

Voltammetric Determination of *N*-Acetylcysteine Using Modified Multiwall Carbon Nanotubes Paste Electrode

Hassan Karimi-Maleh^{1*}, Mohsen Keyvanfard¹, Khadijeh Alizad¹, Masoud Fouladgar², Hadi Beitollahi³, Ali Mokhtari⁴ and Fathali Gholami-Orimi⁵

¹ Department of Chemistry, Majlesi Branch, Islamic Azad University, Isfahan, Iran

² Department of Biochemistry, Falavarjan Branch, Islamic Azad University, Falavarjan, Iran

³ Environment Department, Research Institute of Environmental Sciences, International Center for Science, High Technology & Environmental Sciences, Kerman, Iran

⁴ Department of Engineering and Technology, Golestan University, Aliabad Katool, Iran

⁵ Department of Chemistry, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran

*E-mail: h.karimi.maleh@gmail.com

Received: 28 September 2011 / Accepted: 5 November 2011 / Published: 1 December 2011

A ferrocene modified carbon nanotubes paste electrode (FCMCNTPE) was constructed and used as a fast and sensitive tool for the determination of *N*-acetylcysteine (NAC) at trace level. It has been shown by direct current cyclic voltammetry and double step chronoamperometry that this sensor can catalyze the oxidation of NAC in phosphate buffer solution (PBS) and produces a sharp oxidation peak current. Linear calibration ranges were 1.0-400.0 $\mu\text{mol L}^{-1}$ with detections limit of 0.6 $\mu\text{mol L}^{-1}$ for differential pulse voltammetry. Finally, the sensor was also examined as a selective, simple and precise new electrochemical sensor for the determination of NAC in real samples such as drug, serum and urine.

Keywords: *N*-Acetylcysteine, Ferrocene; carbon nanotubes paste electrode, voltammetry.

1. INTRODUCTION

Acetylcysteine or *N*-acetyl-L-cysteine (NAC) is a pharmaceutical drug and nutritional supplement used primarily as a mucolytic agent and in the management of paracetamol (acetaminophen) overdose [1]. Its action as an antioxidant and a glutathione precursor may also contribute to a protective effect against cancer [2].

Therefore, determination of this compound is very important. Several methods have been proposed for the determination of NAC, including HPLC [3], conductometry [4], spectrophotometry [5], flow injection [6,7], chromatography [8,9], colorimetry [10] and electrochemical methods [11-13].

Carbon nanotubes have been proved to be a novel type of nanostructure with unique structural, electronic and mechanical properties and have drawn extensive attention since their discovery [14-16]. Research over the past decade has revealed that the carbon nanotubes constitute a new form of carbon materials that are finding striking applications in many fields, such as energy conversion and storage [17,18] electrochemical sensing [19-25] and so forth.

In continuation of our studies concerning the preparation of chemically modified electrodes [26-35], in the present work, we described initially the preparation and suitability of a ferrocene-modified carbon nanotubes paste electrode (FCMCNTPE) as a new electrode in the electrocatalysis and determination of NAC in an aqueous buffer solution. Finally, in order to demonstrate the catalytic ability of the modified electrode in the electrooxidation of NAC in real samples, we examined this method for the voltammetric determination of NAC in human urine, tablet and serum samples.

2. EXPERIMENTAL

2.1. Apparatus and reagents

All the voltammetric measurements were performed using an Autolab PGSTAT 302N, potentiostat/galvanostat (Utrecht, The Netherlands) connected to a three-electrode cell, Metrohm (Herisau, Switzerland) Model 663 VA stand, linked with a computer (Pentium IV, 1,200 MHz) and with Autolab software.

A platinum wire was used as the auxiliary electrode. FCMCNTPE and Ag/AgCl/KCl_{sat} were used as the working and reference electrodes, respectively. The electrode prepared with carbon nanotubes and the one with the modifier were characterized by scanning electron microscopy (SEM) (Seron Tech. AIS 2100). A digital pH/mV-meter (Metrohm model 710) was applied for pH measurements. Graphite fine powder, paraffin oil and reagents were analytical grade from Merck. NAC was purchased from Merck. Multi-walled carbon nanotubes (purity more than 95 %) with *o.d.* between 10 and 20 nm, *i.d.* between 5 and 10 nm and tube length from 0.5 to 200 μm were prepared by Nanostructured & Amorphous Materials (USA).

2.2. Preparation of the electrode

20.0 mg of ferrocene was hand mixed with 880.0 mg of graphite powder and 100.0 mg of multiwall carbon nanotubes in a mortar and pestle. Using a syringe, 15 drops of paraffin were added to the mixture and mixed well for 50 min until a uniformly-wetted paste was obtained. The paste was then packed into a glass tube. Electrical contact was made by pushing a copper wire down the glass tube into the back of the mixture. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing it on a weighing paper. The unmodified carbon paste

electrode (CPE) was prepared in the same way without adding ferrocene and multiwall carbon nanotubes to the mixture to be used for the comparison purposes.

2.3. Preparation of a real samples

The urine sample was stored in a refrigerator immediately after collection. Ten milliliters of the sample was centrifuged for 5 min at 2000 rpm. The supernatant was filtered through a 0.45 μm filter and then diluted 10-times with water. The solution was transferred into a voltammetric cell to be analyzed without any further pretreatment.

For determination of NAC in tablet, five effervescent tablets of *N*-acetylcysteine labeled with 600 mg per tablet, were completely ground and homogenized. 140 mg of the powders was accurately weighted and dissolved with ultrasonication in 50 mL water. After mixing completely, the mixture was filtered with an ordinary filter paper. The standard addition method was used for the determination of NAC in real samples.

3. RESULTS AND DISCUSSION

3.1. SEM Characterization of modified electrode

Figure 1 displays a typical morphology of (a) CPE, (b) CNTPE, and (c) FCMCNTPE characterized by scanning electron microscope (SEM).

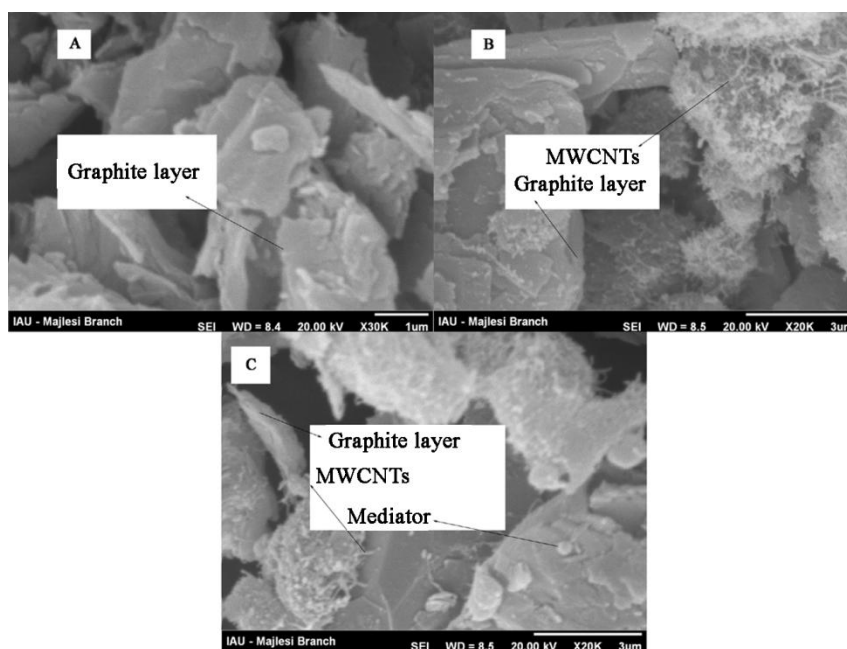


Figure 1. SEM image of A) CPE, B) CNTPE and C) FCMCNTPE.

From Figure 1, it can be seen that ferrocene and multiwall carbon nanotubes distributed almost homogeneously at the surface of carbon paste matrix and exhibiting a special three-dimensional structure.

3.2. Electrochemical behavior of the FCMCNTPE

Cyclic voltammetry was employed for the investigation of the electrochemical properties of the FCMCNTPE in a pure buffered aqueous solution (pH 9.0).

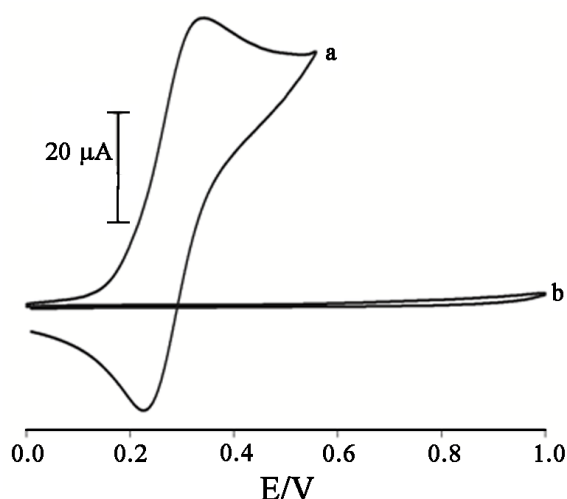


Figure 2. Cyclic voltammograms of a) bare CPE and b) FCMCNTPE in 0.1 mol L⁻¹ PBS (pH 9.0) at a scan rate 20 mV s⁻¹.

The cyclic voltammogram (Figure 2) exhibits an anodic and corresponding cathodic peaks with $E_{pa} = 0.340$ V and $E_{pc} = 0.230$ V vs. Ag/AgCl/KCl_{sat}. The experimental results showed well-defined and reproducible anodic and cathodic peaks related to the Fc/Fc⁺ redox couple with quasi-reversible behavior [36], because the peak separation potential, $\Delta E_p = (E_{pa} - E_{pc})$, was greater than the $59/n$ mV expected for a reversible system. In addition, the result obtained from cyclic voltammetry of this modified electrode in various buffered solutions did not show any shift in the anodic and cathodic peak potentials. Therefore, the electrochemical behavior of the redox process of Fc/Fc⁺ in the FCMCNTPE is independent of the pH of the aqueous solution. The calculated RSDs for various parameters accepted as the criteria for a satisfactory surface reproducibility were 1–3 %. This degree of reproducibility is virtually the same as that expected for a renewed or ordinary carbon paste surface.

3.3. pH effect study

It is well known that the electrochemical behavior of NAC is dependent on the pH value of the aqueous solution [13], whereas the electrochemical properties of the Fc/Fc⁺ redox couple are

independent of pH [36]. Therefore, we studied the electrochemical behavior of NAC in buffered solution (0.1 M phosphate) with various pHs ($3.0 < \text{pH} < 10.0$) at the surface of FCMCNTPE by cyclic voltammetry.

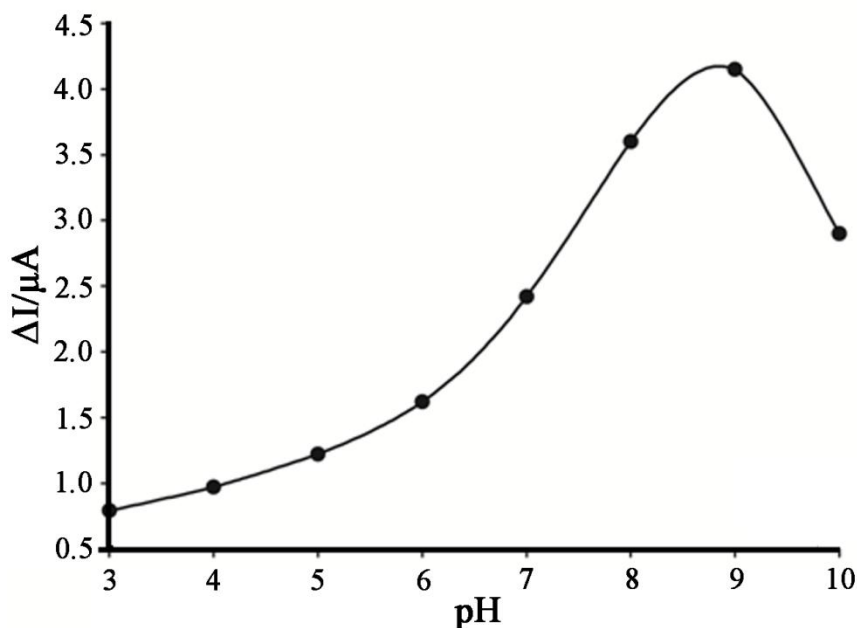


Figure 3. Net current–pH curve for electrooxidation of $400.0 \mu\text{mol L}^{-1}$ NAC in 0.1 mol L^{-1} PBS at the surface of FCMCNTPE at scan rate 20 mV s^{-1} .

Figure 3 shows the variation of ΔI_{pa} versus pH for NAC oxidation at the surface of this modified electrode. As can be seen, the maximum electrocatalytic current was obtained at pH 9.0. Therefore, pH 9.0 was chosen as the optimum pH for electrocatalytic oxidation of NAC at FCMCNTPE and all electrochemical experiments were done at this pH.

3.4. Electrochemistry of NAC at the FCMCNTPE

The cyclic voltammetric responses from the electrochemical oxidation of $600.0 \mu\text{mol L}^{-1}$ NAC at the FCMCNTPE (curve c), the ferrocene modified CPE (FCMCPE) (curve b), the CNTPE (curve d) and the bare CPE (curve e) are depicted in Figure 4. As can be seen, the anodic peak potentials for the oxidation of NAC at the FCMCNTPE (curve c) and the FCMCPE (curve b) are about 340 mV, while at the CNTPE (curve d), the peak potential was about 880 mV. At the unmodified CPE, the peak potential was about 900 mV of NAC (curve e). From these results, it was concluded that the best electrocatalytic effect for NAC oxidation was observed at the FCMCNTPE (curve c). For example, the results showed when the oxidation of NAC at the FCMCPE (curve b) and the FCMCNTPE (curve c) are compared there is an enhancement of the anodic peak current at the FCMCNTPE relative to the value obtained at the FCMCPE. In the other words, the obtained data clearly showed that the

combination of carbon nanotubes and mediator (FC) definitely improve the characteristics of NAC oxidation. The FCMCNTPE in 0.1 mol L^{-1} phosphate buffer (pH 9.0) without NAC in the solution exhibited a well-behaved redox reaction (curve a) upon the addition of $600.0 \mu\text{mol L}^{-1}$ NAC, the anodic peak current of mediator was increased, while the corresponding cathodic peak decrease on the reverse scan of the potential (curve c). This behavior is typical of that expected for electrocatalysis at chemically modified electrodes [37,38].

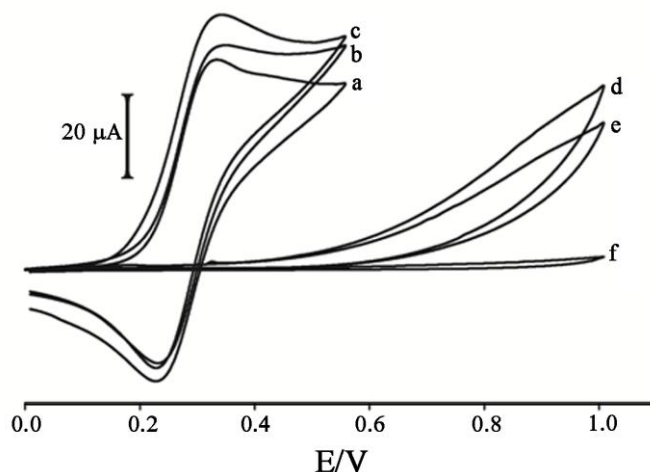


Figure 4. Cyclic voltammograms of a): 0.1 mol L^{-1} PBS (pH 9.0) at FCMCNTPE; c): 0.1 mol L^{-1} PBS (pH 9.0) at FCMCNTPE in the presence of $600.0 \mu\text{mol L}^{-1}$ NAC; b) 0.1 mol L^{-1} PBS (pH 9.0) plus $600.0 \mu\text{mol L}^{-1}$ NAC at FCMCPE; d) is as (c) and (e) is as (b) at CNTPE and CPE, respectively. Curve (f) 0.1 mol L^{-1} PBS at CNTPE. Condition; scan rate, 20 mV s^{-1} .

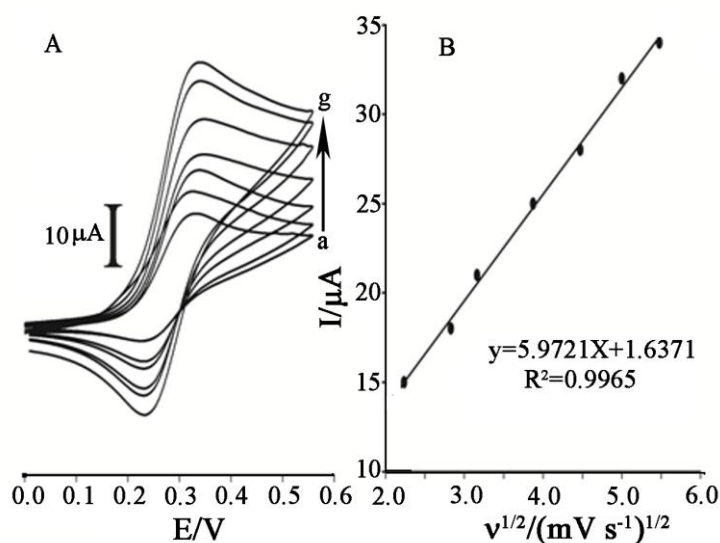


Figure 5. A) Cyclic voltammograms of the FCMCNTPE in 0.1 mol L^{-1} PBS (pH 9.0) containing $200.0 \mu\text{mol L}^{-1}$ NAC at different scan rates. The number of a–g corresponds to 5, 8, 10, 15, 20, 25 and 30 mV s^{-1} , respectively. B) Variation of electrocatalytic current (I_p) with the square root of scan rate.

Figure 5A show the cyclic voltammograms of a 200.0 $\mu\text{mol L}^{-1}$ NAC solution with different scan rates. As indicated by Figure 5A, the catalytic oxidation peak potential gradually shifts toward more positive potentials with increasing the scan rate, suggesting a kinetic limitation in the reaction between the redox site of the FCMCNTPE and NAC. However, the oxidation currents change linearly with the square root of the scan rate (Figure 5B), suggesting that at sufficient overpotential, the reaction is mass transfer controlled.

In order to obtain information on the rate determining step, a Tafel plot was developed for FCMCNTPE in the presence of 200.0 $\mu\text{mol L}^{-1}$ NAC at pH 9.0, using the data derived from the raising part of the current–voltage curve. The slope of the Tafel plot was equal to $n(1-\alpha)F/2.3RT$. $n\alpha$ is thus calculated as 0.46. Assuming $n = 1$, then $\alpha = 0.46$.

3.5. Chronoamperometric studies

The catalytic oxidation of NAC by a FCMCNTPE was also studied by chronoamperometry. Chronoamperometric measurements of different concentrations of NAC at the FCMCNTPE were performed by setting the working electrode potential at 100 and 400 mV. From the chronoamperometric studies, the diffusion coefficient, D , of NAC was determined.

The experimental plots of I vs. $t^{-1/2}$ with the best fits for different concentrations of NAC were employed.

The slopes of the resulting straight lines were then plotted vs. the NAC concentration. From the resulting slope and Cottrell equation the mean value of the D was found to be $2.64 \times 10^{-6} \text{ cm}^2/\text{s}$.

The rate constant for the chemical reaction between NAC and redox sites in FCMCNTPE, k_h , can be evaluated by chronoamperometry according to the method of Galus [39]:

$$I_C/I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (kC_b t)^{1/2} \quad (1)$$

Where I_C is the catalytic current of FCMCNTPE in the presence of NAC, I_L the limited current in the absence of NAC and t is the time elapsed (s). The above equation can be used to calculate the rate constant of the catalytic process k_h . Based on the slope of the I_C/I_L versus $t^{1/2}$ plots, k_h can be obtained for a given NAC concentration. From the values of the slopes an average value of k_h was found to be $k_h = 3.5 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. The value of k_h explains as well as the sharp feature of the catalytic peak observed for catalytic oxidation of NAC at the surface of FCMCNTPE.

3.6. Calibration plot and limit of detection

Differential pulse voltammetry (DPV) was used to determine the concentration of NAC (Figure 6). The responses were linear with NAC concentration in the range from 1.0-400.0 $\mu\text{mol L}^{-1}$ and the current sensitivity was 0.0063 $\mu\text{A}/\mu\text{M}$. The detection limit (3σ) was 0.6 $\mu\text{mol L}^{-1}$.

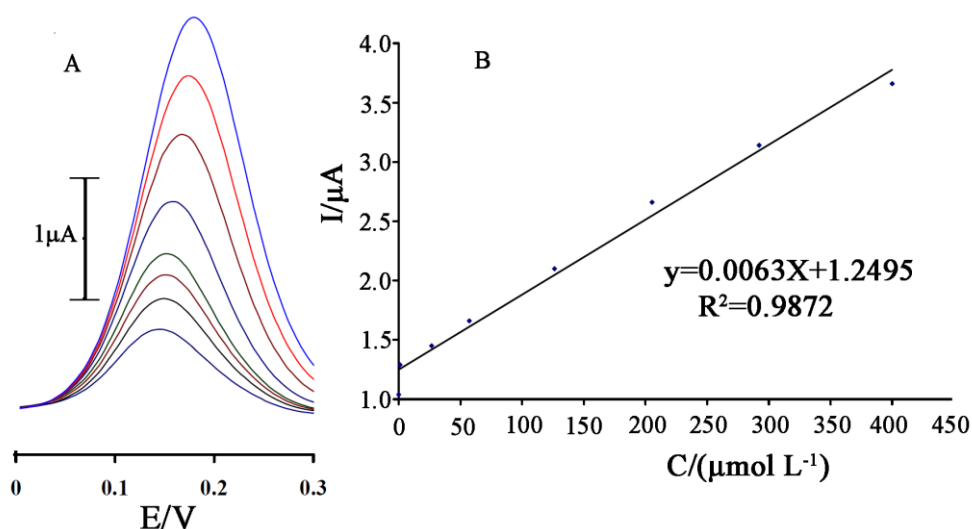


Figure 6. A) Differential pulse voltammograms of the FCMCNTPE in 0.1 mol L⁻¹ PBS (pH 9.0) containing different concentrations of NAC, from inner to outer corresponding to 0.0, 1.0, 26.6, 57.0, 126.0, 205.3, 292.0 and 400.0 μmol L⁻¹ of NAC; B) plots of the electrocatalytic peak current as a function of NAC concentration.

3.7. Interference studies

The influence of various substances as compounds potentially interfering with the determination of NAC was studied under optimum conditions with 3.0 μmol L⁻¹ NAC at pH 9.0. The potentially interfering substances were chosen from the group of substances commonly found with NAC in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than ±5 % in the determination of NAC. According to the results, neither a 600-fold excess of glucose, sucrose, lactose, fructose, Ca²⁺, Mg²⁺, SO₄²⁻, Al₃⁺, CO₃²⁻, Cl⁻, F⁻ affected the selectivity. In addition, neither a saturated starch solution nor a 2-fold excess of ascorbic acid interfered with the determination of NAC. Although ascorbic acid shows minor interference, this can be minimized, if necessary, using the ascorbic oxidase enzyme which exhibits high selectivity to the oxidation of ascorbic acid.

3.8. Determination of NAC in a real sample

To investigate the applicability of the proposed sensor for the catalytic determination of NAC in real samples, we selected urine, serum and tablet samples for the analysis of their NAC contents (see Table 1). The obtained results clearly demonstrate and confirm the capability of the FCMCNTPE in the voltammetric determination of NAC with high selectivity, accuracy, and good reproducibility.

Table 1. The results of determination of NAC in real sample.

Sample	Added ($\mu\text{mol L}^{-1}$)	Expected ($\mu\text{mol L}^{-1}$)	Founded ($\mu\text{mol L}^{-1}$)	Recovery %
Tablet	----	300.0	301.5 \pm 0.7	100.5
	10.0	310.0	311.0 \pm 0.6	100.3
Urine	----	----	<Limit of detection	----
	200.0	200.0	201.6 \pm 0.9	100.8
Serum	----	----	<Limit of detection	----
	350.0	350.0	350.9 \pm 0.4	100.2

4. CONCLUSIONS

This work demonstrates the construction of a FCMCNTPE and its application in the determination of NAC. The results showed that the oxidation of NAC was catalyzed at pH 9.0, whereas the peak potential of NAC was shifted by 540 mV to a less positive potential at the surface of the FCMCNTPE. The catalytic peak currents obtained using DPV, were linearly dependent on the NAC concentrations and the detection limit for NAC was 0.6 $\mu\text{mol L}^{-1}$. The high current sensitivity and high selectivity of the FCMCNTPE for the detection of NAC proved its potential as a sensor. In addition, the FCMCNTPE was employed for the determination of NAC in some drug and urine samples.

ACKNOWLEDGMENT

The authors wish to thank, Majlesi Branch, Islamic Azad University, and the Iranian Nanotechnology Initiative Council for their support.

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