polymers) Biosensors

Before any deposit, the working area of the Au-SPE was washed with ethanol. The Au-SPE surface was incubated in PVC-COOH dissolved in 1, 4 dioxane. The -COOH groups were activated by an incubation of the electrode in a solution of EDC and NHS prepared in water. The activated Au-SPE/PVC-COOH was then washed twice with PBS buffer (pH 7.4) to remove unbound MP. Then, a TRIS solution was deposited to block any unbounded -COOH groups left on the surface of the electrodes. In order to create a polymer matrix around the template, the polymerization stage was carried out by adding APS and TEMED in a solution containing AAM as functional monomer and NNMBA as cross linker in PBS buffer. Methanol and acetic acid are able to allow a successful removal of MP from the imprinted layer. The Au-SPE/MIP was ready to use after washing it with distilled water. As control, the NIPs were prepared in a similar procedure, without the addition of MP.

C. Electrochemical Measurements

The electrochemical measurements were carried out using PalmSens[3]. SPEs were purchased from DROPSENS (8X220AT). Working and counter electrodes were made of gold, whereas reference electrode was made of silver. CV measurements were recorded in 5.0 mmol/L of $[Fe(CN)_6]^{3-/4-}$, prepared in PBS buffer (pH 7.4). For CV assays, the potential was performed in potential range of -0.4 to 0.6 V, at a scan rate of 30 mV/s. In DPV studies, potentials were changed from -0.1 to 0.2 V, at a scan rate of 10 mV/ s. EIS assays were conducted under a number of frequencies equal to 50. The frequency range was 0.1 Hz–50 kHz.

D. Samples Preparation

For real detection, the wastewater samples were collected from Meknes city, Morocco. The samples were directly used to avoid their degradation and consequent change in properties. Besides, shower gel and shampoo were purchased from a supermarket. 0.5 g of each cosmetic products sample was weighed accurately, dissolved in 10 mL of methanol. The supernatant was filtrated. Then, the filtrate was diluted with PBS (pH 7.4) for the detection stage.

III. Results and Discussion

A. Control of the Surface Modification of the Au-SPE and Au-SPE Functionalized with Au-NPs

The same MIP elaboration was done for the both biosensors. CV assays are not as sensitive as EIS but may serve as an additional confirmation of the chemical modification.

For the first biosensor, the modification step of the Au-SPE with PVC-COOH show a low current peak. However, the subsequent immobilization steps on the PVC-COOH layer present an increase of the current peak. In the other words, the deposit of MP displays a more important current peak relative to that of PVC-COOH. After the deposition of the polymer, an increase of current peak is observed again. This is explained by the interaction between the template and the polymer (Fig. 1 (a)).

The Nyquist plots obtained by EIS method are shown in Fig. 1 (b). The Au-SPE coated with a PVC-COOH layer displays a high electron transfer resistance (R_{ct}). The linkage of MP and the polymerization deposit on the modified Au-SPE produced a significant decrease of R_{ct}.

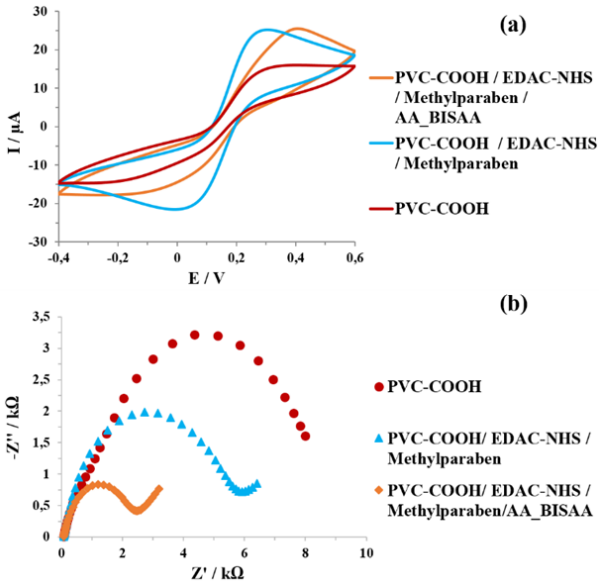

Fig. 1 (a) Cyclic voltammograms and (b) Nyquist plots of sequential immobilization steps onto Au-SPE

In the case of the second biosensor, the same phenomenon was noticed for the Au-SPE functionalized with Au-NPs but with a higher sensitivity. The amplitude of the currents is more important during the second biosensor elaboration (Fig. 2 (a))

compared with the first one. It can be seen from Fig. 2 (b) that the resistance values are also more important.

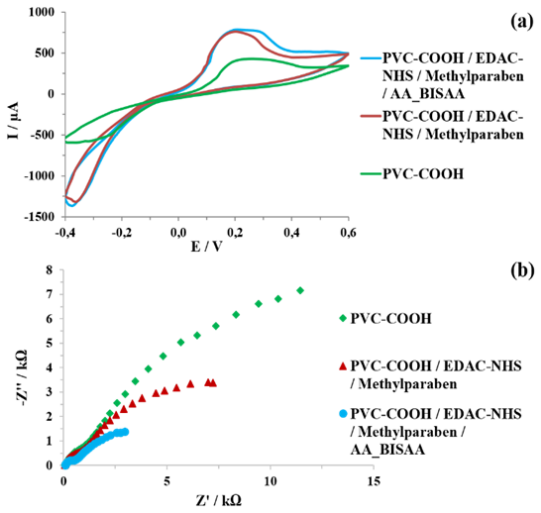

Fig. 2 (a) Cyclic voltammograms and (b) Nyquist plots of sequential immobilization steps onto Au-SPE functionalized with Au-NPs

B. MIP and NIP Biosensors Responses

In order to investigate the retention performance of the MP-biosensors, the imprinted films were dipped into the binding solutions containing various concentrations of MP. DPV and EIS were used to monitor the biosensor responses.

For the first biosensor, it is obvious from DPV results that MP oxidation currents increase with increasing MP concentrations (Fig. 3 (a)). This is in perfect agreement with EIS where the diameter of the semicircles decreased gradually with increasing concentrations of MP (Fig. 3 (b)).

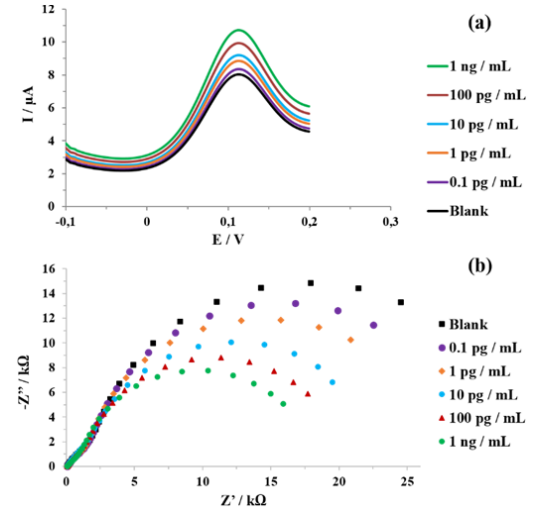

Fig. 3 (a) Differential pulse voltammograms and (b) Nyquist plots of MIP biosensor based on Au-SPE

For the second biosensor (Fig. 4 (a)), DPV technique shows high increasing current values relative to the first. According to EIS method, lower R_{ct} were remarked (Fig. 4 (b)).

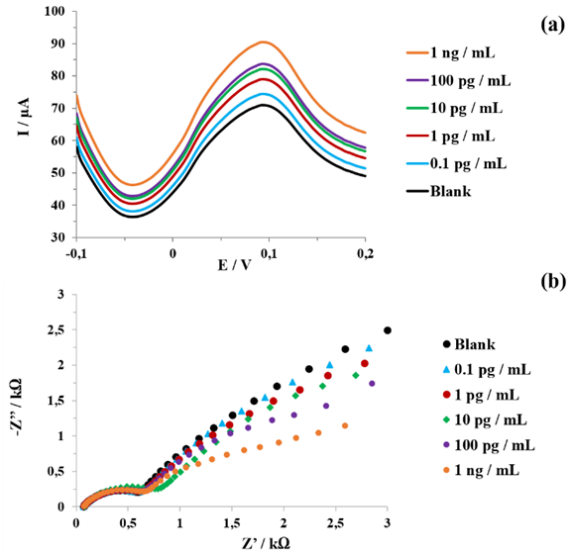

Fig. 4 (a) Differential pulse voltammograms and (b) Nyquist plots of MIP biosensor based on Au-SPE functionalized with Au-NPs

Additionally, NIP biosensors were also prepared with the same manner to confirm the MIP responses. No variation of the signals was observed for both methods explaining the non-fixation of the imprint molecule (Fig. 5).

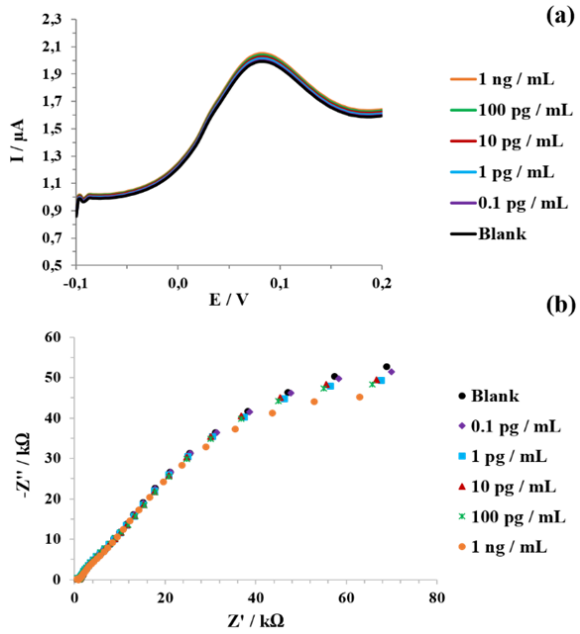

Fig. 5 (a) Differential pulse voltammograms and (b) Nyquist plots of NIP biosensor based on Au-SPE

Fig. 6 shows the variation of the current and resistance responses from the MIP and NIP biosensor against the logarithm of the MP concentrations. Then, in a linear range from 0.1 pg/mL to 1 ng/mL, the biosensor was found to have a low limit of detection of 0.12 fg/mL and 5.18 pg/mL by DPV and EIS, respectively.

C. Selectivity of the MIP Biosensor

Selectivity is an important property of biosensors. Therefore, the cross-reactivity of some MP analogues compounds such as sucrose, citric acid, and glucose was investigated. Fig. 6 indicates the recorded responses of these interfere using the same polymer. DPV curves present little variations of their current amplitudes (Fig. 6 (a)), and EIS plots demonstrate negligible changes of R_{ct} (Fig. 6 (b)). It clearly means that the fabricated MIP biosensor is only selective toward MP.

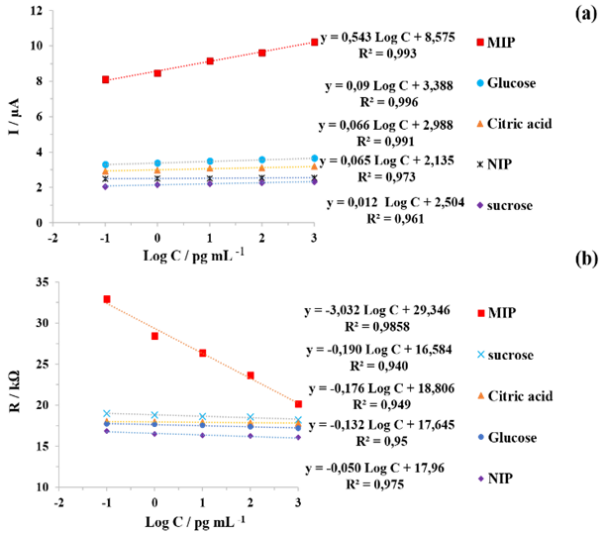

Fig. 6 Calibration curves for MP of MIP and NIP biosensors by (a) DPV and (b) EIS

D. Stability of the MIP Biosensor

The long-term storage stability of MIP biosensor was performed. The MIP biosensor was stored in PBS (pH 7.4) at 4 °C when not used. The electrode retained 97.75% of its initial response for a MP concentration (1 ng/mL) after two months, which indicates that the biosensor had long-term stability.

E. Application of the MIP Biosensor for the Determination of MP in Wastewater and Cosmetic Products Samples

By using this developed biosensor, the MP detection experiments in four wastewater samples were performed. Table I shows the results obtained by DPV and EIS techniques. This presented method for MP assessment described averages relative standard deviation (RSD) less than 0.13% and 0.16% by DPV and EIS, respectively. It demonstrated that the biosensor was suitable for the MP determination in wastewater samples.

Moreover, the fabricated biosensor was also used for MP determination in shampoo and shower gel samples. It was found that MP concentrations determined using the proposed biosensor were 0.351 g/100 mL and 0.38 g/100 mL for shampoo and shower gel, respectively. It was in good agreement with the manufacturer's stated contents of MP (0.4 g/100 mL) (Table II). It concludes that the proposed method can be applied successfully for the determination of MP in cosmetic products.

TABLE I
RESULTS OF MP DETERMINATION IN WASTEWATER SAMPLES

	MP concentrations (ng / mL)			
Wastewater samples	DPV	RSD (%) (n=3)	EIS	RSD (%) (n=3)
Road Kasbah	0.273	0.04	0.275	0.03
Ain karma	8.63	0.06	8.016	0.09
Toulal	6.796	0.1	6.716	0.12
Bab bouamair	0.766	0.13	0.731	0.16

TABLE II
DETERMINATION OF MP IN SHAMPOO AND SHOWER GEL SAMPLES

	MP concentrations (g / 100 mL)		
Cosmetic products samples	DPV	RSD (%) (n=3)	Manufacturer's stated contents
Shampoo	0.351	1.4	0.4
Shower gel	0.377	0.9	

IV. CONCLUSION

The first molecularly imprinted biosensor based on Au-SPE was developed for MP determination, revealing a detection limit of 0.12 fg/mL and 5.18 pg/mL by DPV and EIS, respectively. The biosensor exhibited good selectivity, sensitivity and stability. It has been successfully applied for determination of MP by DPV and EIS in wastewater and cosmetic products samples. Furthermore, the second biosensor functionalized with Au-NPs offered the advantages to enhance sensitivity.

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