Determination of Dopamine and Ascorbic Acid Using Boron Doped Diamond Microelectrode Arrays

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A selective electrochemical method was developed for the determination of dopamine (DA) and ascorbic acid (AA) using boron doped diamond microelectrode arrays (BDD-MEA). The overlap problem of oxidation peaks for determination of DA and AA in their mixture is solved by using BDD-MEA, a good peaks separation of 410 mV between DA and AA was obtained. Thus, the selective determination of DA and AA was carried out with low detection limit, 0.044 \( \mu \)M and 7.5 \( \mu \)M, respectively (S/N=3). The linear range for DA and AA concentration was 0.2 - 1 \( \mu \)M and 20-200 \( \mu \)M, respectively.

Keywords: boron-doped diamond, microelectrodes-arrays, dopamine, ascorbic acid, electroanalysis

The development of electrochemical sensors for the selective determination of neurotransmitters in human fluids has received considerable attention for measurements in biological environments [1-7]. Ascorbic acid (AA) and neurotransmitters are electrochemically active compounds co-existing in body fluids and their concentrations in biological samples vary from species to species, in a wide range, from \( 10^{-7} \) to \( 10^{-3} \) M. Usually, DA concentrations in biological samples vary from species to species, in a wide range, from \( 10^{-7} \) to \( 10^{-3} \) M. Usually, the DA concentration is 10 nM to 1 \( \mu \)M while AA concentration is as high as 0.1 mM in biological systems [8]. Therefore sensitivity and selectivity are very important in the development of any method for the determination of dopamine (DA) and AA. DA play important roles in central nervous system, cardiovascular, renal and hormonal systems functioning as well as in drug addiction. In the case of the conventional electrodes very often appear the fouling effect owing to the accumulation of oxidized product on the electrode surface, leading to a poor selectivity and sensitivity. Several electrochemical procedures based on different electrocatalytic materials have been developed in order to avoid this major drawback and to enhance the simultaneous determination of DA and AA [9-16].

The boron doped diamond microelectrodes are very attractive in electroanalysis field due to the unique properties such as a very low background current, high stability and selectivity, constant current response, enhanced mass transport, and the ability for use in high resistance media [17]. In order to increase the current signal of a single microelectrode, arrays of microelectrodes are often used. In this case, radial diffusion dominates the mass transport of reactants, leading to enhanced mass transport coefficients compared to planar diffusion. The limiting current at microelectrode arrays is known to be independent of potential scan rate owing to pure spherical diffusion. The advantages of boron doped diamond microelectrode arrays (BDD-MEA) are (i) low and stable voltammetric and amperometric background current, (ii) a wide working potential window, (iii) quasi-reversible to reversible electron transfer kinetics for several redox systems without conventional pretreatment and with enhanced signal-to-background ratios due to the low background signal, (iv) long-term response stability, (v) morphological and microstructural stability during anodic polarization, and (vi) weak adsorption of polar molecules [18]. Due to these peculiar properties, BDD MEA electrodes could be used in electrochemical and biological sensing applications [19-21].

The surface termination contributes to the chemical and physical properties of BDD electrode and thus is very important for electroanalysis. Usually, the BDD electrodes obtained in the research laboratories are initially hydrogen-terminated as they are deposited in a hydrogen plasma CVD chamber. In order to achieve an oxygen-terminated BDD electrode is very convenient for electroanalysis to do the oxidation by a simply anodic treatment of the BDD surface at high positive potentials or repetitive cycling in positive potential range. Under these conditions, the OH radicals are produced from water at the electrode surface, which precedes the oxygen evolution having high anodic overpotential at BDD electrode. The oxygen-terminated BDD surface (OBDD) is hydrophilic having a lower conductivity and negative surface charge, while the hydrogen-terminated BDD (HBDD) surface is hydrophobic and have high conductivity [22]. The big advantage of OBDD electrodes is the high surface stability to fouling [23-25].

To develop a reliable method for DA and AA determination, we combined these advantages of OBDD MEA, and the sensitivity of the chronoamperometric technique for biomolecules detection. Thus, the overlaping problem of anodic peaks of DA and AA is solved into two well defined peaks in DPV at 0.85 and 1.26 V, respectively. The sensitivity and DL in the determination of DA and AA using BDD MEA are comparable with other reported results [26-27]. Thereby, the present study provides a new method for selective and sensitive detection of DA and AA, which could be very attractive in biological and chemical fields.

Experimental part

Phosphate buffer solution (0.1 M NaH\(_2\)PO\(_4\) / 0.1 M NaHPO\(_4\), pH=7.4) was used as the supporting electrolyte for voltammetry. All chemicals were of analytical reagent grade and used without any further purification. Deionised water obtained from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used throughout all experiments. Dopamine and ascorbic acid were purchased from Merck. Stock solutions of dopamine (0.01M) and ascorbic acid (0.01M) were prepared daily by dissolving...
DA and AA in phosphate buffer solution (PBS). Dopamine hydrochloride ampoules 0.5% were obtained from S. C. Zentiva S. A. Romania. The content uniformity test of dopamine hydrochloride ampouls was performed taking the DA hydrochloride ampoul (50 mg dopamine hydrochloride) and placed in a 25 mL volumetric flask, buffered with PBS solution of pH=7.4 to the mark. After the obtaining of calibration curves for the applied potential of 0.5 V in Chronoamperometry, the working cell was filled with known concentration solution from DA hydrochloride ampoul and the current developed was measured in order to calculate the recovery of dopamine from pharmaceutical products.

The BDD MEA have been obtained from Adamant Technologies SA, Switzerland. The diamond layer was covered with a patterning Si$_3$N$_4$ layer that was performed by standard photolithography technique through dry etching to form a hexagonal array of 473 microdiscs, 5 mm in diameter and separated by 150 μm. The same BDD MEA was used by Kapalka et al. [28] in their study regarding to the electrochemical oxygen transfer reaction (EOTR) on BDD MEA.

Electrochemical measurements were taken with Autolab PGSTAT 302N (Metrohm Autolab), which was connected to a PC running the GPES software. Electrochemical measurements were carried out in a single compartment cell (10 mL) having as counter electrode a Pt wire and Ag/AgCl/3M KCl (Metrohm) as reference electrode.

DPV was used for the simultaneous determination of DA and AA at BDD MEA because of its higher current sensitivity and better resolution than cyclic voltammetry. The optimised parameters used in differential pulse voltammetry (DPV) were: equilibration time = 3s, initial potential = -0.2V, end potential = 1.8V, step potential = 0.0015 V , modulation amplitude = 0.05V, modulation time = 0.05s, interval time = 0.5s. Chronoamperometric study was performed in unstirred batch conditions. The time for each measurement was 60 s and the current readings for calibration plots were at 50s. These values were used in all subsequent calibration measurements. The pH of the solutions was measured by a pH meter purchased from Metrohm Autolab. The experiments were conducted at room temperature (23°C).

Results and discussions

In order to ensure a reproducible surface and equivalent separation of the oxidation potential peaks within our study concerning to the electrochemical sensing of DA and AA the BDD MEA surface was pretreated in 0.1M KOH by applying a potential of 1.92 V for 60 min, before every series of electrochemical measurement.

**Electrochemical sensing of DA at BDD MEA using DPV**

The behaviour of DA was investigated using both differential pulse voltammetry and chronoamperometry method. In figure 1 are presented the differential pulse voltammograms for BDD MEA in 0.1M PBS (pH=7.4) containing 2, 4, 6, 8, 10, 12, 14, 16 mM DA. The oxidation of DA takes place at 0.72 V and the peak current was increasing for the successive addition of 2 μM DA in PBS solution. The BDD MEA exhibited a linear current response to DA concentration in the range of 2-16 μM, the corresponding equation being $I_p (nA) = 0.035 + 0.0633 x C_{DA} (μM)$.

Considering the BDD capacity to produce hydroxyl radicals at the electrode surface through the electrochemical oxidation of water it should assumed the reaction mechanism that involves the participation of hydroxyl radicals (OH) for the oxidation of DA and AA at BDD MEA. During the production of hydroxyl radicals, the electrode surface of BDD MEA is changed from hydrophobic to hydrophilic character leading to enhanced affinity for DA in cationic form and giving one strong current response.

A possible type of mechanism for electrochemical oxidation of DA at BDD MEA could be ECEC, in which the first step is electron transfer followed by the release of a proton to produce a neutral radical, followed by a second electron transfer, as reported in the literature [29, 30].

$$\text{HO(DA)OH} \rightarrow \text{HO(DA)OH}^+ + e^-$$

$$\text{HO(DA)OH}^2 \rightarrow \text{HO(DA)O}^+ + H^+$$

$$\text{HO(DA)O}^+ \rightarrow \text{H}^+ \text{O(DA)O}^+ + e^-$$

$$\text{H}^+ \text{O(DA)O}^+ \rightarrow \text{O} = \text{(DA)}\text{O} + H^+$$

**Electrochemical sensing of AA at BDD MEA using DPV**

DPV method was used for the oxidation of AA on BDD MEA in PBS solution. Figure 2 reports the DPV traces recorded at BDD MEA for different AA concentrations and the inset corresponds to the calibration plot, showing the linear dependence of the current intensity with AA concentration, within the investigated range. This linear relationship exists between current peak and the AA concentration in the range 0.2 – 1 mM ($r^2 = 0.9988$) with a slope of 0.028 nA/μM. The oxidation of AA in PBS solution, $pH=7.4$ takes place at 1.23 V. At this value of $pH$, DA exists as a cation and AA as an anion. This high oxidation potential of AA could be explained taking in consideration that between the BDD MEA surface and the negatively charged ascorbate anion there is a strong electrostatic repulsion.
Electrochemical sensing of DA in the presence of AA at BDD MEA

There are two issues in sensing of DA and AA, the close oxidation potential values of AA and DA at conventional electrode and the other is the electrocatalytic oxidation of dopamine by ascorbic acid [31]. Oxidized dopamine, (dopamine-o-quinone) is chemically reduced by ascorbic acid. Using BDD MEA a good peak separation (410 mV) was obtained, two well-defined peaks were observed at 0.85 and 1.26 V, corresponding to the oxidation of dopamine and ascorbic acid, respectively. The study has been done by varying the concentration of DA while the concentration of AA was kept constant. The measurements were carried out in the potential range between -0.2 and 1.8 V. Figure 3 exhibits the differential pulse voltammograms obtained for the determination of DA in the presence of 0.5 mM AA. The inset of figure 3 shows the calibration plot for dopamine in the presence of 0.5 mM AA that was linear with a slope of 0.0392 nA/mM. The oxidation of DA in presence of AA takes place at 0.85 V and the voltammetric peak corresponding to this potential was found to increase linearly with the increase of the bulk concentration of dopamine, whereas the peak current for oxidation of ascorbic acid decreased. One possible explanation of this behaviour could be based on the strong attraction between OBDD MEA and DA cationic form that is very close to the surface of the electrode. Far away from the surface of the electrode the electrocatalytic oxidation of dopamine by ascorbic acid takes place. The OBDD MEA enhanced electrochemical activity could be related to the faster electron transfer and the greater surface area of the BDD MEA relative to the BDD microelectrode.

The slopes of the calibration graphs, i.e. the $\Delta I$ vs. DA concentration plots in the presence and the absence of the AA, suggest that the presence of AA in a high concentration as 0.5 mM has a certain influence the DA oxidation.

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in the presence of 0.01 mM AA at 0.5 V is lower than for 0.4 V. For an applied potential of 0.6 V (fig. 4C) the AA interferes more in the oxidation of DA at BDD MEA on the whole concentration range (the values of the pK_{sel}^{amp} are lower than 0.5). At this potential and higher than 0.6 V the DA cationic form is strongly attracted by AA close to the surface of the electrode. At applied potentials higher than 0.6 V the interference of AA increases and for applied potentials lower than 0.4 V the BDD MEA does not respond. For the amperometric determination of DA in pharmaceutical products the best applied potential was 0.6 V and for the determination of DA in biological samples the proper potential could be 0.4 V. The values for pK_{sel}^{amp} when the selectivity over AA was tested for different applied potentials, 0.4, 0.5 and 0.6 V are presented in table 1. These values indicate that AA slightly interfere in the determination of DA when the applied potential was 0.4 V and 0.5 V. For the applied potential higher than 0.5 the interference of AA is high.

The BDD MEA proved to be useful for the content uniformity test of dopamine hydrochloride ampoules, the obtained results indicate that the BDD MEA can be reliably used for the DA determination in pharmaceutical products. Recovery of DA from dopamine hydrochloride ampoules was 102%. The calibration curves were obtained using chronoamperometry method and the applied potential on BDD MEA was 0.6 V.

The best response of BDD MEA for the determination of DA in the presence of 0.01 mM AA was obtained for the applied potential of 0.4 V. The limit of detection based on three times the noise level (S/N=3), was determined to be 0.042 \( \mu \)M, comparable with DL acquired by using polyaniline/Au nanocomposite modified nanoelectrodes or unmodified, exfoliated graphite electrodes [33, 34].

Electrochemical sensing of AA in the presence of DA at BDD MEA

Differential pulse voltammograms for a mixture of AA and DA at BDD MEA in PBS at physiological pH are shown in figure 5. The study has been done by varying the concentration of AA while the concentration of DA was kept constant. The measurements were carried out in the potential range from -0.2 to 1.8 V. The oxidation of AA in the presence of DA takes place at 1.38 V and the voltammetric peak corresponding to this potential was found increased linearly with the increase of the bulk concentration of AA and the peak current for oxidation of DA was also increased. This behaviour could be explained taking into account that close to the surface of the BDD MEA is occurring the chemical reaction of the electrochemically oxidized DA with AA. The calibration curve (inset fig. 5) for sensing of AA in the presence of 100 mM DA shows excellent linearity over a range from 200 to 1600 \( \mu \)M. The slope of the linear calibration curve between the peak current and the concentration was 0.0186 nA / \( \mu \)M and the correlation coefficient was found to be 0.9963.

Determination of AA was also performed using chronoamperometry method. In figure 6 are presented the calibration curves obtained for determination of AA in the presence of 0.02 mM DA in PBS solution of pH = 7.4 containing 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 mM AA at different applied potentials: A) 1 V; B) 1.3 V.
that the presence of DA slightly influences the AA oxidation. The interference of DA at 1.3 V is lower than at 1 V, at this value of the potential is favored the oxidation of AA. Using chronoamperometry method for the determination of AA in the presence of DA the detection limit (S/N=3) was decreased to 7.5 μM.

No irreversible adsorption process of DA and AA at the surface of BDD MEA was observed. When BDD MEA was immersed in a solution containing DA and AA and then subsequently were immersed in a 0.1 M PBS solution, pH = 7.4 not containing these species, no voltammetric signal was observed.

For determination of AA in the presence of DA, amperometric selectivity coefficients, $K_{\text{amp}}$, were also determined following the method proposed by Wang [32]. The values for $K_{\text{amp}}$ when the selectivity over DA was tested are presented in table 2. These values indicate that DA interferes in the determination of AA when the applied potential was both 1 and 1.3 V. However, a very low interference was obtained at applied potential of 1.3 V; the sensitivities of AA determination in the presence and the absence of DA are almost equal (2.14 x 10^-4 mA/ mM respectively 2.139x10^-4 mA/ μM).

**Conclusions**

The BDD MEA exhibits a good electrocatalytic activity for the oxidation of DA and AA. In voltammetric measurements of DA and AA in their mixture solutions, the separation of the oxidation peak potentials is about 410 mV. BDD MEA presented a linear response range between 0.2 and 1 mM and 20 and 200 mM for determining DA and AA, respectively. This work demonstrates the advantage in utilizing of BDD MEA for selective and sensitive determination of DA over the conventional microelectrodes. Thus, BDD MEA proved to be useful for the content uniformity test of dopamine hydrochloride ampoules, the obtained results indicate that the BDD MEA can be reliable used for the DA determination in pharmaceutical products. The present study requires further research concerning to detection of DA and AA in biological samples.

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