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Electrochemical determination of adrenaline in human urine using a boron-doped diamond film electrode

Jozef Sochr, Ľubomír Švorc *, Miroslav Rievaj, Dušan Bustin

Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, Bratislava SK-812 37, Slovak Republic

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ABSTRACT

A simple and sensitive square-wave voltammetric method for the determination of adrenaline on unmodified boron-doped diamond film electrode was developed. Adrenaline exhibited the quasi-reversible behavior with oxidation peak on the forward scan at +0.75 V and smaller reduction peak on the reverse scan at −0.10 V vs. Ag/AgCl electrode in 0.5 M $HClO_4$. The effect of supporting electrolyte, pH and scan rate on the current response of adrenaline was examined to select the optimum experimental conditions. At optimized square-wave voltammetric parameters (amplitude of 100 mV, frequency of 50 Hz and step potential of 5 mV), the linear concentration range from 0.7 to 60 μM ($R^2 = 0.998$, number of measurements $n = 6$), the excellent repeatability (relative standard deviation of 3.5% for $n = 50$) and the detection limit of 0.21 μM were achieved without any chemical modification and pretreatment of working electrode. The practical application of method was demonstrated in the determination of adrenaline in spiked human urine samples with satisfactory recoveries (98 to 102%).

1. Introduction

Adrenaline (also known as epinephrine, ADR) is a hormone and neurotransmitter representing the most important catecholamine (together with noradrenaline) produced by adrenal glands. Despite the many positive functions of ADR in human system such as regulator of the blood pressure, heart rate and glycogen metabolism as well as bronchodilator for asthma, its presence may also signify a serious problem with negative impact on public health [1]. ADR is usually released during physical (muscular exercise, thermal burn) or emotional (trauma) stress which may activate the sympatho-adrenal system followed by an increase of concentration of ADR in blood and urine. Currently, many life phenomena such as increase of incidence and development of cancers are related to the presence of ADR in blood at which its concentration usually varies from 0 to 5 nM [2,3]. The low concentration levels of ADR have also been found in patients with Parkinson's disease [4]. On the other hand, not only stress, but also many foods including coffee, tea, bananas, chocolate and citrus fruits and certain drugs can increase the concentration of ADR in urine where its content usually alters in the range of 1–1 μM. Therefore, the analysis of ADR and other catecholamines in biological fluids plays a significant role in the study of its physiological functions or diagnose of some diseases in clinical chemistry. In respect of these objectives, there is a growing need for continual development of novel, simple and sensitive analytical methods in research and clinical laboratories.

Different analytical methods for the determination of ADR have been reported. High performance liquid chromatography (HPLC) [5–7], capillary electrophoresis [8,9] and spectrophotometric [10] techniques are among popular analytical methods for quantification of ADR and other catecholamines. However, these methods suffer from some disadvantages such as high cost, long analysis time and requirement for sample pretreatment when some procedures as derivatization, extraction and purification are usually included as well as demands for highly skilled personnel often restrict their use in routine analytical practice.

Electrochemical methods have many inherent advantages such as simplicity, low cost, and possibility of miniaturization and sometimes may represent an independent alternative to so far dominant spectrophotometric and chromatographic techniques. Many papers dealing with electrochemical determination of ADR individually or simultaneously with other drugs have been reported. These methods involve the amperometry as a detection technique in HPLC [11–16] and enzyme biosensors [17,18]. Nevertheless, the electrode surfaces covered by miscellaneous type of modifiers such as carbon nanotubes [19–24], polymers [25–30] or platinum [31,32] and gold nanoparticles [27,33,34] as well as the use of microbial biosensor [35] are among the most dominating electrochemical tools for the determination of ADR. Hence, they have appeared to be the sensitive sensors mainly due to good electron transfer ability. Regarding unmodified (bare) carbonaceous electrodes, carbon fiber ultramicroelectrode [36] and glassy carbon electrode [37] have merely been utilized for the determination of ADR. However, an irreversible adsorption of products of electrode reaction resulting in the passivation layer usually occurs in such electrochemical measurements.

* Corresponding author. Tel.: +421 2 59325302; fax: +421 2 59325590.
E-mail address: lubomir.svorc@stuba.sk (Ľ. Švorc).

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Boron-doped diamond film (BDDF) as the new perspective carbon-based electrode material opens new possibilities of electrochemical investigations due to its excellent features such as low adsorption pertinent to sp^3 character of diamond carbon, low and stable background current, good mechanical robustness, stability in several media and the widest usable potential window among all electrode materials (up to 4 V) [38]. These properties are commonly induced by morphologic factors, crystallographic orientation and presence of impurities (non-diamond sp^2 carbon) which make an unmodified (bare) BDDF electrode surface significantly different from those of other conventional carbon electrodes, e.g. glassy carbon or carbon paste. Moreover, the physical and chemical properties of BDDF electrode are affected by surface termination (*H*- and *O*-terminated surfaces with high and low conductivity, respectively) [39]. The development of new electrochemical methods using BDDF electrode can provide the invaluable services in drug monitoring in terms of protection of human health. The recent reports of our working group have revealed that several drugs such as caffeine [40], paracetamol [41], penicillin V [42] and codeine [43] are able to be sensitively and selectively determined using unmodified BDDF electrode.

This paper describes an application of unmodified BDDF electrode as sensitive electrochemical sensor for the determination of ADR. Moreover, to the best of our knowledge, no published report dealing with this topic using BDDF electrode is available until now according to the literature survey. The practical usefulness of proposed method is demonstrated in the determination of ADR in spiked human urine samples. The developed procedure could also find an application in drug monitoring thanks to rapidity, simplicity and sensitivity.

2. Experimental

2.1. Chemicals

ADR standard (CAS No. 51-43-4, purity of 99%), urea, ascorbic acid, uric acid, folic acid, glucose and barbituric acid were obtained from Sigma-Aldrich (Slovak Republic) and used as received without any further purification. The studied supporting electrolytes were acetate buffer solution, hydrochloric acid, nitric acid, sulfuric acid and perchloric acid (Lachema Brno, Czech Republic). All other chemicals were of analytical grade purity. Stock standard solution of ADR (1 mM) was prepared by dissolution of its solid standard in 5 mL of methanol and then diluted with double-distilled deionized water with resistivity above 18 MΩ cm. Working and calibration solutions of lower concentrations of ADR were freshly prepared by diluting with supporting electrolyte. Acetonitrile (Merck, Czech Republic) was used as organic component in mobile phase for the purposes of HPLC analysis.

2.2. Apparatus

Electrochemical measurements were carried out using an AUTOLAB PGSTAT-302N (Metrohm Autolab B.V., The Netherlands) potentiostat/galvanostat controlled with NOVA 1.9 software. The three electrode system consisted of Ag/AgCl/3 M KCl and platinum wire as reference and counter electrode, respectively. BDDF electrode inserted in polyether ether ketone (PEEK) body with inner diameter of disk of 3 mm, resistivity of 0.075 Ωcm and boron doping level of 1000 ppm (declared by Windsor Scientific Ltd., United Kingdom as manufacturer) was used as working electrode. The pH values of solutions were measured using pH meter Model 215 (Denver Instrument, USA) with combined electrode (glass-reference electrode), which was daily calibrated with standard buffer solutions. All the potentials reported in this paper were given against Ag/AgCl/3 M KCl reference electrode at a laboratory temperature of 25 ± 1 °C.

2.3. Measurement procedures

A particular volume of standard solution of ADR was pipetted into a 25 mL volumetric flask, then filled up with the supporting electrolyte and transferred quantitatively into voltammetric cell. Cyclic voltammetry (CV) and square-wave voltammetry (SWV) were employed without deaeration since oxygen did not influence the oxidation of ADR. Five CV voltammograms were obtained for each measurement, and the last scan was always considered for the evaluation and making the figures reported in this paper. SW voltammograms were recorded after optimization of instrumental parameters (amplitude, frequency and step potential). The peak currents (I_p) recorded using CV and SWV were evaluated from the straight lines connecting the minima before and after the peak maximum without background correction. Prior to the first use, BDDF electrode was cleaned by rinsing with deionized water and gently rubbed with a piece of damp silk cloth until a mirror-like appearance of surface was attained. Calibration curve was constructed from the average of six replicate measurements for each calibration solution of ADR and analyzed by linear least-square regression in OriginPro 8.0 (OriginLab Corporation, USA) with the relevant results (slope and intercept) reported with confidence interval for 95% probability. The detection limit was calculated as three times the standard deviation for the blank solution (supporting electrolyte) divided by the slope of the calibration curve.

2.4. Preparation of human urine samples spiked with ADR

Human urine samples were collected from three healthy non-smoking volunteers V1–V3 (co-authors) who did not undergo the treatment by pharmaceuticals containing ADR. The determination of ADR in human urine samples spiked with ADR was performed as follows: the aliquot amount of standard solution of 1 mM ADR was added to the volumetric flask containing 1 mL of fresh urine sample and subsequently was diluted to 25 mL with supporting electrolyte (0.5 M $HClO_4$). The analysis was carried out by standard addition method in order to reduce the matrix effect using the conditions described in previous sections.

3. Results and discussion

3.1. Electrochemical behavior of ADR on BDDF electrode

3.1.1. Effect of supporting electrolyte and pH

It is well-known that determination of electroactive compounds on unmodified electrodes is sometimes difficult due to the absence of electrocatalytic effect of appropriate modifier. The choice of the supporting electrolyte is an important stage in electroanalytical studies because its composition and pH influence the properties of analyzed solution as well as the electrode–solution interface, modifying the thermodynamics and kinetics of the charge transfer process. Cyclic voltammetry (CV) was applied to examine the electrochemical behavior of ADR on unmodified BDDF electrode. All necessary factors influencing the current response of ADR were carefully checked to reach the conditions at which the best analytical performance was achieved.

The electrochemical reaction mechanism of ADR and other catecholamines is familiar and has been elucidated in some papers [44–46]. ADR usually undergoes a quasi-reversible two-electron oxidation in participation of two protons to form adrenalinquinone as evidenced in Scheme 1. In our study, however, the reaction mechanism has not been proved by any experimental data. In order to find the appropriate medium for oxidation of 0.1 mM ADR the different supporting electrolytes such as nitric acid, sulfuric acid, hydrochloric acid, perchloric acid and acetate buffer solution (ABS) were tested. Concerning ABS, two oxidation peaks at + 0.70 V and + 1.25 V were registered at pH 6 with two small cathodic peaks at + 1.15 and − 1.20 V on the reverse scan on unmodified BDDF electrode (Fig. 1). This phenomenon was observed in pH values in the range 3–7 and well corresponded with literature data