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Voltammetric determination of penicillin V in pharmaceutical formulations and human urine using a boron-doped diamond electrode

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ABSTRACT

Simple, sensitive and selective differential pulse voltammetric method for determination of penicillin V on a bare (unmodified) boron-doped diamond electrode has been developed. Penicillin V provided highly reproducible and well-defined irreversible oxidation peak at very positive potential of +1.6 V (vs. Ag/AgCl). The optimum experimental conditions for oxidation of penicillin V were achieved in acetate buffer solution (pH 4.0). The modulation amplitude of 0.1 V, modulation time of 0.05 s and scan rate of 0.05 V s^{-1} were selected as optimum instrumental parameters for differential pulse voltammetry. Linear response of peak current on the concentration in the range from 0.5 to 40 μM with coefficient of determination of 0.999, good repeatability (RSD of 1.5%) and detection limit of 0.25 μM were observed without any chemical modifications and electrochemical surface pretreatment. The effect of possible interferents such as stearic acid, glucose, urea, uric acid and ascorbic acid appeared to be negligible which evidently proved the good selectivity of method. The practical analytical utility of proposed method was demonstrated by determination of penicillin V in pharmaceutical formulations (tablets) and human urine samples with satisfactory recoveries (from 98 to 101% for tablets and 97 to 103% for human urine).

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1. Introduction

Penicillins belong to the most familiar group of β-lactam antibiotics which have been used extensively in both veterinary and human medicine practices to prevent the infections of bacteria and fungi [1–3]. Despite the positive effects of penicillins, their occurrence in food or in aquatic environment after consumption presents a serious health hazard [4]. Additionally, the presence of residues of penicillins in animal feedstuff may signify a serious problem because of their eventual spreading to the human food production chain (milk, meat) exerting a negative impact on public health such as severe allergic responses. Thus the analysis of penicillins plays a significant role in drugs chemistry.

Many analytical techniques for determination of penicillins in various matrices such as biological samples or pharmaceutical formulations have been published. High-performance liquid chromatography with different modes of detection, such as ultraviolet detection [5–7] and mass spectrometry [8–11] as well as spectrophotometric [12–15] techniques are among the most popular analytical methods for detection and quantification of penicillins. Generally, these methods belong to the most sensitive and selective, but on the other hand they are long lasting, expensive and often too laborious, when some procedures such as derivatization, extraction and purification are included [16,17]. The electrochemical methods have many inherent advantages such as simplicity, low-cost and possibility of miniaturization that eliminate limitations of other analytical methods [18,19]. On the basis of literature survey only a few papers dealing with an electroanalysis of penicillins were reported in comparison with other drugs. This is probably because oxidation of penicillins usually occurs as not clearly defined waves at a very high positive potential and thus may be affected with electrochemical reactions limiting potential window from the anodic side [20–22]. Some reports dealing with determination of penicillins are based on pulsed amperometric technique [23,24], flow injection analysis [25] or biosensors [26,27]. Stred'anský et al. [28] published a concise report dealing with amperometric pH-sensing biosensors for urea, penicillin and oxalacetate.

Boron-doped diamond (BDD) is a modern electrode material which opens new possibilities of electrochemical investigations due to its excellent features, such as the low background current, wide potential window in aqueous solutions, long-term stability of response, good resistance to surface fouling due to weak adsorption and low sensitivity to dissolved oxygen [29–31]. The versatility of this electrode material has also been utilized for the development of sensors and biosensors [32].

It is important to remark that there were no reports for determination of penicillins on BDD electrode published yet in literature.

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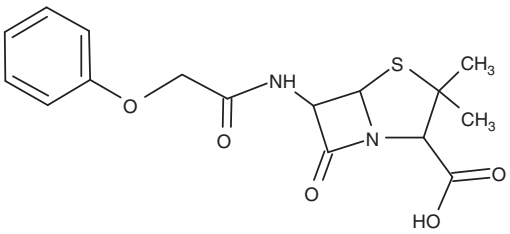

Scheme 1. Structural formula of penicillin V.

This paper demonstrates the application of bare BDD electrode as a sensitive electrochemical sensor for the voltammetric determination of penicillin V (Scheme 1) without any chemical modifications and/or electrochemical pretreatment of electrode surface. This simple, low-cost and practical analytical approach is illustrated on quantification of penicillin V in pharmaceutical formulations (tablets) and human urine samples.

2. Experimental

2.1. Chemicals

Penicillin V (PEN), sugars (glucose, fructose, sucrose, lactose, cellulose and starch) urea, uric acid, stearic acid and ascorbic acid were obtained from Zentiva (Hlohovec, Slovak Republic) and used as received without any further purification. Various supporting electrolytes such as phosphate buffer, Britton-Robinson buffer, acetate buffer, sulfuric acid, sodium hydroxide and acetic acid were of Lachema (Brno, Czech Republic) production. All reagents were of analytical grade. All aqueous solutions were made with double-distilled deionized water with resistivity above 18 MΩ cm. Standard calibration solutions were prepared by appropriate dilution of stock solution with supporting electrolyte.

2.2. Apparatus

All electrochemical experiments were performed in the three-electrode single compartment glass cell. This cell consisted of Ag/AgCl (3 M) reference electrode, platinum wire as counter electrode and bare BDD electrode inserted in an inert polyether ether ketone (PEEK) body with an inner diameter of 3 mm (Windsor Scientific Ltd, United Kingdom) used as working electrode. All potentials reported in this paper were obtained vs. Ag/AgCl electrode at laboratory temperature of 25 ± 1 °C. Voltammetric measurements were carried out using AUTOLAB PGSTAT-302N (EcoChemie, The Netherlands) potentiostat/galvanostat controlled with the NOVA 1.7 software. All pH values were measured with pH meter Model 215 (Denver Instrument, USA), which was calibrated with standard buffer solutions.

2.3. Measurement procedures

20 mL of supporting electrolyte containing an appropriate amount of PEN were added to the electrochemical cell. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were employed without deaeration since oxygen did not influence the oxidation of PEN. Five CV voltammograms were obtained for each measurement, and the last scan was considered for evaluation. The calibration curve was constructed from the average of six consecutive DPV measurements for each calibration standard solution. The detection limit (LOD) was calculated as three times the standard deviation for the blank solution divided by the slope of the calibration curve. The standard addition method was used for analysis of pharmaceutical formulations and human urine spiked with aliquot amounts of PEN.

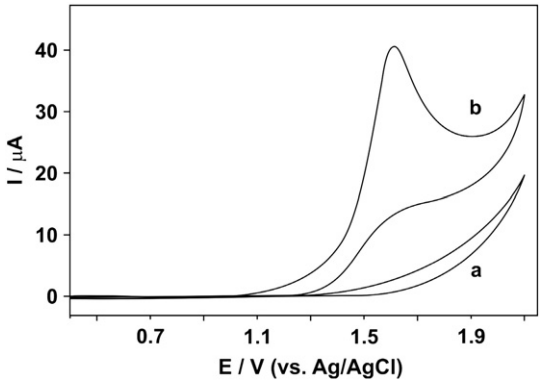

Fig. 1. Cyclic voltammograms of (a) 0 M and (b) 10 μM PEN in ABS at pH 4.0 on bare BDD electrode with scan rate of 0.05 V s^{-1}.

3. Results and discussion

3.1. Cyclic voltammetry

It is well-known that determination of biologically active compounds on bare electrodes is sometimes difficult due to the absence of electrocatalytic behavior of bare electrodes [19]. Cyclic voltammetry was applied to study the electrochemical behavior of PEN on bare BDD electrodes. All necessary factors influencing the current response of PEN were carefully checked to reach the conditions at which the best analytical performance was achieved.

3.1.1. Effects of supporting electrolyte and pH

In order to find the appropriate medium for oxidation of PEN various supporting electrolytes such as sulfuric acid, acetic acid, sodium hydroxide, phosphate buffer solution (PBS), Britton-Robinson buffer solution (BRS) and acetate buffer solution (ABS) were tested. The use of sulfuric acid, acetic acid and sodium hydroxide was inconvenient due to the less sensitive, bad shaped and reproducible current responses (results not shown). The oxidation peak was very well-defined in PBS, BRS and ABS, but the best results were obtained in ABS in which the magnitude of peak current was found to be the 2–3 times higher than in PBS and BRS. Following these facts ABS was chosen in further experiments.

The oxidation peak at about 1.6 V (vs. Ag/AgCl) was registered without any cathodic peak on the reverse scan which clearly indicated that the charge transfer during electrode process is electrochemically irreversible on bare BDD electrode (Fig. 1). It is also apparent that background current appeared to be sufficiently low at higher potentials on bare BDD electrode.

In general, the pH value of supporting electrolyte is a significant factor that affects electrochemical behavior of biologically active

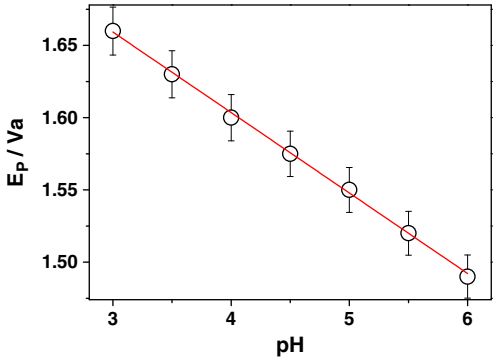

Fig. 2. Effect of pH of supporting electrolyte (ABS) on the peak potential of 10 μM PEN on bare BDD electrode (error bars are constructed for six measurements).

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