Flow injection analysis–direct chemiluminescence determination of ergonovine maleate enhanced by hexadecylpyridinium chloride

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A flow injection analysis–direct chemiluminescence method is proposed for the determination of ergonovine maleate, based on the oxidation of the drug by hexacyanoferrate(III) in sodium hydroxide. The chemiluminescence was enhanced by a cationic surfactant, hexadecylpyridinium chloride. The calibration graph was linear over the range from the limit of detection (70 ppt) to 1 ppm of ergonovine maleate, with an RSD (n = 50, 50 ppb) of 1.8% and a sample throughput of 118 h^{-1}. The influence of foreign compounds was tested. The method was applied to the determination of ergonovine maleate in synthetic samples.

Aim of investigation

Ergonovine is a naturally occurring alkaloid isolated from ergot and it has no α-adrenergic blocking activity, a direct stimulating action on smooth muscle, especially that of the blood vessels and the uterus, and antiserotonin effects. It may be used in obstetrics to prolong uterine contractions in the later stages of labour and to check *post-partum* haemorrhaging.[1]

The analytical profiles of ergonovine maleate have been broadly reviewed elsewhere[2] and no chemiluminescent behaviour was reported. Most of the currently used procedures for the determination of ergot alkaloids, including official recommendations in some pharmacopoeias and in the AOAC Official Methods of Analysis,[3] are spectrophotometric and based on the reaction of the alkaloid with *p*-dimethylaminobenzaldehyde, commonly known as the Van Urk reaction. The earlier procedures have been improved in different ways, usually by employing iron(III), nitrite and even UV irradiation[4] in a flow assembly.

A search for methods in *Analytical Abstracts*, from 1980 to 1997, yielded only 24 papers dealing with ergonovine or ergometrine. The methods reported included HPLC[5] and immunoassay[6,7] and also spectrophotometric, spectrofluorimetric and amperometric[8] methods. Thus, as far as we know, the work described here is the first attempt to determine ergonovine by a direct chemiluminescence (CL) procedure. Two flow injection analysis (FIA)[8,9] procedures have been published.

The analytical applications of liquid-phase CL can be divided into those procedures that involve well known CL reactions and those that involve direct CL of the analyte, which is responsible for light emission.[10,11] The present paper deals with the latter and probably simpler approach. Although a number of papers have been published dealing with FIA–direct CL of pharmaceuticals,[10–12] this topic is still a growing area in analytical chemistry[13] and much experimental effort (screening procedures) has been expended in elucidating the direct chemiluminescent behaviour of different kinds of substances mainly owing to the analytical advantages arising from low detection limits, wide linear dynamic ranges, speed of response and reproducible mixing of sample and reagents near the detector.

As a result of an extensive study of molecular connectivity,[14–16] the chemiluminescent behaviour of ergonovine was predicted; subsequently, this theoretical behaviour was established by empirical evidence, such that ergonovine showed CL on reaction with some common strong oxidants, mainly hexacyanoferrate(III), which has previously been employed in the direct CL determination of thiamine[17] and some tetracyclines,[18] in addition to its use for the oxidation of luminol.[19] Thus, the aim of the present work was to develop a FIA system suitable for the determination of ergonovine.

Experimental

Reagents

All solutions were prepared from analytical-reagent grade materials using distilled, de-ionized water. The following reagents were used: ergonovine maleate, maleic acid and sodium dodecyl sulfate (Sigma); $K_3Fe(CN)_6$, NaOH (pellets), $KMnO_4$, Ce(IV) and ammonium nitrate, 37% HCl, $AgNO_3$, $Ca(ClO)_2$ and boric acid (Panreac); hexadecylpyridinium chloride (HD) (Fluka); $K_2S_2O_8$ and soluble starch (Merck); KIO_4 (Probus); 33% H_2O_2, KOH (pellets), 95–97% H_2SO_4, Triton X-100 and D-(+)–sucrose (Scharlau); L-(+)-ascorbic acid (UCB); sodium saccharin, magnesium stearate, codeine phosphate and paracetamol (Guinama); anhydrous caffeine (Doesder); and D-(+)-glucose (BDH).

Materials and apparatus

The flow injection manifold finally adopted (Fig. 1) consisted of a peristaltic pump (Gilson Minipuls 2) which pumped alkaline medium (Q_1, 4.2 ml min^{-1} of 9 mol l^{-1} NaOH), surfactant (Q_2, 4.2 ml min^{-1} of 0.5×10^{-3} mol l^{-1} HD) and oxidant [Q_3, 3.2 ml min^{-1} of 5×10^{-4} mol l^{-1} $K_3Fe(CN)_6$] solutions through

PTFE tubes (0.8 mm id). The first two solutions were mixed at a W-shaped connector before sample injection (465 μl of aqueous ergonovine maleate solution per injection; injection valve: Rheodyne, Model 5041), then mixed with the reagent stream in a T-shaped junction positioned 2 cm from the entrance to the flow cell, which consisted of a flat spiral-coiled quartz tube (1.0 mm id, total diameter of the flow cell 3 cm, without gaps between loops). The flow cell was placed 2 mm from the photomultiplier tube (end window, type 9902, Thorn–EMI) and backed by a mirror for maximum light collection. The T-piece, flow cell and photomultiplier were placed in a home-made, light-tight box. The photomultiplier was operated at −1273 V, supplied by a PHV-40 programmable photomultiplier high voltage power supply (Acton Research). The output was fed to a voltmeter and a multi-speed variable span recorder (Omniscribe).

Results and discussion

Preliminary work

The reactivity of ergonovine maleate (50 ppm) with different oxidants was studied by means of the manifold and the home-made luminometer depicted in Fig. 2. The oxidants tested were: (a) in 1 mol l^{-1} H_2SO_4 medium: $KMnO_4$, Ce(IV) and $K_2S_2O_8$ (Ag^+); and (b) in 1 mol l^{-1} NaOH medium: $K_2S_2O_8$ (Ag^+), H_2O_2, $Ca(ClO)_2$ and $K_3[Fe(CN)_6]$.

The analytical signal was always calculated as sample output minus blank. The highest signal was obtained when the oxidation took place in alkaline medium with potassium hexacyanoferrate(III). The signal was 318.1 mV *versus* the second highest, 46.8 mV, obtained with potassium persulfate oxidation in alkaline medium. As potassium hexacyanoferrate(III) has an almost constant redox potential between pH 4 and 13 and yields the maximum signal, it was selected as the most suitable oxidant.

Next, and bearing in mind the influence that some trace level impurities present in the reagents might have on the CL, a study of the influence of NaOH or KOH on the reaction was carried out employing the same flow manifold. Sodium hydroxide (as specified under Reagents) was selected as the optimum reagent, because the signal was more than twice that obtained with other alkaline reagents.

The next study was performed with the aim of determining the effect of the alkaline medium on the CL reaction. NaOH solution was merged with either sample and reagent (sodium hydroxide flowing by Q_2 and Q_3, see Fig. 2) or with only one of them (only Q_2 or Q_3), before the CL reaction in the flow cell. The analytical signal ranged from 0.0 (H_2O flowing through Q_2 and Q_3) to 325.0 mV when 1 mol l^{-1} NaOH circulated through both channels, Q_2 and Q_3, which was selected for further work.

Next, the influence of NaOH concentration (Q_2 and Q_3) was tested; the results are depicted in Fig. 3. A concentration of 0.50 mol l^{-1} was selected as the most suitable.

The effect of the oxidant (Q_4) concentration was also tested and, bearing in mind the results depicted in Fig. 4, a concentration of 3×10^{-4} mol l^{-1} was selected.

The influence of the amount of dissolved oxygen in the solutions was also studied by testing two types of treated solutions: (a) solutions from which oxygen was removed by passing a nitrogen stream, and (b) solutions in which the oxygen content was increased to saturation level by passing an air stream. The solutions tested contained only the alkaline medium and oxidant because ergonovine solutions might be degraded by the dissolved oxygen.[2] The CL signal was clearly diminished to 40% when solutions were de-oxygenated and was unaltered when they were oxygenated. Hence, untreated solutions were selected for further work.

Bearing in mind the influence that surfactants might have on the CL emission,[20] which has been studied in a number of cases but without significant results,[21,22] different kinds of surfactant (namely non-ionic, anionic and cationic) were tested at their critical micellization concentration (c.m.c.), merging with the alkaline medium before it merged with the sample. Merging with the oxidant was avoided because a precipitate was formed. While Triton X-100 (non-ionic; c.m.c., 0.24 mM) and sodium dodecyl sulfate (anionic; c.m.c., 8.27 mM) had no influence on the CL signal, hexadecylpyridinium chloride (HD) (cationic; c.m.c., 0.24 mM) gave a signal twice that without surfactant. Hence, HD was selected for further work.

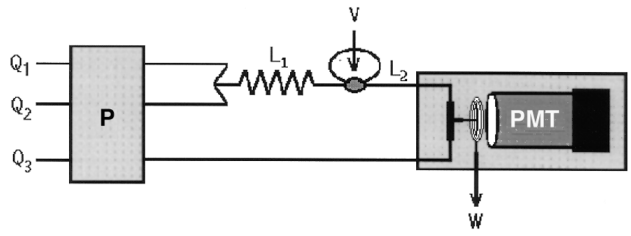

Fig. 1 FIA assembly finally adopted. Q_1, carrier (4.2 ml min^{-1} of 9 mol l^{-1} NaOH); Q_2, surfactant (4.2 ml min^{-1} of 0.5×10^{-3} mol l^{-1} HD); and Q_3, oxidant [3.2 ml min^{-1} of 5×10^{-4} mol l^{-1} $K_3Fe(CN)_6$]; V, injection valve (465 μl of aqueous ergonovine maleate solution); L_1 = 600 cm; L_2 = 99.3 cm; PMT, photomultiplier tube; P, peristaltic pump; and W, waste.

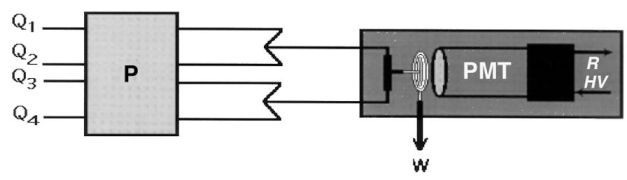

Fig. 2 Flow injection assembly used in the preliminary work. Q_1: oxidant, 0.02 mol l^{-1}; Q_2 and Q_3: medium, 1 mol l^{-1}; Q_4: ergonovine maleate, 50 ppm (or de-ionized water blank). Flow rate: 2.8 ml min^{-1} in all channels. HV: high voltage power supply; R: recorder; P: peristaltic pump; V: injection valve; L_2 injection valve–flow cell distance; and, W: waste.

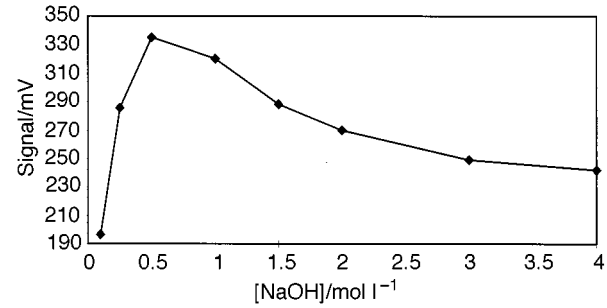

Fig. 3 Influence of NaOH concentration on the oxidant and ergonovine streams.

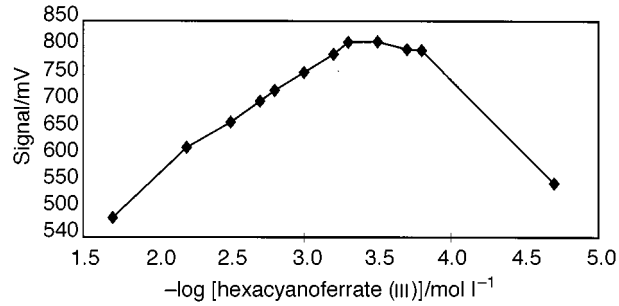

Fig. 4 Influence of potassium hexacyanoferrate(III) concentration on the intensity of chemiluminescent emission.

Finally, the influence of flow rate was studied. This parameter can be critical owing to its influence over the point in the flow manifold at which the excited molecule emits light and consequently over the signal magnitude: too high flow rates can result in emission after the flow cell and too low flow rates in emission before it. It was observed that the flow rate should be as high as possible, which might indicate that reaction speed and emission are fast. Nevertheless, a final flow rate of 14.6 ml min^{-1} (3.7 ml min^{-1} in each channel) was selected in order to avoid pressure build up problems in the manifold, mainly at the flow cell.

A pre-calibration of ergonovine maleate was then carried out and 0.5 ppm was selected for further work.

Studies in a FIA manifold

Next, the continuous flow manifold was replaced by a FIA manifold assembly and the pre-selected variables were re-optimised. Different FIA manifolds were tested so that sodium hydroxide was assayed merging with the sample before its insertion, with the oxidant and as the carrier. The best results (Fig. 1) were obtained when NaOH (Q_1) merged with HD (Q_2) before sample insertion. Table 1 shows the variables studied, with the ranges studied and the values selected. Then, after a new pre-calibration, 0.1 ppm of ergonovine maleate was selected for further work.

At this point, FIA variables were optimised, suppressing the HD solution stream (channel Q_2) to prevent the observed degradation of the HD–NaOH mixture when the time of contact was long, particularly when the flow rate was very low. The NaOH concentration was then duplicated (Q_1) to keep it at the selected value. The modified simplex multivariate optimisation method[23] was applied. The parameters studied and ranges tested were as follows: Q_1 and Q_3 (ml min^{-1}) 0.6–6.9; sample volume (μl) 108–610; and injection valve–flow cell length (cm) 0.0–200.0. The selected apex, after testing 44 apices, presented the following figures: Q_1 5.7; Q_3, 1.9; V, 465; and, L_2 99.3.

Then, the flow through Q_2 was reinstated and the chemical and some FIA variables (*viz.* L_1, Q_1, Q_2 and Q_3), were further re-optimised by a univariate method. Table 2 shows both the tested and selected values.

Analytical applications

With the optimised manifold, the linear (log–log) range was between the limit of detection (LOD) and 1 ppm of ergonovine maleate. The LOD (70 ppt) was experimentally determined as the concentration yielding a signal three times the background noise. The linear (log–log) equation was $\log I = 3.5 + 0.859 \log C$ ($n = 18$, $r = 0.997$), where C is the ergonovine maleate concentration in ppm and I is the CL signal obtained in mV.

When the calibrations were performed without HD, very small differences were observed between the signal outputs corresponding to ergonovine solutions whose concentrations ranged from 0 to 0.01 ppm. However, as the ergonovine concentration rose, the above-mentioned differences grew. Namely, the linear equation obtained for ergonovine maleate concentrations in the range from the LOD to 0.05 ppm, without surfactant, was $I = 3.3 + 4567.9C$, with a slope 25% smaller than with surfactant. The differences increased as the concentrations rose. This might be attributed to an auto-quenching or auto-absorption effect of ergonovine which would be avoided by the surfactant.

A between-day reproducibility study was performed by working each day (6 days) with freshly prepared solutions, with ergonovine concentrations in the range from the LOD to 0.05 ppm. The mean ($n = 6$) slope obtained was 6012.5, with an RSD of 4.3%.

The average insertion rate for 50 peaks (0.05 ppm) was 118 h^{-1}. The RSD thus obtained was 1.8%.

The influence of different foreign compounds (*versus* 0.02 ppm of ergonovine maleate) which can be found accompanying ergonovine in preparations was also studied. Table 3 shows the results obtained; a competitive selectivity can be observed; only paracetamol requires a concentration similar to the analyte; all the other active drugs, excipients or protecting agents studied did not affect the determination.

The proposed method was evaluated by analysing galenic preparations of ergonovine prepared according to ref. 1 (there were no commercial pharmaceutical formulations in Spain). The results obtained were compared with those obtained with the official methods based on the reaction with *p*-dimethylaminobenzaldehyde and absorbance measurement at 545 nm.[24] Table 4 shows a good agreement between the results, with mean relative errors between +2.4 and −0.95%.

Table 1 Univariate pre-optimisation of chemical variables and flow rate

Variable	Range studied	Value selected
C (NaOH, Q_1)/mol l^{-1}	2.0–12.0	8.0
C (hexacyanoferrate(III), Q_3)/mol l^{-1}	(0.06–2.00) × 10^{-3}	3 × 10^{-4}
C (HD, Q_2)/mmol l^{-1})	0.1–3.6	0.9
Flow rate (Q_3)/ml/min^{-1}	2.2–4.4	4.1
($Q_1 + Q_2 = Q_3$ and $Q_1 = Q_2$)		

Table 2 Re-optimisation of chemical and FIA parameters by the univariate method

Variable	Range studied	Value selected
C (NaOH, Q_1)/mol l^{-1}	5.0–10.0	9.0
C (hexacyanoferrate(III), Q_3)/mol l^{-1}	(0.7–7.0) × 10^{-4}	5 × 10^{-4}
C (HD, Q_2)/mmol l^{-1}	0.0–3.6	0.5
$Q_1 = Q_2$/ml min^{-1}	1.6–5.4	4.2
Q_3/ml min^{-1}	0.6–3.8	3.2
L_1/cm)	200.0–700.0	600.0

Table 3 Influence of foreign compounds on the FIA outputs *versus* outputs of 0.02 ppm ergonovine maleate solutions

Foreign compound	Concentration (ppm)	Relative error (%)
Ascorbic acid	2	+0.27
Starch	4	−1.98
Sodium hydrogensulfite	1	−1.07
Caffeine	3	+1.13
Hydrochloric acid	9	−0.96
Sodium chloride	4	+0.89
Codeine	3	−0.95
Magnesium stearate	3	−0.14
Glucose	3	−1.48
Lactose	4	+0.58
Maleic acid	7	−0.38
Paracetamol	0.02	−2.24
Sucrose	5	−0.70
Sodium saccharin	18	+1.22

Conclusions

The direct CL of ergonovine maleate has been reported, from which a rapid (118 h^{-1}) and reproducible (RSD = 0.8%, n = 50, 50 ppb) FIA method is proposed for its determination. The chemiluminescent emission was enhanced by a surfactant, hexadecylpyridinium chloride.

The method has the usual advantages of CL methods, namely a very low LOD (equivalent to 32.6 pg by injection) and a wide

Table 4. Galenic samples prepared in the laboratory according to ref. 1. Relative errors (E_r) were calculated for both methods *versus* the prepared ergonovine amount. All reported results are in ppm

Sample	Results (FIA method)	Results (reference method)	E_r (FIA method) (%)	E_r (reference method) (%)
Ergometrine Injection	0.0128	40.58	2.40	1.45
Injectable Ergometrine	0.0198	39.63	−0.95	−0.93

linear range (from the LOD to 1 ppm). The proposed method also presents for ergonovine determination a competitive sensitivity similar to that presented by HPLC[5] or immunological[6,7] methods, but higher sample throughput and a degree of selectivity (higher than any other published FIA[4,9] procedure) which makes a separation step unnecessary.

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