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Electrochemical Determination of N-acetylcysteine and Folic Acid in Pharmaceutical and Biological Samples Using a Modified Carbon Nanotube Paste Electrode

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2-chlorobenzoyl ferrocene, was synthesized and used to construct a modified carbon nanotube paste electrode. The electrooxidation of N-acetylcysteine at the surface of the modified electrode was studied. Under the optimized conditions, the square wave voltammetric (SWV) peak current of N-acetylcysteine increased linearly with N-acetylcysteine concentration in the ranges of 5.0×10^{-8} to 4.0×10^{-4} M. The prepared modified electrode exhibits a very good resolution between the voltammetric peaks of N-acetylcysteine and folic acid which makes it suitable for the detection of N-acetylcysteine in the presence of folic acid in real samples.

Keywords: N-acetylcysteine, Folic acid, Carbon paste electrode, Carbon nanotubes, Electrocatalysis

1. INTRODUCTION

N-acetylcysteine is a pharmaceutical drug used primarily as a mucolytic agent since it is able to cleave disulfide bonds and converting them into two sulfhydryl groups. This reduces the chain length, which thins the mucus and so makes it easier to eliminate.

N-acetylcysteine can also be very effective as an antidote in cases of acetaminophen poisoning [1]. In addition, this drug has an antioxidant action, and some authors have even suggested that N-acetylcysteine can aid in the complexation and elimination of heavy metals, as well as preventing some types of cancer [2].

There are many methods described in the literature for the quantification of N-acetylcysteine, including titrimetry [3], spectrophotometry [4], chemiluminescence [5], fluorimetry [6], turbidimetry [7], amperometry [8] and cathodic stripping voltammetry [9]. With respect to its relatively large oxidation overpotential, the corresponding voltammetric signals on the surface of unmodified electrodes are usually weak. In order to decrease the undesirable anodic overpotential in the electrochemical oxidation of N-acetylcysteine, various chemically modified electrodes have been constructed [10-17].

Folic acid, often regarded as a part of vitamin B complex, possesses the considerable biological importance for general human health, especially during periods of rapid cell division and growth [18]. The deficiency of folic acid will cause serious diseases, notably for women planning for pregnancy, which can result in malformations of the spine, skull, and brain [19]. Therefore, the determination of folic acid has drawn significant attention, and a reliable and sensitive detection method is highly expected. At present, some measurements, such as spectrophotometry [20], fluorometric [21], high-performance liquid chromatography (HPLC) [22] and flow injection chemiluminescence [23] have been used to detect folic acid. However, these techniques are complex, time-consuming, and require expensive instruments. Electrochemical methods have also been used and attracted enormous interest due to their advantages of simplicity, rapid response, excellent reproducibility, good stability, low cost and low detection limit, etc. [24–39].

Electrochemical techniques in the field of pharmaceutical analysis have developed due to their simplicity, reasonable accuracy and precision, low cost, and rapidity. There is no need for derivatization or time-consuming extraction steps in comparison with other techniques because of less sensitivity of electroanalytical methods to the matrix effects [40-60].

Carbon-paste electrodes (CPEs) are widely utilized to perform the electrochemical determinations of a variety of biological and pharmaceutical species owing to their low residual current and noise, ease of fabrication, wide anodic and cathodic potential ranges, rapid surface renewal, and low cost. Moreover, chemically modified electrodes (CMEs) can be easily prepared by adding different substances to the bulk of CPEs in order to increase sensitivity, selectivity, and rapidity of determinations [61-85]. Application of transition metal Schiff base and phthalocyanine complexes (e.g., cobalt and iron) in preparation of CMEs has shown excellent electrocatalytic properties owing to structural characterization of modifiers, in which the steric and electronic effects of substituent groups can affect the catalytic activity of the complex [86-103].

The integration of nanotechnology with electrochemistry is expected to produce major advances in the field of electrochemical sensors. There is growing interest in developing new enhanced materials and designing novel sensors with controlled features on a nanometric scale. The unique properties of metal nanoparticles (enhanced mass transport, high surface area and improved signal-to-noise ratio) can often be advantageous in electroanalytical techniques [104, 105]. Carbon nanotubes (CNTs) are gaining popularity in the electrochemistry as a viable nanomaterial due to their extraordinary electronic, chemical and structural characteristics. CNTs display intrinsic properties that include high surface areas, high electrical conductivities, and their inherent size and hollow geometry, which make them extremely attractive as substrates for heterogeneous catalysis [106-115].

In this paper, initially the preparation and suitability of a 2-chlorobenzoyl ferrocene (2CBF) modified carbon naotube paste electrode (2CBFCNPE) as a new electrode in the electrocatalysis and determination of N-acetylcysteine in an aqueous buffer solution was described. Then the analytical performance of the modified electrode in quantification of N-acetylcysteine in the presence of folic acid was also evaluated. Finally this new constructed electrochemical sensor was used for determination of N-acetylcysteine and folic acid in real samples.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrode cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and 2CBFCNPE were used as the reference, auxiliary and working electrodes, respectively. Finally a Metrohm 710 pH meter was used for pH measurements.

All solutions were freshly prepared with double distilled water. N-acetylcysteine, folic acid and all of the other reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-11.0.

2.2. Synthesis of 2-chlorobenzoyl ferrocene

To a 100 ml round-bottomed flask under argon atmosphere, 1.86 g (10 mmol) of ferrocene, 1.75 g (10 mmol) of 2-chlorobenzoyl chloride and 20 ml of dichloromethane is added. The reaction mixture is cooled in an ice bath (0-5ºC), then 1.40 g (11 mmol) of anhydrous aluminum chloride added in small portions at such a rate that the reaction mixture remains below 5ºC. The resulting solution is stirred for 30 min at 0-5ºC and 2 h at room temperature. Then, the flask is placed in ice bath again and 20 ml of water is added cautiously to give a two-phase mixture. After stirring for 30 minutes the aqueous layer is extracted with two 15 ml portions of dichloromethane. The combined dichloromethane extracts are washed once with water, twice with 10% sodium hydroxide solution, dried over magnesium sulfide and evaporated at reduced pressure. The crude residue is purified by recrystallization from heptane to afford (2-chlorobenzoyl)ferrocene in 95% yield. M.p. 99-100ºC,

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 4.30 (s, 5H), 4.62 (t, *J*=1.6 Hz, 2H), 4.77 (t, *J*=1.6 Hz, 2H), 7.36 (dt, *J*=1.6, 7.6 Hz, 1H), 7.42 (dt, *J*=2.0, 7.6 Hz, 1H), 7.47 (dd, *J*=1.2, 8.0 Hz, 1H), 7.52 (1.6, 7.2 Hz, 1H).

IR (KBr) ($\nu_{max,}$ cm^{-1}): 3080.8, 3052.5 (C-H Aromatic), 1644.9 (C=O), 1442.7, 1292.8, 1031.5, 827.7.

2.3. Preparation of the electrode

The 2CBFCNPEs were prepared by dissolving 0.01 g 2CBF in 3 mL diethyl ether and then added in 0.89 g graphite powder and 0.1 g CNTs with a mortar and pestle. Then, 0.7 mL of paraffin were added to the above mixture and mixed for 15 min until a uniformly wetted paste was obtained. The paste was then packed into the end of a glass tube. A copper wire inserted into the carbon paste provided the electrical contact. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing with a weighing paper.

For comparison, 2CBF modified CPE electrode (2CBFCPE) without CNTs, CNTs paste electrode (CNPE) without 2CBF, and unmodified CPE in the absence of 2CBF and CNTs were also prepared in the same way. A typical scanning electron microscope (SEM) image for 2CBFCNPE is shown in Fig. 1.

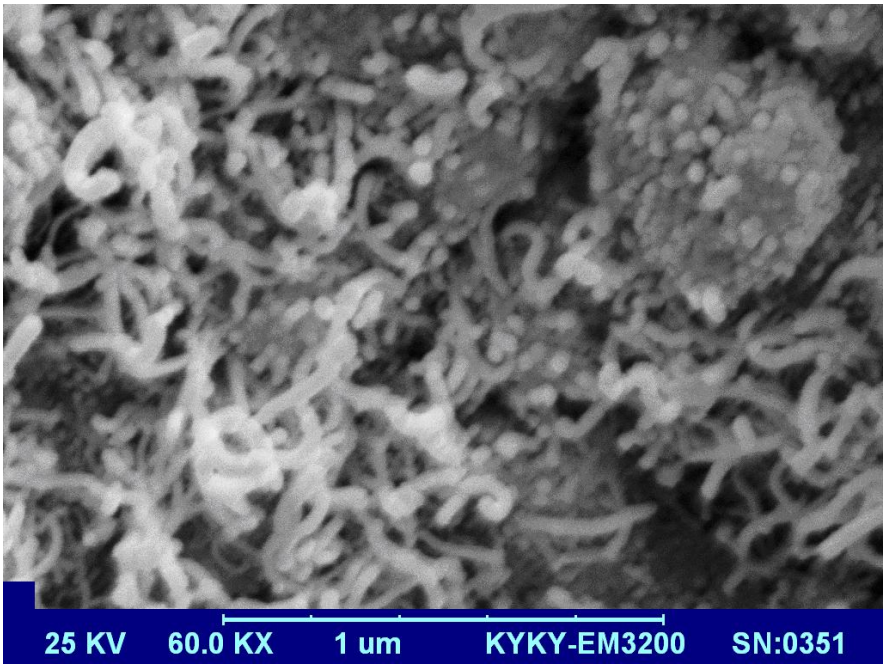

Figure 1. SEM image of 2CBFCNPE.

2.4. Procedure of real samples preparation

Five N-acetylcysteine tablets (labeled 600 mg) were grinded and a N-acetylcysteine solution was prepared by dissolving 600 mg of the powder in 100 mL water by ultrasonication. Then, different volumes of the diluted solution were transferred into a 10 mL volumetric flask and were diluted to the mark with phosphate buffer solution (PBS) (pH 7.0). The N-acetylcysteine content was analyzed by the proposed method using the standard addition method.

Also, five folic acid tablets (labeled 1.0 mg) were grinded and the tablet solution was prepared by dissolving 50 mg of the powder in 100 mL water by ultrasonication. Then, different volumes of the diluted solution were transferred into a 10 mL volumetric flask and were diluted to the mark with PBS

(pH 7.0). The folic acid content was analyzed by the proposed method using the standard addition method.

Urine samples of healthy people were stored in a refrigerator immediately after collection. Ten milliliters of the sample was centrifuged for 15 min at 2000 rpm. The supernatant was filtered out using a 0.45 μm filter. Then, different volume of the solution was transferred into a 25 mL volumetric flask and diluted to the mark with PBS (pH 7.0). The diluted urine sample was spiked with different amounts of N-acetylcysteine and folic acid.

The serum sample of healthy people was prepared from a local laboratory. It centrifuged and after filtering, diluted with PBS (pH 7.0) without any further treatment. The diluted serum sample was spiked with different amounts of N-acetylcysteine and folic acid.

3. RESULTS AND DISCUSSION

3.1. Electrochemical Behavior of 2CBFCNPE

Since the 2CBF complex is insoluble in aqueous solutions; it can be used in the carbon paste without leaching out from the electrode surface, which leads to a stable chemically modified electrode. The electrochemical behavior of the 2CBFCNPE was investigated.

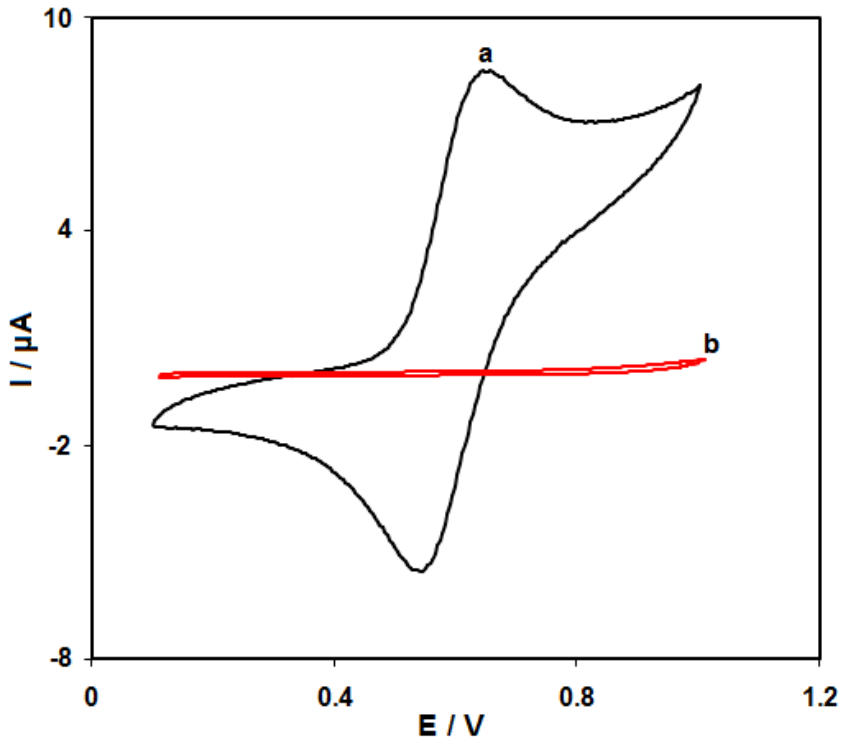

Figure 2. CVs of 2CBFCNPE (a) and CPE (b) in 0.1 M PBS (pH 7.0) In all cases scan rate is 100 mV s^{-1}.

Fig. 2 shows the CVs of the modified electrode at scan rate of 100 mVs^{-1} in 0.1 M phosphate buffer solution (PBS) (pH 7.0). A pair of reversible peaks was observed at E_{pa} = 0.655 V and E_{pc} = 0.540 V vs. Ag/AgCl. Half-wave potential ($E_{1/2}$) and ΔE_p were 0.597 V vs. Ag/AgCl and 0.115 V, respectively. The peak separation potential, ΔEp ($=E_{pa} - E_{pc}$), was greater than the expected one (59/*n*) mV for a reversible system. This suggests a quasi reversible behavior in an aqueous medium [116].

3.2. Electrocatalytic oxidation of N-acetylcysteine at a 2CBFCNPE

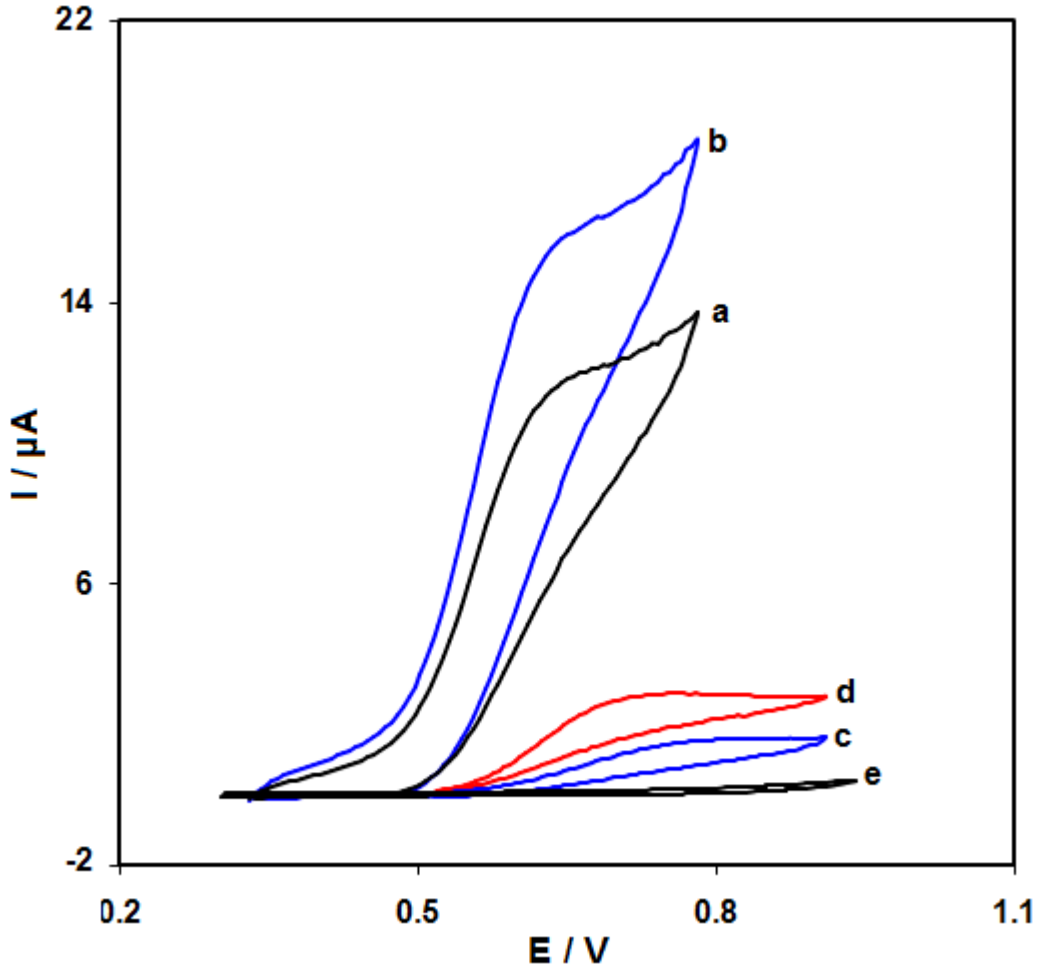

Figure 3. CVs of 2CBFCPE (a) and 2CBFCNPE (b) in 0.1 M PBS (pH 7.0) containing 100.0 µM N-acetylcysteine. (c) and (d) are CVs of CPE and CNPE in 0.1 M PBS (pH 7.0) containing 100.0 µM N-acetylcysteine and (e) is CV of CPE in 0.1 M PBS (pH 7.0) In all cases scan rate is 10 mV s^{-1}.

Fig. 3 depicts the CV responses for the electrochemical oxidation of 100.0 µM N-acetylcysteine at unmodified CPE (curve c), CNPE (curve d), 2CBFCPE (curve a) and 2CBFCNPE (curve b). As can be seen, while the peak potential for N-acetylcysteine oxidation at the CNPE, and unmodified CPE are 740 and 790 mV, respectively, the corresponding potential at 2CBFCNPE and 2CBFCPE is ~ 655 mV. These results indicate that the peak potential for N-acetylcysteine oxidation at

the 2CBFCNPE and 2CBFCPE electrodes shift by ~ 85 and 135 mV toward negative values compared to CNPE and unmodified CPE, respectively. However, 2CBFCNPE shows much higher anodic peak current for the oxidation of N-acetylcysteine compared to 2CBFCPE, indicating that the combination of CNTs and the mediator (2CBF) has significantly improved the performance of the electrode toward N-acetylcysteine oxidation. The 2CBFCNPE, in 0.1 M PBS (pH 7.0) and without N-acetylcysteine in solution (Fig. 2 curve a), exhibited a well-behaved redox reaction and with addition of 100.0 μM N-acetylcysteine, increased the anodic peak current (Fig. 3 curve b), indicating a strong electrocatalytic effect [116].

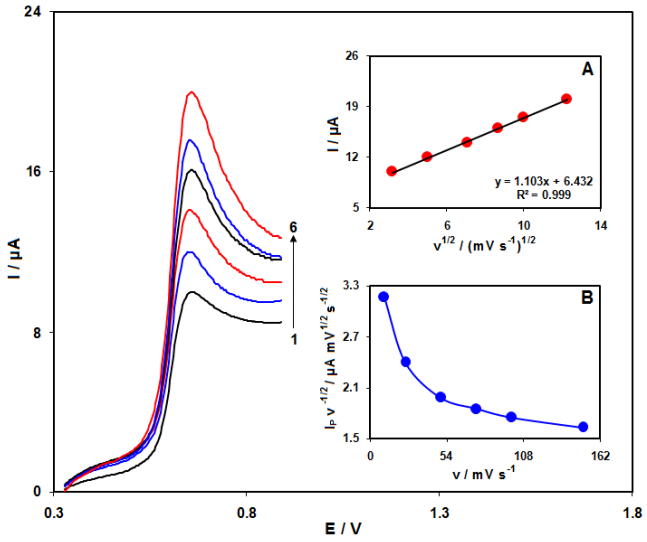

Figure 4. LSVs of 2CBFCNPE in 0.1 M PBS (pH 7.0) containing 50.0 μM N-acetylcysteine at various scan rates; numbers 1-6 correspond to 10, 25, 50, 75, 100 and 150 mV s^{-1}, respectively. Insets: Variation of (A) anodic peak current *vs*. square root of scan rate; (B) normalized current ($I_p/ v^{1/2}$) vs. *v*.

The effect of potential scan rate on the electrocatalytic oxidation of N-acetylcysteine at the 2CBFCNPE was investigated by LSV (Fig. 4). A plot of peak height (I_p) vs. the square root of scan rate ($v^{1/2}$) was found to be linear in the range of 10-150 mV s^{-1}, suggesting that, at sufficient over potential, the process is diffusion rather than surface controlled (Fig. 4A). A plot of the scan rate-normalized current ($I_p/v^{1/2}$) vs. scan rate (Fig. 4B) exhibits the characteristic shape typical of an electrocatalytic (EC') process [116].

Fig. 5 shows the LSV of 2CBFCNPE obtained in 0.1 M PBS (pH 7.0) containing 50.0 μM N-acetylcysteine, with a sweep rate of 10 mV s^{-1}. The points show the rising part of the voltammograms (known as the Tafel region), which is affected by the electron transfer kinetics between N-acetylcysteine and 2CBFCNPE. If deprotonation of N-acetylcysteine is a sufficiently fast step, the number of electrons involved in the rate determining step can be estimated from the slope of the Tafel plot.

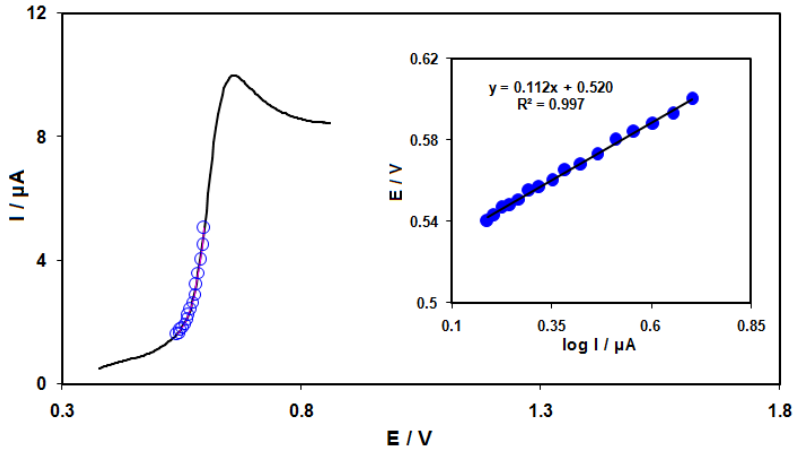

Figure 5. LSV (at 10 mV s^{-1}) of an 2CBFCNPE in 0.1 M PBS (pH 7.0) containing 50.0 µM N-acetylcysteine . The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the linear sweep voltammogram.

The inset of Fig. 5 shows a Tafel plot that was drawn from points of the Tafel region of the LSV. The Tafel slope of 112.0 mV obtained in this case agrees well with the involvement of one electron in the rate determining step of the electrode process, assuming a charge transfer coefficient of $\alpha = 0.47$.

3.3. Chronoamperometric measurements

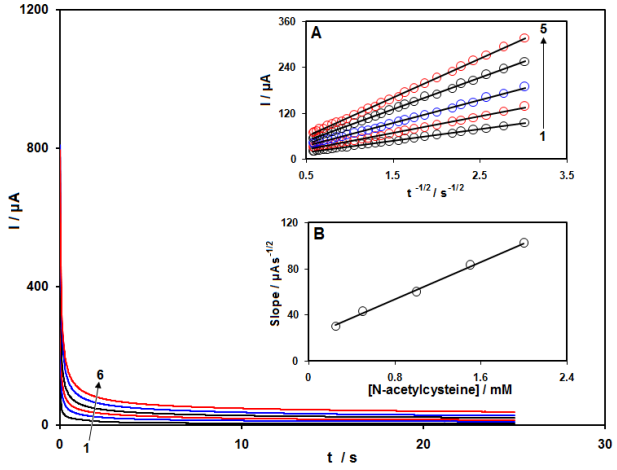

Figure 6. Chronoamperograms obtained at 2CBFCNPE in 0.1 M PBS (pH 7.0) for different concentration of N-acetylcysteine . The numbers 1–6 correspond to 0.0, 0.25, 0.5, 1.0, 1.5 and 2.0 mM of N-acetylcysteine . Insets: (A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 2–6. (B) Plot of the slope of the straight lines against N-acetylcysteine concentration and (C) Dependence of I_c/I_l on $t^{1/2}$ derived from the data of chronoamperograms 1–6.

Chronoamperometric measurements of N-acetylcysteine at 2CBFCNPE were carried out by setting the working electrode potential at 0.75 V for the various concentration of N-acetylcysteine in

0.1 M PBS (pH 7.0) (Fig. 6). For an electroactive material (N-acetylcysteine in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [116]:

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

Where D and C_b are the diffusion coefficient ($cm^2\ s^{-1}$) and the bulk concentration (mol cm^{-3}), respectively. Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of N-acetylcysteine (Fig. 6A). The slopes of the resulting straight lines were then plotted vs. N-acetylcysteine concentration (Fig. 6B). From the resulting slope and Cottrell equation the mean value of the D was found to be 6.8×10^{-5} cm^2/s.

3.4. Calibration plot and limit of detection

The electrocatalytic peak current of N-acetylcysteine oxidation at the surface of the 2CBFCNPE can be used for determination of N-acetylcysteine in solution. Therefore, square wave voltammetry (SWV) experiments were performed using modified electrode in 0.1 M PBS (pH 7.0) containing various concentration of N-acetylcystein.

The plot of peak current vs. N-acetylcysteine concentration consisted of a linear segments with slopes of 0.102 $\mu A / \mu M^{-1}$ in the concentration range of 5.0×10^{-8}-4.0×10^{-4} M. The detection limit (3σ) of N-acetylcysteine was found to be 26.0 nM. This values are comparable with values reported by other research groups for electrocatalytic oxidation of -acetylcysteine at the surface of chemically modified electrodes by other mediators (see Table 1).

Table 1. Comparison of the efficiency of some modified electrodes used in the electrocatalysis of N-acetylcysteine.

Electrode	Modifier	Method	pH	Peak potential Shift (mV)	Scan rate (mV/s)	Limit of detection (M)	Dynamic range(M)	Ref.
Palladized aluminum	Prussian blue film	Amperometry	2.0	-	20	5.4×10^{-7}	2.0×10^{-6}– 4.0×10^{-5}	11
Carbon Nanotube Paste	2,7-BF	Voltammetry	7.0	470	10	5.2×10^{-8}	7.0×10^{-8}– 3.0×10^{-4}	12
Carbon Paste	Catechol	Voltammetry	6.0	400	20	1.0×10^{-5}	3.0×10^{-5} - 2.0×10^{-3}	13
Carbon Paste	Cobalt salophen	Voltammetry	7.0	-	50	5.0×10^{-8}	1.0×10^{-7}– 1.0×10^{-4}	14
Carbon Paste	2CBF	Voltammetry	7.0	135	10	2.6×10^{-8}	5.0×10^{-8}– 4.0×10^{-4}	This Work

3.5. Simultaneous determination of N-acetylcysteine and folic acid

One of the main objectives of the present study was the development of a modified electrode capable of the electrocatalytic oxidation of N-acetylcysteine and separation of the electrochemical responses of N-acetylcysteine and folic acid.

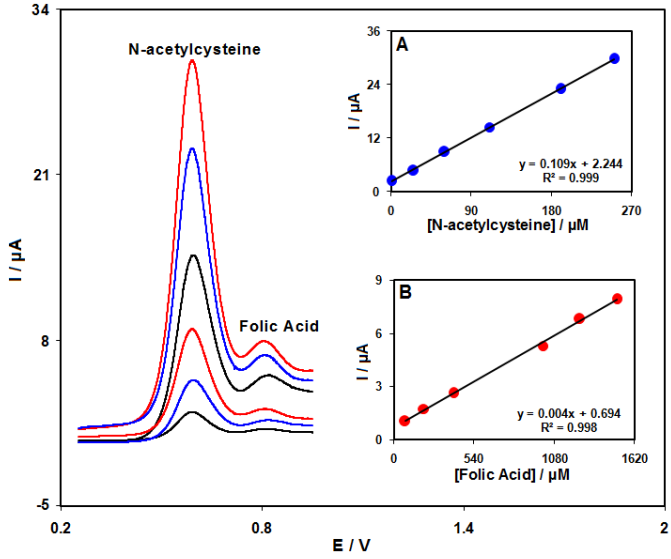

Figure 7. SWVs of 2CBFCNPE in 0.1 M PBS (pH 7.0) containing different concentrations of N-acetylcysteine and folic acid in µM, from inner to outer: 0.1 + 75.0, 25.0 + 200.0, 60.0 + 400.0, 110.0 + 1000.0, 190.0 + 1250.0 and 250.0 + 1500.0 respectively. Insets (A) plots of I_p vs. N-acetylcysteine concentration and (B) plot of I_p vs. folic acid concentrations.

Determination of two compounds was performed by simultaneously changing the concentrations of N-acetylcysteine and folic acid, and recording the SWVs (Fig. 7). The voltammetric results showed well-defined anodic peaks at potentials of 600 and 820 mV, corresponding to the oxidation of N-acetylcysteine and folic acid, respectively, indicating that simultaneous determination of these compounds is feasible at the 2CBFCNPE as shown in Fig. 7.

3.6. Real sample analysis

3.6.1 Determination of N-acetylcysteine and folic acid in pharmaceutical preparations

In order to evaluate the analytical applicability of the proposed method, it was also applied to the determination of N-acetylcysteine and folic acid in their tablets. Based on the repeated square wave voltammetric responses (n=5) of the diluted analytes and the samples that were spiked with specified concentration of N-acetylcysteine and folic acid were made for determination of N-acetylcysteine and folic acid concentrations in the pharmaceutical preparations. The results are listed in table 2.

Table 2. The application of 2CBFCNPE for simultaneous determination of N-acetylcysteine and folic acid in their tablet samples (n=5).

Sample	Spiked (µM)		Found (µM)		Recovery (%)		R.S.D. (%)	
	N-acetylcysteine	Folic Acid	N-acetylcysteine	Folic Acid	N-acetylcysteine	Folic Acid	N-acetylcysteine	Folic Acid

N-acetylcysteine tablet	0	0	25.0	ND	-	-	3.1	-
	5.0	100.0	30.9	98.5	103.0	98.5	2.9	3.4
	7.5	150.0	31.9	150.6	98.1	100.4	2.1	3.1
	10.0	200.0	34.8	195.5	99.4	97.7	2.3	1.9
Folic cid tablet	0	0	ND		-	-	-	2.7
	10.0	30.0	9.9	119.5	99.0	99.6	3.2	1.9
	15.0	50.0	15.5	138.1	103.3	98.6	1.8	2.8
	20.0	70.0	19.6	162.3	98.0	101.4	2.3	3.3

Table 3. Comparison of the total values of N-acetylcysteine and folic acid of some pharmaceutical preparations obtained using 2CBFCNPE with declared values in the lable of the samples (n=5).

Samples	Declared value	Found value	RSD%
N-acetylcysteine tablet (mg per tablet)	600.0	601.0	2.6
Folic Acid tablet (mg per tablet)	1.00	0.99	2.7

The reliability of the proposed modified electrode was also evaluated by comparing the obtained results with those declared in the label of the pharmaceutical preparations (Table 3). The results in table 2 show the relative standard derivations (RSD) and the recovery rates of the spiked samples are acceptable. Also, the data in table 2 indicate that the results obtained by utilizing 2CBFCNPE are in good agreement with those declared in the label of the preparations. Thus, the modified electrode can be efficiently used for individual or simultaneous determination of N-acetylcysteine and folic acid in pharmaceutical preparations.

3.6.2 Determination of N-acetylcysteine and folic acid in human blood serum and urine samples

Table 4. The application of 2CBFCNPE for simultaneous determination of N-acetylcysteine and folic acid in human blood serum and urine samples (n=5).

Sample	Spiked (μM)		Found (μM)		Recovery (%)		R.S.D. (%)	
	N-acetylcysteine	Folic Acid	N-acetylcysteine	Folic Acid	N-acetylcysteine	Folic Acid	N-acetylcysteine	Folic Acid

Human blood serum								
	10.0	125.0	9.9	126.5	99.0	101.2	2.1	3.2
	20.0	150.0	20.6	148.1	103.3	98.7	3.4	1.7
	30.0	175.0	30.4	180.1	101.3	102.9	1.9	1.8
Urine								
	12.5	130.0	12.9	129.2	103.2	99.4	3.5	2.8
	22.5	150.0	22.1	153.5	98.2	102.3	3.2	2.1
	32.5	170.0	32.9	167.2	101.2	98.3	1.8	3.3

In order to evaluate the analytical applicability of the proposed method, it was additionally applied for the determination of N-acetylcysteine and folic acid in human blood serum and urine samples. The results for determination of the two species in real samples are given in table 4. Satisfactory recovery of the experimental results was found for N-acetylcysteine and folic acid. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

4. CONCLUSION

In this paper a carbon paste electrode modified with carbon nanotubes and a ferrocene derivative was fabricated and used for the investigation of the electrochemical behaviors of N-acetylcysteine. The modified electrode successfully resolves the overlapped voltammetric peaks of N-acetylcysteine and folic acid, so that the modified electrode displays high selectivity in the SWV measurement of N-acetylcysteine and folic acid in their mixture solutions. Finally, the modified electrode was also examined for the determination of N-acetylcysteine and folic acid in real samples.

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