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MIP-based screen-printed electrode for Irbesartan sensing†

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Abstract: In this work, the development of a MIP-based electrode for voltammetric detection of Irbesartan is presented. Irbesartan is a drug prescribed to treat hypertension and high blood pressure. Recent studies associated sartans with several forms of cancer, making removing this class of substances from the environment a high priority and the EU has categorized it as an emerging pollutant. Molecularly Imprinted Polymers, MIPs, have already been used to remove pollutants from complex matrixes, so they were also chosen for this work. In particular, a polymer based on polyacrylate moiety was used to functionalize the graphite working electrode of screen-printed cells (SPCs), aiming to develop a voltammetric method for Irbesartan sensing. The prepolymeric mixture was drop coated on the working electrode. The electrochemical technique used to quantify Irbesartan is the square wave voltammetry (SWV); the experiments were carried out in acetate buffer at pH 5.5. A detection limit of 19 μg/L was obtained, and the linearity ranged from 31 μg/L to 432 μg/L. The procedure was replicated with different SPCs obtaining similar results, highlighting good reproducibility. The electrodes were also applied to determine Irbesartan in fortified tap water samples, obtaining high recovery percentages. Since the good results, the electrochemical methods based on MIP-functionalized screen-printed electrodes are promising for quantifying Irbesartan at a trace level.

Keywords: MIPs; Electrochemical sensors; Emerging pollutants; MIP-based sensors; chemosensors

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1. Introduction

Irbesartan is a drug prescribed to treat hypertension, high blood pressure and some kidney diseases linked to type 2 diabetes [1]. The EU has classified many drugs, including sartans, as "emerging pollutants," substances whose effects on the environment and the human population in the short and long term have not been thoroughly studied yet [2]. In the EU, drugs come into contact with the environment through wastewater and sewage sludge, while production plants are secondary sources. Drugs can be found in rivers near highly populated areas and production plants. In the last decades, drug prescription has sharply increased, especially among the older population, and Losartan and Irbesartan are among the most prescribed antihypertensive drugs. They can pose a greater threat than other sub-stances because, as drugs, they are designed to be effective at very low concentrations and because they are left unscathed by wastewater treatment plants making their removal and monitoring of the utmost importance [3]. High-Performance Liquid Chromatography (HPLC) is the analytical technique mainly used to investigate sartans in pharmaceutical formulations, biological samples and environmental ones; coupled with a UV detector or mass spectrometry (MS) [4-5]. For example, Rane et al, described the development of a isocratic reversed-phase HPLC method for simultaneous detection of irbesartan and hydrochlorothiazide in pharmaceutical preparations obtaining a low detection limit (of about 50 nM) and excellent linearity over a range of 20–500 μM [6].

Overall, these techniques are time-consuming and expensive; moreover, a relatively large amount of solvents is employed for every sample, making in situ analysis unavailable.

Compared to conventional techniques, electrochemical sensors have several advantages, such as the low cost of instruments, the low volume of sample required, and the relatively short time of analyses. For obtaining selective and sensitive methods, the employment of specific receptors, both natural and synthetic, can be used to enhance the performances of the electrochemical sensors. Molecularly Imprinted Polymers (MIPs) are the most promising synthetic receptors because they are robust, stable, and resistant to degradation in acidic and alkaline media and at high temperatures. Furthermore, they also show high selectivity and affinity constants suitable for trace analyses [7].

The growing request for disposable and low-cost sensors for *in situ* analyses encouraged the application of screen-printing electrodes, particularly those produced by graphite ink because it is the cheapest [8].

In this scenario, a MIP based on polyacrylate moiety was developed in the present work and applied to functionalize the graphite working electrode of screen-printed cells (SPCs), aiming to develop a voltammetric method for Irbesartan.

2. Materials and Methods

2.1. Materials

Methacrylic acid (MMA) and etilenglycoldimethacrylate (EGDMA) were purchased from Sigma-Aldrich. They were filtered with an aluminum oxide column to remove stabilizers. 2,2-azobisisobutyronitrile (AIBN), concentrated acetic acid, sodium acetate trihydrate, and Irbesartan were used as obtained from Merk Life Science S.r.l. (Milano, Italy). Sodium acetate trihydrate/acetic acid was used to prepare buffer solutions for polymer characterization and voltammetric measurements. Solutions for the electrode surface characterization were prepared from sodium chloride, potassium chloride, and potassium hexacyanoferrate (III) (Merk Life Science S.r.l., Milano, Italy). Tap water from the lab sink (Department of Chemistry, University of Pavia) was used to prepare fortified samples. Screen-printed cells (SPCs) were acquired from Topflight Italia spa (Vidigulfo, Pavia, Italy).

2.2. Instruments

Potentiometric analyses were performed by the potentiostat/galvanostat Em-Stat4s-PalmSens BV (Houten-The Netherlands). UV-Vis spectra were acquired using a Jasco V-750 spectrometer (Jasco Europe S.R.L, Lecco, Italy).

2.3. Prepolymeric mixture preparation and modification of the working electrode surface

The prepolymeric mixture consisted of 86 mg of irbesartan (IRB), 66.8 μL of MMA, 756 μL of EGDMA and 45 mg of AIBN with a molar ratio of 1:4:20 (IRB: MMA: EGDMA). The mixture was deaerated with a gentle flow of N_2 for 5 min and sonicated to dissolve irbesartan and AIBN. The minimum amount of methanol was added to facilitate and completely dissolve the mixture's components. An equal prepolymeric mixture not containing irbesartan was prepared for functionalizing the working electrode with the NIP (non-imprinted polymer). Four electrodes were functionalized with the MIP-prepolymeric mixture and three with the NIP-prepolymeric one. Both polymers were characterized in acetate buffer solutions at pH 5 to determine the sorption kinetics and the maximum sorption capacity of the polymer.

3 μL of MIP or NIP prepolymeric mixture was drop-coated on the SPC's working electrode surface. The thermal polymerization was performed in an oven at 60 °C overnight. Seven cleaning cycles by immersion for one h in 10 mL of a mixture of glacial acetic acid: methanol = 1:4 were carried out to remove the template (IRB) and the eventually unreacted monomers. The so-cleaned functionalized SPC was stored at room temperature before use.

Figure 1 shows a picture of the experimental setup.

Figure 1. Image of the experimental setup.

3. Results

3.1. Polymer characterization

MIP and NIP were characterized by determining their maximum sorption capacity for Irbesartan, derived from the sorption isotherms curves in acetate buffer solutions at pH 5. The Langmuir model well fitted the experimental data, obtaining a maximum sorption capacity, q_{max}, for the MIP of about 0.4 mmol/g and only 0.08 mmol/g for the NIP.

Kinetics experiments were then performed. For example, Figure 2 shows the kinetic profile for the sorption of Irbersartan on the MIP. The results demonstrated that the film diffusion process [9] is the rate-determining step, and the quantitative sorption of the analyte required about 1 h.

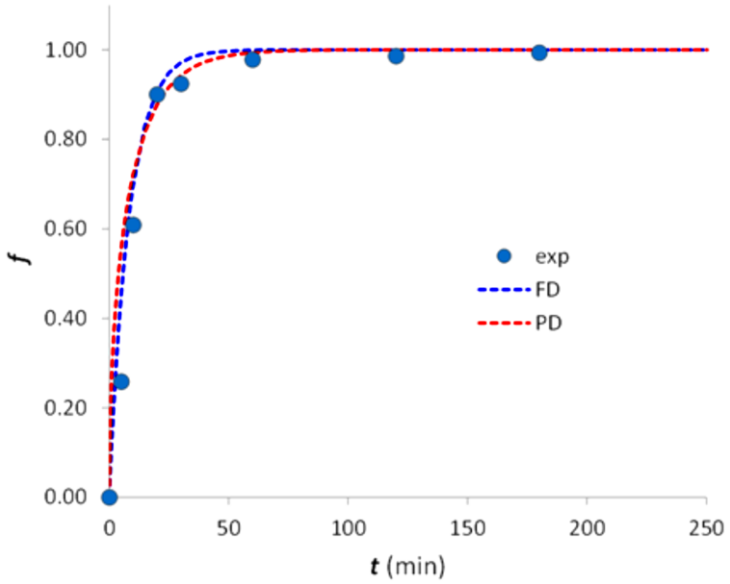


Figure 2. Kinetic sorption profile of Irbesartan on MIP at pH 5.

3.2. Electrode functionalization and measurements

Screen printed cells (SPCs) are an integrated system that contains a working electrode WE, usually made of gold or graphite, a counter electrode CE, usually made of graphite and a reference electrode RE usually made of silver/silver chloride. In the present work, commercial screen-printed cells were used. SPCs with a graphite WE, a graphite CE and an Ag/AgCl RE were used for this work. SPCs are cheap, and the measure is easily made by connecting them to an electrochemical instrument to perform voltammetric measurements. A graphite electrode was chosen to determine Irbesartan as a valid alternative to the classical hanging drop mercury electrode (HDME) previously applied [10-12]. The prepolymeric mixture was drop coated on the working electrode, and polymerization was carried out in an oven at 70°C overnight.

Functionalized and non-functionalized electrodes were characterized by determining surface properties, such as the electroactive area and double-layer capacity. The electroactive area was determined by cyclic voltammetry measurements. It is possible to derive the area, *A*, from the Randles-Sevcik equation [13]:

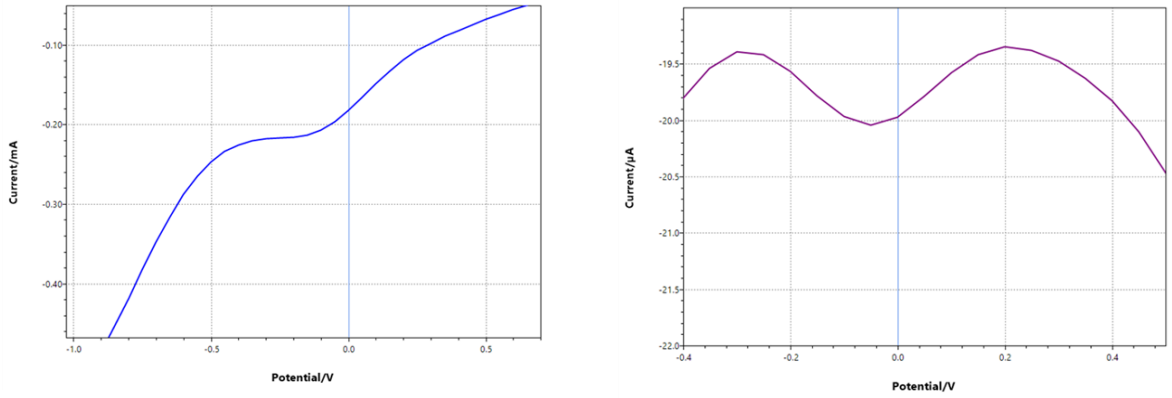
$$i_p = 2.668 \cdot 10^5 \cdot \sqrt{n^3} \cdot A \cdot \sqrt{D} \cdot [C] \cdot \sqrt{v} \quad (1)$$

i_p: peak current in Ampere [A]
n: number of electrons exchanged
A: area [cm^2]
D: diffusion coefficient [cm^2/s]
[C]: electrochemical probe concentration [mol/cm3]
v: scan rate [V/s]

The anodic and cathodic peak current intensities obtained from cyclic voltammetry measurements were plotted against the square root of the potential scan rate, and from the slope of the line, applying the fitted Randles-Sevick equation, the effective area of the working electrode can be computed. For the bare electrode, the area calculated is not very different from the one stated by the manufacturer. However, the area of the electrode functionalized with MIP and with NIP is smaller (9.59(8) mm^2 and 8.80(3) mm^2, respectively). This experimental evidence can be justified considering that the presence of the polymer film above the working electrode "blocks" and reduces the electronic transfer capability of the graphite.

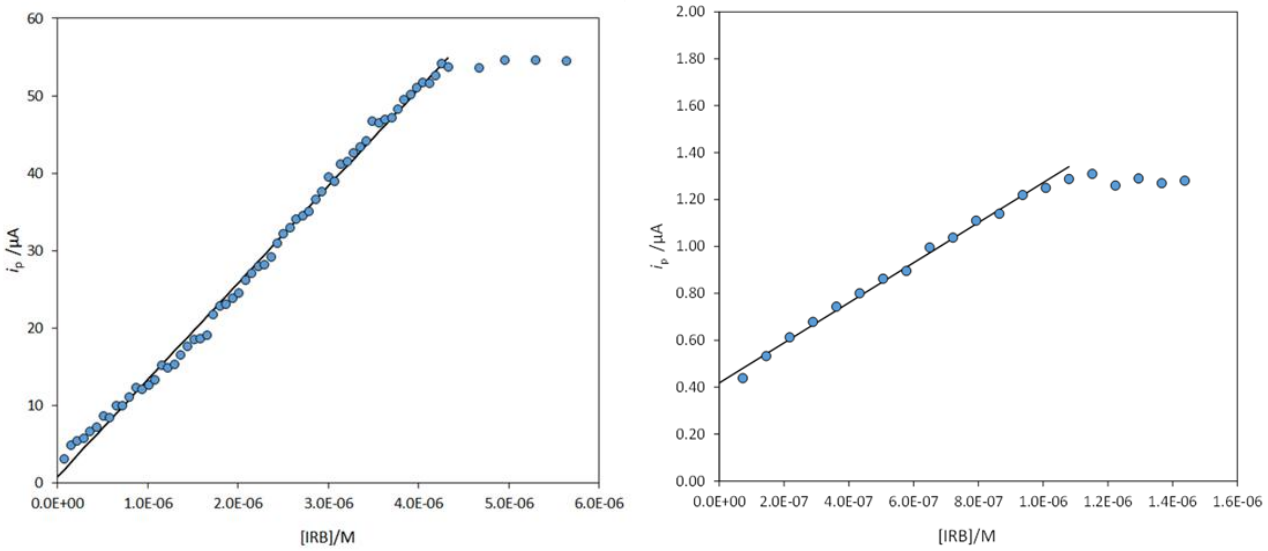
The double layer capacity can also be obtained through cyclic voltammetry experiments: by plotting the capacitive current (obtained as the difference between cathodic peak current and anodic peak current) versus the scan speed, a line is acquired and, by dividing the slope by two, the capacity is determined [14]. The experiments were carried out using NaCl 0.1 M as a supporting electrolyte and increasing the scan speed from 0.025 V to 0.05 V. For the bare electrode, the capacity found is relatively low, and the NIP-electrode's capacity is also low, while still higher than the non-functionalized one. The MIP-electrode possesses a higher capacity than its other counterparts, meaning that the functionalization, in this case, augmented the electrode's ability to accumulate charges.

The electrochemical technique used to quantify Irbesartan is Square Wave Voltammetry (SWV). The experiments were carried out in acetate buffer at pH 5-5.5. Figure 3a shows a voltammogram recorded on a bare electrode, while Figure 3b that recorded on a MIP-functionalized electrode. The difference in shape can be attributed to a better specificity linked to the presence of the polymer film. The calibration curve was obtained by plotting current intensity (μA) versus Irbesartan concentration only after determining the best parameters with an Experimental Design. Figure 4 shows the calibration curves for the bare electrode (without MIP, Fig. 4a) and the MIP-functionalized electrode (Fig. 4b).



(a) (b)

Figure 3. a) Square Wave Voltammogram of Irbesartan reduction on a bare electrode ([IRB] = 0.2 nM). b) Square Wave voltammogram of irbesartan reduction on a MIP-functionalized electrode ([IRB] = 0.2 nM). .



(**a**) (**b**)

Figure 4. (a) Calibration curve of a non-functionalized electrode. (b) calibration curve of a MIP-functionalized electrode.

The linear concentration range for the bare electrode is quite large despite a not well-defined peak; on the contrary, a lower linear range is obtained for the MIP-functionalized electrode, but in this case, the peaks are better defined, and the detection limit is lower ($4.4 \cdot 10^{-8}$ M), i.e., not so different to than that found with traditional techniques.

The MIP-based electrodes were also applied to determine Irbesartan in fortified tap water samples; the results are shown in Table 1.

Table 1. SWV of functionalized (SPC-MIP) and non-functionalized (SPC-bare) for fortified irbesartan samples analysis. The number in parenthesis is the standard deviation on the last digit.

Sensor	[IRB]/ M nominal	[IRB]/ M measured	error %	recovery %
SPC-MIP	$3.6 \cdot 10^{-7}$	$3.37(6) \cdot 10^{-7}$	-6.6	93.4
SPC-bare	$3.6 \cdot 10^{-7}$	$1.2(1) \cdot 10^{-7}$	-66.5	33.5
SPC-MIP	$1.4 \cdot 10^{-6}$	$1.3(2) \cdot 10^{-6}$	-7.4	92.6

SPC-bare	$1.4 \cdot 10^{-6}$	$3.9(5) \cdot 10^{-7}$	-72.8	27.2

A recovery higher than 90% was obtained for all examined samples making the MIP-modified electrode promising for trace analysis in real matrixes. Further ongoing studies aim to optimize the polymer formulation and lower the detection limit.

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Conflicts of Interest: The authors declare no conflict of interest.

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