

Evaluation Screen Printed Carbon Electrode Modified With Poly (O-phenylenediamine) for Etodolac drug Detection

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Abstract:

A modified carbon electrode based on molecularly imprinted poly O-phenylenediamine was developed for the determination of etodolac. The modified electrode was prepared by incorporation of the template molecule etodolac during the electropolymerization of O-phenylene diamine on screen printed carbon electrode using cyclic voltammetry. The performance of the molecularly imprinted (MIP) and non-imprinted (NIP) electrodes were assessed by cyclic voltammetry. Differential pulse voltammetry, the influences of pH, monomer and template concentrations, electropolymerization cycles were optimized. For analytical objective, a well resolved voltammetric peak was obtained in 0.2 M phosphate buffer at pH 4.0 for differential pulse voltammetry. Linear responses in the range of 10.0 to 50.0 μM with detection limits of 5.0 μM were obtained for differential pulse voltammetry. The chemical sensor was applied successfully for the determination of etodolac in serum.

Keywords : Etodolac, O-phenylenediamine, electropolymerization, voltammetry.

تقدير الإيتودولاك على سطح قطب الكربون المطبوع على شريحة من البلاستيك المعدل بواسطة البلمرة الكهروكيميائية لمركب أورثو فينيل داي

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المستخلص:

تم تطوير مستشعر كهروكيميائي بسيط وفعال يعتمد على بوليمر مطبوع جزئياً للكشف الانتقائي عن الإيتودولاك من خلال البلمرة الكهروكيميائية "المولنيمر أورثو فينيل داي أمين" o-phenylenediamine في وجود الإيتودولاك (50) ملي مولر، ومحلول مساعد NaClO_4 تركيزه (10) ملي مولر باستخدام دورتين من تقنية الفولتاميتري الدائري (CV) في المدى من (0 وحتى +1.4) فولت؛ لتكوين بوليمر مطبوع فيه جزيئات الإيتودولاك على سطح قطب الكربون المطبوع على شريحة من البلاستيك والذي يعرف بـ screen printed carbon electrode (SPCE)، وبعد ذلك تم استخلاص الإيتودولاك من البوليمر المتكون باستخدام (10) دورات CV من (0 وحتى +0.8) فولت في وجود محلول الفوسفات المنظم ذو الأس الهيدروجيني (4.0)، وعند استخلاص الإيتودولاك من البوليمر المتكون يترك فراغات في البوليمر لها نفس حجم وشكل الإيتودولاك المستخلص تمثل بصمة له فقط، وهو ما يعرف بـ molecularly imprinted polymer (MIP)، يمكن استخدامها في تحديد الإيتودولاك في عينات حقيقية للعلاج، باستخدام تقنية الفولتاميتري ذو النبض التفاضلي (DPV)، حيث تم الحصول على أعلى إشارة أنودية لـ (الإيتودولاك) في محلول فوسفات المنظم ذو الرقم الهيدروجيني (4.0)، كان نطاق الاستجابة الخطية في مدى (10-50) ميكرومولر، وكان حد الاكتشاف منخفضاً يصل إلى (5.0) ميكرومولر. تشير

نتائج إلى أن المستشعر MIP كان مفيداً لتحديد الإيتودولاك مع انتقائية ممتازة وحساسية عالية وقابلية للتكرار، كما أوضحت التجارب على ذلك، أيضاً في بداية البحث تمت دراسة السلوك الكهروكيميائي لمركب الإيتودولاك على SPCE باستخدام محلول الفوسفات المنظم (0.2) مولر عند قيم مختلفة من الأس الهيدروجيني تتراوح من (3.0 وحتى 10.0).

الكلمات المفتاحية: الإيتودولاك - أورثو فينيل داي أمين - الكهروكيميائية - الفولتاميتري.

1. Introduction

Etodolac (Fig1) [1,8-diethyl-1,3,4,9-tetra hydroprano (3, 4-b) indole-1-acetic acid] is a member of non-steroidal anti-inflammatory drugs that selectively inhibits the activity of prostaglandin synthetase and also used in postoperative pain and rheumatoid arthritis. It is rapidly metabolized in the liver, followed by renal elimination as the primary route of excretion[1]. Various analytical methods have been reported for the quantitation of etodolac in different matrices (viz. pharmaceutical formulations, plasma and urine). These methods include high performance liquid chromatography (HPLC) coupled with UV detector[2-4], hydrophilic interaction liquid chromatography (HILIC) coupled with tandem mass spectrometry (MS/MS)[5], spectro photometry and spectrofluorimetry, and capillary electrophoresis[6].

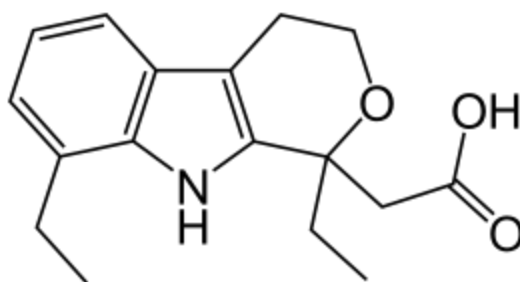


Fig.1: Chemical structure of Etodolac [7].

Most of the previously reported methods are time consuming, require sophisticated instrumentation and extraction of the drug from its formulations and/or biological fluids prior to the analysis which limit their use in quality control laboratories. Voltammetric methods are highly sensitive, convenient and effective tool for the analysis of pharmaceuticals and biomolecules[8-11]. owing to their simplicity, low cost and relatively short time analysis. This paper reports the

electrochemical behaviour of etodolac, and the development of a novel method to determine the etodolac on screen printed carbon electrode[12].

In general, electrochemical biosensors compose mainly of three major parts as the following: (I) the recognition element which is the part responsible for Specific interaction to the analyte and it depends on the nature of the analyte being analyzed, (modified screen printed carbon electrode) (II) Transducer which is the part responsible for the translation of interaction process between the recognition element and the analyte into a measurable electrical signal, and (III) Signal processing using suitable programming process [13].

Screen printed carbon electrode (SPCE) is electrochemical measurement device that are manufactured by printing carbon ink on plastic or ceramic substrates, allowing quick in-situ analysis with high reproducibility, sensitivity and accuracy[14]. The screen printed carbon electrode (SPCE) is a device consisting of three different electrodes[15]. One of the three electrodes is the working electrode, which is typically made of platinum, gold, silver, glassy carbon, nickel, or palladium. The redox process occurs at this electrode. The second electrode is the reference electrode, which provides calibration for the applied potential. Examples of commonly used references are the normal hydrogen electrode, Ag/AgCl electrode, and calomel electrode (e.g. Hg/Hg₂Cl₂). The third electrode is the Auxiliary or counter electrode. It is the electrode that completes the circuit of the three-electrode cell, as it allows the passage of current. It enables the analysis of processes in which electronic transfer takes place[16].

2. Experimental Part

2.1 Chemicals and reagents

Etodolac, potassium dihydrogen phosphate, potassium hydrogen phosphate and were purchased from Sigma-Aldrich and other reagents were commercially available as analytical grade and used without further purification. O-

phenylenediamine (98%, Sigma-Aldrich) . Stock solutions of nicergoline and buffer solutions were prepared by using ultra-pure deionized water.

2.2 Apparatus

All electrochemical experiments, including cyclic voltammetry and differential pulse voltammetry were carried out using a CHI610C Electrochemical Analyzer (CH Instruments, USA), driven by the data processing software (CHI, software version 9.09). three-electrode configuration was composed of a working screen-printed carbon electrode (3.1 mm diameter), printed from a carbon-based ink; a silver–silver chloride pseudo-reference electrode made from a silver-based ink; and the auxiliary electrode from a carbon ink.

All pH measurements were made using a combined glass electrode connected to the digital pH-meter CG 808 (Schott Gerate, Germany).

A micro pipette was used to transfer the analyte solution to the cell throughout the experimental work.

2.3 Electrochemical imprinting of etodolac into O-phenylene diamine on SPCE(Preparation of MIP)

The etodolac imprinted into O-phenylenediamine / screen printed carbon electrode was obtained by Electrochemical polymerization on the surface of SPCE using CV in potential range between 0 and +1.4 V during 2 cycles (scan rate 100 mV s^{-1}) in 0.1 M NaClO_4 supporting electrolyte including 10 mM O-phenylenediamine and 50 mM etodolac.

3. Results and discussion

3.1 Electrochemical behavior of etodolac

The electrochemical behavior of etodolac has been investigated on the surface of screen printed carbon electrode. Fig. 2 shows typical cyclic voltammograms of 50 μM etodolac in phosphate buffer solution pH 4.0 using screen printed carbon

electrode. two anodic peak appeared at a potential sweep rate of 100 mV s^{-1} . In the reverse sweep, no peaks appeared, indicating an irreversible heterogeneous electron transfer process for the oxidation of etodolac. The anodic peak appearing in the voltammogram can be related to the oxidation of hydroxyl group in etodolac. The effect of potential sweep rate was studied using screen printed carbon electrode in the range of 20 to 90 mV s^{-1} . Along with the potential sweep rate increase, the peak currents are proportional to the square root of the scan rate; indicating a mass diffusion-controlled process; and the peak potential shifted to more positive values, confirming the irreversible nature of the reaction processes[17].

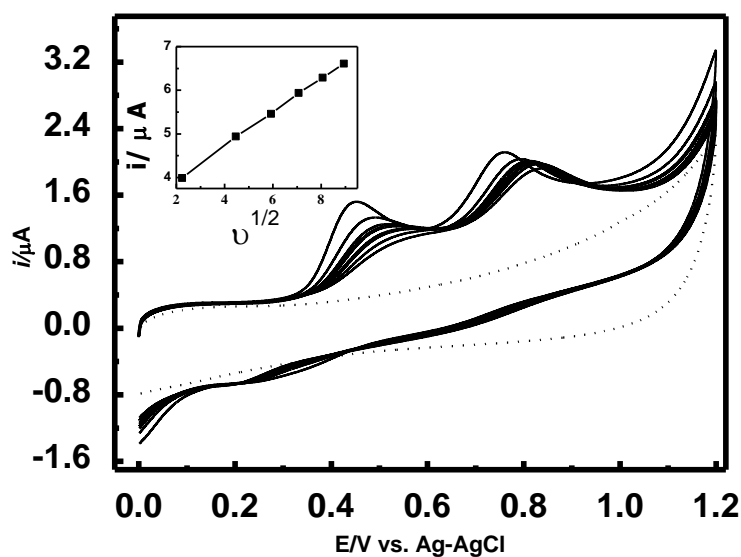


Fig. 2: Cyclic voltammograms obtained for 50 μM etodolac at screen printed carbon electrode in 0.2 M phosphate buffer solution pH 4.0 at Scan rate = 20 (a), 30 (b), 40(c), 50(d), 60(e), 70(f), 80(g) and 90(h) mVs^{-1} .

Differential pulse voltammetry was also used to investigate the effect of pH on the electrochemical oxidation of 50 μM etodolac in aqueous supporting electrolytes over a pH range from 3.0 to 10.0. By preparing a series of a series of phosphate buffer solutions (0.2 M, pH 3.0-10.0) (orthophosphoric acid 85%, potassium dihydrogen phosphate KH_2PO_4 , disodium hydrogen phosphate

Na_2HPO_4 , and sodium phosphate Na_3PO_4), mixed with different amounts and diluted to 200 ml with distilled water to obtain the required pH. (Fig. 3) as already found by cyclic voltammetry. The peak potential of anodic peak of etodolac is shifted linearly towards more negative values and peak current also increased up to pH = 4.0 and afterwards decreased with increasing pH values. but peak potential of pH 4.0 showed two peaks separated.

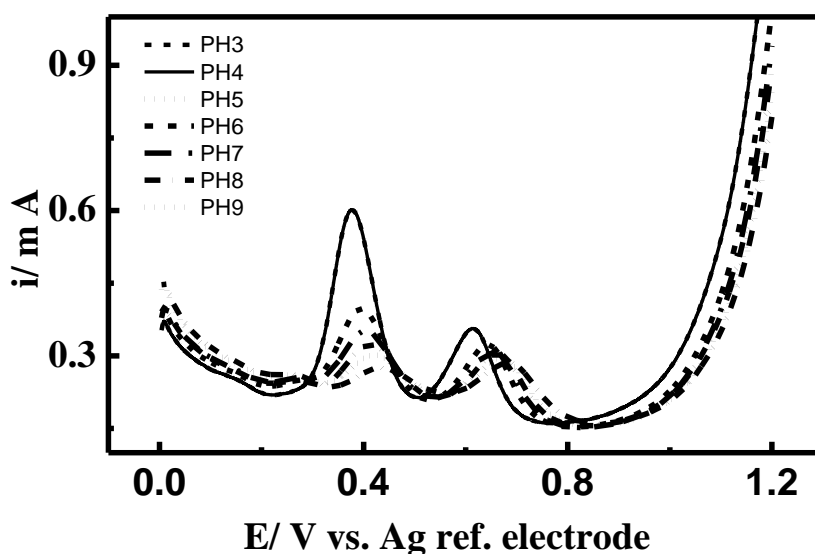


Fig. 3: Effect of pH on determination of 50 μM using Differential pulse voltammetry at the screen printed carbon electrode in 0.2 M phosphate buffer solution.

3.2 Electrochemical polymerization of O-phenylenediamine :

Figure (4b) demonstrates cyclic voltammetry of electropolymerization of O-phenylene diamine in the presence of etodolac. The etodolac imprinted into O-phenylenediamine/SPCE was obtained by Electro chemical polymerization on the surface of SPCE using CV in potential range between 0 and +1.4 V during 2 cycles (scan rate 100 mV s^{-1}) in 0.1 M NaClO_4 supporting electrolyte including 10 mM O-phenylene diamine and 50 mM etodolac. The oxidation peak of etodolac (template) can be seen easily in this figure. This oxidation peak indicates that the template is becoming part of the polymeric chain. etodolac molecules diffuse towards the surface of the screen printed carbon electrode during the

electropolymerization process and trapped into the polymer matrix. The creation of the molecular imprints is favored by the diffusion of the electroactive template, generating a far higher number of recognition sites during the electrodeposition of the polymer. The etodolac template molecules are trapped in the polymer matrix as a result of the ability of these molecules to interact with the O-phenylenediamine units. Hydrogen bonding could occur between the hydrogen in the hydroxyl group of etodolac structure and the nitrogen atom of the N–H group of O-phenylenediamine units. This imprinting process creates a micro environment for the recognition of etodolac molecule based on shape selection and positioning of the functional group.

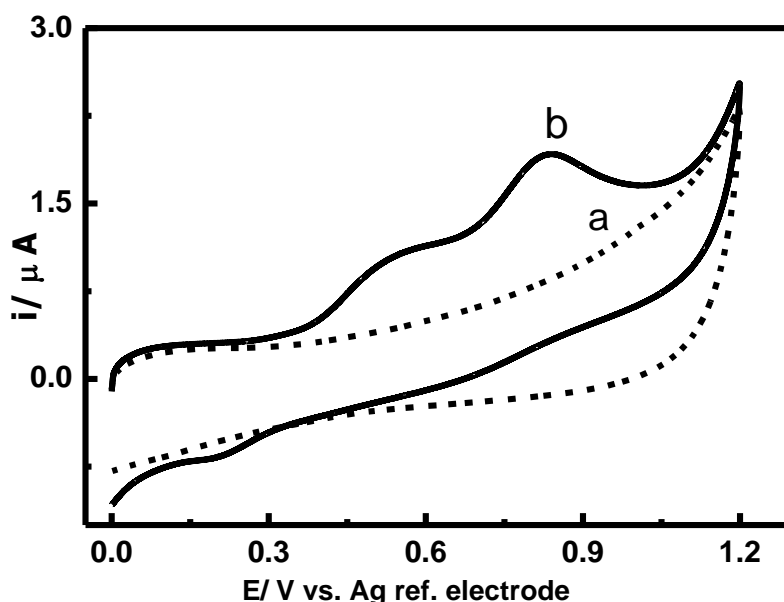


Fig. 4: Cyclic voltammograms taken during the electropolymerization of O-phenylenediamine (0.01M). (a) without and (b) with etodolac (100 μM) onto a screen printed carbon electrode (scan rate: 100 mV/s ; supporting electrolyte: 0.1 M NaClO₄).

3.3 Optimization of electrochemical measurement conditions

3.3.1 Effect of pH

The pH of the medium has important influence on the polymeric film, To research the influence of pH on the MIP sensor, To research the influence of pH on the MIP sensor, differential pulse voltammetry tests were carried out in PBS at different pH values. By preparing a series of a series of phosphate buffer solutions (0.2 M, pH 3.0-10.0) (orthophosphoric acid 85%, potassium dihydrogen phosphate KH_2PO_4 , disodium hydrogen phosphate Na_2HPO_4 , and sodium phosphate Na_3PO_4), mixed with different amounts and diluted to 200 ml with distilled water to obtain the required pH. Because the etodolac molecule has been proved unstable in alkalinity solution [18], According to the results, etodolac molecules which specifically adsorbed to the imprinted binding sites show different electrochemical oxidation behavior from the non-specific etodolac molecules bonded to the polymeric film in different pHs, When pH was greater than 5, the peak current decreased with increasing solution pH. On the contrary, the peak current responses of the MIP electrode increased gradually when the pH was less than 5. Therefore, the test solution with a pH of 4.0 was chosen for all further experiments.

3.3.2 Effect of Incubation Time

The adsorption kinetics of etodolac was investigated by varying the adsorption time from 1 min to 5 min, and the initial concentration of etodolac kept constant at 50 μM . The peak current increased rapidly with the incubation time and then leveled off after 2 min, presumably resulting from reaching the absorption balance between the sample solution and surface of MIPs- screen printed carbon electrode. The result reveals rapid response equilibrium of etodolac molecules to poly(O-phenylenediamine) -screen printed carbon electrode, which might be due to the surface binding sites of screen printed carbon electrode-MIP through π - π stacking between aromatic rings and hydrogen bonds between and nitrogen and oxygen-containing groups of the O-phenylene diamine units and

etodolac. Thus, the absorption time of 2 min was selected as an optimum for further experiments.

3.4 Analytical performance

The differential pulse voltammetry responses of the MIP sensors modified electrode after incubation in the etodolac solution were increased linearly with the concentration in the range of 10 - 50 μM of etodolac. A linear calibration graph has been constructed by plotting the corresponding absolute value of voltammetric peak current versus etodolac concentration[19]. The linear regression equation was expressed as $I (\mu\text{A}) = 0.053[\text{etodolac}] + 0.002$. Detection limit at MIPs based electrochemical sensor was evaluated according to the $3S_b/m$ criteria, where S_b is the standard deviation of the blank and m is the slope of the linear calibration curve 4. The LOD was found to be 5 μM .

3.5 Sample Analysis

To application of the proposed sensor for the determination of etodolac in the real samples conditions such as urine and serum was studied . For the preparation of human serum sample, 5.0 mL of blood sample was kept at 37 °C for 5 min, and then the sample was centrifuged (5 min with 3000 rpm) after the addition of 2.0 mL methanol. The obtained deproteinized human serum was diluted to 20.0 mL by doubly distilled water. The samples were measured by differential pulse voltammetry as shown in Fig 5. the results were listed in Table 1 with the recoveries between 91.55 % - 96.85 % . These results indicated that the screen printed carbon electrode- MIP sensor could be successfully applied for the determination of etodolac in the real samples[20].

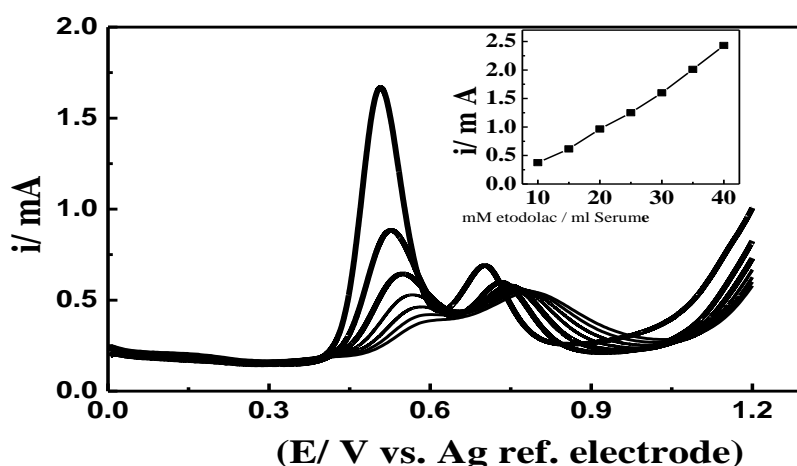


Fig. 5: Differential pulse voltammetry for the determination of etodolac μg in 1ml serum samples spiked with 30.0 -35.0 -40.0 -45.0 - 50.0 and 35.0 μM etodolac per 1mL serum with 0.2 M phosphate buffer solution of pH 4.0 at MIP; inset: calibration curve of etodolac in serum at MIP.

Table 1. Recovery tests of etodolac in serum samples.

Added (μM)	Found(μM)	Recovery(%)
30.00	28.95	96.50
35.00	32.12	91.77
40.00	38.74	96.85
45.00	41.20	91.55
50.00	47.63	95.26

3.5 Conclusion

In conclusion, an electrochemical sensor has been successfully fabricated by the cyclic voltammetric deposition of poly-O-phenylene diamine film on disposable screen printed carbon electrode in the presence of target etodolac. The sensor combines the selectivity shown by molecularly imprinted polymer, with the sensitivity and the real time detection offered by the use of the electrochemical transduction. Molecularly imprinted poly-O-phenylene diamine modified screen printed carbon electrode showed a stable and reproducible response without any influence of interferents commonly existing in real samples. The proposed low cost chemical sensor could find application in the measurement of etodolac level in real samples.

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