Electrochimica Acta 123 (2014) 485–493

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

A flow injection analysis coupled dual electrochemical detector for selective and simultaneous detection of guanine and adenine

Rajendiran Thangaraj [a], Subramanian Nellaiappan [a], Raja Sudhakaran [b], Annamalai Senthil Kumar [a,*]

[a] Environmental and Analytical Chemistry Division, School of Advanced Sciences, Vellore Institute of Technology University, Vellore-632 014, India
[b] Aquaculture Biotechnology Laboratory, School of Bioscience and Technology, Vellore Institute of Technology University, Vellore-632 014, India

ARTICLE INFO

Article history:
Received 1 November 2013
Received in revised form 9 January 2014
Accepted 10 January 2014
Available online 23 January 2014

Key words:
Flow injection analysis
Dual electrochemical detector
Bipotentiostat
Guanine
Adenine.

ABSTRACT

Adenine (**A**) and guanine (**G**), important bases of nucleic acids, are often analyzed by separation coupled spectroscopic detection methods. Herein, we are demonstrated a new flow-injection analysis (FIA) coupled dual electrochemical detector (DECD), where a chitosan-carbon nanofiber (Chit-CNF) modified glassy carbon electrode prepared by a simple technique and pH 7 phosphate buffer solution as a carrier system, for separation-less quantification of **G** and **A**. This method is highly selective and no interference by the presence of the other DNA bases (Thymine and Cytosine). The FIA-DECD was operated at two different operating potentials, E1 = 0.80 V and E2 = 0.95 V vs Ag/AgCl, where **G** and {**G** + **A**} get oxidized, respectively. Amount of **A** was calculated from the difference between the FIA current signals, measured at $E2_{0.95V}$ and $E1_{0.80V}$. The GCE/Chit-CNF was characterized by cyclic voltammetry with potassium ferricyanide system and Raman spectroscopy. The modified electrode showed unique electron-transfer feature with metal like conductivity. Under an optimal condition, FIA-DECD showed linear calibration plots for **G** and **A** in a concentration range, 200 nM—50 μM with current sensitivity values 13.83 ± 0.48 and 4.84 ± 0.11 nA μM^{-1} respectively. Calculated detection limit (signal-to-noise ratio = 3) values were 46.8 nM and 73.8 nM for **G** and **A** respectively. Applicability of the present technique was further demonstrated by detecting **G** and **A** in beef kidney sample and DNA hybridization process.

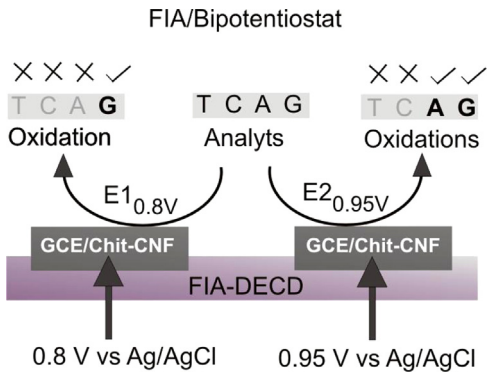
1. Introduction

Adenine (**A**) and guanine (**G**) are the building blocks of both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) that plays a crucial role in protein biosynthesis and the storage of genetic information [1]. Selective and sensitive detection of the purine bases provides valuable insights in fundamental fields such as understanding of DNA sequence, oxidative damage and hybridization and protein metabolism in cells, protein-DNA interactions, etc [2–4]. Commonly used quantification technique for **G** and **A** are separation coupled spectroscopic methods. For instance, ion-pair reversed phase high performance liquid chromatography and capillary electrophoresis coupled UV [1,5,6], micellar electrokinetic chromatography with indirect laser-induced fluorescence detection (ILIFD) [7] and high performance liquid chromatography coupled mass spectrophotometer (HPLC-MS) [8] techniques were reported for the detection of **G** and **A**. Each method has its own strengths and weakness in terms of analytical performance. UV based detectors are versatile; however owing to low extension coefficient, sensitivity of the signals are very low. Similarly, the ILIFD and mass spectroscopy based detection technique allow to detect low concentration of purine bases; beside with respect to the instrumentation cost, off-line sampling preparation, run-time and skilled person requirement, the above techniques are not suitable for routine analytical measurements. Hence, it is highly challenging research to develop a new technique which full fills all majority of the above mentioned criteria. Herein, we introduce a dual electrochemical detector (DECD) based flow injection analysis technique (FIA-DECD) for rapid and simultaneous detection of **G** and **A** without any derivatization and separation procedure.

Electro-analytical techniques offer simple, less-expensive, highly sensitive and selective analytical approach extendable to disposable type screen printed electrode and miniaturization. In the past, there were several electrochemical methods (cyclic and pulse voltammetric techniques), in which various chemically modified electrodes (CMEs) as working systems were reported for simultaneous detection of **G** and **A** with the test sample volume about 10 mL. Following are the representative CMEs:

* Corresponding author. Tel.: +91 416 2202754; fax: +91 416 2243092.
E-mail address: askumarchem@yahoo.com (A.S. Kumar).

0013-4686/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.electacta.2014.01.066



Scheme 1. Illustration for the flow injection analysis coupled with dual electrode system (FIA-DECD) for simultaneous detection of guanine and {guanine + adenine} at two different operating potentials, $E1_{0.80V}$ and $E2_{0.95V}$. Adenine's current can be calculated from the current difference between $i_{E2,0.95V}$ and $i_{E1,0.8V}$.

TiO_2-graphene oxide nanocomposite [9], PbO_2-carboxylated carbon nanotubes (CNT)-ionic liquid composite film [10], CeO_2 nano particles decorated multiwalled carbon nanotube (MWCNT) [11], microwave-assisted prepared carbon nanotube/$La(OH)_3$ nanocomposite [12], polythionine/NPAu/MWNTs modified electrode [13], graphene–nafion composite film [14], β-cyclodextrin(β-CD)/MWCNTs modified electrode [15], potassium/phenanthrene doped MWCNTs incorporated poly(new fuchsin) composite film (MWCNT-PNF)) [16], and f-MWCNT-gold-hydroxypropyl β-CD composite film (f-MWCNT, f = functionalized) (f-MWCNT-β-CD-Au) [17], $NiFe_2O_4$ magnetic nano particles decorated MWCNTs [18], 2,6-pyridinedicarboxylic acid/graphene composite film [19], graphitized mesoporous carbon modified GCE [20], carboxylic acid functionalized graphene modified glassy carbon electrode [21] and TiO_2 nano particles-magnesium doped zeolite Y modified carbon paste electrode [22]. Unfortunately, most of the recently reported working electrodes are either expensive or having tedious preparation routes. For instance, f-MWCNT-β-CD-Au involved with expensive chemicals such as functional carbon nanotube and Au, and the MWCNT-PNF needs over 48 hours time for the electrode preparation [16]. In further, working with 10 mL volume is not practically viable for DNA based real sample analysis; where the samples are expensive and difficulty in getting required volume (10 mL) for the analysis. Alternatively, flow injection analysis (FIA) coupled electrochemical detection (ECD) technique (FIA-ECD; single working electrode) is a suitable one for low volume detection of real sample (5-50 μL) with high sensitivity and selectivity. Few reports were available for the detection of either **G** or **A** individually [23,24] or inbound DNA [25,26] by FIA-ECD.

To the best of our knowledge, simultaneous detection of **G** and **A** without any separation technique by FIA-DECD is not yet attempted. Complication due to the co-electro-oxidation of both the purine bases, **G** and **A** restricts the technique for the selective analysis [27]. In further, electrochemical oxidation of one of the DNA bases, is often influenced by presence of other bases [28]. In this work, we resolved the problem by taking two discrete working electrodes as electrochemical detectors coupled with a bipotentiostat for simultaneous FIA of **G** and **A**. In this technique, **G** was detected at 0.80 V ($E1_{0.80V}$), while **A** along with **G** was determined at 0.95 V vs Ag/AgCl ($E2_{0.95V}$) simultaneously. In further by suitably subtracting the $E1_{0.80V}$'s current signal with $E2_{0.95}$'s value one can easily calculate the **A**'s current contribution (Scheme 1). A new carbon nanofiber (CNF)-chitosan modified glassy carbon electrode (GCE/Chit-CNF) introduced here is a low cost one (ten times lesser price than impure-MWCNT) and can be prepared within 40 ± 2 minutes without any linkers [16,17]. Furthermore, quantification of **G** and **A** in beef kidney and DNA hybridization were successfully demonstrated. Note that CNF has cylindrical nanostructure with different stacking arrangements of graphene sheets such as stacked platelets, ribbon or herringbone [29]. The mechanical strength and electrical properties of CNF are closer to that of the MWCNT [30]. The primary distinguishable character of CNF from MWCNT is the stacking of graphene sheets of varying shapes, producing more edge sites on the outer wall of the CNF than the MWCNT. Such edge plane defects may facilitate the electron-transfer of electro-active analytes [31,32]. In further we found in this work that CNF has relatively less adsorption of aromatic organic compounds than the MWCNTs. Such property is highly desirable for easy reproducible working electrode surface in FIA-ECD.

2. Experimental

2.1. Chemicals and Instruments

Chitosan, graphitized carbon nanofibers (diameter 100 nm, length 20-200 μm), adenine, guanine, single-stranded probe DNA (5′-AACCAGAGTGGTGGATGGAA), complementary probe DNA (5′-TTGGTCTCACCACCTACCTT) and non-complementary DNA (5′-GTCGACGAACTTCACTGGGA) were obtained from Sigma-Aldrich. Aqueous solutions were prepared by using deionized alkaline $KMnO_4$ double distilled (DD) water. Unless otherwise stated pH 7 phosphate buffer solution (PBS) of 0.1 mol L^{-1} ionic strength was used as a supporting electrolyte solution.

Electrochemical measurements were carried out using a CHI model 660 C electro-chemical work station, USA with 10 mL working volume. The three electrode system consisted of GCE with 0.0707 cm^2 geometrical surface area and its CME as a working electrode, Ag/AgCl with 3 M KCl as a reference electrode, and platinum wire as a counter electrode. The Bio-analytical system (BAS, USA) polishing kit was used to polish the GCE surface. Hydrodynamic amperometric measurements were done using a bipotentiostat instrument (CHI 760D electrochemical workstation, Austin, TX, USA). The FIA system consisted of Hitachi L-2130 pump delivery, a Rehodyne model 7125 sample injection valve (20 μL loop) with interconnecting Teflon tube and a conventional electrochemical cell (BAS, USA) [33]. A DECD with two glassy carbon electrodes of similar geometric area (0.0707 cm^2) placed 2 mm apart purchased from BAS was used as a dual working electrode. FEI Quanta FEG 200 instrument was used for FE-SEM analysis. Raman spectroscopic analysis was performed using AZILTRON, PRO 532, (USA) with 532 nm laser excitation. Following optimal DPV parameters were used for electrochemical measurements: increment = 4 mV; amplitude = 50 mV; pulse width = 0.2 s; pulse period = 0.5 s and sampling width = 0.0167 s.

2.2. Preparation of the chemically modified electrode (GCE/Chit-CNF)

Initially, the GCE was cleaned both mechanically (polished with 0.05 micron alumina powder, cleaned with acetone and washed with DD water) and electrochemically (by performing cyclic voltammetry (CV) for 10 cycles in the potential window -0.2 V to 1.0 V vs Ag/AgCl at the potential scan rate (ν) of 50 mV s^{-1} in pH 7 PBS), before each experiment and served as an underlying substrate. For preparation of the GCE/Chit-CNF modified electrode, 1 mg of CNF dispersed in 500 μL of 0.1% chitosan solution, sonicated for 5 ± 2 minutes in a water bath at room temperature, was drop coated on the cleaned GCE surface and allowed to air dry for 30 ± 2 minutes at room temperature (25 ± 2 °C) to get near uniform thin layer on GCE. In total, 40 ± 2 min time required for the electrochemical detector preparation.