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Quantification of N-acetylcysteine in pharmaceuticals using cobalt phthalocyanine modified graphite electrodes

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ABSTRACT

Flow injection analysis (FIA) with amperometric detection was employed for the quantification of N-acetylcysteine (NAC) in pharmaceutical formulations, utilizing an ordinary pyrolytic graphite (OPG) electrode modified with cobalt phthalocyanine (CoPc). Cyclic voltammetry was used in preliminary studies to establish the best conditions for NAC analysis. In FIA-amperometric experiments the OPG–CoPc electrode exhibited sharp and reproducible current peaks over a wide linear working range (5.0×10^{-5}–1.0×10^{-3} mol L^{-1}) in 0.1 mol L^{-1} NaOH solution. High sensitivity (130 mA mol^{-1} cm^2) and a low detection limit (9.0×10^{-7} mol L^{-1}) were achieved using the sensor. The repeatability (R.S.D.%) for 13 successive flow injections of a solution containing 5.0×10^{-4} mol L^{-1} NAC was 1.1%. The new procedure was applied in analyses of commercial pharmaceutical products and the results were in excellent agreement with those obtained using the official titrimetric method. The proposed amperometric method is highly suitable for quality control analyses of NAC in pharmaceuticals since it is rapid, precise and requires much less work than the recommended titrimetric method.

1. Introduction

The utilization of chemically modified electrodes has received increasing interest for many types of applications. The main reason for this preference is settled on the possibility of the user still free to deliberately control and manipulate the properties of the sensor surface. Many areas of analysis have benefited from the unique properties of these new sensors, and species that were previously not easily quantifiable by voltammetric methods can now be studied. The use of modified electrodes for pharmaceutical analyses is a growing field of research [1,2], with the combination of voltammetry and flow injection analysis markedly improving sensitivity and speed of analysis [3]. Transition metal phthalocyanine complexes are widely recognized for their excellent electrocatalytic properties and are used in the detection of many important analytes [4–6]. The electrochemical applications of Schiff base complexes of various metals include the development of novel electrocatalysis-based sensors and their use as ion-carriers in ion-selective electrodes [7].

N-acetylcysteine (NAC) is a pharmaceutical drug used primarily as a mucolytic agent since it is able to cleave disulfide bonds, 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 [8]. In addition, this drug has an antioxidant action, and some authors have even suggested that NAC can aid in the complexation and elimination of heavy metals, as well as preventing some types of cancer [9].

There are many methods described in the literature for the quantification of NAC, including titrimetry [10], spectrophotometry [11,12], chemiluminescence [13], fluorimetry [14], turbidimetry [15], amperometry [16] and cathodic stripping voltammetry [17]. Many of these methods have been associated with chromatographic methods [18,19].

In this paper we describe a very simple and effective system which employs a pyrolytic graphite electrode modified with cobalt phthalocyanine (CoPc) and a FIA-amperometric detection system,

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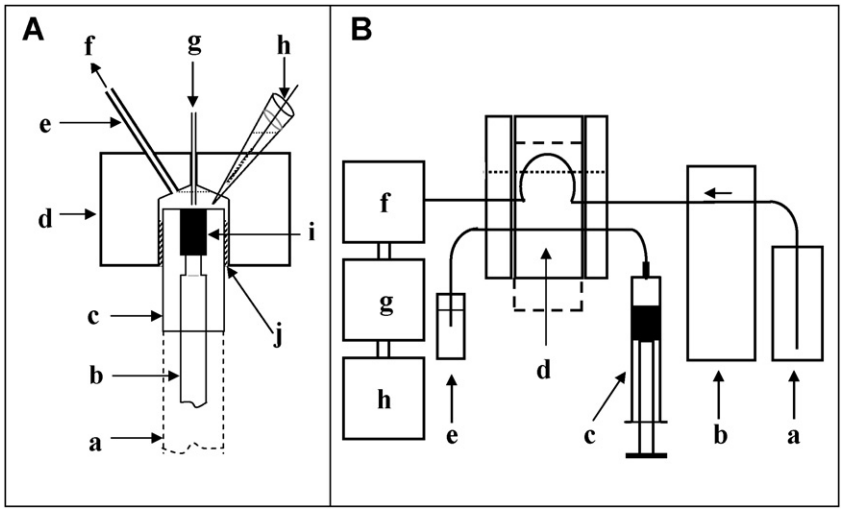

Fig. 1. (A) Details of the flow cell employed in this study: (a) electrode body (Teflon); (b) metallic rod; (c) epoxy resin body; (d) Plexiglas cell body; (e) steel tube (auxiliary electrode); (f) outflow of solution; (g) inflow of solutions; (h) reference electrode; (i) OPG rod; (j) Teflon tape, B) flow injection manifold used for NAC determinations: (a) carrier reservoir (b) peristaltic pump; (c) syringe for sample aspiration; (d) rotary injector; (e) sample reservoir (f) flow cell (detailed on the left side); (g) potentiostat; (h) computer.

for NAC quantification in pharmaceutical products. The characteristics of the proposed system include simplicity, precision and rapid responses, as described in the following sections.

2. Experimental

2.1. Chemicals and solutions

All chemicals were of analytical grade and used without additional purification. Cobalt (II) phthalocyanine (CoPc) and NaOH were obtained from Merck. N-acetylcysteine (NAC) was purchased from Sigma–Aldrich. For both voltammetric and flow analysis experiments, 0.1 mol L^{-1} NaOH solution was used as the supporting electrolyte. Stock solutions of NAC were freshly prepared in 0.1 mol L^{-1} NaOH. All solutions were prepared using deionized water (Millipore Milli-Q Plus, Millipore) with a resistivity not less than 18 MΩ cm.

2.2. Electrochemical measurements

Voltammetric measurements were performed using a BioAnalytical Systems (BAS) Model CV-50W electrochemical workstation. Amperometric measurements were performed using a μ-Autolab Type III potentiostat (Eco Chemie) connected to a microcomputer and controlled by Autolab GPES v. 4.8 software. A conventional three-electrode cell, consisting of a pyrolytic carbon electrode, a platinum wire and a calomel electrode was employed for the voltammetric measurements. The working electrode was constructed using a pyrolytic graphite rod (length 8 mm, diameter 5 mm Union Carbide Co., Cleveland, Ohio, USA), embedded in epoxy resin. For the flow injection analysis experiments, a simple flow cell was built in our laboratory (Fig. 1A) in such a way as to fit the same pyrolytic carbon electrode previously employed in the voltammetric experiments. A miniaturized Ag/AgCl/KCl_{sat} electrode was constructed in the laboratory for use in the same flow cell [20].

2.3. Electrode preparation

Prior to modification, the basal plane of the ordinary pyrolytic graphite electrode (OPG) was polished with 2000 grit emery paper, washed with deionized water, than transferred to a beaker containing deionized water and sonicated for 2 min. After cleaning, the electrode was immersed for 20 min in a 1×10^{-3} mol L^{-1} CoPc dimethylsulfoxide solution. Finally, it was washed with purified water, dried and it was then ready to use.

2.4. Flow analysis

The flow measurements employed a single channel manifold (Fig. 1B) connected to the homemade wall-jet flow cell. A peristaltic pump (Model 78016-30, Ismatec S/A) was used to propel the carrier solutions. A manually operated rotary valve was used to introduce the standards and samples into the flow stream. The carrier solution was 0.1 mol L^{-1} NaOH. This electrolyte was selected based on the voltammetric experiments undertaken previously. Polyethylene tubes (0.8 mm i.d.) were used to connect all parts of the system. All experiments were carried out at room temperature (23 ± 3 °C).

2.5. Analysis of the pharmaceutical samples

The proposed procedure was applied to the analysis of four commercial pharmaceutical samples, two in solid form (sachets containing 100 and 600 mg of N-acetylcysteine) and two in liquid form (ampoules nominally containing 300 mg of N-acetylcysteine), acquired from different suppliers. No special pre-treatment was required for any of these samples prior to analysis. A known amount of each sample was transferred to a volumetric flask (50 or 250 mL), and the volume made up with 0.1 mol L^{-1} NaOH. Further dilutions were used to obtain the final concentrations of N-acetylcysteine used in the analyses.

Quantification of the NAC contents of the pharmaceutical samples was based on an analytical curve constructed from a series of standard solutions. Each solution (standards and samples) was injected at least three times in sequence, and the results were expressed as the mean value of the resulting signals. The percentage content of N-acetylcysteine in each sample was determined by interpolation of the signals on the analytical curve. The results obtained with the modified electrode were compared with those obtained using the iodometric method, recommended in the Brazilian Pharmacopoeia [21].

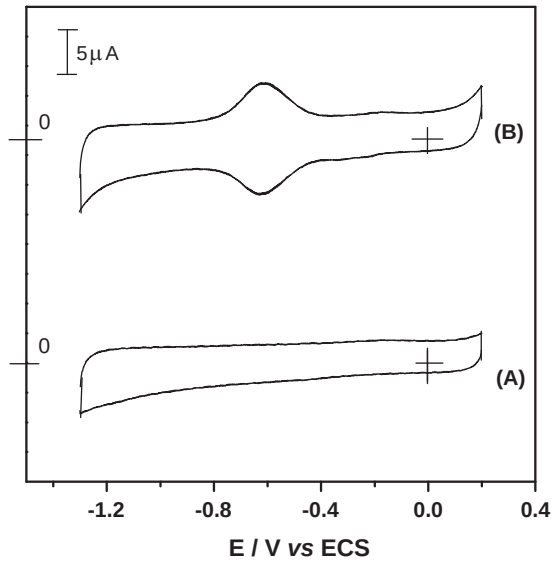

Fig. 2. Cyclic voltammograms of the OPG electrode in N_2 saturated 0.1 mol L^{-1} NaOH (A) without and (B) with adsorbed CoPc. Scan rate, 0.10 V s^{-1}.

3. Results and discussion

3.1. Electrochemical behavior of the CoPc complexes

Fig. 2 illustrates the cyclic voltammograms obtained using the basal plane pyrolytic graphite electrode in the de-aerated 0.1 mol L^{-1} NaOH solution (A) without and (B) with CoPc adsorbed on its surface. The voltammogram recorded with the unmodified electrode shows a low background current in a wide potential window, in agreement with results reported previously [22]. For the electrode modified with CoPc, two well-defined peaks appeared at −0.60 V. According to previous studies, these peaks can be attributed to the presence of the metallic center and are associated with the redox process involving Co(II)Pc/Co(I)Pc [5,23,24]. These peaks became more intense and visible during the second and subsequent scans, probably due to optimization of the phthalocyanine film (data not shown).

Studies involving different scan rates demonstrated the excellent behavior of the phthalocyanine. A linear relationship between the potential scan rate and the current peak was observed between 50 and 500 mV s^{-1} (Fig. 3). Based on the charge under the voltammetric peaks, and assuming that one electron was involved in the redox process, a surface coverage of approximately 3.5×10^{-10} mol cm^{-2} was estimated. These values are close to those obtained by other authors [5,25].

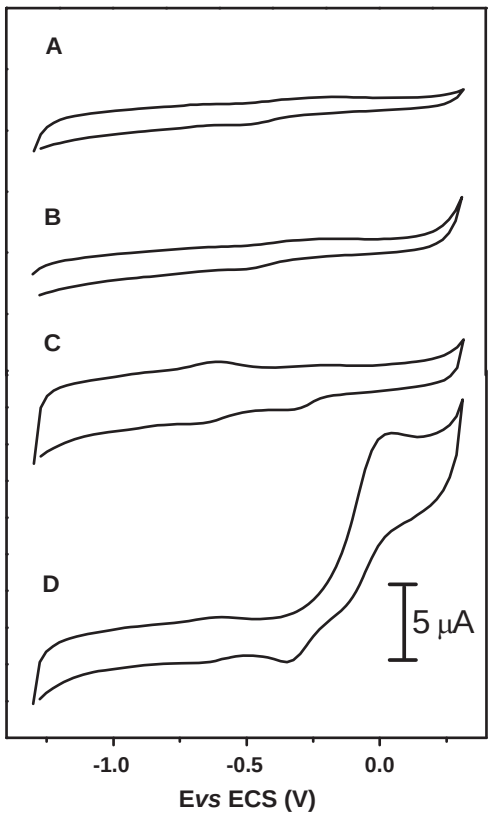

Fig. 4. Cyclic voltammograms for the bare OPG electrode (A and B) and the electrode modified with CoPc (C and D), in the absence of NAC (A and C) or in the presence of 5.0×10^{-4} mol L^{-1} NAC (B and D) in N_2-saturated 0.1 mol L^{-1} NaOH solution. Scan rate: 100 mV s^{-1}.

3.2. Electrocatalytic oxidation of NAC

The need for highly positive potentials to oxidize thiol-containing compounds, including N-acetylcysteine and cysteine, on conventional electrodes has been reported in several studies [6,8,26–28]. The experiments shown in Fig. 4 were performed to demonstrate the electrocatalytic activity of CoPc in the oxidation process involving NAC. Cyclic voltammetric experiments performed with the unmodified electrode (A and B) generated flat

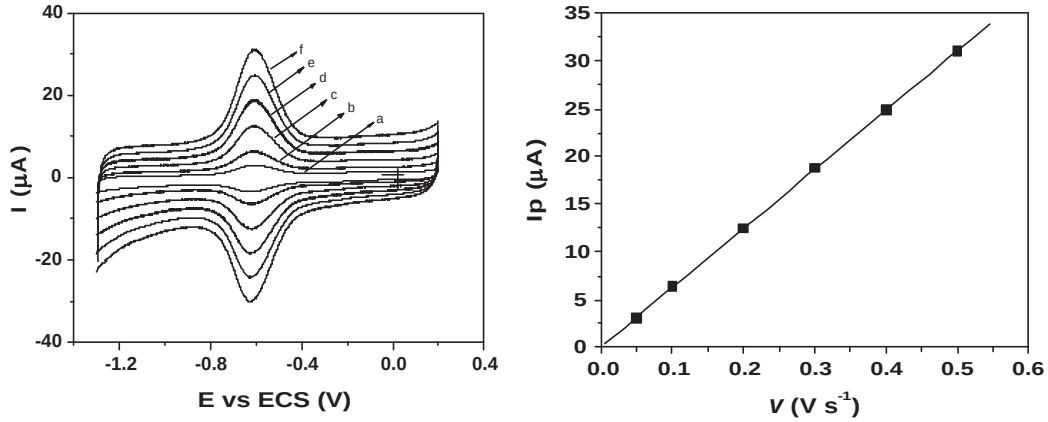

Fig. 3. (A) Cyclic voltammograms for the OPG electrode modified with CoPc in N_2 saturated 0.1 mol L^{-1} NaOH using different scan rates ν (V s^{-1}): (a) 0.05, (b) 0.1, (c) 0.2, (d) 0.3, (e) 0.4, (f) 0.5. (B) Relationship of the peak current (i) and the scan rate (ν).

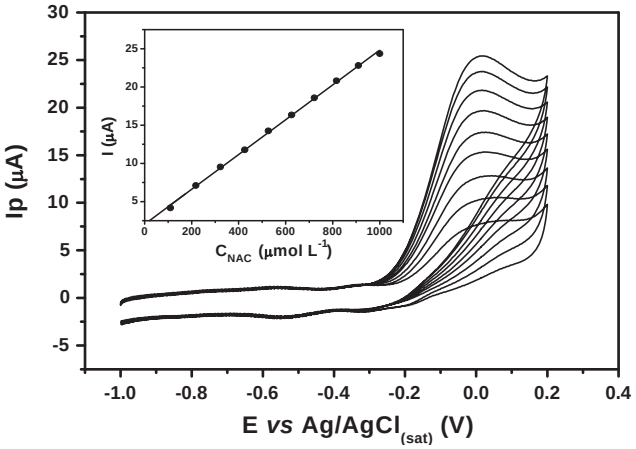

Fig. 5. Cyclic voltammograms for the OPG/CoPc electrode in the presence of 1×10^{-4} mol L^{-1} to 1×10^{-3} mol L^{-1} NAC in 0.1 mol L^{-1} NaOH, scan rate: 100 mV s^{-1}.

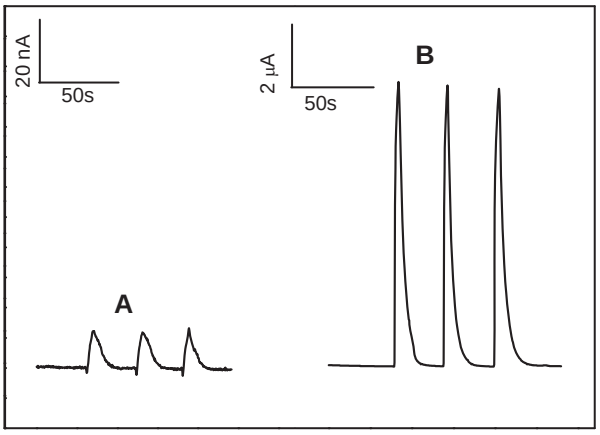

Fig. 6. Flow injection analysis of NAC utilizing a bare OPG (A) and a CoPc modified OPG electrode (B). Each peak corresponds to injections of 150 μL of 5×10^{-4} mol L^{-1} NAC. Flow rate: 2 mL min^{-1}; potential 0.0 V (vs. $Ag/AgCl/KCl_{sat}$).

CVs in a broad potential window (from −1.3 V to 0.3 V). In the presence of 5×10^{-4} mol L^{-1} NAC (B), a small signal appeared at potentials greater than 0.2 V. A different behavior was observed in the experiments using electrodes modified with phthalocyanine. In the absence of NAC, the characteristic pair of peaks from the CoPc film appeared (C), and in the presence of NAC (D) CoPc strongly catalyzed the oxidation of this compound, with a shift of the oxidation potential to 0.0 V. The elevated current recorded in this experiment (130 mA mol^{-1} cm^{-2}) was a clear demonstration of the strong catalysis induced by the phthalocyanine film.

Further experiments confirmed the good performance of the modified electrode. Cyclic voltammetric scans using NAC in the 1×10^{-4} to 10×10^{-4} mol L^{-1} concentration range showed a highly linear response, reflecting the influence of the modification of the OPG with CoPc (Fig. 5). This suggests that this technique should be suitable for the quantification of NAC in many different pharmaceutical products.

3.3. Flow injection analysis (FIA) of NAC

The high current density observed in the previous experiments was a result of the strong catalysis achieved at the OPG–CoPc interface of the modified electrode. These results suggested that the sensor could also be effective for the quantification of NAC by amperometry in a flow analysis system. To investigate this possibility, a new wall-jet cell (shown in Fig. 1) was built in our laboratory and a flow system was assembled. Since the previous experiments had shown that the best results were obtained when 0.1 mol L^{-1} NaOH was used as the supporting electrolyte, the same solution was used here as the carrier solution and to prepare the NAC solutions.

Optimization of the FIA method involved consideration of the influences of flow rate, sample volume and the distance between the nozzle and the detector. A solution of the NAC (5×10^{-4} mol L^{-1}) in 0.10 mol L^{-1} NaOH was monitored at 0.0 V (vs. $Ag/AgCl/KCl_{sat}$). The effect of the injected volume (in the range of 25–100 μL) on the analytical signal was measured. The signal increased with sampling volume from 25 to 75 μL and then remained almost constant for greater volumes. A volume of 75 μL was selected for subsequent experiments since it represented a compromise between the magnitude of the analytical signal and sampling frequency. The influence of flow rate was examined in the range of 0.5–2.5 mL min^{-1}. At lower flow rates, small and broad peaks were obtained, resulting in longer times required for the signal to return to the base line. Flow rates between 2.0 and 2.5 mL min^{-1} resulted in similar peak heights. A flow rate of 2.0 mL min^{-1} was selected since this provided a good response time, moderate consumption of the carrier solution and high sampling frequency. The amperometric signal measured in the wall-jet cell was dependent on the position of the electrode. The same signal was obtained for electrode positions ranging from being almost in contact with the nozzle up to a distance of 2 mm. The signal then decreased rapidly at greater distances.

Fig. 6 presents the responses of the unmodified and modified electrodes when 5×10^{-4} mol L^{-1} standard NAC solution was injected into the flow system. The first series of experiments (Fig. 6A) was obtained using an unmodified OPG electrode. To demonstrate the stability of this system, a high sensitivity was selected and the analyte was injected. The baseline was very stable and the noise was estimated as about 2 nA (peak to peak). A reproducible peak was recorded, but based on the CV studies, at 0.0 V any NAC should be oxidized on the unmodified electrode. The reason for the appearance of this small peak is not clear, but two possibilities must be considered: One possibility could be the oxidation of very low concentrations of impurities accompanying the NAC sample. The second and more probably reason is the charging/discharging process of the electrode's electrical double layer. It is important to consider that even in presence of 0.1 mol L^{-1} NaOH, when 5×10^{-4} mol L^{-1} of NAC (absent in the carrier solution) is injected, it could influence the electrical double layer in this extension.

A similar series of experiments using the modified OPG–CoPc electrode (Fig. 6B) produced very intense peaks. It is important to attend to the different current scales in the two series of experiments in Fig. 6. The current recorded with the modified electrode was around 500 times larger than that obtained using the unmodified electrode. This means that the current recorded for the unmodified electrode when NAC was injected corresponded to $\sim 10^{-7}$ mol L^{-1} of the analyte. This is an estimate, since the active areas of the bare and modified electrodes were not the same.

The geometry of the wall-jet cell ensured a rapid washout of the electrode and the current signal returned to baseline in less than 20 s, so that a frequency in the region of 180 analyses per hour could be achieved. Under the conditions described, the relative standard deviation (R.S.D.%) for thirteen-replicate analyses of 5.0×10^{-4} mol L^{-1} NAC was 1.1%. Linearity was achieved between NAC concentrations in the range of 5×10^{-5} to 1×10^{-3} mol L^{-1}, with a correlation coefficient of 0.999. The limit of detection and quantification was 9.0×10^{-7} mol L^{-1} and 3.0×10^{-6} mol L^{-1} NAC, calculated using $3S_b/m$ and $10S_b/m$, respectively (where S_b is the standard deviation of the blank signal, and m is the slope of the calibration curve, for $n = 5$).

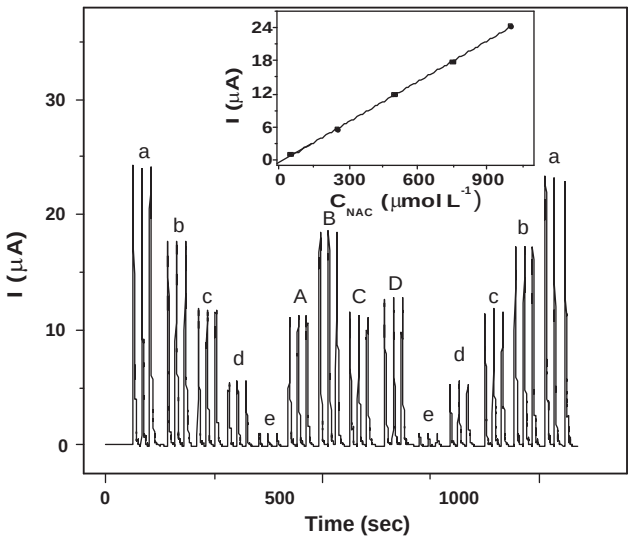

Fig. 7. Utilization of FIA associated with amperometry for the analyses of NAC content in pharmaceutical samples. The analysis of the samples (A_1 to A_4) was preceded and succeeded by injections of series of standard solutions (a to e) containing (a) 1.00, (b) 0.75, (c) 0.50, (d) 0.25, (e) 0.05×10^{-3} mol L^{-1} NAC. The experiments were performed at 0.0 V (vs. Ag/AgCl/KCl_{sat}), flow rate 2.0 mL min^{-1}, sample loop of 75 μL.

3.4. Analytical applications

The performance of the OPG/CoPc electrode for the direct determination of N-acetylcysteine in pharmaceutical formulations, using amperometry combined with flow injection analysis was evaluated using the same conditions previously established for the quantification of the analyte in solid and liquid samples.

A series of NAC standard solutions of different concentrations (0.05, 0.25, 0.50, 0.75 and 1.00 mmol L^{-1}) were injected into the system. Linear regression of the responses gave the equation: $y = 0.035\ (\pm 3.34 \times 10^{-4}) - 0.171\ (\pm 0.157)$ ($r = 0.9999$), where y is the peak height (μA), and x is the NAC concentration (μmol L^{-1}). Fig. 7 presents a series of amperometric signals obtained for the sequential injection (in triplicate) of the five standards solutions (a–e) described above and four pharmaceutical samples (indicated by A, B, C and D) together with a new series of injections corresponding to the same calibration curve.

Table 1 presents the results obtained using the official procedure [21], the proposed FIA method and the label value. Comparison between the official procedures described in the Pharmacopoeia, which is based on iodometric titrations, and the method proposed here showed very good agreement. Advantages of the new method include speed, low consumption of reagents, minor waste generation and ease of operation.

Table 1
NAC determination in pharmaceutical samples using the proposed FIA system and the official method, described in the Brazilian Pharmacopeia [21].

NAC (mg)	Labelled	FIA[a]	Standard[a]	Er_1 (%)	Er_2 (%)
Fluimiucil[b]	300.0	293.9 ± 6.8	303.5 ± 4.9	−2.0	−3.2
Aires[c]	100.0	103.0 ± 3.3	102.3 ± 1.9	3.0	0.7
União Química[b]	300.0	290.6 ± 0.5	308.5 ± 2.5	−3.1	−5.8
Germed[c]	600.0	587.3 ± 5.8	603.0 ± 1.2	−2.1	−2.6

$n = 3$, confidence level 95%.
Er_1 (relative error—FIA vs. labelled).
Er_2 (relative error—FIA vs. reference).
[a] Mean of three determinations ± SD.
[b] mg N-acetylcysteine per ampoule.
[c] mg N-acetylcysteine per sachet.

3.5. Recovery and interferences studies

Besides the comparison with the official method, the use of a recovery experiment is another means of assessing the performance of a new method. Here, the recoveries were investigated using three different pharmaceutical products. Samples were spiked with known amounts of NAC and the new compositions were analyzed. In all cases, the recoveries were in the range of 96.9–101.1%, which indicates that the proposed method should be both reliable and sensitive for the determination of NAC in pharmaceutical formulations.

The influence of different species, such as EDTA, sucrose, and citric acid (which are present in the pharmaceutical products investigated) was evaluated using the same conditions described for the previous experiments. EDTA did not show any effect in a concentration 50 times greater than that of NAC. Sucrose and citric acid caused a positive interference (close to 5%) on the response when using 25:1 (interfering specie/NAC) concentration ratio. These concentration ratio values were much higher than those found in pharmaceutical formulations containing N-acetylcysteine. None of the substances caused any interference at a concentration ratio of 1:1.

4. Conclusion

The results presented in this work provide a strong demonstration of the advantages gathered by the association of voltammetry with flow injection analysis for the determination of NAC in pharmaceutical products. In the proposed method, the samples required a simple dilution in the supporting electrolyte, without the necessity of time-consuming reactions, avoiding the use of toxic and expensive solvents or the requirement of costly instrumentation. The utilization of OPG–CoPc leads to a significant decrease in the oxidation potential and to a large increase of the voltammetric signal. Good repeatability, a wide linear working range, low limits of detection and quantification and a high sampling frequency were easily achieved with the proposed method. Moreover, the substitution of classical iodometric titration by amperometry and FIA is very attractive, especially due the great speed of analysis (120 or even 180 samples h^{-1}), generating fewer residues. In addition, the possibilities of introduce a high level of automation and/or mechanization is another advantage, particularly for applications in routine analysis.

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