Talanta 96 (2012) 202–209

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Chemiluminometric determination of phenothiazines by means of a combined multi-commutated/multi-pumped flow assembly

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ARTICLE INFO

Article history:
Received 18 August 2011
Received in revised form 30 January 2012
Accepted 8 February 2012
Available online 15 February 2012

Keywords:
-Chemiluminescence
Multi-commutation
Multi-pumping
Phenothiazine derivatives

ABSTRACT

A rapid, sensitive and fully automated chemiluminometric method is described for determination of five phenothiazine derivatives, namely, trifluoperazine, fluphenazine, perphenazine, thioridazine and chlorpromazine. The method is based on the chemiluminescence (CL) induced by the oxidation of drugs with Ce(IV) in nitric acid. A flow manifold based on the association of multi-commutation and multi-pumping flow methodologies is proposed. The active operated solenoid devices consisted of a micro-pump (propelling 50 μL per stroke) and a six ports solenoid valve. Reconfiguration of the flow manifold was performed by using software settings, without physical alteration of the instrument manifold. It permits the design of flexible miniaturized networks for flow analysis based on a time-pulse-counting strategy. The proposed method allows the determination of the drugs at the ng mL^{-1} level with a sample throughput of 38 h^{-1} (chlorpromazine) and 40 h^{-1} (trifluoperazine, fluphenazine, perphenazine, and thioridazine). The method was successfully applied to the determination of the phenothiazine derivatives in pharmaceuticals formulations.

1. Introduction

Phenothiazines are a group of tranquilizing drugs with antipsychotic actions thought to act by blocking dopaminergic transmission within the brain. Phenothiazine derivatives used therapeutically (Table 1) are always substituted at position 10 and can carry an additional substituent at position 2.

Phenothiazines are classified into three groups that differ with respect to the substituent on nitrogen: aliphatic compounds (bearing acyclic groups, e.g. chlorpromazine), piperidines (bearing piperidine-derived groups, e.g. thioridazine), and piperazines (bearing piperazine-derived substituents, e.g. trifluoperazine, fluphenazine and perphenazine) [1].

Because flow-injection CL methods have some analytical features such as sensitivity, speed, ease to use and relatively inexpensive instrumentation, they have received much attention for drug analysis [2–4]. Promethazine has been determined by its inhibition of the chemiluminescence (CL) of the luminol hydrogen-peroxide chromium(III) system [5]. The determination of chlorpromazine and promethazine in human urine has been performed using capillary electrophoresis with electrochemiluminescence detection [6,7]. Flow injection analysis and direct chemiluminescence employing strong oxidants have been used for analysing phenothiazine derivatives in dosage forms and biological fluids. Cerium(IV) in acidic medium has been employed with perphenazine [8], fluphenazine, levomepromazine, trimeprazine [9], and chlorpromazine [10]. Thioridazine [11], chlorpromazine [12], and promethazine [13,14] have been determined using potassium permanganate in sulfuric acid as oxidant. A sensitive method using the luminol–$KMnO_4$ system has been described for the determination of chlorpromazine, perphenazine, fluphenazine and thioridazine in injections and urine [15]. $Ru(phen)_3^{2+}$ and Ce(IV) system has been used for determining promazine [16], chlorpromazine and flupheneazine [17] in human serum and drug formulations.

Also liquid chromatography has been employed for phenothiazines analysis combined with different detection strategies, e.g. UV detection [18], fluorescence [18,19], electrogenerated chemiluminescence [20], or coulometric detection [21].

On the other hand, flow analysis systems have shown a remarkable evolution. The developments of FIA have resulted in promising flow modalities such as sequential injection analysis [22], bead injection lab-on-valve [23] and multi-commutation [24], among others. This evolution has arisen from an improvement in manifold design and components for a reliable impulsion and insertion of sample and reagents. In fact, propelling and insertion units markedly determine the potentialities of flow analysis procedures. Multi-commutation flow systems (MCFS) and particularly

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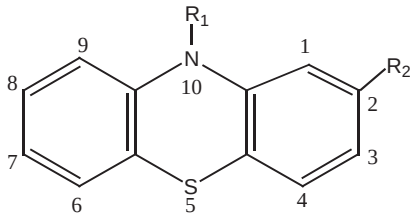
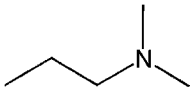
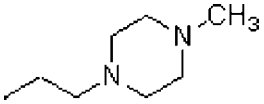
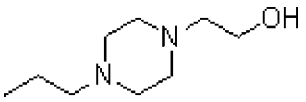
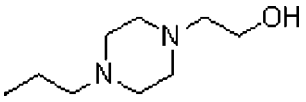
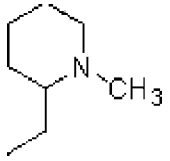
doi:10.1016/j.talanta.2012.02.019

Table 1
Chemical structure of phenothiazines.

Derivative	R_1	R_2
Phenothiazine		
Chlorpromazine		Cl
Trifluoperazine		CF_3
Fluphenazine		Cl
Perphenazine		CF_3
Thioridazine		

multi-pumping flow systems (MPFS) have been recently proposed as new strategies for automation of analytical procedures [25–29]. Based on the use of solenoid operated micro-valves and pumps they enable the design of fully automated systems in which pulsed flow delivered by solenoid pumps causes an effective turbulent mixing [30].

From a conceptual point of view, the main contribution of MCFS and MPFS is the substitution of "volumes" of insertion by "times" of insertion, and flow rates by a "time-pulse-counting" strategy, which allows to develop time-based sampling methods; and, the notion of the flow assembly like a system active, versatile and easy to reshape (flow network). The implementation of micro-solenoid devices in a flow system presents a functional structure controlled by a computer. Moreover, the excellent cost-effective, precision, accuracy and the proved robustness and miniaturization features suggest that MCFS and MPFS could be advantageously used for fulfill the requirements associated to portable fieldwork instruments for in situ environmental analysis.

The aim of the present work is to exploit the analytical features of associated MCFS and MPFS for developing a multi-task flow system for the forward development of flow chemiluminescence methods able to determine phenothiazine derivatives at the ng L^{-1} level. The use of a multi-channel selection solenoid valve combined with an operated solenoid micro-pump enables to perform a set of different analytical tasks, namely, rapid screening multi-tests of chemiluminescent systems, parallel multi-optimization of chemical and physical variables, and multi-standard calibration. Physical reconfiguration of the flow system is not required.

2. 2- Experimental

2.1. Reagents

All reagents were analytically pure unless stated otherwise and prepared in deionized water (18 MΩ cm) using a Sybron/Barnstead Nanopure II water purification system. Aqueous standard solutions of trifluoperazine (Simthkline Beechman Pharmaceuticals, Philadelphia, USA), fluphenazine (Guinama, Valencia, Spain), thioridazine (Sigma–Aldrich Química S.A., Madrid, Spain) and standard solutions of perphenazine, chlorpromazine (Sigma–Aldrich Química S.A., Madrid, Spain) prepared in 0.2 M sulfuric acid were used. Mineral acids, alkalis and oxidants employed in the screening of oxidant systems and optimization procedure were NaOH (Scharlau, Barcelona, Spain), HNO_3 (Merck, Madrid, Spain), H_2SO_4 (from Scharlau), HCl (J.T. Baker, Deventer, Holland), $HClO_4$ and H_3PO_4 (both from Panreac, Barcelona, Spain), $KMnO_4$ (from J.T. Baker), $K_3Fe(CN)_6$ (Panreac), N-bromosuccinimide (NBS, from Scharlau), and $(NH_4)_2Ce(NO_3)_6$ (from Fluka, Buchs, Switzerland).

2.2. Apparatus

Fig. 1 shows a graphical schematic of instrumentation, which comprised a self-priming solenoid operated fixed displacement diaphragm pump (model 120SP12 from Bio-Chem Fluidics, West Caldwell, NJ, USA) that dispensed a solution volume of 50 μL per stroke, and a solenoid operated flow selection valve (model 080T612-32, from Bio-Chem Fluidics) [31]. This type of solenoid valves combine into a single, compact unit six solenoid valve elements, which can be operated independently of each other. For connections between elements a PTFE coil of 0.8 mm i.d. was used. The flow manifold was located in a laboratory-made light-tight box with the detection unit.

The solenoid devices were connected to an electronic interface (KSP, Poland). Its actuation was controlled using a valve and pump controller software (KSP, Poland) running in a Pentium-type computer under XP Windows operating system. The programme and interface allow an independent control of the solenoid devices, namely, the sequence of insertions and the number of cycles according to the number of samples, reagent solutions or standards to be inserted. The flow cell was a flat-spiral quartz tube of 1 mm inner diameter and 3 cm total diameter backed by a mirror for maximum light collection. The photo-detector package was a P30CWAD5F-29 type 9125 photomultiplier tube (PMT) supplied by Electron Tubes operating at 1280 V. The output was fed to a computer equipped with a counter-timer module CT2, also supplied by Electron Tubes.

2.3. Procedures

2.3.1. Preparation of stock solutions

Stock standard solutions of trifluoperazine, fluphenazine, thioridazine (100 μg mL^{-1}) were prepared by exactly weighing and dissolving the drug in deionized water. Perphenazine and chlorpromazine (100 μg mL^{-1}) were dissolved in 0.2 M sulfuric acid. The working standard solutions were freshly prepared by diluting the stock standard solutions in the appropriate volume of deionized water.

2.3.2. Preparation of samples

(a) Mutabase 2–10 dragees (from Schering-Plough) and *Decentán* tablets (from Merck, S.L.).

Five dragees of *Mutabase* 2-10 were dissolved in 100 mL of 0.2 M H_2SO_4. The resulting solution (100 μg mL^{-1} in perphenazine) was diluted with deionized water to obtain a solution in the vicinity of 0.15 μg mL^{-1}.

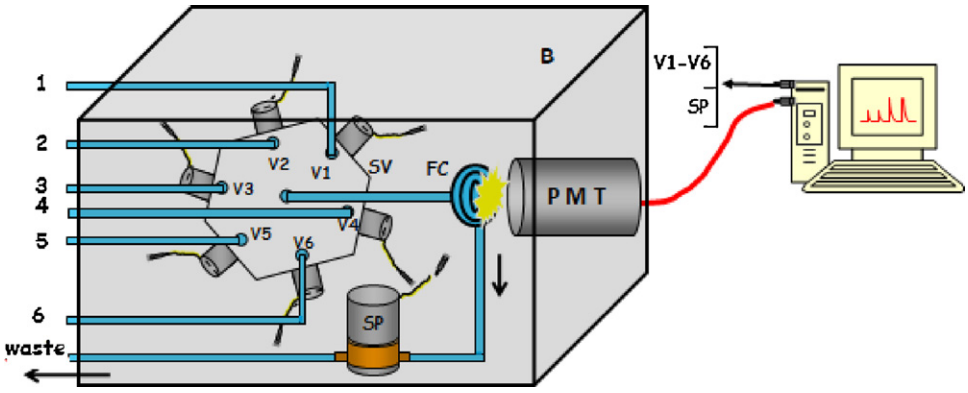


Fig. 1. Flow assembly for the screnning of oxidant systems and determination of phenothiazines.
SV:six-ports solenoid valve; SP: solenoid pump; FC: flow cell; PMT: photomultiplier tube; 1, 2, 3, 4, 5, 6: channels. *For screening of oxidant systems*: (1) (phenothiazine); (2) ($KMnO_4$ 5×10^{-4} M in 2 M H_2SO_4); (3) (Ce(IV) 6×10^{-3} M in 1.5 M H_2SO_4); (4) ($Fe(CN)_6^{3-}$ 10^{-2} M in 2 M NaOH); (5) (NBS 4×10^{-2} M in 2 M NaOH); (6) (air). *For determination of phenothiazines*: (1) (Ce(IV) 2×10^{-3} M in 0.2 M HNO_3); (2) (trifluoperazine); (3) (fluphenazine); (4) (thioridazine); (5) (perphenazine); (6) (chlorpromazine).

Five tablets of *Decentán* (mass of five tablets – 0.5075 g) were powdered by grinding them in a mortar and pestle. An appropriat amount of the powder (0.1269 g) was weighed and dissolved in 100 mL of 0.2 M H_2SO_4. The resulting solution (100 μg mL^{-1} in perphenazine) was diluted with deionized water to obtain a solution in the vicinity of 0.15 μg mL^{-1}.

(b) *Largaltil* 25 mg tablets (from Sanofi-Aventis, S.A.).
One tablet was dissolved in 250 mL of 0.2 M H_2SO_4. The resulting solution (100 μg mL^{-1} in chlorpromazine) was diluted with deionized water to obtain a solution in the vicinity 0.15 μg mL^{-1}.

(c) *Eskazine* 2 mg dragees (from Goldshield Pharmaceuticals Ltd.).
Five dragees of *Eskazine* were dissolved in 100 mL of deionized water. The resulting solution (100 μg mL^{-1} in trifluoperazine) was diluted with deionized water to obtain a solution in the vicinity of 10 ng mL^{-1}.

(d) *Thioridazin* 25 mg tablets (from Jelfa, S.A.)
Five tablets of *Thioridazin* (mass of five tablets – 1.2944 g) were powdered by grinding them in a mortar and pestle. An appropriat amount of the powder (0.2589 g) was weighed and dissolved in 250 mL of deionized water. The resulting solution (100 μg mL^{-1} in thioridazine) was diluted with deionized water to obtain a solution in the vicinity of 2 μg mL^{-1}.

3. Results and discussion

Methodologies known as multi-commutation and multi-pumping, especially when combined into a single flow assembly, present an attractive alternative to classical methods like FIA. Unlike these, MCFS and MPFS enable an easy way to get analytical methods closer to the full and true automation, and a real versatility, as it is possible to reconfigure the system via software, without changing the architecture of the flow system. The physical configuration of the flow system (see Fig. 1) was the same for all experiments performed in the present work. The flow system was developed as a multi-task manifold, in which by changing the composition of solutions (lines 1–6), and altering insertion profiles (sequence, number and duration of electronic pulses operating solenoid elements), is possible to perform a set of different experiments, namely, rapid screening multi-tests of chemiluminescent systems, parallel multi-optimization of chemical and physical variables, and multi-standard calibration.

3.1. Screening of chemiluminescent systems

Screening tests were performed by using oxidants for direct chemiluminescent detection. Several oxidizing systems were examined because chemiluminescence arises most frequently from oxidation reactions involving large energy changes. Four oxidizing systems (potassium permanganate and Ce(IV) in acidic medium; potassium ferricyanide and NBS in basic medium) were tested. Solutions of phenothiazines (8.5×10^{-4} M) were used according to the insertion profile depicted in Fig. 2. The order of aspiration of oxidant solutions was conditioned to avoid cross contamination when changing solution ($KMnO_4$ and Ce(IV) were prepared in acidic medium, whereas that $K_3Fe(CN)_6$ and NBS were prepared in 2 M NaOH). Also for this reason, it was necessary to segment the flow between two consecutive peaks when changing oxidant system (5 micro-segments of air were aspirated at the end of each peak). For each oxidant tested (V2–V5), 21 alternating micro-segments of phenothiazine and oxidant were programed. The peaks of each phenothiazine against oxidants tested were recorded in 124 s. At the end of the peak, 15 s of relaxation time were included to prevent overheating of the solenoid pump, which works continuously throughout the cycle with a frequency of 150 pulses per minute (0.2 s ON, 0.2 s OFF).

Chemiluminescence of phenothiazines was strongly dependent on the oxidizing system employed. The results for screening are summarized in Table 2, in which the intensity of chemiluminescence is showed. Phenothiazines were not detected employing potassium ferricyanide or NBS as oxidant. In all cases the highest analytical signals were obtained for Ce(IV). Relatively high values of standard deviation ca. 15% were observed for trifluoperazine and fluphenazine in acidic cerium. This was caused by the hysteresis associated to outputs closed to overshoot (more than 1.5 millions of counts). Considering also the lowest reported selectivity of $KMnO_4$ as an oxidant in the chemiluminescent detection [11–14], the system Ce(IV) was selected for further experiments.

3.2. Chemiluminescent determination of phenothiazines

The optimized insertion profile for obtaining typical transient analytical signals is depicted in Fig. 3. The insertion pattern

Table 2
Chemiluminescent response of phenothiazines for positive oxidant systems (CL-intensity in counts).

Phenothiazine	$KMnO_4$	$(NH_4)_2[Ce(NO_3)_6]$
Chlorpromazine	35501	97990
Trifluoperazine	16798	2847921
Fluphenazine	25386	1506608
Perphenazine	39949	210539
Thioridazine	17331	30405

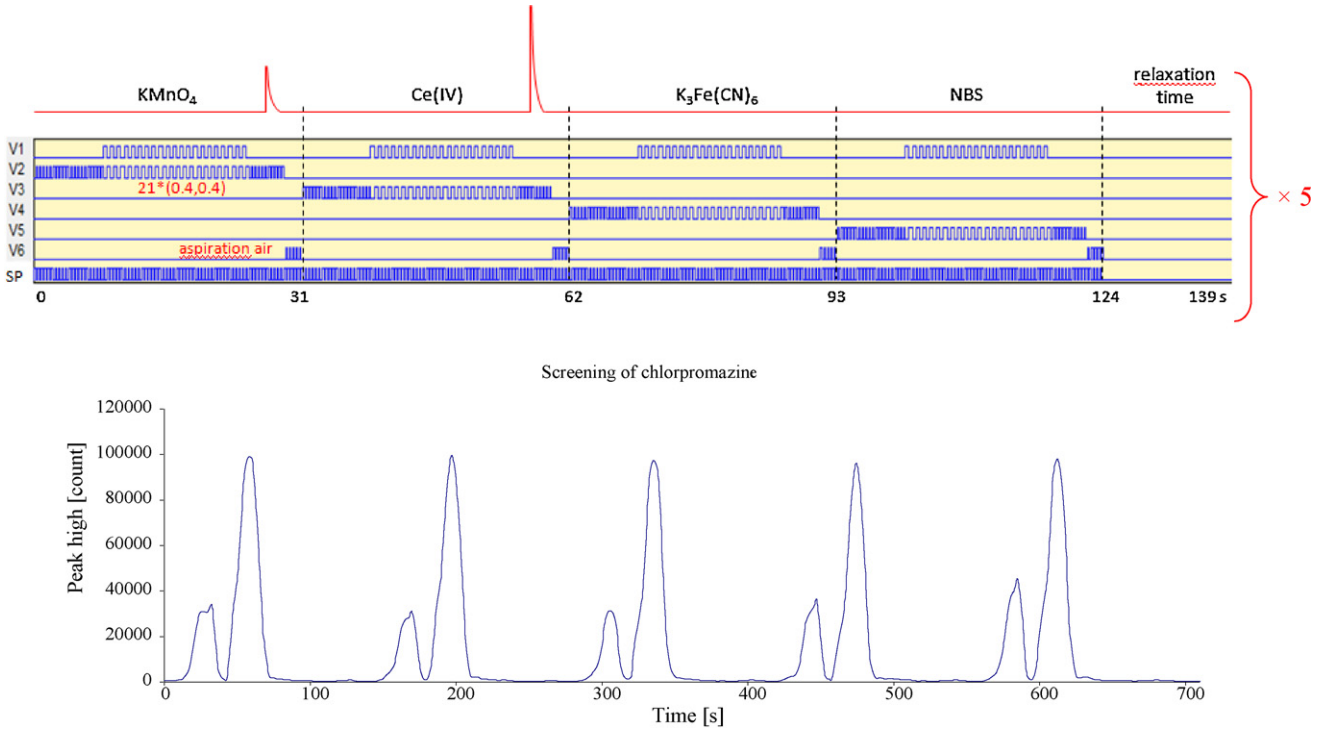


Fig. 2. Insertion profile for the screening of oxidant systems and experimental records for chlorpromazine. Five peaks (×5) were recorded per oxidant.

was the same for trifluoperazine, fluphenazine, thioridazine and perphenazine (Fig. 3II). First, 20 alternated microinsertions of phenothiazine (V2–V5) and oxidant (V1) were performed. During each micro-insertion V1 remains activated during 0.4 s (valve ON, Ce(IV) solution is aspirated), and next deactivated for 0.4 s (valve OFF, phenothiazine is aspirated). Solenoid pump and V1 were synchronized so that cerium acts as carrier solution. A chemiluminescence response is obtained and the peak returns to the base line ready for a new cycle after 15 s relaxation time.

For chlorpromazine determination, changes in the insertion profile were forced due to the different behavior against oxidation and chemiluminescent emission observed (see Fig. 3III). A stopped flow of 20 s was necessary after alternating aspiration of chlorpromazine (V6) and oxidant.

3.3. Optimization of physical parameters

The physical and logical configuration of the described flow system allows a simultaneous optimization process for all phenothiazines. The influence of changes in flowing and chemical parameters needed in the optimization process can be obtained in a single record (five peaks per parameter value under study). First, parameters affecting the pattern flow were studied.

3.4. Effect of volume of sample–oxidant

The volume of sample and oxidant can be controlled by changing the number of alternating commutations of V1 and V2–V6. 5, 10, 15, 20, 25 and 30 micro-insertions of sample–oxidant were tested. The best compromise between CL-intensity, reproducibility and sample consumption was obtained for 20 insertions, equivalent to 666 μL of sample–oxidant (see Fig. 4).

3.5. Effect of flow rate

The influence of flow rate can be studied by altering the pulses frequency of solenoid pump. Pulse duration varied as follows, t seconds ON and t seconds OFF, where t was 0.2, 0.3 and 0.4 s. These values correspond to flow rates of 4.1, 3.2 and 2.5 mL min^{-1}, respectively. Only chlorpromazine was affected by changes in flow rate. The CL-intensity was reduced 45% when the flow rate varied from 2.5 mL min^{-1} to 4.1 mL min^{-1}.

Double peaks were obtained for perphenazine at a flow rate of 2.5 mL min^{-1}. Thus, a flow rate of 3.2 mL min^{-1} was selected.

3.6. Effect of stopped flow

The obtained results have shown that the flow rate influences the kinetics of chlorpromazine oxidation and CL emission. Two stopped-flow experiments were carried out with a 10 μg mL^{-1} solution of chlorpromazine. In the first one, we determined the number of pump pulses needed to drive the bolus of sample–oxidant to the flow cell when the flow was stopped for 10 s. Five pulses provided the highest collection of light by the detector. In the second experiment, after inserting the sample into the carrier oxidant solution, and after five additional pulses of the solenoid pump, the flow stopped for 10, 15 and 20 s. Then the solenoid pump was reactivated and the mixture was conducted to the flow cell. The highest analytical signals were obtained for 20 s of stopped flow.

3.7. Optimization of chemical variables

The chemical optimization affects the composition of oxidant solution, namely, molar concentration of Ce(IV) and acid medium. The concentration of Ce(IV) was studied over the range 4×10^{-4}–4×10^{-2} mol/L using the optimized insertion profile for each phenothiazine. Oxidant solution was prepared in 1.5 M sulfuric acid. Trifluoperazine, fluphenazine, perphenazine and thioridazine yielded higher CL intensity at low concentration of oxidant (2×10^{-3} M), whereas, for chlorpromazine, a cerium concentration of 2×10^{-2} M was the optimal (see Fig. 5).

H_2SO_4, HNO_3, $HClO_4$, and H_3PO_4 were tested at 2 M concentration. $HClO_4$, and H_3PO_4 were discarded due to either insolubility

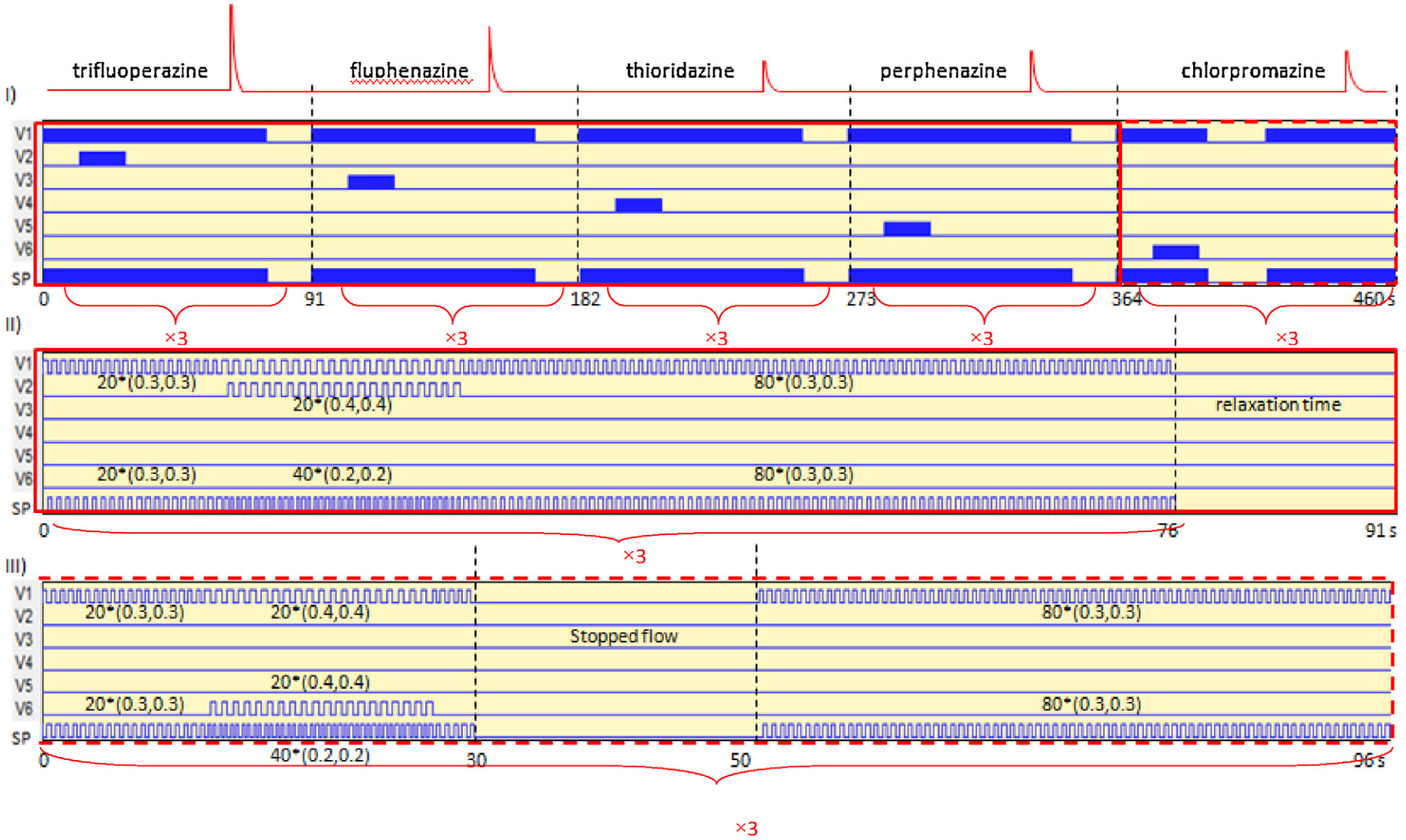


Fig. 3. Optimized insertion profile for determination of phenothiazines.
$N^*(t_1,t_2)$. N: number of insertion (electronic pulses). In each insertion the solenoid element is t_1 seconds ON and t_2 seconds OFF. (I) Sequential determination of phenothiazines; (II) Schematic insertion profile for trifluoperazine, fluofenazine, thioridazine and perphenazine (details); (III) Schematic insertion profile for chlorpromazine (details). Oxidant (V1): (Ce(IV) 2×10^{-3} M in 0.2 M HNO_3). Three peaks (×3) were recorder per drug.

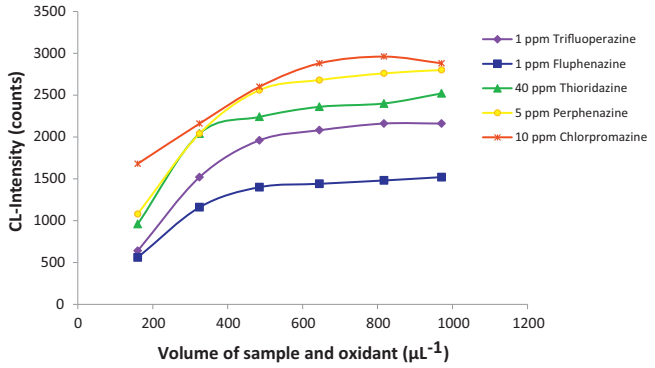


Fig. 4. Influence of volume of sample and oxidant. [Ce(IV)] = 6×10^{-3} mol/L in 1.5 M H_2SO_4.

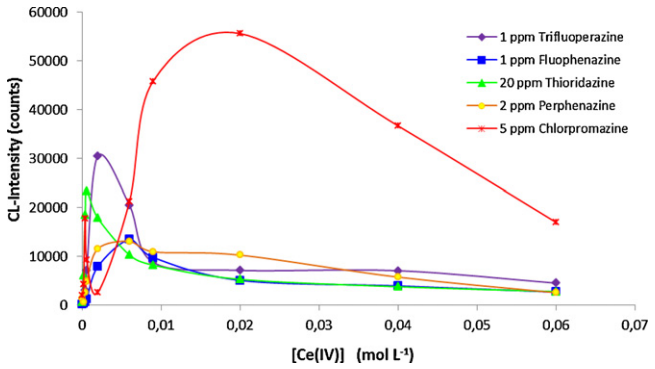


Fig. 5. Influence of concentration of Ce(IV) (in H_2SO_4 1.5 M).

of phenothiazines (turbidity of cerium solution) in these media or lower analytical signals obtained.

Sulfuric and nitric acid were tested over the range 0.05–3 M. Outputs above 80,000 counts were obtained for all phenothiazines, in 0.2 M HNO_3 (e.g. a 2 μg mL^{-1} thioridazine solution yielded CL signals 10 folds those provided by a 20 μg mL^{-1} thioridazine solution with Ce(IV) in sulfuric medium). Moreover, even results obtained for chlorpromazine, showed the same trend as the rest of phenothiazines in solution containing different concentrations of nitric acid (see Fig. 6).

This observation is important, since it allows the use of a single optimized oxidant system for the simultaneous determination of five phenothiazines. By contrast, the use of oxidizing solution prepared in sulfuric acid, provided analytical signals lower than 25,000 counts over the entire concentration range studied. Trifluoperazine, fluphenazine and chlorpromazine increased CL outputs with increasing concentration of sulfuric acid, however, low acid

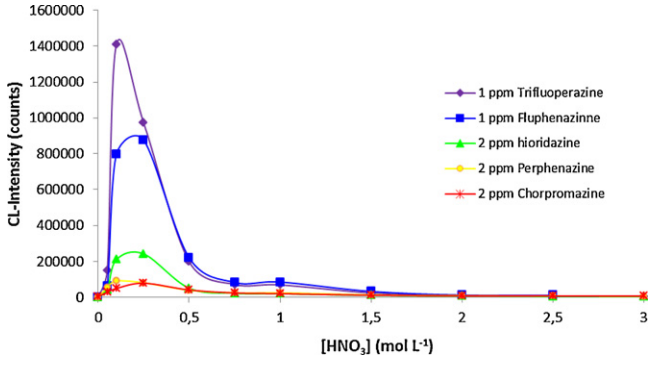


Fig. 6. Influence of nitric acid concentration. [Ce(IV)] = 2×10^{-3} mol/L.

concentrations were more favorable in the case of thioridazine and perphenazine.

Finally, a solution 2×10^{-3} M Ce(IV) in 0.2 M HNO_3 was chosen as oxidant.

3.8. Phenothiazines CL reaction

According to the investigation of CL properties of the fluorophore-sensitized Ce(IV) reaction system by Zhang et al. [32] a possible CL mechanism of the reaction may be attributed to the following reactions:

$$\mathrm{Ce(IV)} + \mathrm{analyte(red)} \rightarrow \mathrm{Ce(III)}* + \mathrm{analyte(ox)}$$

$$\mathrm{Ce(III)}* \rightarrow \mathrm{Ce(III)} + \mathrm{light}$$

$$\mathrm{and/orCe(III)\text{–}analytecomplex}* \rightarrow \mathrm{Ce(III)} + \mathrm{analyte} + \mathrm{light}$$

In the presence of a fluorophore, Ce(III) ions or the Ce(III)–analyte complex transfer the excess energy to the fluorophore which in turn generates CL emission:

$$\mathrm{Ce(III)}* + \mathrm{fluorophore} \rightarrow \mathrm{Ce(III)} + \mathrm{fluorophore}*$$

$$\mathrm{and/orCe(III)\text{–}analytecomplex}* + \mathrm{fluorophore} \rightarrow \mathrm{Ce(III)} + \mathrm{analyte} + \mathrm{fluorophore}*$$

$$\mathrm{fluorophore}* \rightarrow \mathrm{fluorophore} + \mathrm{light}$$

On the other hand, when reducing agents such as ascorbic acid or lactose were injected instead of phenothiazine drugs, CL was not observed with cerium(IV). This suggest that the sulfone derivatives of phenothiazines are the possible CL emitters, not the Ce(III) which is a known luminophore [9]. Therefore, the possible CL mechanism is:

$$\mathrm{Phenothiazine} + \mathrm{Ce(IV)} \rightarrow \mathrm{Phenothiazinesulfone}* + \mathrm{Ce(III)}$$

$$\mathrm{Phenothiazinesulfone}* \rightarrow \mathrm{Phenothiazinesulfone} + \mathrm{light}$$

4. Analytical applications

A study of the analytical features of the proposed methods was carried out to establish the application range, reproducibility, repeatability, detection limit and sample throughput. The results are summarized in Table 3. The reproducibility was obtained as the relative standard deviation of the slopes of three calibration graphs performed in different days with fresh solutions. The limit of detection defined as 3 × RSD of blank/slope of the calibration curve was ≤3 ng mL^{-1}. Repeatability was calculated as the relative standard deviation for a series of 5 insertions of a phenothiazine solution at the ppb level, and was ≤ 4% in all cases. The throughput, calculated using the optimized insertion profile was 38 samples h^{-1} (chlorpromazine) and 40 samples h^{-1} for the rest of phenothiazines.

The proposed methods for phenothiazine derivatives compare favorably with previously reported CL–FIA methods [9,11,17] in terms of sensitivity and limits of detection. Limits of detection obtained were as follows: 0.01 μg mL^{-1} fluphenazine [9], 0.5 μg mL^{-1} thioridazine [11], 0.015 μg mL^{-1} fluphenazine and 0.02 μg mL^{-1} chlorpromazine [17]. Li et al. [15] have determined perphenazine, thioridazine and chlorpromazine at lower LOD than the proposed method (0.7 ng mL^{-1} for perphenazine and thioridazine, and 0.4 ng mL^{-1} for chlorpromazine), but this supposes the use of the highly sensitive luminol system, yielding narrower linear range for thioridazine and chlorpromazine.

Table 3
Analytical features of proposed methods for CL-determination of phenothiazines.

Analytical parameter	Trifluoperazine	Fluphenazine	Thioridazine	Perphenazine	Chlorpromazine
Sample throughput (h^{-1})	40	40	40	40	38
Consumed sample (mL per peak)	0.333	0.333	0.333	0.333	0.333
Consumed reagent (mL per peak)	3.17	3.17	3.17	3.17	3.17
Equation	$I_{CL} = (1761 \pm 50)$ [Phen][a] $- 1200 \pm 700$ ($r^2 = 0.9905$)	$I_{CL} = (1171 \pm 14)$ [Phen][a] $+ 600 \pm 400$ ($r^2 = 0.9987$)	$I_{CL} = (180{,}000 \pm 10{,}000)$ [Phen][b] $- 21{,}000 \pm 4000$ ($r^2 = 0.9992$)	$I_{CL} = (122{,}100 \pm 800)$ [Phen][b] $- 4000 \pm 1900$ ($r^2 = 0.9995$)	$I_{CL} = (131{,}300 \pm 800)$ [Phen][b] $- 400 \pm 800$ ($r^2 = 0.9932$)
Reproducibility[c] RSD (%)	3.0	1.2	5.5	0.7	0.6
Repeatability RSD (%) ($n = 5$)	3.2 (1 ng mL^{-1})	1.0 (2 ng mL^{-1})	3.1 (100 ng mL^{-1})	1.3 (100 ng mL^{-1})	4.1 (50 ng mL^{-1})
Limit of detection[d] (ng mL^{-1})	0.2	0.3	2	3	3
Linear range	Up to 100 ng mL^{-1}	Up to 200 ng mL^{-1}	Up to 5 μg mL^{-1}	Up to 2 μg mL^{-1}	Up to 2.5 μg mL^{-1}

[a] [phenothiazine] in ng L^{-1}.
[b] [phenothiazine] in μg L^{-1}.
[c] RSD of slopes corresponding to three calibrations performed in different days.
[d] $3 \times \sigma_{blank}$/slope calibration.

Table 4
Influence of foreign substances on the determination of phenothiazine derivatives. 0.1 μg mL^{-1} (trifluoperazine), 0.2 μg mL^{-1} (fluphenazine), 2 μg mL^{-1} (thioridazine, perphenazine, chlorpromazine) in the presence of each substance.

Tolerable concentration ratio of substance to phenothiazine					
Substance	Trifluoperazine	Fluphenazine	Thioridazine	Perphenazine	Chlorpromazine
Na_2SO_4	150	75	75	75	37
Na_3PO_4	175	85	0.2	1	10
KNO_3	160	80	65	35	65
NaCl	250	130	125	10	1
KCl	190	95	95	5	1
$CaCl_2$	280	140	30	1.5	1.5
$MgCl_2$	400	200	200	2	2
Lactose	100	50	50	50	50
Saccharose	100	50	50	5	50
Starch	100	50	50	50	50
Urea	100	50	25	25	50
Ascorbic acid	1	0.5	0.5	0.1	1
Na_2SO_3	0.1	0.03	0.2	0.1	0.05

The interference effect of some common ions and excipients in pharmaceutical preparations were studied by recovering 0.1 μg mL^{-1} (trifluoperazine), 0.2 μg mL^{-1} (fluphenazine), and 2 μg mL^{-1} (thioridazine, perphenazine, chlorpromazine) in the presence of each substance. The tolerance of each substance was taken as the largest amount yielding an error less than 5% in the analytical signal by comparing with a reference solution of phenothiazine containing the same concentration. The results are shown in Table 4. It was found a relatively good tolerance for sugars, even for reductant sugars (lactose). Negative errors were observed for almost all reducing agents tested. This can be explained by increased cerium(IV) consumption in their presence. Ascorbic acid and sulfite interfere strongly at concentration ratios ≤1.

Following the procedure detailed in Section 2.3.2, the proposed methods were applied to the determination of phenothiazines in tablets and dragees (see Table 5). The obtained results were compared with those obtained employing a spectrophotometric reference method based on the absorption in the ultraviolet region of phenothiazine derivatives [33].

Used method enabled for determination of four phenotiazines (trifluoperazine, perphenazine, thioridazine and chlorpromazine) in pharmaceutical drugs. However, we cannot determine concentration of fluphenazine. Fluphenazine is available in three different salt forms, which gives three different degrees of antipsychotic potency. Decanoate and enanthate have the highest duration of action, while the hydrochloride salt is of short duration. The drug is currently available only in Spain under the commercial name of Modecate 25 mg (as fluphenazine decanoate) in 1 mL vial. This pharmaceutical preparation contains 15 mg of benzyl alcohol and 1 mL of sesame oil, which is composed of different saturated (palmitic and stearic) and unsaturated (arachidonic, linoleic, and oleic) fatty acids. It also contains lignans sesamin and sesamolin

Table 5
Determination of phenothiazines in pharmaceutical samples.

Phenothiazine	Sample	Claimed (mg/tablet)	Proposed method (mg/tablet)/error (%)	Reference method (mg/tablet)/error (%)
Trifluoperazine	Eskazine 2 mg	2.0	1.94 (−3.0)	2.02 (+1.0)
Thioridazine	Thioridazin 25 mg	25.0	18.92 (−24.3)	26.02 (+4.1)
Perphenezine	Mutabase	2.0	1.95 (−2.5)	3.22 (+61.0)
	Decentán	8.0	7.88 (−1.5)	7.66 (−4.3)
Chlorpromazine	Largaltil	25.0	25.70 (+2.8)	25.80 (+3.0)

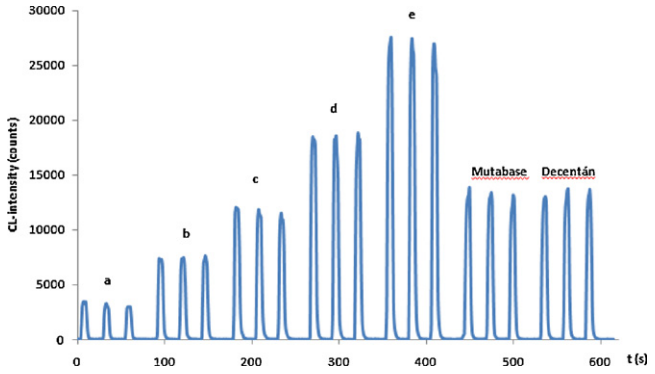


Fig. 7. Example of calibration and sample analysis under optimized conditions a, b, c, d, and e: standard solutions of 50,100,150,200 and 250 ng mL^{-1} in perphenazine.

related compound, but in small quantities. Given the form of presentation as decanoate and the complexity of the matrix, no acceptable results were obtained with the method in the analysis of Modecate. Similar considerations can be applied to the reference method.

Fig. 7 shows an example of calibration and sample analysis (perphenazine in *Mutabase* dragees and *Decentán* tablets) under optimized conditions.

5. Conclusions

A new flow system for CL-determination of phenothiazine derivatives has been proposed. Trifluoperazine, fluphenazine, perphenazine, thioidazine and chlorpromazine can be determined at the ng mL^{-1} level on the basis of the chemiluminometric response of drugs after oxidation with cerium(IV) in acidic medium without using sensitizers.

From authors' knowledge, the chemiluminometric determination of trifluoperazine under flowing conditions has not been reported.

The used manifold was based on the combination of multi-commutation and multi-pumping flow methodologies. The resulting MCFS/MPFS combining solenoid pumps and valves is also benefited from its versatility and viability for miniaturization and the increase of simplicity.

The referred flexibility and simplicity depend on the capability of carrying out an independent control and reconfiguration of the mono-commuting elements without requiring physical reconfiguration of the flow manifold. In fact, the optimization of the flow manifold consists in a fast and easy process via software, in which the only variables involved are the insertion profile of solenoid valves (number and duration of electronic pulses) and the pump pulse frequency, and eventually the stroke volume.

The logical reconfiguration of the flow system enable the use of the manifold for different tasks, namely, rapid screening tests of oxidizing CL-systems, parallel optimization of flow and chemical parameters for all drugs, and calibration using several standards in the flow lines.

Moreover, the excellent day-to-day reproducibility and the proved robustness and miniaturization features suggest that the association of MCFS and MPFS could be advantageously used for developing portable and fully automated fieldwork instruments.

Acknowledgments

This research was supported by EADS CASA. Dr. P. Halaburda is grateful to the CEU- University Cardenal Herrera for the use of instrumentation and installations during the present work.

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