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FIA titrations of phenothiazine derivatives in aqueous micellar and non-aqueous media

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Dedicated to Professor Lumír Sommer on the occasion of his 80th birthday.

Abstract

New methods of flow injection analysis (FIA) neutralization titrations of phenothiazine derivatives in aqueous micellar medium of a cationic surfactant using potentiometric and spectrophotometric detection were proposed; titrations with a mixing gradient chamber and high-speed titrations were compared. The FIA titration method in non-aqueous media based on an official method of determination (titration with perchloric acid in anhydrous acetic acid) was also developed. Under optimized reaction conditions and flow-through parameters, the calibration range and equations, the sensitivity, and the repeatability of all methods were found and discussed. All titrations were assayed for medicinal forms.

Keywords: Phenothiazine derivatives; FIA titrations; Micellar medium; Non-aqueous medium; Potentiometric detection; Spectrophotometric detection

1. Introduction

1.1. FIA titrations

In flow injection analysis (FIA), reproducible sample volumes are injected into the carrier stream or directly into the reaction reagent and analyte concentration is monitored for a constant and very short period of time [1–3].

The flow-through forms of titration determinations are FIA titrations (sometimes also called pseudotitrations), which employ the concentration gradient of the sample formed in the apparatus following injection of the sample into the carrier stream [4]. FIA titrations are carried out in two arrangements: (i) in a mixing gradient chamber and (ii) in a high-speed mode.

FIA titrations with a mixing gradient chamber [5,6] are accompanied by some disadvantages originating in the relative large volume of the chamber. This leads to high consumption of the titration agent and limited sensitivity, caused by high dispersion of the sample ($D > 15$) and relatively low sampling frequency [7]. It has been demonstrated [8] that FIA titration can be carried out without a mixing gradient chamber, as a concentration gradient is formed also in the flow tube. Very short titration time and high sampling frequency are a characteristic feature of these "high-speed" titrations, where the value of the dispersion coefficient D = 3–10 (i.e., medium sample dispersion).

In contrast to batch titration, where the concentration of analyte is proportional to the consumption of the reagent at the equivalence point, at FIA titrations the determination of the concentration of the analyte is based on the calibration dependence between the width of the FIA peak Δt and the analyte concentration [9,10]. The logarithmic calibration dependence, $\log c = f(\Delta t)$, is the most frequently employed in FIA titrations.

1.2. Phenothiazine derivatives

Phenothiazine derivatives are employed as psychopharmaceuticals, antihistamines and anti-Parkinson drugs [11]. In medicinal forms they are present primarily in the form

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of hydrochlorides. As these are very weak acids, p$K_a \approx 9$, which cannot be determined by acid–base titration in aqueous media, these substances are determined by titration with perchloric acid in anhydrous acetic acid medium [12,13].

In our previous publication [14], we demonstrated that the pK_a values of phenothiazine derivatives are shifted to lower values, p$K_a \approx 7$, in aqueous medium in the presence of cationic surfactants. According to the pseudo-phase ion-exchange model the organic substances are bound to the micellar pseudo-phase by hydrophobic interactions; cationic micelles are able to bound OH^- ions to their surface by electrostatic interactions, concentrate them in a small volume and thus, assist dissociation of bound weak acids. Therefore, the hydrochlorides of phenothiazine behave like much stronger acids in this medium and they can be determined by alkalimetric titration. Moreover, the micellar medium solubilizes the formed water insoluble free bases of phenothiazine derivatives. We studied [15] the effect of various cationic surfactants (decylammonium bromide, cetyltrimethylammonium bromide, cetylpyridinium bromide, carbethopendecinium bromide) and we have found that the last of them is the most suitable because of its high solubility and the low value of critical micelle concentration (*cmc* = 1×10^{-4} M in the presence of 0.1 M KCl [16]). The alkalimetric determination must be carried out at surfactant concentration higher then *cmc* and at constant ionic strength, as the pK_a value in surfactants medium also depends on the ionic strength of the solution.

In this work, we studied the conditions for the FIA determination of selected phenothiazine derivatives by neutralization titration in both of the arrangements (with a mixing gradient chamber and in the high-speed mode) in aqueous micellar medium. A comparison was also made for potentiometric and spectrophotometric detection, based on monitoring the colour changes of suitable acid–base indicator, the thymol blue. FIA titration method with perchloric acid in anhydrous acetic acid medium was also developed.

2. Experimental

2.1. Chemicals

Chlorpromazine hydrochloride (Fluka), M_r = 355.32, CAS 69-09-0, diethazine hydrochloride (Interpharma, Czech Republic), M_r = 334.91, CAS 341-70-8, and levomepromazine maleate (Egis, Hungary), M_r = 444.54, CAS 7104-38-3, were used. The tested mass-produced medicinal forms were Deparkin® (50 mg of diethazine·HCl per coated tablet; Léčiva, Czech Republic), Plegomazin® (25 mg of chlorpromazine·HCl per coated tablet; Egis, Hungary), Plegomazin® (100 mg of chlorpromazine·HCl per coated tablet; Egis, Hungary), and Tisercin (25 mg of levomepromazine maleate per coated tablet; Egis, Hungary).

Stock solutions of the phenothiazine derivatives with a concentration of 3×10^{-2} M were prepared in water and, for titration in non-aqueous medium, in anhydrous acetic acid. Determination in medicinal forms was carried out by crushing 10 coated tablets to a powder, homogenization, and the weighed sample was dissolved in anhydrous acetic acid.

A stock solution of NaOH with a concentration of 0.1 M was used for the preparation of titration agents. Its titre was controlled at least once a week.

The Septonex (carbethopendecinium bromide; Zentiva, Czech Republic), M_r = 422.49, CAS 10567-02-9, was employed as cationic surfactant. A stock solution with a concentration of 0.3 M was used for the preparation of titration agents.

All other chemicals used were of analytical grade.

2.2. Titration agents

2.2.1. FIA titrations in aqueous micellar medium

The titration agent for the FIA titration using potentiometric detection was prepared with NaOH concentration of 5×10^{-4} M, Septonex concentration of 3×10^{-3} M, and 0.1 M KCl to adjust the ionic strength.

The titration reagent for the FIA titration using spectrophotometric detection was prepared with NaOH concentration of 5×10^{-5} M NaOH, Septonex concentration of 3×10^{-3} M, and 0.1 M KCl to adjust the ionic strength. The thymol blue was used with the concentration of 4×10^{-3}%: the weighted amount was dissolved in the required amount of 0.1 M NaOH to receive the final (5×10^{-5} M) NaOH concentration.

Fresh titration agents were always used.

2.2.2. FIA titrations in non-aqueous medium

The titration agent was 0.01 M perchloric acid in anhydrous acetic acid. It was prepared and standardized according to [12,13].

2.3. Instrumentation

FIA titrations were carried out in the module apparatus depicted in Fig. 1. The titration agent was pumped using a Masterflex peristaltic pump (Cole–Palmer, USA) and Tygon R-3603 tubes (Cole–Palmer, USA). Sample addition was carried out using a teflon low-pressure six-way valve (Rheodyne, USA).

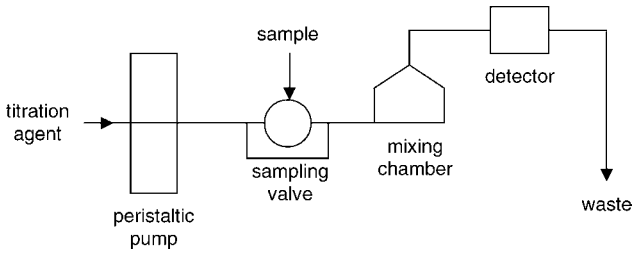

Fig. 1. Schematic diagram of the apparatus for FIA titration used (at high-speed FIA titration the mixing gradient chamber is missing).

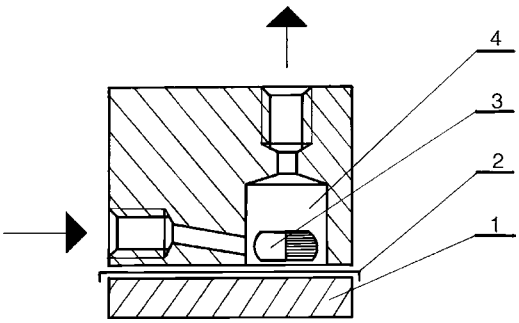

Fig. 2. Scheme of mixing gradient chamber: (1) support, (2) seal, (3) magnetic stirrer, (4) mixing chamber.

The FIA titration in aqueous micellar medium with potentiometric detection were carried out using a 3320 pH meter (Jenway, UK) and a flow-through combined pH electrode (Cole–Palmer, USA). Data were collected using a computer with the Data Acquisition Card AX-5411 (Linseis International, USA).

The FIA titrations in micellar medium with spectrophotometric detection were carried out using a Hewlett-Packard 8453 UV/VIS diode-array spectrophotometer with a flow-through cuvette with an internal volume of 130 μl and absorption layer thickness of 1 cm.

A mixing gradient chamber with an internal volume of 1 ml (this volume was selected on the basis of the literature data) with an electromagnetic stirrer was made from plexiglass (Fig. 2).

For the FIA titration in non-aqueous medium, a teflon detection cell (Fig. 3) with combined pH micro-electrode (Monokrystaly, Czech Republic) was constructed.

2.4. Data processing

The peak Δt width was read off at half height of the maximum concentration peak. The logarithm of the concentration was employed for construction of calibration dependence.

The value of the maximum sampling frequency per hour, which is a practical parameter in FIA analysis [1], was calculated from the relationship

$$S_{\max} = \frac{60}{\Delta t_b} \quad (2.1)$$

where $S_{\max}$ (h^{-1}) is the maximum sampling frequency per hour and Δt_b (min^{-1}) is the width of the peak, which corresponds to the maximum concentration of the calibration dependence at the peak base.

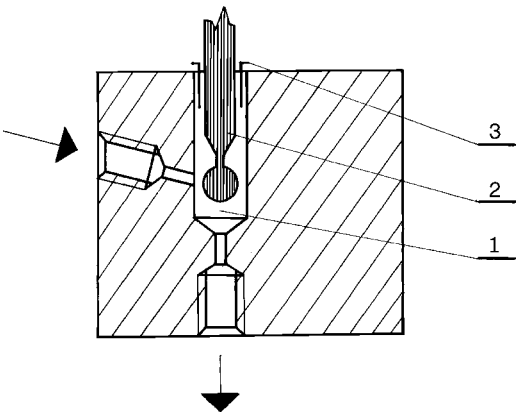

Fig. 3. Scheme of detection cell for high-speed FIA titration in non-aqueous medium: (1) cell, (2) pH microelectrode, (3) seal.

3. Results and discussion

3.1. FIA titration in aqueous micellar medium

3.1.1. Potentiometric detection

The dependences of the signal, i.e., the peak width Δt (min^{-1}), on the flow-rate of the titration agent q (ml min^{-1}) and the sample volume V_S (μl) were monitored in multifactor optimization procedure of parameters of the FIA titrations. The arrangement with mixing gradient chamber and the high-speed arrangement were used. The flow-rate of 0.4 ml min^{-1} and injected sample volume of 250 μl were found to be optimal, yielding a maximum Δt value.

The calibration dependences for diethazine·HCl and chlorpromazine·HCl (levopromazine maleate was not soluble in the medium employed) were measured under the determined optimum conditions in both arrangements; their parameters are given in Table 1. It is apparent that the two studied methods have the same range of linear concentration dependences. The correlation coefficients values are, in all cases, approximately 0.99; the standard deviation values of the calibration dependences are higher for the titrations with the mixing chamber. The comparison of the slopes of the dependences shows that the FIA method with the mixing gradient chamber is more sensitive; however, on the other hand, there is a very low maximum sampling frequency.

3.1.2. Spectrophotometric detection

Thymol blue was chosen (on the basis of the tabulated pK_a values [17] and experiments performed [15]) as a suitable acid–base indicator that changes its colour during the reaction of the analyte with the reagent and enables registration of the FIA peaks. The detection was carried out at a wavelength of λ = 610 nm, which corresponds to the maximum absorbance of the alkaline form of thymol blue. The indicator was part of the titration reagent. At the titration, a decrease in the absorbance at λ = 610 nm first occurs; following completion of the reaction of the hydrochlorides with the titration agent, the absorbance again increases. The FIA peak is registered as the time-dependence of the absorbance at this wavelength.

In both the titration with the mixing gradient chamber and the high-speed mode, the flow-rate of 0.4 ml min^{-1} of titration agent and injected sample volume of 250 μl were found to be optimal. Under these conditions, the calibration dependences were determined for diethazine·HCl and chlorpromazine·HCl; their parameters are given in Table 1.

Table 1
Parameters of the calibration dependences for determination of diethazine·HCl and chlorpromazine·HCl by FIA titration in aqueous micellar medium (q = 0.4 ml min^{-1}, V(sample) = 250 μl, c(NaOH) = 5 × 10^{-4}M (potentiometric detection) or 5 × 10^{-5} M (spectrophotometric detection), c(Septonex) = 3 × 10^{-3} M, c(KCl) = 0.1 M)

Detection	Substance	Arrangement[a]	Calibration range (mg ml^{-1})	Calibration equation	r^2	s_y	S_{max} (h^{-1})
Potentiometric							
	Diethazine·HCl	MC	2.00–20.00	Δt = 266.8 log c + 37.6	0.9994	1.94	7
		HS	2.00–20.00	Δt = 72.8 log c + 2.7	0.9938	1.78	26
	Chlorpromazine·HCl	MC	2.00–20.00	Δt = 209.8 log c + 278.4	0.9897	7.79	8
		HS	2.00–20.00	Δt = 65.9 log c − 0.5	0.9943	1.80	22
Spectrophotometric							
	Diethazine·HCl	MC	0.02–0.50	Δt = 87.2 log c + 199.6	0.9798	6.89	15
		HS	0.02–0.50	Δt = 19.8 log c + 95.6	0.9805	1.54	27
	Chlorpromazine·HCl	MC	0.02–0.50	Δt = 89.4 log c + 211.3	0.9903	4.31	20
		HS	0.02–0.50	Δt = 25.9 log c + 107.9	0.9851	1.17	26

[a] MC: mixing gradient chamber, HS: high-speed titration.

The concentration range is again the same for both experimental arrangements; the correlation coefficients are approximately 0.98 and the standard deviation values of the calibration dependences are higher again for the titrations with the mixing chamber. The sensitivity is lower for high-speed titrations than for titrations with the mixing gradient chamber and the maximum sampling frequency is higher for the high-speed titration. The calibration range is two orders of magnitude lower than for FIA titration with potentiometric detection.

3.1.3. Kinetic effects in studied FIA titrations

It was found in the optimization of experimental conditions that the increase of flow-rate has a negative effect on the sensitivity of determination. As an example, the effect of flow-rate on the calibration dependence of FIA titration of chlorpromazine·HCl with the mixing gradient chamber using potentiometric detection is given in Fig. 4. As increase in the flow-rate leads to a decrease in the "residence time"

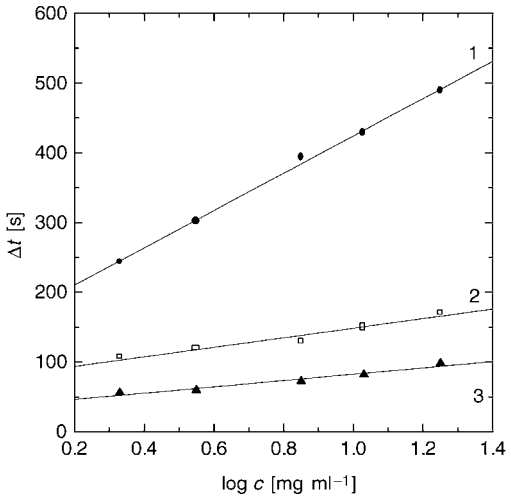

Fig. 4. The effect of flow-rate (1) 0.4 ml min^{-1}, (2) 1.3 ml min^{-1}, (3) 2.5 ml min^{-1} on the calibration dependence of chlorpromazine·HCl at FIA titration in aqueous micellar medium in arrangement with mixing gradient chamber and potentiometric detection (V(sample) = 250 μl, c(NaOH) = 5 × 10^{-4} M, c(Septonex) = 3 × 10^{-3} M, c(KCl) = 0.1 M).

of the reagent in the mixing gradient chamber, the reaction cannot probably proceed to completion. However, because it is known that acid–base reactions are, in general, very fast, the only possible explanation for the decreased reaction rate is slow bounding of the analyte to the surfactant micelles. When the same potentiometric titration was carried out with the classical batch arrangement, equilibrium was also established slowly and the potential could be read off only several minutes after adding the titration reagent.

Kinetic effects can also explain why lower sensitivity of the determination was achieved using high-speed titration, although the sample dispersion is less ($D \approx$ 3–10) than when the mixing gradient chamber is used (D > 15) and thus, theoretically, the sensitivity of the high-speed determination should be higher. The longer residence time of the sample in the mixing gradient chamber apparently allows the reaction to proceed more to completion.

The high sensitivity in the arrangement with the mixing gradient chamber when using optimum flow parameters is accompanied by low maximum sampling frequency. In general, the sampling frequency is directly proportional to the flow-rate. On the other hand, the sensitivity of the determination is decreased with the increase of the flow-rate in our experiments. Thus, we tried to experimentally determine the highest flow-rate at which the sensitivity should be still sufficient. It was found that it is possible to employ a flow-rate of up to 1.6 ml min^{-1}, where the sensitivity is decreased (1.5-fold) but the rate of sample addition can increase significantly (4-fold). It is possible to process up to 30 samples per hour, while only 8 samples per hour can be processed at a flow-rate of 0.4 ml min^{-1}. The calibration ranges do not change in these conditions.

3.1.4. Determination of phenothiazine derivatives in medicinal forms

It was found that FIA determination of phenothiazine derivatives in aqueous micellar medium can be employed only for tablets. For coated tablets, the auxiliary substances in the coating and filling materials substantially interfere in the determination; the samples were not completely soluble

Table 2
Parameters of the calibration dependences for determination of phenothiazines by high-speed FIA titration in non-aqueous medium with potentiometric detection (q = 0.4 ml min^{-1}, V (sample) = 250 μl, c($HClO_4$) = 0.01 M)

Substance	Calibration range (mg ml^{-1})	Calibration equation	r^2	s_y	S_{max} (h^{-1})
Diethazine·HCl	0.2–20.0	$\Delta t = 35.6 \log c + 27.7$	0.9964	0.93	45
Chlorpromazine·HCl	0.2–20.0	$\Delta t = 32.4 \log c + 31.2$	0.9890	4.31	40
Levomepromazine maleate	0.2–20.0	$\Delta t = 35.1 \log c + 32.3$	0.9891	1.54	40

Table 3
Comparison of the results of the determination of phenothiazines in medicinal forms by official and high-speed non-aqueous FIA titration

Substance	Medicinal preparation (mg/tablet)	Found (%)	
		Official method	FIA high-speed
Diethazine·HCl	Deparkin®, 50	100.5 ± 0.3	100.4 ± 0.6
Chlorpromazine·HCl	Plegomazin®, 25	98.4 ± 0.4	98.3 ± 0.9
	Plegomazin®, 100	98.6 ± 0.3	98.7 ± 0.9
Levomepromazine maleate	Tisercin®, 25	98.7 ± 0.2	98.7 ± 0.8

in aqueous medium, the consumption of titration agent was higher as a consequence of the presence of hydrogen carbonates.

3.2. FIA titration in non-aqueous medium

Because of the above-described problems in analysis of medicinal forms in coated tables by the FIA titration method in aqueous micellar medium, a flow-through variant of the official method of determination of phenothiazine derivatives by potentiometric titration with perchloric acid in acetic acid medium was developed. FIA titration in the high-speed arrangement was selected, as because of the corrosive reaction medium, it would be necessary to make the mixing gradient chamber from resistant teflon. Also, the maximum sampling frequency is higher in high-speed arrangement. The potentiometric detection is suitable for the determination of samples with higher concentrations of the substances than the spectrophotometric one.

The calibration dependence for FIA titration in non-aqueous medium was measured for all studied phenothiazines. An analogous procedure as in the previous part was employed in optimising the flow parameters. Values of the flow-rate of the titration agent of 0.4 ml min^{-1} and added sample volume of 250 μl were found to be optimal. The parameters of the calibration dependences are given in Table 2. It follows from Table 2 that the parameters of these FIA titrations are very good; in particular, the maximum sampling frequency is twice as high as for FIA high-speed titration in aqueous micellar medium. However, the low sensitivity of the determination is a disadvantage (the slopes have a value of about 35 ml mg^{-1}, compared to 70 ml mg^{-1} in aqueous medium).

3.2.1. Determination of phenothiazine derivatives in medicinal forms

The developed method of FIA high-speed titration with perchloric acid in non-aqueous medium was employed for analysis of mass-produced medicinal preparations. The results obtained were compared with the results of determinations carried out by official batch titration method. The results of the determination are given in Table 3. It can be seen that the contents obtained are comparable. The determination time is decreased to almost one half when FIA titrations are used and the working efficiency is thus much higher. Moreover, using well-sealed FIA apparatus, there are none of the problems associated with corrosive vapours, which are a characteristic for the work with acetic acid in classical batch arrangement.

4. Conclusions

The potential for using aqueous micellar medium for FIA titrations of phenothiazine derivatives was tested. A comparison was made of the experimental arrangement with the mixing gradient chamber and in the high-speed mode, in both cases, with potentiometric and spectrophotometric detection. It was found that, in both cases, FIA titration with the mixing gradient chamber is more sensitive; however, it has the disadvantage of a low sampling frequency. A possible explanation of the discrepancy between the results obtained and the literature data, according to which FIA high-speed titrations should be more sensitive than titrations with a mixing gradient chamber, could probably lie in the kinetic effects occurring in the micellar medium (the reaction time for high-speed titrations is not sufficient for establishing equilibrium on the surface of the micelles). In comparison of the two types of detection, the spectrophotometric detection enables work in a lower concentration range than potentiometric one, which is suitable for higher analyte concentrations.

Because of the impossibility of employing the developed FIA titration in aqueous micellar medium in analysis of medicinal forms in the form of coated tablets, FIA titration with perchloric acid in anhydrous acetic acid medium were developed as a flow-through variant of the batch

titration determination of phenothiazines as described in official methods. The non-aqueous medium eliminates the effect of the auxiliary substances. The results of the determination of the phenothiazine derivatives in practical samples by the FIA titration in non-aqueous medium agree with the results obtained by official batch titration. The developed FIA in non-aqueous medium reduces the time of titration and decreases the disadvantages of work with corrosive acetic acid medium.

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