



# Performing enzyme-free $\text{H}_2\text{O}_2$ biosensor and simultaneous determination for AA, DA, and UA by MWCNT–PEDOT film

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## ABSTRACT

An enzyme-free hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) biosensor based on MWCNT–PEDOT film modified electrode has been successfully performed on glassy carbon electrode (GCE) and indium tin oxide (ITO) electrode. At an applied potential of  $-0.5$  V vs. Ag/AgCl, the MWCNT–PEDOT electrode exhibited linearly dependence on  $\text{H}_2\text{O}_2$  concentration in the range of 0.1–9.8 mM. It can be observed two significantly linear sections. One shows sensitivity of  $943 \mu\text{M mM}^{-1} \text{cm}^{-2}$  with signal/noise of 6; and the other one shows sensitivity of  $174 \mu\text{M mM}^{-1} \text{cm}^{-2}$  with signal/noise of 4 in pH 7 PBS. It also presented excellent stability at room temperature, with a variation of response current less than 5% over 30 days. Moreover, the sensor was characterized by cyclic voltammetry (CV), scanning electronic microscopy (SEM), atomic force microscopy (AFM), and different pulse voltammetry (DPV). This sensor also can simultaneously detect AA, DA, and UA and can be utilized to develop multifunctional biosensors.

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## 1. Introduction

In recent years, biosensors represent a new trend emerging in terms of technological and theoretical achievements for the development exploitation of analytical devices for detection, quantification and monitoring of specific chemical species for clinical, environmental and industrial analysis (Albareda-Sirvent et al., 2000).

An accurate and reliable method for the determination of hydrogen peroxide is of interest both biomedical and environmental particularly in biosensing studies; because it forms the diagnostic response for several medical sensing devices such as blood glucose monitors (ThomeDuret et al., 1996). Hydrogen peroxide plays a significant role in the chemical and pharmaceutical industries as an oxidizing, bleaching and sterilizing agent. At high concentrations, hydrogen peroxide causes irritation to the eyes and skin and affects human health (Lobnik and Cajlakovic, 2001). Further, the detection of hydrogen peroxide is an important task in many biological, medical and clinical studies (Westbroek et al., 1996; Liu et al., 2005).

Most of the modified electrodes for amperometric determination of hydrogen peroxide are based on enzymes such as horseradish peroxidase (Lvovich and Scheeline, 1997; Wang and

Zhang, 2006; Lin et al., 2000; Kafi et al., 2008). However, there still exist some practical problems related to the use of enzyme in these analytical devices, due to the short operational lifetimes and low reusability of these biocatalysts (Gavalas and Chanotakis, 2000, 2001). The employment of non-enzymatic sensors for determination of hydrogen peroxide is an important priority in chemical, food and environmental. Many researchers have developed non-enzymatic hydrogen peroxide sensors based on  $\text{Fe}_3\text{O}_4$  (Lin and Leu, 2005), vanadium-doped zirconias (Doménecha and Alarcón, 2002), modified carbon fiber microelectrode (Wang et al., 1998), etc. Among different modifiers, polynuclear mixed-valence compounds such as hexacyanoferrates also used for amperometric determination of hydrogen peroxide (Kotzian et al., 2007; Yu et al., 2007; Pauliukaite et al., 2008). Therefore, investigating materials for detection of hydrogen peroxide must be one key part to develop enzyme-free hydrogen peroxide biosensor.

Materials like metal (gold, silver), carbon and polymers (especially conducting polymers) have been used to prepare nanomaterials such as nanoparticles (Walker et al., 2001; Wiley et al., 2004), nanotubes (Iijima, 1991; Nath and Rao, 2001) and nanowires (Qiu et al., 2003; Walter et al., 2003; Liang et al., 2002). These materials are promising for a variety of applications including optical and electronic nanodevices, and chemical and biological sensors (Nalwa, 2003). There are several reviews available which are based on electrochemical nanobiosensor (Wang, 2005; Jain, 2005; Patolsky et al., 2006; Pumera et al., 2007; Xiao and Li, 2008). Recently, Reshetilov and Bezbodrov discussed the fundamental

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nature of interpenetration of nanotechnology and biosensor technology (Reshetilov and Bezborodov, 2008). The development of nanomaterials for the ultra sensitive detection of biological species (Tuutijärvi et al., 2009; Rassaei et al., 2008a) has received great attention because of their unique optical, electronic, chemical and mechanical properties.

Ulrich et al. (2009) have reviewed conducting polymers applied in chemical sensors and arrays (Rassaei et al., 2007, 2008b,c). Among the conducting polymers, poly(3,4-ethylenedioxythiophene), PEDOT, is a very attractive material due to its low band gap, high conductivity and stability, and transparency in the doped state (Ouyang et al., 2005; Groenendaal et al., 2000; Heywang and Jonas, 1992). PEDOT has been proposed as an alternative to traditional polymers as the electroactive component in biosensors (Kros et al., 2001, 2005). It is valuable to keep further researching for application in biosensors development.

The developments in nano-structured conducting polymers and polymer nanocomposites have large impact on biological research (Rajesh et al., 2009). Carbon nanotubes (CNTs) have been of great interest, both from a fundamental point of view and for potential applications. Their mechanical and unique electronic properties open a broad range of applications including nanoelectronic devices, composites, chemical sensors, biosensors and more (Bustero et al., 2006). Carbon nanotubes can be classified as single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs). SWNTs consist of a cylindrical single sheet with a diameter between 1 and 3 nm and a length of several micrometers. They possess a cylindrical nanostructure formed by rolling up a single graphite sheet into a tube. MWNTs consist of a coaxial arrangement of concentric single nanotubes like rings of a tree trunk separated from one another by 0.34 nm. CNTs are promising as an immobilization substance because of their significant mechanical strength, high surface area, excellent electrical conductivity and good stability (Iijima, 1991; Wang and Musamch, 2003). Due to these properties, CNTs have the ability to promote electron transfer reactions when used as an electrode.

With the recent development in nanoscience and nanotechnology, conducting polymer nano-structures received an ever-increasing attention and keeping this in view. We present the preparation and characterization of MWCNT and PEDOT composites and their potential applications in the field of nanosensors/biosensors. Particularly, it would be interesting to perform a multifunction biosensor with enzyme-free  $H_2O_2$  detection and simultaneous determination for AA, DA, and UA mixture by MWCNT and PEDOT.

In this paper, we report a study of the preparation and characterization of nano/conducting polymer composites (MWCNT–PEDOT nanoparticles) which is expected to form an enzyme-free  $H_2O_2$  biosensor. The MWCNT–PEDOT is prepared on electrode surface to study by means of cyclic voltammetry, SEM, AFM, different pulse voltammetry, and amperometry. The surface morphology and electrochemical property of MWCNT–PEDOT film are observed and significantly compared with bare GCE, MWCNT/GCE, and PEDOT/GCE. Electrocatalytic reactions including electrocatalytic reduction for hydrogen peroxide and simultaneously electrocatalytic oxidation for AA, DA, and UA by MWCNT–PEDOT are also examined and discussed.

## 2. Materials and methods

### 2.1. Fabrication of MWCNT, PEDOT, and MWCNT–PEDOT films

Prior to the electrochemical deposition process, the GCE was well polished with the help of BAS polishing kit with aqueous

slurries of alumina powder (0.05  $\mu\text{m}$ ), rinsed and ultrasonicated in double distilled deionized water. To have functional carboxylic group, the acidic treatment of MWCNT is necessary and has following procedure:

Step 1: Baking MWCNT to carbonate impurity in the oven at 350 °C for 1 h.

Step 2: Baking MWCNT in hydrochloric acid (12 M) to be dried out, keeping MWCNT in double distilled deionized water (DDDWW) with ultrasonic dispersion for 4 h after.

Step 3: Repeatedly filtering MWCNT by filter (porosity = 1  $\mu\text{m}$ ) in the ultrasonic and DDDWW till neutral condition (pH 7).

Step 4: Drying the neutralized MWCNT in the oven at 100 °C for 1 h.

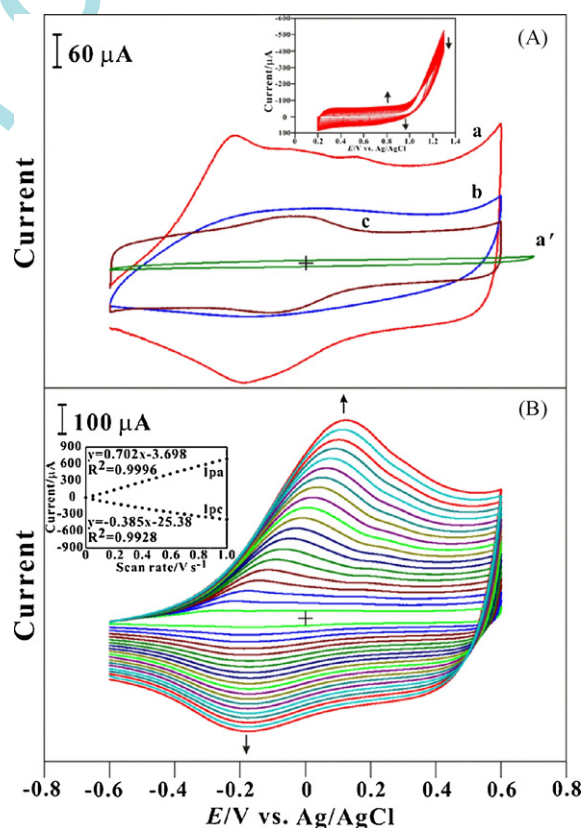
Step 5: Keeping MWCNT in the acidic solution containing sulfuric acid (18 M) and nitric acid (18 M) (v/v% = 3:1) by ultrasonic dispersion for 6 h.

Step 6: Repeatedly filtering MWCNT by filter (porosity = 1  $\mu\text{m}$ ) in the ultrasonic and DDDWW till neutral condition (pH 7).

Step 7: The neutralized MWCNT is dried out in the oven at 60 °C for 1 h.

Step 8: Finally, the carboxylic MWCNT is kept in DDDWW to be utilized.

The carboxylic MWCNT was added to electrode surface and dried out to immobilize on electrode surface due to the MWCNT



**Fig. 1.** (A) CVs of (a) MWCNT–PEDOT/GCE, (b) PEDOT/GCE, (c) MWCNT/GCE, and (a') bare GCE tested in 0.1 M pH 7 PBS solution, scan rate = 0.1 V/s. The inset shows CVs of the PEDOT film growth coated on GCE in pH = 1.5  $H_2SO_4$  solution containing 0.01 M EDOT, scan rate = 0.1 V/s, 20 cycles. (B) Different scan rate study of MWCNT–PEDOT film modified GCE in 0.1 M PBS solution (pH 7). Scan rate in the range of (a) 0.05 V/s, (b) 0.10 V/s, (c) 0.15 V/s, (d) 0.20 V/s, (e) 0.25 V/s, (f) 0.30 V/s, (g) 0.35 V/s, (h) 0.40 V/s, (i) 0.45 V/s, (j) 0.50 V/s, (k) 0.55 V/s, (l) 0.60 V/s, (m) 0.65 V/s, (n) 0.70 V/s, (o) 0.75 V/s, (p) 0.80 V/s, (q) 0.85 V/s, (r) 0.90 V/s, (s) 0.95 V/s, and (t) 1 V/s. Inset:  $I_{pa}$  and  $I_{pc}$  are plot of anodic and cathodic peak current vs. scan rate, respectively.

is not able to be directly modified on GCE by electrodeposition method. The method is applied for the preparation of not only MWCNT/GCE but also MWCNT–PEDOT/GCE film modified electrodes. And the PEDOT/GCE film modified electrode was performed by electropolymerization of EDOT monomers.

## 2.2. Chemicals

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), ascorbic acid (AA), dopamine (DA), uric acid (UA), multi-wall carbon nanotube (MWCNT), and 3,4-dioxyethylenethiophene (EDOT) monomer were purchased from Sigma–Aldrich (USA). All other chemicals (Merck) used were of analytical grade (99%). Double distilled deionized water (DDDW) was used to prepare all the solutions. A phosphate buffer solution (PBS) of pH 7.0 was prepared using  $\text{Na}_2\text{HPO}_4$  ( $0.05 \text{ mol L}^{-1}$ ) and  $\text{NaH}_2\text{PO}_4$  ( $0.05 \text{ mol L}^{-1}$ ).

## 2.3. Apparatus

The pretreatment of MWCNT is using ADVANTEC filter (porosity =  $1 \mu\text{m}$ ) to purify MWCNT. All electrochemical experiments were performed using CHI 1205a potentiostats (CH Instruments, USA). The BAS GCE (0.3 cm in diameter, exposed geometric surface area  $0.07 \text{ cm}^2$ , Bioanalytical Systems, Inc., USA) was used. A conventional three-electrode system was used which consists of an Ag/AgCl (saturated KCl) as a reference electrode, a bare GCE or MWCNT/GCE or PEDOT/GCE or MWCNT–PEDOT/GCE as a working electrode, and platinum wire as a counter electrode. For the rest of the electrochemical studies, Ag/AgCl (saturated KCl) was used as a reference. Electrochemical impedance studies (EIS) were performed using ZAHNER impedance analyzer (Germany).

The morphological characterization of composite films was examined by means of SEM (S-3000H, Hitachi) and AFM images were recorded with multimode scanning probe microscope (Being Nano-Instruments CSPM-4000, China). The buffer solution was entirely altered by deaerating using nitrogen gas atmosphere. The electrochemical cells were kept properly sealed to avoid the oxygen interference from the atmosphere. For our convenience, GCE substrates have been used for the AFM analysis.

## 3. Results and discussion

### 3.1. Preparation of MWCNT–PEDOT film

The MWCNT–PEDOT can be performed on GCE with electropolymerization of EDOT monomers and further loading purified MWCNT to immobilize on the electrode surface to form MWCNT–PEDOT/GCE. The inset of Fig. 1A shows the PEDOT electrodeposition voltammogram which is well known and has a redox couple with a formal potential of approximate 0.8 V in pH = 1.5  $\text{H}_2\text{SO}_4$  solution containing 0.01 M EDOT. Further applying functionalized MWCNT to coat on PEDOT to perform MWCNT–PEDOT. Fig. 1A shows the cyclic voltammogram comparison of (a) MWCNT–PEDOT/GCE, (b) PEDOT/GCE, (c) MWCNT/GCE, and (a') bare GCE in 0.1 M PBS (pH 7), respectively. The redox couples were obviously found the similar formal potential of 0.05 V between (b) PEDOT and (c) MWCNT. However, (a) MWCNT–PEDOT shows interesting redox couples type different from those of unique (b) PEDOT or (c) MWCNT. This result gives a hint that carboxylic MWCNT might react with PEDOT to form the MWCNT–PEDOT and shows more negative formal potential.

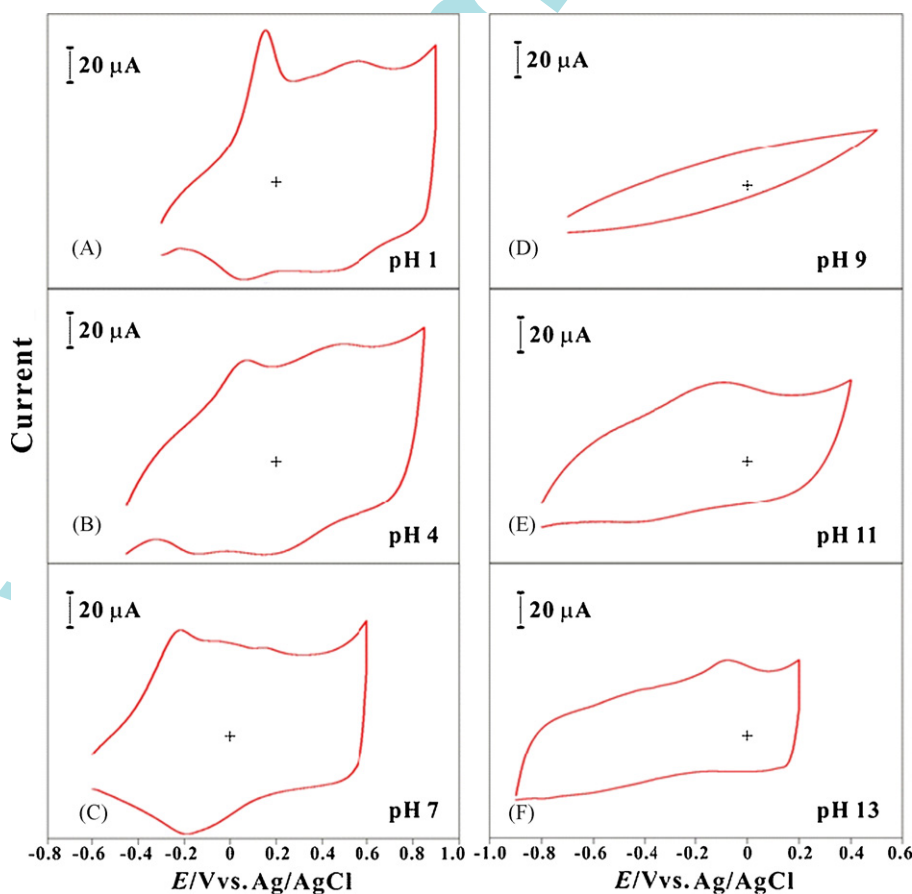


Fig. 2. CVs of MWCNT–PEDOT film modified GCE transferred to various pH solutions including pH: (a) 1, (b) 4, (c) 7, (d) 9, (e) 11, and (f) 13, scan rate: 0.1 V/s.



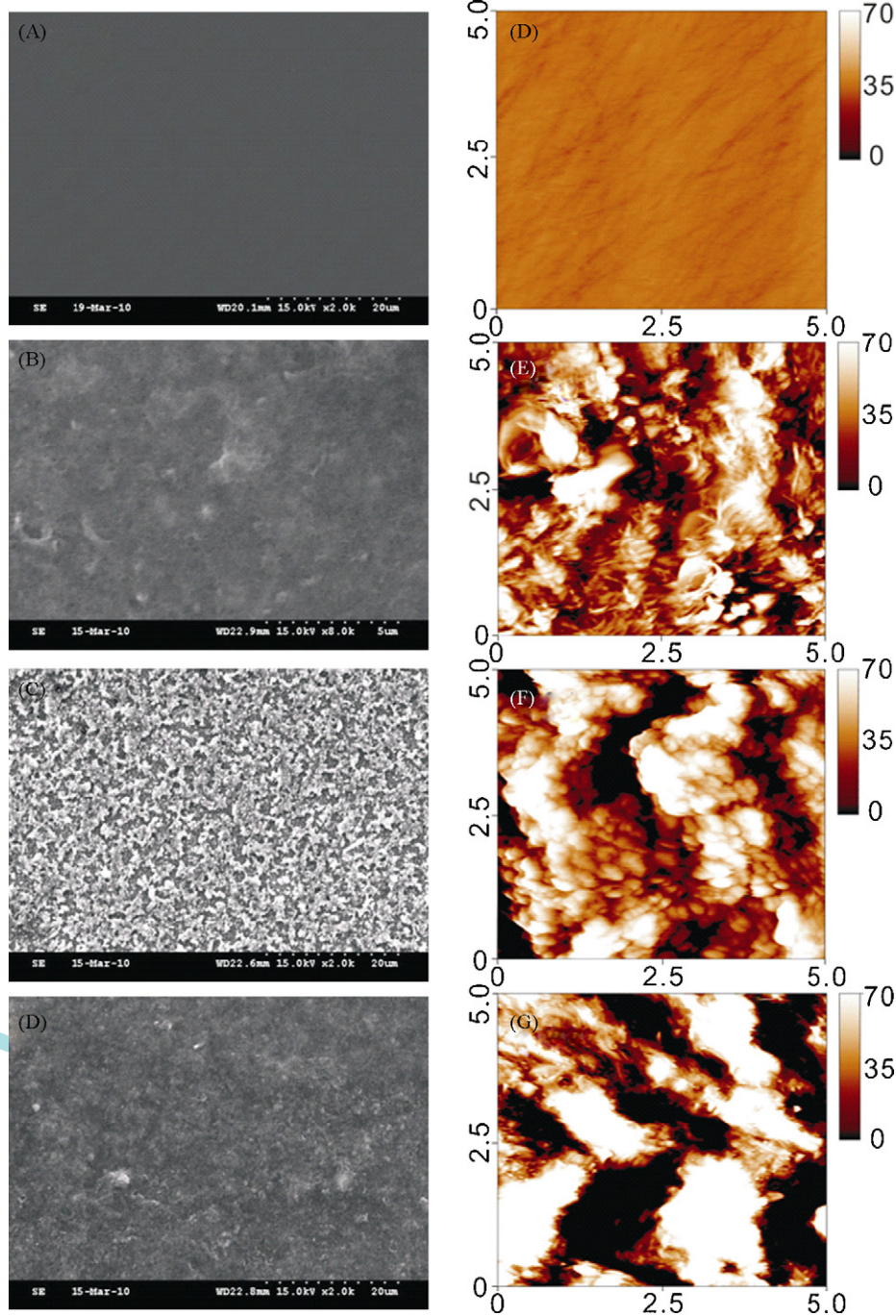
### 3.2. Characterization of MWCNT–PEDOT film

#### 3.2.1. Cyclic voltammogram of MWCNT–PEDOT film with various scan rate and pH conditions

To confirm the MWCNT–PEDOT film was successfully immobilized on the electrode surface, it was transferred to pH 7.0 PBS for CV studies. By the way, the influence of scan rate on the electrochemical response of MWCNT–PEDOT/GCE modified GCE was also investigated. Fig. 1B shows cyclic voltammograms of MWCNT–PEDOT/GCE were performed for the different scan rate studies. According to the obvious redox couple, it represents MWCNT–PEDOT can be modified on GCE in 0.1 M PBS (pH 7) at different scan rate in the range of 0.05–1 V/s. In the potential range

of  $-0.6$  to  $0.6$  V, the MWCNT–PEDOT film exhibits a cathodic peak and a broad anodic peak at the range of  $-0.18$  to  $-0.19$  V and  $-0.26$  to  $0.12$  V, respectively. The redox peak current develops as increasing the scan rate. Both the anodic and cathodic peak current and peak positions were affected by scan rate. By the inset of Fig. 1B, it can be found the linearly correlation of anodic and cathodic peak currents with scan rate, respectively. It indicates that the surface reaction of MWCNT–PEDOT/GCE was a surface-controlled process. At the same time, it is noticed with the anodic peak becomes much broader as increasing scan rate.

To ascertain the effect of pH, the voltammetric response of MWCNT–PEDOT/GCE was recorded in solutions of different pH in the range of 1–13. As can be seen in Fig. 2, the redox couples of



**Fig. 3.** SEM images of (A) bare GCE, (B) MWCNT/GCE, (C) PEDOT/GCE and (D) MWCNT–PEDOT/GCE; tapping mode AFM images of (E) bare GCE, (F) MWCNT/GCE, (G) PEDOT/GCE and (H) MWCNT–PEDOT/GCE.

MWCNT–PEDOT were shifted more negative potential as increasing pH value of solution. The MWCNT–PEDOT shows different redox peaks with various pH conditions. It represents interesting redox couple numbers and can be classified by three types. At the lower pH condition from pH 1 to 4, MWCNT shows two redox couples. At the higher pH condition from pH 7 to 13 except of pH 9, it shows only one redox couple. Particularly, MWCNT–PEDOT shows unique cyclic voltammogram neither obvious anodic peak nor cathodic peak at the condition of pH 9. Even though repeatedly examine MWCNT–PEDOT and change testing order of pH condition, the results are the same. This interesting result might be caused by complicated proton exchange with carboxylic group of MWCNT.

### 3.2.2. SEM and AFM analysis of MWCNT–PEDOT film

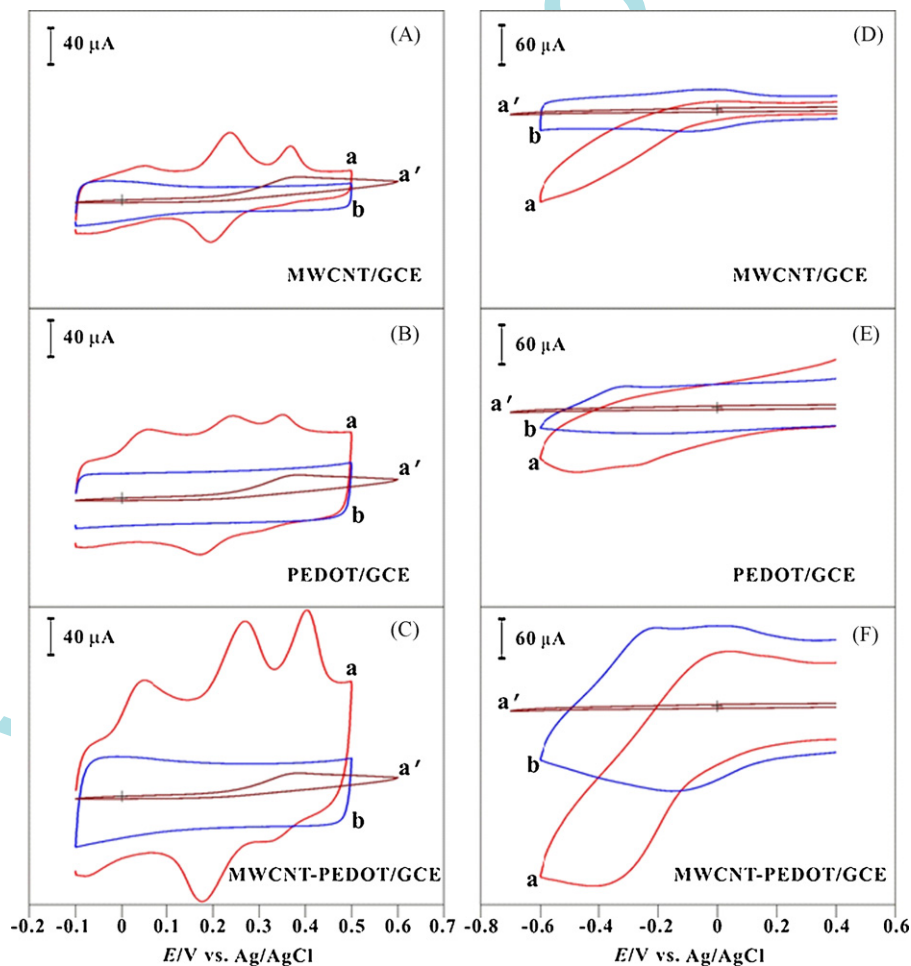
Scanning electron microscopy (SEM) and atomic force microscopy (AFM) were utilized to image the morphology of the active surface of the electrodeposited PEDOT films with/without MWCNT, and compared with the bare GCE, as shown in Fig. 3A–H. The bare GCE appears flat surface, in contrast with the MWCNT, PEDOT, and MWCNT–PEDOT film modified GCE electrodes, which has a relatively smooth surface. The AFM images of these films showed average diameter of 60.6 nm, 76.3 nm, and 65.7 nm MWCNT, PEDOT, and MWCNT–PEDOT, respectively. And the height of nano-grains was found in 20.9 nm, 47.7 nm, and 22.7 nm. By comparison, the PEDOT polymerized layer has much thicker and

extremely rough surface in SEM image. Moreover, it is found that MWCNT–PEDOT shows the average diameter size between PEDOT and MWCNT. It might be caused by the static adsorption between positive charge of PEDOT and carboxylic group of MWCNT. The MWCNT–PEDOT is further found its smoother SEM image compared with the SEM image of PEDOT due to the static adsorption of PEDOT and MWCNT makes film surface more compatible.

### 3.3. Electrocatalytic properties of MWCNT–PEDOT film

#### 3.3.1. Electrocatalytic reduction of hydrogen peroxide by MWCNT, PEDOT, and MWCNT–PEDOT film modified electrodes, respectively

The electrocatalytic reduction of hydrogen peroxide is studied by cyclic voltammetry and compared to different film modified electrodes in the deaerating PBS solution. Fig. 4D–F shows the electrocatalytic reduction cyclic voltammograms of hydrogen peroxide by (D) MWCNT/GCE, (E) PEDOT/GCE, (F) MWCNT–PEDOT/GCE and bare GCE electrodes, respectively. The electrocatalytic property of film modified electrodes is examined in the (a) presence and (b) absence of  $5 \times 10^{-2}$  M hydrogen peroxide in a pH 7.0 PBS solution. Comparing to bare GCE electrode (a'), which shows almost no electrocatalytic current response for  $5 \times 10^{-2}$  M hydrogen peroxide during scanning potential range from 0.4 to  $-0.6$  V, these film modified electrodes can show their uniquely electrocatalytic



**Fig. 4.** (A)–(C) are CVs of (A) MWCNT/GCE, (B) PEDOT/GCE, and (C) MWCNT–PEDOT/GCE examined in the pH 7 PBS mixing solution containing  $2 \times 10^{-3}$  M AA,  $3 \times 10^{-4}$  M DA, and  $2.5 \times 10^{-4}$  M UA. The (a') is bare GCE examined in same mixture and (b) is the film modified GCE examined in pH 7 PBS blank solution without AA, DA, and UA. (D)–(F) are CVs of (D) MWCNT/GCE, (E) PEDOT/GCE, and (F) MWCNT–PEDOT/GCE examined in the pH 7 PBS solution containing  $5 \times 10^{-2}$  M  $\text{H}_2\text{O}_2$ . The (a') is bare GCE examined in  $5 \times 10^{-2}$  M  $\text{H}_2\text{O}_2$  solution and (b) is the film modified GCE examined in pH 7 PBS blank solution without  $\text{H}_2\text{O}_2$ . Scan rate = 0.1 V/s.



potential and current. Particularly, MWCNT–PEDOT can be found its obviously higher electrocatalytic reduction current for hydrogen peroxide with a sharp peak at about  $-0.4$  V (shown in Fig. 4F). It means that MWCNT–PEDOT has its specific electrocatalytic property with stronger electrocatalytic ability of lower overvoltage and higher electrocatalytic reduction current for hydrogen peroxide. As the examined results, MWCNT–PEDOT can be a good choice to detect hydrogen peroxide especially for enzyme-free biosensor development.

### 3.3.2. Electrocatalytic oxidation of AA, DA, and UA mixture by MWCNT, PEDOT, and MWCNT–PEDOT film modified electrodes, respectively

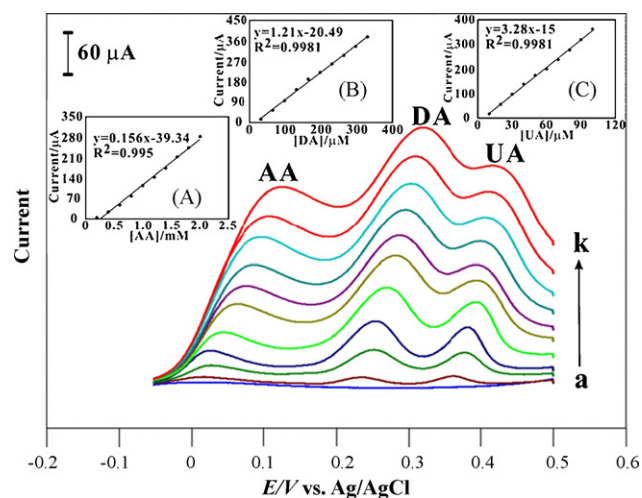
The electrocatalytic oxidation of AA, DA, and UA mixture is studied by cyclic voltammetry and compared to different film modified electrodes in the deoxygenating PBS solution. Fig. 4A–C shows the electrocatalytic oxidation cyclic voltammograms of AA, DA, and UA mixture containing  $2 \times 10^{-3}$  M AA,  $3 \times 10^{-4}$  M DA, and  $2.5 \times 10^{-4}$  M UA by (A) MWCNT/GCE, (B) PEDOT/GCE, (C) MWCNT–PEDOT/GCE and bare GCE electrodes, respectively. The electrocatalytic property of film modified electrodes is examined in the (a) presence and (b) absence of AA, DA, and UA in a pH 7.0 PBS solution. Comparing to bare GCE electrode (a') which always shows only one electrocatalytic peak of about 0.382 V for AA, DA, and UA mixture during scanning potential range from  $-0.1$  to 0.5 V, these film modified electrodes show their significantly electrocatalytic potential and current. Particularly, MWCNT–PEDOT can be found its obviously higher electrocatalytic oxidation current for AA, DA, and UA mixture (shown in Fig. 4C). It means that MWCNT–PEDOT has its specific electrocatalytic property with higher electrocatalytic oxidation current for AA, DA, and UA mixture. As the examined results, MWCNT–PEDOT can be a good choice to simultaneously determine AA, DA, and UA in the mixture without sensing interference.

### 3.3.3. Different pulse voltammetric response of AA, DA, and UA mixture determined by MWCNT–PEDOT Film

The next attempt was taken to simultaneously determine AA, DA, and UA mixture by MWCNT–PEDOT film modified electrode. Since the charging current contribution to background current is a limiting factor in analytical determination of any electroactive species, experiments were carried out using different pulse voltammetry (DPV) in the range of  $-0.05$  to 0.5 V. As can be seen in Fig. 5, three well-defined peaks at about 0.1, 0.3, and 0.4 V were observed, corresponding differential pulse voltammograms of AA, DA, and UA, respectively. These three well distinguished peaks allow us to detect of AA, DA, and UA simultaneously by using DPV. The linear ranges for determination of AA, DA, and UA were  $1 \times 10^{-4}$  to  $2 \times 10^{-3}$ ,  $1 \times 10^{-5}$  to  $3.3 \times 10^{-4}$ , and  $1 \times 10^{-5}$  to  $2.5 \times 10^{-4}$  M respectively. The detection limits of proposed method defined as ( $2\sigma$ ) for AA, DA, and UA were  $1 \times 10^{-4}$ ,  $1 \times 10^{-5}$ , and  $1 \times 10^{-5}$  M, respectively. The relative standard deviation (RSD) for determining AA, DA, and UA ( $n=10$ ) was 3.1%, 2.3%, and 3.9%, respectively. It indicates that the sensor had very good reproducibility at pH 7.

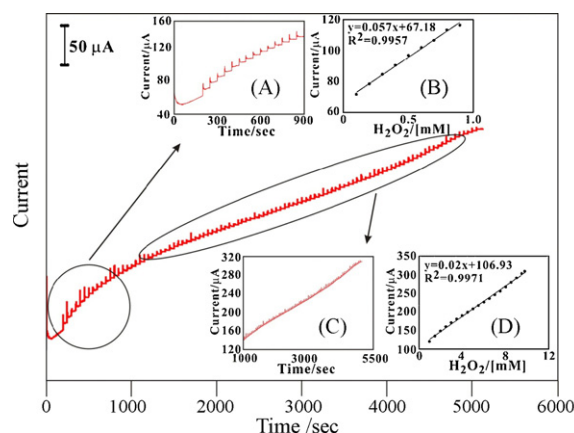
### 3.3.4. Amperometric response of hydrogen peroxide electrocatalysis by MWCNT–PEDOT film

Amperometric response with additions of hydrogen peroxide was tested to study electrocatalytic reduction of hydrogen peroxide by MWCNT–PEDOT film modified GCE electrode in the deaerating PBS solution. Fig. 6 shows amperometric response of hydrogen peroxide that was evaluated at MWCNT–PEDOT/GCE. Applied potential is set at  $-0.5$  V with electrode rotation speed of 2000 rpm. Initial period of 0–200 s, amperometric response of MWCNT–PEDOT/GCE is tested for blank. As sequential additions of  $10^{-4}$  M hydrogen peroxide per 50 s by micro-syringe during 201–5250 s, the



**Fig. 5.** Differential pulse voltammograms of MWCNT–PEDOT/GCE for AA, DA, and UA mixture containing: (a) blank, (b)  $2 \times 10^{-4}$  M AA,  $3.3 \times 10^{-5}$  M DA,  $1 \times 10^{-5}$  M UA, (c)  $4 \times 10^{-4}$  M AA,  $6.6 \times 10^{-5}$  M DA,  $2 \times 10^{-5}$  M UA, (d)  $6 \times 10^{-4}$  M AA,  $9.9 \times 10^{-5}$  M DA,  $3 \times 10^{-5}$  M UA, (e)  $8 \times 10^{-4}$  M AA,  $1.3 \times 10^{-4}$  M DA,  $4 \times 10^{-5}$  M UA, (f)  $1 \times 10^{-3}$  M AA,  $1.7 \times 10^{-4}$  M DA,  $5 \times 10^{-5}$  M UA, (g)  $1.2 \times 10^{-3}$  M AA,  $2 \times 10^{-4}$  M DA,  $6 \times 10^{-5}$  M UA, (h)  $1.4 \times 10^{-3}$  M AA,  $2.3 \times 10^{-4}$  M DA,  $7 \times 10^{-5}$  M UA, (i)  $1.6 \times 10^{-3}$  M AA,  $2.6 \times 10^{-4}$  M DA,  $8 \times 10^{-5}$  M UA, (j)  $1.8 \times 10^{-3}$  M AA,  $3 \times 10^{-4}$  M DA,  $9 \times 10^{-5}$  M UA, and (k)  $2 \times 10^{-3}$  M AA,  $3.3 \times 10^{-4}$  M DA,  $1 \times 10^{-4}$  M UA in pH 7 PBS solution,  $E_{\text{pulse}} = -0.05$  to 0.5 V. Insets: plot of  $I_{\text{pa}}$  vs. concentration for (A) AA, (B) DA, and (C) UA, respectively.

correlative amperometric response of MWCNT–PEDOT/GCE can be found. As tested result, MWCNT–PEDOT/GCE has detection limit of 0.05–10 mM and shows linearly amperometric responses for hydrogen peroxide and can be observed in two linearly sections. The first one during 201–900 s shows sensitivity of  $943 \mu\text{M mM}^{-1} \text{cm}^{-2}$  and signal/noise of 6 as shown in Fig. 6A and B. After 900 s, it shows sensitivity of  $174 \mu\text{M mM}^{-1} \text{cm}^{-2}$  and signal/noise of 4 with another linearly amperometric response of hydrogen peroxide additions as shown in Fig. 6C and D. The relative standard deviation (RSD) for determining  $\text{H}_2\text{O}_2$  ( $n=10$ ) in the section of 201–900 s and 901–5250 s was 4.1% and 4.5%, respectively. It indicates that the sensor had very good reproducibility at pH 7. It seems that MWCNT–PEDOT/GCE would have higher response in early additions (during 201–900 s) for hydrogen peroxide and



**Fig. 6.** Amperometric responses of sequential additions of  $\text{H}_2\text{O}_2$  (each  $10^{-4}$  M per time; time interval = 50 s) at the MWCNT–PEDOT/GCE in pH 7 PBS. Rotating speed = 2000 rpm,  $E_{\text{app}} = -0.5$  V. The blank amperometric response of MWCNT–PEDOT/GCE is tested during 0–200 s. Inset: (A) scale-up amperometric responses of first  $\text{H}_2\text{O}_2$  additions during 201–900 s and shows (B) initial plot of  $\text{H}_2\text{O}_2$  concentration vs. catalytic current; (C) scale-up amperometric responses of  $\text{H}_2\text{O}_2$  additions during 901–5250 s and shows (D) plot of  $\text{H}_2\text{O}_2$  concentration vs. catalytic current.

more sensitive correlation between current and concentration of hydrogen peroxide. Having above information, it would be easier to compensate the electronic signal of hydrogen peroxide sensor for practical fabrication.

### 3.4. Stability study of MWCNT–PEDOT film

Repetitive redox cycling experiments were done to determine the extent of stability relevant to MWCNT–PEDOT modified CCE in 0.1 M PBS solution (pH 7). This investigation indicated that after 100 continuous scan cycles with scan rate of  $100 \text{ mV s}^{-1}$ , the peak heights of the cyclic voltammograms decreased less than 5%. On the other hand, the MWCNT–PEDOT modified GCE kept its initiate activity for more than one month as kept in 0.1 M PBS solution (pH 7). A decrease of 5% was observed in current response of the electrode at the end of 30th day. And the electrocatalytic response current of MWCNT–PEDOT for  $\text{H}_2\text{O}_2$ , AA, DA, and UA can keep more than 90% of original current response.

## 4. Conclusions

Here we report a method to form an enzyme-free hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) biosensor based on MWCNT–PEDOT nanoparticles. It has good electrocatalytic reduction for hydrogen peroxide without HRP enzyme and shows lower over-potential and higher current response compared with MWCNT/GCE and PEDOT/GCE. Amperometric response of MWCNT–PEDOT/GCE is linearly dependent on concentration of hydrogen peroxide. The proposed film also can simultaneously detect AA, DA, and UA and can be utilized to develop multifunctional biosensors. As the results, the proposed method has excellent advantages of enzyme-free, multifunction, low cost, low over-potential, high current response, high sensitivity, and high selectivity.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bios.2010.07.019](https://doi.org/10.1016/j.bios.2010.07.019).

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