An Integrative Medical Perspective on Novel Dopamine Detection Method

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Dopamine is very important neurotransmitter and the rapid and effective methods for its determination are of great importance in fundamental medical research. A rapid voltammetric assay for dopamine (DA) detection in presence of ascorbic acid (AA) based on an electrochemically pretreated pencil graphite electrode has been investigated. Differential pulse voltammetry results showed two well-distinguished oxidation peaks for DA and AA at 0.369 V and 0.142 V, respectively, and the method was applied for DA determination without previous separation. The obtained detection limit for DA was 5.17 × 10^{-8} M. The method has been successfully applied for determination of DA in a pharmaceutical sample.

Keywords: disposable sensor, dopamine, surgery, pharmaceutical samples, medicine

Dopamine (DA), is an important catecholamine neurotransmitter existing in the brain and central nervous system of mammals, and is essential in neural communication [1,2]. Altered central dopaminergic synaptic transmission has been implicated in several neurological and psychiatric disorders, obesity and depression [3-6, 40]. Obese people have fewer receptors for dopamine, a neurotransmitter that helps produce feelings of satisfaction and pleasure. Improving the dopamine function, in future might be an exciting strategy in the treatment of obese people [41-45]. Individuals with these diseases demonstrate dramatic sleep disturbances, such as excessive daytime sleepiness [7], rapid-eye-movement (REM), sleep behavior disorder [8], and disturbed sleep architecture [9]. Dramatic changes in neurotransmitter levels are known to occur as the brain progresses through the sleep-wake cycle. Evidence from human clinical disorders, suggests that DA significantly modulates aspects of sleep state and may carry clinical implications for a number of sleep-related disorders (e.g. depression and Parkinson's disease) [10]. Obstructive sleep apnea (OSA) is a condition characterized by repetitive collapse of the upper airway during sleep. The efficient screening, diagnosis and treatment of sleep apnea will lead to a reduction of the costs associated with the aggravation of disorders (brain or heart attack) [11]. Generally, the diagnosis of OSA is made based on overnight polysomnography, which is both time and labor intensive. Thus, the quest for OSA biomarkers (e.g. dopamine) is critical.

At the same time it must be mentioned the dopamine role in perioperative blood pressure management, especially in plastic and reconstructive surgery. In free flap surgery, the control of free flap perfusion and local arterial pressure is very important.

Different analytical methods have been established to determine DA including high performance liquid chromatography-MS [12], gas chromatography-MS [13], chemiluminescence [14], fluorimetry [15], and spectrophotometry [16]. Many electrochemical strategies have been designed and applied for the detection of DA in samples [17-23]. DA and AA have very close oxidation potentials, and due to the fact that AA is in higher concentration than DA in biological samples they strongly interfere, and so selective determination of DA and AA remain a major goal of electroanalytical research [19].

A drawback of voltammetric analysis based on conventional electrodes, is their surface contamination during the measurements, and a cleaning step of the electrode surface is necessary before each voltammetric recording. The use of disposable electrodes eliminates this time-consuming stage [17]. Pencil graphite electrode (PGE) presents some advantages compared with conventional electrodes [24], and its suitability in multiple applications has been demonstrated in the literature [25]. Electrochemical pre-treatment of carbon materials has shown significant enhancement in electrochemical properties with an improved electrocatalytic effect and a better electron transfer rate of analyte toward the electrode surface [24,26,27].

The present paper describes the voltammetric behavior of DA and AA at the PGE*. The analytical performance of the PGE* towards DA determination has been evaluated. There are few examples in the literature for DA determination based on PGE* [28-31], this paper being the first example of a disposable PGE* used for the determination of DA in presence of ascorbic acid (AA). Determination of DA by a differential pulse voltammetric (DPV) method was successfully realized by using the PGE*, and thus a novel approach for selective DA detection

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is proposed. The method was applied to the analysis of DA in pharmaceutical probe.

Experimental part

Regents and solutions

Dopamine hydrochloride and ascorbic acid (both from Sigma-Aldrich) were respectively dissolved with ultra-pure water to obtain 10^{-2} M of standard stock solutions. As supporting electrolyte, Britton-Robinson buffer (BRB) solution was used.

Equipment

Voltammetric measurements were performed with Autolab PGSTAT 128N (Ecochemie B.V., Netherlands) controlled by Nova 1.8 software. A pencil graphite electrode (PGE) or an electrochemically pre-treated pencil graphite electrode (PGE*) as the working electrodes, Pt wire and Ag/AgCl as auxiliary and reference electrodes were used. PGEs were commercially Rottring HB pencil-leads, and were prepared as described elsewhere [32]. In order to enhance the heterogeneous electron-transfer rate, the PGEs were activated by an electrochemical pre-treatment described in detail elsewhere [24,27], this step assuring the electrode activation and stabilization. Each voltammetric recording was carried out on a new graphite pencil lead.

Electrochemical measurements

Cyclic voltammetry (CV) studies were done in the potential range -0.2 to + 0.6 V, at a scan rate of 100 mV s^{-1}, unless otherwise stated. Differential pulse voltammograms (DPVs) were recorded for different concentrations of DA solution prepared in BRB solution of *p*H 3.29, under optimized instrumental parameters (scan rate 0.01 V s^{-1}, pulse amplitude 0.025 V, sampling width 17 ms, pulse width 100 ms, pulse period 500 ms). The oxidation peak of DA at +0.369 V was used for its quantification.

Analytical sample preparation

The applicability of the DPV method based on PGE* was assessed for the DA quantification in a pharmaceutical product (dopamine hydrochloride, 5 mg mL^{-1}, Zentiva, perfusions injections). 0.25 mL from the pharmaceutical probe was diluted with 10 mL water, and an aliquot of 1 mL from this solution was diluted further with 10 mL BRB solution of *p*H 3.29; finally, 0.1 mL from this last solution was introduced in the electrochemical cell and diluted again with 10 mL BRB solution and analyzed. DPVs were recorded for the sample solution before and after 3 additions of 0.1 mL from the 10^{-4} M DA final stock solution.

Results and discussion

Surface characterization of PGE and PGE* by atomic force microscopy and their electrochemical behavior in a solution of 1 x 10^{-3} M $K_3[Fe(CN)_6]$ prepared in 1 M KCl were presented in a previous paper [24]. The electrochemical results indicated an easier electronic transfer at the PGE* surface compared with PGE due to the pre-treatment of the electrode.

*Electrochemical behavior of DA and AA at PGE**

CV studies of DA in presence of AA at PGE and PGE*, revealed that in case of using PGE the cyclic voltammogram has almost no electrochemical significance (fig. 1), while at PGE* exhibits a well-defined irreversible anodic peak for AA oxidation at about 0.237 V, and a pair of well-defined quasi-reversible redox peaks for

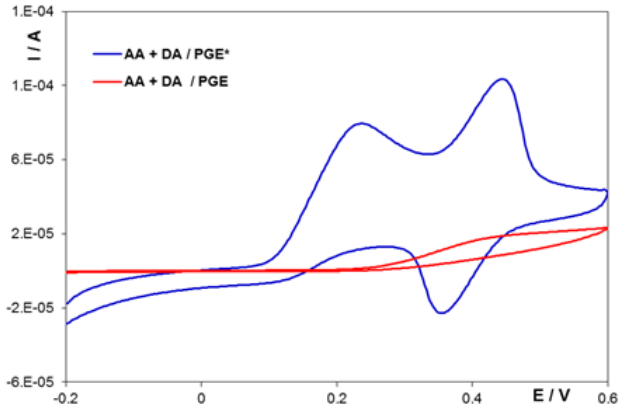

Fig. 1. Cyclic voltammograms for a mixture of 1.0 × 10^{-3} M AA and 1.0 × 10^{-4} M DA in BRB solution (*p*H 3.29) at PGE and PGE*; scan rate 100 mV s^{-1}

DA electrochemical process with E_{pa} = 0.437 V and E_{pc} = 0.362 V (ΔE_p = 0.075 V) and a ratio of I_{pa}/I_{pc} = 1.15. It is supposed that the difference between DA and AA electrochemical behavior might be caused by the charge of the analytes at the measured acidity (*p*H 3.29) which is related to their different pK_a values [33], on one hand, and by the presence of some un-oxidized aromatic rings with rich delocalized π electrons on the PGE* surface, which make the material to present strong ability to interact with DA special aromatic ring through π-π staking mode [22], on the other hand, which finally resulted in an increased electrochemical response for DA compare with AA.

It can be concluded that the electrochemical pre-treatment process makes possible an important improvement in the voltammetric behavior of both compounds at PGE* compared with PGE. These good features are enough to allow the voltammetric quantification of a mixture of DA and AA, since the analytical signals were resolved at PGE*. Based on this feature, the electrode was applied as a highly selective sensor for the detection of DA in the presence of AA.

Method optimization

In order to better understand the electrochemical mechanism of DA and to obtain the optimal electrochemical response at PGE*, some electrochemical parameters (the *p*H and scan rate) effect on peaks currents and potentials of DA and AA were investigated.

As shown in figure 2, with gradually decreasing of *p*H, the oxidation peak potential of DA shifted toward more positive values, and the peak current increased slightly. Moreover, by decreasing the pH, both associated anodic and cathodic peak currents for DA increase with about 2.62 μA per *p*H unit, presenting a maximum at *p*H 3.29. From the E_p = f(*p*H) plot (fig. 2) two linear relationships were obtained for the oxidation and reduction processes of DA, with regression expressed by the equations: E_{pa} (V) = –0.058 *p*H + 0.6409 (R^2 = 0.9917) and E_{pc} (V) = –0.052 *p*H + 0.5273 (R^2 = 0.9942) respectively. The slopes of the two regression equations for DA are close to the theoretical (Nernstian) value of 59 mV pH^{-1} indicating that an equal number of protons and electrons take part in the electrochemical reaction [34]; the electrochemical redox process of DA at PGE* should be two electrons and two protons process. In case of AA oxidation, the regression equation was: Epa (V) = –0.046 *p*H + 0.3857 (R^2 = 0.9946).

Considering the determination sensitivity, a BRB solution of *p*H 3.29 was further used as the optimal supporting electrolyte for all further experiments.

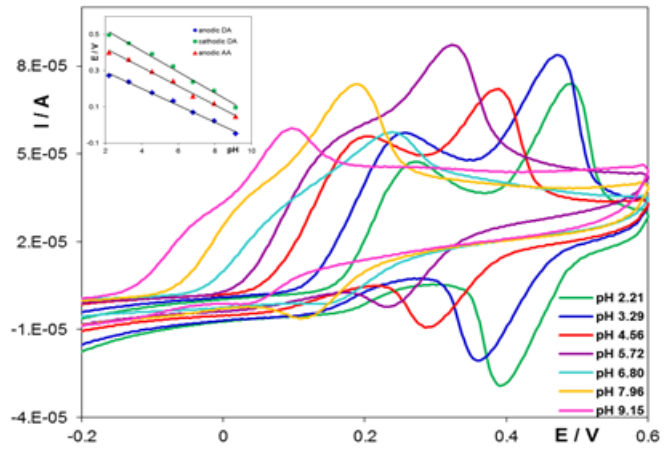

Fig. 2. Cyclic voltammograms for a mixture of 1.0×10^{-3} M AA and 1.0×10^{-4} M DA in BRB solution of different pH values at PGE*. Inset, the effect of pH on peak potentials; scan rate 100 mV s^{-1}.

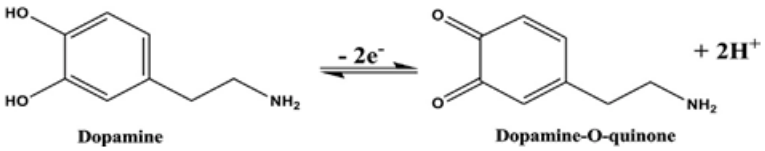

Fig. 3. The electro-oxidation mechanism of DA at PGE*.

Based on the formula [35]: dEp/d*p*H = 2.303 mRT/nF in which, m is the number of protons and n is the number of electrons, m/n was calculated to be 0.98 and 0.93 for the oxidation and reduction process of DA, respectively. Thus, the electrochemical oxidation of DA at the PGE* should be a two-electron and two-proton process [36] which is well in accordance with typical electro-oxidation mechanism of DA shown in figure 3.

In case of DA the anodic and cathodic peak currents increase linearly with the square root of the scan rate (inset fig. 4) and the regression equations were: I_{pa}(μA) = 5.2894 $v^{1/2}$ (mV s^{-1}) + 8.8718 (R^2 = 0.9960) and I_{pc}(μA) = 4.1332 $v^{1/2}$ (mV s^{-1}) + 6.5007 (R^2 = 0.9953), which demonstrate that the electrochemical redox process of the DA is diffusion-controlled. At the same time, by increasing the scan rate (fig. 4), the oxidation and reduction peak potentials observed in CVs at PGE*, shifted towards more positive and negative values, respectively, thus confirming the kinetic limitation in the electrochemical reaction. The anodic and cathodic peak potential showed a linear relationship with the decimal logarithm of scan rate between 10 mV s^{-1} to 500 mV s^{-1}. For oxidation and reduction processes of DA the linear regression equations were: E_{pa} (V) = 0.0881 log *v* + 0.8197 (V s^{-1}, R^2 = 0.9959) and E_{pc} (V) = –0.0589 log *v* + 0.0953 (V s^{-1}, R^2 = 0.9933), respectively.

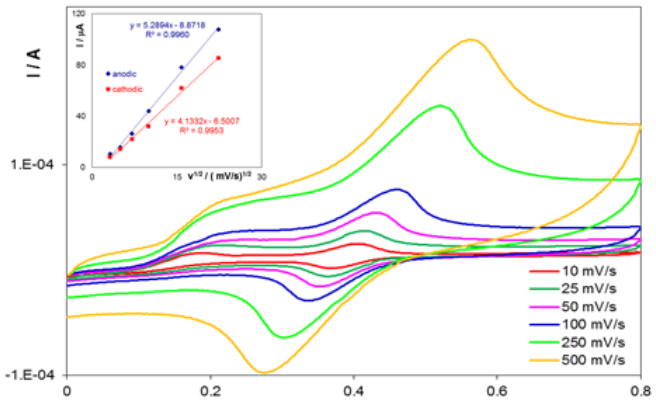

Fig. 4. Cyclic voltammograms for a mixture of 1.0×10^{-3} M AA and 1.0×10^{-4} M DA in BRB solution *p*H 3.29 at different scan rates on PGE*; Inset, the graph of I_p vs. $v^{1/2}$ for DA

The electro-transfer kinetic parameters such as the electron transfer coefficient (α) and the standard electron transfer rate constant (k_s) of DA on the PGE* were also investigated. According to Laviron theory [37], the charge transfer coefficient (a) was calculated based on the equation, $K_a/K_c = \alpha/1-\alpha$, where K_a and K_c is the slope of the straight lines for E_{pa} versus log v and E_{pc} versus log v, respectively; the value for α was 0.60 and the calculated electron transfer number (n) was about 2. The heterogeneous electron transfer rate constant (k_s) can be calculated from equation [37]:

$$\log k_s = \alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nFv} - \frac{\alpha(1-\alpha)nF\Delta E_p}{2.3RT},$$

where *n* is the number on electrons involved in the reaction, ΔE_p is the peak potential separation (E_{pa}-E_{pc}), α is the charge transfer coefficient, v is the scan rate, R, T and F having their usual meaning. As the number of electrons involved in electrochemical redox process of DA is about 2, the calculated value for k_s was 0.330 ± 0.082 s^{-1}, which is comparable with those reported in the previous literatures [38,39]. These results indicate that the PGE* in this work has good catalytic capacity to promote electron transfer kinetics of DA.

DPV studies

For the quantitative determination of dopamine DPV method was used as a suitable electroanalytical technique due to the low background currents and low detection

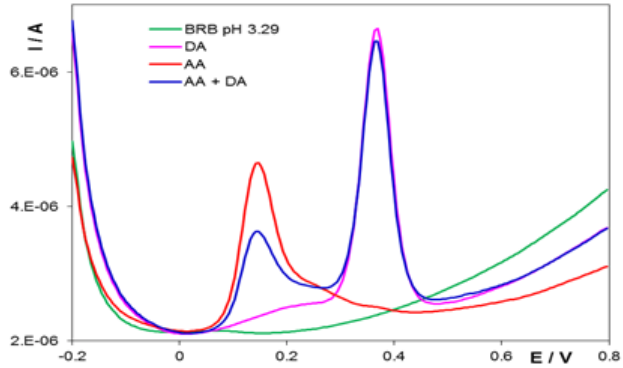

Fig. 5. Differential pulse voltammograms of solution containing 1×10^{-4} M AA, 1×10^{-6} M DA, and a mixture of 1.0×10^{-4} M AA and 1.0×10^{-6} M DA in BRB solution *p*H 3.29 at PGE*

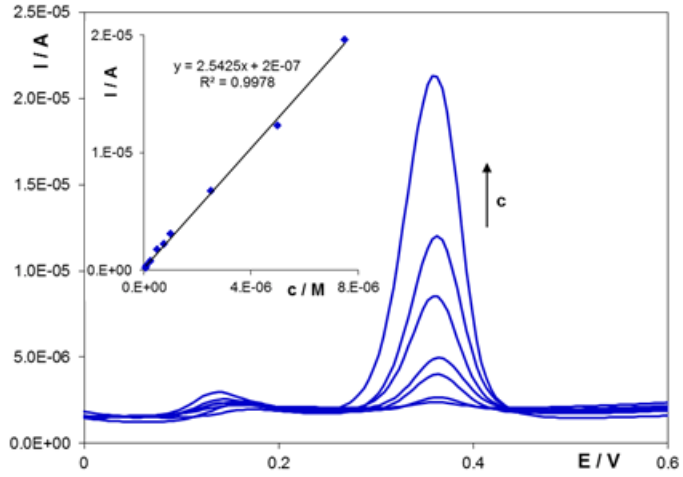

Fig. 6. Differential pulse voltammograms of DA solution at different concentrations in presence of 1 × 10^{-4} M AA in BRB solution of *p*H 3.29 at PGE*

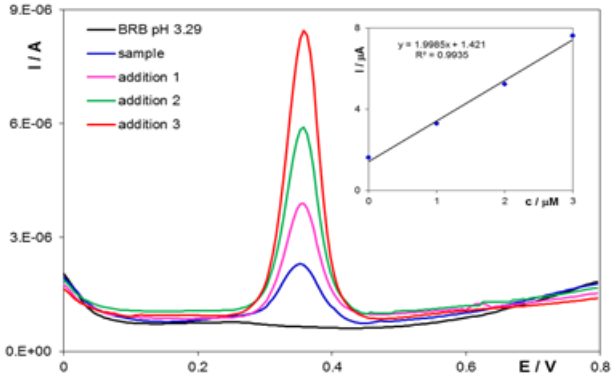

Fig. 7. Differential pulse voltammograms for a DA sample and 3 additions of 0.1 mL 10^{-4} M DA at PGE*. Inset, the calibration graph

Added [µM]	Intra-day			Inter-day		
	NP found ± SD [µM]	Precision (RSD%)	Accuracy bias(%)	NP found ± SD [µM]	Precision (RSD%)	Accuracy bias (%)
0.10	0.10±0.0025	2.46	0.02	0.11±0.0035	3.33	0.05
1.00	1.01±0.0208	2.05	0.01	0.99± 0.0265	2.67	-0.01
7.50	7.52± 0.0300	0.40	0.00	7.55± 0.0737	0.98	0.01

Bias, [(Found – Added)/Added] × 100; SD: standard deviation, % RSD: relative standard deviation

Table 1
RESULTS FOR THE EVALUATION OF THE INTRA-DAY AND INTER-DAY PRECISION AND ACCURACY OF DA DETERMINATION IN PRESENCE OF AA BY DPV

Sample	Content (mg mL^{-1})	Found±SD (mg mL^{-1})	RSD (%)	Recovery±SD (%)
1	5.0	5.05±0.06	1.20	101.07±1.21
2	5.0	5.07±0.10	1.97	101.38±2.00
3	5.0	4.93±0.05	0.97	98.52±0.96

Table 2
DA DETECTION IN PERFUSIONS INJECTIONS
(n = 3)

limits. DPV results (fig. 5) showed two well-distinguished anodic peaks for AA and DA at 0.142 V and 0.369 V, respectively, and therefore the simultaneous determination of the two compounds was possible at the surface of PGE*.

It was found that the variation of the oxidation peak current for DA is linear to its concentration in the range of 7.5 × 10^{-8} M to 7.5 × 10^{-6} M, having the following regression equation I(A) = 2.5456 C_{DA}(M) + 2x10^{-7}, R^2 = 0.9979 (fig. 6); the detection and quantification limits for DA (from linear regression analysis) were 5.17 × 10^{-8} M and 1.57 × 10^{-7} M, respectively. The limit of detection was better or almost the same when compared with others in the literature [28,29]; moreover, compared with PGE chemically modified electrodes [30,31], the sensor developed in this work is easy to be prepared.

The accuracy and precision of the method were evaluated by analysis of DA in presence of AA at three levels of low, moderate and high concentrations by performing three replicate analysis of standard solution over one day (intra-day assay), and for three successive days (inter-day assay). The results in table 1 confirmed both good precision and accuracy of the method. The intra-day % RSD no more than 2.24% and the inter-day % RSD smaller than 3.33% indicated that the method is precise and confident.

In order to prevent any matrix effect, standard addition method was applied for the DA determination by DPV. The oxidation peak currents (fig. 7) were measured and used to calculate DA contents and the % recoveries of DA from the pharmaceutical product.

To evaluate the practicability of the sensor, the fabricated electrochemically pretreated graphite/nafion composite modified SPCE was used to determine DA content in commercially available dopamine Hydrochloride Injection and the results are presented in table 2. Recovery values close to 100% demonstrated the ability of PGE* for the selective determination of DA in real samples. It can be concluded that the voltammetric method can be efficiently used for the determination of DA in pharmaceutical samples. The good recovery results of the fabricated electrode validate that it can be used as a potential candidate for the effective determination of DA in pharmaceutical samples.

Conclusions

In this work, an electrochemically pre-treated pencil graphite electrode and differential pulse voltammetric method were first used to determine DA quantitatively in presence of AA. The PGE* showed an enhanced electrocatalytic activity toward the oxidation of DA when compared with non-treated ones. The oxidation peak potentials of DA and AA were separated with 0.227 V at *p*H= 3.29. The new DPV method is simple, rapid and was successfully applied in the determination of the DA in pharmaceutical samples. The disposable electrochemically pre-treated pencil graphite electrode used as the working electrode offers the advantage of simple electrode preparation steps with no need to use chemical modifiers, is easy to be replaced, cheap and commercially available. The identification of a rapid and effective method of dopamine determination may contribute on the one hand to the improvement of perioperative management of the free flap perfusion and on the other hand to develop a novel treatment strategy for obese patients with sleep apnea syndrome.

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