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Preparation and Characterization of Nanofilm-Coated Modified Electrodes and Their Use in Valsartan Drug Analysis

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The use of nanomaterials is a modern trend in electrochemical analysis because of the ease and accuracy of the analysis that, in addition, being non-destructive to the samples, gives the possibility of repeating the analysis to obtain better results. In this research, films of a co-conductive polymer of pyrrole and one of its derivatives are fabricated by docking on a graphite substrate. The film and the substrate form a modified electrode described by EIS and CV in the presence and absence of valsartan. Using a modified electrode, the concentration of valsartan is determined in titres and blood samples of patients with a standard deviation (SD = 0.6). The quantitative and detection limit are LOQ = 6 μM and LOD = 1.8 μM, respectively. DPV with standard addition method has standard deviation (SD = 0.34). The new method succeeds in being more accurate with LOQ = 3.4 μM and LOD = 1.1 μM, respectively. *F*-test proves that the HPLC method is not better than DPV and DPV with modified standard addition methods in determining the drug concentration of valsartan. The modified standard addition method is probably the best.

Використання наноматеріялів є сучасною тенденцією в електрохемічній аналізі через легкість і точність аналізи; крім того, вони не руйнують зразки, що дає можливість повторної аналізи для одержання ліпших результатів. У цьому дослідженні плівки провідного полімеру піролу й одного з його похідних були виготовлені шляхом стикування на графітовій підкладинці. Плівка та підкладинка утворили модифіковану електроду, описану електрохемічною імпедансною спектроскопією та циклічною вольтамперометрією, у присутності та за відсутности валсартану. За допомогою модифікованої електроди визначено концентрацію валсартану в титрах і зразках крові пацієнтів зі стандартним відхилом (SD = 0,6). Кількісна межа та межа виявлення становлять LOQ = 6 μM і

LOD = 1,8 μM відповідно. Диференційна імпульсна вольтамперометрія з методом стандартного додавання має стандартний відхил (SD = 0,34). Новий метод став більш точним із LOQ = 3,4 μM і LOD = 1,1 μM відповідно. *F*-тест довів, що метод високоефективної рідинної хроматографії не є кращим, аніж диференційна імпульсна вольтамперометрія та диференційна імпульсна вольтамперометрія з модифікованими стандартними методами додавання у визначенні концентрації препарату валсартану. Модифікований стандартний метод додавання, ймовірно, є найліпшим.

Key words: polymer thin films, anchoration, modified electrode, differential pulse voltammetry, valsartan.

Ключові слова: полімерні тонкі плівки, анкерування, модифікована електрода, диференційна імпульсна вольтамперометрія, валсартан.

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1. INTRODUCTION

Electrochemical analysis is a modern, accurate, non-destructive sampling technique [1], which can be used on water-soluble solid or liquid samples [2]. Electrochemical analysis has many characteristics and advantages [3]. All methods work through the contact of the sample solution with a working electrode, and the circuit is completed with an auxiliary electrode and the potential is measured with respect to a comparator electrode within the triple cell [4].

Electrochemical analysis involves measuring current (ampere), potential (volt), or amount of electricity (charge) (coulombs) against time, potential, or current [3]. During the analysis, precise electrical devices measure the very slight changes in the analytical signal values against the changes of the corresponding factor in the experiment. The analysis depends mostly on electrical conductivity and oxidation–reduction reactions within the cell, in which the measurement is made [5]. Working electrodes are the base for cell used in an analytical or chemical application [6].

Electrodes are often composed of a surface of a chemically inert material that is a good conductor of electric current; this material forms the main body of the electrode in electrical operations and transfers the electric current to the circuit outside the solution [5]. Many layers can be added to the surface of the electrode for work [6]. Recently, there is a tendency to use nanofilms on electrodes in many applications, such as electrolysis, electrolysis, and photoelectrolysis [7]. The thickness of the film, the size of its particles, the type of its constituent material, and the electrode material affect the application of the film and the method of its manufacture on

the surface of the electrode [8]. Nanofilms are made chemically by anchoring and electrodeposition and physically by dipcoting and spincoting. Dipcoting and spincoting were preferred for fabrication of films, which are used for analytical applications, due to the ease of fabrication of very thin, well-covered, homogeneous films [9].

Films in analytical applications are very thin and easily damaged; so, we need to renew constantly them to reuse them again [10]. Conductive polymers can form thin films by an anchoring method. Concentration, temperature and time all affect the film thickness and particle size [11]. Electrodeposition is used in the preparation of many thin films of metal oxides and conductive polymers. The potential and current of deposition and the film material often affect the thickness of the film, the size of its particles and its stability. Many researchers made thin films by electrodeposition and studied their analytical properties [12].

The drug valsartan is widely used in patients with chronically elevated arterial pressure at different doses that suit the patient's condition alone or in synergy with one or more other drugs. Many researchers have developed analysis methods that meet the need, but need more development [13]. In this research, we present a method for analysing the valsartan in both pure samples and capsules and in blood samples, based on measurements of the cyclic potential of the aqueous solution on a working electrode, whose surface is covered with a film of poly(pyrrole-formylpyrrol) by anchoring.

2. EXPERIMENTAL

2.1. Materials

Pyrrole-2-carboxaldehyde 98% sigma, pyrrole > 98% sigma trichloroacetic acid sigma, and graphite electrodes sigma.

2.2. Measurements

Thin films were characterized by electrochemical impedance spectroscopy (EIS) applied using in $KClO_4$ (1 M) solution, 1 mA/cm^2 and 0.58 V in the range of 0.1 Hz–10 kHz, cycle voltammetry (CV) method at a scan rate of 100 $mV \cdot s^{-1}$ within the potential range of –0.2–1 V (AMEL model 2550). Thin film morphologies were examined with AFM (Nanosurf model eseyscan2) and SEM (TESCAN model MIRA3).

2.3. Analysis Protocol

The differential pulse voltammetry (DPV) was in a potential range

of 0.01 to 0.6 V at scan rate of 5 $mV \cdot s^{-1}$ and the DPV input data are as follow: step potential of 0.004 V, modulation amplitude of 0.08 V, modulation time of 0.2 s, and interval time 0.5 s. The DPV oxidation peaks of valsartan were at 227 ± 5 mV against the Ag/AgCl electrode. HPLC [13] used mobile phase acetonitrile and water in the ratio 55:45 (v/v), and the pH adjusted to 3.6 with 88% orthophosphoric acid with a flow rate of 1.0 $ml \cdot min^{-1}$ and C18 column (250×4.6 mm id, 5 μm particle size).

2.4. Thin Films Fabrication

The film was fabricated by anchoration in the reaction solution (pyrrole and 2-formyl pyrrole (10 mmol)) was dissolved in the 25 ml of ethanol, and 3 g of trichloroacetic acid was added [14]. The electrodes were immersed in the solution of reaction, and left in the solution for 30 min and removed from the solution. The films (on electrodes) were washed with deionized water and alcohol (do not touch the film surface).

2.5. Film Deposition

Graphite electrodes were sanded to great fineness. Electrode was isolated with electrical insulator coating except for a precisely defined surface (1 cm^2). The films were fabricated by docking: the electrode was immersed completely in the reaction solution to prepare a polymer.

2.6. Sample Analysis

The valsartan samples were analysed in two methods for comparison; the first method is by HPLC as the reference method, and the second method is by DPV. Standard series were prepared from standard analysis using the same method. Samples from three sources of valsartan were obtained for the purpose of the study.

2.6.1. Capsule Sample

The capsule was obtained from the pharmacy outlets in the city of Baghdad for a drug produced by a national company. According to the attached leaflet, it contains a substance containing (160 mg) an active substance loaded with excipients and film-coated. Ten capsules were taken and finely ground. Transfer to a 1000 ml volumetric flask and add water to the mark. A solution of theoretical con-

centration of 100 mg/L was prepared from the mother liquor.

2.6.2. Pure Substance Sample

The active substance of the valsartan drug was taken from the manufacturing laboratory; the data sheet for the substance was reviewed, and the data of the substance was verified before working on it. From the material, weigh accurately, transfer to a beach, add the solution to it, stir until dissolution, transfer to a volumetric flask, and complete the volume to the mark.

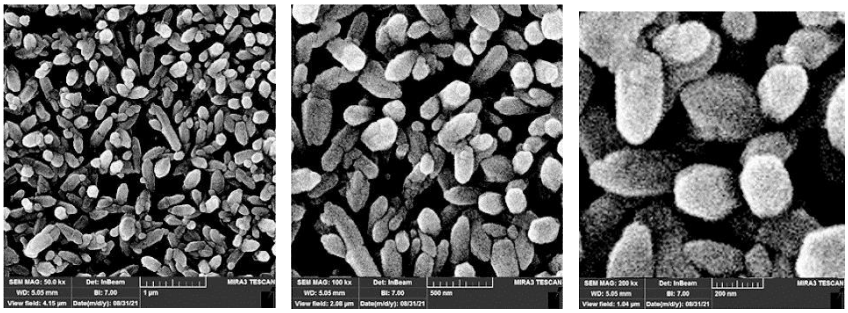
2.6.3. Patient Blood Sample

A blood sample (5 ml) was taken from a patient's left hand vein after the patient's consent by a medical specialist with sterile instruments after 2, 4 and 6 hours of taking the drug. Samples were subjected to primary treatment to obtain blood serum and sedimentation of soluble proteins. The treated serum sample was transferred to a 25 mL calibrated flask and filled with distilled water to the mark.

3. RESULTS AND DISCUSSION

3.1. SEM of Polymer

Electron microscopy images give information about the shape and size of nanoparticles. Figure 1 shows pictures of the electronic microscope of the polymer. Polymer particles are bacillus in close sizes and the size of the particles is of around 70 nm. The particles on a rough surface are clogged to be coral.



a *b* *c*

Fig. 1. Scanning electron microscopy images of polymer.

3.2. AFM of Polymer Thin Films

Figure 2 shows the atomic force microscopy images for two areas of the film.

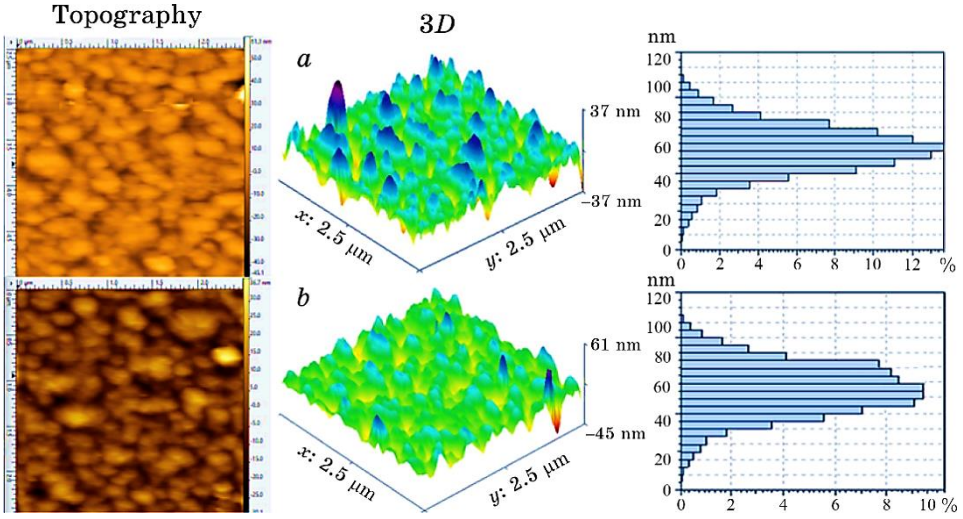


Fig. 2. The atomic force microscopy images for two areas of the film.

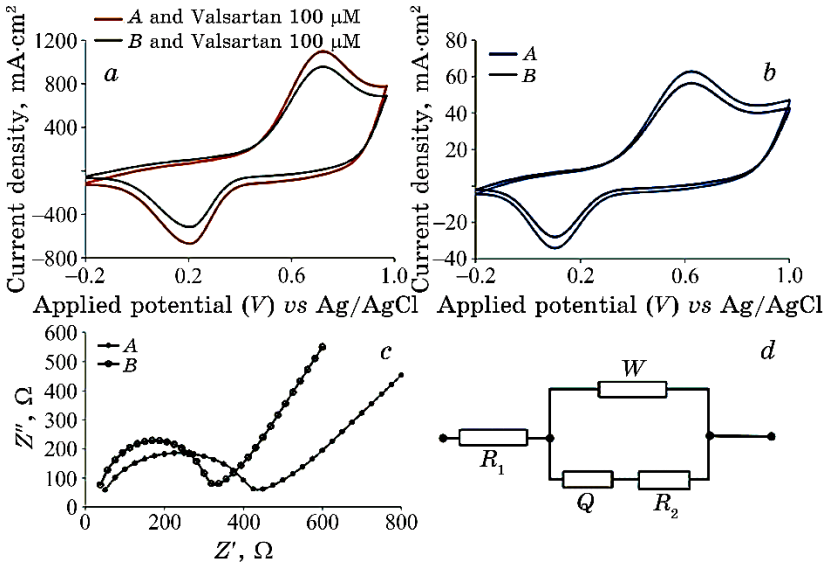


Fig. 3. (*a*) Cyclic voltammetry of the electrodes with valsartan 100 μM; (*b*) cyclic voltammetry of the electrodes without valsartan; (*c*) Nyquist plot for films; (*d*) equivalent electrical circuits for the films.

From the comparison of Fig. 2, *a* and *b*, it appears that there is a good homogeneity in the structure of the films forming the surface of the electrode, the peaks are close in height up to 80 nm, the surface is rough, and the particle size is of about 60 nm in both images. This approximates the particle size in electron microscopy images, and the particle shape is similar too.

3.3. CV and EIS of Polymer Thin Films

The cyclic voltammetry of the electrodes (Fig. 3, *b*) shows two clear return oxidation peaks on two electrodes, the peak around 0.582 V, when using the electrodes (Fig. 3, *b*) valsartan solution. The oxidation peak differs, which becomes at 0.679 V.

Figure 3, *c* shows the electrochemical impedance spectrum for electrodes. The curves consist of two parts. Semi-circular at high frequencies is the first part, and the other one is linear at low frequencies. In order to analyse the EIS results, we fitted the impedance data to equivalent electrical circuits (Fig. 3, *d*), which consist of resistance (solution resistance R_1) of 46 ± 5 Ohm. Warburg Impedance (noted *W*) or capacitance is, therefore, ascribed to the diffusive capacitance (*a*: 54 ± 7 and *B*: 43 ± 5 $(\text{m}\cdot\text{Ohm})^{-2}$). Warburg Impedance is analogous to resistance (R_2) and capacitance (*Q*). Resistance (R_2) is (*a*: 154 ± 18 and *b*: 185 ± 42 Ohm), while *Q* is the double-layer capacitance (*a*: 0.23 ± 0.02 and *b*: 0.183 ± 0.02 $\mu\text{F/cm}^2$).

3.4. Valsartan Analysis by HPLC

A standard method was used to determine the valsartan using HPLC according to Ref. [13], where a titre series (100–600 μM) was prepared and analysed according to the conditions of the aforementioned method. Figure 4 shows: the peak area *versus* valsartan concentration, ($y = 898870x + 1 \cdot 10^6$)-regression equation with noise rate (0.013) and LOD = 0.039 μM and LOQ = 0.132 μM.

3.5. Valsartan Analysis by DPV

Figure 5 shows the current measurement curves in DPV and the linear regression curve of current density *versus* concentration. In Figure 5, *a*, the current density increases exponentially with increasing valsartan concentration. The linear regression equation is ($y = 0.0368x + 6.99$) with a correlation coefficient ($R_2 = 0.998$), with a standard deviation (SD = 0.6). The limit of detection (LOD) and limit of quantification (LOQ) were calculated by standard deviation (signal to SD ratio of 3:1) and quantified (signal to SD ratio of

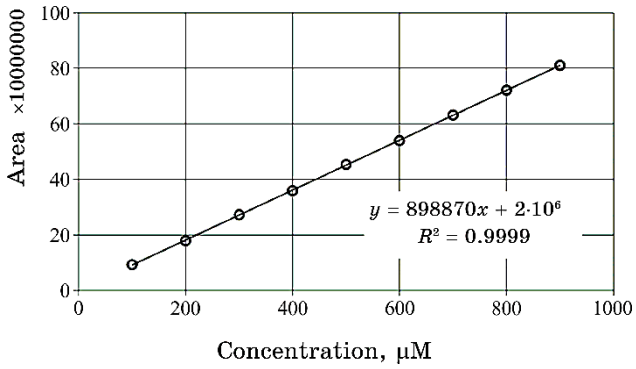


Fig. 4. The peak area *versus* valsartan concentration.

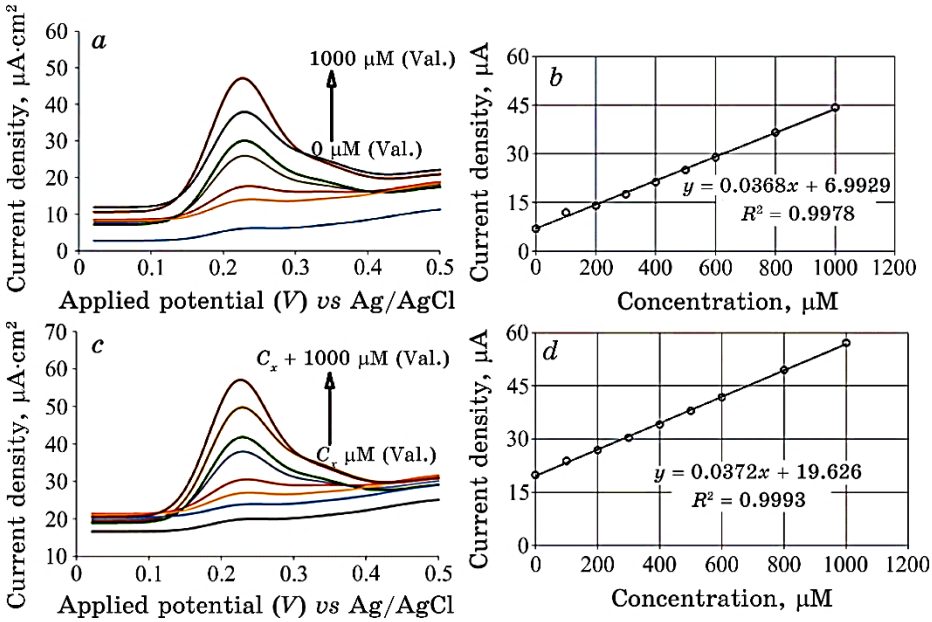


Fig. 5. (*a*) current measurement curves in DPV; (*b*) current density *vs* valsartan concentration; (*c*) current measurement curves in DPV by the modified standard addition method; (*d*) current density *vs* valsartan concentration.

10:1), respectively: LOD = 1.8 μM and LOQ = 6.0 μM.

Tables 1 and 2 show the results of valsartan analysis. Retrospective titre samples reached (58.1 μM) blood samples gave results with good reproducibility.

Figure 5 shows the current measurement curves in DPV by the modified standard addition method, where a concentrated titre solu-

TABLE 1. The results of the analysis of samples of valsartan.

Level, %	*n*	Concentration, μM	Found concentration, μM	Recovery	RSD
50	5	150	147.9	99.2	0.584
100	5	300	298.5	99.5	0.486
150	5	450	449.1	99.8	0.569

TABLE 2. Shows the results of valsartan analysis by DPV and DPV-S.A.M.

Method	Level, %	*n*	Concentration, μM	Found concentration, μM	Recovery	RSD
PDV	50	5	175	177.6	101.5	0.459
	100	5	350	349.3	99.8	0.658
	150	5	525	519.2	98.9	0.662
PDV-S.A.M.	50	5	175	173.8	99.3	0.349
	100	5	350	344.8	98.5	0.417
	150	5	525	528.2	100.6	0.394

tion of valsartan (525 μM) was used as an unknown solution, and an amount of a high-concentration titre solution was added to it according to the titre series. The concentration was calculated from the regression equation. The linear regression equation is ($y = 0.0372x + 19.63$) with a correlation coefficient ($R^2 = 0.998$), with a standard deviation (SD = 0.34). The limit of detection (LOD) and limit of quantification (LOQ) were calculated by standard deviation (signal to SD ratio of 3:1) and quantified (signal to SD ratio of 10:1), respectively: LOD = 1.0 μM and LOQ = 3.4 μM.

Table 2 shows the results of valsartan analysis retrieved from titre samples (58.2 μM) in blood samples; the concentration of valsartan was determined by the modified standard addition method, and we got good results with a good reproducibility.

The concentration of valsartan was determined by three methods: HPLC, DPV, and DPV with modified standard addition. The methods were compared with a paired *F*-test to determine the most accurate method. The *F*-value for (0.05, 9, 9) is of 3.117. Table 3 shows F_{exp} for comparison of methods. From this table, HPLC method is not batter than DPV and DPV with modified standard addition.

4. CONCLUSIONS

Conductive polymer thin film was fabricated on a graphite substrate and characterized by SEM and AFM. The film and substrate formed

TABLE 3. F_{exp} shown for comparison of methods.

Method *vs* Method	n	F_{exp}
DPV *vs* HPLC	9	2.250
DPV-S.A. *vs* HPLC	9	0.723
DPV *vs* DPV-S.A.	9	3.114

a modified electrode. The modified electrode was described by CV and EIS in the presence and absence of valsartan. Using a modified electrode, the concentration of valsartan was determined in titres and blood samples of patients, with a standard deviation (SD = 0.6). The quantitative and detection limit are LOQ = 6 μM and LOD = 1.8 μM, respectively; DPV with standard addition method has been modified to suit the use of limited volume samples such as human blood samples and non-destructive measurement methods. The new method has standard deviation (SD = 0.34). The new method succeeded in being more accurate with LOQ = 3.4 μM and LOD = 1.1 μM respectively. Fisher's test proved that the HPLC method is not superior to the electrochemical methods in determining the drug concentration of valsartan; by comparing the two methods, it was found that the modified standard addition method is more accurate, especially with its ability to exclude the effect of the matrix.

5. HIGHLIGHTS

A nanofilm of a carrier polymer was fabricated and characterized by SEM and AFM.

Preparation of a conducting electrode, characterization by electrical impedance spectrum, and study by CV are used to determine the possibility of using it for the analysis of valsartan.

It is introduced a modified standard addition method for limited-source samples and non-destructive analytical methods.

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