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Studies and analytical application of reaction of imipramine with chrome azurol S

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Abstract

Chrome azurol S (CAS) was tested as a spectrophotometric reagent for the determination of imipramine (IMP). It reacts in aqueous media with IMP forming pink–red, sparingly soluble in water ion association compound. This compound was quantitatively extracted with chloroform and the absorbance of organic phase was measured at 510 nm. The extraction conditions were studied using a batch method. On the basis of the results obtained with batch method, three-line flow-injection system was constructed. Batch and flow-injection methods were successfully applied for the determination of IMP in pharmaceutical preparation.

Keywords: Imipramine; Chrome azurol S; Extractive spectrophotometric analysis; Flow injection analysis

1. Introduction

Imipramine (IMP, 10,11-dihydro-*N*,*N*-dimethyl-5H-dibenz[b,f]-azepin-5-propanamine) hydrochloride, biologically active heterocyclic compound, is extensively employed as antidepressant in clinical practice of psychiatry. A wide application of IMP requires efficient methods for its quantitative determination in body fluids and pharmaceuticals.

Several methods have been reported recently for the determination of IMP including high-performance liquid chromatography (HPLC) [1–4], gas chromatography-mass spectrometry (GC-MS) [5], spectrophotometry [6–9], derivative spectrophotometry [10,11] spectrofluorimetry [12,13], atomic-absorption spectrophotometry (AAS) [14] chemiluminescence [15], voltammetry [16], titrimetry [17] and flow-injection [9,13]. Some of these methods are not simple for routine analysis and they need sophisticated instruments, not yet available in many control laboratories. Therefore it seems necessary to develop a simple, rapid and sensitive method for the determination of IMP.

The review of methods for the determination of this drug [1–17] show that the literature on its extractive spectrophotometric determination is very scanty though these methods are very useful for the determination of IMP in pharmaceuticals and body fluids.

Conventional batch process solvent extraction is a tedious and time-consuming procedure, and many efforts have been directed towards the de-

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velopment of mechanization of this procedure. Flow-injection technique has advantages that make it suitable also for this purpose. This technique eliminates direct contact of the worker with the toxic organic solvent and is faster and more economical than conventional procedure. First application of solvent extraction in flow-injection analysis (FIA) has been developed by Karlberg and Thelander [18], and since then several FIA methods, including pharmaceuticals determination, have been applied and reviewed [9,13,19–28].

The present method is based on the reaction of IMP with chrome azurol S (CAS). It was found that IMP reacts with CAS forming pink–red compound. The composition of this compound was established and spectroscopic studies in UV-VIS and IR region were performed. The ^{13}C NMR method was used for the confirmation of the structure of this compound. The compound obtained is sparingly soluble in water but fairly soluble in methanol, ethanol, and acetone and quantitatively extracted from aqueous solutions into chloroform. The intensively coloured extracts are stable for about 24 h. These properties have been exploited for the extractive spectrophotometric determination of IMP.

In this work both batch and flow-injection procedures for the determination of IMP in association with extraction of ion pair IMP–CAS have been described.

2. Experimental

2.1. Reagents

A standard 1.0×10^{-2} M solution of IMP hydrochloride was prepared by dissolving a commercial product (Sigma, St. Louis, MO) in double distilled water; this solution remained stable for half a month if kept refrigerated. Working solutions of lower concentrations were freshly prepared by appropriate dilution of the standard solution.

A 1.0×10^{-2} M CAS stock solution was prepared by dissolving the required amount of dye (Sigma) in double distilled water. Solutions of lower concentration were prepared by dilution of the stock solution with double distilled water.

Working solutions of HCl, H_2SO_4 and CH_3COOH (P.O.Ch., Gliwice, Poland) were prepared by successive dilution appropriate volume of concentration acids in double distilled water.

Chloroform (P.O.Ch., Gliwice, Poland) was used without further purification.

All reagents used were of analytical grade.

2.2. Apparatus

A Hewlett Packard Model 8452A diode-array spectrophotometer.

A Spekol–11 spectrophotometer (Carl Zeiss Jena, Germany).

A model 5021 rotary injection valve (Rheodyne, Cotati, CA).

A Gilson Minipuls 2 peristaltic pump (Villiers Le Bell, France).

A Tecator flow through cell—18 μl internal volume (Höganäs, Sveden).

A Nicolet spectrometer ST-IR, Magna 550.

A Bruker AC 200F spectrometer. The chemical shifts were measured in DMSO-d_6 with tetramethylsilane as the internal standard.

2.3. Procedure

The course of the formation and extraction of the coloured compound of IMP with CAS depends on the acidity of the solutions and reagents concentration. These factors were investigated as follows: a fixed amount of IMP (2.0 ml of 5.0×10^{-4} M) and suitable amounts of CAS and acid or buffer (HCl, H_2SO_4, CH_3COOH or acetic buffer–pH 3.0–5.5) were mixed in 25 ml separating funnels, and diluted to 10 ml with water. The mixture was shaken 2 min and extracted with two successive 4–5 ml portions of chloroform. The extracts were combined in a 10-ml volumetric flask and diluted to the mark with chloroform. The absorbance was measured at $\lambda = 510$ nm against the reagent blank.

It was noticed that formation of a coloured product was most efficient in the presence of acetic acid—the absorbance of the extracts was maximal and constant in the range 1.5×10^{-3}–

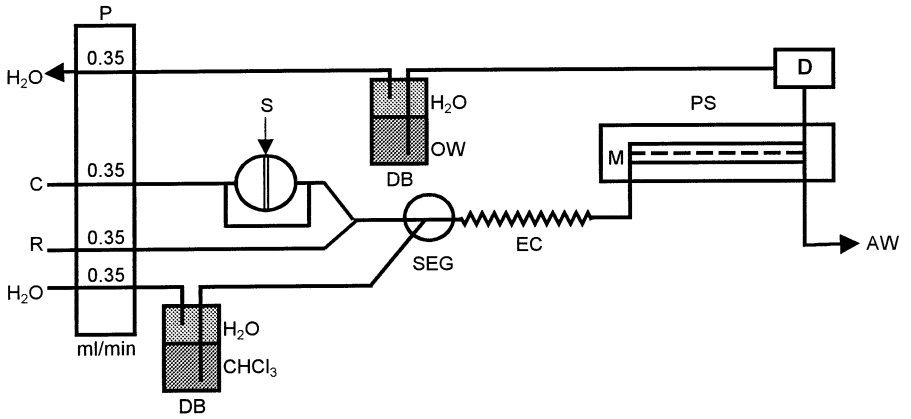


Fig. 1. Schematic diagram of the extraction-flow injection system for imipramine determination. P, peristaltic pump; C, acetic acid; R, chrome azurol S; S, sample injection valve; DB, displacement bottle; SEG, phase segmentor; EC, extraction coil; PS, phase separator; M, separation membrane; D, spectrophotometer (510 nm); AW, aqueous-phase waste; OW, organic-phase waste.

3.0×10^{-3} M acetic acid. A concentration of acetic acid of 2.5×10^{-3} M was selected for further work. The effect of CAS on the colour intensity of the extracts was maximal and constant when 2–8-fold excess of dye with respect to IMP was used. The absorbance of the extracts was stable for 24 h.

2.4. Batch extractive spectrophotometric determination of imipramine

Aliquots of the standard IMP hydrochloride solution (0.5–4.0 ml of 5.0×10^{-4} M) were transferred into a series of 25 ml separating funnels. Then 2.5 ml of 1.0×10^{-2} M CH_3COOH, and 3–4-fold excess of CAS with respect to IMP were added to each separating funnel. The total volume of the aqueous phase was adjusted to 10 ml by the addition of distilled water. Then the mixture was shaken for 2 min with two 5 ml portions of chloroform and two phases were allowed to separate. The absorbance of combined extracts was measured at 510 nm against a reagent blank and the standard calibration graph for IMP was constructed.

2.5. FIA extractive spectrophotometric determination of imipramine

On the basis of conditions selected for the batch method, the solvent extraction flow–injection system was arranged for the automation of the proposed procedure. A schematic diagram of the flow system used is shown in Fig. 1.

A multichannel peristaltic pump was used to deliver acetic acid, CAS and water. Water was pumped to closed vessel, which was filled with organic solvent that was forced to the FIA system where the continuous liquid–liquid extraction took place. The 300 μl sample of analyte was introduced into acidified solution of the CAS by means of Rheodyne rotary valve to which a volume control loop was attached. All connecting tubing was made of poly (tetrafluoroethylene) (PTFE). A segmentor, in which the aqueous and organic solvent merged at 120° angle, was used for mixing both phases to form regular segments of each phase. The extraction coil where the extraction process took place was 100 cm long. The

Table 1
^{13}CNMR data for the compound and reagents in $DMSO_{d6}$

Compound	Chemical shift, ppm
IMP–CAS	16.32; 23.62; 32.53; 47.56; 56.15; 120.58-170.23
IMP	22.23; 31.42; 41.59; 47.31; 54.45; 119.69;122.54; 126.35; 129.67; 133.56; 147.74
CAS	16.08; 16.42; 127.05–138.22; 169.96

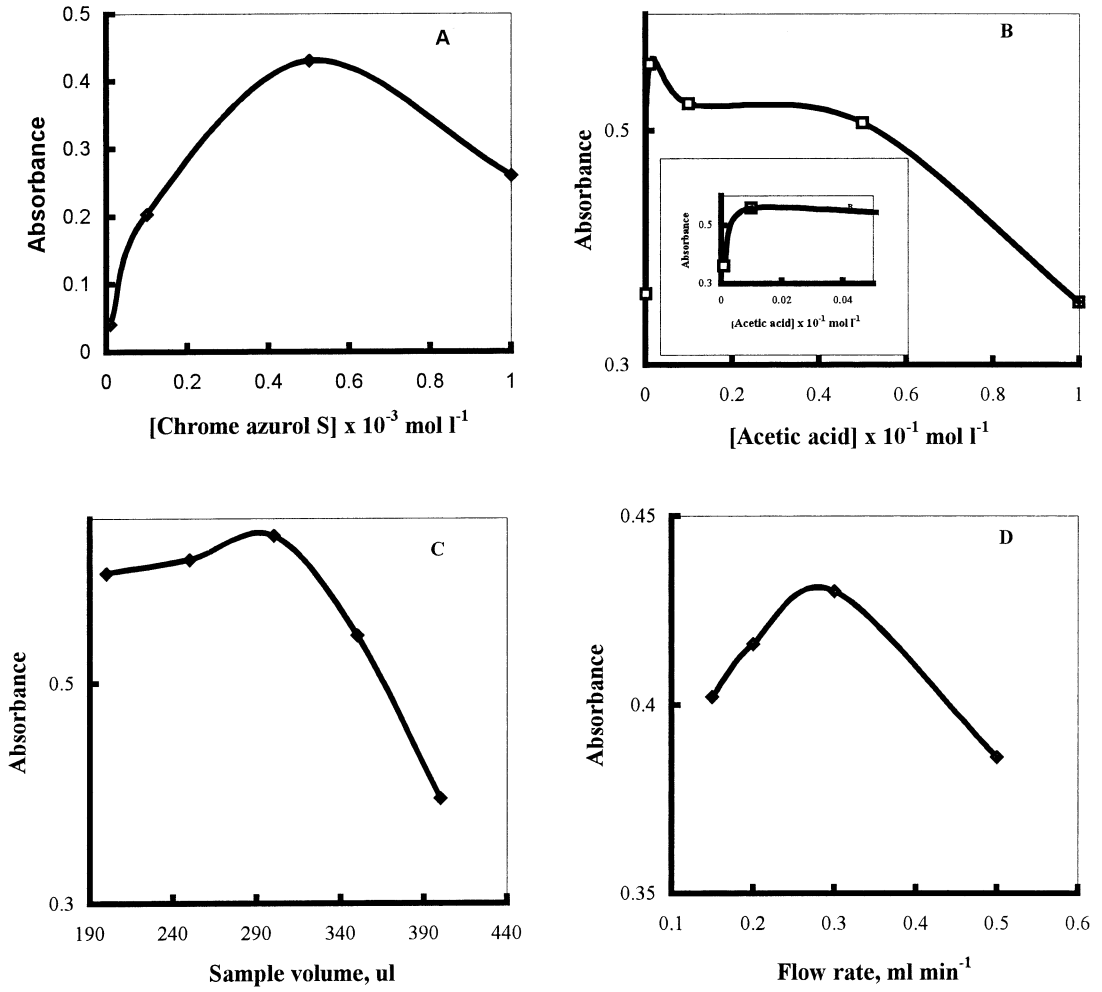


Fig. 2. Effect of the concentration of chrome azurol S as reagent stream (A), acetic acid as carrier stream (B), sample injection volume (C) and flow rate (D) on peak height in flow-injection measurements.

home-made phase separator was built with two PTFE blocks (1 cm thick, 3.8 cm wide and 11.5 cm long) and had an inlet and two outlets (bore 2 mm i.d.). The distance from the inlet to the outlet was 9.5 cm in a straight line. Each block had a groove of 1 mm depth and 2 mm width. The PTFE membrane with 1 μm pore size permeable to chloroform but impermeable to the aqueous solution was placed between the two blocks pressed together with the aid of stainless-steel screws. For improved separation, the organic phase was fed from the flow-through cell through a peristaltic pump to the waste. The absorbance of the organic phase was measured at 510 nm with a Hewlett Packard 8452A a diode array spectrophotometer with a Tecator flow though cell (18 μl inner volume and 10 mm light path length).

3. Results and discussion

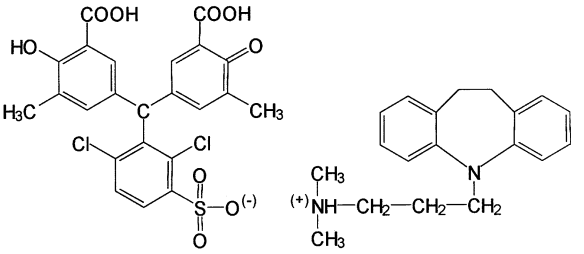
3.1. Batch method

The composition of the compound of IMP with CAS was established by Job's continuous varia-

tion method and by spectrophotometric titration. It was found that molar ratio IMP:CAS = 1:1.

The absorption spectra in UV-VIS region of the compound formed in the IMP-CAS system was examined. It was found that the characteristic for CAS bands of absorption at 300 and 492 nm and for IMP at 260 nm were practically preserved in the compound spectra. On this basis it can be concluded that the compound studied was ion-association complex. The ion-association character of the formed compound was confirmed by IR and ^{13}CNMR spectroscopy.

Infrared spectra of compound and the reagents were measured (KBr disc) in the region 400–4000 cm^{-1}. In the IR spectrum of IMP hydrochloride, the presence of a strong broad band in the 2360–2760 cm^{-1} region is attributable to the interaction of the quaternary ammonium ion $(R_3NH)^+$ with chloride ions [29]. In the spectrum of the compound studied, this band disappears. It indicates a change in the strength of the hydrogen bonding between the nitrogen atom and the chloride ion. This suggests that the protonated amine nitrogen reacts with the anionic form of CAS forming ion-association complex. That confirms the spectra of the compound studied in the region 600–1800 cm^{-1} which is a sum of the spectra of reagents. The band in the range 2780–2990 cm^{-1}, attributable to the heterocyclic nitrogen atom show a relatively small shift upon reaction of complexation [29]. The ^{13}C NMR data for the IMP, CAS and their compound, taken in DMSO-d_6 are presented in Table 1. This data indicate the ion-association character of the compound. On the basis of the results it can be concluded the amino nitrogen of aliphatic chain of IMP is responsible for formation of the compound studied.



This interpretation is in fair agreement with the results of the examination compounds of IMP with their aliphatic and aromatic amines [30].

It was found that the compound studied precipitated from acidic aqueous solutions in the form pink–red sediment, can be quantitatively extracted with chloroform ($\log K_{ex} = 3.99$ established by Likussar method [31]). Taking advantage of this property the extractive spectrophotometric method for the determination of IMP was elaborated. Extracted compound in chloroform obey Beer's law in the range 2.4–24.0 μg ml^{-1} of IMP. The linear calibration equation was $Y = 0.043x - 0.0052$ (where Y is the absorbance and x is the concentration of IMP in μg ml^{-1}) with the correlation coefficient 0.9994. The reproducibility of the measurements, expressed as relative standard deviation (RSD), was 0.8–6.5% depending on the IMP concentration. Molar absorptivity and Sandell's sensitivity were 1.4×10^4 l mol^{-1} cm^{-1} and 0.0226 μg cm^{-2}, respectively. The over-all extraction efficiency was 99.3%. The influence of the foreign compounds that can commonly accompany IMP in pharmaceutical preparations was investigated by preparing solutions containing 5 μg ml^{-1} of the drug and increasing concentration of potential interferent. The tolerance concentration of each foreign compound was taken as the largest amount yielding an error of less than $\pm 5\%$ in the analytical signal of IMP.

Table 2
Analytical features of the proposed methods

Parameter	Bath method	FIA method
Concentration range (μg ml^{-1})	2.4–24	3.0–30.0
Regression equation		
Slope	0.043	0.0139
Intercept	0.0052	0.0124
Correlation coefficient	0.9994	0.9998
Detection limit (μg ml^{-1})	0.6	1.0
Reproducibility (%RSD)	1.64[a]	1.54[b]
Molar absorptivity (l mol^{-1} cm^{-1})	1.4×10^4	
Sandell's sensitivity (μg cm^{-2})	0.0226	

[a] For five replicates samples (15.8 μg ml^{-1}).
[b] For ten replicates samples (15.0 μg ml^{-1}).

Table 3
The results of the determination of imipramine hydrochloride in pharmaceutical preparations

Taken for the detm. IMP	Found (mg)[a]				
	Pharmacopoeial method	Batch method	Error (%)	FIA method	E (%)
1ampoule content 25 mg	25.00 ± 0.13	25.10 ± 0.06	0.40	24.95 ± 0.15	0.20
1 tablet content 50 mg	50.12 ± 0.15	49.93 ± 0.28	0.37	49.78 ± 0.41	0.68

[a] Mean of three determination ± S.D.

The results obtained for different interfering compounds were as follows (concentration in μg ml^{-1}): sodium chloride, 500; magnesium nitrate, 500; starch, 250; acetylsalicylic acid, 250; sodium citrate, 250; ascorbic acid, 100; sucrose, 1000; glucose, 1000. The proposed method was successfully applied to the determination of IMP hydrochloride in 'Imipramin' injections and 'Tofranil' tablets.

3.2. FIA method

The optimization of the main manifold parameters with respect to sensitivity, reproducibility and efficiency of extraction was carried out in univariate method using a fixed sample volume and sample concentration (5 μg ml^{-1}). The parameters investigated were: concentration CAS as a reagent stream, concentration of acetic acid as a carrier stream, injected sample volume, flow-rate, and the length of the extraction coil.

The effect of CAS concentration was tested within the range 1.0×10^{-5}–1.0×10^{-3} M with the concentration of acetic acid as carrier stream fixed at 1.0×10^{-3} M. The peak height was maximal at reagent concentration 5.0×10^{-4} M and decreased outside this value (Fig. 2A).

With the optimal concentration of CAS solution the concentration of the acetic acid was investigated over the range 1.0×10^{-4}–1.0×10^{-1} M. Optimal concentration was found to be 1.0×10^{-3} M (Fig. 2B).

The volume sample injected was varied by changing the length of sample loop in the injection valve. Initially the peak height increased slightly with increasing sample size up to 300 μl above which it decreased (Fig. 2C). The volume injected was selected as 300 μl.

The flow-rate (q) of the streams (acetic acid, CAS, extraction solvent) was varied from 0.15 to 0.50 ml min^{-1}. In order to obtain a regular-segment flow, the flow rates of the organic solvent and the carrier stream and the reagent stream were identical. The peak height increased as q increased from 0.20 to 0.35 ml min^{-1} and then distinctly decreased (Fig. 2D). The decrease in the signal at $q > 0.35$ ml min^{-1} was probably caused by an insufficient phase contact time.

The effect of the length of extraction coil (100 and 200 cm) was also examined. When the 200 cm coil was used, the return of the peak to the baseline was slow, because of diffusion. The baseline was noisy with extraction coil shorter than 100 cm. Considering these results an extraction coil length of 100 cm (0.8 i.d.) was selected.

Standard solutions of IMP were injected into manifold under the optimized conditions to establish the linearity of calibration graph, the application range, reproducibility, detection limit and sample throughput.

The calibration graph was linear from 3.0 to 30.0 μg ml^{-1} of IMP. The linear calibration equation was: $Y = 0.0139x - 0.0124$ (where Y is the peak height in absorbance unit, and x is concentration of imipramine in μg ml^{-1}) with the correlation coefficient 0.9998. Ten different samples containing 15 μg $ml^{-1}$of IMP were injected into the carrier stream in order to calculate the reproducibility of the method and the sample throughput: the obtained results were 1.54% and 10 h^{-1}, respectively. The detection limit defined as three times the baseline was 1.0 μg ml^{-1}. Important features of the proposed bath and FIA methods for the determination of IMP are summarized in Table 2.

3.3. Determination of imipramine in commercial pharmaceuticals

In order to confirm the applicability of the proposed procedures, IMP hydrochloride was determined in injection 'Imipramin' (from POLFA, Poland) and tablets 'Tofranil' (from Ciba Geigy, Switzerland). One ampoule of the injection preparation with a certified amount of 25 mg IMP was diluted up to 250 ml with distilled water. For the determination in tablets, five tablets were weighed and powdered. The powder equivalent to single tablet was dissolved in methanol, then the obtained solution was filtered and evaporated to dryness. The residue was dissolved with distilled water and make up to volume in a 100 ml calibrated flask. The stock solutions were further diluted to the requisite concentrations. Then followed according to the manual and FIA procedures above described. Interference from the injection matrix was not a problem. The results in Table 3 show that the IMP content determined by the proposed manual and FIA procedures were in excellent agreement with those obtained using pharmacopoeial method [32]. This method includes the titration of the drug in acetone–chloroform solution containing about 6% mercury (II) acetate solution against perchloric acid using crystal violet indicator. Comparison of the results obtained with the manual and FIA procedures with those obtained by the pharmacopoeial method is shown in Table 3.

4. Conclusion

The above results indicate that imipramine can be determined by batch and FIA procedures based on the extraction of ion pair with CAS. The proposed procedures are very favourable for rapid and precise quantification of imipramine in pharmaceutical preparations, and can be alternative to procedures utilizing other ion-association complex [9,13]. The method is advantageous when compared to others of the reported recently extractive-spectrophotometric methods [6,8] in having higher sensitivity and stability of the coloured extracts of the ion-association complex. The applied flow methodology can be easily automated and has all the advantages of FIA methods. Additional advantages are the avoiding problems and hazards involved in handling toxic organic solvents and their relatively low consumption.

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