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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

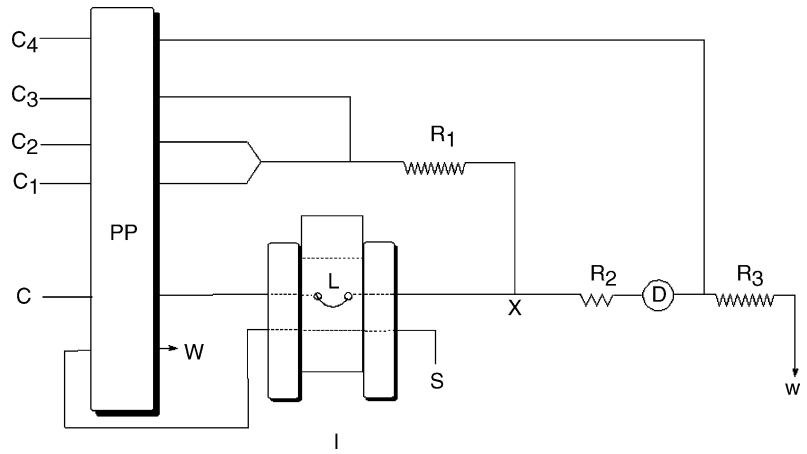

Fig. 1. Schematic diagram of flow injection system for acetylcysteine determination. (PP) peristaltic pump; (C) deionized water; (C_1) 0.22 mol/l sodium bromide solution; (C_2) 0.24 mol/l hydrochloric acid solution; (C_3) 1.25×10^{-2} mol/l bromate solution; (C_4) 5% (w/v) ascorbic acid solution; (I) 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.