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Generation and destruction of unstable reagent in flow injection system: determination of acetylcysteine in pharmaceutical formulations using bromine as reagent

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Abstract

A flow injection spectrophotometric procedure was developed for determination of acetylcysteine in sachets and liquid formulations. The determination of this drug was carried out by reacting it with bromine chemically generated in flow injection system monitored continuously at 400 nm. Acetylcysteine reacts with bromine causing a decrease in the absorbance that is proportional to the analyte concentration. The bromine in excess was destroyed on-line by an ascorbic acid solution before the discard. The calibration curve for acetylcysteine determination was linear in the concentration range from 1.6×10^{-4} to 1.6×10^{-3} mol/l with a detection limit of 8.0×10^{-5} mol/l. The relative standard deviation (R.S.D.) was lesser than 1.2% for a solution containing 5.3×10^{-4} mol/l acetylcysteine ($n = 10$), and 60 determinations per hour were obtained.

Keywords: Acetylcysteine; Flow injection spectrophotometry; Bromine

1. Introduction

Acetylcysteine (*N*-acetyl-L-cysteine, L-α-acetamide-β-mercaptopropionic acid), is an important mucolytic agent used to reduce the viscosity of pulmonary secretions in respiratory diseases and when administrated intravenously is an effective antidote in the treatment of paracetamol poisoning [1–3].

The United States Pharmacopoeia [2] recommended high performance liquid chromatography for pharmaceutical formulations analysis. Other reported methods described in the literature comprise: titrimetry [3], spectrophotometry [4–6], fluorimetry [7–10], polarography [11], stripping voltammetry [12], liquid chromatography [13,14], chemiluminescence [15] and capillary electrophoresis [16,17] for determination of acetylcysteine in pure form, in dosage forms and/or in biological samples.

Flow injection system has been proposed in the literature for determining the acetylcysteine in pharmaceutical products. A flow injection with spectrophotometric detection was described based on the formation of a yellow complex between acetylcysteine and palladium ions [18]. A flow injection system containing a chemically modified silver electrode as a potentiometric detector was also proposed for the determination of this analyte in pharmaceutical formulations [19].

Flow injection systems allow the use of unstable reagents in analytical determinations, avoiding thus periodical standardization of prepared solutions [20].

This paper describes a flow injection procedure for the determination of acetylcysteine in pharmaceutical formulations. The proposed method is based on the oxidation of acetylcysteine by bromine generated on-line. Thus, the reaction between analyte and bromine causes a decrease in the absorbance, owing bromine concentration decrease, which

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was monitored spectrophotometrically at 400 nm. This decrease in the absorbance was related with the concentration of acetylcysteine in the sample. Before the discard, the excess of Br_2 solution was destroyed on-line using an ascorbic acid solution. Bromine was reduced to bromide and ascorbic acid was oxidized to dehydroascorbic acid.

2. Experimental

2.1. Reagents

Acetylcysteine and all reagents used in this work were of analytical grade and all solutions were prepared with deionized water from a Millipore (Bedford, MA) Milli-Q system (model UV Plus Ultra-Low Organics Water).

Acetylcysteine (Aldrich, Milwaukee, WI, USA) stock solution at concentration of 2.0×10^{-3} mol/l was freshly prepared in deionized water and the reference solutions were prepared by appropriate dilution of this stock solution with water.

2.2. Apparatus

The optimized manifold (Fig. 1) consisted of a 12-channel peristaltic pump Ismatec IPC-12 (Zurich, Switzerland) supplied with Tygon® tubing. Sample and reference solutions were inserted in the flow system with the aid of a three-piece manual injector-commutator made of Perspex®, containing two fixed bars and a sliding central bar [21]. A Femto model 435 spectrophotometer (São Paulo, Brazil) equipped with a glass flow-cell (optical path of 1.00 cm) was used for the spectrophotometric measurements. Transient signals were recorded using a Cole-Parmer (Chicago, IL, USA) model 1202-0000 two-channel strip-chart recorder.

2.3. Preparation of pharmaceutical samples

The determination of acetylcysteine of Brazilian commercial sachets and liquid formulations using the proposed flow injection procedure was performed. Ten sachets were weighted and known accurate amounts in the range from 100.0 to 300.0 mg were dissolved with deionized water in 100.0-ml calibrated flask. Additional dilutions were necessary to obtain final concentrations around 6.3×10^{-4} mol/l in 100.0-ml calibrated flask. These sample solutions were inserted into flow injection system with the aid of an injector-commutator. The content of acetylcysteine in these samples was determined using a calibration curve obtained with several reference solutions in the concentration range from 1.6×10^{-4} to 1.6×10^{-3} mol/l.

The liquid formulation was determined using the standard addition method [25]. An accurate volume was diluted with deionized water in 100 ml calibrated flask. An additional dilution was necessary to obtain a final concentration of 2.0×10^{-4} mol/l and different aliquots of reference solutions of acetylcysteine were added in the concentrations of 2.0×10^{-4}, 3.3×10^{-4} and 5.2×10^{-4} mol/l.

2.4. Flow injection procedure

The bromine generation was based on the oxidation of bromide by bromate as shown in Eq. (1) (Scheme 1).

The sample or reference solutions (S) were inserted into deionized water carrier by the aid of an injector-commutator and merged downstream with the bromine produced by the reaction between potassium bromide (C_1), hydrochloric acid solution (C_2) and sodium bromate solution (C_3) (Eq. (1); Scheme 1). The bromine was generated in sufficient amount for sensitive detection with a spectrophotometer at 400 nm. When the acetylcysteine consume the bromine, Br_2, in the flow system cause a decrease in the absorbance (analytical

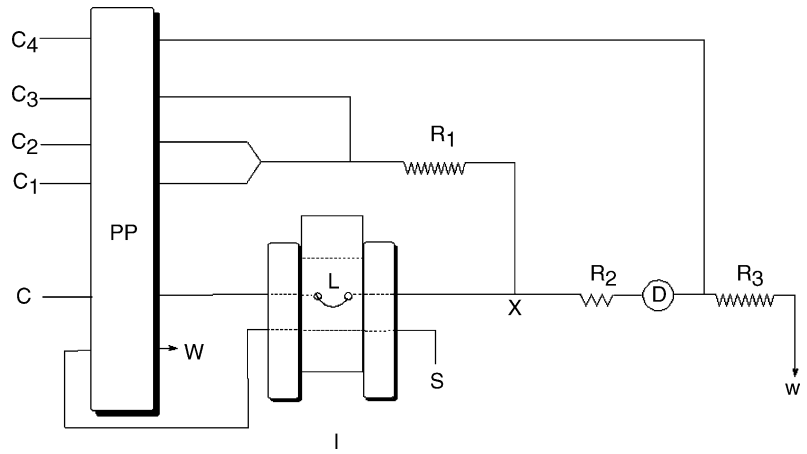
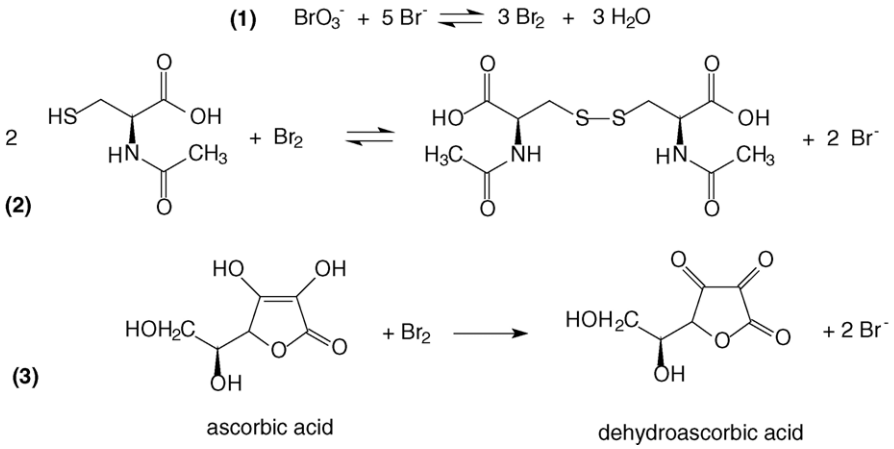


Fig. 1. Schematic diagram of flow injection system for acetylcysteine determination. (PP) peristaltic pump; (C) deionized water; (C_1) 0.22 mol/l sodium bromide solution; (C_2) 0.24 mol/l hydrochloric acid solution; (C_3) 1.25×10^{-2} mol/l bromate solution; (C_4) 5% (w/v) ascorbic acid solution; (I) injector-commutator; (L) sample volume (250 μl); (S) reference or sample solutions; (R_1), (R_2) and (R_3) 140, 60 and 60 cm helicoidal coils length, respectively; (D) detector and (W) waste.



Scheme 1. Schematic reactions. (1) Bromine generation; (2) acetylcysteine oxidation by bromine and (3) bromine destruction by ascorbic acid.

signal) is proportional to the acetylcysteine concentration in the solution as shown in Eq. (2) (Scheme 1). Thus, the bromine was completely destroyed by a 5% (w/v) ascorbic acid solution (C_4) before discard (W) (Eq. (3); Scheme 1).

3. Results and discussion

The proposed flow injection system is based on the ability of oxidation of acetylcysteine by bromine (Br_2) generated on-line. This proposed system allows the use of bromine in flow injection systems with spectrophotometric detection because the excess of bromine was destroyed on-line by a 5% (w/v) ascorbic acid solution. This on-line treatment is necessary because of the high toxicity of bromine, which vapors irritates the respiratory system and eyes [23]. This flow procedure can be considered a greener analytical method, once it produces a waste of low toxicity to people and environment [24].

3.1. Bromine generation

Initially, to optimize the bromine generation, a fixed flow rate of 1.7 ml/min for reagent solutions (C_1, C_2 and C_3) and 1.5 ml/min for carrier solution (C) were employed. In the study of effect of HCl concentration (C_2) on the baseline signal (absorbance), 0.23 mol/l potassium bromide (C_1) and 1.2×10^{-2} mol/l sodium bromate (C_3) were used. The baseline signal increased with the increase of the HCl concentration up to 0.24 mol/l. Furthermore, this concentration was chosen for further experiments.

The effect of potassium bromide concentration was investigated using a 1.9×10^{-2} mol/l sodium bromate solution in C_3 and 0.24 mol/l HCl solution in C_2. The results showed that the concentration of bromine generated increases with increasing of the bromide solution concentration used up to 0.35 mol/l. A 0.22 mol/l potassium bromide solution was selected due to the lower R.S.D. of baseline obtained in this concentration solution.

The effect of sodium bromate concentration (C_3) on the baseline signal was performed by passing a 0.22 mol/l potassium bromide through C_1 and 0.24 mol/l hydrochloric acid solution in C_2. The baseline signal increased with the increases in the bromate concentration up to 1.9×10^{-2} mol/l, above which, it remained almost constant. Therefore, a 1.25×10^{-2} mol/l sodium bromate solution was selected.

3.2. Flow injection parameters

Acetylcysteine, when injected into carrier stream (C), resulted in a negative peak after emerging with the generated bromine stream.

The effect of R_1 coil length on bromine generation was studied in the range from 45 to 160 cm monitoring the baseline signal. The baseline absorbance increased until 140 cm, above which, it remained constant. Then, a 140 cm length was selected to flow injection system. The effect of R_2 coil length was also investigated from 50 to 120 cm with a 1.2×10^{-3} mol/l acetylcysteine solution. In this coil, the acetylcysteine is oxidized by bromine causing a decrease in the bromine concentration in the stream. As the analytical signal was unchanged in all length, a 60 cm coil was selected due to a better solution homogenization. The effect of R_3 coil length in the destruction of bromine was also investigated. As a R_3 does not modify the analytical performance of the flow system and the 60 cm coil length was sufficient to permit a total consume of bromine in the flow system, this length was selected to further experiments.

The effect of injection sample volume from 100 to 300 μl on the analytical signal (negative peak) was studied using the 1.2×10^{-3} mol/l acetylcysteine reference solution. The increase of sample volume resulted in an increase of analytical signal. A better compromise between sensibility and analytical frequency was obtained using the 250 μl sample volume. Thus, this sample volume was used in the optimized flow injection procedure.

In this flow injection procedure the reagent solutions and the carrier were pumped through the flow injection system at same flow rate in the range from 1.2 to 2.5 ml/min. The best analytical response (signal/noise) was obtained with 1.5 ml/min flow rate in each channel. Thus, this flow rate was established to further experiments.

3.3. Interferences and recovery studies

The selectivity of the flow injection system was investigated by studying the effect of excipients commonly found in pharmaceutical preparations such as EDTA, NaCl, citric acid and sucrose by comparison of the response for the reference solution containing 5.2×10^{-4} mol/l acetylcysteine with those produced by a similar acetylcysteine solution with additions of the investigated excipients with final concentration of 5.2×10^{-5} and 5.2×10^{-3} mol/l. No interference in the response of flow system procedure was observed up to a 10-fold excess of these studied substances.

To study the recovery of the acetylcysteine from pharmaceutical solutions, three commercial samples of pharmaceutical formulations were used. The recovery of acetylcysteine was examined by adding three different aliquots of acetylcysteine to samples (40.5, 60.7 and 80.9 mg/l) and the results obtained were compared with those without the additions. Recoveries of 95.7 and 104% of acetylcysteine from samples of three pharmaceutical formulations ($n = 3$) were obtained with the flow injection system. These results obtained suggest a small matrix effect for one of these pharmaceutical formulations analyzed on the flow injection procedure response. Thus, in this samples was employed the standard addition method for the determination of acetylcysteine as discussed below.

3.4. Analytical characteristics and applications

The flow injection system shows a calibration curve for acetylcysteine in the concentration range from 1.6×10^{-4} to 1.6×10^{-3} mol/l ($\Delta A = 0.002 + 289.5C$; $r = 0.9973$, where ΔA is the decrease in the absorbance signal and C is the acetylcysteine concentration in mol/l). The relative standard deviation (R.S.D.) for a solution containing 5.3×10^{-4} mol/l was lesser than 1.2% ($n = 10$) and the detection limit obtained was 8.0×10^{-5} mol/l. The analytical frequency was 60 determinations per hour. In order to check the reproducibility of this system, calibration curves were obtained with freshly prepared solution on different days and the R.S.D. obtained was 3.7% ($n = 8$). Fig. 2 shows the transient signals obtained for reference solutions of (1) 1.6×10^{-4} mol/l, (2) 3.3×10^{-4} mol/l, (3) 6.6×10^{-4} mol/l, (4) 9.8×10^{-4} mol/l and (5) 1.6×10^{-3} mol/l followed by three samples (A, B and C) and reference solutions again in decreasing concentrations. The sample C was determined using the standard addition method [25]. In Fig. 2, Cs is the sample solution and RS_1, RS_2 and RS_3 are the reference solutions added to the sample.

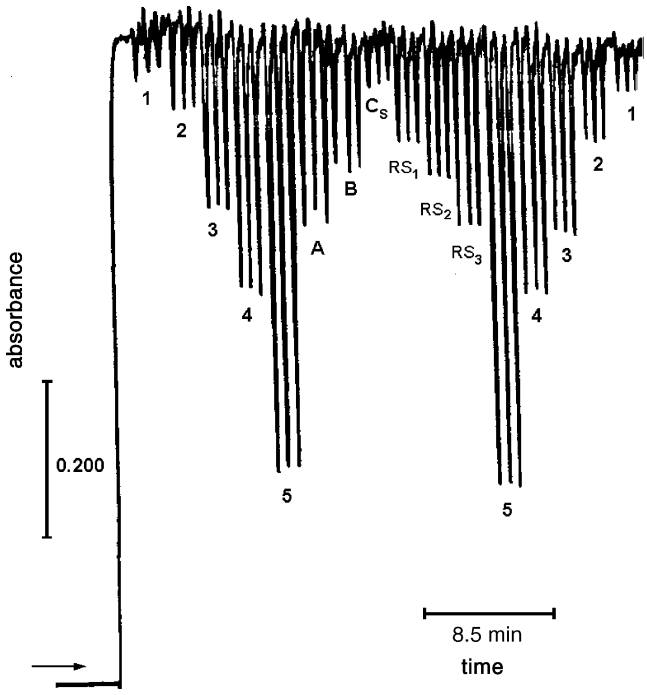


Fig. 2. Transients signals obtained in triplicate for acetylcysteine determination: (1) 1.6×10^{-4} mol/l, (2) 3.3×10^{-4} mol/l, (3) 6.6×10^{-4} mol/l, (4) 9.8×10^{-4} mol/l and (5) 1.6×10^{-3} mol/l acetylcysteine reference solutions, followed by three samples A, B and C and reference solutions again in decreasing concentrations. The sample C was determined by standard addition method (C_s: sample solution; RS_1: 2.0×10^{-4} mol/l; RS_2: 3.3×10^{-4} mol/l; and RS_3: 5.2×10^{-4} mol/l acetylcysteine reference solutions added).

Table 1
Determination of acetylcysteine in pharmaceutical products using the reference method [22] and the flow injection procedure proposed

Samples	Acetylcysteine[c]			Error (%)	
	Labelled	Reference method	Flow procedure	E_1	E_2
Genérico[a] (mg/g)	37.2	41.0 ± 0.5	36.5 ± 0.6	−1.9	−10.9
Flumicil[a] (mg/g)	39.3	39.9 ± 0.3	44.2 ± 0.3	12.4	10.7
Flumicil[b] (mg/ml)	11.5	11.3 ± 0.4	11.4 ± 0.4	−0.9	0.9

E_1: flow procedure vs. labelled value; E_2: FIA vs. reference method.
[a] Sachets.
[b] Liquid formulations.
[c] $n = 3$, confidence level 95%.

The acetylcysteine in commercial pharmaceutical formulations was determined by the proposed and reference method [22] and the results are presented in Table 1. The results obtained with the proposed flow procedure are in a good agreement with those obtained with the reference method at confidence level of 95% [25].

4. Conclusions

In this work, the generation and destruction of an unstable reagent in a flow injection system was successfully applied

in the determination of acetylcysteine in pharmaceutical formulations without the production of toxic waste. This flow system is reliable, simple to use, rapid, low cost, and precise and does not require extensive preliminary sample preparation.

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