



Polyaniline and poly(flavin adenine dinucleotide) doped multi-walled carbon nanotubes for p-acetamidophenol sensor

Ying Li, Yogeswaran Umasankar, Shen-Ming Chen*

Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No. 1, Section 3, Chung-Hsiao East Road, Taipei 106, Taiwan, ROC

ARTICLE INFO

Article history:

Received 7 March 2009

Received in revised form 6 April 2009

Accepted 7 April 2009

Available online 16 April 2009

Keywords:

Multi-wall carbon nanotubes

Biocomposite film

Modified electrodes

Electrocatalysis

Electroanalysis

Para-acetamidophenol

ABSTRACT

A conductive biocomposite film (MWCNTs–PANIFAD) which contains multi-walled carbon nanotubes (MWCNTs) along with the incorporation of poly(aniline) and poly(flavin adenine dinucleotide) copolymer (PANIFAD) has been synthesized on gold and screen printed carbon electrodes by potentiostatic methods. The presence of MWCNTs in the MWCNTs–PANIFAD biocomposite film enhances the surface coverage concentration (Γ) of PANIFAD and increases the electron transfer rate constant (k_s) to 89%. Electrochemical quartz crystal microbalance studies reveal the enhancements in the functional properties of MWCNTs and PANIFAD present in MWCNTs–PANIFAD biocomposite film. Surface morphology of the biocomposite film has been studied using scanning electron microscopy and atomic force microscopy. The surface morphology results reveal that PANIFAD incorporated on MWCNTs. The MWCNTs–PANIFAD biocomposite film exhibits promising enhanced electrocatalytic activity towards the oxidation of p-acetamidophenol. The cyclic voltammetry has been used for the measurement of electroanalytical properties of p-acetamidophenol by means of PANIFAD, MWCNTs and MWCNTs–PANIFAD biocomposite film modified gold electrodes. The sensitivity value of MWCNTs–PANIFAD film ($88.5 \text{ mA mM}^{-1} \text{ cm}^{-2}$) is higher than the values which are obtained for PANIFAD ($28.7 \text{ mA mM}^{-1} \text{ cm}^{-2}$) and MWCNTs films ($60.7 \text{ mA mM}^{-1} \text{ cm}^{-2}$). Finally, the flow injection analysis (FIA) has been used for the amperometric detection of p-acetamidophenol at MWCNTs–PANIFAD film modified screen printed carbon electrode. The sensitivity value of MWCNTs–PANIFAD film ($3.3 \text{ mA mM}^{-1} \text{ cm}^{-2}$) in FIA is also higher than the value obtained for MWCNTs film ($1.1 \text{ mA mM}^{-1} \text{ cm}^{-2}$).

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Electropolymerization is a simple but powerful method in targeting selective modification of different type electrodes with desired matrices. However, the materials on the matrices do not possess peculiar properties when compared with those materials which are chemically synthesized by traditional methods. The electroactive polymers and carbon nanotubes (CNTs) matrices have received considerable attraction in recent years. Numerous conjugated polymers have been electrochemically synthesized for their application in the fabrication of chemical and biochemical sensor devices [1]. These conjugated polymers for sensor devices exhibits interesting enhancement in the electrocatalytic activity towards the oxidation or reduction of several biochemical and inorganic compounds [2] where, some of the functional groups in polymers will act as catalyst [3–5]. In this article, the word “enhanced electrocatalytic activity” could be explained as both; increase in peak current and lower overpotential [6]. The wide variety of applica-

tions of matrices made of CNTs for the detection of inorganic and bioorganic compounds such as IO_3^- , ascorbic acid, etc., have already been reported in the literature [7–9].

Even though the electrocatalytic activity of conjugated polymers and CNTs matrices individually shows good results; some properties like mechanical stability, sensitivity for different techniques, and electrocatalysis for multiple compound detections are found to be poor. To overcome this difficulty, new studies have been developed in the past decade for the preparation of composite films composed of both CNTs and conjugated polymers. The rolled-up graphene sheets of carbon exhibit π -conjugative structure with highly hydrophobic surface. This unique property of the CNTs allows them to interact with organic aromatic compounds through π – π electronic and hydrophobic interactions to form new structures [10,11]. There were past attempts in the preparation of composite and sandwiched films made of polymer adsorbed on CNTs, and used them for electrocatalytic studies such as, selective detection of dopamine in the presence of ascorbic acid [12]. The sandwiched films were also been used in the designing of nanodevices with the help of non-covalent adsorption, electrodeposition, etc. [13].

Among conjugated polymers, polyaniline (PANI) is one of the most widely used conducting polymer [14]. The synthesis of PANI

* Corresponding author. Tel.: +886 2270 17147; fax: +886 2270 25238.
E-mail address: smchen78@ms15.hinet.net (S.-M. Chen).

was first reported by Letheby [15], where aniline was oxidized on Pt-sheet in acidic media. PANI layers have been used as gas sensors [16,17], electrode material for redox supercapacitor [18], shield material for electromagnetic interference control [19], corrosion control [20], etc. Similarly, flavin adenine dinucleotide (FAD) is a flavoprotein coenzyme that plays an important biological role in many oxidoreductases and in reversible redox conversions in biochemical reactions. FAD has an isoalloxazine ring as a redox-active component. The adsorption of FAD has been studied on Hg electrode and titanium electrodes [21,22], and the electrochemical reaction of FAD was also studied on various electrodes in aqueous solutions [23,24]. Further, the electropolymerization of FAD to form poly(flavin adenine dinucleotide) (PFAD) was also reported in the literature [25].

p-Acetamidophenol is a well-known drug which has extensive applications in pharmaceutical industries. It is an antipyretic and analgesic compound that has high therapeutic value. It is also used as a precursor in penicillin, and as stabilizer for hydrogen peroxide, photographic chemical, etc. Various studies were reported for the determination of p-acetamidophenol in drug formulations using different techniques [26–33]. Among these several methods of determination techniques, the electrochemical methods have more advantages over the other in sensing p-acetamidophenol. However in these reports, the same principle, that is, oxidation of p-acetamidophenol by metal ion oxidants has been used for the estimation.

The literature survey reveals that there were no previous attempts made for the synthesis of biocomposite film composed of CNTs, PANI and PFAD for the use in sensor application. In this article, we report about a novel biocomposite film (MWCNTs–PANIFAD) made of multi-walled carbon nanotubes (MWCNTs) which has been incorporated with PANIFAD co-polymer. MWCNTs–PANIFAD biocomposite film's characterization, enhancement in functional properties, peak current and electrocatalytic activity have also been reported along with its application in the determination of p-acetamidophenol. The film formation processing involves the modification of gold electrode with uniformly well dispersed MWCNTs, and which is then modified with PANIFAD co-polymer.

2. Experimental

2.1. Materials

Aniline, FAD, MWCNTs (OD = 10–20 nm, ID = 2–10 nm and length = 0.5–200 μm), potassium hydroxide and p-acetamidophenol obtained from Aldrich and Sigma–Aldrich were used as received. All other chemicals used were of analytical grade. The preparation of aqueous solution was done with twice distilled deionized water. Solutions were deoxygenated by purging with pre-purified nitrogen gas. Solutions were prepared from 1 M HClO_4 for the pH 2.28 aqueous solution.

2.2. Apparatus

Cyclic voltammetry (CV) was performed in analytical system models CHI-611, CHI-400 and CHI-1205A potentiostats. A conventional three-electrode cell assembly consisting of Ag/AgCl reference electrode and a Pt wire counter electrode were used for the electrochemical measurements. The working electrode was either an unmodified gold or a gold modified with PANI, PANIFAD, MWCNTs–PANI or MWCNTs–PANIFAD biocomposite films. In all experiments, the potentials are reported vs. the Ag/AgCl reference electrode. The working electrode used for EQCM measurements was an 8 MHz AT-cut quartz crystal coated with gold electrode. The diameter of the quartz crystal is 13.7 mm; the gold elec-

trode diameter is 5 mm. The flow injection analysis (FIA) of the p-acetamidophenol at screen printed carbon electrode (SPCE) was done using Alltech 426 HPLC pump containing an electrochemical cell with electrodes. This above-mentioned electrochemical cell was connected with an inlet from the pump and an outlet, which were used for the carrier stream flow in the electrochemical cell. The morphological characterizations of the films were examined by means of scanning electron microscopy (SEM) (Hitachi S-3000H) and atomic force microscopy (AFM) (Being Nano-Instruments CSPM4000). All the measurements were carried out at $25^\circ\text{C} \pm 2$.

2.3. Preparation of MWCNTs dispersion and MWCNTs–PANIFAD modified electrode

There was an important challenge in the preparation of MWCNTs. Because of its hydrophobic nature, it was difficult to disperse it in any aqueous solution to get a homogeneous mixture. Briefly, the hydrophobic nature of the MWCNTs was converted in to hydrophilic nature by following the previous studies [34]. This was done by weighing 10 mg of MWCNTs and 200 mg of potassium hydroxide in to a ruby mortar and grained together for 2 h at room temperature. Then the reaction mixture was dissolved in 10 ml of double distilled deionized water and it was precipitated many times in to methanol for the removal of potassium hydroxide. Thus obtained MWCNTs in 10 ml water was ultrasonicated for 6 h to get uniform dispersion. This functionalization process of MWCNTs was done to get hydrophilic nature for the homogeneous dispersion, in water. This process not only converts MWCNTs to hydrophilic nature but this helps to breakdown larger bundles of MWCNTs in to smaller ones also. This process was confirmed using SEM, which is not shown in figures.

Before starting each experiment, gold electrodes were polished by BAS polishing kit with 0.05 μm alumina slurry and rinsed and then ultrasonicated in double distilled deionized water. The gold electrodes studied were uniformly coated with $50 \mu\text{g cm}^{-2}$ of MWCNTs and then dried at 35°C . The concentrations of homogeneously dispersed MWCNTs were exactly measured using micro-syringe. The electropolymerization of aniline with FAD to form PANIFAD co-polymer was performed using the mixture 50 mM aniline and 1 mM FAD present in pH 2.28 aqueous solution by consecutive CV over a suitable potential region of -0.35 to 1.0V . Then the MWCNTs–PANIFAD modified gold electrode was carefully washed with double distilled deionized water. For detailed study, comparison of different modified gold electrodes such as PANI, PANIFAD, MWCNTs–PANI and MWCNTs–PANIFAD have been performed. These characterization studies were done to reveal the obvious necessity for the usage of MWCNTs and PANIFAD in the MWCNTs–PANIFAD biocomposite film. For the FIA of p-acetamidophenol oxidation (at 726 mV), carrier stream used was pH 2.28 aqueous solution with the flow rate of 2 ml min^{-1} , and the volume of p-acetamidophenol injected at each cycle was $10 \mu\text{l}$ at the time interval of 50 s.

3. Results and discussions

3.1. Electrochemical synthesis of MWCNTs–PANIFAD biocomposite film and its characterization

The electropolymerization of aniline (50 mM) with FAD (1 mM) mixture on MWCNTs modified gold electrode present in pH 2.28 aqueous solution has been performed by consecutive CVs for the preparation of MWCNTs–PANIFAD biocomposite film. Fig. 1(a) shows the reduction and electropolymerization of aniline and FAD at MWCNTs modified gold electrode. Four redox couples (formal potential $E^{0'}$ = -14.8 , 202.15 , 511.9 and 718.4 mV vs. Ag/AgCl) are

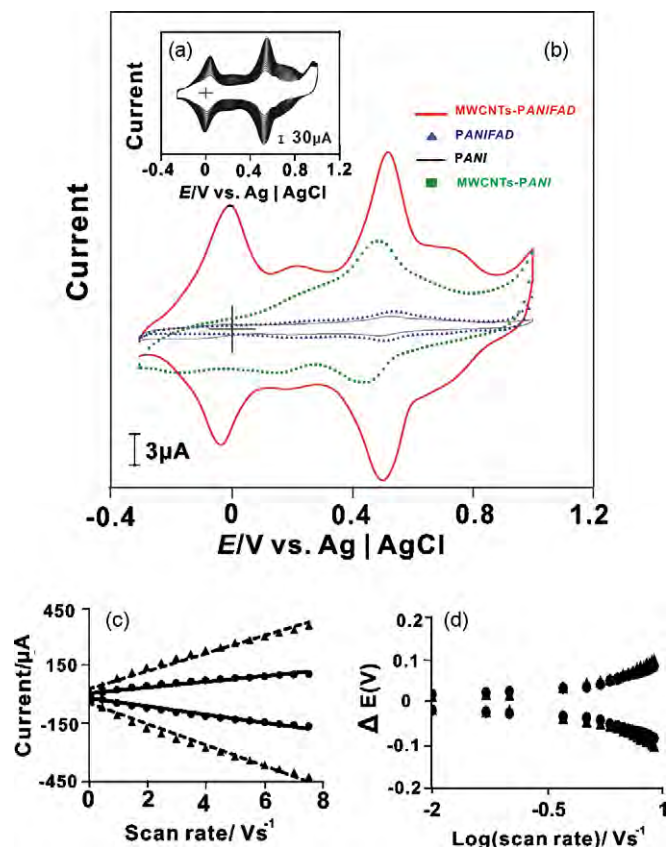


Fig. 1. (a) Repetitive CVs of MWCNTs–gold electrode modified from 50 mM aniline with 1 mM FAD present in pH 2.28 aqueous solution, scan rate 100 mV s⁻¹. (b) Comparison of CVs of PANI, PANIFAD, MWCNTs–PANI and MWCNTs–PANIFAD biocomposite films at gold electrode in pH 2.28 aqueous solution, scan rate 20 mV s⁻¹. (c) The plot of I_{pa} and I_{pc} vs. different scan rate for PANIFAD (continuous line) and MWCNTs–PANIFAD (dotted line) present in pH 2.28 aqueous solution and (d) the plot of ΔE vs. log scan rate for the same films.

obtained for PANIFAD during electropolymerization, which represents the electrochemical redox reactions of PANIFAD co-polymer present in MWCNTs–PANIFAD biocomposite film. The increase in peak current of the same redox couples at each cycle reveals the continuous deposition of PANIFAD during cycling. The electropolymerization has been carried out at a suitable potential range of -0.3 to 1 V. In Fig. 1(a) result, the $E^{0'}$ at -14.8 and 202.15 mV represents PFAD redox reactions, then the $E^{0'}$ at 511.9 mV represents PANI redox reaction, and then the $E^{0'}$ at 718.4 mV represents the redox reaction of PANIFAD co-polymer. In the following experiments, each newly prepared MWCNTs–PANIFAD biocomposite film on gold electrode has been washed carefully in deionized water to remove the loosely bounded aniline and FAD on the modified gold electrode. It was then transferred to pH 2.28 aqueous solution for other electrochemical characterizations.

Fig. 1(b) represents the electrochemical redox signals of PANI, PANIFAD, MWCNTs–PANI and MWCNTs–PANIFAD modified gold electrodes present in pH 2.28 aqueous solution; scan rate 20 mV s⁻¹. Among these four films, PANI has a redox couple at $E^{0'} = 511.9$ mV vs. Ag/AgCl, where as for PANIFAD two new redox couples appear at $E^{0'} = 202.15$ and 718.4 mV along with the enhancement in the redox couple's peak current at $E^{0'} = 511.9$ mV. Similarly in MWCNTs–PANI film, the presence of MWCNTs abruptly enhances the PANI's redox couple at $E^{0'} = 511.9$ mV. These above results show that the presence of PFAD and MWCNTs in the film enhances the redox reaction of PANI. In the same figure, MWCNTs–PANIFAD biocomposite film have four redox couples at $E^{0'} = -14.8, 202.15, 511.9$ and 718.4 mV. These four redox couple for MWCNTs–PANIFAD biocomposite

Table 1

Surface coverage concentration (Γ) of various species present in PANIFAD at different gold modified electrodes.

Modified films ^a	Γ (nmol cm ⁻²)				
	b	c	d	e	
PANI	–	–	0.13	–	
PANIFAD	0.37	0.03	0.15	–	
MWCNTs–PANI	–	–	0.92	–	
MWCNTs–PANIFAD	1.82	0.18	2.03	0.93	

Species b, c, d, and e representing redox couples at $E^{0'} = -14.8, 202.15, 511.9$ and 718.4 mV, respectively.

^a Studied using CV technique in pH 2.28 aqueous solution.

film represents the redox reactions of the compounds as we discussed above for Fig. 1(a). Among these four redox couples, $E^{0'}$ at -14.8 mV do not appear for other three films, and the peak currents of each redox couple in MWCNTs–PANIFAD biocomposite film is higher than the other films. From these CVs in Fig. 1(b), the surface coverage concentration (Γ) values have been calculated and given in Table 1. In this above calculation, the charge involved in the reaction (Q) has been obtained from CVs and it has been applied in the equation $\Gamma = Q/nFA$, where the number of electron transfer involved in PANIFAD redox reactions are assumed as two. These values in Table 1 indicates that the concentration increase of MWCNTs increases the Γ of PANIFAD. The Γ increase can be explained as, the concentration of MWCNTs directly proportional to the geometric area, where the MWCNTs modified gold electrode contains more surfaces to hold PANIFAD when comparing bare gold electrode surface. In general, the geometric area of nanomaterials is higher than the bulk materials. The slope value obtained from Table 1 reveals the increase in Γ of PANI and PFAD per μg of MWCNTs is ≈ 16 and $29 \text{ pmol cm}^{-2} \mu\text{g}^{-1}$ respectively.

The CVs of PANIFAD and MWCNTs–PANIFAD biocomposite films on gold electrode in pH 2.28 aqueous solution at different scan rates have been studied (CVs not shown). In these results, the redox peaks intensity increases linearly with the increase of scan rates up to 7.8 V s⁻¹. The scan rate vs. PANI redox couple peak current in Fig. 1(c) shows that, the ratio of I_{pa}/I_{pc} remains almost in unity as expected for surface type behavior. These above results demonstrate that the redox process is not controlled by diffusion up to 7.8 V s⁻¹. From the slope values of ΔE_p vs. log scan rate given in Fig. 1(d) plot, by assuming the value of $\alpha \approx 0.5$, the electron transfer rate constant (k_s) has been calculated based on Laviron theory [35]; and the k_s for PANI redox reaction at PANIFAD and MWCNTs–PANIFAD biocomposite films are ≈ 6.1 and 11.5 s^{-1} respectively. From these k_s values, we have calculated the increase in the ability of electron transfer between the electrode surface and PANIFAD in presence of MWCNTs, and it is $\approx 89\%$. These results show the enhanced functional property of MWCNTs–PANIFAD biocomposite film in the presence of MWCNTs.

3.2. EQCM and pH studies of MWCNTs–PANIFAD biocomposite film

The EQCM experiments have been carried out by modifying the gold electrochemical quartz crystal with and without uniformly coated MWCNTs, which was then dried at 35° C. Fig. 2(a) and (b) shows the consecutive CVs from EQCM studies for the PANIFAD formation at MWCNTs modified and unmodified gold coated quartz crystal electrodes respectively. In these results, increase in the voltammetric peak currents of PANIFAD redox couples and the frequency decrease, or mass increase (figure not shown) are found to be consistent with the growth of PANIFAD film on both modified and unmodified gold electrode. From the frequency change, change in the mass of PANIFAD and MWCNTs–PANIFAD biocom-

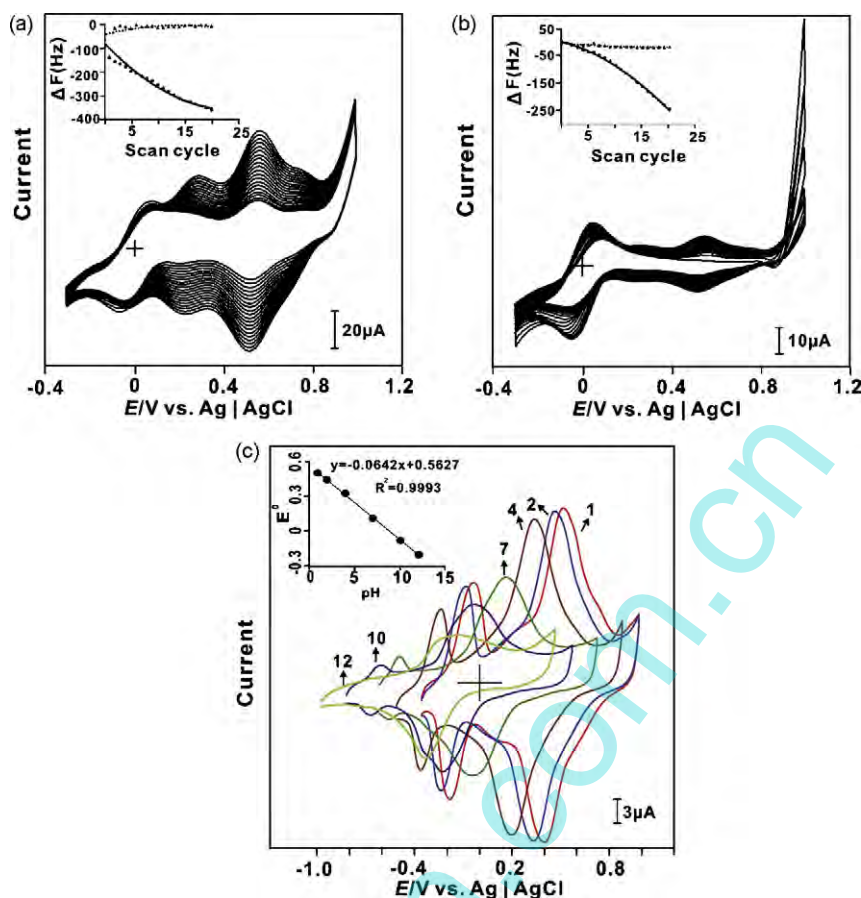


Fig. 2. Consecutive potential CVs of electrochemical quartz crystal gold electrode modified with PANIFAD at 1.0 to -0.3 V (scan rate 20 mV s^{-1}), where (a) and (b) is the presence and absence of MWCNTs on the gold electrode respectively. Insets in both (a) and (b) are the variation of frequency change with the increase of scan cycles (continuous line) and every cycle frequency change with the increase of the scan cycles (dotted line). (c) CVs of MWCNTs–PANIFAD biocomposite film synthesized at pH 2.28 on gold electrode and transferred to various pH solutions; inset shows formal potential vs. pH.

posite film at gold coated quartz crystal has been calculated using Sauerbrey equation. From this calculation for the above-mentioned experimental conditions, it has been found that 1 Hz frequency change is equal to 1.4 ng of mass change [36–39]. Therefore, mass change during PANIFAD incorporation on the MWCNTs modified and unmodified gold electrodes for total cycles are 504 and 341 ng cm^{-2} respectively. The insets in Fig. 2(a) and (b) indicates the every cycle frequency change with the increase of the scan cycles (dotted lines) and variation of frequency change with the increase of scan cycles (continuous lines) for MWCNTs–PANIFAD biocomposite and PANIFAD films respectively. From these plots, it is clear that there is a higher deposition of PANIFAD in presence of MWCNTs, which is similar to the I results given in Table 1. These results show that the deposition of PANIFAD on MWCNTs film and bare gold electrode is stable and homogeneous. Previous studies on CNT composites have shown also the necessity of CNTs for improving the functional properties such as orientation, enhanced electron transport, high capacitance, etc. [40,41]. Fig. 2(c) shows the CVs of MWCNTs–PANIFAD on gold electrode at various pH aqueous solutions in which the aniline and FAD are absent. In these experiments, the MWCNTs coated gold electrode has been modified with PANIFAD using pH 2.18 aqueous solution, and it has been washed with deionized water and then transferred to various pH aqueous solutions for CV measurements. These CV results show that the PFAD present in the film is stable in the pH range between 1 and 10, and the E_{pa} and E_{pc} values of both PANI and PFAD present in the film depends on the pH value of the aqueous solution. The decrease of PFAD peak current in pH 7 or greater could be due to

the decomposition of PFAD in higher pH, which in turn affects the PANI redox peak current also. The inset in Fig. 2(c) shows the formal potential of PANI redox couple present in MWCNTs–PANIFAD plotted over the pH range of 1–12. The response shows a slope of -64 mV pH^{-1} , which is close to that given by Nernstian equation for equal number of electrons and protons transfer [42,43].

3.3. Topographic characterization of MWCNTs–PANIFAD biocomposite film

Three different films MWCNTs, PANIFAD and MWCNTs–PANIFAD have been prepared on gold electrode with similar conditions and similar potential as mentioned in the previous sections, and were characterized using SEM and AFM. It is a well-known fact that the prolonged exposure to electron beam will damage the PFAD present in PANIFAD and MWCNTs–PANIFAD films, so an at most care has been taken to measure these images. Comparison of Fig. 3(a) and (b) SEM images reveals significant morphological difference between PANIFAD and MWCNTs–PANIFAD films. The top views of nanostructures in Fig. 3(a) on the gold electrode surface show grains of PANIFAD deposited on the electrode. The MWCNTs–PANIFAD biocomposite film in Fig. 3(b) shows that the plateaus of PANIFAD deposited over MWCNTs to form MWCNTs–PANIFAD biocomposite modified gold electrode. Similarly, (a') and (b') represents bare gold electrode and only MWCNTs, where MWCNTs are more visible in (b') when comparing (b). The same modified gold electrodes have been used to measure the AFM topography images shown in Fig. 4(a) PANIFAD and (b) MWCNTs–PANIFAD, where (a') is the bare

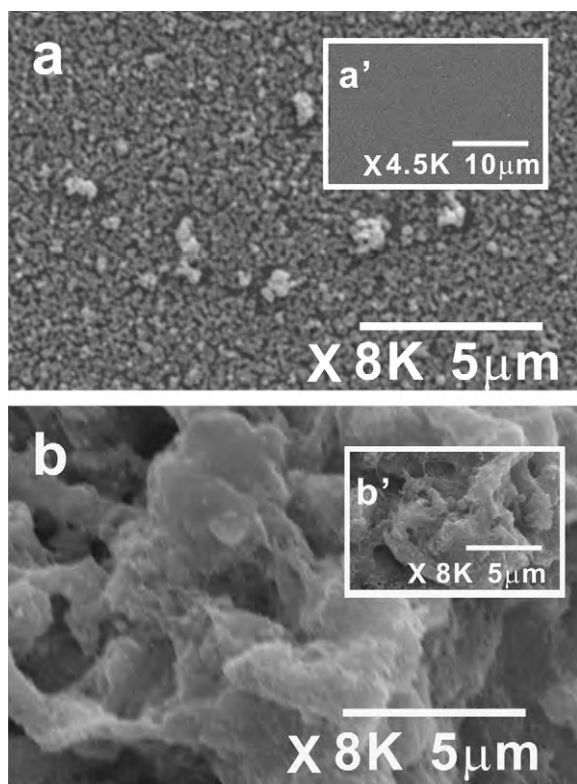


Fig. 3. SEM images of (a) PANIFAD and (b) MWCNTs–PANIFAD biocomposite films, where (a') is bare gold electrode and (b') is only MWCNTs.

gold electrode. The higher magnification ($10\ \mu\text{m} \times 10\ \mu\text{m}$) of AFM images when comparing SEM images reveal that disk shaped grain structures of PANIFAD formed on the gold electrode. However in Fig. 4(b), elongated deposition of PANIFAD formed over MWCNTs instead of disk shaped grain structure. This result reveals that higher concentration of PANIFAD has been deposited on MWCNTs modified gold electrode, which is consistent with the I values given in Table 1. Further, the thicknesses of PANIFAD and MWCNTs–PANIFAD obtained from AFM results are 300 and 1500 nm respectively. These values too show MWCNTs–PANIFAD is thicker than PANIFAD, which is due to the presence of MWCNTs covered under PANIFAD film. These SEM and AFM results reveal the coexistence of MWCNTs and PANIFAD in the biocomposite film.

3.4. Electroanalytical response of *p*-acetamidophenol at MWCNTs–PANIFAD biocomposite film

The MWCNTs–PANIFAD biocomposite film has been synthesized on gold electrode at similar conditions as given in experimental section. Then the MWCNTs–PANIFAD biocomposite film modified electrode has been washed carefully in deionized water and transferred to pH 2.28 aqueous solution for the electrocatalysis of *p*-acetamidophenol. All the CVs have been recorded at the constant time interval of 1 min with nitrogen purging before the start of each experiments. Fig. 5 shows the electrocatalytic oxidation of *p*-acetamidophenol ($60\ \mu\text{M}$) at various film modified and unmodified gold electrodes; scan rate $20\ \text{mV s}^{-1}$. The various film modified electrodes are PANIFAD, MWCNTs and MWCNTs–PANIFAD biocomposite films, where the MWCNTs–PANIFAD biocomposite film is shown with highest concentration (60 μM) and also in the absence of *p*-acetamidophenol (blank). The schematic representation of *p*-acetamidophenol electrochemical oxidation at MWCNTs–PANIFAD biocomposite film is given in Scheme 1. The CVs for MWCNTs–PANIFAD biocomposite film exhibits reversible

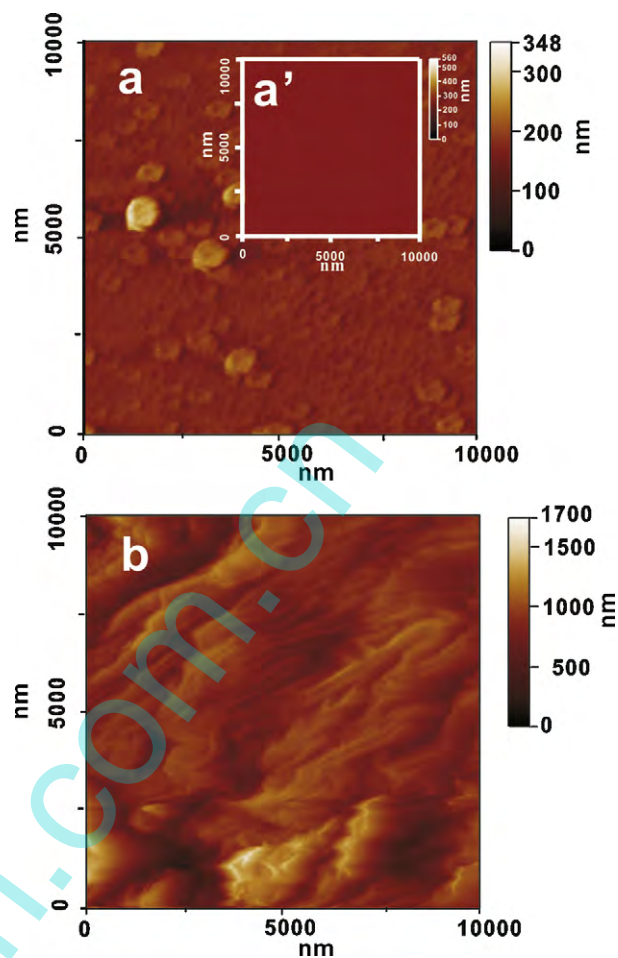


Fig. 4. AFM images of (a) PANIFAD and (b) MWCNTs–PANIFAD biocomposite films, where (a') is bare gold electrode.

redox couples in the absence of *p*-acetamidophenol, and upon addition of *p*-acetamidophenol a new growth in the oxidation peak of *p*-acetamidophenol appeared at $E_{\text{pa}} = 726\ \text{mV}$. This above said anodic peak shows that the electrocatalytic oxidation of

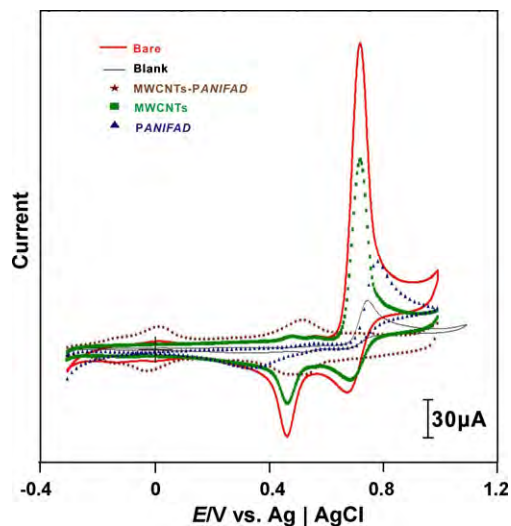
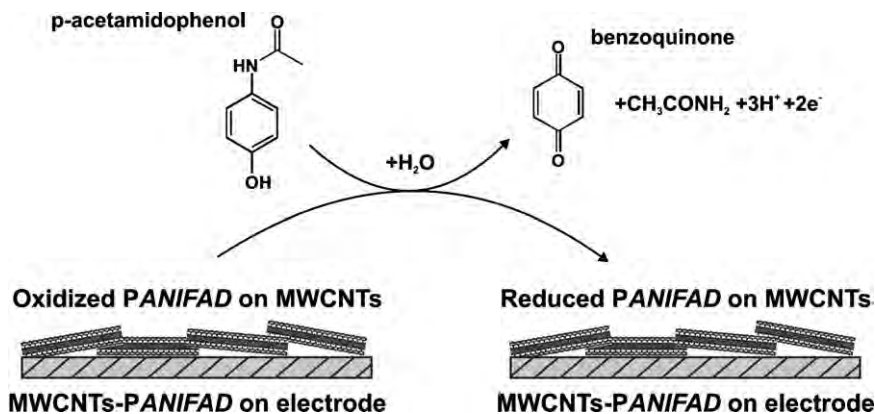


Fig. 5. CVs of *p*-acetamidophenol ($60\ \mu\text{M}$) at bare, PANIFAD, MWCNTs and MWCNTs–PANIFAD biocomposite film modified gold electrodes using pH 2.28 aqueous solution at $20\ \text{mV s}^{-1}$, where MWCNTs–PANIFAD biocomposite film is shown with both the absence and presence of $60\ \mu\text{M}$ *p*-acetamidophenol.



Scheme 1. Electrochemical oxidation of p-acetamidophenol at MWCNTs–PANIFAD biocomposite film modified electrode.

p-acetamidophenol takes place at PANIFAD co-polymer's redox couple $E^{0'} = 718.4$ mV. In these above electrocatalysis experiment, an increase in concentration 2–60 μM of p-acetamidophenol simultaneously produces a linear increase in the oxidation peak current of p-acetamidophenol with good film stability at PANIFAD, MWCNTs and MWCNTs–PANIFAD biocomposite films. However, there is no increase at bare gold electrode. The anodic peak current is linear with the concentration of p-acetamidophenol for all the three films.

It is obvious that the MWCNTs–PANIFAD biocomposite film shows higher electrocatalytic activity for p-acetamidophenol. More specifically, the enhanced electrocatalytic activity of MWCNTs–PANIFAD biocomposite film can be explained in terms of both lower in overpotential and higher peak current than that of PANIFAD film, MWCNT film and bare gold electrode. These results can be observed from the I_{pa} and E_{pa} values given in Table 2. Where, the increase in peak current and decrease in overpotential; both are considered as the electrocatalytic activity [6]. In detail, the enhancement in electrocatalytic activity at MWCNTs–PANIFAD biocomposite film is due to presence of both MWCNTs and PANIFAD, where the anodic peak potential is lowered by MWCNTs, and the peak current is enhanced by both MWCNTs and PANIFAD. From the slopes of linear calibration curves, the sensitivities of PANIFAD, MWCNTs and MWCNTs–PANIFAD biocomposite film modified gold electrodes and their correlation co-efficient have been calculated and given in Table 3. It is obvious that the sensitivity of MWCNTs–PANIFAD biocomposite film is higher for p-acetamidophenol when comparing PANIFAD and MWCNTs films. The overall view of these results reveals that MWCNTs–PANIFAD biocomposite film is efficient for p-acetamidophenol analysis.

3.5. FIA of p-acetamidophenol at MWCNTs–PANIFAD biocomposite film

The MWCNTs–PANIFAD biocomposite film has been synthesized on SPCE at similar conditions to that of gold electrode. Then the modified electrode has been washed carefully in deionized water and used for FIA of p-acetamidophenol as shown in Fig. 6. The

Table 2

Comparison of E_{pa} and I_{pa} of p-acetamidophenol in electrocatalytic oxidation reaction using CV technique at different modified electrodes in pH 2.28 aqueous solution.

Modified films ^a	E_{pa} (mV)	I_{pa} (μA)
Bare gold electrode	753.5	36.91
PANIFAD	765.3	69.88
MWCNTs	728.2	152.1
MWCNTs–PANIFAD	726.0	246.1

^a Modified at gold electrode.

Table 3

Sensitivities and correlation coefficient of different modified electrodes for p-acetamidophenol oxidation in pH 2.28 aqueous solution using various techniques.

Modified films	Sensitivity ($\text{mA mM}^{-1} \text{cm}^{-2}$) [correlation coefficient]	
	CV ^a	FIA ^b
PANIFAD	28.7 [0.9976]	–
MWCNTs	60.7 [0.8941]	1.1 [0.9326]
MWCNTs–PANIFAD	88.5 [0.7843]	3.3 [0.9957]

^a Films modified at gold electrode.

^b Films modified at SPCE.

carrier stream used was pH 2.28 aqueous solution with the flow rate of 2 ml min^{-1} , and the volume of p-acetamidophenol injected at each cycle was $10 \mu\text{l}$ at the time interval of 50 s. Fig. 6 shows the successive addition of p-acetamidophenol in the concentration range from $10 \mu\text{M}$ to 0.1 M at the potential of 726 mV. This above-mentioned potential is the optimized potential obtained from CV study. The rapid amperometric response of the MWCNTs and MWCNTs–PANIFAD biocomposite film is proportional to the respective analytes concentration. From the slopes of linear calibration curves, sensitivities of MWCNTs and MWCNTs–PANIFAD biocomposite film modified SPCE and their correlation co-efficient have been calculated and given in Table 3. The comparison of slope values of both the films show that the MWCNTs–PANIFAD biocom-

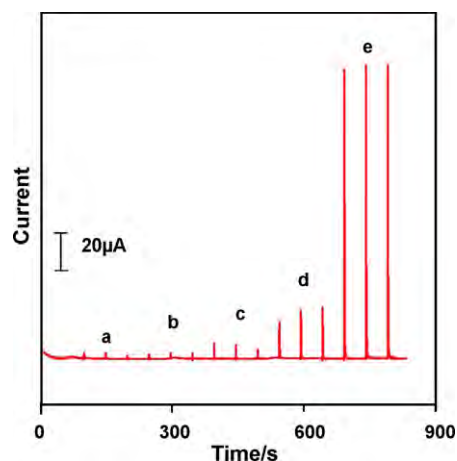


Fig. 6. FIA of p-acetamidophenol at MWCNTs–PANIFAD biocomposite film with five different concentrations of p-acetamidophenol = (a) 1×10^{-5} ; (b) 1×10^{-4} ; (c) 1×10^{-3} ; (d) 1×10^{-2} and (e) $1 \times 10^{-1} \text{ M}$; potential = 726 mV, where the carrier stream used was pH 2.28 aqueous solution; flow rate = 2 ml min^{-1} and injected volume = $10 \mu\text{l}$.

Table 4

Electroanalytical values obtained for the p-acetamidophenol tablet's determination using FIA in pH 2.28 aqueous solution at MWCNTs–PANIFAD biocomposite film modified SPCE.

Added (mM)	Found (mM)	RSD (%)	Recovery (%)
0.01	0.0102	1.94	102
0.1	0.107	2.61	107
1	1.08	2.78	108
10	10.86	1.28	109
100	109.49	1.71	109

posite film possess good reproducibility with higher sensitivity for p-acetamidophenol.

3.6. Analysis of p-acetamidophenol tablet

The performance of MWCNTs–PANIFAD biocomposite film modified SPCE has been tested by applying it to the determination of the p-acetamidophenol present in p-acetamidophenol tablets. The technique used for the determination was FIA. These p-acetamidophenol tablets were obtained from GlaxoSmithKline Dunganvaran Ltd., Ireland. The tablet's labeled composition is 500 mg of acetamidophenol and 65 mg of caffeine. In these experiments, the concentration added, found and the relative standard deviation (RSD) are given in Table 4. From these results in Table 4, the recovery of p-acetamidophenol is $\approx 107\%$. These above results show that MWCNTs–PANIFAD biocomposite film is efficient for p-acetamidophenol detection.

4. Conclusions

We developed a novel biocomposite material using MWCNTs and PANIFAD (MWCNTs–PANIFAD) at gold electrode and SPCE, which are more stable in pH 2.28 aqueous solution. The developed MWCNTs–PANIFAD biocomposite film for the electrocatalysis combines the advantages of ease of fabrication, high reproducibility and sufficient long-term stability. The EQCM results confirmed the incorporation of PANIFAD on MWCNTs modified gold electrode. The SEM and AFM results have shown the difference between PANIFAD and MWCNTs–PANIFAD biocomposite films morphology. Further, it has been found that MWCNTs–PANIFAD biocomposite film has excellent functional properties along with good electrocatalytic activity on p-acetamidophenol. The experimental methods of CV and FIA with MWCNTs–PANIFAD biocomposite film sensor integrated into the gold electrode and SPCE which are presented in this article provide an opportunity for qualitative and quantitative characterization. Therefore, this work establishes and illustrates in principle and potential, a simple and novel approach for the devel-

opment of a voltammetric and amperometric sensor which is based on modified gold electrode and SPCE.

Acknowledgement

This work was supported by the National Science Council and the Ministry of Education of Taiwan (Republic of China).

References

- [1] C.P. McMahon, G. Rocchitta, S.M. Kirwan, S.J. Killoran, P.A. Serra, J.P. Lowry, R.D. O'Neill, *Biosens. Bioelectron.* 22 (2007) 1466.
- [2] I. Becerik, F. Kadirgan, *Synth. Met.* 124 (2001) 379.
- [3] T. Selvaraju, R.R. Ramaraj, *J. Electroanal. Chem.* 585 (2005) 290.
- [4] M. Mao, D. Zhang, T. Sotomura, K. Nakatsu, N. Koshiba, T. Ohsaka, *Electrochim. Acta* 48 (2003) 1015.
- [5] M. Yasuzawa, A. Kunugi, *Electrochem. Commun.* 1 (1999) 459.
- [6] C.P. Andrieux, O. Haas, J.M. SavGant, *J. Am. Chem. Soc.* 108 (1986) 8175.
- [7] J. Wang, M. Musameh, *Anal. Chim. Acta* 511 (2004) 33.
- [8] H. Cai, X. Cao, Y. Jiang, P. He, Y. Fang, *Anal. Bioanal. Chem.* 375 (2003) 287.
- [9] A. Erdem, P. Papakonstantinou, H. Murphy, *Anal. Chem.* 78 (2006) 6656.
- [10] Q. Li, J. Zhang, H. Yan, M. He, Z. Liu, *Carbon* 42 (2004) 287.
- [11] J. Zhang, J.K. Lee, Y. Wu, R.W. Murray, *Nano Lett.* 3 (2003) 403.
- [12] M. Zhang, K. Gong, H. Zhang, L. Mao, *Biosens. Bioelectron.* 20 (2005) 1270.
- [13] R.J. Chen, Y. Zhang, D. Wang, H. Dai, *J. Am. Chem. Soc.* 123 (2001) 3838.
- [14] M. Trojanowicz, *Microchim. Acta* 143 (2003) 75.
- [15] H. Lethbey, *J. Chem. Soc.* 15 (1862) 161.
- [16] D. Nicolas-Debarnot, F. Poncin-Epaillard, *Anal. Chim. Acta* 475 (2003) 1.
- [17] J. Huang, S. Virji, B.H. Weiller, R.B. Kaner, *J. Am. Chem. Soc.* 125 (2003) 314.
- [18] V. Gupta, N. Miura, *Electrochem. Solid-State Lett.* 8 (2005) A630.
- [19] S.K. Dhawan, N. Singh, S. Venkatachalam, *Synth. Met.* 125 (2002) 389.
- [20] D. Sazou, M. Kourouzidou, E. Pavlidou, *Electrochim. Acta* 52 (2007) 4385.
- [21] V.I. Birss, S. Guha-Thakurta, C.E. McGarvey, S. Quach, P. Vanyssek, *J. Electroanal. Chem.* 456 (1998) 71.
- [22] R. Garjonyte, A. Malinauskas, L. Gorton, *Bioelectrochemistry* 61 (2003) 39.
- [23] M.M. Kamal, H. Elzanowska, M. Gaur, D. Kim, V.I. Birss, *J. Electroanal. Chem.* 318 (1991) 349.
- [24] L.T. Kubota, L. Gorton, A.R. Lanzilotta, A.J. McQuillan, *Bioelectrochem. Bioenerg.* 47 (1998) 39.
- [25] S.A. Kumar, S.M. Chen, *J. Solid State Electrochem.* 11 (2007) 993.
- [26] J.E. Wallace, *Anal. Chem.* 39 (1967) 531.
- [27] F.M. Plakogiannis, A.M. Saad, *J. Pharm. Sci.* 64 (1975) 1547.
- [28] K.K. Verma, A.K. Gulati, S. Palod, P. Tyagi, *Analyst* 109 (1984) 735.
- [29] S.M. Sultan, I.Z. Alzamil, A.M. Aziz Alrahman, S.A. Altamrah, Y. Asha, *Analyst* 111 (1986) 919.
- [30] F.A. Mohamed, M.A. AbdAllah, S.M. Shammatt, *Talanta* 44 (1997) 61.
- [31] J.F. van Staden, M. Tsanwani, *Talanta* 58 (2002) 1095.
- [32] M. Oliva, R.A. Olsina, A.N. Masi, *Talanta* 66 (2005) 229.
- [33] M.K. Srivastava, S. Ahmad, D. Singh, I.C. Shukla, *Analyst* 110 (1985) 735.
- [34] Y. Yan, M. Zhang, K. Gong, L. Su, Z. Guo, L. Mao, *Chem. Mater.* 17 (2005) 3457.
- [35] E. Laviron, *J. Electroanal. Chem.* 101 (1979) 19.
- [36] S.M. Chen, M.I. Liu, *Electrochim. Acta* 51 (2006) 4744.
- [37] S.M. Chen, C.J. Liao, V.S. Vasantha, *J. Electroanal. Chem.* 589 (2006) 15.
- [38] U. Yogeswaran, S.M. Chen, *Electrochim. Acta* 52 (2007) 5985.
- [39] U. Yogeswaran, S.M. Chen, S.H. Li, *Electroanalysis* 20 (2008) 2324.
- [40] J. Wang, J. Dai, T. Yarlagadda, *Langmuir* 21 (2005) 9.
- [41] M. Tahhan, V.T. Truong, G.M. Spinks, G. Wallace, *Smart Mater. Struct.* 12 (2003) 626.
- [42] T. Komura, G.Y. Niu, T. Yamaguchi, M. Asano, A. Matsuda, *Electroanalysis* 16 (2004) 1791.
- [43] U. Yogeswaran, S. Thiagarajan, S.M. Chen, *Carbon* 45 (2007) 2783.