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Flow-injection chemiluminometric analysis of some benzamides by their sensitizing effect on the cerium-sulphite reaction

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Abstract

A simple, highly sensitive chemiluminescent method using flow injection is described for the determination of three substituted benzamides, namely: sulpiride, sultopride and tiapride. The method is based on the sensitizing effect of these drugs on the chemiluminometric oxidation of sulphite by cerium(IV). The different experimental parameters affecting the chemiluminescence intensity were carefully studied and incorporated into the procedure. The method permits the determination of 0.05–2.5 μg ml^{-1} sulpiride, 0.01–2.5 μg ml^{-1} sultopride hydrochloride and 0.01–1.5 μg ml^{-1} tiapride hydrochloride with minimum detectability of 0.01 μg ml^{-1}. The method was applied to the determination of these benzamides in pharmaceutical preparations and biological fluids.

Keywords: Benzamides; Chemiluminescence; Cerium(IV)-sulphite reaction; Pharmaceuticals; Biological fluids

1. Introduction

Sulpiride, sultopride and tiapride (Fig. 1) are substituted benzamides which exhibit antipsychotic properties [1]. They are antagonist of the dopamine D_2 receptors, which distinguishes these compounds from other anti-psychotic agents. This singular feature may explain the very low incidence of side-effects on the extrapyramidal system. In addition to their anti-psychotic action, substituted benzamides present anti-emetic, anti-dyskinetic and anti-hypertensive action [1].

The therapeutic importance of these compounds required the development of sensitive, rapid and industrial quality control and clinical monitoring. A review of the literature revealed that few methods have been described for their determinations and only sulpiride is official in BP [2], while sultopride and tiapride are not official yet. The BP [2] recommends a non-aqueous titration method with potentiometric detection of the end-point for the evaluation of the raw material

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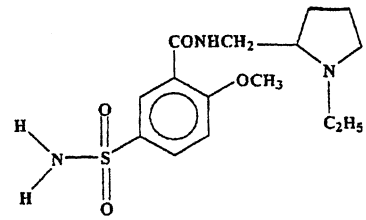
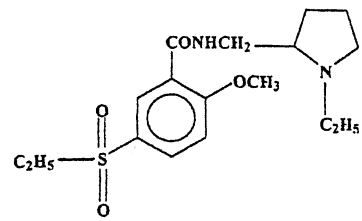
1. Sulpiride



2. Sultopride



3. Tiapride

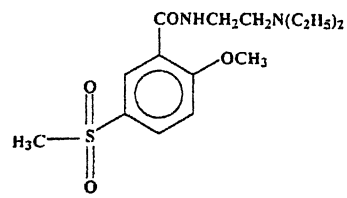


Fig. 1. Structures of the studied substituted benzamides.

of sulpiride. Most analytical methods employed for the determination of benzamides in formulations and biological fluids are chromatographic, including HPLC [3–8], GC [9] and TLC [10]. Other reported methods are titrimetric [11], spectrophotometric [11,12], fluorimetric [13], oscillopolarographic [14] and radioimmunoassay [15].

Reviewing the literature revealed that, up to the present time, nothing has been published concerning the chemiluminescence (CL) determination of the substituted benzamides.

The oxidation of sulphite in acidic solutions is a well known chemiluminescent reaction and the analytical properties of the reaction have been thoroughly studied by using potassium permanganate [16–19] or cerium(IV) [20]. The emission has been attributed to the formation of excited sulphur dioxide molecules which radiate during de-excitation [17]. The emission intensity is greatly enhanced by the presence of some compounds (sensitizers) in the reaction solution. Typical examples of sensitizers are riboflavin for the reactions with permanganate [21,22] and cerium(IV) [23], flavinmononucleotide for the reaction with permanganate [24] and 3-cyclohexylaminopropanesulphonic acid (CAPS) for the reactions with permanganate [25] and cerium(IV)

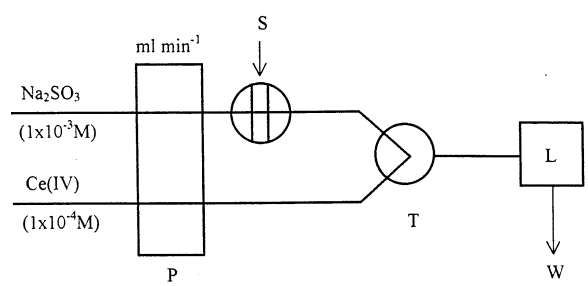


Fig. 2. FI manifold for the chemiluminescent determination of the substituted benzamides: p, peristaltic pump; S, sample port; T, perspex T-piece; L, luminometer; W, waste.

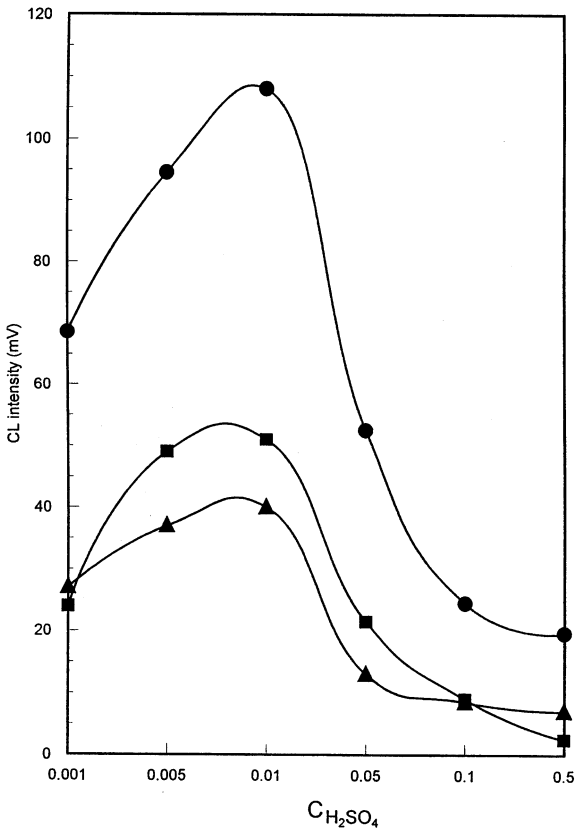


Fig. 3. Effect of H_2SO_4 concentration as a diluent for Ce(IV) on CL intensity. Injected drug solution 1.0 μg ml^{-1}. ●—●, tiapride hydrochloride; ■—■ sulpiride; ▲—▲, sultopride hydrochloride. Ce(IV) 1×10^{-4} M, Na_2SO_3 1×10^{-3} M, loop size 500 μl for tiapride HCl and 200 μl for the other two drugs.

[26]. Quinine and quinidine act as sensitizers on the oxidation of sulphite by cerium(IV) owing to their fluorescent properties. Both compounds have been determined in dosage forms [27]. Also, bile acids were found to enhance the emission intensity from the chemiluminogenic oxidation of sulphite by cerium(IV), potassium permanganate, bromate and dichromate [28].

This paper describes the assay of some benzamides in bulk, in dosage forms and biological fluids by their sensitizing effect on the CL oxidation of sulphite by cerium(IV).

2. Experimental

2.1. Apparatus and flow system

The flow system used for the determination of the substituted benzamides with CL detection is shown schematically in Fig. 2. A Gilson Minipuls 3MP4 peristaltic pump (two channels, variable speed), was used to drive the carrier and the reagent streams through the flow system. Each stream was pumped at a constant flow rate of 3.75 ml min^{-1} using PTFE tubing (0.8 mm i.d.). Benzamides solutions were injected through the sample injection valve which allows mixing of the sample with the sulphite solution and then combination with Ce(IV) solution just before the detec-

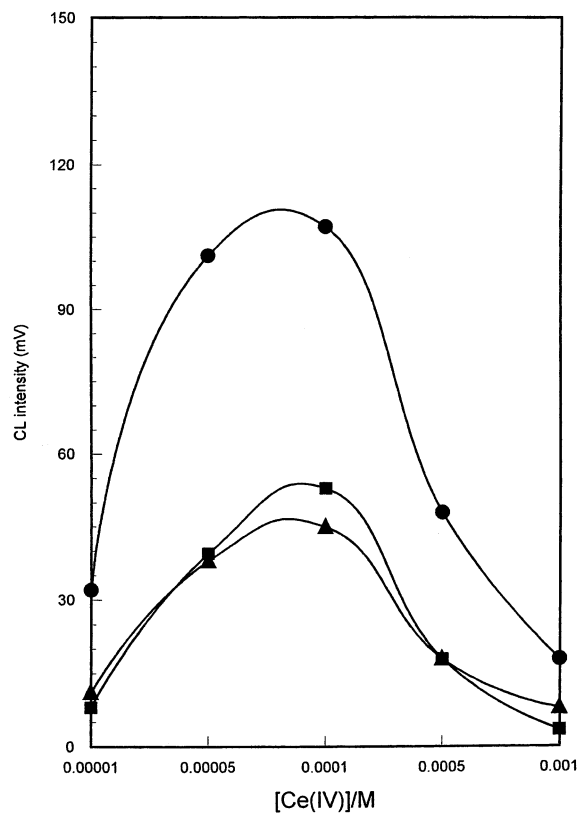


Fig. 4. Effect of Ce(IV) concentration on CL intensity. Injected drug solution 1.0 μg ml^{-1}. ●—●, tiapride hydrochloride; ■—■, sulpiride; ▲—▲, sultopride hydrochloride. Na_2SO_3 1×10^{-3} M, loop size 500 μl for tiapride HCl and 200 μl for the other two drugs.

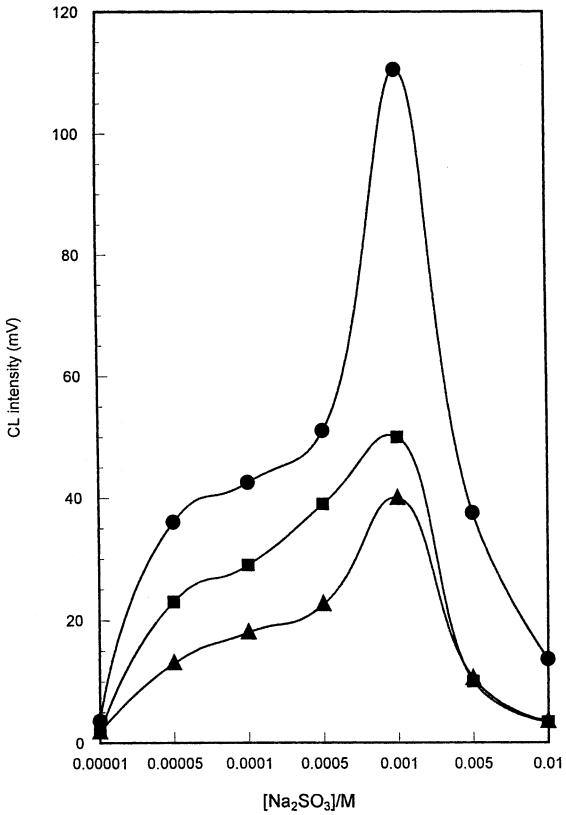


Fig. 5. Effect of Na_2SO_3 concentration on CL intensity. Injected drug solution 1.0 μg ml^{-1}. ●—●, tiapride hydrochloride; ■—■, sulpiride; ▲—▲, sultopride hydrochloride. Ce(IV) 1×10^{-4} M, loop size 500 μl for tiapride HCl and 200 μl for the other two drugs.

tor. The emitted light was measured by a photomultiplier tube (Thorn EMI, 9789QB) which was operated at 1200 V. The signal was recorded by a Yokogawa model 3021 recorder (Yokogawa, Japan). Peak heights were measured.

2.2. Reagents and materials

Benzamides were kindly provided by Synthelabo Recherche (Bagneux Cedex, France) and used as received. Dosage forms of benzamides were obtained from local commercial sources. Stock standard solutions (1 mg ml^{-1}) of all substituted benzamides were prepared in distilled water, except for sulpiride, which was prepared in 1 ml of 0.1 M sulphuric acid and further diluted with distilled water. Working standard solutions of benzamides were prepared by appropriate dilution immediately before use. Other reagents used were: aqueous sodium sulphite solution (Merck, Germany), 1×10^{-3} M; aqueous cerium sulphate solution (Merck), 1×10^{-4} M; sulphuric acid solution (BDH Ltd., Poole, UK), 0.1 and 1×10^{-2} M; sodium hydroxide solution (Merck), 0.5 M; and ethyl acetate (BDH).

2.3. Procedures

2.3.1. General procedure

Working solutions of substituted benzamides in the range cited in Table 1 were prepared from the stock solutions. A 200 μl portion of sulpiride or sultopride or a 500 μl portion of tiapride was injected into a stream of 1×10^{-3} M sodium

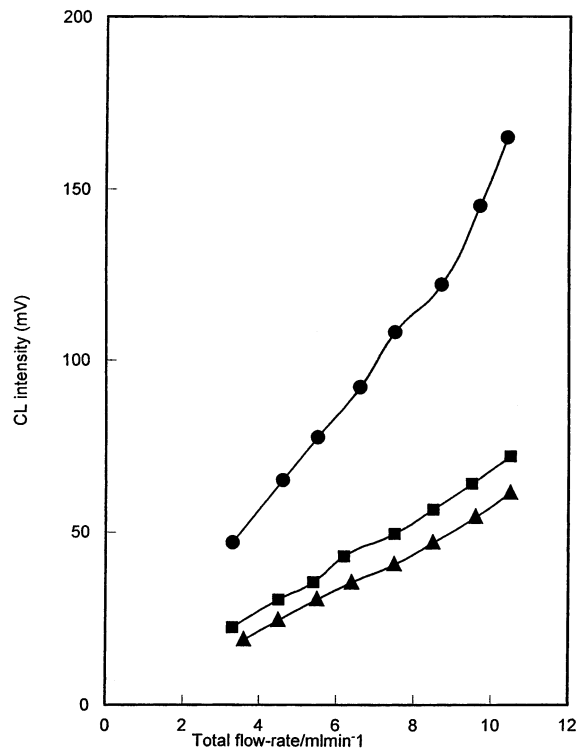


Fig. 6. Effect of total flow rate on CL intensity of substituted benzamides. Injected drug solution 1.0 μg ml^{-1}. ●—●, tiapride hydrochloride; ■—■, sulpiride; ▲—▲, sultopride hydrochloride. Ce(IV) 1×10^{-4} M, Na_2SO_3 1×10^{-3} M, loop size 500 μl for tiapride HCl and 200 μl for the other two drugs.

Table 1
Analytical characteristics for the chemiluminometric determination of the studied benzamides

Compound	Linear calibration range (μg ml^{-1})	Limit of detection[a] (μg ml^{-1})	Regression equation $I^{b} = a + bC$	δ^{c} (a)	δ^{c} (b)	Correlation coefficient[d]	Found (%)[e]	
							Proposed method	Fluorimetric method[13]
Sulpiride	0.05–2.5	0.01	$I = 4.82 + 109$ C	0.3720	0.2830	0.99998	99.8 ± 0.5	99.3 ± 0.8
Sultopride hydrochloride	0.01–2.5	0.01	$I = 2.38 + 91$ C	0.3042	0.2456	0.99997	100.1 ± 0.5	99.6 ± 1
Tiapride hydrochloride	0.01–1.5	0.01	$I = 3.82 + 178$ C	0.5417	0.7600	0.99995	99.3 ± 0.7	98.9 ± 0.4

[a] s/n = 3.
[b] Intensity (mV).
[c] $\delta(a)$ and $\delta(b)$ are S.D. for coefficients a and b.
[d] Eight data points.
[e] Mean ± S.D. ($n = 5$).

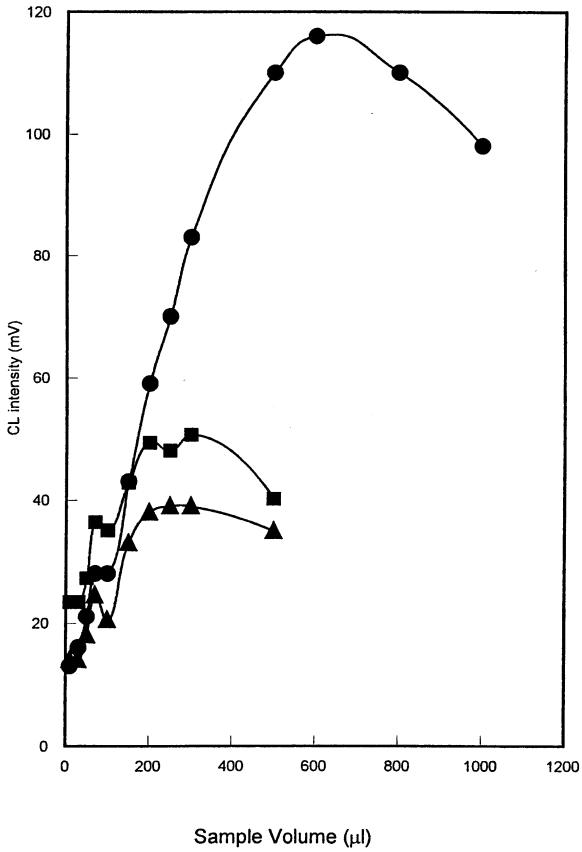


Fig. 7. Effect of sample volume of the substituted benzamides on CL intensity. Injected drug solution 1.0 μg ml^{-1}. ●—●, tiapride hydrochloride; ■—■, sulpiride; ▲—▲, sultopride hydrochloride. Ce(IV) 1×10^{-4} M and Na_2SO_3 1×10^{-3} M.

sulphite solution which was then combined with a stream of 1×10^{-4} M cerium(IV) solution and the resulting peak heights were measured. Calibration graphs were prepared by plotting the peak heights against the drug concentration over the range cited in Table 1.

2.3.2. Procedure for tablets and capsules

An accurately weighed amount of ten powdered tablets or mixed contents of ten capsules, equivalent to 10.0 mg of the drug, was transferred into a 100-ml calibrated flask. Then, 1.0 ml of 0.1 M sulphuric acid was added to the flask and completed to the mark with distilled water. The flask with its contents were sonicated for 5 min and filtered. Proceed as described in Section 2.3.1. The nominal content was calculated from the corresponding calibration graph or regression equation.

2.3.3. Procedure for ampoules

The contents of 20 ampoules were mixed. An accurately measured volume of the mixed solution, equivalent to 10.0 mg of the drug, was transferred into 100-ml calibrated flask and made up to the mark with distilled water. Proceed as described in Section 2.3.1. The nominal content was calculated from the corresponding calibration graph or regression equation.

2.3.4. Procedure for spiked biological fluids

An aliquot of urine or plasma (1.0 ml) in a 15-ml centrifuge tube was spiked with an aliquot of aqueous solution of the benzamide containing 250 μg. A total of 0.2 ml of 0.5 M sodium hydroxide was added and the solution was mixed gently. Three 10-min extractions with 5.0 ml ethyl acetate were carried out using a laboratory shaker. The upper organic layers obtained after centrifugation at 2500 rpm for 5 min were combined and evaporated to dryness under a stream of nitrogen at ambient temperature. The residue was dissolved in 2.0 ml of 0.1 M sulphuric acid and the solution was transferred into a 25-ml calibrated flask and completed to volume with distilled water. The general procedure was then followed (Section 2.3.1). A blank experiment was carried out adopting the above procedure.

3. Results and discussion

The sensitizing effect of the studied benzamides on the chemiluminescent reaction of the oxidation of sulphite in acidic solutions was studied using different oxidants. A very weak CL was obtained with some oxidants, such as potassium persulphate, potassium permanganate, potassium dichromate, potassium bromate and sodium hypochlorite. Other oxidants, such as potassium iodate, sodium periodate and *N*-bromosuccinimide gave no CL signals. Maximum CL intensity was obtained when cerium(IV) was used as an oxidant in an acidic medium. In the absence of

Table 2
Analysis of substituted benzamides in their dosage forms by the proposed and fluorimetric methods

Preparation	Concentration taken (μg ml^{-1})	Recovery (%)	
		Proposed method	Fluorimetric method[13]
Dogmatil capsules* (50 mg sulpiride/capsule)	0.2	100.0	
	0.5	101.1	
	1.0	99.8	
	1.5	99.4	
Mean ± S.D.		100.1 ± 0.7	100.4 ± 1.5
Barnetil ampoules* (100 mg sultopride/2 ml)	0.2	98.8	
	0.5	99.8	
	1.5	100.8	
	1.8	100.5	
Mean ± S.D.		100.0 ± 0.9	100.2 ± 1.2
Tipridal tablets* (100 mg tiapride/tablet)	0.1	98.0	
	0.2	101.5	
	0.6	100.0	
	1.0	100.0	
Mean ± S.D.		99.9 ± 1.4	100.1 ± 0.9
Tipridal ampoules* (100 mg tiapride/2 ml)	0.1	99.2	
	0.2	101.2	
	1.0	101.3	
	1.2	101.4	
Mean ± S.D.		100.8 ± 1.1	100.3 ± 0.8

* Products of Delagrange (France).

sulphite, no CL signals were obtained with all the studied oxidants in acidic or basic media.

3.1. Configuration designs

The flow injection configuration used for the determination of the studied benzamides was so designed to provide different reaction conditions for magnifying the CL signal generated by the sensitizing effect of the benzamide on the oxidation of sodium sulphite by cerium(IV). CL signal was obtained only when the sample was injected into a stream of 1×10^{-3} M sodium sulphite and then mixed with cerium(IV) prior to the detector.

The studied benzamides increase the weak radiation emitted during the CL oxidation of sulphite–cerium(IV) in sulphuric acid medium. The two reactants are continuously mixed and introduced into the flow cell and the weak CL radiation emitted from this reaction is continuously recorded as the baseline. When a benzamide drug is injected into the sulphite stream, the intensity is enhanced in proportion to its concentration.

3.2. Optimization of experimental variables

The effect of experimental conditions on the CL intensity was studied. A series of experiments were conducted to establish the optimum analytical variables. The parameters optimized included reagent concentrations and some physical variables, including the flow rate and the sample volume.

3.2.1. Effect of sulphuric acid concentration as a diluent for cerium(IV)

Ce(IV) is not readily soluble in water, but becomes stable when dissolved in dilute sulphuric acid. Therefore, the effect of H_2SO_4 concentration in the range 1×10^{-3} to 0.5 M was studied;

Table 3
Analytical characteristics for the chemiluminometric determination of the studied benzamides in urine and plasma

Compound	Plasma					Urine				
	Linear calibration range (μg ml^{-1})	Regression equation[a] $I=a+bC$	δ^{b} (a)	δ^{b} (b)	Correlation coefficient[c]	Linear calibration range (μg ml^{-1})	Regression equation[a] $I=a+bC$	δ^{b} (a)	δ^{b} (b)	Correlation coefficient[c]
Sulpiride	0.1–1.0	$I=1.13+64.6$ C	0.3856	0.6764	0.99989	0.1–1.0	$I=2.06+61.1$ C	0.2918	0.5118	0.99993
Sultopride hydrochloride	0.1–1.0	$I=1.27+47.0$ C	0.1703	0.2987	0.99996	0.1–1.0	$I=2.69+52.3$ C	0.1301	0.2282	0.99998
Tiapride hydrochloride	0.1–0.5	$I=-1.72+106$ C	0.3160	0.9990	0.99996	0.1–1.0	$I=3.08+80.0$ C	0.1988	0.3487	0.99998

[a] Intensity (mV).
[b] $\delta(a)$ and $\delta(b)$ are S.D. for coefficients a and b.
[c] Five data points.

Table 4
Determination of the studied benzamides in spiked urine and plasma

Compound	Concentration taken (μg ml^{-1})	Found (%)	
		Urine	Plasma
Supiride	0.1	101.4	98.9
	0.2	98.0	98.3
	0.5	101.5	101.9
	1.0	100.0	99.8
Mean ± S.D.		100.1 ± 1.5	99.7 ± 1.6
Sultopride hydrochloride	0.1	100.8	
	0.2	98.5	98.3
	0.5	100.5	101.1
	1.0	99.9	100.0
Mean ± S.D.		100.1 ± 1.2	100.1 ± 1.3
Tiapride hydrochloride	0.1	99.0	98.1
	0.2	99.5	100.7
	0.5	100.8	99.5
	1.0	99.9	100.8
Mean ± S.D.		99.8 ± 0.8	99.4 ± 1.3

1×10^{-2} M chosen for further studies as it gave the maximum CL intensity for each drug (Fig. 3).

3.2.2. Effect of cerium(IV) concentration

Fig. 4 shows the effect of Ce(IV) concentration on the CL intensity of each drug. The greatest CL response was obtained with 1×10^{-4} M. Higher concentrations of Ce(IV) lowered the CL intensity. Therefore, 1×10^{-4} M Ce(IV) was used for further investigation.

3.2.3. Effect of sodium sulphite concentration

The effect of sodium sulphite concentration on the CL intensity is shown in Fig. 5. The CL intensity was increased as the sulphite concentration increased from 1×10^{-5} up to 1×10^{-3} M, after which the intensity started to decrease. Therefore, the optimum sulphite concentration was chosen to be 1×10^{-3} M.

3.2.4. Effect of flow rate

The solutions of oxidant and sulphite were introduced into the manifold at equal flow rates. The effect of total flow rate on the CL intensity of each drug is shown in Fig. 6. The intensity increased with increasing flow rate. However, a total flow rate of 7.5 ml min^{-1} (3.75 ml min^{-1} for each channel) is recommended because of greater precision and economy in the use of reagents.

3.2.5. Effect of sample volume

The variation of the CL emission with the injected sample volume in the range 10–800 μl was studied. The obtained results showed an increase in the CL intensity up to 200 μl for sulpiride and sultopride and up to 500 μl for tiapride, above which the intensity was almost constant (Fig. 7). For tiapride, large sample volume is needed. This may be due to the absence of pyrrolidine ring in its structure (Fig. 1).

3.2.6. Effect of some micellar solutions

The effect of some organized systems, including neutral surfactants (Triton X-100), cationic surfactants (cetyltrimethylammonium bromide, cetylpyridinium bromide and cetylpyridinium chloride), and anionic surfactants (sodium dodecyl sulphate) on the CL reaction were investigated. All these surfactants had no effect on the CL intensity.

3.3. CL mechanism

Benzamides are fluorogenic compounds in aqueous solutions, therefore, they can be excited by energy transfer. So, the possible CL mechanism is:

$$HSO_3^- + Ce^{4+} \rightarrow HSO_3^{\bullet} + Ce^{3+}$$
$$2\ HSO_3^{\bullet} \rightarrow S_2O_6^{2-} + 2H^+$$
$$S_2O_6^{2-} \rightarrow SO_4^{2-} + SO_2^*$$
$$SO_2^* + \text{Benzamide} \rightarrow \text{Benzamide}^* + SO_2$$
$$\text{Benzamide}^* \rightarrow \text{Benzamide} + \text{light}$$

Sulphite acts as the reductant and the energy from the chemical reaction is released as chemiexcites sulphur dioxide, which emits radiation at wavelengths >300 nm [29]. The studied benzamides increase the weak radiation emitted during the CL oxidation of sulphite by cerium(IV) in sulphuric acid medium, so they act as sensitizers. This was further confirmed by injecting quinine instead of the benzamide and a very intense CL signal was obtained.

3.4. Determination of the studied benzamides

Under the described experimental conditions, a series of standard solutions over the concentration ranges cited in Table 1 was pumped, each as three replicates, to test the linearity of the calibration graph. A plot of the CL intensity versus concentration of the studied benzamides was linear over the ranges given in Table 1. The precision of the method was evaluated by analyzing pure samples of benzamides. The results in Table 1 were in accord with those obtained by the fluorimetric method [13].

3.5. Application of the method

3.5.1. Analysis of pharmaceutical preparations

In order to evaluate the analytical usefulness of the chemiluminescent method, the studied benzamides were determined in pharmaceutical preparations. The results in Table 2 are in accordance with those obtained by the fluorimetric method [13].

Statistical analysis [30] of the results by using the Student's t-test and the variance ratio F-test showed no significant difference between the two methods as regards accuracy and precision.

3.5.2. Analysis of spiked urine and plasma samples

The high sensitivity attained by the proposed method allows the determination of the studied benzamides in biological fluids. Recent studies have demonstrated that metabolism appears to be of limited importance in the urine excretion of some benzamides [31]. As a consequence, the proposed method appears convenient for therapeutic drug monitoring in urine. In addition, the combination of this CL technique with flow injection, requiring smaller volumes of samples, may be valuable for routine drug screening of patients under treatment.

The extraction procedure for biological fluids was performed by using ethyl acetate which was the best extraction solvent, as reported by Nicolas et al. [32]. Tables 3 and 4 show the performance data and the results of determination of studied benzamides in urine and plasma.

4. Conclusion

The proposed method is simple, accurate and precise. It allows the determination of the studied benzamides in pharmaceutical preparations and biological fluids. Solutions can be analyzed at a rate of 190 (sulpiride), 150 (sultopride hydrochloride) and 144 samples per hour (tiapride hydrochloride).

Acknowledgements

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