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Short communication

Flow injection chemiluminescent determination of amiodarone in pharmaceutical preparations using photogenerated *tris*(2,2′-bipyridyl)ruthenium(III)

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Abstract

A flow injection configuration was developed and evaluated for the chemiluminescent determination of amiodarone. The method is based on the reaction of the drug with *tris*(2,2′-bipyridyl)ruthenium(III), which was generated through the on-line photo-oxidation of *tris*(2,2′-bipyridyl)ruthenium(II) with peroxydisulfate. Under the optimum experimental conditions, a linear calibration graph was obtained over the range 3.0–60.0 μg ml^{-1} with a detection limit of 0.28 μg ml^{-1}. The proposed method allows 120 injections h^{-1} with excellent repeatability and precision (R.S.D. less than 0.5% and 2.8%, respectively) and a reagent consumption of only 0.37 μmol (0.27 mg) of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ per determination. The method was successfully applied to the determination of amiodarone in commercial pharmaceutical formulations.

Keywords: Amiodarone; Chemiluminescence; Flow injection; *Tris*(2,2′-bipyridyl)ruthenium(III) photogenerated

1. Introduction

Amiodarone, chemically known as 2-butyl-3-benzofuranyl [4-(2-diethyl amino) ethtoxy]-3,5-diiodophenylketone, is an antiarrhythmic drug used for treating supraventricular and ventricular arrhythmia and tachycardia associated with the Wollf–Parkinson-White syndrome [1–4].

Several methods have been developed for the determination of amiodarone in different matrices. For the analysis of biological fluids, high performance liquid chromatography [5–8] and capillary electrophoresis [9,10] methods have often been proposed for the determination of amiodarone. For the determination of this drug in pharmaceutical preparations, potentiometric titration [11], spectrophotometry [12,13], infrarred spectroscopy [14], fluorimetry [15], liquid chromatography [16,17] and electrochemical methods [18,19] have been reported.

Interest in using chemiluminescence (CL) for pharmaceutical determination has increased in recent years [20–22]. CL detection offers many advantages such as very simple instrumentation needed and the wide dynamic concentration range that can be measured. In addition, the theoretically zero background expected in the absence of analyte means that the CL technique is extremely sensitive. When coupled to flow injection analysis (FI), the CL based FI method has received considerable attention due to its being an inexpensive technique that involves the reproducible and rapid mixing of reagents. To the best of our knowledge, however, nothing exists in the literature concerning the CL assay of amiodarone. The aim of this work, therefore, was to investigate the use of CL in the detection of amiodarone in pharmaceutical preparations with no need for sample pretreatment.

Tris(2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$) has been used as the basis for the CL detection of a wide range of compounds after oxidation to the ruthenium(III) complex ($Ru(bpy)_3^{3+}$) [23,24]. The analyte reacts with the ruthenium(III) complex reducing it to the ruthenium(II) complex in an excited state, which then emits CL as it returns to the ground state. In the present work, an FI method with CL detection was developed for the rapid determination of trace amounts of amiodarone. The proposed approach used the oxidation of the drug by $Ru(bpy)_3^{3+}$, which was generated by the on-line oxidation of $Ru(bpy)_3^{2+}$ with peroxydisulfate in a PTFE reactor incorporating a low pressure

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mercury lamp. The proposed method is simple, rapid and inexpensive and has been satisfactorily applied to the determination of amiodarone in pharmaceutical formulations.

2. Experimental

2.1. Reagents

All chemicals were of analytical reagent grade and obtained from Sigma (Madrid, Spain) unless stated otherwise. Ultrapure water from a Milli-Q Plus System (Millipore Ibérica, Madrid, Spain) was used in all the experiments. A stock standard solution of amiodarone (100 μg ml^{-1}) was prepared by dissolving 10.0 mg of amiodarone hydrochloride in 10 ml of 0.1 mol l^{-1} sodium dodecylsulfate and diluting to 100 ml with ultrapure water; this solution remained stable for at least 4 weeks if kept refrigerated. Working standard solutions were prepared from the stock solution by appropriate dilution with ultrapure water. The working standard solutions of amiodarone were stable for at least 5 days at room temperature.

Tris(2,2′-bipyridyl)ruthenium(II) (5×10^{-3} mol l^{-1}) stock solution was prepared by dissolving an appropriate amount of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ (Fluka, Buchs, Switzerland) in ultrapure water. The stock solution of peroxydisulfate (0.1 mol l^{-1}) was prepared weekly by dissolving potassium peroxydisulfate in ultrapure water.

2.2. Apparatus

Measurements of the emitted light were performed with a Bio Orbit (Turku, Finland) luminometer connected to a personal computer functioning with the Bio Orbit Software. A Gilson (Villiers-le-Bel, France) Minipuls 3 peristaltic pump was used to pump all the reagent solutions. Two Omnifit (Cambridge, UK) rotary valves were used to inject the sample and $Ru(bpy)_3^{2+}$ solutions. Except for the pump tubing (Tygon), PTFE tubing (0.5 mm, i.d.) was used throughout the manifold. The CL cell was a Hellma 170.QS (Müllheim, Germany) flow cell with a mirror behind. The aperture to the adjacent photomultiplier tube was 3.5 mm × 18.5 mm. The UV source was a Spectronic (Westbury, NY, USA) rod-shaped low-pressure mercury discharge lamp (50 mm × 5 mm Ø) that had a major emission line at 254 nm.

2.3. Determination of amiodarone in commercial dosage forms

Ten tablets of Trangorex® tablet were accurately weighed to obtain the mean tablet weight and ground to homogenized powder; a portion of the powder corresponding to 100 mg of amiodarone was accurately weighed and dissolved with 1 ml of 1 mol l^{-1} hydrochloric acid and 100 ml of 0.1 mol l^{-1} sodium dodecylsulfate, cleaned with an ultrasonic cleaner for 20 min and further diluted to 1000 ml with ultrapure water. After filtering, aliquots of the filtrate were further diluted with ultrapure water so that the concentration of amiodarone was in the working range of determination of amiodarone. One hundred and eighty five microliters of this solution was injected in the flow injection system.

Trangorex® ampoules were analysed by diluting with ultrapure water to 1000 ml in a calibrated flask. Aliquots of this solution appropriately diluted with ultrapure water were used for analysis.

3. Results and discussion

Initially attempts were made using a continuous flow manifold with three channels. Peroxydisulfate and $Ru(bpy)_3^{2+}$ solutions were pumped by separate lines and mixed before the photoreactor. The amiodarone stream was merged with the premixed $Ru(bpy)_3^{2+}$ and $S_2O_8^{2-}$ streams at a confluence point which was situated just before or after the photoreactor. It was found that the highest light emission was obtained when the three solutions were mixed before passing through the photoreactor.

3.1. Manifold design

A reverse FI system was initially used in order to minimise consumption of the expensive $Ru(bpy)_3^{2+}$ solution. This consisted of two streams, one of the sample, into which the $Ru(bpy)_3^{2+}$ was injected, and the other of the peroxydisulfate solution buffered to the pH selected. Both streams were mixed before they reached the photoreactor, which was constructed from PTFE tubing (0.5 mm, i.d.; length, 100 cm) formed into a spiral with 35 turns around the lamp. The photoreactor-lamp assembly was housed in a fan-ventilated metal box covered with aluminium foil to increase photon flux by reflection. The tubing from the photoreactor to the flow cell was covered with black insulating tape to prevent a fibre optic effect from introducing stray light into the detector.

At a later stage the manifold was changed for one considered more suitable for analysis (Fig. 1). In this new manifold, the sample and $Ru(bpy)_3^{2+}$ were inserted simultaneously with the aid of two rotary valves. Amiodarone was introduced into the buffer stream and $Ru(bpy)_3^{2+}$ was injected into the peroxydisul-

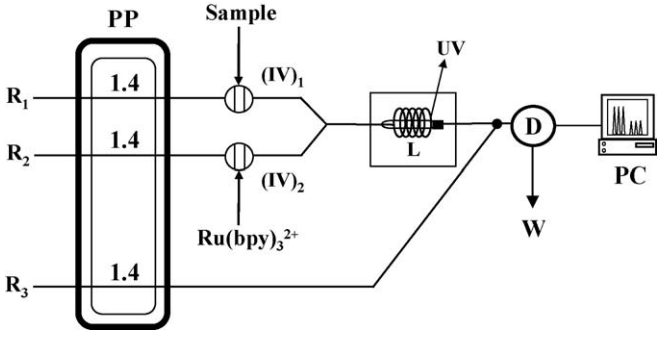

Fig. 1. Schematic diagram of the flow injection manifold. PP peristaltic pump (with the flow rates given in ml min^{-1}); $R_1 = 0.02$ mol l^{-1} phosphate buffer of pH 5.7; $R_2 = 1 \times 10^{-2}$ mol l^{-1} peroxydisulfate and 0.02 mol l^{-1} phosphate buffer of pH 5.7; $R_3 = 0.05$ mol l^{-1} phosphate buffer of pH 7; $(IV)_1$ = sample injector; $(IV)_2$ = $Ru(bpy)_3^{2+}$ injector; L = photoreactor (0.5 mm, i.d.; length, 100 cm); *D* = luminometer; W = waste.

fate stream. Both streams merged just before the photoreactor. The results of both manifolds were compared using the optimised conditions and no difference was found in performance in terms of linear range and detection limit.

3.2. Optimisation of the flow injection system

The CL reaction between amiodarone and $Ru(bpy)_3^{3+}$ is affected by the following chemical, photochemical and hydrodynamic variables:

3.2.1. Effect of pH

The efficiency corresponding to the on-line photogeneration of the Ru(III) complex and its subsequent reduction by amiodarone was measured at different pH values using phosphate and acetate buffers in the pH range 3.0–8.0. The peak height increased with increasing pH up to 5.5, remained constant up to 6.0 and then decreased at higher pH values. The buffer constituents (phosphate or acetate) had little influence on the emission time response. Phosphate buffer provided higher peaks, and 0.02 mol l^{-1} phosphate buffer adjusted to pH 5.7 was used in all subsequent experiments.

The CL intensity was also affected by the pH in the flow cell. CL signal increased with increasing pH, but values higher than 7.5 are not recommended because high backgrounds were obtained with this reagent [27]. By pumping 0.05 mol l^{-1} phosphate buffer solution of pH 7 through channel R_3, which was then merged with the solution emerging from the photoreactor, a pH of about 6.8 was achieved in the flow cell (Fig. 1).

3.2.2. Effect of peroxydisulfate concentration

The efficiency of the on-line photoxidation of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$ was found to increase with increasing peroxydisulfate concentrations until a plateau was reached between 9.0×10^{-3} and 1.3×10^{-2} mol l^{-1}, above which it decreased, probably due to the quenching of $[Ru(bpy)_3^{2+}]^*$ by $S_2O_8^{2-}$ [25]. The concentration selected was 1.0×10^{-2} mol l^{-1}.

3.2.3. Effect of the Ru(II) complex concentration

The influence of $Ru(bpy)_3^{2+}$ concentration on the emission intensity at various amiodarone concentrations was studied. The highest intensity in each instance was obtained with the highest $Ru(bpy)_3^{2+}$ concentration tested, 5×10^{-3} mol l^{-1}. An 2×10^{-3} mol l^{-1} was chosen as a compromise between signal intensity and consumption of this expensive reagent. The volume of $Ru(bpy)_3^{2+}$ injected was also studied. The peak height steeply increased with increasing loop size in the injection up to 185 μl, above which it slightly increased. The volume selected was 185 μl.

3.2.4. Effect of flow rate and photoreactor coil length

Flow rate and photoreactor coil length had a strong influence both on the irradiation time, and hence on the photoconversion of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$, and on the residence time of the light emitting species in the flow cell which determines the CL intensity. Because both variables were interdependent, they could not easily be optimised by the univariate method. The Yates method [26] of factorial design was used for the optimisation of these two manifold parameters and the concentration of $Ru(bpy)_3^{2+}$ solution injected, which was also considered an interrrelated variable. The low and high levels of each variable were selected from the results obtained for the univariate method. The results of the first set of experiments showed that the flow rate and $Ru(bpy)_3^{2+}$ concentration had a positive impact and the effect of flow rate was lower than that of the concentration of the Ru(II) complex solution; the length of the photoreactor had little effect and negative impact. In the second set of experiments, the increase in flow rate had a small positive effect, which meant that the optimum parameter value was near. An increase in $Ru(bpy)_3^{2+}$ concentration had a positive effect but a lower photoreactor length was not necessary. A final set of experiments was then carried out and the optimum values found are listed in Table 1. Using the conditions obtained by the Yates method, an increase of 180 mV in the CL signal was observed.

Table 1
Optimum parameters for the FI system

Variable	Univariate method	Multivariate method
Flow rate (ml min^{-1})	3.8	4.2
Coil length (cm)	100	100
$Ru(bpy)_3^{2+}$ (mol l^{-1})	2.0	2.7

3.2.5. Effect of sample volume

CL emission increased with increasing sample volume injected up to 185 μl, above which it remained virtually constant. The volume selected was 185 μl.

3.3. Validation of the method

Appropriate validation information concerning new methods for analysing pharmaceuticals is required by regulatory authorities. The proposed method was validated in compliance with the guidelines Q2A and Q2B [28] issued by the International Conference on Harmonization.

3.3.1. Stability of the solutions

The response factors of standard solutions were found to be unchanged for 4 weeks at least. Less than a ±3% concentration difference was found between the solutions freshly prepared and those aged 4 weeks. The solutions can therefore be used within this period without affecting the results.

3.3.2. Linearity

Using the selected conditions, the effect of the concentration of amiodarone on CL intensity was studied by measuring the peak height when 185 μl of amiodarone standard solutions of different concentrations (at least 12 samples covering the whole range of concentrations) were injected. The calibration graph was found to be linear in the range 3–17 μg ml^{-1}. However, a log–log calibration line exhibited linearity from 3 to 60 μg ml^{-1}

Table 2
Recovery and precision data in the determination of amiodarone in synthetic samples

Concentration added ($\mu g\,ml^{-1}$)	Concentration found ($\mu g\,ml^{-1}$)[a]	Recovery
7.0	7.08 ± 0.03	101.1
10.0	9.98 ± 0.16	99.8
15.0	14.94 ± 0.10	99.6
20.0	20.10 ± 0.26	100.5
50.0	49.40 ± 0.22	98.8

[a] Mean of six determinations ± S.D.

and this was used instead in order to increase the linear range. The regression equation obtained was:

$$\mathrm{Log}(\Delta V) = (2.02 \pm 0.04)\ \log C + (0.01 \pm 0.02), \quad (r^2 = 0.998)$$

where ΔV is the CL signal in mV, C the concentration of amiodarone in $\mu g\,ml^{-1}$ and r is the correlation coefficient.

3.3.3. Accuracy and precision

The accuracy of the method was tested with several synthetic samples of amiodarone with different concentrations. The results obtained are shown in Table 2, from which it is clear that both recovery and repeatability are excellent.

The inter-day precision of the method was studied by analysing three identical samples containing 15.0 $\mu g\,ml^{-1}$ of amiodarone. On 5 consecutive days, each sample was injected 10 times every day. The R.S.D. was 2.8%.

3.3.4. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD was studied from the equation $\mathrm{LOD} = kS_B/m$, where the standard deviation (S_B) from 12 blank determinations, the slope of the calibration curve (m) and $k = 3$ were used. The calculated LOD was 0.28 $\mu g\,ml^{-1}$.

The LOQ was estimated using the above equation but taking $k = 10$. The LOQ was 0.93 $\mu g\,ml^{-1}$.

3.3.5. Specificity

As the procedure described is for application to pharmaceutical preparations, the presence of excipients was evaluated. The criterion for interference was a relative error of less than ±3% of the average CL signal taken for an amiodarone concentration corresponding to 10.0 $\mu g\,ml^{-1}$. The excipients found in the pharmaceutical preparation analysed lactose, povidone, starch, magnesium stearate and bencilic alcohol as well as polyvinylpirrolidone, agar, talc, glucose, saccharin sodium, sucrose, sorbitol and benzalkonium chloride had no effect on the amiodarone CL response at higher concentrations than those usually present in pharmaceutical products.

3.3.6. Applications

The proposed FI method was applied to the determination of amiodarone on two commercially available formulations.

Table 3
Determination of amiodarone in pharmaceutical preparations

Brand name	Proposed FI method[a]	Reference method[b]
Trangorex ampoules (150 mg per 3 ml; mg ml^{-1})	49.1 ± 0.2	49.2 ± 0.1
Trangorex tablets (200 mg per tablet; mg)	198.5 ± 0.3	199.1 ± 0.2

[a] Mean of four determinations ± S.D.
[b] Official method [11]. Values are the mean of three determinations ± S.D.

Table 4
Recovery of amiodarone in pharmaceutical preparations

Sample	Amount added (g)	Amount found[a] (mg)	Recovery
Trangorex (ampoule)	50	49.7 ± 0.1	99.4
	60	60.2 ± 0.2	100.3
	80	79.2 ± 0.1	99
Trangorex (tablet)	45	44.1 ± 0.2	98
	80	78.2 ± 0.2	97.7
	120	119.3 ± 0.1	99.4

[a] Mean of four determinations ± S.D.

Table 3 shows the results obtained which are in good agreement with the pharmacopeia reference method and the declared content.

The recoveries were determined by adding various amounts of amiodarone to each pharmaceutical preparation and substracting the results obtained for pharmaceuticals to which no amiodarone had been added. The obtained recoveries were in the range 97.7–100.3% (Table 4), indicating that the proposed FI method is sufficiently accurate and no interfering substances were encountered.

4. Conclusions

The CL reaction of amiodarone with $Ru(bpy)_3^{3+}$ photogenerated on line is analytically useful. The present method offers several advantages, which are inherent in the FI technique: low reagent consumption, high sampling frequency (120 injections h^{-1}) and ease of automation. In addition, it is not expensive because the merging zones approach provides a reagent consumption of only 0.37 μmol (0.27 mg) of $Ru(bpy)_3Cl_2{\cdot}6H_2O$ per determination. The proposed method is suitable for the precise, accurate and rapid determination of amiodarone in pharmaceutical dosage forms.

When this method is compared with other methods proposed for determining of this drug in pharmaceuticals (Table 5) it can be seen that its sensitivity is higher than the most of them. Only methods with sample preconcentration [9,10] yielded higher sensitivity but the instrumental set-up is much more complex and expensive.

Table 5
Summary of methods for the determination of amiodarone in pharmaceutical formulations

Method	Characteristics	Concentration range ($\mu g\,ml^{-1}$)	References
Titrimetry	Potentiometric titration in non-aqueous media with a 0.1N $HClO_4$ solution		[11]
Spectrophotometry	Charge-transfer complex with: *p*-chloroanilic acid	10.0–360	[13]
	DDQ	2.0–65	[13]
Spectrophotometry	Indirect	2.0–12	[12]
Fluorimetry	Native fluorescence in 0.1N sulphuric acid medium	40–200	[15]
Sensor	Membrane electrode based on an amiodarone-dipicrylamine ion-pair complex	6.45–6450	[19]
HPLC	Column: C_{18}. Mobile phase: methanol–THF–0.1 M triethylamine. UV-detection	12.3–37	[17]
HPLC	Column: nitrile. Mobile phase: acetonitrile–ammonia acetate. UV-detection		[16]
CE	Electrokinetic injection with field-amplified sample stacking. UV detection	0.004–0.1	[9]
MEKC	Electrokinetic injection with field-amplified sample stacking. UV detection	0.08–20	[10]
FIA	$Ru(bpy)_3{}^{3+}$ generated on-line and CL detection	3–60	This work

CE, capillary electrophoresis; MEKC, micellar electrokinetic chromatography; FIA, flow injection analysis; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

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