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Electrooxidation of catecholamines at carbon nanotube-modified indium tin oxide electrodes

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ABSTRACT

In this study, we prepared carbon nanotube (CNT)/Nafion-modified ITO electrodes and investigated their electrochemical behavior. The CNTs were dissolved in a solution of the ionic polymer Nafion and then CNT/Nafion composite films were deposited onto ITO electrodes through spin-coating of this homogeneous solution. We studied the effects of chemical pretreatment of the CNTs and the pH of the buffer on the electroanalytical behavior of the CNT/Nafion-modified ITO electrodes toward catecholamines. The modified electrodes enhanced the peak current and lowered the overpotentials. We observed high electrooxidative performance for the modified ITO electrodes: the oxidative currents of the catecholamines were up to 125-fold higher than those obtained using bare ITO electrodes.

1. Introduction

Since their initial discovery by Iijima in 1991 and subsequent report of their synthesis by Ebbesen and Ajayan in 1992, carbon nanotubes (CNTs) have been the subject of many experimental and theoretical investigations [1–3]. Two kinds of CNTs are known: single-wall carbon nanotubes (SWNTs) and multiwall carbon nanotubes (MWNTs). They have attracted much attention as a result of their unique structures and chemical and physical properties, which make them suitable for use in a range of potential applications [1,4–7], such as high-sensitivity microbalances [8], gas detectors [9,10], catalyst supports [11,12], electron sources in field emission-mode displays [13], tiny tweezers for nanoscale manipulation [14], and probe tips for scanning probe microscopy [15]. Theoretical calculations have indicated that, depending on their symmetry and diameter, CNTs can exhibit metallic or semiconductive behavior [16–18]. Furthermore, CNTs are more highly conductive than graphite [19]. The large surface areas and abundances of functional groups presented on nanomaterials make them suitable for specific electroanalytical reactions of certain substances when incorporated into electrodes. For example, CNTs have the ability to mediate electron transfer reactions of electroactive species in solution, exhibiting catalytic effects on the electrochemical behavior of dopamine [20], proteins [21], and oxygen [22].

A conventional approach toward fixing CNTs onto the surfaces of working electrodes is through spin-coating of a film of, for example, Nafion mixed with CNTs [23]. Nafion, a cation-exchange polymer, forms films that are highly permeable to cations but almost impermeable to anions. CNTs lacking any additional functional groups can be dissolved completely in Nafion solutions. Using glassy carbon electrodes (GCEs) modified with CNTs increases the sensitivity of cyclic voltammetry (CV) toward the detection of analytes [23].

Modified GCEs are, however, still susceptible to poor reproducibility because of the need to clean the GCE surface. One approach to solving this problem is the use of a disposable

material as an electrode base; this material must have low cost and be readily available and easily modified. Carbon paste is a widely used material for fabricating disposable electrodes [24]; screen-printing techniques can be employed for their mass production. Indium tin oxide (ITO) is another possible material that has recently become widely used industrially for thin display panels; thus, it is easy to purchase at standard specifications [25]. ITO is an excellent photoelectric material because of its high conductivity and photo-penetrability. Although it can decrease electrical resistance when used as an electrode substrate, the electroanalytical activity of ITO is relatively low when compared with most other types of detectors. Surface modification of ITO glass is one means to increasing its electroanalytical activity. We suspected that modifying ITO with CNTs would provide a substrate exhibiting the pure properties of CNTs without any interference from graphite.

Because catecholamines are very important neurotransmitters in mammalian central nervous systems [26], intensive efforts have been made to determine their levels in vivo and in vitro using selective electrochemical techniques [27–29]. Ideally, we would like to establish simple, rapid, and selective methods for the routine analysis of catecholamines.

In this study, we describe the electroanalytical performance of CNT/Nafion-modified ITO electrodes for the detection of catecholamines, including dopamine, epinephrine, and 3,4-dihydroxyphenylalanine (DOPA). Nafion is an effective solubilizing agent for CNTs, and the resulting CNT/Nafion films exhibited very good adhesion on the ITO surface. Because various activation procedures influence the electrochemical reactivity of electrodes, we studied the effects of chemical pretreatment of the CNTs and the pH of the buffer on the electroanalytical behavior of our CNT/Nafion-modified ITO electrodes. The electrooxidation toward catecholamines at CNT/Nafion-modified ITO electrodes was investigated.

2. Experimental

2.1. Reagents

The ITO glass (ITO thickness: 125 ± 20 nm; $2.32 \, \Omega \, \text{cm}^{-2}$) was purchased from Merck Display Technologies Ltd. (Taoyuan, Taiwan). A 5 wt% solution of Nafion in a mixture of lower aliphatic alcohols and 10% water was obtained from Sigma–Aldrich. The analytes for detection were catechol (Sigma–Aldrich), dopamine (Acros), epinephrine (ICN Biomedicals), and DOPA (Tokyo Chemical). Potassium hexacyanoferrate (ferric cyanide) was purchased from Riedel-de Haën. The MWNTs (C150P, Bayertubes, Bayer) were synthesized through chemical vapor deposition (CVD). Phosphate buffer solutions for electrochemical analyses were formulated from disodium hydrogen phosphate and sodium dihydrogen phosphate (Fluka). All solutions were prepared using deionized (DI) water obtained from a Milli-Q system (Millipore, USA).

2.2. Apparatus

The detection system was an 8021b electrochemical analyzer (CHI, USA) coupled with a three-electrode detection system, i.e., working, auxiliary, and reference electrodes connected through sockets. The surface areas of the ITO working electrodes were 0.25 cm². Scanning electron microscopy (SEM) images were obtained using an S-4700I microscope (Hitachi, Japan). Fourier transform infrared (FT-IR) spectra were measured using an Excalibur HE Series FTS3100 spectrometer (Digilab, USA). The PM-490 spin-coater (Synrex, Taiwan) featured a two-step speed controller.

2.3. Working electrode preparation

CNTs were functionalized through ultrasonic bathing for 1, 2, or 4 h in a mixture of concentrated sulfuric acid and nitric acid (3:1, v/v). This process results in the formation of carbonyl moieties on the surfaces of the CNTs [30] while simultaneously removing metallic impurities [31]. A mixture of ethanol and water (3:1, v/v) was used to dilute the 5 wt% Nafion solution to 0.5 wt%. The treated CNTs were dissolved in this 0.5 wt% Nafion solution under ultrasonication; the concentration of CNTs was 10 mg mL^{-1}.

The ITO glass was washed with neutral detergent and then cleaned through ultrasonication in isopropanol, acetone, and DI water, sequentially, for 10 min each. The ITO glass was cut into 3 cm × 4.5 cm pieces and polyepoxide was used to control the surface of the detection area. Modification of the ITO glass was then performed through spin-coating (1500 rpm, 45 s) of the CNT/Nafion solution. The ITO glass was then dried at 140 °C for 1 h and cut into 3 cm × 0.5 cm pieces. The detection area of each modified ITO electrode was 0.5 cm × 0.5 cm.

3. Results and discussion

3.1. Characterization of CNT/Nafion films

We used FT-IR spectroscopy to characterize the carbonyl moieties present on the surface of the CNTs. The signals at 1200 (C–O), 1700 (C=O), and 3400 (O–H) cm^{-1} in the FT-IR spectrum of the acid-treated CNTs reflected the presence of carbonyl groups on their surfaces [30]. Fig. 1 displays SEM images of the ITO electrodes coated with Nafion and CNT/Nafion films. These images indicate clearly that the CNT/Nafion film was homogeneous on the ITO surface.

Fig. 2a displays the CV traces of a CNT/Nafion-modified ITO electrode that had been scanned for 100 cycles in 50 μM ferric cyanide. Each scan was almost identical. Fig. 2b presents the SEM image of a CNT/Nafion-modified ITO electrode that had been subjected to 100 CV scanning cycles; the modified CNTs appear to have undergone no apparent loss. According to these results, we conclude that the CNT/Nafion films exhibit very good adhesion toward ITO surfaces, with high durability for use in subsequent experiments. Thus, Nafion is a useful solubilizing agent for CNTs when preparing CNT-modified ITO electrodes.

Treatment of MWNTs with strong acids causes segmentation and carboxylation at their termini [32]. We investigated the electrochemical behavior of catechol on CNT/Nafion-modified ITO electrodes functionalized with CNTs that had been subjected to the acidic conditions for various treatment times. The cyclic voltammograms in Fig. 3 indicate that the redox peak currents were highest for the CNT/Nafion-modified
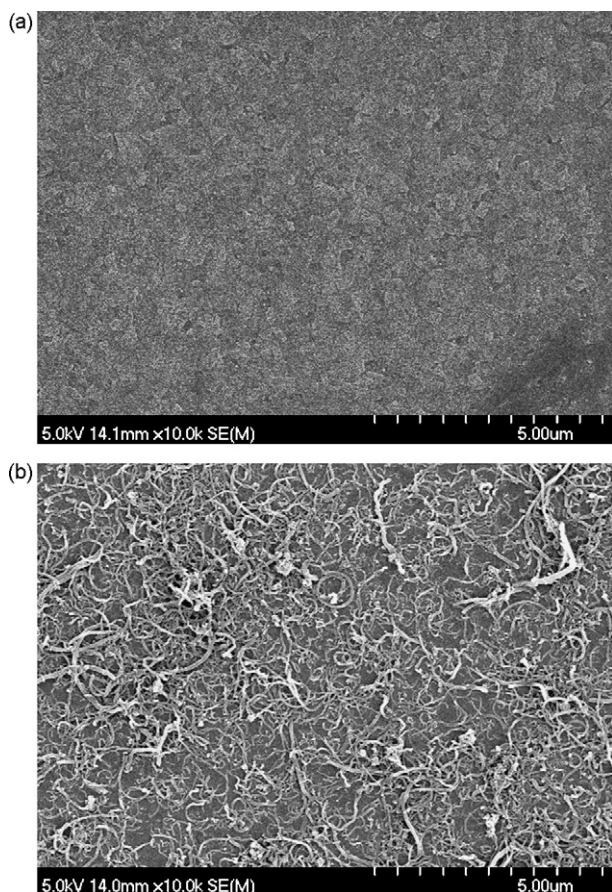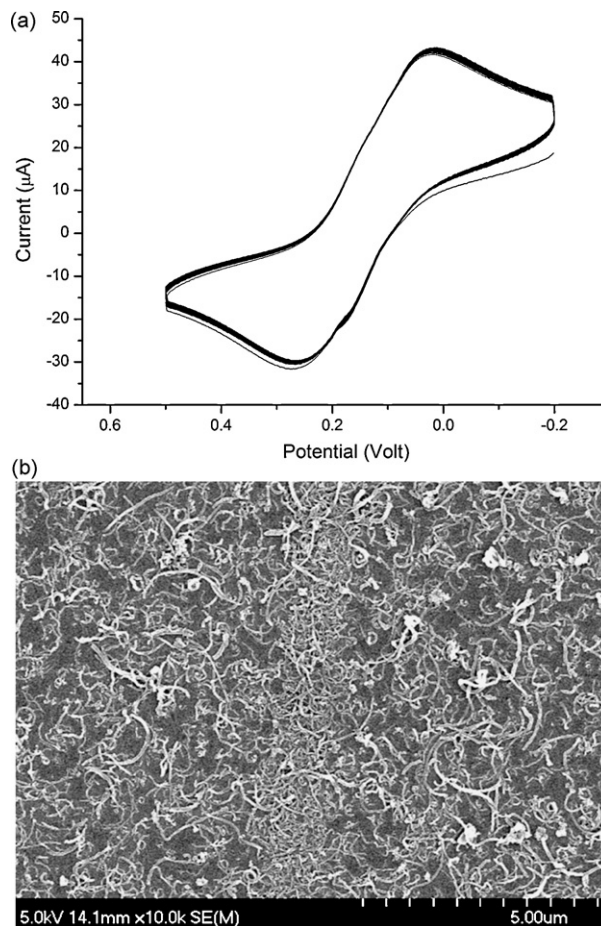
Fig. 1 – SEM images of (a) Nafion and (b) CNT/Nafion films on ITO electrodes; magnified by a factor of 10,000.

Fig. 2 – (a) Cyclic voltammograms of a CNT/Nafion-modified ITO electrode in 0.05 M $K_3Fe(CN)_6$; scan rate: 0.1 V s^{-1}, between −0.2 and +0.5 V (vs. Ag/AgCl). (b) SEM image of a CNT/Nafion-modified ITO electrode surface after performing 100 CV scans.
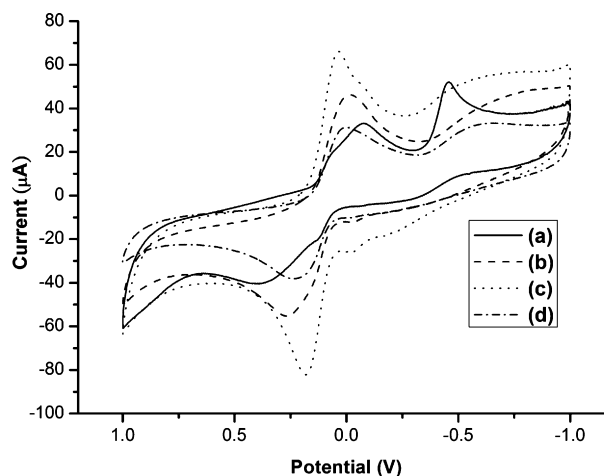
ITO containing the 2-h-treated CNTs. The CNTs were probably too long to be distributed evenly on the ITO surface after acidic treatment for 1 h; in contrast, they were probably too short to exhibit high electroanalytical activity when treated for more than 2 h. Therefore, we utilized the 2-h-treated CNTs in all subsequent experiments. Varying the scan rate would also affect the redox peak current and the background current. After evaluating the electroanalytical performance of CNT/Nafion-modified ITO electrode, 0.1 V s^{-1} scan rate was selected.

### 3.2. Electrooxidative ability for catecholamine

We used CV to study the effect of the solution pH on the electrooxidative activity of the CNT/Nafion-modified ITO electrodes toward dopamine. Using 0.1 M phosphate buffer at values of pH ranging from 5.0 to 9.0, we found that the oxidation peak potential was pH-dependent. The effect of the pH of the solution on the oxidation current revealed that the largest oxidation current was obtained at ca. pH 7.0. We suspect that dopamine was attracted to the surface of CNTs in acidic solution, thus enhancing the oxidation current. Under basic conditions, however, the oxidation current decreased rapidly upon increasing the pH, probably because the anionic form of dopamine was repelled from the surface CNTs.

Fig. 3 – Cyclic voltammograms of 0.5 mM catechol detected by ITO electrodes containing CNTs that had been treated with strong acid for (a) 0 h, (b) 1 h, (c) 2 h, and (d) 4 h; 0.1 M phosphate buffer (pH 7.4); scan rate: 0.1 V s^{-1}, between +1.0 and −1.0 V (vs. Ag/AgCl).
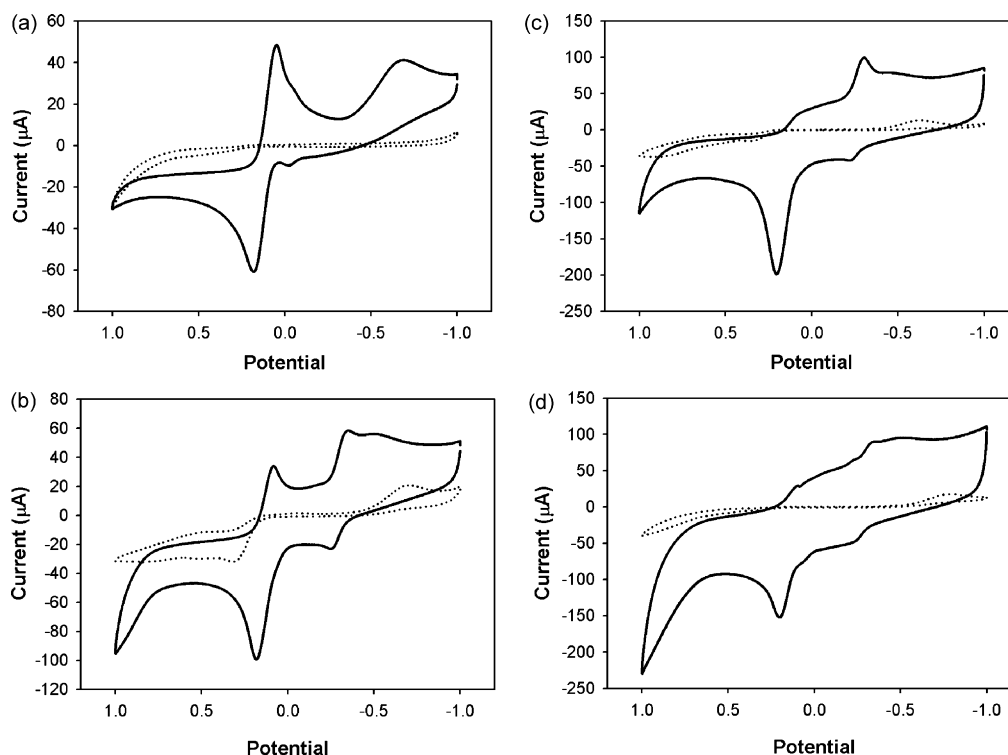
Fig. 4 – Cyclic voltammograms of 0.5 mM (a) catechol, (b) dopamine, (c) epinephrine, and (d) DOPA, as detected by CNT/Nafion-modified (solid lines) and bare (dotted lines) ITO electrodes. The conditions were the same as those used to obtain Fig. 3.

We employed four catecholamines to demonstrate the analytical performance of the CNT/Nafion-modified ITO electrodes. Fig. 4 presents the CV traces of 0.5 mM catecholamine solutions as detected by the CNT/Nafion-modified and bare ITO electrodes. These cyclic voltammograms were obtained using 0.1 M phosphate buffer solution (pH 7.4) containing each analyte; a scan rate of 0.1 V s^{-1} was employed between +1.0 and −1.0 V. The redox peak currents of the analytes were all enhanced significantly in the presence of the CNT/Nafion-modified ITO electrode. In addition, the oxidation peak potentials all decreased to ca. 0.20 V. The enhanced peak currents and decreased overpotentials are clear evidence for the electroanalytical effect of the CNTs during the catecholamine redox reaction and the cation-exchange properties of the Nafion film. Taking the CV trace of dopamine as an example, Fig. 4b indicates that the CNT/Nafion-modified ITO electrode improved not only the redox peak currents but also the reversibility of the redox reaction of dopamine.

The electrochemical behavior of dopamine on conventional electrodes is dependent on the solution pH [33]. In the cyclic voltammogram of dopamine at pH 7.4 (Fig. 4b), we observe two oxidation peaks. The oxidation of dopamine was highly reversible, suggesting that intramolecular cyclization of dopamine orthoquinone was occurring. In other words, dopamine probably underwent a two-step oxidative reaction. In a solution having a slightly basic pH, the carboxylate groups on the surface of the CNT/Nafion-modified ITO electrode form hydrogen bonds with the hydroxyl groups of dopamine, which increases the electrooxidative efficiency. When dopamine

loses its first pair of electrons, dopamine orthoquinone is formed. This compound loses a second pair of electrons to form aminochrome, a cyclization product of dopamine ortho-quinone, in basic media [34–37]. Thus, two enhanced oxidative peaks appeared for dopamine when using the CNT/Nafion-modified ITO electrode.

We also employed the CNT/Nafion-modified ITO electrode to detect 0.5 mM ascorbic acid in 0.1 M phosphate buffer. A small oxidative current appeared at +0.8 V that was quite distinct from the catecholamines' oxidation potentials. Because ascorbic acid exists in anionic form at pH 7.4, the reduced oxidative current might also be due to the cation-exchange properties of the Nafion film. Therefore, the CNT/Nafion-modified ITO electrode could be used to detect catecholamine neurotransmitters in the presence of ascorbic acid, a common interfering agent present in biological samples.

To demonstrate the performance of our modified electrode, Table 1 lists the correlation coefficients (r^2), linear ranges, limits of detection (LODs), linear regression equations, and enhancement factors for the detection of the series of catecholamines. This modified electrode exhibited high stability and good repeatability for the analyses of these catecholamines. The LODs were all in the nanomolar range. The modified electrode displayed a larger linear range for dopamine than the result from a CNT/Nafion-modified carbon nanotube microelectrode [38] Through modification with the CNTs, the oxidative detection of the catecholamines was amplified by up to 125-fold relative to those of the bare ITO electrode.

Table 1 – Figures of merit for the determination of catecholamines

	Catechol	Dopamine	Epinephrine	DOPA
Correlation coefficient (r^2)	0.997	0.995	0.998	0.997
Linear range (μM)	$1-10^3$	$1-10^3$	$10^{-1}-10^3$	$1-10^3$
Linear regression of concentration	$y = 0.122x + 0.380$	$y = 0.208x - 0.789$	$y = 0.409x - 1.676$	$y = 0.309x - 0.966$
LOD (nM)[a]	380	220	39	200
Enhancement factor[b]	96.2	75.7	97.1	124.6

N = 10.

[a] S/N = 3.

[b] Enhancement factor was calculated for the detection current, relative to that from a bare ITO electrode, at 0.5 mM of the analyte (as indicated in Fig. 4).

4.   Conclusions

CNT/Nafion-modified ITO electrodes exhibit excellent electroanalytical activity towards the redox reactions of catecholamines. The modified electrode enhanced the peak currents and lowered the overpotentials. Nafion was a useful solubilizing agent for preparing the CNT-modified ITO electrodes. Such CNT/Nafion-modified ITO electrodes are easy to prepare and exhibit good repeatability, remarkable electroanalytical performance, and low detection limits for catecholamines. Consequently, these modified ITO electrodes could be employed as electrochemical sensors exhibiting high selectivity and sensitivity toward catecholamines, without interference from the presence of ascorbic acid. Furthermore, photolithography can be used to fabricate ITO glass into specific electrode patterns that are readily integrated into disposable biosensors.

Acknowledgment

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