Scientific Paper

Flow Injection Spectrophotometric System for *N*-Acetyl-L-Cysteine Determination in Pharmaceuticals

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Abstract

A flow injection system with spectrophotometric detection is proposed for determining *N*-acetyl-L-cysteine in pharmaceutical formulations. In this system, *N*-acetyl-L-cysteine was oxidized by Fe(III) and the Fe(II) produced is spectrophotometrically monitored as Fe(II)-1,10-phenantroline complex at 510 nm. Under the optimum analytical conditions, the linearity of the calibration curve for *N*-acetyl-L-cysteine ranged from 3.5×10^{-6} to 4.3×10^{-4} M. The detection limit of 6.3×10^{-7} M and recoveries between 98.5 to 110% were obtained.

Key words: Flow injection, spectrophotometry, *N*-acetyl-L-cysteine, pharmaceuticals

Introduction

N-Acetyl-L-cysteine is a mucolitic agent and is effective as an antidote in paracetamol overdoses in the first 12 hours after the ingestion of drug.[1,2]

Numerous analytical methods have been developed for quantitative determination of this analyte in pharmaceutical formulations as titrimetry,[3–5] spectrophotometry,[6,7] fluorimetry[8] and chemiluminescence.[9] The United States Pharmacopeia (USP)[10] describes a chromatographic procedure to *N*-acetyl-L-cysteine determination in pharmaceutical formulations.

Flow injection systems are adequate procedures to use in routine analysis in pharmaceutical laboratories control due their simplicity, high analytical frequency and capacity to reduce reagent consumption when compared with batch procedure.[11,12]

Few reports are described for *N*-acetyl-L-cysteine determination in pharmaceuticals using flow injection systems, such as: spectrophotometric[13] and potentiometric detection.[14]

Bergamin *et al*. extended the concept of merging zones in flow injection analysis by introducing the sample and reagent into inert carrier streams with synchronized merging zones with aid of double injector commutador.[15,16]

The flow injection system with symmetric merging zones procedure proposed in this article is based on the oxidation of *N*-acetyl-L-cysteine by iron(III) and the iron(II) produced was determined spectrophotometrically as a stable tris(1,10-phenantroline)iron(II) complex at 510 nm[7].

Experimental

Apparatus

The flow injection system, showed in the Figure 1 consisted of a twelve-channel Ismatec (Zurich, Switzerland) model IPC-12 peristaltic pump supplied with Tygon™ tubes, an injector laboratory-constructed three-piece manual injector-commutator made of Perspex™, with two fixed side bars and a sliding central bar was used to introduce sample or reference solutions into the flowing stream,[15,16] and a Femto model 435 spectrophotometer (São Paulo, Brazil) equipped with a glass flow-cell (optical path of 1.00 cm) was used. All system was constructed using polyethylene tubing with 0.8 mm i.d.

In the Figure 1, the injector-commutator (I) is in the loading position, the sample (S) and the reagent ($Fe(NO_3)_3.9H_2O$) is pumped to fill the reagent and sample loop (L1 and L2, respectively); its excess of these solutions were discarded in the waste vessel (W). When the injector-commutator is commutated to injection position, the selected volumes of sample and reagent ($Fe(NO_3)_3.9H_2O$) are pushed by carrier streams (1.3 M acetate buffer (pH 4.0), merging in the point X simultaneously, and then going to point Y to receipt by confluence the reagent stream (1,10-phenantroline). The chromophore produced was monitorated in the spectrophotometer (D) at 510 nm.

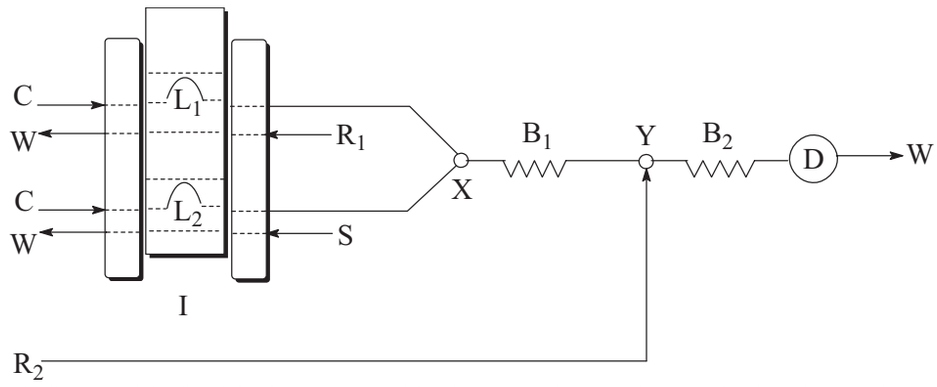
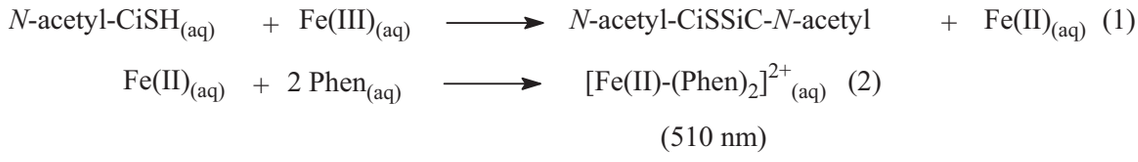


Figure 1. Schematic diagram of the flow injection system used for the spectrophotometric determination of *N*-acetyl-L-cysteine. The central bar of the manual injector–commutator (I) shows the sample position. S, sample or reference solutions; L_1, reagent loop (200 μL); L_2, sample loop (350 μL); C, carrier solution (1.3 M acetate buffer solution (pH 4.0) at flow rate of 1.6 mL min^{-1}); R_1, Ferric Nitrate solution (1.0×10^{-3} M in 2.0×10^{-3} M acid nitric); R_2, 1,10-phenantroline solution (1.8×10^{-3} M at flow rate of 0.6 mL min^{-1}); B_1, reactor coil length (15 cm); B_2, reactor coil length (70 cm); X and Y, confluence point; D, spectrophotometer (510 nm) and W, waste. X is the confluence point placed 5 cm from the injector-commutator.



Scheme 1. Reduction of iron(III) by *N*-acetyl-L-cysteine. *N-acetil-CiSH*: represents the analyte and *Phen*: 1,10-phenantroline.

Reagents

All reagents were of analytical grade and all solutions were prepared with water from a Millipore (Bedford, MA) Milli-Q system model UV plus ultra-low organics water.

The 1.3 M acetate buffer solution (pH 4.0) was prepared by mixing appropriate volumes of 0.2 M sodium acetate and 1.1 M acetic acid, and diluting to 500 mL with deionized water.

A 5×10^{-2} M stock solution was prepared by dissolving *N*-acetyl-L-cysteine in 100 mL calibrated flask with same acetate buffer solution.

Reference solutions containing from 3.5×10^{-6} to 4.3×10^{-4} M of *N*-acetyl-L-cysteine were prepared before the analysis by serial dilutions of appropriate volume of the standard stock solution in 1.3 M acetate buffer solution (pH 4.0).

A 4.0×10^{-4} M 1,10-phenanthroline solution was prepared by dissolving 0.0362 g of this reagent (Synth) in 200 mL of deionised water heated to approximately 70 °C to aid in dissolving the reagent and after cooling this volume was transferred to 500 mL calibrated flask and the volume was completed with deionized water.

A 1.0×10^{-3} M $Fe(NO_3)_3.9H_2O$ was prepared in 2.0×10^{-3} M HNO_3 using a 100 mL calibrated flask.

Sample preparation

The commercially samples analyzed by proposed flow injection procedure are available in oral powder that are administered mixed in liquids as water, beverage or juice. The contents of five oral powder units were powdered in a mortar and an accurately weighed portion of the homogenized powder containing 20 mg of *N*-acetyl-L-cysteine was transferred to a 100 mL volumetric flask and diluted to volume with deionized water. Working solutions were prepared by appropriate dilution of an aliquot of 2.0 mL in 100 mL calibrated flask with water, so that the final concentration was in the working range.

Results and discussion

The proposed flow method is based on the ability of *N*-acetyl-L-cysteine to reduce iron(III), which is converted to stable tris(1,10-phenantroline)iron(II) complex that was monitored at 510 nm (Scheme 1).[7] The chemical parameters investigated were Fe(III) concentration, carrier solution and 1,10-phenantroline concentration.

The effect of ferric ion concentration upon the analytical response of the flow system was examined in the concentration range from 5.0×10^{-4} to 4.0×10^{-2} M in 0.15 M nitric acid in order to prevent the formation of $Fe(OH)_3$ in the solutions. Maximum response was obtained at 1.0×10^{-3} M ferric nitrate solution. Thus, the influence of concentration of the 1,10-phenantroline solution was studied in the concentration range from 6.0×10^{-3} to 4.9×10^{-3} M. A 1.8×10^{-3} M 1,10-phenantroline

solution was selected for showing better engagement between analytical signal (absorbance) and reagent consumption. To avoid Fe(III) hydrolysis, a 2.0×10^{-3} M nitric acid solution (final concentration) was used in the ferric nitrate solution, without affect in the blank signal.

The parameters of flow injection system used in the determination of *N*-acetyl-L-cysteine were optimised by the univariated method with the purpose of maximizing the analytical frequency and reproducibility. The parameters studied were: sample and reagent volumes, reactor coil lengths, carrier and reagent flow rates, as showed in the Table 1.

The effect of varying sample and reagent loop from 75 to 500 μL on the analytical signal was evaluated by injection of 2.2×10^{-5} M *N*-acetyl-L-cysteine solution. The result is shown in the Figure 2. The absorbance increased with increases of Fe(III) volume up to 200 μL and it was maintained constant to volumes higher than 200 μL. Thus, this volume was selected for the further studies. When the effect of the sample loop on the analytical signal was evaluated in the same range, the signal increased with sample volume up to 500 μL. Considering the sensibility and analytical frequency, a 350 μL sample volume was selected to further optimisation experiments.

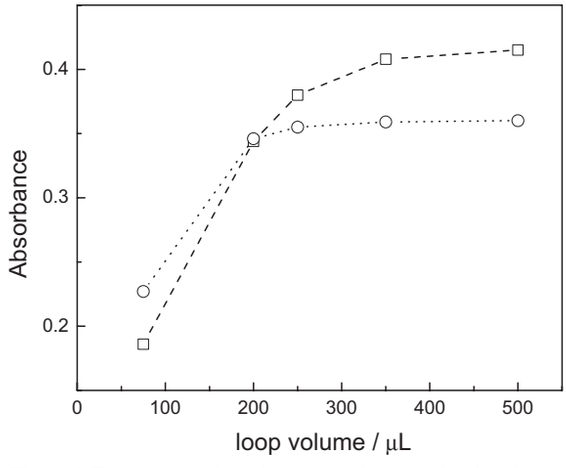


Figure 2. Effect of sample and reagent volume on the absorbance signal for 5×10^{-4} M *N*-acetyl-L-cysteine. (o) reagent volume; (□) sample volume.

The effect of carrier flow rate in the analytical response was studied in the range from 1.2 to 2.4 mL/min. It was found that the sensitivity increased up to 1.6 mL/min and it was maintained constant in higher flow rates. Therefore, a flow rate of 1.6 mL/min was selected.

The 1,10-phenantroline solution stream flow rate was studied in the range from 0.6 to 1.5 mL/min with carrier stream flow rate of 1.6 mL/min. It was found that the sensitivity showed a decrease with increase of flow rate stream. Thus, a 1,10-phenantroline solution stream flow rate of 0.6 mL/min was selected as optimum.

The reactor coil length B_1 was studied in the range from 15 to 70 cm. Better analytical signal was obtained (higher signal/noise ratio) using a 15 cm-reactor. Thus, this reactor coil length was selected. The reactor coil length B_2 was studied in the range from 40 to 150 cm. The analytical signals increased gradually with the length up to 70 cm. Longer length did not show increase of signal. A 70 cm length reactor coil was selected as optimum.

The flow injection system shows a linear calibration curve for *N*-acetyl-L-cysteine in the concentration range from 3.5×10^{-6} to 4.3×10^{-4} M (Abs=0.011 + 4810.56 x [NAC]; r=0.9995, where Abs is the absorbance and [NAC] is *N*-acetyl-L-cysteine concentration in M). The relative standard deviation of five calibration curves obtained on different work days was smaller than 1.5%.

The detection limit of 6.3×10^{-7} M (three times blank standard deviation/slope of calibration curve) was achieved.

The analytical frequency of 60 determinations per hour was estimated considering the injection of five reference solutions in the range 3.5×10^{-6} to 4.3×10^{-4} M of *N*-acetyl-L-cysteine and the two pharmaceuticals formulations solutions in triplicate.

Studying the effect of common substances such as saccharine, benzoate, saccharose and citric acid, on the determination of 3.5×10^{-4} M *N*-acetyl-L-cysteine, we assessed the selectivity of the proposed procedure. No interference in the flow injection system procedure was observed up to 10-fold excess for the substances studied. A slight negative interference of more than 5% was obtained for citric acid when this acid was present at same concentration of *N*-acetyl-L-cysteine. It was therefore not possible to determine *N*-acetyl-L-cysteine in syrups containing citric acid due to the excess of this substance in the samples.

Table 1. Optimization of chemical and flow injection parameters.

Parameter	Studied range	Selected
[Fe(III)] / 10^{-3} (M)	0.05 to 4.0	1.0
[1,10-phenantroline] / 10^{-3}(M)	0.6 to 4.9	1.8
Sample volume (μL)	75 to 500	350
Fe(III) volume (μL)	75 to 500	200
Reactor coil 1 length (cm)	15 to 70	15
Reactor coil 2 length (cm)	40 to 150	70
Carrier flow rate[a] (mL/min)	1.2 to 2.4	1.6
1,10-phen. flow rate (mL/min)	0.6 to 1.5	0.6

[a] each channel of peristaltic pump.

Recoveries of 98 to 110% of *N*-acetyl-L-cysteine from three pharmaceutical formulations were obtained

Table 2. Determination of *N*-acetyl-L-cysteine in pharmaceuticals by Brazilian Pharmacopeia[5] and proposed flow injection procedure.

Samples	*N*-acetyl-L-cysteine (mg/g)[a]			Error (%)		$t_{calc.}$
	Label Value	Comparative procedure	Flow injection Method	E_1	E_2	
Fluimucil	20	20 ± 1	21.1 ± 0.3	5.5	5.5	1.83
Genérico	20	19 ± 1	19.7 ± 0.1	–1.5	3.7	1.03

[a] Mean ± standard deviation. E_1: relative error of flow injection procedure *vs.* label value. E_2: relative error of flow injection procedure *vs*. Brazilian Pharmacopoeia volumetric procedure[5]. t_{theor} 2.78 (95%).

using the flow injection procedure. In this study, 3.7×10^{-5}, 5.5×10^{-5} and 7.4×10^{-5} M of *N*-acetyl-L-cysteine solutions were added to each solution product containing 2.5×10^{-5} M of *N*-acetyl-L-cysteine. The recovery results obtained show no significant matrix effect of the sample. The analyses of commercial samples were performed using a calibration curve.

Table 2 presents the results obtained using the proposed flow procedure and the titrimetric procedure proposed by Brazilian Pharmacopeia.[5] Applying paired *t*-test it was found that all results are in agreement at the 95% confidence level and within an acceptable range of errors.[10,17]

Conclusions

The proposed flow injection procedure for determining *N*-acetyl-L-cysteine in pharmaceutical is simple, precise, accurate, and has high analytical frequency. When compared with batch spectrophotometric procedure,[7] the developed procedure reduced the consumption of Fe(III) from 1.4 mg to 0.2 mg per determination and the consumption of 1,10-phenatroline was reduced from 6300 μg to 43 μg per determination.

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Povzetek

Za določanje *N*-acetil-L-cisteina v farmacevtskih pripravkih smo uporabili pretočno injekcijsko analizo s spektrofotometrično detekcijo. V tem sistemu oksidiramo *N*-acetil-L-cistein z Fe(III) in nastali Fe(II) določimo spektrofotometrično v obliki koordinacijske spojine z 1,2-fenantrolinom z detekcijo pri 510 nm. Pri optimalnih pogojih je bila umeritvena krivulja linearna v koncentracijskem območju $3{,}5\times10^{-6}$ do $4{,}3\times10^{-4}$ M. Meja zaznave je $6{,}3\times10^{-7}$ M, izkoristki pa se gibljejo med 98,5 in 110%.