ELECTROCHEMICAL DETERMINATION OF ACETAMINOPHEN AT THE SURFACE OF A GLASSY CARBON ELECTRODE MODIFIED WITH MULTI-WALLED CARBON NANOTUBE

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ABSTRACT

A multi-walled carbon nanotubes (MWCNTs) film coated glassy carbon electrode (GCE) was fabricated, and the electrochemical behavior of acetaminophen (AAP) was studied in Britton–Robinson (BR) buffer (pH 7.0), using cyclic voltammetry (CV), square-wave voltammetry (SWV) and differential pulse voltammetry (DPV). Cyclic voltammograms of AAP showed enhanced redox responses for the MWCNT/GCE with respect to the bare GCE. The anodic peak potential shifted to less positive values by increasing the pH. The electrochemical oxidation of AAP at MWCNT/GCE was quasi-reversible and diffusion-controlled. Experimental parameters, such as pH, scan rate, SW frequency and step height, were optimized for AAP measurement. Under the optimum pH of 7.0 in 0.1 M BR buffer solution, the DPV anodic peak current showed a linear relation versus AAP concentration in the range of 7.9×10^{-7} to 3.4×10^{-4} M with a detection limit of 3.3×10^{-7} for DPV, and from 6.5×10^{-7} to 3.5×10^{-4} M for SWV with a detection limit of 2.1×10^{-7} (signal to noise = 3). Moreover, this method demonstrated good reproducibility (RSD = 1.3%, n = 10) and long-term stability. These have been applied to the determination of AAP in effervescent dosage samples.

Keywords: Acetaminophen; Carbon nanotubes; Glassy carbon electrode; Electrochemical determination

INTRODUCTION

Carbon nanotubes (CNTs) are a novel nanoscale material, mainly consisting of single-wall carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs)^{1,2}. CNTs have attracted considerable attention due to their extraordinary structural, mechanical, electrical, and electrochemical properties as well as their promise in the field of materials science³. Due to their unique structure, CNTs can behave electrically as a metal or as a semiconductor⁴. The subtle electronic behavior of CNTs demonstrates that they have the ability to promote electron-transfer reactions when used as electrode materials⁵. Several types of CNT electrodes have been reported, including CNT paste electrodes^{6,7}, CNT film-coated electrodes^{8,9}, CNT powder microelectrodes¹⁰ and CNT paper electrodes¹¹.

Acetaminophen (N-acetyl-p-aminophenol or paracetamol) (AAP) is a common analgesic and antipyretic drug that is used for the relief of fever and headaches, as well as other minor aches and pains. It is an attractive alternative drug for children and adults that are sensitive to aspirin¹². At the recommended dosage, there are no side effects. However, overdoses due to liver and kidney damage can be harmful. Therefore, it is very important to establish a simple, fast, sensitive and accurate detection method for the determination of AAP. Many methods have been described for AAP determination, such as titrimetry¹³, spectrophotometry¹⁴, liquid chromatography¹⁵ and UV–Vis spectrophotometry^{16,17,18}. Electrochemical methods, owing to their low cost and reduced time consumption, are also popular for this application. Among electrochemical methods, AAP has been assessed by voltammetry using several techniques, including C₆₀-modified glassy carbon electrodes (GCEs)¹⁹, GCEs coated with a SWCNT-dicetyl phosphate film²⁰, PANI-MWCNT modified electrodes²¹ and carbon-coated nickel-modified electrodes²².

Electrode surface modification and pretreatments have been widely used to improve the electrochemical responses of biological compounds²³. For example, CNT-modified GCEs provide a stable and sensitive electrochemical response for phenols²⁴, NADH²⁵, estrogenic phenolic compounds^{26,27}, dopamine²⁸, uric acid²⁸ and tetracycline²⁹.

In the current study, a MWCNTs modified GE was fabricated and used to determination of AAP. The electrochemical behavior of AAP suggested that MWCNTs modified GE exhibits an obvious electrocatalytic effect on the oxidation of AAP, since it greatly enhances the oxidation peak current of AAP as well as lowering its oxidation overpotential. After optimizing the experimental parameters, this electrode has used for the direct measurement of AAP in tablets and syrups. This method possesses many advantages such as a low detection limit, fast response, low cost and simplicity.

MATERIAL AND METHODS

Reagents and chemicals

AAP was purchased from the Farabi Pharmaceutical Company. The

Britton Robinson (BR) buffer solution was comprised of phosphoric acid, boric acid and glacial acetic acid and its pH was adjusted with NaOH. MWCNTs with purity >95% (40–60 nm in diameter) were obtained from the Chinese Academy of Sciences. Generally, pristine MWCNTs contained 3% amorphous carbon, SWCNTs and graphite, requiring some purification pre-treatment before electro-analytical characterization. A 1.0×10^{-3} M AAP standard solution was simply prepared by dissolving AAP in distilled water. Working solutions were prepared with this standard solution and using suitable dilutions with a 0.1 M BR buffer solution. BR buffer solution was also used as the supporting electrolyte.

Apparatus

Electrochemical measurements were carried out on a M273A Electrochemical Workstation (America, EG&G Corporation). A conventional three-electrode system was employed, consisting of a MWCNTs modified GE as a working electrode (Metrohm), a saturated calomel reference electrode (SCE) (Metrohm) and a Pt wire counter electrode (Metrohm). Solution pH values were determined using a 691 pH meter (Metrohm Swiss made) combined with glass electrode (Metrohm). Deionized water was formed with an ultrapure water system (smart 2 pure, TKA, Germany). MWCNTs was dispersed with an ultrasonic bath (SONOREX DIGITAL, 10P, BANDELIN). Data were collected at room temperature.

Fabrication of the modified electrode

Using ultrasonic agitation, 0.5 mg of purified MWCNTs was dispersed into 5 ml of redistilled water for 30 min. Prior to modification, the GCE was polished with 0.05 mm alumina slurry and then cleaned ultrasonically in double-distilled water. Subsequently, the GCE was coated with 20 μ L of MWCNT suspension and the solvent was evaporated in air.

RESULTS AND DISCUSSION

Voltammetric behavior of AAP on the MWCNT/GCE

The electrochemical behavior of AAP at unmodified GCE and MWCNT film coated GCE was examined by CV. Figure 1 shows the cyclic voltammograms of 5.0×10^{-5} M AAP on a modified (a) and unmodified GCE (b) in the potential range of 0.0 to 0.7 V in 0.1 M BR buffer (pH 7.0). Figure 1c shows the cyclic voltammogram of the modified electrode in the absence of AAP. As can be seen, at the unmodified GCE (Figure 1b), AAP shows irreversible behavior with a relatively weak oxidative peak at $E_p = 0.447$ V, and no reduction peak in reverse scan.

Figure 1: Cyclic voltammograms of 5.0×10^{-5} M AAP in 0.1 M BR buffer (pH 7.0) at scan rates of 100 mV s^{-1} at (a) MWCNT/GCE; (b) unmodified electrode; and (c) absence of AAP in solution on modified electrode.

When comparing the unmodified GCE to a MWCNT/GCE (Figure 1a), a pair of well-defined redox waves was observed for AAP on MWCNT/GCE. Because of the i_p/i_c ratio is approximately equal, then the electrochemical behavior of AAP at MWCNT/GCE is a quasi-reversible process. The anodic (E_{pa}) and cathodic (E_{pc}) peak potentials were 0.32 V and 0.29 V (versus SCE), respectively. The obvious peak current enhancement and negative shift in the oxidation peak potential reveal that MWCNT films exhibit catalytic effects toward the oxidation of AAP.

Effect of scan rate

The oxidation peak current was measured with CV, at different scan rates ($50 - 200 \text{ mV s}^{-1}$), for 5.0×10^{-5} M AAP (Figure 2). By increasing the scan rate, the heights of oxidation and reduction peaks was increased. Also, peak potentials shifted towards more positive and negative values for oxidation and reduction processes, respectively. Oxidation and reduction peak currents varied linearly with the square root of scan rate ($v^{1/2}$) (Insets in Figure 2). These demonstrated an $r > 0.998$, indicating that the electrochemical reaction was a diffusion-controlled process²².

Figure 2: Cyclic voltammograms of 5.0×10^{-5} M AAP at the modified electrode at different scan rates from $50 - 200 \text{ mV s}^{-1}$ in 0.1 M BR buffer (pH 7.0). (Insets) linear relationship between the anodic and cathodic peak currents and the square root of scan rate.

Effect of pH

The electrochemical behavior of AAP was investigated over the pH range from 4 to 10 in 0.1 M BR buffer solution. Figure 3 shows cyclic voltammograms of 5.0×10^{-5} M AAP in 0.1 M BR buffer solution. As can be seen the peak current in pH=7.0 is maximum, therefore pH=7.0 was selected as the optimum pH and this pH was used in all following experiments. After that, the oxidation potential was observed to decrease by increasing the pH. This behavior was

attributed to an increasing amount of hydrolysis occurring as the pH increased, leading to the formation of reducing compounds, such as *p*-hydroxyaniline³⁰. The relationship of the peak potential versus pH can be expressed by following equation:

$$E_{pa} \text{ (mV vs. Ag/AgCl)} = +716.3 - 51.5 \text{ pH}$$

Figure 3: Cyclic voltammograms of 5.0×10^{-5} M AAP in 0.1 M BR buffer at different pHs (from 4 to 10). Inset, plot of E_{pa} versus pH. Scan rate = 100 mV s^{-1} .

Effect of DPV and SWV parameters

The peak current obtained in DPV and SWV is dependent on several instrumental parameters, including pulse height, frequency, scan increment and scan rate. These parameters are interrelated and have a combined effect on the response. In this work, these parameters were found to have little effect on the peak potential. For SWV, when the pulse height was varied in the range between 10 and 300 mV, the peak currents increased with increasing pulse height. However, the peak width was also increasing at the same time. This was particularly apparent when the pulse height was greater than 100 mV. Hence, 100 mV was chosen as the square-wave pulse height. The scan increment together with the frequency defines the effective scan rate. Hence, an increase in either the frequency or the scan increment results in an increase in the effective scan rate. The response for AAP increased with a SW frequency up to 30 Hz, above which the peak current was obscured by a large residual current. By fixing the frequency at 30 Hz, the effect of scan increment was studied. At scan increments greater than 3 mV, the resolution and sensitivity decreased. However, at a scan increment of 3 mV, a response was recorded. Overall, the optimized parameters are summarized in Table 1.

Table 1. Optimum experimental condition for SWV and DPV.

Variable	Range studied		Optimum value	
	SWV	DPV	SWV	DPV
Pulse height (mV)	10-300	50-300	100	200
Frequency (Hz)	10-100	---	30	---
Scan increment (mV)	2-10	2-10	3	2
Scan rate (mVs^{-1})	---	50-200	---	150

Calibration curve

The relationship between the oxidation peak current and AAP concentration was examined using DPV and SWV and two linear dynamic ranges were observed. In DPV method, the first linear dynamic range was between $7.9 \times 10^{-7} - 5.7 \times 10^{-5}$ M and the second was between $5.7 \times 10^{-5} - 3.4 \times 10^{-4}$ M (Figure 4). The relationship between peak current and concentration can be described with the following linear regression equations for the aforementioned concentration ranges for the first and second linear dynamic ranges:

$$\begin{aligned} i_{pa} \text{ (}\mu\text{A)} &= 0.165c \text{ (}\mu\text{M)} + .0126 & (r = 0.9996) \\ i_{pa} \text{ (}\mu\text{A)} &= 0.0447c \text{ (}\mu\text{M)} + 12.365 & (r = 0.9989) \end{aligned}$$

The first linear dynamic range was chosen for the determination of AAP with a detection limit of 3.3×10^{-7} M.

Figure 4: DPV of AAP on MWCNT/GCE in 0.1 M BR buffer (pH 7.0) at different AAP concentrations from 7.9×10^{-7} to 3.4×10^{-4} M; Inset, Linear relationship between i_{p_a} and the concentration of AAP (7.9×10^{-7} to 5.7×10^{-5} M).

Figure 5 shows SW voltammograms of AAP at various concentrations. The oxidation peak current of AAP was found to be proportional to AAP concentration over the ranges of 6.5×10^{-7} to 5.8×10^{-5} M and 5.8×10^{-5} to 3.5×10^{-4} M. The linear regression equations for the first and second linear dynamic ranges are expressed as:

$$\begin{aligned} i_{p_a} (\mu A) &= 0.0581c (\mu M) + 0.3236 & (r = 0.9998) \\ i_{p_a} (\mu A) &= 0.0298c (\mu M) + 2.906 & (r = 0.9987) \end{aligned}$$

When the signal-to-noise ratio was 3, the detection limit was 2.1×10^{-7} M, which is lower than the DPV value of 3.3×10^{-7} M. Which values of detection limit is comparable to values reported by other research groups for oxidation of AAP at the surface of modified electrodes (Table.2). SWV calibration curves of AAP for the first and second linear dynamic ranges are showed in Figures 6A and 6B, respectively.

Table 2. Comparison of the efficiency of some modified electrodes used in the electro-oxidation of AAP.

Electrode	Method	pH	Detection limit (M)	Dynamic range (M)	Ref.
MWCNT/paste electrode	SWV	7.0	1.1×10^{-6}	1.0×10^{-5} - 1.0×10^{-4}	31
5-amino-3',4'-dimethyl-biphenyl-2-ol-CNT/paste electrode	SWV	7.0	2.0×10^{-7}	4.0×10^{-7} - 9.0×10^{-4}	32
Nano Pt-MWCNT/paste electrode	DPV	7.0	1.7×10^{-7}	5.0×10^{-7} - 1.0×10^{-4}	33
Poly(acid yellow 9)-TiO ₂ /GCE	DPV	7.0	2.0×10^{-6}	1.2×10^{-5} - 1.2×10^{-4}	34
poly (calconcarboxylic acid)/GCE	DPV	6.0	1.0×10^{-8}	1.0×10^{-7} - 1.0×10^{-5}	35
MWCNT/ GCE	DPV	7.0	3.3×10^{-7}	7.9×10^{-7} - 3.4×10^{-4}	This work

Figure 5: SWV of AAP on MWCNT/GCE in 0.1 M BR buffer (pH 7.0) at different AAP concentrations from 6.5×10^{-7} to 3.5×10^{-4} M.

Figure 6: SWV calibration curves of AAP covering different concentration ranges with a MWCNT/GCE. A, 6.5×10^{-7} to 5.8×10^{-5} M; B, 5.8×10^{-5} to 3.5×10^{-4} M.

Stability and reproducibility of the MWCNT/GCE

The reproducibility of MWCNT-film modified GCEs was examined by repeating the measurement of 1.0×10^{-5} M AAP. After each determination, the used modified electrode underwent five successive cyclic voltammetric sweeps between 0.20 and 1.00 V at a scan rate of 50 mVsec^{-1} in 0.1 M BR (pH 7.0), to remove any adsorbents and yield a reproducible electrode surface. The relative standard deviation (RSD) of ten measurements was 1.3%. The long-term stability of the MWCNT film-coated GCE was also tested by measuring the reduction peak current at a fixed AAP concentration of 1.0×10^{-5} M over a period of 3 weeks. After 3 weeks, the current response only decreased by 3.2%. These results indicate that the electrode has excellent reproducibility and long-term stability, making it attractive for the fabrication of chemical sensors.

Interference

The effect of possible interference was investigated by the addition of other compounds to a solution containing 1.0×10^{-5} M AAP in 0.1 M BR electrolyte. Each possible contaminant was first added to yield a concentration identical to that of AAP. Subsequently, another addition was made so that the interferent concentration was 10 times that of AAP. Ascorbic acid, uric acid, penicillin, aspirin and amoxicillin were tested as interferents. Uric acid, penicillin, aspirin and ascorbic acid were found to cause less than a 5% increase in the AAP current peak height, even when present in 10-fold excess. The presence of amoxicillin in 10-fold excess of AAP was sufficient to cause a 10% decrease in AAP signal.

Sample analysis

The MWCNT-modified GCE was used to determine AAP in tablets. AAP content was calculated by the standard addition method and the results are shown in Table 3. Results obtained by SWV are in good agreement with DPV results, as well as with the declared AAP content. The recovered ratio indicates that the determination of AAP using MWCNT/GCEs is effective and sensitive.

Table 3. Determination of AAP in tablets.

Sample	Declared (μ M)	Detected (μ M)		Recovery (%)	
		SWV	DPV	SWV	DPV
Acetaminophen (325 mg)	7.00	7.06	6.40	100.9	91.4
Acetaminophen codeine (300 mg)	7.00	7.20	6.60	102.8	94.3
Acetaminophen (500 mg)	10.00	9.50	9.30	95.0	93.0
Acetaminophen Syrup	80.00	86.00	87.00	107.5	108.7

CONCLUSION

Utilizing the unique properties of MWCNTs, such as their high specific surface area and subtle electronic properties, MWCNT-modified glassy carbon electrodes in DPV and SWV techniques were used for the determination of AAP. The detection limit for SWV was 2.1×10^{-7} M, which is lower than the DPV detection limit of 3.3×10^{-7} M. Based on this work, a simple, rapid and sensitive method (i.e., SWV) was developed for AAP determination.

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