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Sequential injection-adsorptive stripping voltammetric quantitation of purine nucleobases using an electrochemically activated carbositall electrode

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ABSTRACT

The present paper describes an electroanalytical application of an activated carbositall electrode (ACSE) for the detection of purine nucleobases (adenine and guanine) and their derivatives by sequential injection-adsorptive stripping voltammetric approach (SI-AdSVA). It was found that ACSE characterized by significant electrocatalytic and adsorptive abilities toward investigated compounds over a wide pH range, good stability and reproducibility under the fixed hydrodynamic conditions. Utilizing on-line adsorption accumulation of an analyte during 120 s, the limit of detection (S/N = 3σ) was achieved down to 8 ng/mL (50 nM) and 10 ng/mL (75 nM) for guanine and adenine, respectively. It was demonstrated that the proposed SI-AdSVA method could be efficiently used for the routine analysis of real samples with satisfactory results.

1. Introduction

The purine nucleobases, guanine (**Gua**) and adenine (**Ade**), are derivatives of purine, consisting of a fused pyrimidine-imidazole ring system with conjugated double bonds (Scheme 1). Gua and Ade are important chemical fragments of DNA and RNA, which play a significant role in the storage of genetic information and protein biosynthesis [1].

High demand in the quantitative determination of these compounds and their derivatives is caused by the need to solve a variety of fundamental and applied problems in medicine and pharmacology, mainly for analysis of biological samples and for evaluating the quality of pharmaceutical formulations [2]. The use of automated flow-based methods seems to be the most advanced strategy for these purposes. To date, the application of high-performance liquid chromatography [3], capillary electrophoresis [4] and flow injection (**FI**) chemiluminescence [5] for the quantitation of Gua and/or Ade in biological and pharmacological samples has been reported.

In recent years, a new FI-method for the simultaneous determination of purine bases has been proposed by using dual electrochemical detector containing a chitosan carbon nanofiber modified glassy carbon electrode (**GCE**) [6]. A novel chemically modified GCE has been also applied to develop an automatic procedure for the determination of Gua and Ade based on the sequential injection (**SI**) concept in lab-on-valve format [7]. Remarkable advances in electroanalytical chemistry based on FI- and SI-principles have been demonstrated, especially in automation and miniaturization of laboratory assays [8–14].

The main problem of the electrochemical detection of purine nucleobases is connected with their direct electrooxidation at bare solid electrodes at high overvoltage. Therefore, there has been intensive research interest in the determination of Gua and Ade by means of different techniques based on novel bare electrodes or various chemically modified electrodes (**CMEs**). In particular, modification-free electrochemical approach for sensitive determination of purine DNA bases in biological samples using boron-doped diamond electrode has been described [15]. Numerous CMEs have been constructed for enhancing electrochemical signals of Gua and/or Ade by using single-walled carbon nanotubes (**CNTs**), multi-walled carbon nanotubes (**MWCNTs**) and nanostructured composites [16,17], including microwave-assisted prepared $La(OH)_3$/CNTs [18], β-cyclodextrin/CNTs [19], CeO_2 nanoparticles/MWCNTs [20], polythionine/gold nanoparticles/MWCNTs [21]. However, technologies of the majority of CMEs are complicated, time consuming and costly. Furthermore, the operating time of such sensors is usually rather short, which limits their application in automated systems. Voltammetric sensors produced by electrochemical activation of the carbon seem to be more suitable for these purposes. Compared to other modification procedures,

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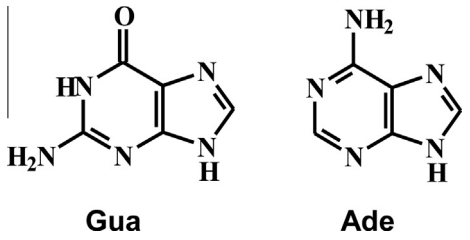

Scheme 1. Molecular structures of Gua [2-amino-1*H*-purin-6(9*H*)-one] and Ade [9*H*-purin-6-amine] considered in this study.

electrochemical activation bears several advantages. Typically, the unique properties of the activated carbon, such as the large surface area and the presence of redox and acid-base functional centers, provide electrocatalytic response toward many biologically active compounds [22–25].

In the present work, we report the results of electrochemical investigation of the redox behavior of a carbositall electrode (**CSE**) previously activated by using ultrasonic irradiation and cyclic voltammetry techniques. The utility of an activated CSE (**ACSE**) as sensor for detection of Gua and Ade was first time investigated by incorporating it in a computer-controlled SI-system coupled with adsorptive stripping voltammetry (**AdSVA**). In light of the results obtained to date, carbositall is a synthetic pyrocarbon material that consists of nanocarbon phase containing large and small monocrystalline inclusions of boron (4–5%). The production process of this material is based on oriented crystallization under pyrolysis of hydrocarbons in the presence of halides of refractory metals [26]. The remarkable properties of carbositall (e.g. high surface-volume ratio, high electrical conductivity, wide potential window in the positive range, chemical inertness and strong mechanical strength) created interest in its electrochemical applications [27].

2. Materials and methods

2.1. Reagents and solutions

Reagents used were purchased from Sigma–Aldrich Chemical Corp. USA (purity: ACS). Stock solutions of Ade and Gua (0.01 M and 1.0 mg/L) were prepared by dissolving their hydrochloride salts in NaOH (0.05 M or 5.0 mM). All solutions were stored in the dark at 4 °C. Working standard solutions were prepared by stepwise dilution of the respective stock solution with a supporting electrolyte. These solutions should be used within 12 h. Different supporting electrolytes such as acetate buffer solutions (ABSs), Britton–Robinson buffer solutions (BRBSs) and phosphate buffer solutions (PBSs) containing 0.1 M KCl were tested. PBSs of various pH values were prepared from 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 and adjusting pH with 0.1 M H_3PO_4 or 5.0 mM NaOH. All of the other compounds were of analytical grade and used without further purification. Doubly distilled water with resistivity of 3–5 MΩ cm^{-1} at 25 °C was used throughout all the experiments. The solutions were deoxygenated by passing nitrogen gas.

2.2. Apparatus

All voltammetric measurements were performed by using an Ecotest-VA analyzer (Econix-Expert, Russia) controlled by means of MDEV software. A conventional cell with three electrodes was employed for cyclic voltammetric experiments. A carbositall dick electrode (Volta, Russia) was used as a working electrode; an Ag/AgCl/KCl (3 M) electrode as a reference electrode and a platinum wire as an auxiliary electrode.

An automatic FIALab 3500-analyzer with programmable flow (FIAlab Instruments, Inc., WA, USA) was used for the development of the SI-AdSVA method. The operation manifold was composed of a 2.5 mL syringe pump (**SP**), a 600 μL holding coil (**HC**), a multi-position selection valve (**MPV**) with eight ports and a mixing chamber (**MC**) connected to a home-made three electrode flow cell (**FC**). 0.1 M PBS (supporting electrolyte) was linked with the in-position mode. A sample solution was linked with port-1 in the MPV. Four standard solutions were attached to ports 2–5. $HClO_4$ was linked with port-7.

Scanning electron microscopy (**SEM**) measurements in ambient conditions were performed with a Carl Zeiss NVision 40 electron microscope (Carl Zeiss Group, Germany). pH values were tested by using a pH-meter Model OP-110 (Radelkis, Hungary). The ultrasonic bath Elmasonic One (Germany) was used for irradiation of a pure CSE with 35-kHz ultrasound.

2.3. CSE surface activation

The activation procedure of a pure CSE surface was carried out by using ultrasonic irradiation and cyclic voltammetry techniques. Before activation, an electrode surface was polished with 0.3 and 0.05 μm alumina powders to mirror like. The polished electrode was ultrasonically irradiated subsequently in anhydrous ethanol for 3 min and doubly distilled water for 2 min. Then, it was dried with highly purified nitrogen gas in air. The pre-treated CSE was electrochemically activated in 0.1 M $HClO_4$ by scanning potential in the range of (0.0 to +1.5) V for 20 cycles (voltage scan rate $\boldsymbol{\nu}$, 50 mV/s). Further, this electrode was scanned between +0.2 to +1.4 V in 0.1 M PBS until a steady-state current–voltage curve was obtained. The activated CSE was stored in air at room temperature.

2.4. Operating procedure

Fig. 1 shows a scheme of the SI-AdSVA system, which was constructed to perform on-line controlled adsorptive accumulation of an analyte at the electrode, followed by linear-sweep stripping voltammetric measurements of the surface species in a supporting electrolyte medium under the stopped-flow conditions.

A protocol controlling the analytical cycles of the proposed SIA procedure was programmed. Following this protocol, a sample solution (600 μL) was aspirated into the HC using the SP. After flow reversal, the sample zone was transported to the electrochemical FC at the required flow rate (2–20 μL/s). During the contact time between the sample and the working electrode, the analyte accumulated onto the electrode surface at an open circuit. After accumulation step, 0.1 M PBS was propelled through the FC. A stripping voltammogram was recorded under the stopped-flow conditions in the anodic potential range from 0.2 V to 1.4 V. The resulting stripping peak current of purine nucleobases was measured. Before the beginning of a new sample pre-treatment step, for removing adsorbed compounds, the electrode surface was regenerated by scanning potential (number of scans = 5) in the blank PBS for about 7 cycles. $HClO_4$ was used to on-line activate the electrode surface, when necessary.

For calibration measurements, standard solutions were used instead of a sample solution. For standard addition procedure, the sample and a standard solution (10–40 μL) were aspirated sequentially into the HC and then they were directed into the MC for a faster homogenization. The resulting mixture solution was then propelled toward the FC to be deposited. To study the accuracy of the proposed method and to check the interferences from excipients of the dosage forms, recovery experiments were carried out. All currents were corrected for the current measured for the blank solution.