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Utilization of Acetaminophen as a Homogeneous Electrocatalyst for N-acetyl-L-cysteine Oxidation at the Surface of Carbon Paste Electrode

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Abstract- The utilization of acetaminophen (AC) as a homogeneous electrocatalyst in the presence of N-acetyl-L-cysteine (NAC) at the surface of carbon paste electrode (CPE) modified with multi-walled carbon nanotubes (MWCNT) has been surveyed in aqueous medium using double step potential chronoamperometry (CHA), cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods. Though, NAC itself shows a very negligible electrochemical response at bare CPE, the response could be greatly increased in the presence of acetaminophen at MWCNT paste electrode (MWCNT-CPE) which enables a sensitive electrochemical determination of the NAC. The kinetic parameters such as transfer coefficient (α), the apparent diffusion coefficient (D_{app}) and the chemical reaction rate constant (k_h) were calculated 0.33, 5.56×10^{-6} cm^2 s^{-1} and 1.35×10^{2} cm^3 mol^{-1} s^{-1}, respectively. A linear calibration range of 1.0×10^{-6} M-3.0×10^{-5} M and detection limit of 3.9×10^{-7} M were obtained for determination of NAC at MWCNT-CPE using DPV. The offered method is useful for the routine analysis of NAC in real sample.

Keywords- N-acetyl-L-cysteine, Acetaminophen, Multi-Walled Carbon Nanotubes, Homogeneous electrocatalyst

1. INTRODUCTION

N-acetyl-L-cysteine (NAC) which usually known as acetyl cysteine is one of the homologues of L-cysteine [1]. NAC is a mucolytic agent and leads to reducing the pulmonary spatters in chronic respiratory disease [2]. This drug has an antioxidant action and some authors have even suggested that NAC can bestead in the complexation and elimination of heavy metals, as well as preventing some species of cancer [3]. Many methods have been used for determination of NAC such as chromatography [4], flow injection analysis [5], spectrophotometry [6], fluorimetry [7], and electrochemical methods [2]. Compared to these options, electroanalysis has the advantages of simplicity, fast response, high sensitivity, low power requirement and low cost.

Acetaminophen, N-acetyl-p-aminophenol or AC, is extensively used as an anti-pyretic and painkiller. Also it is regarded as a convenient alternative for aspirin in patients sensitive to aspirin [8]. Over doses of AC agent is caused liver and kidney damage and lead to death [9]. There are many methods for determination of AC such as spectrophotometry, [10] HPLC, [11] titrimetry, [12] capillary electrophoresis, [13] chemiluminesance [14] and flow–injection [15]. This drug due to hydroxyl and -NH groups on its aromatic ring is electroactive molecule [16]. Thus, in this study, we used as a homogeneous electrocatalyst for electrochemical oxidation of NAC. Chemical structures of NAC and AC are shown in Fig. 1.

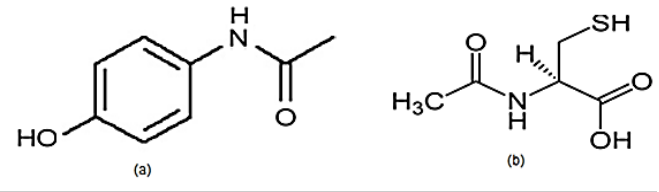


Fig. 1. Chemical structures of acetaminophen (A) and N-acetyl-L-cysteine (B)

Carbon nanotube (CNT) is a novel material which was originally discovered by Iijima [17] and in recent years significant endeavors have been made to construct various CNT morphologies and explore their applications in different fields such as electrochemical equipment, sensors and composites [18,19]. These materials having a high active surface property, high conductance, the ability to accelerate electrode reactions electrocatalytic effect on redox processes [20]. CNTs are known to show extremely properties by promoting electron transfer between the electrode surface and the electroactive species [21]. There are two groups of CNTs: single-walled (SWCNTs) and multi-walled (MWCNTs) carbon nanotubes [22]. Both SWCNTs and MWCNTs have the ability to aid electron-transfer reactions while used as an electrode material in the electrochemical reactions [23]. Generally, multi-walled carbon nanotubes have better sorption capabilities than SWCNTs owning to the existence of concentric layers of grapheme [24].

We introduced a carbon paste electrode modified with MWCNTs and used this electrode as an electrochemical sensor for voltammetric determination of NAC in the presence of AC as a homogeneous electrocatalyst.

2. EXPERIMENTAL

2.1. Instrumentation

The electrochemical measurements were carried out on potentiostat/galvanostat (SAMA 500 Electro-analyzer System, Iran). The platinum wire and MWCNT-CPE, Ag │AgCl │KCl (3.0 M) electrode were used as the auxiliary, working and reference electrodes, respectively.

2.2. Reagents and materials

Whole chemicals and reagents were of analytical grade quality. The Multi-walled carbon nanotubes (with purity>95%, outer diameter 5-20 nm, inner diameter 2-6 nm, length 1-10 μm, number of walls 3-15, from obtained Tehran, Iran) were used as the working electrode substrates. N-acetyl-cysteine (NAC), paraffin oil (density=0.88 g cm^{-3}), sulfuric acid and nitric acid were obtained from Fluke. Graphite powder (particle diameter=0.1 mm) and acetaminophen were obtained from Merck. Buffer solutions were prepared from ortho-phosphoric acid and its salts in the pH values of 4.00-9.00.

2.3. Preparation of working electrodes

For purification of multi-walled carbon nanotubes, 1.0 g of the MWCNT was dispersed in 3 ml nitric acid and 9 ml sulfuric acid, then was diluted with distilled water several times and placed at the magnetic stirring for 24 h and filtered on 4 filter paper and washed with doubled distilled water until the pH of the solution reached to neutral (pH=7.00) [25]. The MWCNT-CPE was prepared by hand mixing of 0.425 g graphite powder and 0.075 g MWCNT with a mortar and pestle. Then, 2.5 ml of paraffin oil was added to the above mixture and mixed for 30 min until a uniformly wetted paste was obtained. The paste was then packed into the end of a glass tube (inner diameter 3.4 mm) by pushing an excess of the paste out of the tube and polishing with a paper. Electrical contact was established via a copper wire. The MWCNT-CPE was applied as a working electrode.

2.4. Real samples preparation

For determination of NAC in tablet, one tablet of N-acetyl-L-cysteine labeled with 600 mg per tablet (from Avicenna, Iran), were completely ground and homogenized. An enough amount of ground table accurately weighted and dissolved with ultra-sonication in 10 ml

distilled water. After mixing completely, the mixture was filtered with an ordinary filter paper. The serum sample was centrifuged and then after filtering, diluted with phosphate buffered solution (PBS) (pH=7.00) without any further treatment. The diluted serum sample was spiked with different amounts of NAC.

3. RESULTS AND DISCUSSION

3.1. Characterization of working electrodes

Scanning electron microscopy (SEM) was used for characterization of prepared working electrode. Fig. 2 shows typical SEM images of bare and modified CPE. It can be seen, on the surface of CPE (Fig. 2A), the layer of irregular flasks of graphite powder was present. After multi-walled carbon nanotubes (MWCNTs) added to the carbon paste, it can be seen that MWCNTs were distributed on the electrode with special three-dimensional structure (Fig. 2B).

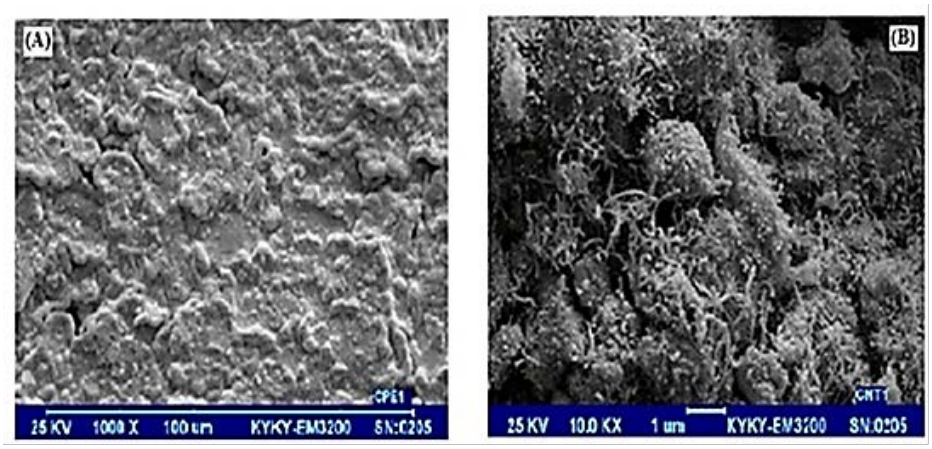


Fig. 2. SEM image of (A) CPE and (B) MWCNT/CPE

3.2. Electrochemical behavior of NAC at the surface of MWCNT modified CPE

The electrochemical properties of NAC at the surface of unmodified and modified carbon paste electrodes were studied in the absence and presence of AC in 0.1 M phosphate buffered solution (pH=7.00) using cyclic voltammetry method (Fig. 3A and B). As can be seen, the bare CPE and MWCNT-CPE do not show any anodic and cathodic peaks in the absence of NAC or AC in 0.1 M PBS (Fig. 3A (a) and (b)). On the other hand, the cyclic voltammogram of acetaminophen at MWCNT-CPE in the absence of NAC shows the anodic and cathodic peaks (E_{pa}=0.362 V and E_{pc}=0.275 V. ($E_{1/2}$)=0.318 V *vs*. Ag| AgCl| KCl (3.0 M) and $\Delta E_p=(E_{pa}-E_{pc})$=0.87 V related to the acetaminophen/N-acetyle-p-quinine immine redox

couple (Fig. 3A(curve d)). The electrode process was quasi-reversible, with ΔE_p, greater that the expected value (59/n mV) for a reversible system.

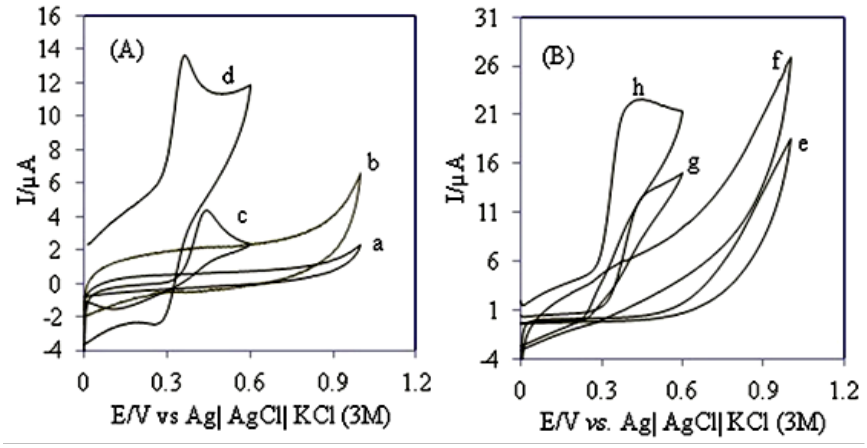
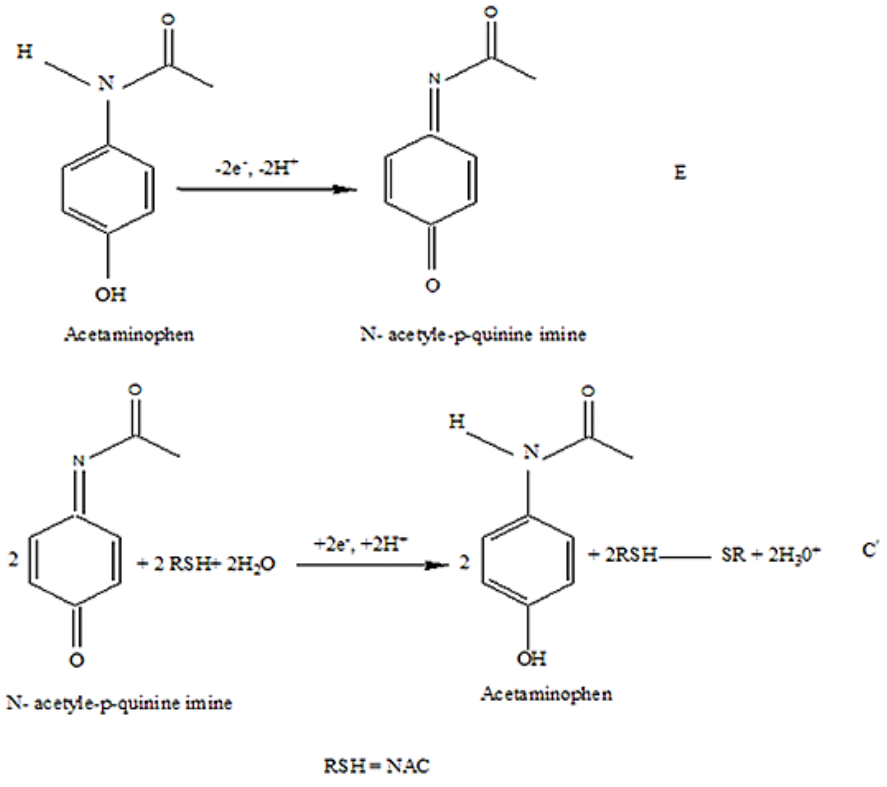


Fig. 3. (A) Cyclic voltammograms of (a) bare CPE, (b) MWNCT-CPE in 0.1 M PBS (pH=7.00), (c) as (a) and (d) as (b) in the presence of 100 µM AC in 0.1 M PBS (pH=7.00); (B) Cyclic voltammograms (e) as (a) , (f) as (b) in the presence of 1000 µM NAC, (g) as (a) and (h) as (b) in the presence of 1000 µM NAC and 100 µM AC in 0.1 M PBS (pH=7.00). Scan rate of potential is 20 mV s^{-1}



Scheme 1. Proposed mechanism for electrooxidation of acetaminophen in the presence of NAC at the surface of MWCNT-CPE

However, in the presence of NAC (Fig. 3B (e) and (f)), the oxidation of NAC occurs irreversibly with a broad peak at potential of nearly 1.0 V *vs*. Ag| AgCl| KCl (3.0 M) at the surface of CPE and MWCNT-CPE, respectively. The electrocatalytic oxidation of NAC in the presence of AC occurs at about 448 mV and 420 mV *vs*. Ag │AgCl │KCl (3.0 M) at the surface of CPE and MWCNT-CPE, respectively which is very close to the formal potential of the AC/ N-acetyl-p-quinine immine redox couple. Therefore, the overpotential of NAC oxidation decreases about 552 mV and 580 mV in the presence of acetaminophen at pH=7.00 at the surface of bare CPE and MWCNT-CPE, respectively (comparison of curves (g) with (e) and (f) with (h) of Fig. 3B).

As can be seen the anodic peak current of NAC enhances remarkably, while the corresponding cathodic peak disappears on the reverse scan rate of potential. These results show that under these conditions, acetaminophen acts as an efficient redox mediator for the homogeneous electrocatalysis of the NAC oxidation. This process corresponds to an EC´ mechanism, where N-acetyl-p-quinine immine was electrochemically formed and reacted with NAC for conversion to acetaminophen, according to below: proposed mechanism (Scheme 1).

3.3. Effect of pH

The electrochemical behavior of NAC and AC are affiliate on the pH value of the aqueous solution [26]. Thus, pH optimization of the solution seems to be essential in order to obtain the electrocatalytic oxidation of NAC in the presence of AC. Therefore, the electrochemical behavior of NAC was studied in 0.1 M phosphate buffer solutions in different pH values (pH=4.00-9.00) at the surface of MWCNT-CPE in the presence of acetaminophen by cyclic voltammetry method (Fig. 4A).

As can be seen, the anodic peak potential of NAC was shifted to a less positive potential with enhancing of pH at the surface of MWCNT-CPE in the presence of AC (Fig. 4B). Fig. 4C shows the variation of I_{pa} versus pH for NAC oxidation at the surface of MWCNT-CPE in the presence of AC. As can be seen, the maximum electrocatalytic current was obtained at pH=7.00.Therefore, pH=7.00 was chosen as the optimum pH for electrooxidation of NAC in the presence of acetaminophen at MWCNT/CPE due to the high oxidation current obtained in this pH.

3.4. The effect of scan rate

The effect of scan rate (10-300 mV s^{-1}) on the electrocatalytic oxidation of NAC in the presence of AC at the surface of MWCNT-CPE was considered by cyclic voltammetry (Fig. 5). As can be observed in Fig. 5A, the oxidation peak potential shifted to more positive potentials with enhancing scan rate of potential, confirming the kinetic limitation in the

electrochemical reaction. Also, the variation of I_{Pa} versus the square root of scan rate ($\upsilon^{1/2}$) was found to be linear in the range of 10-300 mV s^{-1}, (Fig. 5B), suggesting that at sufficient over potential, the process is diffusion rather than surface controlled [27]. Figure 5C shows the Tafel plot for the sharp rising part of the voltammogram at the scan rate of 20 mV s^{-1}. The slope of Tafel plot was found to be 5.554 V. This slope exhibits a transfer coefficient of α=0.33 for a one electron transfer process, which is rate-determining step.

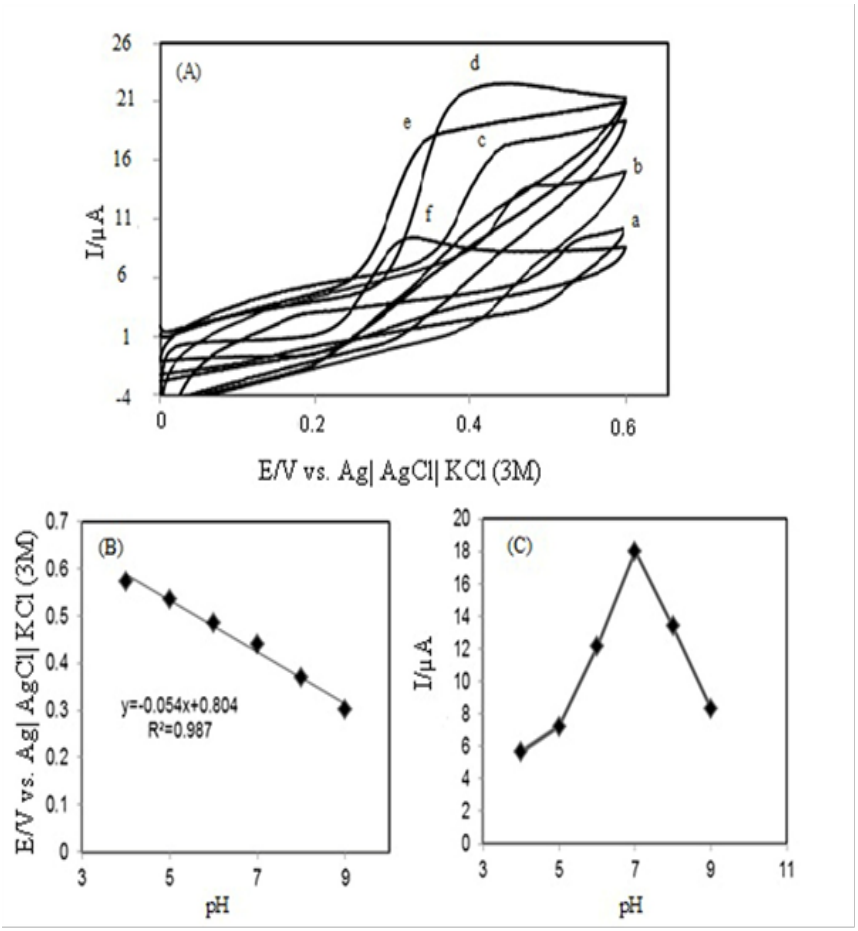


Fig. 4. (A) Cyclic voltammograms of 100 µM acetaminophen in the presenc of NAC in 0.1 M phosphate buffer solution at the surface of MWCNT-CPE in the various pHs: (a) 4.0, (b) 5.0, (c) 6.0, (d) 7.0, (e) 8.0 and (f) 9.0 at scan rate 20 mV s^{-1}; The variation of (B), NAC oxidation peak potentials and (C) electrooxidation peak currents of NAC versus pH values

3.5. Chronoamperometry studies

We studied the electrochemical behavior of NAC in an aqueous buffered solution (pH=7.00) in the presence of 100 µM acetaminophen at the surface of MWCNT-CPE using double-step potential chronoamperometry method (Fig. 6A). for an electroactive material (NAC in this case) with a diffusion controlled electron transfer, the current observed for the electrochemical reaction is described by the Cottrell equation [28]:

$$I = nFAD^{1/2}{}_{app}C_o\pi^{-1/2}t^{-1/2} \quad (1)$$

Where C_o and D_{app} are the bulk concentration (mol cm^{-3}) and the apparent diffusion coefficient (cm^2 s^{-1}), respectively. The plot of I versus $t^{-½}$ was linear (not shown), and from its slope, the mean values of the D_{app} for NAC was calculated 5.56×10^{-6} cm^2 s^{-1}.

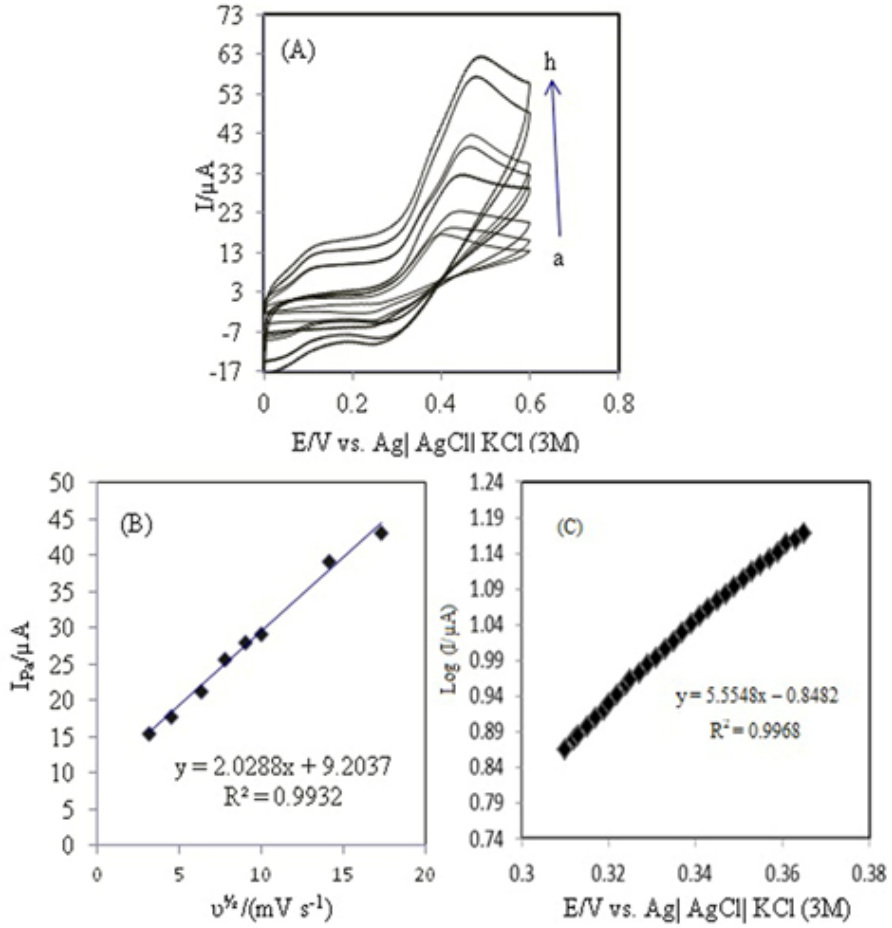


Fig. 5. (A) Cyclic voltamograms of NAC in the presence of AC at MWCNT-CPE in 0.1 M phosphste buffer solution (pH=7.00) at various scan rates: (a) 10, (b) 20, (c) 40, (d) 80, (e) 100, (f) 200 and (g) 300 mVs^{-1}; (B) Plot of anodic peak currents versus $\upsilon^{½}$ from cyclic voltammograms of (A); (C) Variationt of Log I *vs*. Oxidation peak potential (Tafel plot)

As can be seen acetaminophen can act as a homogeneous electrocatalysis for electrooxidation of NAC at the surface of MWCNT-CPE, the rate constant for the chemical reaction (k_h) can be evaluated by chronoamperometry according to the method described by Gallus [29]:

$$\frac{I_C}{I_L} = \pi^{1/2}(k_hC_ot)^{1/2} \quad (2)$$

where I_C is the catalytic (anodic) current of NAC at the surface of MWCNT-CPE, I_L is the limited current of NAC in the absence of AC and $\gamma=k_h C_o t$, C_o is the initial concentration of NAC in bulk solution and t is elapsed. The above equation can be used to calculate the rate constant, k_h, of the catalytic process from the slop of I_C/I_L *vs*. $t^{1/2}$ at a given NAC concentration. The average value of k_h was found to be 1.35×10^2 cm^3 mol^{-1} s^{-1} from the values of the slope.

Fig. 6B shows the double-potential step chronocolugrams of NAC in the absence (a´) and presence (d´) of AC at the surface of MWCNT-CPE. The results show that forward and backward potential step chronocolumetry yields very symmetrical chronocolugrams for NAC in the absence of AC. However, in the presence of AC, the charge for forward step is greater than that observed for backward step. This behavior is typically expected for electrocatalysis at chemically modified electrodes [30,31].

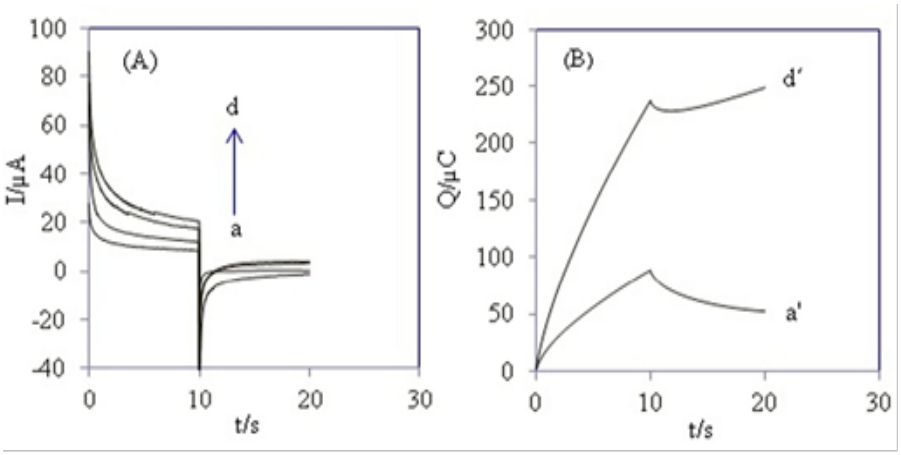


Fig. 6. (A) Chronoamperograms obtained for different concentration of NAC in the presence of AC in 0.1 M phosphate buffer solution (pH=7.00) with applied potential of 0.4 V and 0.2 V *vs*. Ag| AgCl| KCl (3M) at forward and backward step at MWCNT-CPE,respectively. The numbers a-d correspond to 0.0, 50, 250 and 300 µM of NAC; (B) chronocolougrams of (a) and (d) of plot (A)

3.6. Analytical measurement

The electrooxidation of NAC in the presence of acetaminophen was used for voltammetric determination of this compound. Therefore, we studied the electrochemical properties of 100 µM of acetaminophen in the presence of different concentrations of NAC at the surface of MWCNT-CPE using CV and differential pulse voltammetry methods (Figs. 7 and 8).

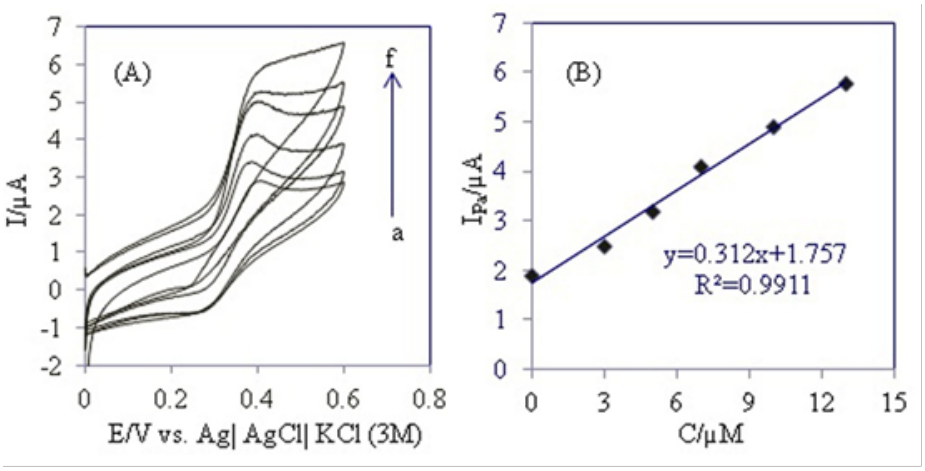


Fig. 7. (A) Cyclic voltammograms of 100 µM acetaminophen in the absence (a) and presence of NAC at different concentrations: (b) 3, (c) 5, (d) 7, (e) 10 and (f) 13 µM in 0.1 M phosphate buffer solution (pH=7.00) at a scan rate of 20 mV s^{-1} at MWCNT-CPE; (B) The plot of peak currents versus NAC concentrations

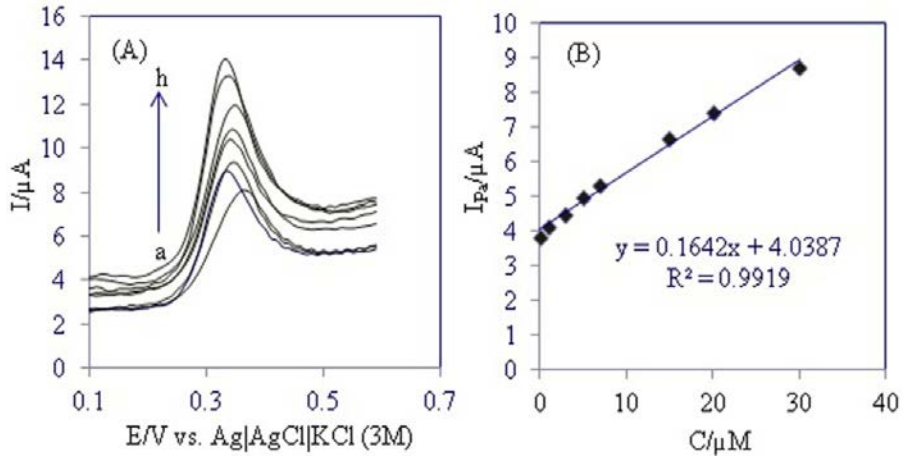


Fig. 8. (A) Differential pulse voltammograms of 100 µM acetaminophen in the absence (a) and in the presence of different concentrations of NAC: (b) 1, (c) 3, (d) 5,(e) 7, (f) 15, (g) 20 and (h) 30 µM in 0.1 M phosphate buffered solution (pH=7.00) at MWCNT-CPE; (B) The plot of peak current versus NAC concentrations

As can be seen under the optimum conditions, pH=7.00 and scan rate of 20 mV s^{-1}, the anodic peak currents were linear versus NAC concentrations from 3×10^{-6} M to 1.3×10^{-5} M (with R^2=0.991) and 1×10^{-6} M to 3×10^{-5} M (with R^2=0.993) in the CV and DPV methods, respectively (Figs. 7B and 8B). The detection limits (3σ) were 4.5×10^{-7} M and 3.9×10^{-7} M using CV and DPV, respectively. Therefore, this proposed method can readily be applied for voltammetric determination of NAC. These values are comparable with values reported by

other investigation groups for electro catalytic oxidation of NAC at the surface of other chemically modified electrodes (Table1).

Table 1. Comparison of the efficiency of some methods in the electrochemical determination of NAC

Electrode	Modifier	Methods	pH	LOD (M)	Scan rate (mVs^{-1})	LRD (M)	Ref.
CPE	CuHCF[a]	LSV	6.00	3.6×10^{-7}	-	1.2×10^{-4}-8.3×10^{-6}	[32]
PB/Pd-AlME[b]	Prussian blue	AMP	2.00	5.4×10^{-7}	20	4×10^{-5}-2×10^{-6}	[33]
GC	Naphtoquinone	DPV	7.00	8×10^{-7}	20	1.3×10^{-4}-4×10^{-6}	[34]
MWCNT-CPE	AC	DPV	7.00	3.9×10^{-7}	20	3×10^{-5}-1×10^{-6}	This work

[a] Copper(II) hexacyanoferrate(III)

[b] Palladized aluminum modified electrode

3.7. Determination of NAC in real samples

In order to evaluate the analytical applicability of the proposed method, it was applied to determination of NAC in tablet (from Avicenna, Iran) and human blood serum samples. The result for determination of NAC is given in Table 2.

Table 2. Determination on NAC in real sample using purposed method (n=2)

Sample	NAC added (μM)	found (μM)	Recovery	%RSD
Tablet[a]	9.0	9.3	103	0.48
	6.0	5.3	88.3	0.89
Serum	7.0	6.9	98.57	3.5
	3.0	3.2	106	2.67

[a](Avicenna, Iran)

Satisfactory recovery of the experimental results was found for NAC. The reproducibility of the method was demonstrated by the mean relative standard deviation (RSD). The obtained results clearly exhibit the capability of the proposed method in the voltammetric determination of NAC with high selectivity, and accuracy.

4. CONCLUSION

In present study, carbon-paste electrode modified with multi-walled carbon nanotubes used for the determination of NAC in the presence of AC as a homogeneous electrocatalyst. This mo dified electrode was very beneficial for exact determination of NAC in real samples and showed to be promising for NAC determination with many favorable properties including high sensitivity, low detection limit, decrease in over-voltage for the electrochemical oxidation of this compound and reproducible responses.

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