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Determination of guanine and adenine by high-performance liquid chromatography with a self-fabricated wall-jet/thin-layer electrochemical detector at a glassy carbon electrode

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ABSTRACT

A sensitive wall-jet/thin-layer amperometric electrochemical detector (ECD) coupled to high-performance liquid chromatography (HPLC) was developed for simultaneous determination of guanine (G) and adenine (A). The analytes were detected at a glassy carbon electrode (GCE) and the HPLC – ECD calibration curves showed good linearity ($R^2 > 0.997$) under optimized conditions. Limits of detection for G and A are 0.6 nM and 1.4 nM ($S/N=3$), respectively, which are lower than those obtained with an UV–vis detector and a commercial electrochemical detector. We have successfully applied this HPLC – ECD to assess the contents of G and A in hydrochloric acid-digested calf thymus double-stranded DNA. In addition, we compared in detail the analysis of G and A by cyclic voltammetry (CV) and by the HPLC – ECD system on both bare GCE and electroreduced graphene oxide (ERGO) modified GCE. We found that the adsorption of G and A on the electrode surfaces can vary their anodic CV peaks and the competitive adsorption of G and A on the limited sites of the electrode surfaces can cause crosstalk effects on their anodic CV peak signals, but the HPLC – ECD system is insensitive to such electrode-adsorption and can give more reliable analytical results.

1. Introduction

Nucleic acid is a kind of biomacromolecules of organism, which store and transmit genetic information in protein biosynthesis. Thus, it takes a critical part in great life phenomena such as growth, heredity, variation, and so on. Guanine (G) and adenine (A) are two important components found in nucleic acid and play crucial roles in physiological and pathological activies. Their levels are considered as important parameters for diagnosis of tumor, AIDS, epilepsy, myocardial cellular energy status, disease progress, and therapy responses [1–3]. Hence, the determination of G and A has great significance in clinical research and molecular biology investigation [4,5].

Until now, a large number of methods have been established for G and/or A analysis, including electrochemical methods [6–14], capillary electrophoresis [15], flow injection-chemiluminescence [16], micellar electrokinetic chromatography [17], resonance Rayleigh scattering [18], fluorimetric detection [19,20], and so on. High-performance liquid chromatography (HPLC) is one of the most widely used techniques for quantitative analysis in complicated matrices [21–27]. Due to its excellent selectivity and sensitivity, HPLC coupled with electrochemical detector (HPLC – ECD) may be a good choice for the quantitative analysis of electroactive compounds in complicated biological samples. As far as we know, the amperometric ECD, one of the main ECDs for HPLC (another commonly encountered ECD is the coulommetric ECD), can be classified into four categories, namely, thin–layer mode, wall–jet mode, open–tubular electrode mode, and in–tube electrode probe mode, according to the configuration of detection electrode against the eluent [28]. Among those modes, the thin–layer and wall–jet modes are the most common in the reported HPLC–ECD systems. However, inconvenience of the electrode rinse in the thin–layer mode and a limited response signal resulted from the transience of the HPLC liquid jetted onto the electrode surface in the wall–jet mode can restrict their effectiveness in electrochemical detection. Hence, a combination of wall–jet and thin–layer modes, namely, a wall–jet/thin–layer amperometric ECD, can well integrate the advantages of both modes and weaken their respective shortcomings, yielding an ECD with enhanced sensitivity and more convenient washing/refreshment of the working electrode

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surface [28]. To the best of our knowledge, no investigation on such a self-fabricated wall–jet/thin–layer amperometric ECD for quantitative analysis of G and/or A has been reported to date.

Herein, we report a home-made integrated wall-jet/thin-layer amperometric ECD coupled to HPLC for the simultaneous determination of G and A at a glassy carbon electrode (GCE), as depicted in Scheme S1. Our detector here possesses the following advantages and significance. (1) The analytes are ejected from the HPLC outlet tube and reach the center of the working electrode, then flow around the electrode surface, thus we could improve the electrolysis efficiency and enhance the sensitivity by increasing the electrode area; (2) its simplicity and facile operation make the working electrode be treated easily and modified conveniently with any conductive and/or electrocatalytic material for wider applications. We will apply it to determine purine bases in hydrochloric acid-digested calf thymus double-stranded DNA (dsDNA) and compare its performance with an UV–vis detector and a commercial electrochemical detector. Furthermore, we will compare the influences of electrode adsorption of G and A in conventional cyclic voltammetry (CV) electroanalysis and HPLC – ECD analysis.

2. Materials and methods

2.1. Instruments and materials

The HPLC system comprised a LC-20AT binary high-pressure pump (Shimadzu, Kyoto, Japan), a UV–vis spectrophotometric detector SPD-20AV (Shimadzu, Kyoto, Japan) and a manual injector with a 20.0 μL sample loop. The commercial L-ECD-6 A electrochemical detector (Shimadzu, Kyoto, Japan) for HPLC, consisted of a glassy carbon plate working electrode, an Ag/AgCl reference electrode and a stainless steel nipple auxiliary electrode. The mobile phases were filtered and degassed by a SHB-3 type vacuum pump (Zhengzhou Dufu Instrument Factory, Zhengzhou, Henan, China) and the pH of buffer solution was measured with a Leici PHS-3C pH meter (Shanghai Precision & Scientific Instrument Co. Ltd., Shanghai, China). A self-fabricated wall-jet/thin-layer ECD and a CHI660A electrochemical workstation (Shanghai Chenhua Instrument Co. Ltd., Shanghai, China) was used for HPLC analysis, as schematically depicted in Scheme S1. We employed a three–electrode configuration consisting of a GCE as the working electrode (5 mm in diameter), a KCl-saturated calomel electrode (SCE) as the reference electrode and a carbon rod as the counter electrode. A plexiglass plate (0.25 cm in thickness) was pasted on the bottom of a plastic vessel and a hole of ca. 0.10 cm diameter was drilled in the plexiglass plate. The HPLC outlet tube was passed through the hole and its end was just horizontal to the upper surface of the plate. We pressed the working electrode firmly against the hole in plexiglass plate and the HPLC outlet tube was placed against the center of the working electrode. Thus the space between the working electrode surface and the plate can form a thin layer. All potentials are reported versus SCE unless otherwise specifically stated. Amperometric current-time curves were generated using a potentiostatic method with the accompanying software of the CHI660A electrochemical workstation.

G and A were obtained from Amresco (Solon, OH, USA). Calf thymus dsDNA and graphene oxide (GO) were purchased from Sigma (St. Louis, Missouri, USA) and Xianfeng Nanotechnology Inc. (Nanjing, Jiangsu, China), respectively. HPLC-grade methanol was purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Phosphate-buffered saline (PBS) solution was prepared with KH_2PO_4 and $K_2HPO_4 \cdot 3H_2O$ (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) unless otherwise specified, the pH of which was adjusted to the desired value by concentrated phosphoric acid (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China). Other chemicals used were of analytical grade or better quality and Milli-Q ultrapure water ($>$ 18 MΩ cm, Millipore, Boston, Massachusetts, USA) was used throughout the experiments.

2.2. Chromatographic conditions

The HPLC analysis was carried out on a Shim-pack VP-ODS (i.d. 5 μm, 150 × 4.6 mm) column (Shimadzu, Kyoto, Japan) and the flow rate was 1.0 mL min^{-1}. An isocratic elution of the mobile phase consisting of 0.01 M PBS (pH 7.00)-methanol (85:15, v/v) was used. Prior to use, the mobile phase was filtered through a 0.22 μm membrane and degassed by a vacuum pump. All sample solutions should be filtered through 0.22 μm filters before injection. The column temperature was ambient and the electrochemical detector was placed behind the UV detector operating at 254 nm.

2.3. Preparation of electroreduced graphene oxide modified GCE

The accurately weighed amount of GO was dispersed in 0.10 M PBS (pH 7.40) consisting of $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 12H_2O$ and subsequently ultrasonicated for 30 min to obtain 1 mg mL^{-1} GO dispersion. Prior to use, the GCE was first polished with 1.0 and 0.05 μm alumina slurry sequentially and then ultrasonically washed in ethanol and water for 5 min, respectively. The electroreduced graphene oxide modified GCE (ERGO/GCE) was prepared by immersing the cleaned GCE into GO dispersion and then scanning 10 cycles in a potential range from 0 to −1.50 V with a scan rate of 25 mV s^{-1}.

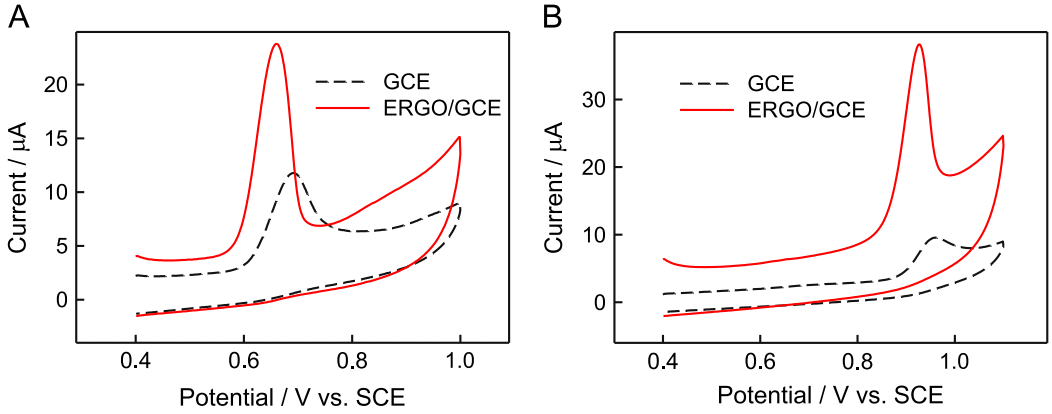

Fig. 1. Cyclic voltammograms of 50.0 μM G (panel A) and 50.0 μM A (panel B) in 0.10 M PBS (pH 7.00) obtained at the bare GCE (black dashed curves) and the ERGO/GCE (red solid curves). Scan rate: 100 mV s^{-1}.