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Sensitive Determination of the Indole Alkaloid Reserpine Using a Glassy Carbon Based Electrochemical Sensor

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In the present paper a glassy carbon electrode was successfully applied for the sensitive and selective determination of the indole alkaloid reserpine by differential pulse voltammetry (DPV). The electrochemical behavior of reserpine was investigated using cyclic voltammetry and best conditions for its quantification were examined by differential pulse voltammetry. Cyclic voltammetry has shown that the oxidation process is irreversible over the pH range 1 - 12 and that the electrode process is mainly diffusion controlled. The proposed procedure has wide linear concentration range for determination of reserpine by DPV from 2 µM to 75 µM with a detection limit 0.6 µM. The sensor in combination with optimized electrochemical parameters was successfully applied for the determination of reserpine in model human urine with good recovery.

Keywords: Reserpine, glassy carbon electrode, differential pulse voltammetry, human urine sample

1. INTRODUCTION

Methyl(3β,16β,17α,18β,20α)-11,17-dimethoxy-18-[(3,4,5-trimethoxybenzoyl)oxy]yohimban-16-carboxylate, also known as reserpine, is an indole alkaloid and one of the most important Rauwolfia alkaloids, a well-known sedative, anti-hypertensive and sympatholytic agent. It has been isolated from the roots of *Rauwolfia serpentina*. The administration of reserpine to rodents has been suggested as a pharmacological model of Parkinson's disease (PD) based on its effects on depleting monoamines with consequences on the motoric activity [1]. Reserpine interferes with the storage of monoamines in intracellular vesicles, causing monoamine depletion in nerve terminals and transient hypolocomotion and muscular rigidity, depending on the dose [1, 2]. Reserpine is an irreversible inhibitor of the vesicular monoamine transporter 2 (VMAT-2). The blockage of dopamine vesicular uptake results in

the accumulation of neurotoxic dopamine oxidation byproducts [3]. Dopamine (DA) reacts with molecular oxygen to form dopamine-quinones which can deplete the antioxidant glutathione, generating reactive oxygen species (ROS) during this process [4]. In addition, the enzymatic metabolic breakdown of dopamine (via monoamine oxidase) increases the formation of ROS [5]. When the production of ROS exceeds the ability of the antioxidant system to eliminate them, oxidative damage occurs [6]. Reserpine also causes depletion of brain catecholamines leading to an akinetic state in experimental animals [7]. It has been observed that L-DOPA administration also alleviated the reserpine induced akinetic state, indicating that behavioral recovery is dopamine dependent [7]. The model of reserpine-induced orofacial dyskinesia shows an important aspect of face validity with PD [8]. It has been reported that repeated treatment with low doses of reserpine progressively induces alterations in motoric functions and provoke an increase in the striatal oxidative stress, indicating its application in the study of the neuroprogressive nature of the motor signs in PD [9]. The typical initial dose is 0.5 mg daily for 1 or 2 weeks whereas the maintenance dose in adults is 0.05 to 0.25 mg once daily. Therefore, the determination of reserpine is of great importance and interest in current analytical chemistry. So far, the literature describes several methods for determining reserpine like electrochemical detection with functionalized single-walled carbon nanotube with polyaniline [10], adsorption stripping voltammetry with multi-walled carbon nanotube [11], flow injection spectrofluorometric determination [12], spectrofluorometric [13], fluorimetric determination with photochemical derivatization [14], photochemical spectrofluorimetry [15], liquid chromatographic-tandem mass spectrometry [16], but most of them require several time-consuming manipulation steps, sophisticated instruments and special training. Differential pulse voltammetry as one of the most sensitive electrochemical techniques can offer a low detection limit and selective and simple determination of reserpine without any pre-treatment of samples.

In this paper we demonstrate the application of the glassy carbon electrode as carbonaceous electrochemical sensor for a sensitive determination of reserpine by differential pulse voltammetry. The voltammetric behavior of reserpine and the optimization of experimental conditions are also presented. The practical usefulness of method is do the determination of reserpine in model human urine samples with good recoveries. This newly proposed procedure has following advantages: high sensitivity, low cost, rapid response and simplicity.

2. EXPERIMENTAL

2.1. Chemicals and reagents

Highly pure water (18.3 MΩ cm) prepared with a cartridge system (Milli-Q) was used throughout. Reserpine, ascorbic acid, uric acid, dopamine, boric acid, sodium hydroxide, acetic acid and phosphoric acid were purchased from Sigma Aldrich and used as received without any further purification. Stock solutions (10^{-3}M) of the analyte and interferences were prepared with water. Solutions with lower concentration were made from the stock solution by appropriate dilution with

supporting electrolyte. Britton-Robinson buffer solutions were used as supporting electrolyte. The pH value of the Britton-Robinson buffer was adjusted with sodium hydroxide (0.2 M).

Cyclic voltammetric measurements and differential pulse voltammetric measurements were performed with a hand-held potentiostat (Palm Sense, the Netherlands). The cell (10mL) contained three electrodes, a glassy carbon working electrode, an Ag/AgCl (saturated KCl) reference electrode and a Pt counter electrode. All potentials reported in this paper are given versus this Ag/AgCl reference electrode at an ambient temperature. All pH values were measured with a pH meter (model 1230, Orion). Prior to each experiment the working electrode was polished with alumina (0.05 μm particle size) followed by rinsing with water and sonication.

The potential was swept over the range from 0 to +1.2V at different scan rates for CV, and from 0 to +1.2V vs. (Ag/AgCl) with optimized parameters (scan rate 25 mV/s, pulse amplitude 50 mV, step potential 5mV and pulse width 40 ms) for DPV.

2.2. Sample preparation

Urine samples were collected from three different persons and spiked with proper aliquots of the stock solution of the alkaloid.0.1 mL of the sample were diluted to 10 mL with Britton-Robinson buffer (pH 5) and then analyzed directly.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of reserpine

The influence of pH on the peak potential and on the peak current of reserpine is shown in Figure 1. Both are strongly dependent on this parameter. The peak potential of reserpine shifted linearly with increasing pH to less positive potential. From these results and the slope of the peak potential dependence on pH it can be concluded that protons are participating in the oxidation reaction. There is no constant slope over the whole investigated pH-range; the reason for this could be that due to the varying presence of protonated species at different pH values the number of protons involved changes. The US pharmacopoeial convention reports a pKa value of 6.6 [17]. This is congruent with pH dependence found in this work where the slopes changes significantly at around pH 7. At pH <4 there seems to be an influence of the second protonated nitrogen, which seems to be rather acidic. The proposed overall mechanism of the electrode oxidation of reserpine is given in Scheme 1. yielding dehydroreserpine [12]. The height of peak reaches a maximum at pH 5 and then starts to decrease. Therefore, pH5 was chosen as the optimum pH value of the Britton-Robinson buffer for the quantitative determination of reserpine. Subsequently, the effect of the scan rate ($v^{1/2}$) on the peak current at pH 5 was examined. As the scan rate was increased from 10 to 100 mV/s at a fixed concentration (0.1mM) of reserpine, the peak current increased steadily and the peak potential was at the same value without any significant shift. A linear Randles-Sevcik plot was obtained (Figure 2) with a regression equation I_p (μA)=0.6621$v^{1/2}$(mV/s) - 1.0963, r^2=0.9923, indicating a somehow diffusion-

controlled nature of the electrode process, but the intercept of the graph with the horizontal axis indicates that there are also other effects superimposed.

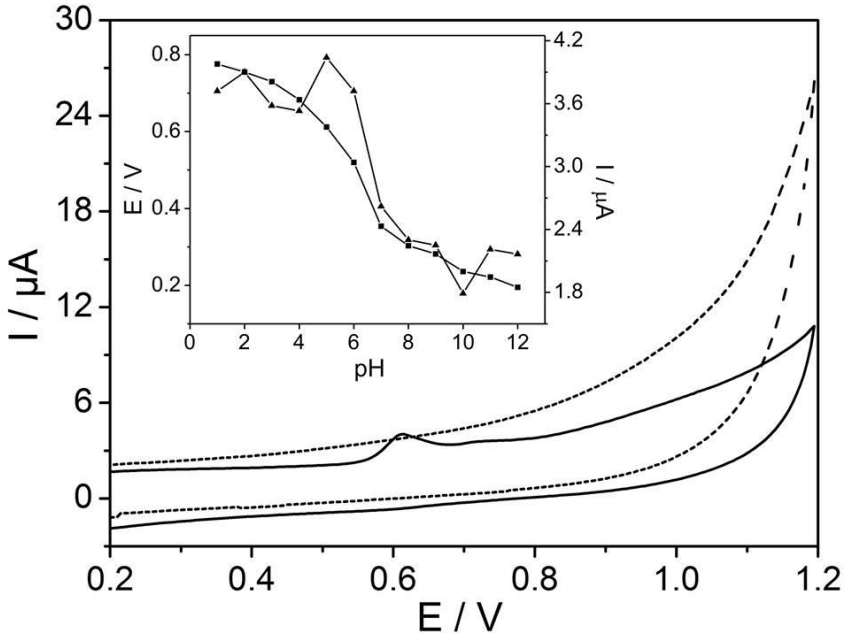


Figure 1. Cyclic voltammogram of 0.1mM of reserpine in BR buffer pH5 on a glassy carbon electrode; the insert shows the dependence of the peak potential (■) and the peak current (▲) on the pH of the supporting electrolyte, BR buffer.

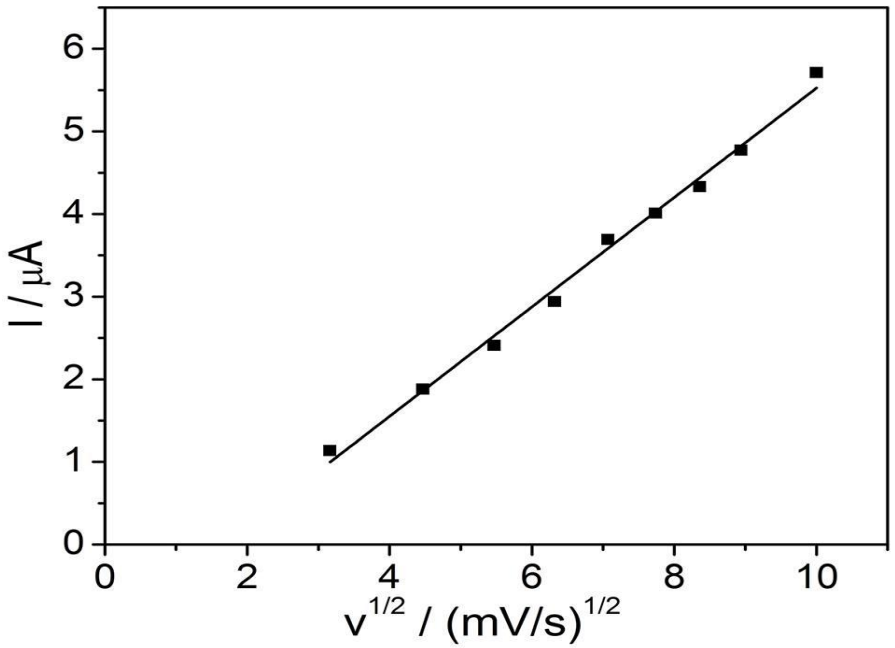
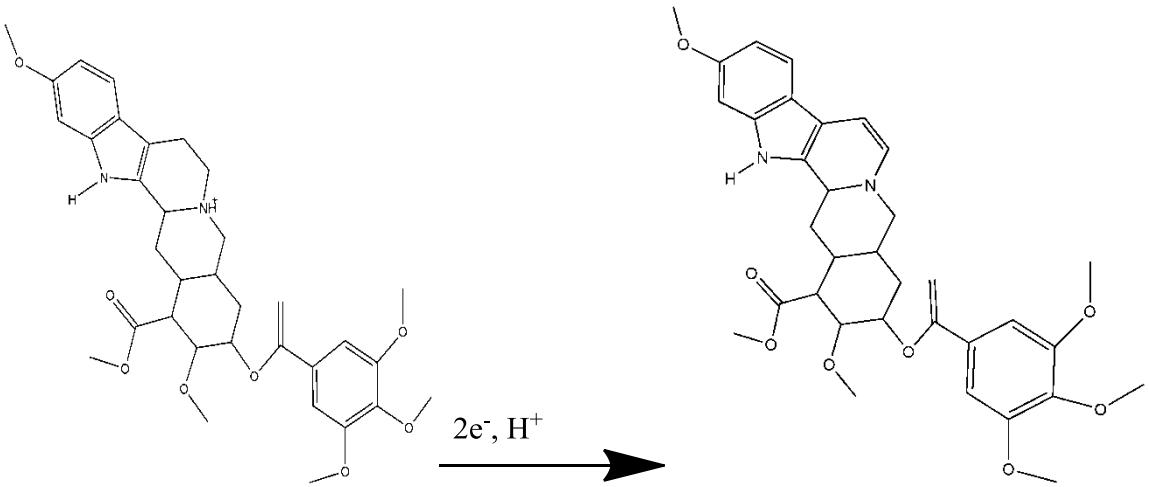


Figure 2. Dependence of the peak current on the square root of the scan rate, from 10 to 100 mV/s for reserpine concentration 0.1 mM, in BR buffer pH 5.



reserpine 3,4- dehydroreserpine

Scheme 1. Electrode oxidation mechanism of reserpine.

3.2. Optimization of DPV parameters

The parameters for differential pulse voltammetric measurements such as pulse amplitude, pulse time and scan rate were optimized. During this procedure the investigated parameter was changed while the others were kept constant. The peak currents increased with the pulse time in the range of 10-40 ms and decreased beyond, so the most suitable peak current was observed at 40 ms. Varying the pulse amplitude from 10 to 75 mV leads to an increase but also to a widening of the current peaks. The best peaks with respect to peak height and width were obtained for pulse amplitude of 50 mV. 25 mV/s was chosen as the most appropriate value of scan rate.

3.3. Interferences

In order to assess the possible analytical applications of the proposed method, the effect of some substances that often accompany reserpine were studied by adding them indifferent amounts to 35 μM of reserpine. Ascorbic acid, dopamine and uric acid were investigated as interferences in a urine matrix. As can be seen from the Figure 3the absolute current practically remains unchanged, and also the standard deviation in current of 5 repetitive measurements increased only slightly from 0.09μA in the absence of interferences to 0.11 in their presence. Thus, it can be concluded that there is no substantial influence of these three interfering compounds on the determination of reserpine.

Some structurally similar substances, such as ajmalicine ((19α)-16,17-didehydro- 19-methyloxayohimban- 16-carboxylic acid methyl ester) and yohimbine (17α-hydroxy-yohimban-16α-carboxylic acid methyl ester) have hardly any influence on reserpine in same concentration as reserpine because the oxidation potential of this compound is on higher potential (Fig. 4). In brief, the proposed sensor with the elaborated method has excellent selectivity for reserpine.

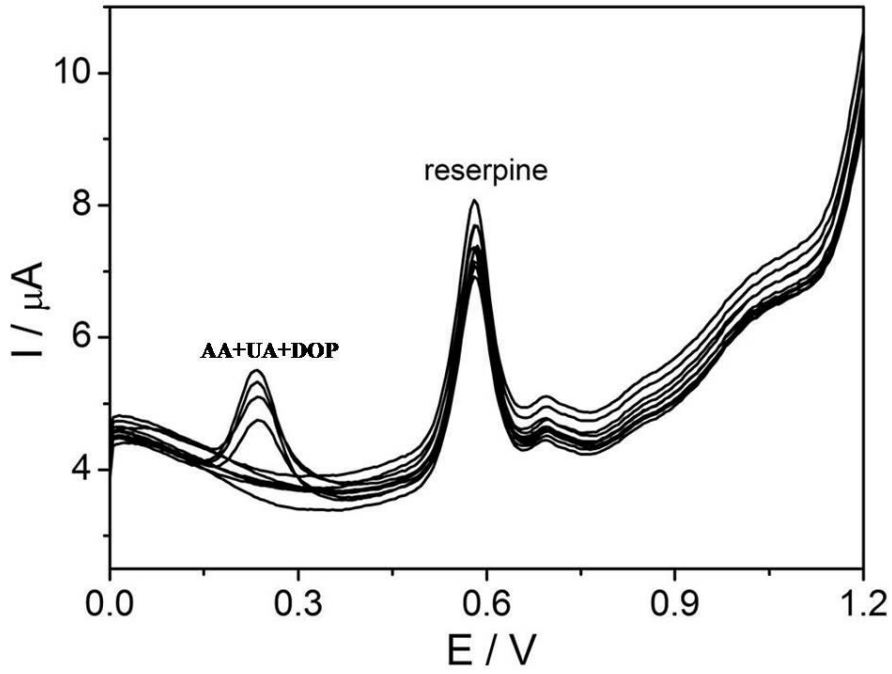


Figure 3. The DP voltammograms of 35μM of reserpine under optimized DPV parameters, without and with influence of ascorbic acid, dopamine and uric acid.

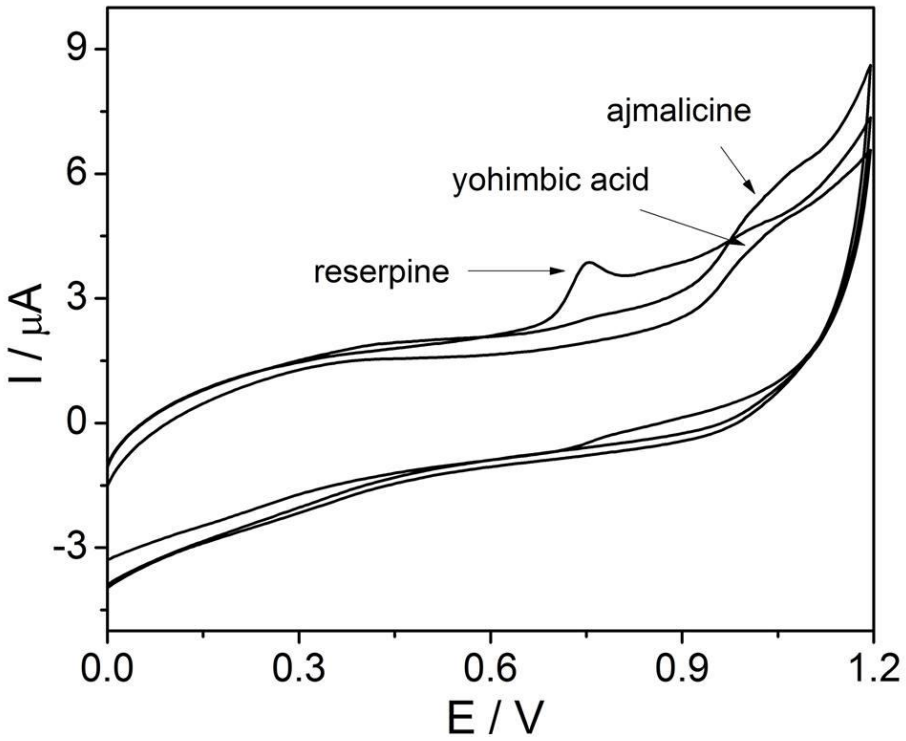


Figure 4. Cyclic voltammograms of reserpine, ajmalicine and yohimbine (all 0.1 mM) on a glassy carbon electrode, supporting electrolyte BR buffer pH 5.

3.4. Calibration data and analytical applications

DP voltammograms for different concentrations of reserpine under optimized parameters are presented in Figure 5. The insert displays the calibration curve which exhibits two linear regimes for the current dependence on the concentration c, the first one from 2 to 10 μM (I=0.078c-0.05, R^2=0.9902) and the second from 10 to 75 μM (I=0.361+037c, R^2=0.9927), where c is concentration in μM and I the current in μA. The detection limit of the method (3σ) was evaluated as 0.6 μM. The repeatability of 5 repetitive measurements was 0.09 μA for 35 μM of reserpine. From the results it can be concluded that the proposed method has good sensitivity and repeatability to be applied to the determination of the target compound in proper matrices.

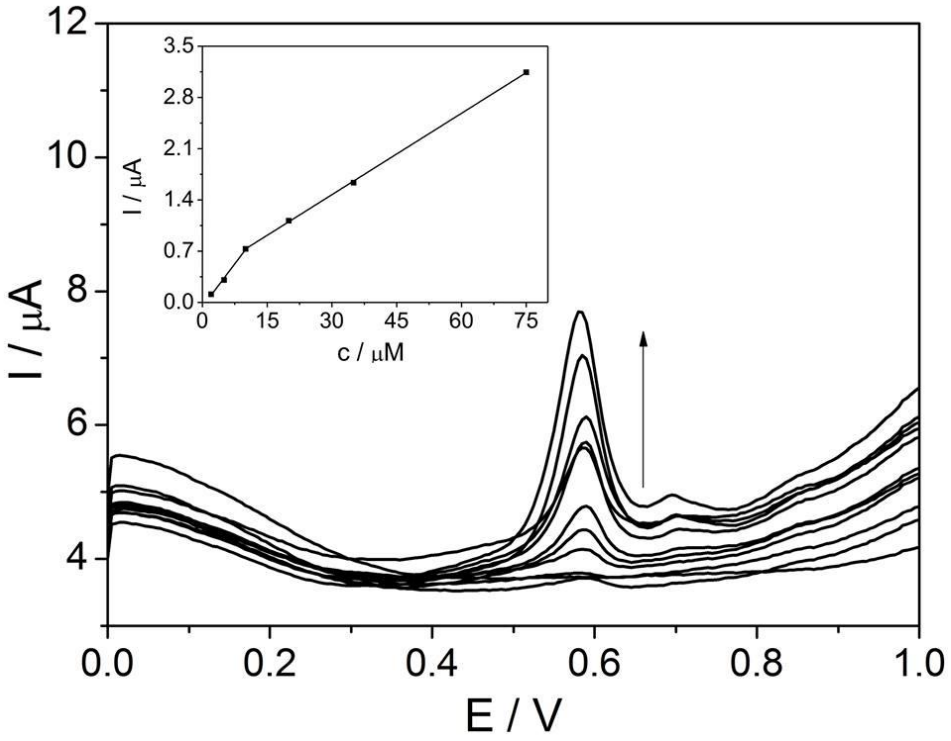


Figure 5. Differential pulse voltammograms of different concentrations of reserpine, from 2 to 75 μM, at optimized parameters; calibration curves for determination of reserpine under the optimized parameters.

Application of the sensor to model samples was one of the primary requirements for the validation of the analytical method. The concentration was estimated by DPV (Figure 6.) from the calibration curves of the proposed method. The sensor was used for the determination of reserpine in human urine samples. Spiking of the sample with 10, 15 and 20 μM was used to assess the correctness with recovery experiments (Table 1). All the recovery rates are within acceptable limits and document that the proposed method can be applied to the determination of the alkaloid in urine.

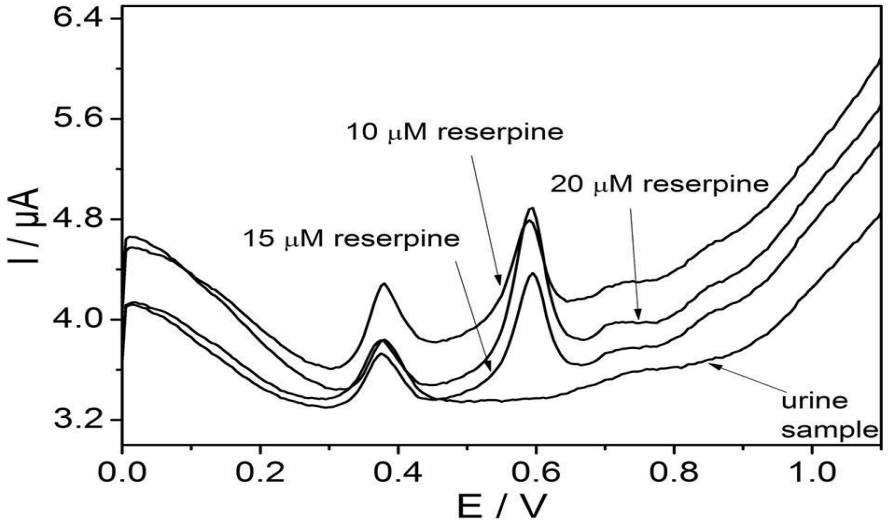


Figure 6. DP voltammograms for determination of reserpine in human urine sample, under optimized electrochemical parameters, with proposed sensor

Table 1. Recovery values of reserpine in spiked urine samples

Sample	Added, μM	Found, μM	Recovery %
1	10	10.28	102.8
	15	16.00	106.7
	20	21.85	109.3
2	10	10.18	101.8
	15	16.15	107.7
	20	20.87	104.4
3	10	10.30	103.0
	15	15.92	106.1
	20	21.44	107.2

4. CONCLUSIONS

In this study the glassy carbon electrode was applied for the first time for the determination of the indole alkaloid reserpine by differential pulse voltammetry. For elucidation of the electrochemical behavior of reserpine cyclic voltammetry was used. With optimum parameters the linear concentration range for determination of reserpine was from 2 to 75 μM with two linear segments, first from 2 to 10 μM and second from 10 to 75 μM of reserpine. The analytical application of sensor consisted in determination of reserpine in model human urine samples without any pre-treatment except simple dilution, and without significant influence of the most common urinary compounds. The proposed procedure with the glassy carbon electrode offers a simple, low cost and rapid alternative for the determination of investigated alkaloid in comparison with other analytical techniques previously described in the literature.

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