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ORIGINAL PAPER

Catechol as an electrochemical indicator for voltammetric determination of *N*-acetyl-L-cysteine in aqueous media at the surface of carbon paste electrode

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Abstract The utilization of catechol as an electrochemical indicator in the presence of *N*-Acetyl-L-cysteine (NAC) at a carbon paste electrode (CPE) has been investigated in aqueous media using cyclic voltammetry (CV), differential pulse voltammetry (DPV), and double-step potential chronoamperometry methods. The results show that NAC participates in Michael type addition reaction with electrogenerated quinone from electrooxidation of catechol at CPE to form the corresponding thioquinone derivative. The reoxidation of the adduct leads to increase in the oxidative current which is proportional to the concentration of NAC. Therefore, in the optimum condition (pH = 6.00) by CV, the oxidation of NAC occurs at a potential about 400 mV versus Ag|AgCl|KCl_{sat} in the presence of catechol at the surface of CPE. The practical utility of the method showed that low detection limit and high sensitivity for voltammetric determination of NAC. The proposed method is useful for the routine analysis of NAC in pharmaceutical formulations.

Keywords Catechol · *N*-Acetyl-L-cysteine · Michael addition · Carbon paste electrode · Differential pulse voltammetry · Cyclic voltammetry

1 Introduction

N-Acetyl-L-cysteine (NAC) is an acetylated derivative of the L-cysteine, as an amino acid. NAC is commonly used as pharmaceutics. It has first been managed as a mucolytic agent reducing the viscosity of pulmonary secretions in chronic respiratory illness as well as an antidote for hepatotoxicity due to acetaminophen overdose. It has also been efficient in the treatment of Sjogren's syndrome, smoking cessation, influenza, hepatitis C, and myoclonus epilepsy [1]. Another less expanded chemical property of NAC concerns its antioxidant activity. As a matter of fact, NAC constitutes an excellent source of sulphydryl groups (SH), which is converted in organism into metabolites able to stimulate the synthesis of reduced glutathione (GSH). It also acts as a scavenger of free radicals and reactive oxygen species (ROS), consuming directly super oxide anion [2], or hypochlorous acid [3]. This reactivity makes NAC as a powerful antioxidant and as a potentially therapeutic agent in the treatment of cancer [4], cardiovascular and respiratory diseases [5], human immunodeficiency virus (HIV) infection [6, 7], acetaminophen toxicity [8], neurodegenerative disorder [9], and other diseases characterized by free radicals production and oxidative damage.

N-acetyl-L-cysteine has been determined by numerous chemical and instrumental techniques, such as chromatography [10–12], spectrophotometry [13–16], and fluorimetry [17]. Compared to these options, electroanalysis has the advantages of simplicity and high sensitivity. The determination of thiols such as NAC provides a substantial challenge to the electroanalytical community. The direct oxidation of the thiols functionality is generally hampered by poor voltammetric behavior at solid electrodes [18, 19], while thiol oxidation is very complex at mercury, gold, and platinum electrodes [20]. It is desirable to have an unmodified electrode to oxidize NAC at neutral pH with high efficiency, also the requirement of electrode modification or electrochemical pre-treatment steps should be avoided to use the electrode frequent

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repeatedly [19]. In order to propose a specific electrochemical detection, different chemically modified carbon electrodes were developed [18, 21] as well as the use of a mediator [19].

Catechol derivatives have been used as electron transfer mediators in electrochemical processes due to their high electron transfer efficiency, excellent redox reversibility, and low cost [22–24]. These compounds as mediators were immobilized on the electrodes surface by various methods, such as adsorption [25], mixing into carbon paste electrode [26], or simply added to the test solution [27]. On the other hand, the oxidation of catechol provides a reactive species which sulphydryl thiols will readily bind through 1,4-addition reaction [27]. In this study, the activity of catechol as an electrochemical indicator for electrooxidation of NAC was investigated at the surface of a carbon paste electrode (CPE) in buffered aqueous media using various electrochemical methods. Here, a simple and sensitive method was reported for voltammetric determination of NAC based on its reaction with catechol corresponding quinone.

2 Experimental

2.1 Reagents and materials

All solutions were prepared using twice distilled water. Buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 5.00–8.00. High viscosity paraffin (density = 0.88 g cm^{-3}) from Fluka was used as the pasting liquid for the CPE (surface area = 0.09 cm^2). Graphite powder (particle diameter = 0.1 mm) from Merck was used as the working electrode (WE) substrate. Potassium chloride from Fluka was used as a supporting electrolyte for all experiments. Catechol and NAC were from Fluka, and were used as received. A 1.5 mM of NAC solution was prepared daily. All other reagents were analytical grade.

2.2 Instrumentation

The electrochemical measurements were carried out using a Potentiostat/Galvanostat (SAMA 500; Electroanalysis System, Iran) coupled with a Pentium IV personal computer. The experiments were performed in a three compartment cell. The carbon paste, platinum disk, and $Ag|AgCl|KCl_{sat}$ (Azar electrode) were used as the working electrode, auxiliary electrode, and reference electrode, respectively. Also, a pH-meter (Ion Analyzer 250, Corning) was used to read the pH of the buffered solution.

3 Results and discussions

3.1 Electrochemical oxidation of catechol in the absence and in the presence of NAC

Cyclic voltammetry (CV) of 1.0 mM catechol in 0.1 M phosphate buffer solution (pH 6.00) containing 0.1 M KCl as the supporting electrolyte at the surface of the CPE with scan rate of potential (υ) 20 mV s^{-1}, shows one anodic peak and a corresponding cathodic peak which corresponds to transformation of catechol (C) to *o*-benzoquinone (B) and vice versa within a quasi-reversible process (Fig. 1b). The cyclic voltammogram of bare CPE in pure supporting electrolyte does not show any anodic or cathodic peaks (Fig. 1a). The electrode capability for the generation of a reproducible surface was examined using CV data from five separately prepared CPEs obtained in optimum solution pH in the presence of catechol (Table 1). The calculated RSD for various parameters was accepted as the criteria for a satisfactory surface reproducibility (about 1–4%). The results show that well-defined and reproducible anodic and cathodic peaks related to catechol redox system can be used as an electrochemical indicator for determination of some important biological compounds with slow electron transfer. Also, the long term stability of the CPE was tested over a 2-week period. When cyclic voltammograms were recorded after the electrode was stored in atmosphere at room temperature (after repetitive scanning), the peak potential for oxidation of catechol was unchanged, and the oxidation current showed that <2.4% decrease relative to the initial response.

In addition, the effect of potential scan rates (10–600 mV s^{-1}) on the electrochemical properties of the catechol in the absence of NAC was studied at the surface of

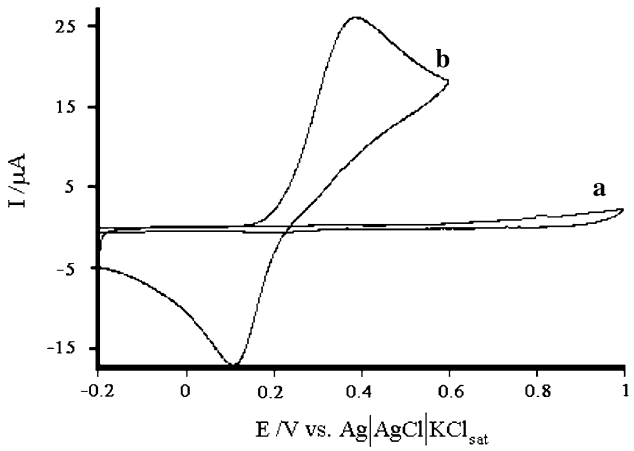


Fig. 1 Cyclic voltammograms of CPE in 0.1 M phosphate buffer solution (pH 6.00) containing KCl 0.1 M as supporting electrolyte in the (*a*) absence and (*b*) presence of 1.0 mM catechol at a scan rate 20 mV s^{-1} at the surface of CPE

Table 1 Cyclic voltammetric data for CPEs in the presence of catechol in phosphate buffer solution (pH = 6)

E_{pa} (V)[a]	E_{pc} (V)[a]	$E_{1/2}$(V)[a]	ΔE_p(V)[a]	I_{pa}(μA)	I_{pc}(μA)
0.387 (1.4)[b]	0.107 (2.2)[b]	0.247 (1.7)[b]	0.280 (3.6)[b]	26.06 (2.3)[b]	−17.2 (2.1) [b]

[a] Versus Ag|AgCl|KCl_{sat} as reference electrode

[b] The values in *parentheses* indicated the calculated RSD

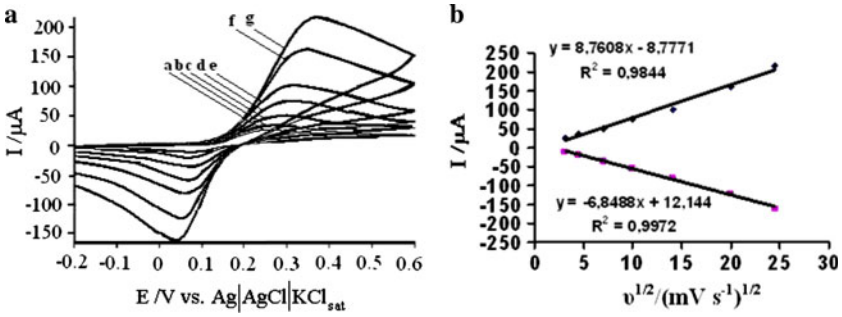


Fig. 2 **a** Cyclic voltammograms of 1.0 mM catechol in 0.1 M phosphate buffer solution (pH 7.00) at various scan rates: (*a*) 10, (*b*) 20, (*c*) 50, (*d*) 100, (*e*) 200, (*f*) 400, and (*g*) 600 mV s^{-1} at CPE. **b** Plot of anodic and cathodic peak currents versus $\upsilon^{1/2}$ from cyclic voltammograms of (**a**)

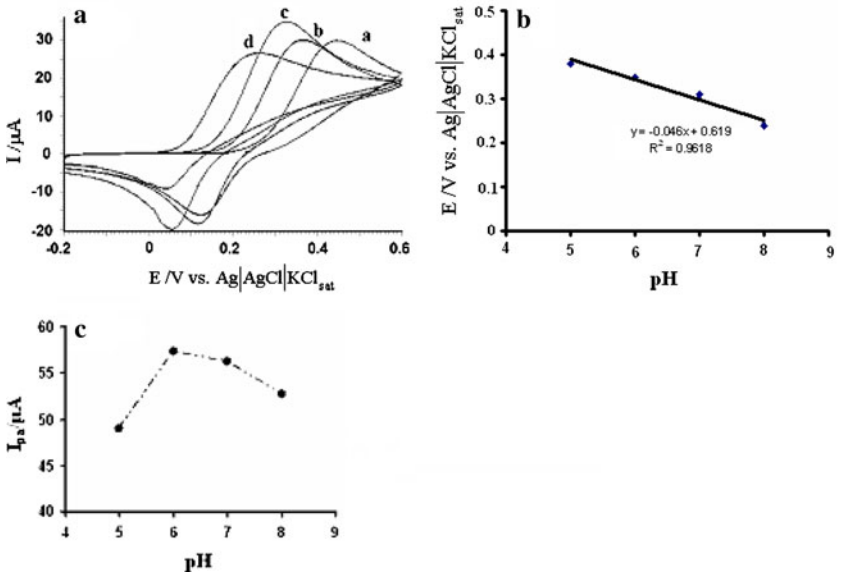


Fig. 3 **a** Cyclic voltammograms of 1.0 mM catechol in 0.1 M phosphate buffer solution at the surface of CPE in the various pHs: (*a*) 5.0, (*b*) 6.0, (*c*) 7.0, and (*d*) 8.0 at scan rate 20 mV s^{-1} in the absence of NAC. **b** The variation of catechol oxidation peak potentials versus pH values. **c** The variation of electrooxidation peak currents for 1.0 mM catechol in the presence of 1.5 mM NAC in 0.1 M phosphate buffer solution at the surface of CPE versus pH values

CPE (Fig. 2a). The plots of the anodic and cathodic peak currents were linearly depended on the square root of the scan rate ($\upsilon^{1/2}$) at all scan rates (Fig. 2b). This behavior indicates that the nature of this redox process is diffusion controlled.

It is well-known that the electrochemical behavior of catechol and NAC are dependent on the pH value of the aqueous solution [28, 29]. Therefore, pH optimization of the solution seems to be necessary to obtain the electrooxidation of NAC in the presence of catechol at the surface of CPE. Thus, we studied the electrochemical behavior of 1.0 mM catechol in 0.1 M phosphate-buffered solution at various pH values (5.00–8.00) in the absence and in the presence of NAC at the surface of CPE. Figure 3a shows pH effect on the electrooxidation of catechol in the absence of NAC at the surface of CPE. As seen, the anodic peak potential of catechol was shifted to a less positive potential with increasing of pH at the surface of CPE (Fig. 3b). Figure 3c shows the variation of oxidation peak currents of catechol electrooxidation in the presence of NAC versus pH values. As the solution pH was lowered, the thiol functionality was increasingly protonated and hence the nucleophilicity character of the thiol moiety diminished. Therefore, pH 6.00 was chosen as the optimum pH for electrooxidation of NAC in the presence of catechol at CPE due to the high oxidation current obtained in this pH.

A comparison of the cyclic voltammograms of catechol in the 0.1 M phosphate buffered solution (pH 6.00) in the absence (Fig. 4a) and in the presence of 1.0 mM of NAC (Fig. 4b) with the cyclic voltammogram of 1.5 mM of NAC

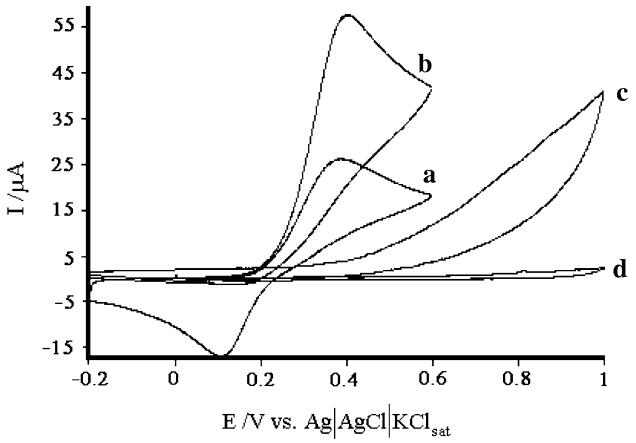


Fig. 4 (*a*) Cyclic voltammogram of CPE in the 1.0 mM catechol in 0.1 M phosphate buffered solution (pH 6.00) at scan rate 20 mV s^{-1}, *b*) as (*a*) in the presence of 1.5 mM NAC, *c*) Cyclic voltammogram of 1.5 mM of NAC in the 0.1 M phosphate buffered solution (pH 6.00) at the surface of CPE, *d*) as (*c*) in the absence of NAC at scan rate 20 mV s^{-1}

(Fig. 4c) at the CPE in the same pH, demonstrated that the electrooxidation of NAC can be catalyzed by catechol as a homogenous electrochemical indicator. Also, Fig. 4c shows that NAC oxidation occurs irreversibly with a broad peak at potential of nearly 1.0 V versus Ag|AgCl|KCl_{sat} at the CPE, whereas the cyclic voltammogram of supporting electrolyte at the CPE did not appear any anodic or cathodic peaks (Fig. 4d). Therefore, the oxidation of NAC occurs at a potential about 400 mV versus Ag|AgCl|KCl_{sat} in the presence of catechol at the surface of CPE. This value is comparable with values reported by other research groups for electrooxidation of NAC using the other modified electrodes (see Table 2) [19, 21].

The electrooxidation product of catechol subsequently undergoes a chemical addition–reduction process (Michael type addition) with NAC as a nucleophile agent to produce the reduced adduct compound. In the presence of the thiol moiety in the structure of the product, due to its electron donating property, facilitates the electrooxidation of the additional product and decreases its anodic potential toward less positive potential, through the route as proposed in Scheme 1 (ECE type mechanism). As discussed above, this also represented that catechol can be electrochemically converted to *o*-benzoquinone and will readily undergo reaction with thiols possessing sulphydryl group. Hence, the increase in the oxidation peak height is attributed to the oxidation of catechol–NAC adducts that arises through the electrochemically initiated reaction previously shown in Scheme 1. In fact, once *o*-benzoquinone is formed, it could react with a variety of nucleophile reagents, as those possessing sulphydryl (–SH) groups [30]. Moreover, compounds with sulphydryl groups appear to be far more reactive toward *o*-quinones, than amines [31]. For this reason, in the case of catechol oxidation in the presence of NAC, only the thioether (*S*-adduct) is formed, but no N-adduct are observed [32].

The effect of potential scan rates (10–600 mV s^{-1}) on the electrochemical oxidation of NAC in the presence of catechol was also studied using cyclic voltammetry (not shown). The result showed that the oxidation current of NAC increased linearly with the square root of the potential scan rates, which demonstrates a diffusion-controlled electrochemical process.

3.2 Chronoamperometry studies

Double potential step chronoamperometry as well as other electrochemical methods was employed for the investigation of electrochemical processes. Therefore, we studied the electrochemical behavior of 1.0 mM catechol in an aqueous buffered solution (pH 6.00) in the presence of NAC at the CPE using chronoamperometry method. Figure 5 shows current–time curves of 1.0 mM catechol in 0.1 M phosphate buffered solution (pH 6.00) by setting the working electrode potential at 0.55 V (first potential step) and 0.06 V (second potential step) versus Ag|AgCl|KCl_{sat} in the presence of various concentrations of NAC. There is not any net cathodic current corresponding to the reduction of *o*-benzoquinone to catechol in the presence of NAC, when the second potential step is employed. The oxidation current value increases with increasing NAC concentration at first potential step.

The current corresponding to the electrochemical reaction of an electroactive compound (under diffusion controlled) is described by Cottrell equation [33]:

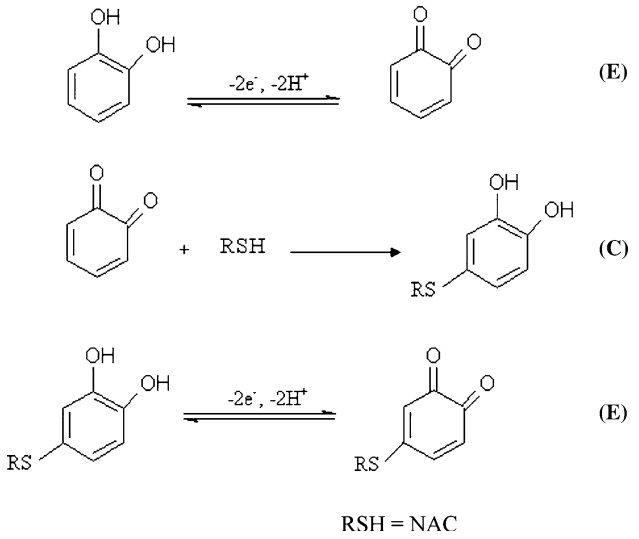
$$I = \mathrm{nFA}D_{\mathrm{app}}^{1/2}\mathrm{C_O}\pi^{-1/2}t^{-1/2} \quad (1)$$

where D_{app} and C_O are the apparent diffusion coefficient (cm^2 s^{-1}) and the bulk concentration (mol cm^{-3}),

Table 2 Comparison of the efficiency of some modified electrodes in the electrooxidation of NAC

Electrode	Modifier	pH	Electrooxidation peak potential in the presence of modifier (mV)	References
GCE	Acetylferrocene	6.00	500	[19]
CPE	Copper(II) hexacyanoferrate(III)	6.00	800	[21]
CPE	[a]	6.00	400	This study

[a] Without modifier (using catechol as electrochemical indicator based on Michael addition reaction, ECE type mechanism)



Scheme 1 Proposed mechanism for electrooxidation of catechol in the presence of NAC at the surface of CPE

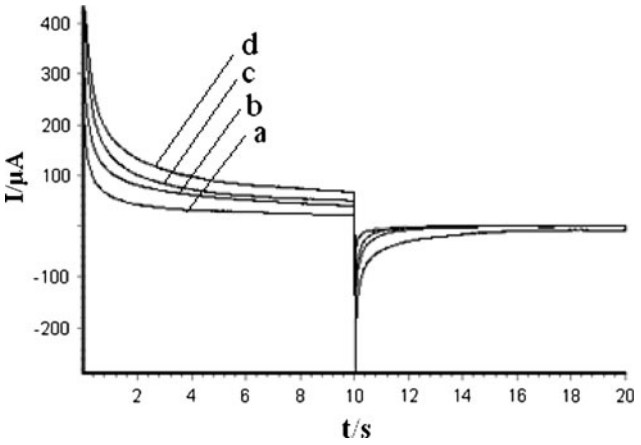


Fig. 5 Double step potential chronoamperograms of 1.0 mM catechol in the (*a*) absence and presence of (*b*) 0.2, (*c*) 0.4, and (*d*) 1.0 mM of NAC in 0.1 M phosphate buffer solution (pH 6.00) at the surface of CPE. First and second potential steps were 0.55 and 0.06 V versus Ag|AgCl|KCl_{sat}

respectively. The plot of I versus $t^{-1/2}$ was linear (not shown), and from its slope, the mean values of the D_{app} of NAC was calculated 3.4×10^{-5} cm^2 s^{-1}.

As catechol can act as a homogeneous electrochemical indicator for electrooxidation of NAC, the rate constant for the chemical reaction (k_h) can be evaluated by chronoamperometry according to the method described in [34]:

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

where I_C is the anodic current in the presence of NAC, I_L is the diffusion-limited current in the absence of NAC, and C_O is the initial concentration of NAC in bulk solution. From the slop of I_C/I_L versus $t^{1/2}$ plot, the value of rate constant for chemical reaction between NAC and *o*-benzoquinone produced from electrooxidation of catechol, k_h can be simply calculated for a given concentration of substrate. The calculated value of k_h for NAC is 2.9×10^3 M^{-1} s^{-1} using the slop of I_C/I_L toward $t^{1/2}$ plot. The value of k_h explains as well as the sharp feature of peak observed for electrochemical oxidation of NAC in the presence of catechol at the surface of CPE.

3.2.1 Analytical measurements

The electrooxidation of NAC in the presence of catechol was used for voltammetric determination of this compound. Thus, we studied the electrochemical properties of 1.0 mM of catechol in the absence and in the presence of various concentrations of NAC at the CPE using CV and DPV (Figs. 6 and 7). Under the optimum conditions, pH 6.0, and scan rate of 20 mV s^{-1}, the anodic peak currents were linear versus NAC concentrations from 8.0×10^{-5} M to 1.0×10^{-3} M (with the correlation coefficient of 0.9952) and 3.0×10^{-5} M to 2.0×10^{-3} M (with the correlation coefficient of 0.993) in the CV and DPV methods, respectively (Figs. 6b, 7b). The detection limits (2δ) were 6.0×10^{-5} M and 1.0×10^{-5} M using CV and

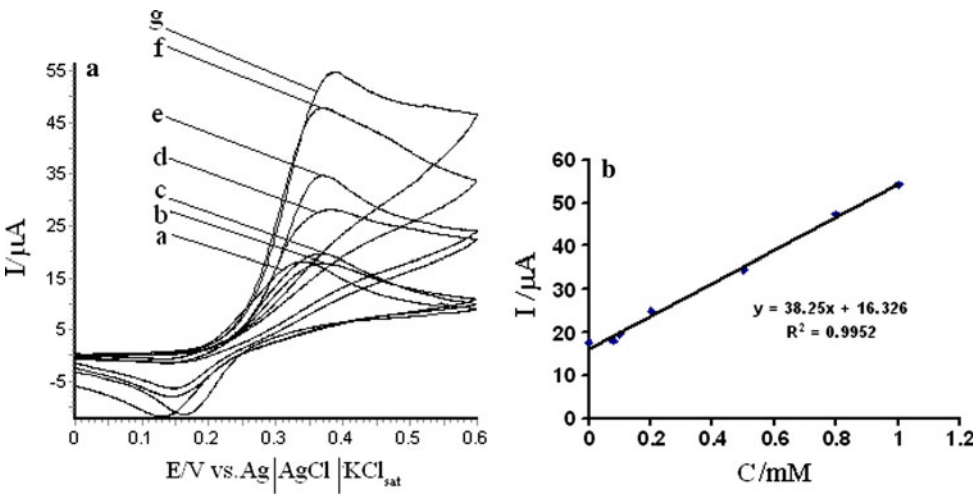


Fig. 6 **a** Cyclic voltammograms of 1.0 mM catechol in the presence of NAC at different concentrations: (*a*) 0.00, (*b*) 0.08, (*c*) 0.10, (*d*) 0.20, (*e*) 0.50, (*f*) 0.80, and (*g*) 1.00 mM in 0.1 M phosphate buffer solution (pH 6.00) at a scan rate of 20 mV s^{-1} at CPE. **b** Plot of peak current versus NAC concentrations

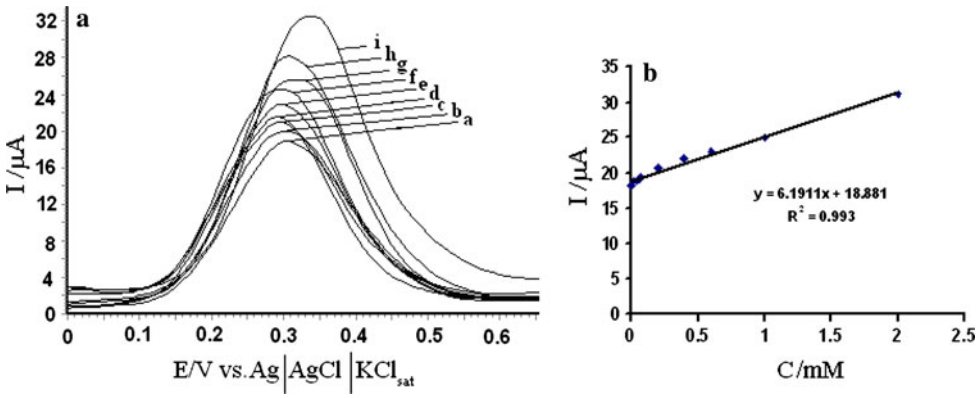


Fig. 7 **a** Differential pulse voltammograms of 1.0 mM catechol in the presence of (*a*) 0.00, (*b*) 0.03, (*c*) 0.04, (*d*) 0.07, (*e*) 0.20, (*f*) 0.40, and (*g*) 0.60, (*h*) 1.00, and (*i*) 2.00 mM of NAC in 0.1 M phosphate buffer solution (pH 6.00) at CPE. **b** Plot of peak current versus NAC concentrations

Table 3 Results obtained for *N*-acetyl-L-cysteine determination in pharmaceutical formulation

Sample	Label value	Proposed Voltammetric method[a]	E_r%
A*	200	203 ± 1.3	+1.5
B**	15	14.2 ± 0.6	−5.3

E_r relative error

[a] Mean value of three repeat determinations

* mg *N*-acetylcysteine/tablet

** mg *N*-acetylcysteine/mL

DPV. Thus, this proposed method can readily be applied for voltammetric determination of NAC.

3.3 Determination of NAC in pharmaceutical preparations

The proposed method was applied to voltammetric determination of NAC in pharmaceutical formulations. Table 3 presents the results obtained using an official procedure [35], the proposed voltammetric method and label value. The statistical calculations for the assay results showed that good precision of the method. According to the *t*-test, there are no significant differences between the results obtained by either procedure at the 95% confidence level, indicating that the proposed method can be used for voltammetric determination of NAC in such samples (Table 3).

4 Conclusions

An electrochemical study step by step was presented on electrooxidation of catechol as an electrochemical indicator for determination of NAC at the surface of CPE in buffered aqueous medium. The reaction was applied successfully for the selective voltammetric determination of NAC in aqueous media; due to an increase in the anodic current attributed to the reoxidation of the thiol addition product also it could be exploited as means of quantifying the concentration of the NAC. This indicator showed to be promising for NAC detection with many desirable properties including high sensitivity, low detection limit, decrease in over-voltage for the electrochemical oxidation of this compound, many reproducible responses, and fast response time. Nevertheless, the electrode was reliable, simple, and rapid to prepare, low cost, precise, and did not require extensive preliminary sample treatment.

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