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Novel electroanalyical method for the determination of andrographolide from *Andrographis paniculata* extract and urine samples

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ABSTRACT

This paper presents, for the first time, electrochemical behavior of diterpene lactone andrographolide and its voltammetric determination using differential pulse voltammetry (DPV) at boron-doped diamond electrode, in human urine and oil extract from *Andrographis paniculata* seeds. Andrographolide, due to its chemical structure, provides well defined peak at + 1.55 V vs. Ag/AgCl electrode (3 M) in Britton–Robinson buffer solution pH 12. The proposed method, under optimum experimental parameters displays excellent selectivity towards andrographolide in the presence of some interference, as ascorbic acid, uric acid and dopamine, with detection limit of 0.75 μM. Calibration curves were found to be linear in the range from 2 to 40 μM, with two linear ranges, first from 2 to 10 μM and second from 10 to 40 μM with corresponding equations I (μA) = (4.00 ± 0.12) c (μM) + (13.42 ± 0.66) (R^2 = 0.9975) and I (μA) = (0.87 ± 0.05) c (μM) + (46.33 ± 1.53) (R^2 = 0.9767), respectively. Relative standard deviation of 7 measurements was 4% which verified excellent repeatability of proposed method and this method was successfully applied for the determination of andrographolide in human urine sample and extract from the *A. paniculata* seeds. The Prussian blue method was used for validation of the proposed elactroanalytical method.

1. Introduction

Andrographis panuculata, from the family Acanthaceae, is a well known plant in Asia, one of the world's most important plants, especially used for centuries in China as folklore remedy for a wide spectrum of ailments, is nowadays incorporated into a number of herbal medicinal preparations [1–3]. A number of animal studies have shown that extract of *Andrographis paniculata* is biologically active. *A. paniculata* extract showed a protective effect against liver toxicity produce in mice by given them carbon tetra-chloride, alcohol, or other toxic chemicals [4]. This extract was also used for gastric disorders, colds, influenza and other infectious diseases [5–11].

The active components of *A. paniculata* extract are diterpene lactones collectively known as andrographolides found in the aerial parts of the plant and in addition to these compounds some flavonoids and polyphenols were identified to be the major components. Among these components andrographolide was the most important one, showing anti-inflammatory and antimicrobial activity [12–14]. Until now there are several analytical procedures for the determination of andrographolide in *A. paniculata* extract, and in urine samples collected after oral administration. Most of them require expensive equipment like liquid chromatography-tandem mass spectrometry [12], NMR spectroscopy [15], high-performance liquid chromatography [3], and several manipulating steps.

Electroanalytical methods are one of the most sensitive techniques for determination of traces of numerous compounds because of its remarkably low detection limit [16–18]. Other advantageous features of electroanalytical methods include relatively low cost instrumentation and the capability for simultaneous determination.

Based on this, the aim of this work was to develop a new, cheap and sensitive method for the determination of andrographolide, using boron-doped diamond (BDD) electrode as one of the best electrode materials [19], environmental friendly (green) and widely used electrode for determination of biologically active molecules. Under the optimal parameters this method was successfully applied for determination of andrographolide in urine samples and in the extract of *A. paniculata*.

2. Experimental

2.1. Apparatus and reagents

Andrographolide, boric acid, sodium hydroxide, ethanol, acetic acid and phosphoric acid were purchased from Sigma Aldrich and used as received without any further purification. Stock solution 10^{-3} M of the

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tested compound was prepared in ethanol/ double distilled water. Calibration standard solutions were prepared from the stock solution by appropriate dilution with supporting electrolyte. Britton–Robinson buffer solution was prepared in the usual way by mixing 40 mM of all necessary components (phosphoric acid, acetic acid and boric acid). The pHs of different Britton–Robinson buffers were adjusted with sodium hydroxide (0.2 M).

Cyclic voltammetric and differential pulse voltammetric measurements were performed using an electrochemical system AUTOLAB PGSTAT 302N, Methrom Autolab B.V. (The Netherlands), controlled by the corresponding software (NOVA 1.10). The cell (10 ml) consisted of three-electrode system, boron-doped diamond electrode (inner diameter of 3 mm; Windsor Scientific Ltd., Slough, Berkshire, United Kingdom), an Ag/AgCl (saturated KCl) reference electrode and Pt counter electrode. All potentials reported in this paper were obtained vs. Ag/AgCl reference electrode at an ambient temperature. All pH values were measured with pH meter model Orion 1230.

The potential was swept over the range from 0 to +1.8 V (vs. Ag/AgCl) at different scan rates for CV, and from 0.5 to +1.8 V vs. (Ag/AgCl) at the optimized instrumental parameters (step potential 5 mV, modulation amplitude 50 mV, modulation time 40 ms) for differential pulse voltammetry.

For the Prussian blue method the different volumes of andrographolide were prepared in range as for DPV. The following mixture was added to each dilution of control solutions: 400 μl of 0.0008 M $K_4Fe(CN)_6$ and 400 ml of $FeCl_3$ in 0.1 M HCl solution. The final volume was 10 ml. Seven minutes later absorbance was measured at 700 nm.

2.2. Sample preparation

Dried seeds of *A. paniculata* were purchased from a local herbal store. The plant material was ground into coarse powder by electrically driven grinder and was soaked in 70% aqueous-methanol for 1 h under reflux. The soaked material was filtered and the procedure was repeated one more time. The combined filtrate was evaporated and obtained oil was stored in the fridge at 4 °C. Human urine samples were collected from two different persons, and 100 μl of urine was added in cell and diluted with supporting electrolyte to make 10 ml of tested solution. Standard addition of andrographolide caused current increment at the sample potential and made the determination of andrographolide in the urine sample possible.

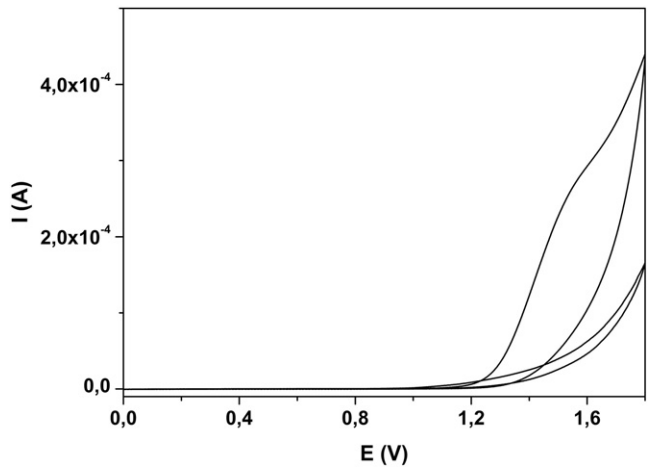

Fig. 1. Cyclic voltammograms of BR buffer solution pH 12 in the presence and absence of 0.1 mM andrographolide at BDD electrode, scan rate 100 mV/s.

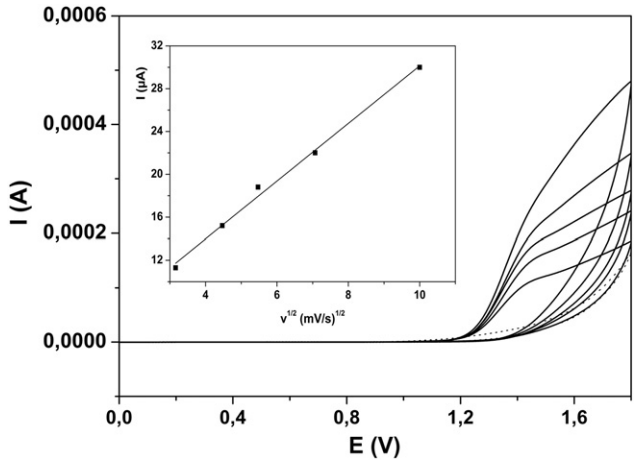

Fig. 2. Cyclic voltammograms of andrographolide at different scan rates (10, 20, 30, 50 and 100 mV/s) using 0.1 mM andrographolide in BR buffer solution pH 12 at BDD electrode. Inset figure presents the dependence between peak current and square root of scan rate under the same experimental conditions.

3. Results and discussion

3.1. Electrochemical behavior of andrographolide

Andrographolide as diterpene lactone, due to its complicated structure, provides well defined peak at very high potential and in string basic medium. Probably because of that, until now no one in the literature has dealt with the behavior and determination of andrographolide using electrochemical methods. In Britton–Robinson buffer solution at pH lower than 11 peak of andrographolide does not occur or it happens at very high potential, higher than 1.8 V, and cannot be observed with proposed sensor and in suggested supporting electrolyte. This study confirms advantages of BDD electrode compared with other solid and unmodified electrodes and could be an interesting topic for the field of the modified electrodes and their application in bioelectrochemistry. In buffer solution pH 12 this diterpene provides a well defined and singe peak at +1.55 V (Fig. 1). At this pH effect of scan rate (Fig. 2) and dependence of peak current from square root of scan rate (inset of Fig. 2) were evaluated. Peak current started to increase with increasing scan rate but shifts in peak potential were not significant. The linear Randles–Sevcik plot indicates the diffusion-controlled nature of the

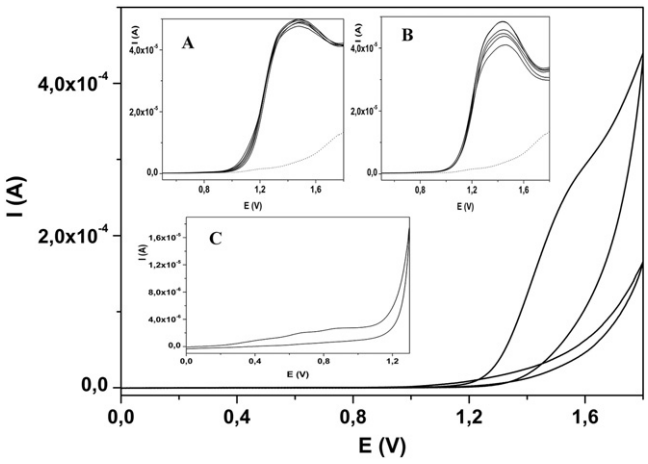

Fig. 3. Cyclic voltammogram of andrographolide obtained under optimized experimental conditions. Inset A: 7 measurements of andrographolide (0.01 mM) without interferences; B: 7 measurements of andrographolide (0.01 mM) with AA, UA and DOP as interferences in the same concentration level; C: CV voltammograms of AA, UA and DOP under the same experimental conditions.