A SOLID-PHASE REACTOR AS THE CATALYST SOURCE IN A FLOW-INJECTION ASSEMBLY. SPECTROPHOTOMETRIC DETERMINATION OF THIORIDAZINE.

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Summary.- A flow injection assembly is proposed in which the sample solution containing thioridazine was forced through a solid-phase reactor containing immobilized PbO_2. By subsequent reduction of PbO_2 by thioridazine, Pb (II) is released into the system. The liberated Pb(II) is then merged with a Mn (II)-EDTA complex solution to unmask Mn(II) which in turn acts as a catalyst in the IO_4^- - *N,N*-diethylaniline reaction at pH 7. The product of this last redox reaction is spectrophotometrically monitored at 470 nm. The calibration graph is linear over the range 0.25 - 5.0 $\mu g\ ml^{-1}$ with an RSD of 1.3% and a sample throughput of 39 h^{-1}. The influence of foreign compounds is also studied. The proposed method is applied to the determination of thioridazine in a pharmaceutical formulation and is also applicable to determination of other relative compounds such as phenothiazines.

Key-words.- FIA, thioridazine, spectrophotometry, solid-phase reactors.

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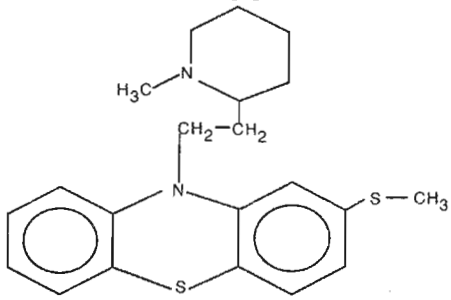
1. Introduction

Thioridazine (10-[2-(1-methyl-2-pyperidyl)ethyl]2-methylthiophenothiazine) hydrochloride is a member of the antipsycotic - phenothiazine group which is prescribed for the symptomatic management of psycotic disorders [1].



Thioridazine

The titration of thioridazine in glacial acetic acid and acetic anhydride against perchloric acid solution is officially recommended as the determination of thioridazine [2]. Spectrophotometric and fluorimetric batch procedures have been reported [3]. Two FIA procedures have been reported; one is based on the oxidation by iron(III) perchlorate in perchloric acid medium [3], in which the reaction product is monitored spectrophotometrically. The other deals with the on-line photodegradation by a UV lamp; detection is also spectrophotometric.

Solid-phase reactors are proved to have excellent features to be included in FIA assemblies [6]. They are even known to have advantages over the use of the similar systems in solution [7]. The solid-phase reactors have been used for analytical purposes for various reasons, pre-treatment of samples or reagents, preparation of unstable reagent solution, derivatization of samples, and coupling with optical detectors [8]. The present work focused on the study of the degree of reproducibility of the chemical behavior of the solid-phase reactors. With this goal in mind, a new application of these reactors on the indirect-catalytic determination of thioridazine in pharmaceuticals in FIA system has been tested; the solid-phase reactors act as a source of a constant concentration of metal cations. Provided that these reactors are capable of supplying with a constant and controlled concentration of metal ions, the resulting solution can be used as a catalyst solution for an indicator reaction which can be monitored spectrophotometrically.

2. Experimental

2.1.Reagents.

Aqueous solutions were prepared in pure distilled and deionized water; 100 mg ml^{-1} stock solution of thioridazine was prepared by dissolving 0.0546 g of powdered reagent in water and

this solution was stable at least for eight days. An aliquot of 10^{-3} M H_2SO_4 added to the solution to make the resulting solution of 10^{-4} M in H_2SO_4 . A stock solution of citric acid (Panreac, a.r.) was prepared by dissolving 42.0656 gr. of the reagent in water, and 50 ml of 2.2 M H_3PO_4 (Panreac, a.r.) was added to the solution, and pH was finally potentiometrically ajusted to 7.0 by 2 M NaOH (Panreac,a.r). A Mn(II) stock solution was prepared by dissolving 0.1982 g of metallic manganese (Merck, a.r.) in 2 ml of 1:1 HNO_3 (Panreac, a.r.) and diluting to 200 ml with 1% HCl. The working solutions were prepared by diluting 17 ml and 25 ml of the stock solution to 250 ml and 500 ml, respectively. Other reagents used were $NaIO_4$ (Panreac, a.r.) *N,N*-Diethylaniline, DEA, (Aldrich 99%); lactose (Guinama), sucrose (Guinama) and ethanol (Panreac, a.r.).

For determination of thioridazine in a pharmaceutical formulation, a sample solution was prepared as follows. Five tablets were powdered using an agate mortar and a pestle. About 0.05 g of the powder was exactly weighed and mixed with 200 ml of water and magnetically stirred in darkness. The resulting mixture was filtered and was finally diluted to 250 ml.

2.2. Apparatus

The proposed FIA assembly is depicted in Fig. 1. The assembly was provided with a Gilson Minipuls 2 peristaltic pump and a sample injector (Rheodyne, Model 5041). The product of the IO_4^--DEA redox reaction was monitored at 470 nm by a Perkin-Elmer Lambda 16 UV-vis spectrophotometer with a flow-cell of 18 µl (Hellma). The PTFE tubes were of 0.8 mm i.d. except for the solid-phase reactor which was made of tubes of 1.5 mm i.d.

2.3.Preparation of the solid-phase reactors

The immobilization of PbO_2 by means of polyester resin beads was carried out according to the procedure formerly published [9]. The solid PbO_2 (Probus) was added to a solution of polyester resin Al-100 (Glasspol Lacomba C.B.) and then methylketone peroxide (Glasspol Lacomba C.B.) was added as a catalyst. The resulting mixture was manually stirred up to hardness. The solid was then broken with a hammer and powdered to the required particle size by means of a coffee mill. The particles were washed with distilled water and dried at room temperature and kept dry until use.

3. Results and discussion

Preliminary experiments were performed with a two-fold purpose; 1) to investigate the catalytic action of Mn(II) on the IO_4^-/DEA system under continuous-flow conditions and 2) to determine whether the Pb(II) ions released from a solid-phase reactor by reaction with thioridazine could be used for its indirect determination of the reagent.

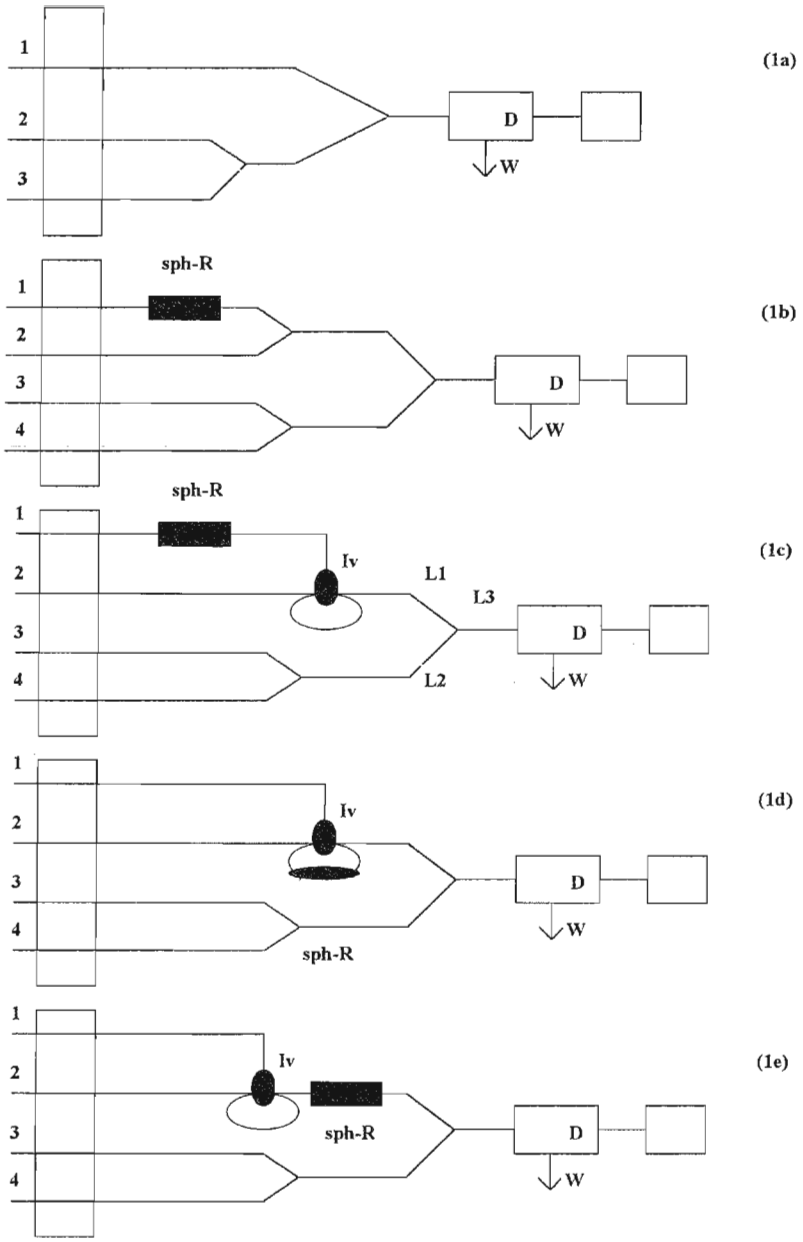


Figure 1.- Flow manifolds

1a) and 1b) were used for study of chemical system.

1c), 1d) and 1e) were the FIA manifolds tested. Selected FIA manifold was 1c).

P= pump; sph-R= solid phase reactor; Iv= Injection valve; D= Detector; W= Waste; R=Recorder; L1, L2,L3 = Length reactors.

To this end, we first assembled a system for solution tests (Fig. 1*a*) and another including a solid-phase reactor containing PbO_2 immobilized on a polymer resin (Fig. 1*b*). Channel 1 of the system depicted in Fig. 1a was used to successively circulate 10^{-5} M and 10^{-6} M solutions of Mn(II), the Mn(II)–EDTA complex, 10^{-5} and 10^{-6} M Pb(II) and distilled water. Channels 2 and 3 were used to circulate $7x10^{-3}$ M IO_4^- and 10^{-3} M DEA (buffered at pH 7), respectively. The results revealed that only Mn(II) acted as a catalyst for the reaction between IO_4^- and DEA, and that it allowed the reaction product to be monitored at 470 nm. Also, the presence of Pb(II) and the Mn–EDTA complex showed a catalytic effect on the reaction as Pb(II) acted as an unmasking agent and released Mn(II) ions from the EDTA complex.

The next test batch, performed in the assembly of Fig. 1*b*, was intended for the study of the influence of various media on the redox reaction of immobilized PbO_2 in the reactor and the subsequent release of Pb(II). For this purpose, channel 1 was used to circulate the following media: H_2SO_4, $HClO_4$, HNO_3, H_3PO_4 (all at a concentration of either 10^{-3} or 10^{-4} M) and distilled water. Channel 2 was used to circulate (*a*) 2×10^{-6} M Mn(II) or (*b*) a mixture of 2×10^{-6} M Mn(II) and 4×10^{-6} M EDTA. Channels 3 and 4 are the corresponding to channels 2 and 3 of the former figure. When blank solution (such as water, perchloric acid, sulfuric acid, and so on) was circulated, the reactor was removed in order to ensure the possible maximum absorbance difference (with and without reactor). Sulfuric acid proved the best medium for the intended purpose.

Next, the proposed procedure was applied to the indirect determination of a reagent in a FIA assembly (Fig. 1*c*). The reagents tested were isoniazid, ondansetron, paracetamol, promethazine, emetine, captopril and thioridazine. All were used at a 20 ppm concentration in 10^{-4} M H_2SO_4 and the obtained signals were compared with injection of sulfuric acid at the same concentration. Ondansetron, promethazine and thioridazine showed a positive result. The last reagent was used for subsequent experiments as it provided the highest signals relative to the blank.

The optimum FIA manifold was chosen among that depicted in Fig. 1*c* and two others: one with the reactor located in the loop of the sample injection valve (Fig. 1*d*) and other with the reactor following the sample injection point (Fig. 1*e*). All injected samples contained 2.5 ppm thioridazine in 10^{-4} M H_2SO_4 (with 10^{-4} M H_2SO_4 as the blank). The reactor was made by packing 150–200 μm particles at a resin/PbO_2 w/w ratio of 3:1 and was 9.7 cm long.

The other solutions used were as follows: (*a*) 10^{-4} M H_2SO_4 (channel 2); (*b*) 0.014 M IO_4^-; (channel 3) and (*c*) a mixture of 10^{-3} M DEA, 1.2×10^{-5} M Mn(II) and 1.6×10^{-5} M EDTA

(channel 4); length reactors and flow rates were the same in all cases. The signals obtained from the assembly of Fig. 1*d* were roughly one-third of those provided by the manifolds of Fig. 1*c*; also, baseline restoration with the manifold of Fig. 1*d* was rather slow. This was checked by injecting variable concentrations of thioridazine and various Mn(II)/EDTA solutions with different metal-ligand concentration ratios at a various flow rate in order to vary the time of contact between the drug and immobilized PbO_2. In addition, two different valve actuation time of 1 and 2 min were used with the assembly of Fig. 1*d*. The results provided by the manifold of Fig. 1*c* always excelled those of the other assemblies, so it was finally chosen for subsequent work.

After the optimization of the FIA configuration, experimental variables that potentially influence the FIA signals were optimized. By using the assembly shown in Fig. 1c, the concentration of buffer circulated through channel 4 was optimized first. The buffer was prepared from various concentrations of citric and phosphoric acid (with citric and phosphoric acid concentration ratio = 2:1) and the pH was potentiometrically adjusted to 7.0 by adding 2.0 M NaOH as required. The effect of concentration of the buffer was studied over the range 0.01 to 0.05 mol citric acid in 0.5 l. The analytical signal was found to increase significantly with increasing buffer concentration from 0.010 to 0.035 M and to remained virtually constant between 0.0420 - 0.0455M. A concentration of 0.0430 M was selected as optimum.

We then optimized the dimensions and characteristics of the solid-phase reactor. The column initially used was 9 cm long, being 1.5 mm and 150-200 μm the inner diameter and the particle size, respectively. PbO_2/resin weight ratio was 3:1. The variables studied were, in order of investigation, particle size, reactor inner diameter, PbO_2/resin weight ratio and reactor length. Table 1 gives the tested ranges of the parameters (with two different concentrations of thioridazine), the corresponding peak heights (in absorbance units (corrected for the blank value) and the values chosen as optimal.

A PbO_2/resin weight ratio of 4 was selected since a ratio of 5 was near the mechanical stability limit for the solid polymer. The effect of the reactor length was then tested at various thioridazine concentration (pre-calibration) in order to ensure not only the maximum possible sensitivity, but also the widest linear range. Of the lengths initially studied (6. 10, 18, 30 cm, giving absorbance of 0.3701, 0.3720, 0.7027 and 0.6277, respectively), 18 and 10 cm were further tested for the linearity of the calibration graphs. The equations obtained were $A = 0.037 + 0.464\,C$ for the 18 cm reactor, and $A = 0.128 + 0.445\,C$ for the 10 cm reactor, where A and C represent the peak height in absorbance units and concentration of the reagent in $\mu g\ ml^{-1}$. The reactor length of 10 cm was chosen because it provided a considerably wider range over 18 cm reactor despite the slightly lower sensitivity (slope of the calibration graph).

The average column lifetime was estimated by circulating the thioridazine in a continuous manner rather than injecting it. The signal remained stable for 50 hr during which 2.4 ppm thioridazine in 10^{-4} M H_2SO_4 was passed at a flow-rate of 1.89 ml/min^{-1}.

Table 1. Optimization of the solid-phase reactor parameters

Parameter	Tested range	Peak- height		Selected value
		1 ppm	2.3ppm	
Particle size (μm)	150-200	0.2769	0.7189	150-200
	200-250	0.2071	0.5950	
	250-300	0.1795	0.5190	
	300-400	0.1592	0.5072	
Reactor i.d. (mm)	0.5	0.0931	0.2988	1.5
	0.8	0.2370	0.5522	
	1.5	0.2941	0.6760	
PbO_2/resin	1:1	0.1345	0.4921	4:1
Weight ratio	2:1	0.1396	0.5016	
	3:1	0.1823	0.5753	
	4:1	0.1649	0.5614	
	5:1	0.2284	0.5880	
Reactor length (cm)	6	0.3701	-	10
	10	0.3720	-	
	18	0.7027	-	
	30	0.6177	-	

The FIA variables were optimized by using the modified Simplex Method (MS) [10,11,12] in three steps. First, the ranges to be studied for each experimental variable were established according to the results of preliminary experiments. After 14 vertices, a new simplex was started with narrower ranges. The studied parameters are listed in Table 2 with the tested ranges. Finally, the vertices that gave the highest signals were selected and repeated with the various concentrations of thioridazine in order to obtain the linear range of the calibration curves in addition to peak widths and reproducibility. The linear range obtained was 0.2–1 ppm for vertex 8 in the second simplex. For vertex 8 in the first simplex and vertex 10 in the second simplex, the obtained linear ranges were 0.25–3.5 ppm ($A = 0.054 + 0.461C$, $r = 0.9975$) and 0.5–3 ppm ($A = 0.139 + 0.487C$, $r = 0.9982$), respectively. Based on these results, vertex 8 in the second simplex was selected as it provided a wider linear range and a similar sensitivity (the slope of the calibration graph being only 5% lower) to vertex 10 in the second simplex. As a result, the optimal FIA parameter values were as shown in Table 2.

The optimized FIA system was used to study the influence of the concentrations of the reactants involved in the second reaction (IO_4^-/DEA). The periodate (IO_4^-) ion concentration was found to strongly influence the height of the transient signal. The peak height increased sharply with increasing the periodate concentration from 0.001 - 0.025 M and remained virtually constant up to 0.090 M and then decreased slightly up to the highest concentration tested (0.1 M). A concentration of 0.03 M was chosen as an intermediate value in the plateau obtained from the peak height–IO_4^- concentration plot.

The influence of the DEA concentration on the transient signal was found to be critical; observed peak heights given in absorbance units (with the DEA concentration given in parentheses) were: 0.0103 (10^{-4} M); 0.3758 (10^{-3} M) and 0.5781 ($1.1x10^{-3}$ M). However, with higher concentration of DEA, dissolution of the reagents was hindered and formation of emulsion was observed. A DEA concentration of 10^{-3} M was thus chosen as optimal.

Table 2. Optimization of the FIA parameters by the Simplex method

Parameter	Tested range		Selected value
	1st Simplex	2nd Simplex	
Sample flow rate (ml/min)	1.42 - 4.32	1.42 - 2.38	1.67
Carrier flow rate (ml/min)	0.45 - 4.32	0.45 - 3.35	0.97
Reagent flow rate (ml/min)	0.45 - 4.32	0.45 - 3.35	1.20
Sample volume (μ l)	90 - 392	140 -291	141
Coil length L_1 (cm)	10 - 30	10 - 30	15.1
Reactor length L_2 (cm)	20 - 200	30 - 100	48
Reactor length L_3 (cm)	100 - 600	100 - 900	528.5

4. Analytical figures of merit

The calibration graph was linear over the range 0.25 - 5.0 μg ml^{-1} of thioridazine; $A = 0.2636 + 0.5578C$ and the regression coefficient of 0.9998; the detection limit (defined as three times the background noise) was 0.05 μg ml^{-1}.

The reproducibility was satisfactory with rsd of 1.27 % for 40 replicates of 2 ppm of thioridazine and the sample throughput was 39 hr^{-1}. The day-to-day reproducibility was tested by obtaining calibration graphs on four different days; all solutions were freshly prepared and the identical solid-phase reactor was used. The rsd calculated from the slopes of the calibration graphs was 3.2%.

The influence of foreign compounds that are commonly co-formulated with thioridazine in pharmaceutical formulations was investigated. To solution containing 2 μg ml^{-1} of thioridazine, various concentrations of the possible interefering substance were added until the relative error (compared with pure thioridazine solution) exceeded 2% or the maximum of 500/1 (foreign compound/thioridazine) concentration ratio. The results given as relative error % (with the concentration ratio, foreign compound/thioridazine, given in the parentheses) are: sucrose, 1.8% (100/1), ethanol, 1.3% (100/1), and, lactose, 0.3% (500/1).

Thioridazine was successfully determined by the present method in a pharmaceutical formulation, Meleril 200 retard (from Sandoz), with the relative error of 2.0 % compared with the claimed value on the label .

6. Conclusions

This paper deals with a new strategy in the application of solid-phase reactors, where they act as a constant and controlled source of metal ions by reaction with the analyte. The strategy is applied to the indirect-catalytic determination of thioridazine in pharmaceutical formulations in a flow-injection assembly.

The present method allows the spectrophotometric determination of thioridazine hydrochloride by using a solid-phase reactor as a source of Pb(II) ions; the released Pb(II) acts as a demasking agent to liberate Mn(II) from a Mn(II)-EDTA complex and Mn(II) then acts as a catalyst on the oxidation of DEA by periodate ions. The spectrophotometric output is related to the concentration of thioridazine hydrochloride.

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