ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 823–828

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Determination of tiopronin in pharmaceuticals using a chemiluminescent flow-injection method

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Received 9 September 1997; received in revised form 13 November 1997; accepted 15 November 1997

Abstract

A flow-injection method for the determination of tiopronin in the range 1×10^{-7}–7×10^{-5} M is described. The procedure is based on the chemiluminescent reaction of tiopronin with cerium(IV) in sulphuric acid medium using rhodamine 6G and quinine as fluorophors. The flow-injection method is rapid and precise and allows measurements of up to 80 solutions per hour. The applicability of the method to the determination of tiopronin in pharmaceutical preparations was demonstrated by investigating the effect of potential interferences and by analysing commercial preparations.

Keywords: Tiopronin; Cerium(IV) oxidation; Chemiluminescence; Flow-injection; Pharmaceuticals

1. Introduction

Tiopronin [*N*-(2-mercaptopropionyl)-glycine] is a sulphydryl compound with properties similar to those of penicillamine. It is used in the management of cystinuria and as an antidote to heavy metal poisoning. Tiopronin is also used in the treatment of rheumatoid arthritis, hepatic disorders and as a mucolytic in respiratory disorders, administered by inhalation in the latter case.

Various methods have been proposed for the determination of thiol-containing drugs such as spectrophotometry based on the formation of complexes with metal ions, mainly Co^{2+} and Pd^{2+} [1,2], liquid chromatography [3] and kinetic methods [4]. The reaction with an aroylacrylic acid to give stable fluorescent thiol adducts has also been proposed for the spectrophotometric and fluorometric determination of these drugs [5].

Interest in the use of chemiluminescence (CL) to determine trace and ultra-trace concentrations of inorganic and organic species has increased in the past few years [6,7]. The most notable characteristics of CL methods are the better sensitivity and wider dynamic ranges compared with those from other analytical methods. CL analysis may also be useful as the instrumentation is relatively simple. Further, the introduction of flow injection

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0731-7085/98/$19.00
PII S0731-7085(98)00013-2

analysis (FI) has made CL methods even more attractive, because it is possible to mix samples and reagents rapidly and with a high degree of reproducibility [8,9].

Some thiol containing drugs have been determined either by their inhibitory effect on the copper(II)-catalysed luminol-hydrogen peroxide CL reaction [10] or by their CL reaction with cerium(IV) in sulphuric medium using Rhodamine B as a fluorophore [11]. Recently, a CL-FI method has been described for the determination of tiopronin based upon the oxidation by cerium (IV) in diluted sulphuric acid medium and sensitized by quinine [12,13].

The aim of the work presented here has been to study the effect of different fluorophors on the oxidation of thiol containing drugs by cerium (IV) in order to increase the weak CL of these reactions. The pilot substance selected was tiopronin. Our results showed that a mixture of quinine and rhodamine 6G yielded a synergetic effect on the CL emission providing about a six and four times improved sensitivity when compared to the reaction using only quinine or rhodamine 6G, respectively. Based on this finding, a very sensitive FI-CL method for tiopronin was developed. Levels as low as 1.0×10^{-7} M tiopronin can easily be determined by measuring the light produced.

2. Materials and methods

2.1. Apparatus

The measurements of the emitted light were made with a laboratory-built apparatus. A shield and separately housed SLM-Aminco Model JD 490 photomultiplier tube (PMT) was used as a light detection system; the PMT output was amplified and quantified by an SLM-Aminco Bowman Series 2 spectrofluorimeter connected to a personal computer fitted with the SLM-Aminco Bowman Manager software. A Gilson Minipuls-8 peristaltic pump and Omnifit rotary valve were also used. Except for the pump tubing (Tygon) poly(tetrafluoroethylene) (PTFE) tubing (0.5 mm I.D.) was used throughout the manifold. The CL cell was a Helma 170 QS flow cell placed in a thermostated cell holder immediately in front of the PMT, with a mirror behind; the volume of the cell was 45 μl, exposing a large surface area to the adjacent PMT. Extreme precautions were necessary to ensure that the sample compartment and PMT were light-tight.

2.2. Reagents

All chemicals were analytical-reagent grade and were used as received. All solutions were prepared with doubly distilled water.

Tiopronin (Sigma) stock solution (1.0×10^{-3} M) was prepared in water. Working solutions of lower concentrations were prepared daily by appropriate dilution of the stock solution with water. All solutions were kept in dark bottles at 4°C.

Cerium(IV) stock solution (1.0×10^{-2} M) was prepared by dissolving cerium(IV) sulphate ($Ce(SO_4)_2 \cdot 4H_2O$) (Merck) in 1 M sulphuric acid. This solution was kept in a dark bottle.

Stock solutions (1.0×10^{-3} M) of rhodamine 6G (C.I. 45160), rhodamine B (C.I. 45170), fluorescein (C.I. 45350), erythrosine B (C.I. 45430), phloxin (C.I. 45410), eosin (C.I. 45380), Rose Bengal (C.I. 45440), phenosafranine (C.I. 50200), safranine T (C.I. 50240), methylene blue (C.I. 52015), diquat, quinine and 8-hydroxyquinoline were prepared by dissolving the appropriate amount of the product (Merck or Sigma) in water.

2.3. Manifold

The flow manifold finally adopted is shown in Fig. 1 with optimum conditions as stated. Rhodamine 6G, quinine and polyvinyl alcohol (PVA) solutions were pumped separately and combined prior to merging with the sample stream. The mixed streams travelled 20 cm downstream before the Ce(IV) solution was injected by means of a rotary valve with a 35-μl loop. The distance between the injection valve and the flow cell was 6 cm (minimum distance achieved). The light emitted by the CL reaction was detected with no wavelength discrimination.

The tube connecting the injection valve and the flow cell was covered with black insulating tape to

prevent a fibre optic effect from introducing stray light into the detector.

2.4. Determination of tiopronin in pharmaceutical preparations

The tablets or pills (five or more) were finely powdered. An amount of this powder, equivalent to about 5 mg of tiopronin was accurately weighed and shaken with 200 ml of distilled water in a water-bath at 50°C for 10 min. After cooling, the solution was filtered into a 1000-ml calibrated flask, the residue was washed several times and the solution diluted with water to the mark to obtain a solution of 5 μg ml^{-1}, which was pumped into the flow system.

3. Results and discussion

3.1. Batch study

Preliminary work was carried out to select oxidants for the possible generation of CL emission with tiopronin. No detectable signal was observed with the following oxidising agents in either 0.1 M sulphuric acid or 0.1 M sodium hydroxide: potassium iodate, potassium permanganate, potassium bromate, potassium dichromate, hydrogen peroxide and potassium persulphate. Only cerium(IV) sulphate in 0.1 M sulphuric acid gave an detectable response.

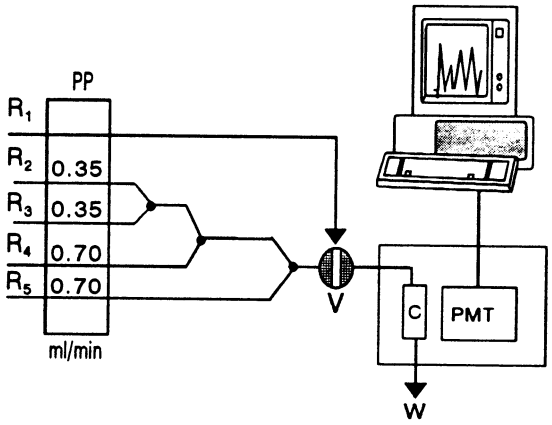

Fig. 1. Schematic diagram of the flow-system. PP, peristaltic pump (with flow-rate given in ml min^{-1}); T, PTFE-Y pieze; V, injection valve; C, flow cell; PMT, photomultiplier tube; $R_1 = 1 \times 10^{-3}$ M Ce(IV) in 0.1 M H_2SO_4; $R_2 = 7 \times 10^{-5}$ M Rh6G; $R_3 = 3 \times 10^{-4}$ M quinine; $R_4 = 0.1\%$ (w/v) PVA; R_5 = sample solution.

Table 1
Effect of various fluorescers upon the Ce(IV)-tiopronin CL reaction

Compound[a]	Relative CL emision
None	100
Fluorescein	114
Erythrosine B	50
Eosine	124
Phloxin	106
Rose Bengal	62
Phenosafranine	103
Safranine T	160
Methylene Blue	144
Rhodamine B	1820
Rhodamine 6G	3740
Quinine	2412
Rhodamine 6G + Quinine	14 840
Diquat	144
8-Hydroxyquinoline	75

[a] Concentration: 2×10^{-5} M.

Based on the study of the CL properties of fluorophore-sensitized Ce(IV) reactions with sulphite [14,15] and some thiol-compounds [11–13] in sulphuric acid medium, a wide variety of different classes of fluorescent molecules were investigated with respect to the amplification of the weak radiation emitted during the oxidation of tiopronin by cerium(IV). Among those screened were fluorescent dyes such as the rhodamines, fluoresceins, thiazines and phenazines, heterocyclic compounds such as 8-hydroxyquinoline, diquat and quinine. An important practical consideration in the selection of an optimum fluorescer is that it must show a high degree of stability to both cerium(IV) and photo-oxidation in the envisaged reaction. Table 1 shows that quinine, rhodamine 6G (Rh6G) and rhodamine B (Rh B) are good fluorophors, respectively increasing about 24-fold, 37-fold and 18-fold the CL efficiency of the system

On the other hand, the simultaneous presence of two of the fluorescers listed in Table 1 were

also investigated. It was found that the simultaneous presence of Rh6G and quinine gave rise to the most intense emission, which was about four times greater than from the reaction using only Rh6G, the fluorophor more effective. The mixture Rh6G-quinine was used in all subsequent studies.

The sensitized CL emission from cerium(IV) and tiopronin is most intense when the oxidant is dissolved in sulphuric acid as opposed to nitric or perchloric acids. The latter acids, especially nitric acid, obviously are to be avoided because of their strong oxidising properties Therefore sulphuric acid was selected in all experiments.

The effect of the aqueous micellar system of each charge type (i.e. cationic, anionic, zwitterionic, or non-ionic) on the CL cerium(IV)-tiopronin reaction sensitized by quinine and RH6G was studied. No enhancement in CL emission could be detected with cationic, anionic or zwitterionic surfactants. However, non-ionic surfactants such as triton X-100 and PVA slightly increased the CL signal, and so further experiments were carried out in the presence of PVA.

In order to measure the CL at maximum intensity, the effect of mixing reagents on the CL signal and on its time profile were examined. It was found that the emission intensity was dependent on the way of mixing the reagents. The greatest CL signal was obtained when the Ce(IV) solution was added to the premixed tiopronin and Rh6G and quinine solutions. The light emission occurred very rapidly and reached maximum intensity within 2 s from adding the Ce(IV) solution.

3.2. Manifold design

A preliminary investigation based on the batch experiments was carried out to design the FI configuration to be used in order to determine tiopronin. The best results were obtained when the sample, PVA and fluorophors solutions were propelled by separate lines and mixed within the flow system before injecting the Ce(IV) solution containing 0.1 M sulphuric acid. This reverse mode configuration resulted in increased sensitivity and a very low baseline, which recovered very quickly. An additional advantage of this reverse mode was that it prevented blockage of the tubes caused by the hydrolysis of the cerium(IV)salt.

3.3. Influence of flow-rate and volume of cerium(IV) solution injected

The solutions of the sample, PVA and fluorophors were always pumped at equal flow-rates. The effect of the flow-rate is critical. Excessively low or high flow-rates result in the absence of CL in the flow cell. Variation of the total flow-rate over the range 1–3 ml min^{-1} led to an increase in the CL of the flow cell (Fig. 2). The increasing sensitivity suggested that the CL reaction was completed inside the cell. A total flow-rate of 2.1 ml min^{-1} was selected to avoid extensive consumption of sample and reagents with no pronounced increase in sensitivity.

The variation of the CL emission with the volume of Ce(IV) injected was studied using an 1.0×10^{-3} M cerium(IV) sulphate solution in 0.1 M sulphuric acid. The peak height decreased slightly with increasing volumes of Ce(IV) solution over the 35–185 μl range. The chosen volume to be injected was 35 μl.

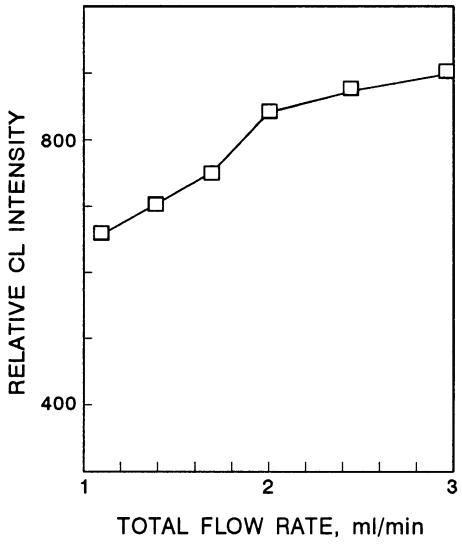

Fig. 2. Effect of flow rate on the emission intensity.. Conditions: Rh6G 5×10^{-5} M; quinine 5×10^{-4} M; 1×10^{-5} M tiopronin, 0.1% (w/v) PVA, 5×10^{-5} M;Ce(IV) in 0.1 M H_2SO_4.

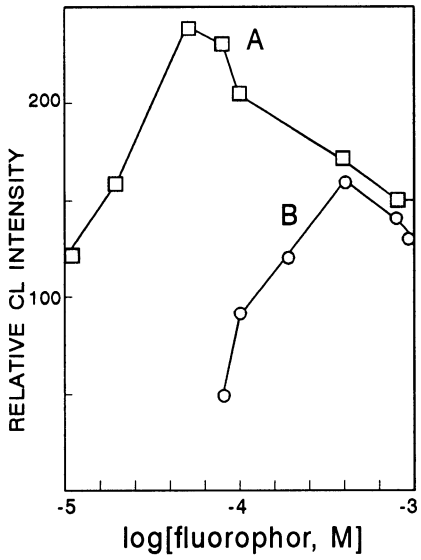

Fig. 3. Effect of the fluorophors concentration on the emission intensity. Fluorophors: A, Rh6G; B, quinine. Conditions: 1×10^{-5} M tiopronin; 0.1% PVA; 1×10^{-3} M Ce(IV) in 0.1 M H_2SO_4, total flow rate 2.1 ml min^{-1}.

3.4. Influence of reagent concentrations

CL intensity was affected by the concentration of sulphuric acid, the optimum concentration being 0.1 M. The effect of the cerium(IV) concentration in 0.1 M sulphuric acid upon the emission intensity were studied over the range 2×10^{-4}–8×10^{-3} M. The CL increased with increasing cerium(IV) concentration up to 8×10^{-4} M, above which it decreased. This decrease in CL intensity at concentrations higher than 1×10^{-3} M might be attributed to the absorption of the emitted light by the excess of cerium(IV).

The effect of the Rh6G and quinine concentrations on the emission intensity induced by the oxidation of tiopronin with cerium(IV) is shown in Fig. 3. The emission intensity increased with increasing fluorophor concentrations up to 7.0×10^{-5} M for Rh6G and 3.0×10^{-4} M for quinine [12,13], and then decreased in both cases.

The CL signals yielded in the presence of 7.0×10^{-5} M Rh6G and 3.0×10^{-4} M quinine were four and six times greater than those obtained using only Rh6G or quinine, respectively.

3.5. Calibration graph and reproducibility

A series of standard tiopronin solutions (at least 15 samples covering the whole range of concentrations) was injected into the manifold under the selected conditions to test the linearity of the calibration graph. A linear relationship between drug concentration and CL signal was obtained in the range 1.0×10^{-7}–7.0×10^{-5} M. The sampling rate was about 80 samples per hour. The precision was tested by using ten samples of tiopronin at two concentration levels. The relative standard deviations were 1.8% and 2.6% at the concentrations 3×10^{-5} M and 3×10^{-6} M, respectively. The detection limit calculated according to IUPAC recommendations [16] was 3.6×10^{-8} M.

3.6. Interference studies

In order to apply the proposed method to the analysis of pharmaceutical dosage forms, the influence of commonly used excipients and additives was studied by preparing solutions containing 2×10^{-5} mol l^{-1} of tiopronin and different amounts of the foreign compound. No interference was found for glucose, fructose, lactose, maltose, cellulose, starch, metoclopramide, propylenglycol, cetyl alcohol, sorbitol and cyclobutyrol at [interferent]/[tiopronin] ratios of up to 20:1. The tolerance limit was taken as the concentration causing an error of not more than $\pm$ 3% in the determination of the drug. Hence, the proposed method may be considered as sufficiently selective.

3.7. Applications

The proposed FI method was applied to the determination of tiopronin in two pharmaceutical preparations. The results obtained and the labelled contents are summarized in Table 2. There were no significant differences between labelled contents and those obtained by the proposed method. Recovery studies were also performed on each of the analyzed samples by adding a known amount of tiopronin to the sample before the recommended treatment. Recoveries ranged from

Table 2
Determination of tiopronin in pharmaceutical preparations and recovery experiments

Sample[a]	Amount (mg)		Added	Recovered (mg)	Mean recovery (%) ($n = 4$)
	Label	Found ± S.D. ($n = 4$)			
Hepadigest® (pills)	100	102 ± 2.3	25	24	96
			50	52	104
			100	98	98
Sutilan® (pills)	100	97 ± 3.1	25	26	104
			50	52	104
			100	97	97

Hepadigest and Sutilan are the trade mark of tiopronin manufactured by Uriach (Barcelona, Spain) and Cusí (Barcelona, Spain) laboratories, respectively

[a] Composition of samples: Hepadigest: 100 mg tiopronin, 10 mg metoclopramide hydrochloride, 100 mg cyclobutyrol calcium, excipient. Sutilan: 100 mg tiopronin, excipient.

96–104%. Table 2 also summarizes recovery results.

4. Conclusions

The cerium(IV)-tiopronin CL reaction sensitized by Rh6G and quinine has been shown to be applicable to the determination of tiopronin. The CL measurement was readily automated in a flow system.

The proposed method is very simple, selective, sensitive and rapid (80 samples per hour). The utility of the method has been illustrated by the results obtained in the analysis of commercial pharmaceutical preparations. The reagents and instrumentation for this analysis are inexpensive.

A comparison of FI-CL methods using only quinine [12] or a mixture of Rh6G and quinine for enhancing the weak CL of the reaction tiopronin-cerium (IV) shows an advantage for the latter: the higher sensitivity (about one decade) than that obtained using the previously reported procedure [12].

Acknowledgements

This research was supported by a grant from the Dirección General de Investigación Científica y Técnica de España (PB96-1101).

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