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Journal of Pharmaceutical and Biomedical Analysis
22 (2000) 85–91

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Chemiluminescence determination of thioridazine hydrochloride by flow-injection analysis

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Received 17 June 1999; received in revised form 22 September 1999; accepted 4 October 1999

Abstract

Hydrogen peroxide, potassium permanganate, potassium dichromate, potassium hexacyanoferrate(III), cerium(IV) sulphate and sodium peroxidisulphate have been tested as potential reagents for chemiluminescence generation from the oxidation of phenothiazine derivatives. Only with potassium permanganate in acidic medium were satisfying results achieved. A total of 13 different phenothiazine derivatives produce luminescence of different intensities on oxidation. Thioridazine hydrochloride was chosen to develop a rapid and simple method for its determination in pharmaceutical formulations. The limit of detection is 1.2×10^{-6} mol l^{-1}, and 110 samples per hour can be determined. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Chemiluminescence; Flow-injection analysis; Phenothiazine derivatives; Thioridazine hydrochloride

1. Introduction

Solution phase chemiluminescence produced by a redox reaction is well known and finds application as flow-injection analysis (FIA) and liquid chromatography (LC) postcolumn detection mode [1]. Substances like luminol, oxalate esters and acridinium salts are most widely used, especially in indirect determinations of organic and inorganic species, but only few mechanisms of chemiluminescent reactions are fully clear [2]. Another group of applications of chemiluminescence detection are reactions, where the analyte is directly oxidized to produce light. Because they are usually fast reactions, a flow-injection method is favourable for a fast and reproducible way of mixing and transporting the sample into the detector.

Among the few oxidizing agents used in direct chemiluminescence methods, potassium permanganate in acidic medium is the one most widely used. Serotonin [3], buprenorphine hydrochloride [4], morphine [5,6], and naphtols [7] have been determined using simple two-line flow-injection manifolds with a flow cell placed directly near the photomultiplier (PM) window. Li et al. [8] used

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PII: S0731-7085(99)00270-8

the sensitizer octylphenyl polyglycol ether (OP) for enhancing chemiluminescence from tetracyclines oxidation in a micellar system. Another frequently used oxidizing agent in direct chemiluminescence determinations is *N*-bromosuccinimide. Its oxidizing properties are attributed to hypobromous acid, produced on hydrolysis. Isoniazid [9], tetracyclines [10], other antibiotics [11], amiloride [12] and hydrazine [13] have been determined. Other oxidants used to induce chemiluminescence are: cerium(IV) ions in acidic solution [14–16], sodium hypochlorite [17], bromine [18], and hexacyanoferrate(III) [19] in alkaline solution.

Among the methods used for the determination of phenothiazine drugs in pharmaceutical preparations and body fluids, spectrophotometric and chromatographic [20–22] methods are most often used, although electroanalytical detection systems can also be employed [23]. Alwarthan et al. [24] determined promethazine by an indirect chemiluminescence method, using its inhibitory effect on the luminol-hydrogen peroxide-chromium(III) system. Paz and Townshend [25] developed direct chemiluminescence method for the determination of chlorpromazine using potassium permanganate as an oxidant.

This work is concerned with the study of the chemiluminescence generated by ten phenothiazine derivatives using various oxidizing agents. A two channel flow-injection manifold was used to develop a fast and simple assay procedure for thioridazine hydrochloride.

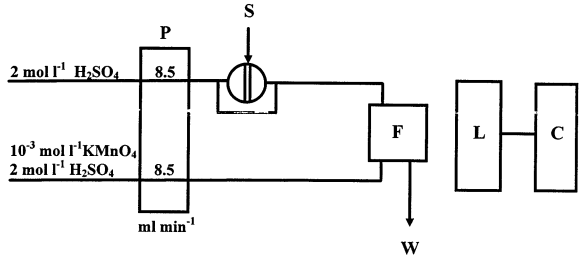

Fig. 1. Schematic diagram of optimized flow-injection system used for determination of thioridazine hydrochloride. C, computer; F, flow cell; L, flow-luminometer; P, peristaltic pump; S, sample injection point; W, waste.

2. Experimental

2.1. Apparatus

The flow-injection set-up, shown schematically in Fig. 1, consisted of an Ismatec MS-Reglo peristaltic pump, a Model 5021 rotary injection valve (Rheodyne, Cotati, CA), and a flow luminometer (KSP, Poland) with a coiled PTFE tube of 1 mm i.d. (length of 25.0 cm in six windings). The photomultiplier was operated at 650 V, and the detector response was recorded on a 386-series personal computer with KSP software. The flow system was made of PTFE tubing of 0.8-mm i.d. The reagent and carrier streams were merged in a Perspex T-piece.

Reference spectrophotometric measurements were performed using a Hewlett-Packard Model 8452A diode array spectrophotometer.

2.2. Reagents

Propericiazine maleate, levomepromazine maleate, frenolon difumarate and frenolon diethanosulphonate were obtained from United Works of Pharmaceutical and Dietetic Products (Budapest, Hungary), thioridazine hydrochloride from Sigma (USA) and all the other phenothiazine derivatives from Polfa (Jelenia Góra, Poland). All the other reagents used were of analytical grade and were obtained from POCh (Gliwice, Poland).

The stock standard solutions of all phenothiazine derivatives were prepared with bidistilled/deionized water, and with 1.0 ml of 2.0 mol l^{-1} H_2SO_4 in 100.0 ml for diethazine hydrochloride, levomepromazine maleate, frenolon difumarate, frenolon diethanosulphonate, methophenazine difumarate and alimemazine tartrate.

The oxidizing solution of potassium permanganate was prepared daily by dissolving 0.0395 g of $KMnO_4$ in 0.25 l of 2.0 mol l^{-1} H_2SO_4.

Table 1
The results of use of different oxidizing reagents in flow-injection manifold for the generation of chemiluminescence from 100.0-mg l^{-1} solution of thioridazine hydrochloride

Oxidizer	Concentration/medium (mol l^{-1})	Signal intensity (nA)	Stability of baseline
$KMnO_4$	1.0×10^{-3}/acidic[b]	132.3	Good
$Ce(SO_4)_2$	1.0×10^{-3}/acidic[b]	0.0	Good
$K_2Cr_2O_7$	1.0×10^{-3}/acidic[b]	0.0	Good
$K_2S_2O_8$	1.0×10^{-3}/acidic[b]	0.0	Good
KIO_3	1.0×10^{-3}/acidic[b]	0.0	Good
H_2O_2	1.0×10^{-3}/acidic[b]	0.0	Good
H_2O_2	1.0×10^{-3}/alkaline[c]	0.0	Good
NCS[a]	1.0×10^{-3}/alkaline[c]	0.0	Very bad

[a] *N*-Chlorosuccinimide.
[b] 2.0 mol l^{-1} H_2SO_4.
[c] 1.0×10^{-1} mol l^{-1} NaOH.

3. Results and discussion

3.1. Effect of different oxidants

In order to find the most efficient oxidizing agent for the determination of phenothiazine drugs, various chemical systems have been tested. As is shown in Table 1, only with the acidic potassium permanganate solution were positive results achieved. With acidic solutions of potassium dichromate, cerium sulphate, potassium periodate, sodium peroxydisulphate, hydrogen peroxide and alkaline hydrogen peroxide and *N*-chlorocuccinimide, no signals were obtained even for 100.0-mg l^{-1} solutions of thioridazine hydrochloride and promazine hydrochloride.

Results obtained in our experiment seem to be quite different from those published by Aly et al. [26], who oxidized fluphenazine hydrochloride, levomepromazine hydrochloride and trimeprazine tartrate, and measured resulting chemiluminescence. The authors stated that the best oxidant for these drugs is Ce(IV) sulphate in 2.0 mol l^{-1} $HClO_4$, and they obtained no positive result using acidic potassium permanganate solution as an oxidizing agent, which was the only working reagent in our experiment. This difference was probably due to the specific properties of the different phenothiazine derivatives used in both experiments. In our experiment with Ce(IV) sulphate as an oxidant, we used sulphuric acid instead of perchloric acid, and in this medium, according to Aly et al. [26], no or little chemiluminescence is produced.

All the experiments in our work were carried out in the manifold shown schematically in Fig. 1. The sample was injected into the carrier stream of sulphuric acid or sodium hydroxide of the same concentration as in the reagent stream.

3.2. Chemiluminescence activity of different phenothiazines using $KMnO_4$

The signal magnitude for 13 different phenothiazine derivatives was measured using potassium permanganate solution in 2.0 mol l^{-1} sulphuric acid, and the results are given in Table 2. The most efficient chemiluminescence was observed for thioridazine hydrochloride and frenolon diethanosulponhate with flow rates of 8.0 ml min^{-1} for both carrier- and reagent stream. Changing of the flow-rate in broad range results in no signal enhancement for all phenothiazine derivatives. The conclusion is that differences in the intensities are due to the specific mechanisms of the reaction of each phenothiazine and its acidic radical, and not to the kinetic factor. Consideration of the particular structures of examined phenothiazines shows that functional group substituted in position 2 is of crucial importance for intensity of produced chemiluminescence. Thioridazine with $-SCH_3$ functional group produces the most intensive chemiluminescence, followed by levomepromazine ($-OCH_3$) and chloropromazine (–Cl).

When position 2 is substituted by hydrogen, chemiluminescence is less intensive like in case of diethazine, promazine, alimemazine or profenamine. Relatively high chemiluminescence produced by frenolon diethanosulphonate compared with frenolon difumarate is due to its acidic radical, since position 2 is substituted in frenolon by hydrogen atom.

All preliminary experiments described above were performed in the not completely optimised manifold, where the sample volume was 250 μl, potassium permanganate and sulphuric acid concentrations were 1.0×10^{-3} and 2.0 mol l^{-1}, respectively and flow-rate in both channels was 8.0 ml min^{-1}.

Table 2
Chemiluminescence arising from the oxidation of 20.0-mg l^{-1} solutions of some phenothiazine derivatives with 1.0×10^{-3} mol l^{-1} potassium permanganate in 2.0 mol l^{-1} sulphuric acid

Phenothiazine	Signal intensity (nA)	Relative signal intensity (%)[a]
Thioridazine hydrochloride	83.9	100
Promazine hydrochloride	13.3	15.9
Chlorpromazine hydrochloride	21.6	25.7
Diethazine hydrochloride	16.1	19.2
Perphenazine hydrochloride	20.9	24.9
Profenamine hydrochloride	6.8	8.1
Promethazine hydrochloride	12.9	15.4
Levomepromazine maleate	62.8	74.9
Frenolon difumarate	19.5	23.2
Frenolon diethanosulphonate	77.6	92.5
Methophenazine difumarate	17.7	21.1
Alimemazine tartrate	7.1	8.5
Propericiazine maleate	18.6	22.2

[a] Chemiluminescence intensity for thioridazine hydrochloride is 100%.

3.3. Nature of chemiluminescence emission

Mellinger and Clyde [27,28] observed considerable increase of fluorescence intensity of phenothiazines as a result of their oxidation with acidic potassium permanganate. After oxidation their spectra form very distinct four-wave excitation pattern and their fluorescence shifts toward lower wavelengths. This effect is due to the formation of sulphoxides or sulphones of phenothiazines, while the sulphur atom in position 5 is oxidized. Comparing fluorescence spectra of sulphoxides of trifluoroperazine or chlorpromazine with fluorescence spectra of potassium permanganate-treated drugs, the authors suggest their oxidation to sulphoxides as the reason for enhancement of fluorescence intensity. What is important that all phenothiazine drugs with the same radical on position 2 of the phenothiazine nucleus produce the same excitation and fluorescence spectra.

Thioridazine represents a special case, since it has two oxidizable sulphur atoms. This is the reason for more intensive fluorescence than by phenothiazines with hydrogen or chlorine atom in position 2. The spectrum of oxidized thioridazine is apparently due to the oxidation of the sulphur of both the ring and the side chain, since it is similar to that of thioridazine disulphoxide and thioridazine disulphone.

In our study we suggest formation of sulphones of thioridazines as the possible source of chemiluminescence. In case of thioridazine it would be 2,5-disulphone molecule in excited form, which is produced as a product of oxidation process. This would explain the high chemiluminescence activity of thioridazine.

3.4. Optimisation of flow-injection determination of thioridazine hydrochloride.

Concentrations of potassium permanganate and sulphuric acid solutions, flow-rates, sample volume and temperature are the factors influencing the obtained signal magnitude. All of them were optimised for the maximum signal-to-noise ratio and the best reproducibility.

Increase of temperature does not affect the signal magnitude to a significant degree until 55°C

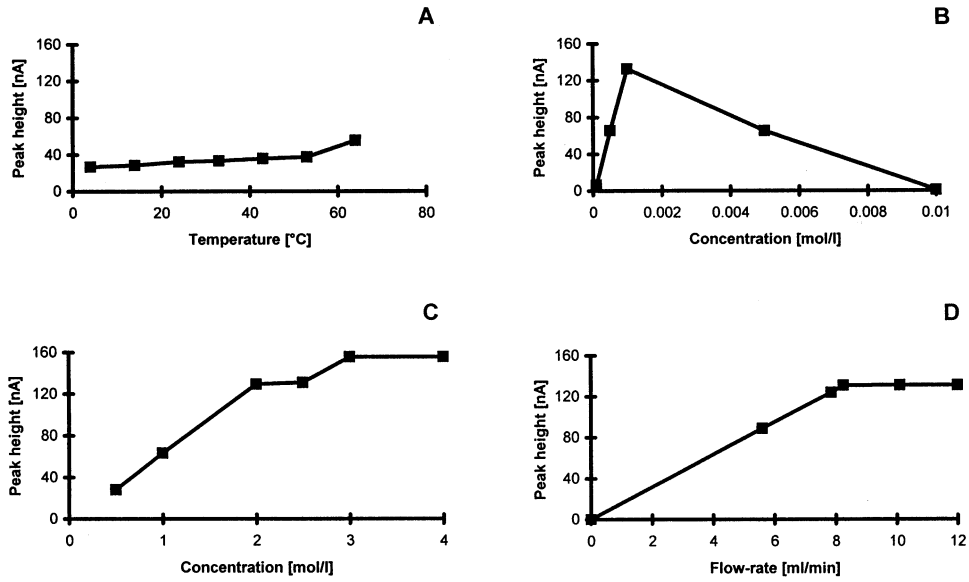

Fig. 2. A: Effect of temperature. B: Effect of concentration of $KMnO_4$. C: Effect of concentration of H_2SO_4. D: Effect of flow-rate on peak height.

(Fig. 2A). Room temperature was chosen for further experiments because of increasing noise with increasing temperature. Fig. 2B and C shows changes of the signal magnitude with the increase of the $KMnO_4$ and H_2SO_4 concentrations in the reagent solution. With concentrations of H_2SO_4 higher than 2.0 mol l^{-1}, precision of the method was rather poor, and this concentration was used in further experiments. The curve for $KMnO_4$ concentration goes through the maximum at the value of $\sim 1.0 \times 10^{-3}$ mol l^{-1} because of decreasing transparency of the solution with increasing concentration (effect of the inner filter).

The flow-rate of both streams is the critical parameter because of the high reaction rate (Fig. 2D). The flow rate of 8.5 ml min^{-1} was chosen for both channels, since further increasing it resulted in no signal increase. Relatively high flow-rate influences the consumption of the reagent, which is not very important in this case.

A total of six sample loops with inner volumes of 100, 200, 250, 300, 350, 400 μl were tested. The increase of the signal magnitude while increasing sample volume was observed until 300 μl, so this sample volume was injected in further experiments.

As a result of the optimization process the optimal parameters of the flow- injection manifold were found as shown in Table 3. Because of a very short reaction time ($t < 4$ s), the reaction coil was not necessary. Mixing and reaction occurred in a planar coil placed very near the photomultiplier tube.

A log-log calibration graph of the optimized system was linear over the range of 1.0–30.0 mg l^{-1} of thioridazine hydrochloride solutions with the equation $y = 1.0522 \times -0.4116$, where y is log (peak height), and x is log (concentration); the correlation coefficient was 0.9974. Fig. 3 shows a typical recording of calibration curve for this

Table 3
Optimized conditions for flow-injection determination of thioridazine hydrochloride in the system shown in Fig. 1

Optimized parameter	Optimum value
$KMnO_4$ concentration, mol l^{-1}	1.0×10^{-3}
H_2SO_4 concentration, mol l^{-1}	2.0
Flow-rate, carrier stream, ml min^{-1}	8.5
Flow-rate, reagent stream, ml min^{-1}	8.5
Sample volume, μl	300.0
Temperature, °C	20.0

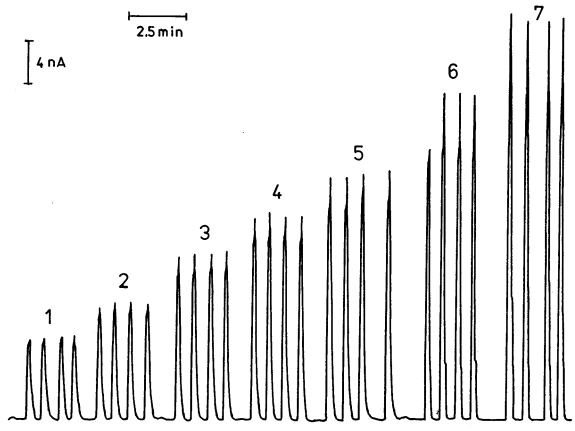

Fig. 3. Example of a recording of flow-injection signal under optimized conditions for injections of 4.0- (1), 6.0- (2), 8.0- (3), 10.0- (4), 12.0- (5), 14.0- (6), and 16.0- (7) mg l^{-1} thioridazine hydrochloride solutions.

Table 4
Effect of concomitants on determination of thioridazine hydrochloride

Coexisting substance	Tolerable concentration (mg l^{-1})
NaCl	7.0
Na_2SO_3	5.0
Lactose	3.0
$NaHSO_3$	3.0
EDTA	2.0
Formaldehyde	2.0
Ascorbic acid	2.0
$CaCl_2$	2.0
Glucose	2.0

system. The detection limit (S/N = 3) was 0.5 mg l^{-1}. The precision of the method calculated for 15 injections of 20.0-mg l^{-1} thioridazine hydrochloride solution was 1.8% (RSD).

3.5. Effect of sensitisers

Addition of different potential sensitisers suggested by Nakagama et al. [29] such as Ce(IV), Cu(II), Fe(II), Co(II), Ag(I)-ions and rodamine-B to the carrier solution resulted in no signal enhancement.

3.6. Interference study

Thioridazine hydrochloride occurs in drug formulations as a rule without interfering components. It was interesting however, how different components typically occurring with other phenothiazines in pharmaceutical formulations do affect the signal magnitude. A foreign substance was considered not to interfere if it caused a relative error of less than 5% for the determination of 20.0 mg l^{-1} of thioridazine hydrochloride. The results are shown in Table 4. The negative error was observed for almost all reducing agents tested. This can be explained by increased permanganate consumption in their presence. Only for formaldehyde, which acts as sensitizer in this case, were slightly increased signals observed. The enhancement of the signal magnitude does not exceed 10% even by concentration of formaldehyde of 20.0 mg l^{-1}. Positive errors were also caused by the presence of calcium chloride and sodium chloride.

3.7. Sample analysis

The possibility of using the developed method in real samples was examined for Melleril tablets which contain 10.0 mg of thioridazine hydrochloride, ferrous oxide and pigment Blue V.

For the purpose of extracting the active ingredient ten tablets were weighed, powdered and a precisely defined quantity of the powder ($\sim 1/10$), was mixed with 100.0 ml of methanol and stirred well for 10 min. The suspension was filtered and diluted 1:10 with bidistilled/deionized water before injection into the manifold. All the thioridazine hydrochloride solutions used for the calibration of the system contained the same amount of methanol, because of enhancing the signal magnitude by this solvent. The obtained results were compared with a spectrophotometric reference method [30] based on the absorption measurements at 254 nm in the methanol solution. The relative error of the proposed method was 1.3%.

4. Discussion

Proposed flow-injection chemiluminescence

method for the determination of thioridazine hydrochloride in tablets is fast, simple and easy to automate. Compared to standard titrimetric method in non-aqueous solution it is less time-consuming, and its cost per assay is lower. When compared to other flow-injection methods for phenothiazine drug determination it is one of the simplest in respect of used equipment and reagents, and has comparable parameters like detection limit, sample throughput and linear calibration range. One should realize however, that the detection method used here is not very selective. Many organic substances can give rise to the analytical signal and many others can consume reagent without chemiluminescent effect or act as inhibitors of chemiluminescence. Therefore if the sample matrix is more complicated, some pretreatment steps are often required.

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