

Fabrication of Cu–CeO₂ Coated Multiwall Carbon Nanotube Composite Electrode for Simultaneous Determination of Guanine and Adenine

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Abstract: A copper nano particles and cerium (IV) oxide modified carbon nanotube based composite on glassy carbon electrode (Cu–CeO₂/MWCNT/GCE) was fabricated for simultaneous determination of guanine and adenine. The surface morphology, chemistry and conductance of the prepared electrodes were characterized by scanning electron microscopy (SEM), energy dispersion X-ray (EDX), X-Ray photoelectron spectroscopy (XPS) and electrochemical impedance spectroscopy (EIS). The Cu–CeO₂/MWCNT/GCE improved electrochemical behaviour of guanine and adenine compared to other electrodes. The modified electrode was also used for

individual and simultaneous determination of guanine and adenine. Under optimized conditions, the calibration curves were obtained linearly in the range of 0.20 to 6.00 µM for the guanine and 0.10 to 8.0 µM for the adenine by differential pulse voltammetry. The limits of detection of guanine and adenine were calculated as 0.128 and 0.062 µM, respectively. Interferences studies were also performed in the presence of inorganic and organic compounds. Moreover, the determination of guanine and adenine contents were carried out in a calf thymus DNA sample by the developed method with satisfactory results.

Keywords: metal nanoparticle • metal oxide • multiwall carbon nanotubes • adenine, guanine

1 Introduction

Guanine and adenine, two essential purine bases of deoxyribonucleic acid (DNA), play main role in life processes such as protein synthesis and storage of heritagenetic information in living organism. Both two bases have well-known effects on coronary system, cerebral circulation, blood flow, cardiac arrhythmias and neurotransmitter release [1–4]. The abnormal changes in the ratio of these compounds in organism may cause some diseases such as cancer, HIV, epilepsy, tumor-genesis, mental retardation, Parkinson's disease, and liver diseases [5–7]. Therefore; simple, cheap and fast analytical methods are needed for sensitive and selective determination of these two bases. For this purpose, various analytical methods such as high performance liquid chromatography [8], laser induced fluorescence detection [9], capillary zone electrophoresis [10], chemiluminescence [11], spectroscopic methods [12] and mass spectrometry [13] have been employed for the determination of guanine and adenine in recent years.

As compared to mentioned analytical methods, electroanalytical techniques especially voltammetry has attracted due to its high sensitivity, simple and cheap equipment, fast response in-situ conditions [14–17]. However, simultaneously analysis of adenine and guanine on the bare electrodes is too difficult due to slow electrochemical oxidation, high oxidation potential and overlapping of their oxidation peaks which lead to low sensitivity and selectivity. In order to overcome these problems various kinds of materials for electrode mod-

ification have been developed and used for analysis of guanine and adenine in DNA [7,18–21]. In recent years, varieties of materials have been used for this purpose such as, carbon nanotubes [1,5], graphenes [2,7,14], metal nanoparticles [20,22], metal oxides [3], polymer films [15,19,21], metal phthalocyanines [23,24], nanofibers [6,7] etc.

Carbon nanotubes (CNTs) have frequently been used as modifier in recent analytical applications [25–28] because of unique advantages which are high stability, conductivity and mechanical strength and fast electron transfer [1,5]. The attachment of some materials in CNTs frameworks have gained interest because of these composite electrodes possess properties of individual components with synergetic effect. The various CNT based composite electrodes were successfully applied to simultaneously determination of guanine and adenine. CeO₂ nanoparticles decorated MWCNT surface has been prepared by Wei and coworkers for detection of guanine and adenine [29]. In this study, the results showed that CNTs could provide huge locations and simplifies the adsorptive deposition of guanine and adenine, CeO₂ nanoparticles

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increase of sensitivity. The another composite electrode used for simultaneously determination of guanine and adenine by Deng et al. was poly(2,6-pyridinedicarboxylic acid) combined with MWCNT modified electrode [30]. The polymer-CNT composite electrode exhibited higher electrocatalytic activity for guanine and adenine electro-oxidation with the catalytic and adsorptive function of the composite electrode. In another study, Liu et al. examined the electrochemical oxidation of guanine and adenine on PbO_2 modified CNT-ionic liquid composite electrode which have showed high sensitivity for the guanine and adenine determination [31]. Rezaei et al. developed an electrode for simultaneous electrochemical determination of guanine and adenine using MWCNTs with the hybrid Ni–Al covered graphene oxide on GCE surface [32]. The oxidation peak currents of guanine and adenine on the composite electrode were greatly enhanced due to hybrid structure of modified electrode. These studies showed that carbon nanotubes have increased the sensitivity of the electrode towards adenine and guanine detection.

To our best knowledge, there is no report about the determination of guanine and adenine on MWCNT modified with Cu– CeO_2 . In this paper, Cu– CeO_2 particles were incorporated on the MWCNT modified GCE surface by electrochemical deposition. The composite electrodes surface morphology and chemistry were identified by SEM, EDX, EIS and XPS techniques. The electrochemical behaviors of guanine and adenine on the Cu– CeO_2 /MWCNT/GC composite electrode were also investigated with cyclic voltammetry (CV) and then the simultaneous determination of guanine and adenine were performed using differential pulse voltammetry (DPV). The studies indicated that the Cu– CeO_2 /MWCNT/GC composite electrode exhibited tremendous electrocatalytic activity for the electrooxidation of guanine and adenine, which lead to the sensitive, easy and cheap method was established to analyze trace levels of DNA.

2 Experimental

2.1 Chemicals

Adenine ($\text{C}_5\text{H}_5\text{N}_5$), guanine ($\text{C}_5\text{H}_5\text{N}_5\text{O}$), sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), multiwalled carbonnanotube (MWCNT), copper sulphate ($\text{Cu}(\text{SO}_4)_2 \cdot 5\text{H}_2\text{O}$), cerium (IV) sulphate ($\text{Ce}(\text{SO}_4)_2$), alumina (Al_2O_3), Acetic acid (CH_3COOH), sodium acetate (NaCH_3COO), boric acid (H_3BO_3), sodium meta borate (NaBO_2), sodium dihydrogen phosphate (NaH_2PO_4), disodium monohydrogen phosphate (Na_2HPO_4), *N,N*-dimethylformamide (DMF), calf thymus DNA sample were supplied from Merck, Sigma or Aldrich. All reagents were analytical grade. Buffer solutions used as supporting electrolyte were prepared from $\text{CH}_3\text{COOH}/\text{NaCH}_3\text{COO}$, $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$, $\text{H}_3\text{BO}_3/\text{NaBO}_2$ in the pH range from 3.76 to 9.02. Ionic strength was kept nearly constant for each buffer solution as 0.1 mol L^{-1} . Stock standard solutions of adenine and guanine (1.0 mmol L^{-1}) were

prepared in 0.1 mol L^{-1} sodium hydroxide. Multiwall carbon nanotube was pretreated with HNO_3 and final suspension was sonicated in DMF. The metal solutions ($5.0 \text{ mmol L}^{-1} \text{ Ce}^{4+}$ and $1.0 \text{ mmol L}^{-1} \text{ Cu}^{2+}$) in H_2SO_4 (0.1 mol L^{-1}) were prepared by dissolution of metal salt with acids. Ultrapure water was produced from Millipore Milli Q system (18.2Ω). During all electrochemical experiments, high purity nitrogen kept flowing over the solution at ambient temperature.

2.2 Apparatus

Autolab 302 N Electrochemical Analyzer system was used for cyclic voltammetry (CV) and differential pulse voltammetric studies (DPV). All Voltammetric measurements with an Ag/AgCl (sat.KCl), a Pt wire and GCE as reference electrode, counter, and working electrode, respectively. CV and DPV techniques were used throughout the electroanalytical studies. Cyclic and differential pulse voltammetric measurements were carried out in 0.1 mol L^{-1} pH 7.0 phosphate buffer solution (PBS). The potential ranging of CV and DPV studies were selected as from 0.5 to 1.3 V and from 0.4 to 1.2 V, respectively. The following DPV conditions were used: 25 mV amplitude modulation, 50 ms pulse width and a scan rate 10.0 mV s^{-1} . The pH of the solution was adjusted by WTW handheld 330i ion analyzer. Before each experiment, solutions were purged with high-purity N_2 for 10 min to remove oxygen.

The SEM and EDX analysis were carried out using the Phillips XL-30S for the surface characterization of CNT and metal and metal oxide particles on the GCE. SEM images were obtained with $\times 10000$ magnitude on all electrodes (15 kV). XPS of the Cu– CeO_2 /MWCNT/GC composite electrode were obtained via Thermo Scientific Spectrometer with K-Alpha surface analysis. The EIS studies were also performed with Autolab 302N Electrochemical Analyzer. The typical Nyquist plots for the all used electrodes were obtained in the $5.0 \text{ mmol L}^{-1} \text{ K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6] + 0.1 \text{ mol L}^{-1} \text{ KNO}_3$ mixture at the frequencies range 0.05 to 75,000 Hz with amplitude 0.005 V. The potential was selected as 0.24 V, which was the formal potential of the redox potential.

2.3 Preparation of MWCNT Modified Glassy Carbon Electrode

In order to purification and activation of MWCNTs, pretreatment was carried out with concentrated HNO_3 as follows: 0.08 g MWCNT was boiled in nearly 7.5 mL concentrated HNO_3 and then it was washed with ultrapure water until obtaining neutral solution. The pretreated MWCNTs was added into DMF to obtain a black suspension, and then the suspension was sonicated for 30 minutes. Before the modification, GCE was polished with Al_2O_3 slurry ($0.05\text{--}3.0 \mu\text{m}$) on a polishing pad, then electrode was rinsed with pure water. The GCE was sonicated in ultrasonic bath for 3 min in absolute ethanol

solution and ultra-pure water, respectively. A 10 μL of MWCNT suspension was injected on the GCE surface. The electrode kept in the drying oven at 70°C for one hour in order to evaporate the solvent (DMF) of the suspension.

2.4 Preparation of Cu–CeO₂ Modified MWCNT/GCE

The Cu nanoparticles and CeO₂ particles were electrochemically deposited on the MWCNT/GCE by cyclic voltammetry (CV) with consecutive scans. For preparation of CeO₂ modified MWCNT/GCE, CeO₂ particles were deposited on MWCNT by CV scanning between -0.6 to 1.0 V in 1.0 mmol L^{-1} Ce(SO₄)₂ in 0.1 mol L^{-1} H₂SO₄ with a scan rate of 50 mV/s for 20 cycles and then Cu nanoparticles were deposited by CV scanning between -0.6 V and 0.5 V in 1.0 mmol L^{-1} CuSO₄ with 5 cycles at the same conditions. The obtained Cu–CeO₂ modified MWCNT/GCE electrode was cleaned with ultra-pure water. The prepared electrode was denoted as Cu–CeO₂/MWCNT/GCE.

2.5 Preparation of the Calf Thymus DNA Samples and the Determination of the Guanine Adenine

The calf thymus DNA sample was hydrolyzed for quantification of guanine and adenine. The 3.0 mg of calf thymus DNA sample was digested using 1.0 mL of 1 mol L^{-1} HCl in a sealed 10 mL glass tube. After heating in a boiling-water bath for 60 minutes, the pH of the solution was adjusted with 1.0 mL of 1 mol L^{-1} NaOH. The standard addition method was used for guanine and adenine concentration in calf thymus DNA. Required amount of sample solution was added to the electrochemical cell consisting of 5.0 mL of buffer solution (pH 7.0 PBS) and the DPV was applied. The spiking of a standard concentration of respective analytes resulted in a systematic increase in the peak current values of guanine and adenine.

3 Results and Discussions

3.1 Preparation of Cu–CeO₂ Modified MWCNT/GC

In Figure 1A, a reduction peak was appeared at -0.40 V related to the reduction of Ce(IV) to Ce(III) during the deposition of CeO₂ particles on the MWCNT/GCE. On the reverse current-potential scan, an oxidation peak was appeared at 0.5 V due to the oxidation of the Ce(III) to Ce(IV) on the electrode surface. As can be seen from the Figure 1B, a cathodic peak was formed at -0.11 V as a result of reduction of Cu²⁺ to Cu metal with the deposition of Cu nanoparticles on the CeO₂/MWCNT/GC electrode. A re-oxidation of metallic formed Cu was appeared at 0.10 V during the anodic potential scan.

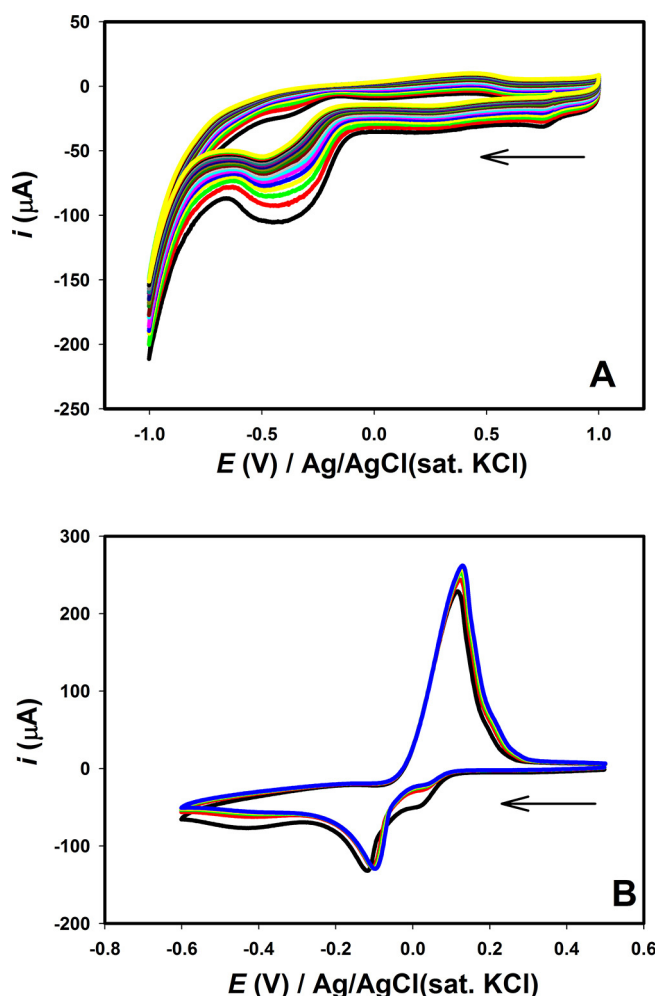


Fig. 1. Consecutive cyclic voltammograms of A) CeO₂ deposition on MWCNT/GCE, in 5.0 mmol L^{-1} Ce(SO₄)₂ + 0.1 mol L^{-1} H₂SO₄ with 20 cycles and B) Cu nanoparticles on CeO₂/MWCNT/GCE in 1.0 mmol L^{-1} CuSO₄ + 0.1 mol L^{-1} H₂SO₄ solutions, at 50 mV/s scan rate.

3.2 Characterization of the Cu–CeO₂/MWCNT Modified GCE

The characterization of morphology and structure of the Cu–CeO₂/MWCNT modified GC surface was investigated with SEM. As shown in Figure 2A, a smooth and tubular surface was observed for the acid pretreated MWCNTs. The typical SEM images of the synthesized CeO₂/MWCNT composite was showed in Figure 2B, which reveal that the MWCNTs was coated with the spherically shaped CeO₂ particles. In contrast, for Cu–CeO₂/MWCNTs surface, a large number of particles as similar to a needle structure were loaded with homogeneous distribution on the MWCNTs surface (Figure 2C). The existence of Ce, O and Cu atoms on MWCNTs were also confirmed by EDX spectroscopy analysis. SEM images indicated that uniformly covered layer of CeO₂ and Cu nanoparticles on the MWCNT surface can be easily synthesized by using of electrodeposition technique.

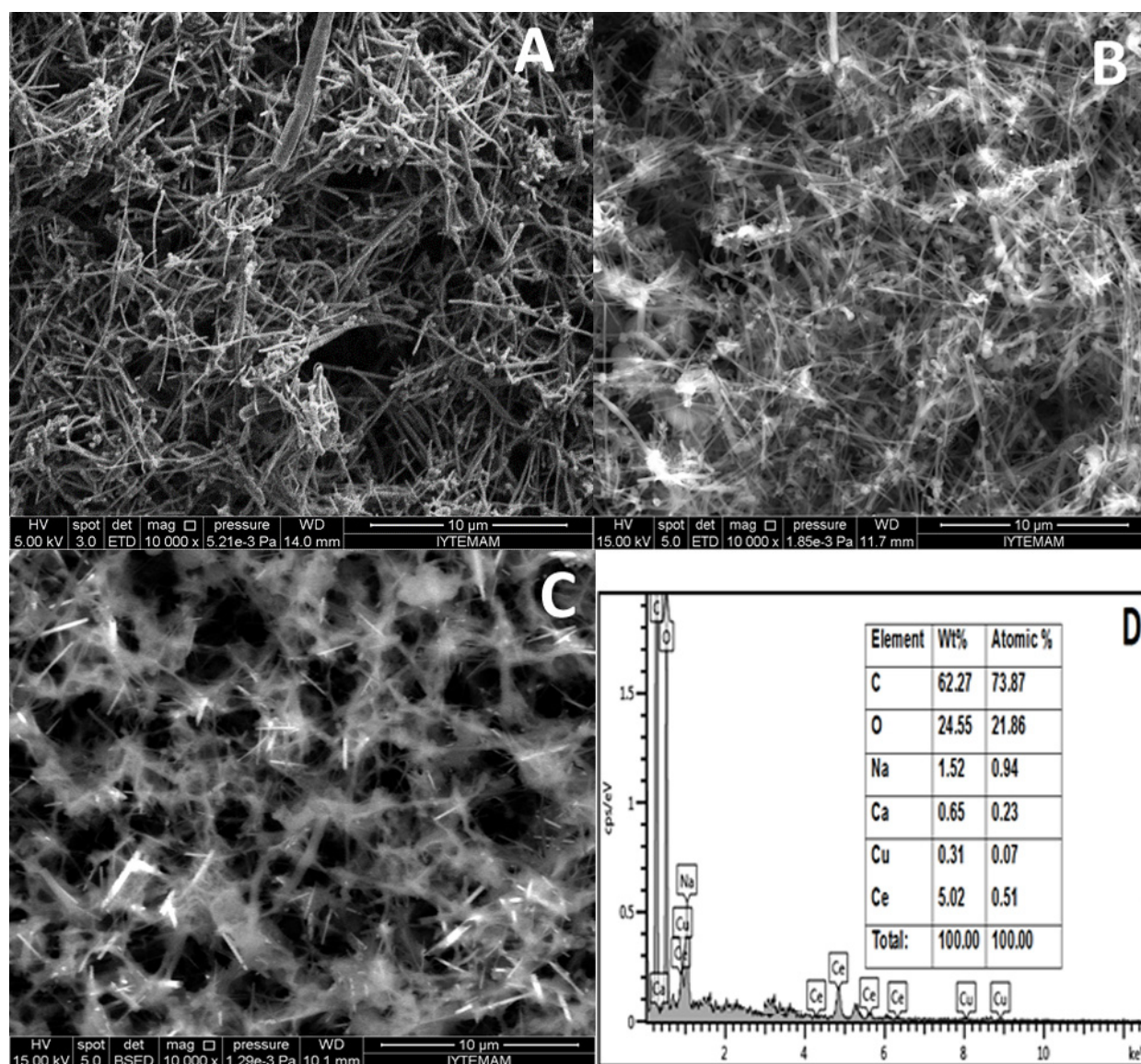


Fig. 2. SEM images of (A) MWCNT/GCE, (B) $\text{CeO}_2/\text{MWCNT}/\text{GCE}$, (C) $\text{Cu}-\text{CeO}_2/\text{MWCNT}/\text{GCE}$ and (D) EDX results of the $\text{Cu}-\text{CeO}_2/\text{MWCNT}/\text{GCE}$.

Furthermore, XPS was used to determine the chemical structure of the $\text{Cu}-\text{CeO}_2/\text{MWCNT}$ and the oxidation state of the Cu and Ce atoms. Figure 3A displays the XPS survey spectrum of $\text{Cu}-\text{CeO}_2/\text{MWCNT}/\text{GCE}$. The survey spectrum showed only Ce, Cu, C and O peaks at certain binding energy values on the composite electrode surface. The C1s and O1s peaks are assigned to the MWCNT structure on modified electrode surface. Figure 3B shows the Ce3d core level XPS spectra of $\text{Cu}-\text{CeO}_2/\text{MWCNT}$. From the spectrum, it can be seen that the Ce 3d_{5/2} and Ce 3d_{3/2} peaks exist mainly as the Ce (IV) oxidation state (885.7 eV and 904.5 eV). These characteristic peaks of Ce3d core level are splitted to multi-peak structure and fitted, which can be deconvoluted into four main peaks. The peaks denoted as U1 and V1 located at 883.7 and 901.8 eV, respectively are the

characteristic peaks of Ce^{3+} states while U2 and V2 peaks located at 886.6 and 905.4 eV are related to Ce^{4+} states. The results indicate that the co-existence of Ce^{3+} and Ce^{4+} states in $\text{Cu}-\text{CeO}_2/\text{MWCNT}$ which are in good agreement with published data [33,34]. The Cu2p line peaks are fitted using fitting procedure from which Cu 2p_{1/2} and Cu 2p_{3/2} peaks were detected (Figure 3C). The Cu 2p_{3/2} spectrum displays a main peak at 933.1 eV attributed to Cu(0) species. XPS spectrum of Cu2p_{1/2} at 953.7 eV gives two different copper oxide species of binding energy allocated to CuO and $\text{Cu}(\text{OH})_2$ [35–37].

EIS was also used to obtain detailed information on the change in the electrochemical property of the glassy carbon electrode modified with $\text{Cu}-\text{CeO}_2/\text{MWCNT}$. Figure 3D displays the typical Nyquist plots for the all used electrodes in the $5.0 \text{ mmol L}^{-1} \text{ K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6] +$

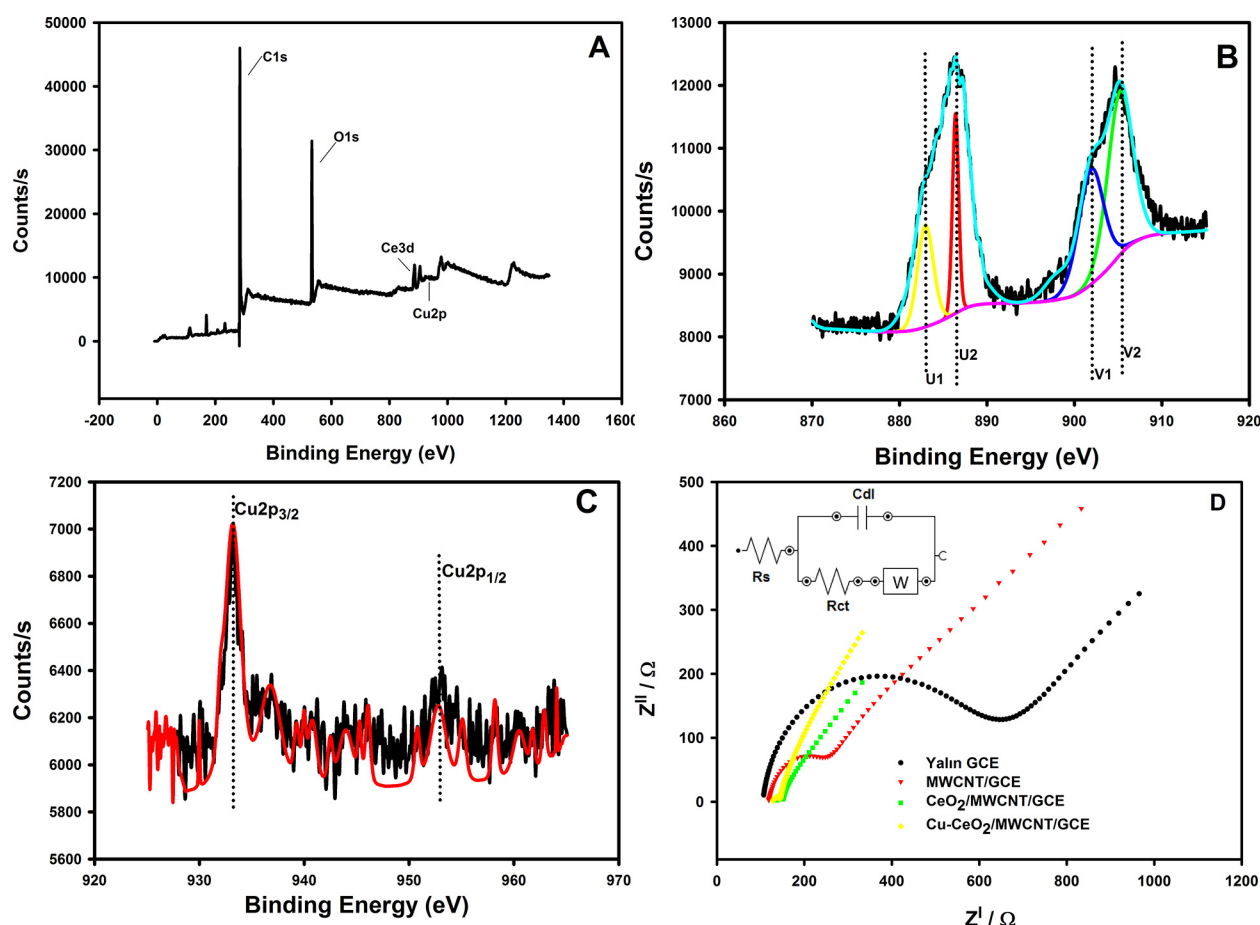


Fig. 3. (A) XPS survey spectrum of Cu–CeO₂/MWCNT/GC composite electrode; core level spectra of (B) Ce3d and (C) Cu2p. (D) The Nyquist plots of the bare and modified electrodes in the 5.0 mmol L^{−1} K₃[Fe(CN)₆]/K₄[Fe(CN)₆] containing 0.1 mol L^{−1} KNO₃ solution at varying frequencies range 0.05 to 75,000 Hz at the formal potential. Inset is the Randles circuit model for the modified electrodes.

0.1 mol L^{−1} KNO₃ mixture. As you can see from the plots, a semi-circular and linear portion was formed at all electrodes. An equivalent circuit were obtained by fitting of EIS data as given in Figure 3D inset. The standard Randles equivalent-circuit model include charge transfer resistance (R_{ct}), electrolyte resistance (R_s) and Warburg impedance (W) and the double layer capacitance (C_{dl}). It was clearly seen that glassy carbon electrode shows a large charge transfer resistance of about 428 ohm. This value showed that the electron transfer rate between electrode interface and redox probe was very slow. The lowest R_{ct} value (20 Ω) for the Cu–CeO₂/MWCNT/GCE implies the relatively fast charge transfer compared to the MWCNT/GCE (119 Ω), CeO₂/MWCNT/GCE (24 Ω) and bare GCE (428 Ω). These results indicated that the excellent electro-conductivity was proved with Cu–CeO₂/MWCNT/GCE surface. Moreover, the Cu–CeO₂/MWCNT was successfully formed on the GCE surface.

3.3 Electrochemical Behavior of Guanine and Adenine

The electrochemical behavior of bare and modified composite electrodes in 0.1 mol L^{−1} pH 7.0 phosphate

buffer solution were investigated in the absence (Figure 4A-inset) and in the presence of 3.0×10^{-5} mol L^{−1} guanine and 3.0×10^{-5} mol L^{−1} adenine mixture (Figure 4A). The CVs were recorded from 0.50 V to 1.30 V potential ranges with 0.05 V s^{−1} scan rate. In the first instance, there was no oxidation or reduction signal for adenine and guanine in the case of the bare and all modified electrodes at studied potential range in 0.1 mol L^{−1} pH 7.0 PBS. On the other hand, two oxidation peaks without the reduction were observed at on bare and all modified electrodes in the presence of 3.0×10^{-5} mol L^{−1} guanine and 3.0×10^{-5} mol L^{−1} adenine in 0.1 mol L^{−1} pH 7.0 PBS which indicates an irreversible electrochemical process for both compounds at all electrodes (Figure 4). Relatively, two weak oxidation peaks at the bare GCE surface (curve a) were obtained at 0.760 V and 1.06 V for guanine and adenine, respectively. In the cases of MWCNT, CeO₂/MWCNT and Cu–CeO₂/MWCNT modified GCE (curve b, c, d), the peak potentials of guanine and adenine were observed at low positive values as about 0.700 V and 0.970 V, respectively. When the oxidation peak current values on the all electrodes were compared, the highest peak currents for

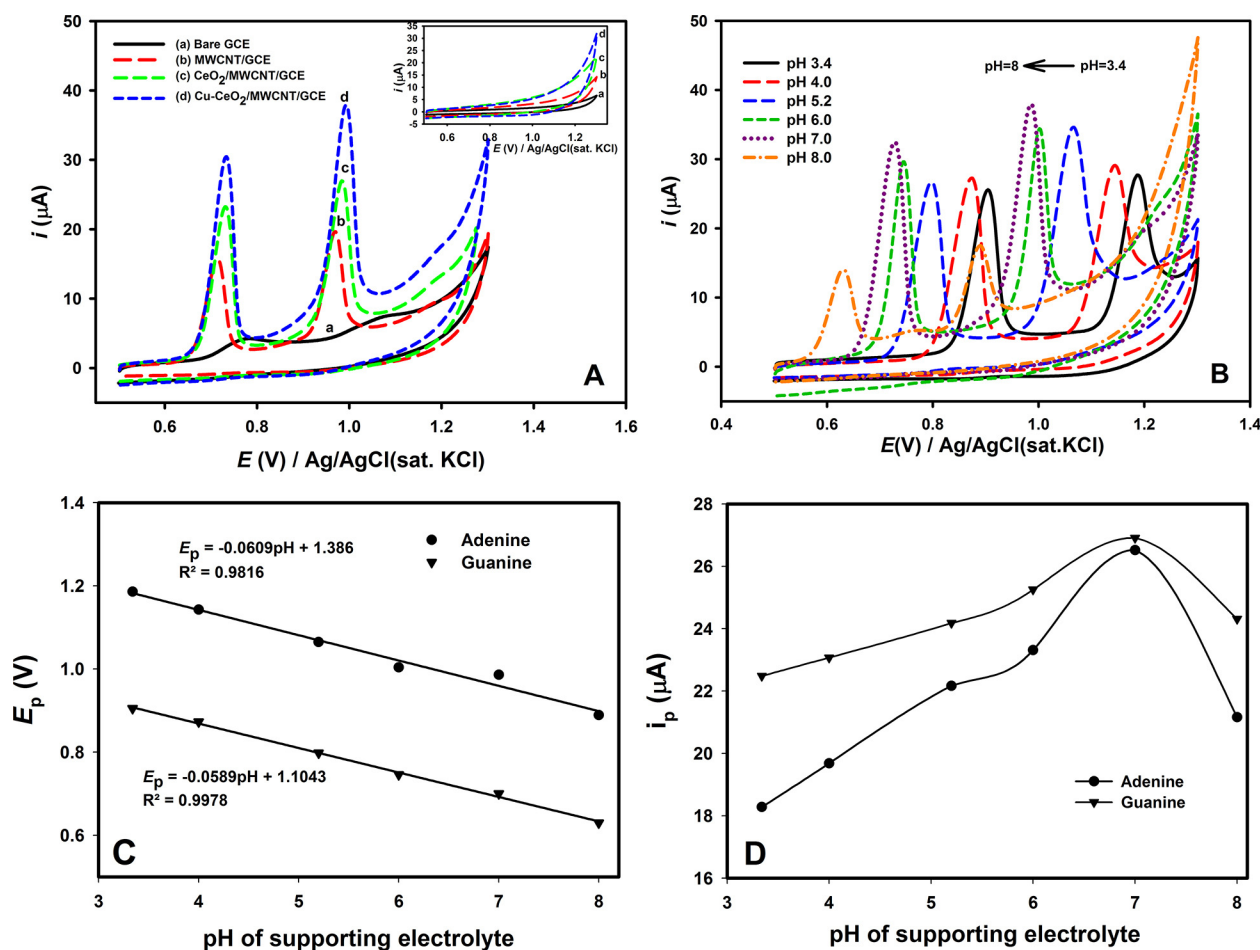


Fig. 4. (A) CV curves of a) Bare GCE, b) MWCNT/GCE, c) CeO₂/MWCNT/GCE and d) Cu-CeO₂/MWCNT/GCE in presence of 3.0×10^{-5} mol L⁻¹ guanine and adenine mixed solution (1:1) in 0.1 mol L⁻¹ pH 7.0 PBS, inset: absence of guanine and adenine in 0.1 mol L⁻¹ pH 7.0 PBS. (B) CVs of 3.0×10^{-5} mol L⁻¹ guanine and adenine in the mixed solution (1:1) on the Cu-CeO₂/MWCNT/GCE at various pH values (3.4, 4.0, 5.2, 6.0, 7.0 and 8.0) with a scan rate of 50 mV s⁻¹. The effect of pH on the (C) anodic peak potential and on (D) the anodic peak current of guanine and adenine.

guanine and adenine oxidation in 0.1 M pH 7.0 PBS solution were obtained with Cu-CeO₂/MWCNT/GC electrode. The enhancement of peak currents and shifting of oxidation peak potentials to less positive values of guanine and adenine indicated that the modification of GCE surface with carbon nanotube and Cu-CeO₂ were gained an electrocatalytic activities toward both guanine and adenine oxidation. The reason of this improvement of peak characteristic can be explained that the formation of carboxyl groups on the carbon nanotube surface during the conditioning of CNT and synergetic effects between MWCNT and Cu-CeO₂.

3.4 Optimization of Cu-CeO₂/MWCNT Modified GCE Surface

To obtain optimum conditions for sensitive and selective determination of guanine and adenine, some main parameters associated with the preparation of Cu-CeO₂/MWCNT modified GC electrode and solution features

were assessed with cyclic voltammetry. The parameters to be optimized were the Ce(SO₄)₂ concentration, cycle number of current-potential scan for the preparation CeO₂ on the electrode surface, CuSO₄ concentration, cycle number of current-potential scan for electrochemical deposition of Cu nanoparticles and MWCNT + DMF containing suspension amount (volume) on the electrode surface. The effects of these parameters on the peak current of guanine and adenine were shown in Figure S1–5 (See supplementary material). Optimum parameters were specified by evaluating the peak current of guanine and adenine which were determined as follows: 5.0 mmol L⁻¹ Ce(SO₄)₂, 20 cycles number for deposition of CeO₂, 1.0 mmol L⁻¹ CuSO₄ concentration, 5 cycles number for Cu nanoparticles deposition and 10 μL of MWCNT respectively.

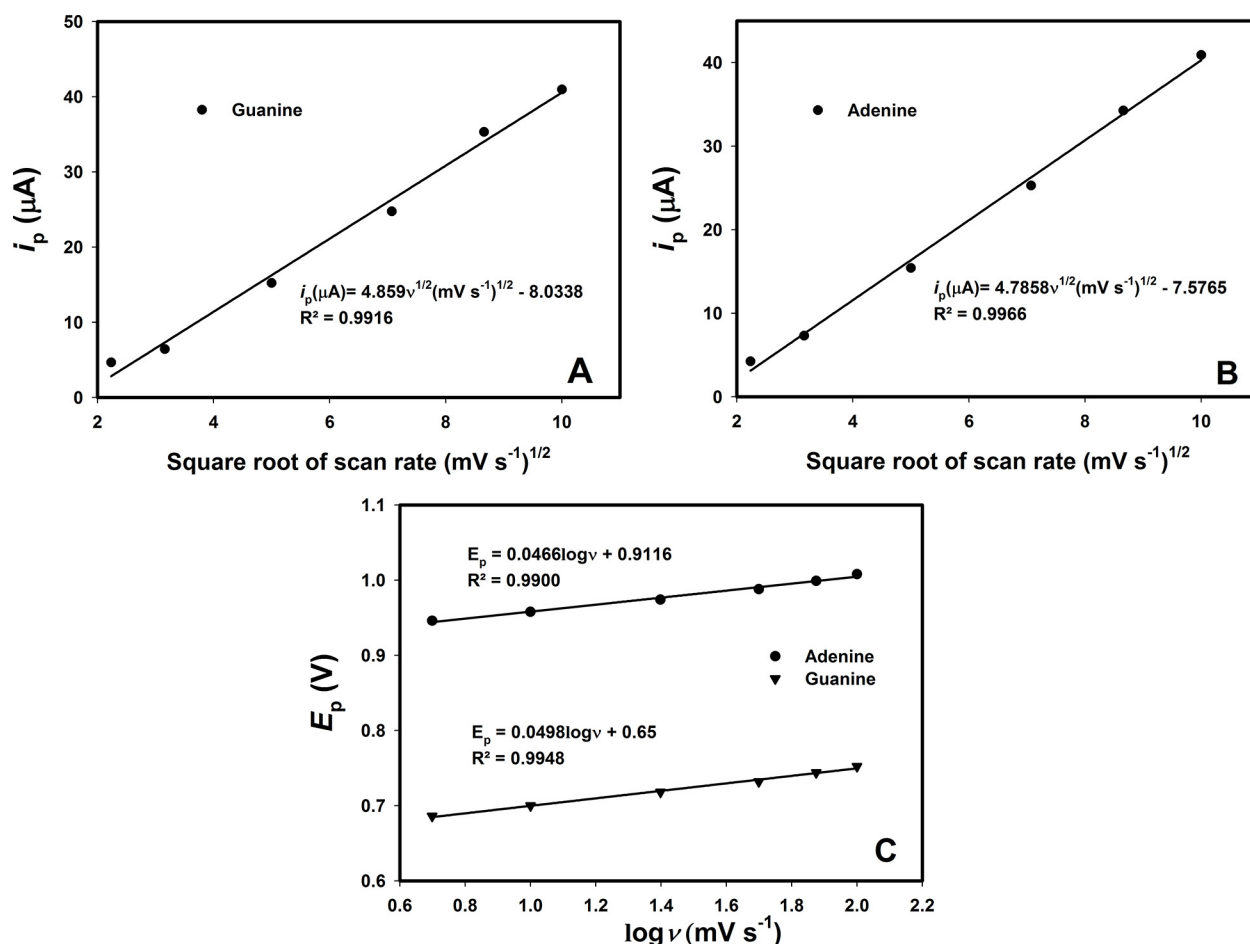
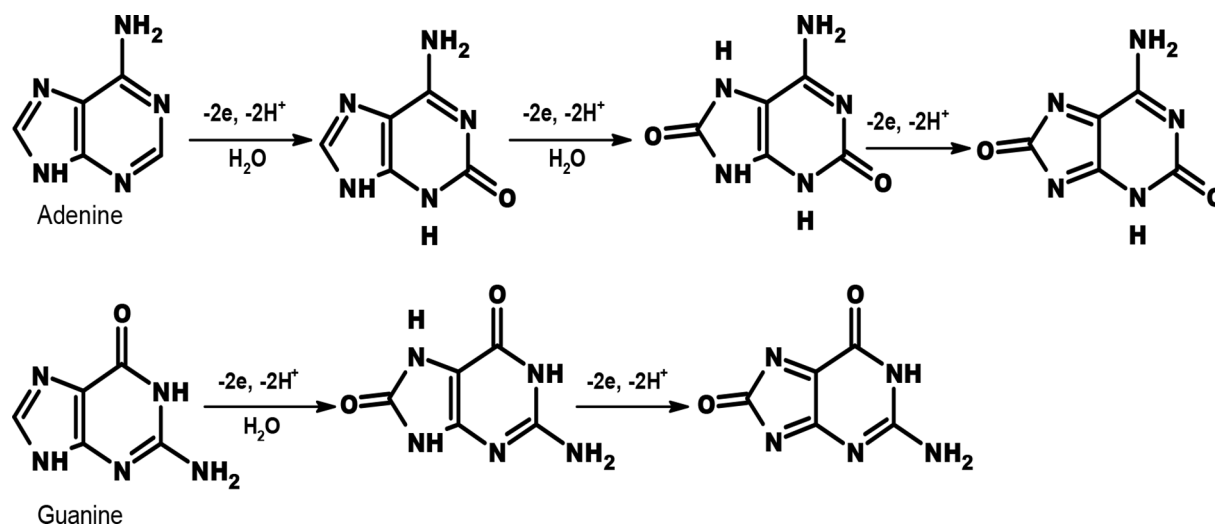


Fig. 5. The plot of oxidation peak current of guanine (A) and adenine (B) versus the square root of scan rate (5.0–100 mV s^{-1}), variation of guanine and adenine oxidation peak potential versus the logarithm of the scan rate (C). ($3.0 \times 10^{-5} \text{ mol L}^{-1}$ guanine and adenine in pH 7 PBS).

3.5 Influence of Supporting Electrolyte pH

The effect of pH changing of supporting electrolyte on the electrochemical behavior of organic compounds should be examined because proton ions are generally taking part in the reactions. Consequently, the effect of supporting electrolyte pH on the electrooxidation of $3.0 \times 10^{-5} \text{ mol L}^{-1}$ Gu and Ad in the mixed solution (1:1) was investigated at Cu–CeO₂/MWCNT modified GCE by CV in pH 3.4–8.0 buffer solution. As demonstrated in Figure 4B, the peak potential of both guanine and adenine oxidation peak potentials shifted toward more negative values with the increasing of supporting electrolyte pH. The shifting of guanine and adenine oxidation peak potentials with varying of pH claim that the oxidation process has proton transfer and the de-protonation process can be enhanced with hydroxide ions [38]. Linear relationships of the peak potential of guanine electrochemical oxidation and supporting electrolyte pH were obtained with slope of about $-58.85 \text{ mV pH}^{-1}$, which was close to the theoretical Nernst theoretical value of 59.10 mV pH^{-1} . (Figure 4C). This value indicate that

electrochemical oxidation process of guanine have equal numbers of electrons are protons. It was described that the electrochemical oxidation of guanine follows a two-step mechanism including the total loss of $4e^-$ and the first $2e^-$ oxidation is rate-determining step as shown in Scheme 1 [1,19,22,24] and 2 H^+ take part in the rate-determining step, which is in good agreement with previously literature data [5–7,19]. On the other hand, similar aspect of good linearity between peak potential of adenine electrooxidation and pH of supporting electrolyte was also obtained with slope of $-60.92 \text{ mV pH}^{-1}$ which is also very close to the theoretical value (Figure 4C). The slope of $-60.92 \text{ mV pH}^{-1}$ suggested that e^- and H^+ numbers in the oxidation mechanism of adenine should be equal. The electrochemical oxidation mechanism of adenine suggests in three steps with six protons and six electrons (Scheme 1) by transferring of $2e^-$ and 2 H^+ in each step. Therefore, the electrooxidation mechanism of adenine on the Cu–CeO₂/MWCNT modified GCE surface can be explained with the first step involving $2e^-$ and 2 H^+ . Investigation of the effect of the pH range 3.4–8 on the peak current of guanine and adenine at the Cu–CeO₂/



Scheme 1. Possible electrochemical oxidation mechanism of Adenine and Guanine.

MWCNT modified GCE revealed that the maximum peak currents of electrooxidation of both were achieved at pH 7.0 (Figure 4D). The oxidation peaks of guanine and adenine disappeared in alkaline media after pH 8. Considering largest peak currents and the satisfactory peak separations, the phosphate buffer solution at pH 7 was chosen as the most suitable value for the subsequent voltammetric analysis also from the point of the mimicking the physiological environment.

3.6 Effect of Potential Scan Rate

In order to obtain detailed information involving electrochemical mechanism of the guanine and adenine on the Cu–CeO₂/MWCNT modified GCE, CVs were recorded in pH 7.00 PBS supporting electrolyte at different scan rates (5.0–100 mV s^{−1}) and were shown in Figure S6 (See supplementary material). The oxidation peak current of 3.0×10^{-5} mol L^{−1} Gu and Ad were increased linearly with the square root of scan rate and the equation can be expressed as $i_p(\mu\text{A}) = 4.859v^{1/2}(\text{mV s}^{-1})^{1/2} - 8.0338$, $R^2 = 0.9916$ for guanine and $i_p(\mu\text{A}) = 4.7858v^{1/2}(\text{mV s}^{-1})^{1/2} - 7.5765$, $R^2 = 0.9966$ for adenine (Figure 5A–B). These results indicated that the process is similar to those controlled by diffusion at the Cu–CeO₂/MWCNT modified GCE. Furthermore, it is seen that the peak potentials of guanine and adenine oxidation were shifted more positive value by the increasing of $\log v$ (Figure 5C) and the regression equation $E_p = 0.0498 \log v + 0.65$, $R^2 = 0.9948$ for guanine; $E_p = 0.0466 \log v + 0.9116$, $R^2 = 0.9900$ for adenine. These results suggested that the oxidations of guanine and adenine on Cu–CeO₂/MWCNT surface were irreversible.

3.7 Individual and Simultaneous Determinations of Guanine and Adenine

The sensing performance of Cu–CeO₂/MWCNT modified GCE towards guanine and adenine detections was investigated using of differential pulse voltammetry (DPV) in 0.1 mol L^{−1} pH 7.0 PBS under the optimal experiment conditions. In the beginning of the study, the selective determinations of guanine and adenine with Cu–CeO₂/MWCNT/GCE were performed in guanine and adenine mixture with one compound retained at a fixed concentration. As shown in Figure 6A, the DPVs of different concentrations of guanine (0.1–25.0 μmol L^{−1}) were obtained in a fixed concentration of adenine (10.0 μmol L^{−1}). The oxidation peak current of guanine located at 0.67 V increased when increasing of the guanine concentration. By the way, the peak current of adenine oxidation located at 0.93 V remained almost constant. The relationship between oxidation peak current and guanine concentration was observed linearly with regression equation $i_p(\mu\text{A}) = 1.2436C_{\text{guanine}}(\mu\text{mol L}^{-1}) + 1.8351$, $R^2 = 0.9974$ (Figure 6A-inset). Similar phenomenon appeared to be the case when adenine coexists with guanine (10.0 μmol L^{−1}) in the range of 0.1–25.0 μmol L^{−1}. The regression equation can be explained as $i_p(\mu\text{A}) = 1.0508C_{\text{adenine}}(\mu\text{mol L}^{-1}) + 0.4181$, $R^2 = 0.9994$ (Figure 6B). The DPV studies have shown that guanine and adenine can be determined independently from each other.

On the other hand, the simultaneous determination of guanine and adenine was also performed by changing both compounds concentrations on Cu–CeO₂/MWCNT modified GCE. As shown in Figure 6C, all the peak currents of guanine and adenine oxidation increased linearly within the range 0.2–6.0 μmol L^{−1} for guanine and 0.1–8.0 μmol L^{−1} for adenine. The regression equations were obtained as $i_p(\mu\text{A}) = 3.0378C_{\text{guanine}}(\mu\text{mol L}^{-1}) - 1.0547$ with $R^2 = 0.9933$ for guanine and $i_p(\mu\text{A}) = 2.9581C_{\text{adenine}}(\mu\text{mol L}^{-1}) + 1.7277$ with $R^2 = 0.9939$ for adenine. The limit

Table 1. Comparison of different modified electrodes for guanine and adenine simultaneous determination.

Electrodes	Mode	Supporting electrolyte	Adenine ($\mu\text{mol L}^{-1}$)		Guanine ($\mu\text{mol L}^{-1}$)		Sample	Reference
			LR	LOD	LR	LOD		
CILE	CV	pH 7.0 B-RBS	0.3–50.0	0.0787	1.5–70.0	0.25	Herring sperm DNA	[39]
Graphene-Nafion/GCE	DPV	pH 4.0 ABS	2.0–120.0	0.58	5.0–170.0	0.75	Herring Sperm DNA	[2]
MWCNT/Ch/GCE	DPV	pH 7.0 PBS	0.2–450	0.06	0.4–500.0	0.15	Herring sperm DNA	[5]
CeO ₂ /MWCNT/GCE	DPV	pH 7.0 PBS	5.0–35.0	0.02	5.0–50.0	0.01	Calf tymus DNA	[29]
SiCNP/GCE	DPV	pH 7.4 PBS	0.1–11.0	0.015	0.1–11.0	0.015	-	[40]
GS/IL/CS/GC	DPV	pH 5.0 PBS	2.5–150.0	0.75	1.5–350.0	0.45	Fishing DNA	[18]
PIlox-GO/GCE	DPV	pH 3.0 PBS	3.3–103.3	0.48	9.6–215.0	1.28	-	[15]
NiCNF/GCE	DPV	pH 4.0 PBS	0.05–2.0	0.03	0.05–2.0	0.03	Fish sperm DNA	[6]
PXA-MoS ₂ /CPE	DPV	pH 7.0 B-RBS	0.5–10.0	0.027	0.5–10.0	0.032	d-GTP	[21]
TNFs/GONs/SPCE	DPV	pH 4.5 PBS	-	-	0.1–10.0	0.0017	p-BudCE4.1 plasmid	[7]
porphyrin/RGO/GC	DPV	pH 2.0 PBS	0.05–150.0	0.016	0.2–200.0	0.06	Calf tymus DNA	[17]
PMEL/BDD	DPV	pH 4.5 PBS	0.076×10^{-3} –50.0	0.02×10^{-3}	0.2–200.0	0.2	Calf tymus DNA	[16]
Cu–CeO ₂ /MWCNT	DPV	pH 7.0 PBS	0.2–6.0	0.128	0.1–8.0	0.062	Calf tymus DNA	This work

CILE: carbon ionic liquid electrode; MWCNT/Ch/GCE: multiwalled carbon nanotube/choline modified glassy carbon electrode; SiCNP/GC: silicon carbide nanoparticles/GCE; GS/IL/CS/GCE: graphene sheets/ionic liquid/chitosan/GCE; BDD: boron-doped diamond electrode; PIlox-GO/GCE: overoxidized polyimidazole/graphene oxide; PXA-MoS₂/CPE: poly(xanthurenic acid)-MoS₂/carbon paste electrode; TNFs/GONs/SPCE: Titanium dioxide nanofibers/graphene oxide nanosheets/screen printed carbon electrode; porphyrin/RGO/GC: reduced graphene oxide/GC; PMEL/BDD: poly-melamine/boron-doped diamond; d-GTP: 20-Deoxyguanosine-50-triphosphate trisodium salt; B-RBS: Britton-Robinson buffer solution; ABS: Acetate buffer solution; PBS: phosphate buffer solution; GCE: Glassy carbon electrode; CV: cyclic voltammetry; DPV: Differential pulse voltammetry

of detections (LOD) of guanine and adenine on Cu–CeO₂/MWCNT modified GCE were calculated as 0.128 and 0.062 $\mu\text{mol L}^{-1}$, respectively. The LOD values were calculated using the equation $\text{LOD} = 3.3 \sigma/m$, where σ is the standard deviation of the response for blank solution, m is the slope of the calibration graph. The analytical performance comparison of some methods for guanine and adenine determination reported by previous studies and the present study were given in Table 1. The Cu–CeO₂/MWCNT composite electrode has both higher sensitivity and lower detection limit than in those of some previously published studies according to values from Table 1.

3.8 Reproducibility and Long Term Stability

The relative standard deviations (RSD) were obtained from the DPV analyses on inter-day ($n=7$) and intra-day ($n=6$) with Cu–CeO₂/MWCNT/GCE for two different concentrations of guanine and adenine. The inter-day RSD values for 0.4 and 2.0 $\mu\text{mol L}^{-1}$ guanine are 3.21 and 2.43 %, respectively and the inter-day RSD values for 0.4 and 2.0 $\mu\text{mol L}^{-1}$ adenine are 2.33 and 1.43 %, respectively. The intra-day RSD values for 0.4 and 2.0 $\mu\text{mol L}^{-1}$

guanine are 4.52 and 3.19 %, respectively and the intra-day RSD values for 0.4 and 2.0 $\mu\text{mol L}^{-1}$ adenine are 4.12 and 3.96 %, respectively. These RSD values indicate that the Cu–CeO₂/MWCNT modified GCE showed good reproducibility. The long term stability of the modified electrode was investigated by examining peak current response of 2.0 $\mu\text{mol L}^{-1}$ guanine and adenine in the mixed solution (1:1) and the modified electrode was stored in 0.1 M PBS at pH 7.0 at room temperature during the stability measurements. The peak current response of electrode retained about 95.3 % and 96.2 % of their initial current after 7 days for guanine and adenine, respectively. The 80 % current response was still retained after 3 weeks. The results of the modified electrodes demonstrate that the modified electrode stability was good enough as long life voltammetric sensor.

3.9 Interferences

To assess the selectivity of the suggested differential pulse voltammetric method for the determination of DNA bases, possible interferences were investigated by adding various foreign species which different concentrations in to pH 7.0 PBS containing 10 $\mu\text{mol L}^{-1}$ Gu and Ad in the

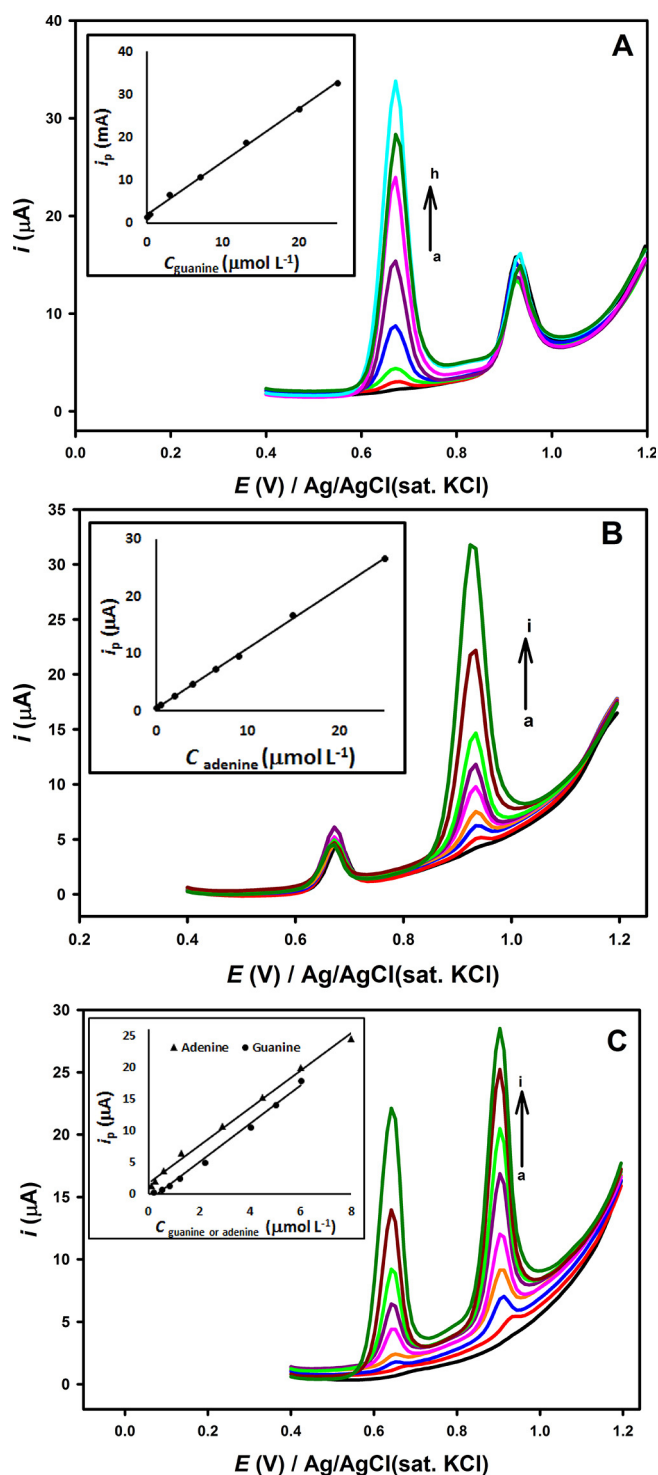


Fig. 6. (A) DPV of different concentrations of guanine (a–h: 0.1–25.0 $\mu\text{mol L}^{-1}$) in the presence of 10.0 $\mu\text{mol L}^{-1}$ adenine. (B) DPV of different concentrations of adenine (a–i: 0.1–25.0 $\mu\text{mol L}^{-1}$) in the presence of 10.0 $\mu\text{mol L}^{-1}$ guanine. (C) DPV of different concentrations of guanine (0.2–6.0 $\mu\text{mol L}^{-1}$) and adenine (0.1–8.0 $\mu\text{mol L}^{-1}$). Inset: the plots of oxidation peak current versus different concentrations of purine bases.

mixed solution (1:1) on the Cu–CeO₂/MWCNT modified GCE. The results showed in Table 2 indicated that

common inorganic ions K⁺, Na⁺, Mg²⁺, Fe³⁺, Ni²⁺, Cu²⁺, Ca²⁺, Cl[−], SO₄^{2−} and NO₃[−] had no significant interference on the determination of guanine and adenine with signal changes less than 6.0%. Other potential electroactive organic interferences such as ascorbic acid, dopamine, uric acid, glucose, L-cysteine which may exist in biological samples were also examined. As can be seen from Table 2, the oxidation peak currents of Gu and Ad were not meaningfully changed by organic compounds when their concentration increased until the about 1000 fold excess amount (signal change < 3.5%). Therefore, the Cu–CeO₂/MWCNT modified electrode can be used for the selective determination of guanine and adenine contents in real-life samples.

3.10 Analytical Application of the Cu–CeO₂/MWCNT/GCE

In order to evaluate the validity of the proposed method, the Cu–CeO₂/MWCNT/GCE was applied to determine guanine and adenine concentration in calf thymus DNA by the standard addition method under the optimized conditions. Required amount of the DNA sample solution prepared as described at section 2.5 was added to the electrochemical cell consisting of 5.0 mL of buffer solution (pH 7.0 PBS) and the DPV measured two well defined oxidation peaks. The spiking of a standard concentration of respective analytes resulted in a systematic increase in the peak current values of guanine and adenine. Calculated guanine and adenine concentrations are 2.07 $\mu\text{mol L}^{-1}$ and 2.82 $\mu\text{mol L}^{-1}$, respectively ($n=3$). These values correspond as 21.16% and 28.82% for guanine and adenine, respectively. The ultimate result showed that the ratio of [guanine]/[adenine] was 0.73. The obtained ratio is comparable to and matches with the previously reported value of 0.77 [38].

4 Conclusions

In conclusion, we have successfully fabricated a new electrochemical sensor for guanine and adenine determination based on multiwall carbon nanotube, Cu nanoparticle and CeO₂ composite film. Combining the unique properties of these materials, the Cu–CeO₂/MWCNT/GCE showed excellent electrocatalytic properties in terms of fast current response, low detection limit, good selectivity, reproducibility and long term stability toward the electrochemical oxidation of guanine and adenine. The present modified electrode has also some advantages such as easy fabrication, sufficiency of small volume of analytes and sample. Under optimized conditions, the peak currents increased linearly within the range 0.2–6.0 $\mu\text{mol L}^{-1}$ for guanine and 0.1–8.0 $\mu\text{mol L}^{-1}$ for adenine. The limit of detections for guanine and adenine were calculated as 0.128 and 0.062 $\mu\text{mol L}^{-1}$, respectively. The studies with proposed method indicated that the Cu–CeO₂/MWCNT/GCE showed great electrochemical activity on simultaneous determination of gua-

Table 2. Relative (%) change of the analytical signal of 10 $\mu\text{mol L}^{-1}$ guanine and adenine in the mixed solution (1:1).

Interfering species	Molar ratio ([Interfering species]/[A + G])	Change on the analytical signal of Adenine (%)	Change on the analytical signal of Guanine (%)
Uric acid	100	1.2	2.1
	500	1.1	2.0
	1000	1.5	2.3
Ascorbic acid	100	1.5	2.2
	500	1.4	2.3
	1000	1.3	2.4
Dopamine	100	3.1	2.2
	500	3.3	2.5
	1000	3.5	2.6
Glucose	100	2.2	3.1
	500	2.4	3.2
	1000	2.5	3.2
L-cysteine	100	3.2	3.6
	500	3.4	3.6
	1000	3.5	3.7
Cl^-	100	0.1	0.1
	500	0.5	0.3
	1000	1.2	0.4
Ca^{2+}	100	-3.7	-1.2
	500	-3.5	-1.4
	1000	-4.2	-2.0
SO_4^{2-}	100	4.4	3.2
	500	4.8	3.5
	1000	5.1	4.2
NO_3^-	100	2.2	2.1
	500	2.4	2.6
	1000	4.3	4.6
K^+	100	1.1	0.5
	500	1.3	0.6
	1000	1.4	0.8
Mg^{2+}	100	3.2	3.3
	500	3.7	4.3
	1000	5.3	6.2
Ni^{2+}	100	5.2	4.4
	500	5.5	5.4
	1000	7.2	6.1
Fe^{3+}	100	5.3	4.9
	500	5.8	5.8
	1000	6.1	6.0

nine and adenine, which lead to developing the selective, sensitive, easy and cheap method for determine trace levels of DNA.

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