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Bead injection spectroscopy-flow injection analysis (BIS-FIA): an interesting tool applicable to pharmaceutical analysis Determination of promethazine and trifluoperazine

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Abstract

A bead injection spectroscopy-flow injection analysis (BIS-FIA) system for the spectrophotometric detection of promethazine and trifluoperazine is developed. The sensor is based in the oxidation of the phenothiazines by Fe(III) which is later determined by formation of the complex between Fe(II) and Ferrozine, $[FeFz_3]^{4-}$. Immediately, this complex is retained on a homogeneous bead suspension of Sephadex QAE A-25 resin (500 μl) which has been injected previously in the system to fill a commercial flow-cell (Hellma 138-OS). The use of BI with respect to the use of a reusable flow-through sensor is justified because the complex is so strongly retained on the beads that the regeneration of the solid support becomes extraordinarily difficult in the proposed method. At the end of the analysis, beads are automatically discarded from the flow-cell, by reversing the flow, and transported out of the system. The analytical signals are measured at a wavelength of 567 nm, corresponding to the absorbance of the complex. Using a sample volume of 600 μl, the analytical signal showed a very good linearity in the range 0.5–8.0 μg ml^{-1} and 0.5–10.0 μg ml^{-1}, with detection limits of 0.09 and 0.14 μg ml^{-1} for promethazine and trifluoperazine, respectively. R.S.D.s (%) lower than 2% were obtained for both analytes. The proposed method is highly selective in the presence of other species that are normally encountered with these analytes. The sensor was satisfactorily applied to pharmaceutical preparations.

Keywords: Bead injection spectroscopy; Flow injection analysis; Promethazine; Trifluoperazine; Pharmaceuticals

1. Introduction

When beads of an active surface are placed in the detection zone of a spectroscopic detector in an appropriate flow-cell of a flow injection analysis (FIA) manifold, the system obtained is a continuous sensing device which allows on line species monitoring. The bead surface has to be regenerated after measurements to achieve the sensing system to be reusable. These systems are called flow-through optosensors [1]. The potentiality of these systems in pharmaceutical analysis has been demonstrated [2]. Nevertheless, several problems can arise in the necessary regeneration steps: (a) the surface deactivation after sensing a certain number of measurements always occurs, and (b) when the monitored species is strongly retained,

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the solid surface regeneration becomes extraordinarily difficult, drastically reducing the life time of the sensing surface. In these cases, flow-injection renewable surface sensing methodology can be applied successfully [3]. The principles of these flow-through sensors with solid phase disposable beads, also called BIS-FIA systems, have been explained previously [4–6].

Phenothiazines are a group of drugs used as neuroleptics in the treatment of schizophrenia and other psychotic illness [7], and they also possess analgesic and antipyretic properties [8]. Massive overdose of phenothiazines may cause coma, miosis, respiratory depression, among other disorders. Two of these compounds have been studied: trifluoperazine and promethazine. Trifluoperazine (10-[3-(4-methyl-1-piperazinyl)-propyl]-2-(trifluoromethyl)-10*H*-phenothiazine) is widely used for the treatment of various psychotic disorders, such as acute or chronic catatonic, hebephrenic and paranoid schizophrenia and the manic phase of manic-depressive illness, and promethazine (*N*,*N*,α-trimethyl-10*H*-phenothiazine-10-ethanamine) has anticholinergic, sedative, and antiemetic effects and some local anesthetic properties, and it is used predominantly as an antiemetic or to prevent motion sickness.

Numerous methods have been proposed for determination of phenothiazines in pure form in pharmaceutical preparations and urine: titrimetric methods [9–11], kinetic methods [12–14], HPLC [15–17] and GC [18] procedures, and many spectrophotometric methods based on the oxidation of the drug with salts [19–21] in acid medium to yield coloured species with maximum absorption at 500–650 nm. Other methods are based on conventional fluorimetry [22–24], UV spectroscopy [25], or complexation with bromocresol green [26]; in most cases sample preparation is the most time consuming step. Flow-injection analysis (FIA) has also been applied to the determination of these compounds. This technique increases sample throughput and minimises sample pre-treatment generally with chemical [27,28] or photochemical [29,30] oxidation and using photometric and fluorimetric [31] detection of their oxidized form. Other procedures use chemiluminiscence [32,33], biamperometric [34] or amperometric [35] detection.

In previous papers, we demonstrated that commercial flow-cells are compatible with BIS-FIA systems [3,36]. In this work we show that BIS-FIA systems are applicable to pharmaceutical analysis. As analyte models, phenathiazines are used. The determination is based on the oxidation of these drugs by Fe(III), and the Fe(II) generated is later determined by complexation of this cation with the reagent Ferrozine (Fz). In order to increase selectivity and sensitivity, the magenta anionic complex $[Fe(II)Fz_3]^{4-}$ is retained on beads placed in the detection area. The renovation of the sensing surface (beads) is carried out for each individual sample analysis due to the difficulty of regenerating the sensing surface. The procedure is very simple, inexpensive and fast and allows the determination of these drugs in pharmaceutical preparations as an illustrative example of alternative to reusable flow-through sensors.

2. Experimental

2.1. Chemicals

All reagents were of analytical-reagent grade, and solutions were prepared in pure solvents and deionized water.

Promethazine hydrochloride (Fluka, Buchs, Switzerlands) and trifluoperazine dihydrochloride (Fluka, Buchs, Switzerlands) stock standard solutions (100 μg ml^{-1}) were prepared by dissolving 10 mg in deionized water. The chromogenic reagent used for Fe(II) was Fz (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′-4″-disulfonic acid disodium salt) (FLUKA, Buchs, Switzerland). All solutions were stable more than 1 month when they were protected from sunlight and kept at about 5 °C in a refrigerator. Working solutions were daily prepared by appropriate dilution of the stock solutions with deionized water.

Carrier solution in FIA experiments was a 1.79×10^{-3} M Fe(III) solution with 0.1 M NaCl (Panreac, Barcelona, Spain) at pH 1. Fe(III) solution was prepared from ammonium ferric sulphate $(NH_4)Fe(SO_4)_2 \cdot 12H_2O$ (Probus, Badalona, Spain) adjusting the pH value with $HClO_4$ (Panreac, Barcelona, Spain).

Sephadex QAE A-25 anion exchanger gel (Aldrich, Madrid, Spain) was used in the chloride form as solid support (40–120 μm, capacity: 3.1 meq g^{-1}). Another tested support was Sephadex DEAE A-25.

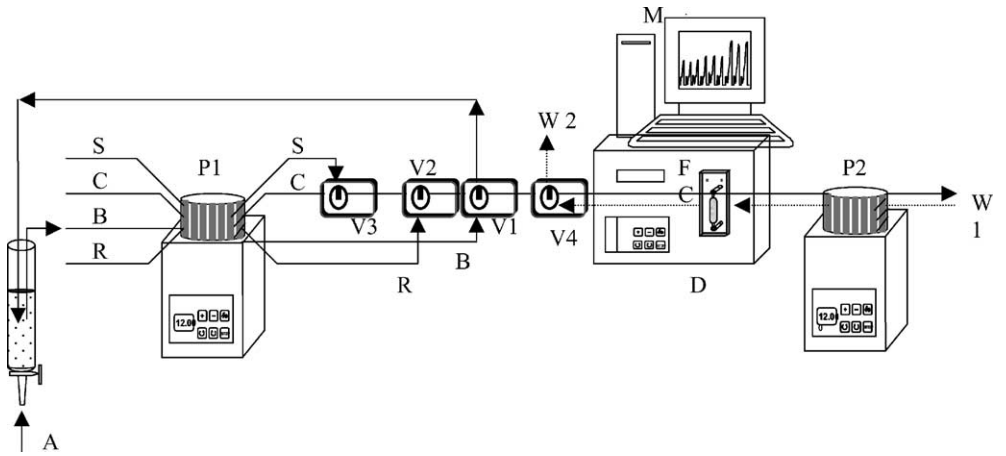

Fig. 1. Manifold. A: air; S: sample; P1,P2: peristaltic pumps; C: carrier solution; R: reagent solution; B: bead suspension; V1, V2, V3: injection valves; V4: selection valve; W1, W2: waste; D: detector; FC: flow-cell; M: computer.

2.2. Apparatus

Manifold is shown in Fig. 1. A chromatography column (i.d.: 16 mm, $l = 30$ cm) was used to obtain a homogeneous aqueous suspension of beads (Sephadex QAE A-25) by purging air gently through it, which was required for injection of a constant amount of beads.

Two four-channel Gilson Miniplus-3 peristaltic pumps with rate selector were used to generate the flow stream and to perform bead discharge. A Hellma 138-OS flow-cell (1 mm light path, 50 μl inner volume) was used to accommodate the resin beads. Some glass-wool was used in the outlet of the flow-cell to retain the resin beads.

Four variable-volume Rheodyne Model 5041 rotary valves with a single tube loop were used: (V1) for beads injection, (V2) for reagent injection, (V3) for sample injection and the last one (V4), connected as selection valve, was used for beads discharge by flushing them out of the cell. Teflon tubing of 0.8 mm i.d. was also used in all cases.

Absorbance was continuously measured with a Lambda 2-UV-Vis spectrophotometer (Perkin–Elmer, Bucks, England) which was interfaced to a compatible PC running Perkin–Elmer Computerized Spectroscopy Software (PECSS V 4.1). Other apparatus consisted of a Crison Model 2002 pH-meter with a glass/saturated calomel combination electrode and a Selecta (Barcelona, Spain) Ultrasons ultrasonic bath.

2.3. Procedure

In the chromatography column, initially, 0.5 g of beads Sephadex QAE A-25 were added to 30 ml of deionized water. This mixture was maintained continuously by purging air to obtain homogeneity. Five hundred microliters of this bead suspension were aspirated and transported to the flow-cell by pumping through the injection valve (V1). Pump 1 and 2 work simultaneously maintaining the flow rate at 1.25 ml min^{-1}. Beads are trapped in the cell and perfused with the carrier stream, and the baseline for the subsequent absorbance measurements is established.

Next, valves V2 and V3 are rotated simultaneously. Eight hundred microliters of Fz and 600 μl of sample solution are both injected at the same time into the carrier, respectively. The carrier, Fe(III), is the oxidizing agent and reacts with phenothiazines to form Fe(II), taking into account that Fz only reacts with Fe(II) to form a complex of magenta colour. This complex was retained on the beads developing the analytical signal, and the absorbance was recorded and measured continuously at 567 nm. When the analytical signal is maintained constant, Pump 1 was switched off and Pump 2 used in flow reversal, so the cell was emptied and the beads are automatically discarded from the flow-cell at the end of the assay cycle by rotating the selecting valve (V4). The Renewable Surface Fiagram is registered along all the process. All samples were analysed by triplicate.

2.4. Treatment of samples

In pharmaceutical preparations, tablets (not less than four) were finely powdered and an accurately weighed portion was dissolved and filtered through a 0.45 μm pore size. From this sample solution, working solutions were prepared by appropriate dilution, so that their concentration was in the respective phenothiazine working range. For the determination in syrup or cream, an accurately measured volume or weight, respectively, was appropriately dissolved in an ultrasonic bath and diluted with deionized water.

The pharmaceuticals analysed were: (1) Fenergan (cream) (2 g of promethazine per 100 g and excipients: estearic acid, cholesterol, lanoline, trietanolamine, glycerine, lavande aromatique, wax, methyl *p*-hydroxybenzoate, etc., Ltd. Aventis); (2) Fenergan (syrup) (composition per 5 ml: 5.65 mg promethazine, 3.75 g saccharose, 5 ml ethanol (4%), 3 mg extract of ipecacuanha, 45 mg potassium sulfoguaiacollate, Ltd. Aventis), (3) Frinova (tablets) (25 mg promethazine and excipients: starch, lactose, wax, saccharose, levilite, magnesium stearate, Ltd. Aventis), (4) Eskazine (tablets) (1 mg of trifluoperazine and excipients: saccharose, Ca sulphate, stearic acid, starch, gelatin, talc, wax, glycerine, Ti dioxide, paraffin, indigotine, Ltd. Goldshield Pharmaceuticals).

3. Results and discussion

3.1. Absorption spectra and volume of bead suspension

Fe(II) reacts with Fz to form a stable anionic magenta complex (ratio 1:3, respectively) with maximum absorbance at 562 nm. Due to the anionic nature of the complex $[Fe(II)Fz_3]^{4-}$, two anionic exchange bead types were tested: Sephadex QAE A-25 and Sephadex DEAE A-25. Sephadex QAE A-25 proved to be the best with regard to both retention capacity and reproducibility in the bead suspension injection, so it was chosen for next experiments. The maximum absorption wavelength of the complex sorbed on the beads was 567 nm. This small difference in the wavelength can be attributed to the modification of the surrounding environment of the analyte in the solid phase with respect to solution. The sorption of complex on the beads for a sample volume of 600 μl resulted in a signal approximately 25 times higher than that obtained in aqueous solution (in the same flow-cell and working conditions). It shows the big increasing obtained in sensibility with the proposed system respect to conventional spectrophotometry.

The homogeneity and the volume of beads suspension are important to be considered. By purging air continuously through the suspension placed in a chromatographic column could obtained easily the homogeneity of the bead suspension. The volume of beads suspension injected was optimised by varying between 300 and 800 μl of a mixture with 0.5 g of beads Sephadex QAE A-25 and 30 ml of deionized water in the column. A volume of 500 μl of homogenized suspension was chosen as appropriate volume because: (1) below 500 μl, beads did not cover completely the whole flow-through irradiated zone of the detection unit, and (2) beyond 500 μl, the absorbance started to decrease due to the dispersion of the analyte on the sensitive beads.

The uniformity of beads is also another important parameter. Beads may be packed in the cell differently each time, and the amount of exchanged complex could be different if they are too much different in size, but the resin beads used in our case (Sephadex QAE A-25) shows satisfactory uniformity.

3.2. Nature and concentration of the carrier and pH of the sample

Of the various oxidants used, Fe(III) was selected for its mild oxidation power. The oxidation of phenotiazines occurs in two steps: (1) phenothiazine is reversibly oxidized to a colour-free radical or semiquinone, and (2) this derivate is further and irreversibly oxidized to a colourless sulphoxide. The Fe(III)/Fe(II) couple only allows the oxidation of phenothiazine to the coloured free radical [37]. This reaction is very rapid and completed in a few seconds. So, phenothiazines can be determined by measuring the absorbance after the complexation between Fe(II) produced and Fz. Although different acids were tested (HNO_3, HCl, $HClO_4$ and H_2SO_4) in order to produces the oxidation together with Fe(III), perchloric acid was found to be the most suitable. The oxidation reaction was facilitated by increasing $HClO_4$ concentration, but high values of this concentration could

not be used because the complexation of Fe(II) with Fz was not possible in this conditions. Below pH 1 the complexation reaction was not carried out, so, the pH of the carrier solution was adjusted at this value with perchloric acid (0.1 M $HClO_4$).

The fixation of complex on the beads was so quick and strong that the beads area with the retained analyte kept above the irradiated zone. An electrolyte (NaCl) was added to the carrier solution for displacement of the complex to the zone of the beads which was irradiated. Various concentration levels of NaCl were tested, between 0.05 and 0.30 mol l^{-1}. NaCl concentrations higher that 0.10 mol l^{-1} did not increase the analytical signal, obtaining the best result with this value. By other hand, Fe(III) concentration was tested in the range 1.79×10^{-4} to 5.37×10^{-3} M, using 10 μg ml^{-1} of analytes. This concentration must be enough for allowing the total reaction of the analytes. The absorbance increased up to a Fe(III) concentration of 1.79×10^{-3} M; higher concentrations did not increase the signals significantly.

The sample pH value did not influence the analytical signal when it was neutral or acid. At basic pH the complex Fe:Fz was not formed, but taking into account the high acidity of the carrier solution it was not necessary to adjust the sample pH to allow the oxidation reaction.

3.3. Concentration and volume of reagent

Firstly, the reagent was immobilized on beads surface in order to avoid the injection of this one. Although its fixation was suitable, the reaction with the analyte was not complete and the formation of the complex did not produce reproducible analytical signals. For this reason, Fz was injected (V2) into the carrier stream, so producing stable and reproducible signals. The influence of Fz concentration was studied by injecting 800 μl of 10 μg ml^{-1} phenothiazine sample solution and 800 μl of Fz at different concentration levels (200–3000 μg ml^{-1}). Absorbance increased up to a Fz concentration of 2000 μg ml^{-1}; higher concentrations did not increase the signal significantly, therefore this concentration was used in all experiments.

The volume of Fz injected was optimised by varying between 600 and 1600 μl. For this experience, 800 μl of 10 μg ml^{-1} phenothiazine sample solution and a Fz concentration of 2000 μg ml^{-1} were used. The absorbance increased up to a reagent volume of 800 μl, beyond it was not increased the analytical signal significantly, so 800 μl was chosen as Fz volume optimum value.

3.4. FIA variables

The effect of the flow-rate was investigated by injecting sample solution of analytes at different flow-rates. An increase in the flow rate resulted in decreasing peak heights and caused over-pressure in the system. As a compromise between sensitivity and throughput, it was adopted a flow-rate of 1.25 ml min^{-1} working with both peristaltic pumps, simultaneously. The reversed flow rate of P2 can be increased to 2.25 ml min^{-1} in the elimination of the beads from the flow-cell.

Due to the reactions which are produced in the method, a reaction coil next to V3 in the system could be necessary. The effect of the length of the reaction coil was tested in the range 0–250 cm (teflon tubing of 0.8 mm i.d.). From the results it can be seen that the influence of the length was not very significant. It indicated that the reaction between Fe(III) and phenothiazines was rapid and complete so, it was not necessary to use a reaction coil in the system.

The effect of the sample volume was also studied. It is known that one of the main advantages of BI-FIA is its potential increase in sensitivity with an increase in the sample volume used for the analysis. This effect was studied with sample volumes between 40 and 1000 μl of 5 μg ml^{-1} standard solutions. Increasing sample volume resulted in a proportional increase in the absorbance as a result of a higher amount of compound retained on the beads. The only inconvenience of using large volumes of sample is that a higher time is required for each determination because of the increase in the signal development times. For two phenothiazines the increase of absorbance was linear in the range 40–800 μl (higher volumes did not increase the signal significantly). The volume chosen in order to calibrate the sensors for promethazine and trifluoperazine determination was 600 μl.

3.5. Analytical parameters

Calibration graphs were obtained for each analyte treated according to the procedure described above.

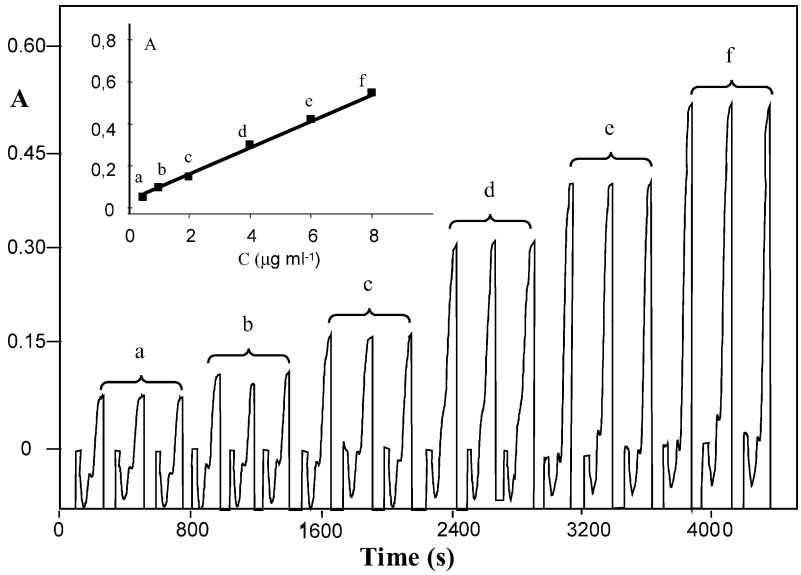

Fig. 2. Fiagram obtained in the calibration of the sensor for promethazine (600 μl of sample volume). Inset: corresponding calibration line.

Fig. 2 shows the fiagram obtained with its corresponding calibration graph for promethazine. Table 1 contains the figures of merit of the proposed method using a sample volume of 600 μl. The data were fitted by standard least-squares treatment and the calibration equations are shown.

The reproducibility was established for ten independent analyses of solutions containing 8 μg ml^{-1} (600 μl) of promethazine and trifluoperazine, respectively. The detection limit [38] was estimated according to IUPAC recommendations as the concentration of analyte producing an analytical signal equal to three times the standard deviation of the blank absorbance signal. The quantification limit [39] ($K = 10$) and the sampling frequency were also evaluated.

Table 1
Figures of merit for phenothiazines determination (600 μl)

Parameter	Promethazine	Trifluoperazine
Linear dynamic range (μg ml^{-1})	0.5–8.0	0.5–10.0
Calibration graph		
Intercept	0.044	0.059
Slope (ml μg^{-1})	0.067	0.046
Correlation coefficient	0.9994	0.9995
Detection limit ($K = 3$) (μg ml^{-1})	0.09	0.14
Quantification limit ($K = 10$) (μg ml^{-1})	0.31	0.46
R.S.D. (%) ($n = 10$)	1.8	2.0
Sampling frequency (h^{-1})	12	12

3.6. Effect of foreign ions

The effect of foreign ions, substances and excipients generally present in pharmaceutical preparations in the determination of 8 μg ml^{-1} of promethazine or trifluoperazine was studied to determine the tolerance of the method to these foreign species. A foreign species was considered not to interfere if it produces an error smaller than ±3% in the analytical signal. If any interference was observed, the ratio interference:phenothiazine (w:w) was reduced progressively until this interference ceased. It is necessary to point out that the maximum tested tolerance of both phenotiazines to saccharose, glucose, starch, talc, gelatin, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} and Zn^{2+} is 1250 (w:w). The amount of other interfering species tolerated in the proposed method such as saccharine, fructose, oxalate, tartrate, magnesium stearate, sodium sulphite, salicylic acid, paracetamol, acetylsalicylic acid and caffeine is at least 100 (w:w), so allowing the determination of phenotiazines in presence of amounts of these compounds much higher than those found

Table 2
Analytical applications (pharmaceuticals)

Sample	Promethazine		
	Amount added ($\mu g\,ml^{-1}$)	Amount found ($\mu g\,ml^{-1}$)	Recovery ± R.S.D.* (%)
Fenergan (syrup), Ltd. Aventis	–	0.976	–
	1	1.951	97 ± 3
	3	4.012	101 ± 3
	5	6.055	102 ± 1
Fenergan (cream), Ltd. Aventis	–	1.478	–
	1	2.460	99 ± 3
	3	4.551	102 ± 1
	5	6.449	99 ± 1
Frinova, Ltd. Aventis	–	0.953	–
	1	1.922	97 ± 3
	3	4.079	103 ± 2
	5	6.015	101 ± 2
	Trifluoperazine		
Eskazine, Ltd. Goldshield Pharmaceuticals	–	2.819	–
	1	3.801	99 ± 3
	3	5.877	102 ± 2
	5	7.856	101 ± 2

* R.S.D.: relative standard deviation (average of three determinations).

in pharmaceuticals. It demonstrates that the proposed method is highly selective if we compare with the homogeneous solution method, due to both the fixation of the analytes on the beads and the exclusion from them of the coexisting species in working conditions.

3.7. Applications

In order to assess the utility of the proposed method, it was applied successfully to the determination of promethazine and trifluoperazine in four pharmaceutical preparations: Fenergán (cream), Fenergán (syrup), Frinova (tablets) and Eskazine (tablets). All applications were performed using 600 μl of sample volume, and after treatment and suitable dilution to fit the concentration of the analyte within the linear calibration range, the samples were injected in triplicate.

Recovery studies were carried out to check the accuracy of the proposed method, by adding known amount of the analyte at three levels of concentration: 1, 3 and 5 $\mu g\,ml^{-1}$. Table 2 shows achieved results in such analysis. As can be seen, good recoveries were reached in all analysed samples, between 97.0 and 103.1%, with relative standard deviations lower than 3% in all cases, so indicating the utility of the proposed method for routine analytical control in pharmaceuticals.

4. Conclusions

The developed BIS-FIA system with spectrophotometric detection proposed for phenothiazines determination is an alternative to other methodologies. This one, besides being simple, accurate and precise, is free from many disadvantages that are common in other spectrophotometric methods: complex sample treatment, critical working conditions, heating of the reaction mixture, expensive chemicals and instrumentation, high time consuming, etc. Moreover, the system presents a high sensitivity, good quality results (R.S.D., 2%) and wide range of linear response. Two of the most important advantages of this sensor are: (1) the use of a commercially available quart flow-cell which allows the selective and sensible determination of the analytes, and (2) the automatic regeneration of the sensing detection area for each measurement. Hence, the proposed method can be recommended for

the routine determination of phenothiazines in their pure form or their preparations.

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