

## Flow-injection chemiluminometric determination of some phenothiazines in dosage forms and biological fluids

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### Abstract

A rapid and sensitive flow-injection chemiluminometric method is described for the determination of three phenothiazine derivatives, namely, fluphenazine hydrochloride, levomepromazine hydrochloride and trimeprazine tartrate. The method is based on the chemiluminescence (CL) induced by the oxidation of drugs with cerium(IV) in an acidic medium. The CL intensity is greatly enhanced when rhodamine B is used as a sensitizer in the case of levomepromazine hydrochloride and trimeprazine tartrate. The proposed method allows the measurement of 0.5–90  $\mu\text{g ml}^{-1}$  fluphenazine and 0.1–6.5  $\mu\text{g ml}^{-1}$  levomepromazine and trimeprazine. The limits of detection ( $3\sigma$ ) were 0.01  $\mu\text{g ml}^{-1}$  fluphenazine hydrochloride and 0.1  $\mu\text{g ml}^{-1}$  for the other two drugs. The method was applied successfully in determining the drugs in dosage form as well as in biological fluids. © 1998 Elsevier Science B.V.

**Keywords:** Phenothiazines; Chemiluminescence; Flow injection; Rhodamine-B; Cerium(IV); Pharmaceutical analysis; Biological fluids

### 1. Introduction

The phenothiazines – as a class – are among the most widely used drugs in medical practice. They are primarily employed in the management of patients with serious psychiatric diseases. In addition, many members of the group have other clinically useful properties, including antiemetic and antihistaminic effects and the ability to potentiate analgesics, sedatives and general anaesthetics [1].

Several methods have been reported for the determination of phenothiazines and their formulations. Both the BP [2] and USP [3] recommend a non-aqueous titration method for analysis of the raw

material of the studied drugs and spectrophotometric methods in the dosage form except for levomepromazine hydrochloride where the BP [2] recommends an acid–base titration for the analysis of its raw material. Other procedures for the determination of phenothiazines involving titrimetry, spectroscopy, polarography, fluorimetry and chromatography, have been reported in the literature. These have been reviewed by Blazek et al. [4,5], Fairbrother [6], Belikov and Moiseeva [7], and Tarasiewicz and Karpinska [8].

Regarding the determination of phenothiazines by chemiluminescence (CL), it is known that promethazine hydrochloride has been determined by the suppression of CL of the luminol–hydrogen peroxide–chromium(III) system [9]. Similarly, some phenothiazines have been determined by using the *bis*(2,4,6-

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trichlorophenyl) oxalate (TCPO)/hydrogen peroxide system [10].

CL analysis has some advantages such as sensitivity, speed, ease of use and simple instrumentation. Also, it can be further improved, with respect to reproducibility, by combining it with a flow-injection (FI) technique which has been used for the determination of many drugs [11,12].

FI chemiluminometric methods based on cerium(IV) oxidation have been reported for the determination of paracetamol [13], tryptophan [14], captopril [15] and folic acid [16]. Rhodamine-B has been used as the sensitizer for the CL reaction of cerium(IV) with captopril [15] and folic acid [16].

This paper describes the development of an FI method based on the CL, generated during the oxidation of some phenothiazine drugs by cerium(IV) in an acidic medium. The analytical procedure is simple, fast, and accurate. It has been satisfactorily applied for the determination of these phenothiazines in pharmaceutical preparations and biological fluids.

## 2. Experimental

### 2.1. Instrumentation

The CL measurements were made with a flow-injection CL analyser which features two basic components, a detector housing and a flow-through system which allows mixing of the sample with the acid and then combination with the Ce(IV) solution just before the detector. Both components were assembled in our laboratory and have been described in detail elsewhere [17].

### 2.2. Reagents

All the reagents used were of analytical-reagent grade and the solutions were prepared with doubly distilled water. Aqueous cerium(IV) sulphate (BDH, Poole, UK) stock solutions  $1 \times 10^{-2}$ ,  $1 \times 10^{-3}$  and  $5 \times 10^{-3}$  M were prepared in  $5 \times 10^{-2}$  M sulphuric acid, and the aqueous perchloric acid (Riedel de-Haen, Germany) was 2.0 M. An aqueous  $70 \mu\text{g ml}^{-1}$  rhodamine-B (Riedel de-Haen, Germany) was prepared from 0.01% stock solution.

### 2.3. Materials

Pure drug samples were kindly provided by the following pharmaceutical companies: fluphenazine hydrochloride (Squibb, Cairo, Egypt); levomepromazine hydrochloride (Specia) and trimeprazine tartrate (May and Baker, Dagenham, U.K.). Dosage forms were obtained from local sources.

### 2.4. Sample preparation

Stock solutions of each drug were prepared daily by dissolving 100 mg of the drug in 100 ml of water. This solution was further diluted with water to give the appropriate concentration for the working solutions. In case of levomepromazine hydrochloride and trimeprazine tartrate, the working drug – rhodamine-B solutions were prepared daily in  $70 \mu\text{g ml}^{-1}$  rhodamine-B from the stock solutions to give a drug solution.

### 2.5. Recommended procedure for calibration

The FI manifold described in Fig. 1 was used. Inject  $200 \mu\text{l}$  of drug solution into a stream of 2 M perchloric acid which was then combined with a stream of cerium(IV) solution, and measure the resulting peak height. Prepare a calibration graph by plotting the peak height vs. drug concentration over the range cited in Table 1.

### 2.6. Procedure for dosage forms

Weigh and pulverize twenty tablets. Transfer an accurately weighed amount of the powder equivalent to 20.0 mg of the drug into a small conical flask, add about 60 ml of 0.1 M HCl, stir for 45 min, filter into a 100 ml volumetric flask, wash the residue with 0.1 M HCl and complete to volume with the same solvent. Analyse a suitable volume as described above. Read the content from the calibration graph or calculate it from the regression equation (Table 1).

### 2.7. Procedure for spiked urine and plasma

Add an aliquot of standard aqueous solution of the phenothiazine to 3.0 ml of plasma or urine sample.

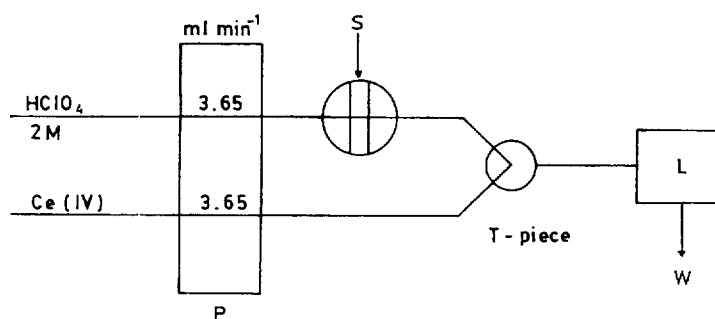


Fig. 1. FI manifold for the CL determination of phenothiazines: P, peristaltic pump; S, sample port; T, Perspex T-piece; L, luminometer; W, waste.

Table 1  
Performance data for the chemiluminometric determination of the studied phenothiazines

Compound	Conc. of Ce(IV) (M)	Conc. of HClO <sub>4</sub> (M)	Linear Calib. range (µg ml <sup>-1</sup> )	Limit of detection <sup>a</sup> (µg ml <sup>-1</sup> )	Correlation coefficient <sup>b</sup>	Regression equation C=a+bl <sup>c</sup>
Fluphenazine hydrochloride	1 × 10 <sup>-2</sup>	2	0.5–90	0.01	0.9999	C=0.397+0.250l
Levomepromazine hydrochloride	5 × 10 <sup>-3</sup>	2	0.1–6.5	0.1	0.9998	C=-0.629+0.0214l
Trimeprazine tartrate	1 × 10 <sup>-3</sup>	2	0.1–6.5	0.1	0.9999	C=-0.207+0.0279l

<sup>a</sup> 3σ.

<sup>b</sup> 8 data points.

<sup>c</sup> Intensity (mV).

Mix for 5 min. Extract with 20 ml of heptane-isoamyl alcohol (98.5+1.5) after rendering alkaline with 2 ml saturated solution of sodium carbonate. Evaporate the organic extract to dryness and dissolve the residue in 0.1 M HCl. Transfer the solution to a 100 ml volumetric flask and complete the volume with 0.1 M HCl. Proceed as described above. The absolute recovery was determined for each drug by comparing the representative peak height of extracted plasma or urine samples with the peak height of the standard drug in 0.1 M HCl at the same concentration.

### 3. Results and discussion

The possible FI chemiluminometric determination of phenothiazines was studied using different oxidants, potassium permanganate, potassium dichromate, sodium peroxodisulphate, potassium iodate, cerium(IV) sulphate and *N*-bromosuccinimide in acidic or basic media. The CL of phenothiazines

was obtained only when Ce(IV) was used as an oxidant in an acidic medium.

#### 3.1. Configuration designs

The FI configuration used for the determination of phenothiazines was designed to provide different reaction conditions for enhancing the CL signal generated by reaction of phenothiazines with Ce(IV). Maximum CL intensity was obtained when the sample was injected into a stream of 2.0 M perchloric acid and then mixed with Ce(IV) prior to the detector.

#### 3.2. Optimization of experimental variables

A series of experiments was conducted to establish the optimum analytical variables. The parameters optimized included reagent concentrations and some physical variables, including the total flow rate and the sample volume. Table 1 shows the performance data for the determination of the studied drugs.

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

Ce(IV) is not readily soluble in water, but becomes stable when dissolved in dilute sulphuric acid. Therefore, the effect of  $\text{H}_2\text{SO}_4$  concentration in the range  $5 \times 10^{-3}$ –1.0 M was studied;  $5 \times 10^{-2}$  M chosen for further studies gave the maximum peak height for each drug (Fig. 2).

### 3.2.2. Effect of cerium(IV) concentration

Fig. 3 shows the effect of Ce(IV) concentration on the CL intensity of each drug. The greatest CL response was obtained with  $1 \times 10^{-2}$ ,  $5 \times 10^{-3}$  and  $1 \times 10^{-3}$  M Ce(IV) for fluphenazine, levomepromazine and trimeprazine, respectively.

### 3.2.3. Effect of different acid concentrations

Five different acids (i.e. HCl,  $\text{HNO}_3$ ,  $\text{HClO}_4$ ,  $\text{H}_3\text{PO}_4$ , and  $\text{H}_2\text{SO}_4$ ) of different concentrations in the range  $5 \times 10^{-2}$ –5.0 M were tested in order to ascertain which was the most suitable. 2 M perchloric acid was found to give the highest intensity (Table 2). This was further established by using different con-

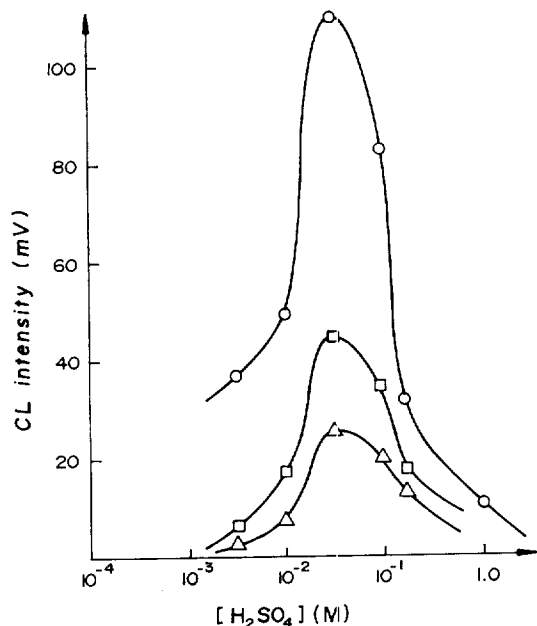


Fig. 2. Influence of  $\text{H}_2\text{SO}_4$  concentration as diluent for Ce(IV) on peak height. Injected drug solution (200  $\mu\text{l}$ ),  $25 \mu\text{g ml}^{-1}$ .  $\circ$ - $\circ$ , fluphenazine hydrochloride;  $\square$ - $\square$ , trimeprazine tartrate;  $\triangle$ - $\triangle$ , levomepromazine hydrochloride.

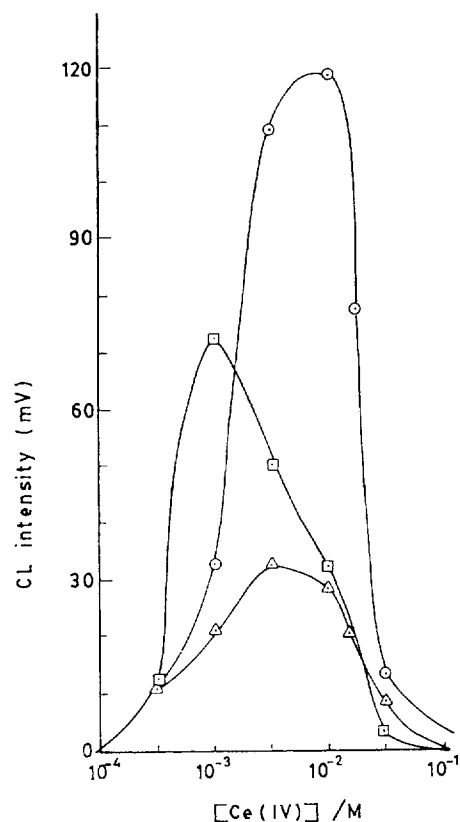


Fig. 3. Influence of Ce(IV) concentration on peak height. Injected drug solution (200  $\mu\text{l}$ ),  $25 \mu\text{g ml}^{-1}$ .  $\circ$ - $\circ$ , fluphenazine hydrochloride;  $\square$ - $\square$ , trimeprazine tartrate (without adding rhodamine-B);  $\triangle$ - $\triangle$ , levomepromazine hydrochloride (without adding rhodamine-B).

centrations of perchloric acid with different concentrations of the drug ( $1$ – $40 \mu\text{g ml}^{-1}$ ).

### 3.2.4. Effect of sensitizers

Potentially chemiluminescent molecules can transfer their excitation energy to a fluorophore (sensitizer) with subsequent emission of energy by the fluorophore (indirect CL), often resulting in an enhancement of the intensity [18,19]. To study their effect as potential sensitizers on phenothiazine CL, different concentrations ( $0.1$ – $100 \mu\text{g ml}^{-1}$ ) of rhodamine-B, fluorescein, and quinine sulphate dissolved in the drug solution, in the carrier or in the Ce(IV) solution, were investigated. It was found that only rhodamine-B enhanced the CL signal when dissolved in a solution of levomepromazine or trimeprazine;  $70 \mu\text{g ml}^{-1}$  rhodamine-B gave

Table 2

Effect of different acid concentrations on the CL intensity of fluphenazine HCl ( $25 \mu\text{g ml}^{-1}$ )

Conc. of acid (M)	CL intensity <sup>a</sup> (mV)				
	HCl	H <sub>2</sub> SO <sub>4</sub>	HNO <sub>3</sub>	HClO <sub>4</sub>	H <sub>3</sub> PO <sub>4</sub>
0.05	45.0	26.2 <sup>b</sup>	45.5	46.0	0.0
0.1	50.2	24.0	51.0	51.5	0.0
1.0	81.9	6.7	104.0	105.0	0.0
2.0	90.5	3.0	112.0	114.3	0.0
3.0	83.2	—	101.0	108.0	0.0
5.0	57.1	—	66.4	91.0	0.0

<sup>a</sup> Each result is the average of three separate determinations.<sup>b</sup> When water is used instead of acid, the average intensity is the same.

rise to the most intense emission, therefore it was used in all subsequent studies of these two drugs. Quinine sulphate had no effect on the CL signal but fluorescein suppressed the CL signal. This quenching effect might be due to the fact that fluorescein mainly provides fluorescence emission in alkaline media [15], whereas the Ce(IV)-phenothiazines reaction occurred in an acidic medium.

### 3.2.5. Effect of flow rate

The flow rates of the solutions are very important and should be regulated. At flow rates that are too low or too high, CL is not emitted in the flow cell and hence the emitter is not detected. Fig. 4 shows the effect of total flow rate on the CL intensity of each drug. The intensity increased with increasing flow rate. However, a total flow rate of  $7.3 \text{ ml min}^{-1}$  ( $3.65 \text{ ml min}^{-1}$  for each channel) is recommended because of the greater precision and economy in the use of reagents.

### 3.2.6. Effect of sample volume

The variation of the CL emission with the injected sample volume in the range  $10\text{--}500 \mu\text{l}$  was studied. The obtained results showed an increase in the emission intensity up to  $200 \mu\text{l}$  for each drug, above which the intensity was almost constant (Fig. 5).

### 3.3. Investigation of the reaction products

The reaction products are suggested to be the sulphone derivatives of phenothiazines by analogy with the oxidation pathway reported for the use of cobalt(III) ions [20]. This was further confirmed from

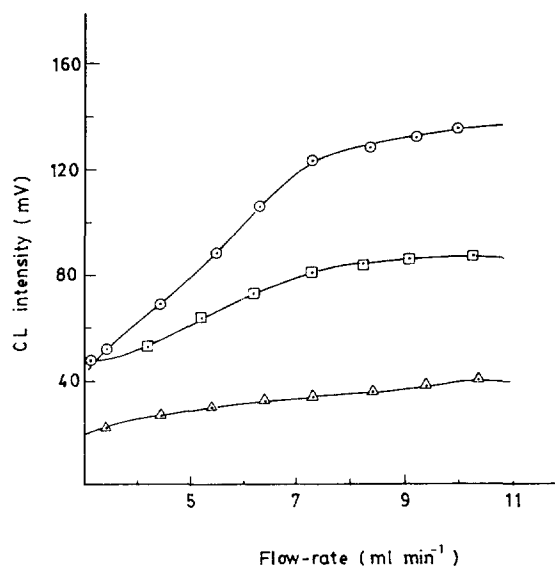


Fig. 4. Effect of total flow rate on CL intensity of phenothiazines. Injected drug solution ( $200 \mu\text{l}$ ),  $25 \mu\text{g ml}^{-1}$ . ○-○, fluphenazine hydrochloride; □-□, trimeprazine tartrate (without adding rhodamine-B); △-△, levomepromazine hydrochloride (without adding rhodamine-B).

UV and IR spectra of the reaction products extracted from the waste with 20% (v/v) tertiary butanol in hexane after rendering the waste alkaline with saturated sodium carbonate solution. The UV spectrum shows a very similar spectrum, with maximum absorbance at  $272 \text{ nm}$ , to that previously reported with cobalt(III) [20]. The IR spectrum shows a strong band at  $1130\text{--}1150 \text{ cm}^{-1}$ , characteristic of sulphone absorption [21].

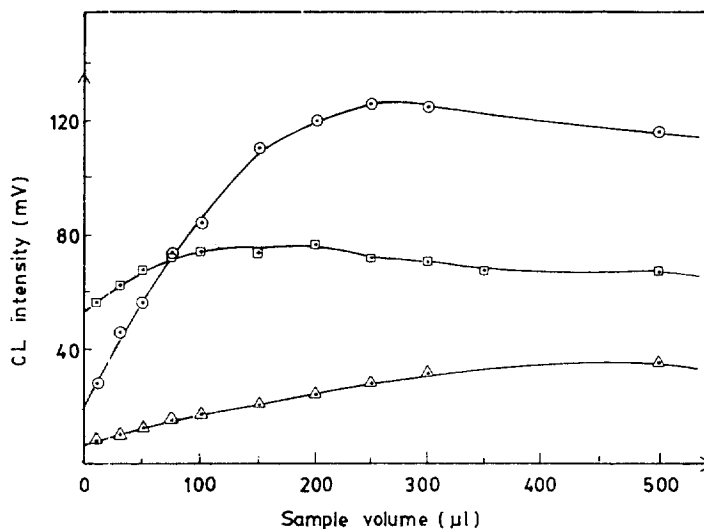
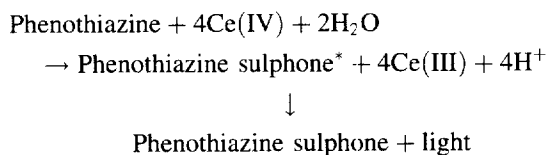


Fig. 5. Effect of sample volume of the studied phenothiazines on peak height. Injected drug solution  $25 \mu\text{g ml}^{-1}$ . ○-○, fluphenazine hydrochloride; □-□, trimeprazine tartrate (without adding rhodamine-B); △-△, levomepromazine hydrochloride (without adding rhodamine-B).

### 3.4. Nature of CL emission

Some reducing agents such as iron(II), cobalt(II) and ascorbic acid were injected instead of phenothiazine drugs, but did not generate CL with cerium(IV). This suggests that the sulphone derivatives of phenothiazines are the possible CL emitters, not the Ce(III), which is a known luminophore. Therefore, the possible CL mechanism is:



In the presence of rhodamine-B, the energy resulting from the redox reaction can effectively be transferred to rhodamine-B which in turn generates CL emission. This occurs in case of levomepromazine hydrochloride and trimeprazine tartrate.

Rhodamine-B is not effective with fluphenazine hydrochloride.

### 3.5. Determination of the studied phenothiazines

Once chemical and instrumental variables had been optimized to achieve maximum CL emission, a series

of standard solutions over the concentration range cited in Table 1 was pumped, each as three replicates, to test the linearity of the calibration graph. A plot of the emission intensity vs. concentration of the studied phenothiazines was linear over the ranges given in Table 1. To test the validity of the method, it was applied to pure samples of the phenothiazines. The average % recoveries ranged from  $99.8 \pm 0.67$  to  $100.7 \pm 1.22$ .

### 3.6. Analysis of pharmaceutical preparations

The proposed method was further applied to the analysis of certain dosage forms containing phenothiazines. The results in Table 3 are in accordance with those obtained by the official methods [2].

Statistical analysis [22] of the results by using the student t-test and the variance ratio *F*-test showed no significant difference between the performance of the two methods as regards to accuracy and precision.

### 3.7. Analysis of spiked urine and plasma samples

The high sensitivity obtained by the proposed method allowed the determination of the studied phenothiazines in biological fluids. The extraction process was carried out with 1.5% (v/v) isoamyl

Table 3  
Analysis of dosage forms containing phenothiazines by the proposed and official methods

Preparation	Recovery (%) <sup>c</sup>	
	Proposed method	Official method [2]
Moditen tablets <sup>a</sup> (1 mg fluphenazine hydrochloride/tablet)	94.1±0.8	93.1±0.6
Levomepromazine tablets <sup>b</sup> (25 mg levomepromazine hydrochloride/tablet)	99.1±0.2	99.3±0.5
Trimeprazine tablets <sup>b</sup> (10 mg trimeprazine tartrate/tablet)	99.3±0.5	99.6±0.5

<sup>a</sup> Squibb, England.

<sup>b</sup> Prepared tablets containing the drug and the tablet excipients lactose (57 mg), starch (50 mg), magnesium stearate (0.9 mg) and talc (8.1 mg) per tablet.

<sup>c</sup> Mean±S.D. (*n*=3).

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

Compound	Concentration taken (µg ml <sup>-1</sup> )	Found (%)	
		Urine	Plasma
Fluphenazine hydrochloride	20.0	95.6	91.5
	30.0	95.0	90.4
	40.0	95.4	90.4
	50.0	95.0	89.4
	60.0	95.2	90.9
Mean±S.D.		95.2±0.26	90.5±0.77
Levomepromazine hydrochloride	1.0	100.0	92.7
	2.0	100.0	92.1
	3.0	97.3	88.6
	4.0	98.5	90.2
	5.0	98.8	89.6
Mean±S.D.		98.9±1.13	90.6±1.72
Trimeprazine tartrate	1.0	97.4	86.8
	2.0	97.4	90.9
	3.0	97.3	88.2
	4.0	97.9	87.3
	5.0	97.0	88.5
Mean±S.D.		97.4±0.32	88.3±1.58

alcohol in heptane after alkalization with a saturated solution of sodium carbonate [23]. Table 4 shows the results of the recovery studies of phenothiazines from spiked plasma and urine.

#### 4. Conclusions

The proposed automated method is simple, accurate, and precise. It allows the determination of the studied phenothiazines in pharmaceutical preparations and biological fluids. It requires no sample pretreat-

ment and solutions can be analyzed at a rate of 180 (fluphenazine hydrochloride), 129 (levomepromazine hydrochloride), and 138 samples h<sup>-1</sup> (trimeprazine tartrate).

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