Table I—Determination of Sorbitol and Mannitol in Various Samples

Sample	Milligrams Taken	Sorbitol Found, mg	Mannitol Found, mg
Sorbitol			
1	34.2	30.6	3.7
2	33.7	30.2	3.6
3	31.6	28.3	3.3
4	29.9	26.8	3.2
5	30.8	27.6	3.4
6	30.4	27.2	3.3
Mannitol			
1	36.5	3.6	32.7
2	36.0	3.5	32.2
3	33.4	3.3	39.9
4	32.6	3.1	29.2

Approximately 10 mg of *n*-butylboronic acid[6] and exactly 1.00 ml of internal standard solution were added to the dried samples. The reaction was completed almost immediately, and 1-μl portions were injected onto the column.

RESULTS AND DISCUSSION

The chromatogram shown in Fig. 1 is of the tris-*n*-butyldiboronate esters (6) of mannitol and sorbitol under the described conditions. No mannitol or sorbitol samples obtained were free of mutual contamination. It is advisable to observe the precaution offered by Eisenberg (6), *i.e.*, to ascertain the purity of the *n*-butylboronic acid reagent by GLC of a pyridine solution on the OV-17 column at 92°. It follows, then, that excess reagent elutes with the solvent front during hexitol analysis. The reagent used here was essentially free of any impurities.

The response of the analytical system was linear to a concentration four times that described in Fig. 2. The residual standard deviations calculated for five replicate samples of mannitol and sorbitol, analyzed separately, were both ±1.2% (Table I). These values compare favorably with those using the method of Manius *et al.* (3). The advantages of this method are the ease of derivatization, the shorter analysis time, and the greater thermal stability of the liquid phase.

[6] Pierce Chemical Co.

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Chemistry of Nonaqueous Titration of Chlorpromazine

SOBHI A. SOLIMAN ˣ, HASSAN ABDINE, and NASHAAT A. ZAKHARI

Abstract □ The chemistry of the red color formed during perchloric acid titration of chlorpromazine hydrochloride in acetic acid in the presence of mercuric acetate is discussed. Addition of ascorbic acid prevents the color formation and allows titration using a crystal violet end-point. Ascorbic acid addition also sharpens the potentiometric end-point. Ascorbic acid and its oxidation product, dehydroascorbic acid, being neutral to perchloric acid, do not interfere with the titration.

Keyphrases □ Chlorpromazine hydrochloride—chemistry of nonaqueous titration, method proposed, compared to official methods □ Nonaqueous titration—chlorpromazine hydrochloride, chemistry □ Titration, nonaqueous—chlorpromazine hydrochloride, chemistry

Chlorpromazine hydrochloride, a phenothiazine derivative, is extensively used as a psychopharmacological agent in various dosage forms. Chlorpromazine hydrochloride is official in the USP XVIII (1), the BP 1963 (2), and the BP 1968 (3) as a pure compound and as injection and tablet dosage forms.

The pure compound is titrated potentiometrically in glacial acetic acid medium, according to the USP XVIII and the BP 1963, and in acetone using methyl orange indicator according to the BP 1968. The injection is assayed spectrophotometrically in the three compendia. In the BP 1968 and the BP 1963 methods, the injection solution is diluted with 0.1 *N* hydrochloric acid solution to a suitable concentration and then the absorbance is measured directly. In the USP XVIII method, several extraction processes are carried out before measurement.

Tablets are determined spectrophotometrically according to the USP XVIII and the BP 1968. The BP 1963, however, recommends the titration of chlorpromazine hydrochloride content of tablets against standard ceric ammonium sulfate solution.

DISCUSSION

Several methods of analysis have been proposed for chlorpromazine hydrochloride involving gravimetry, titrimetry, spectroscopy, colorimetry, and chromatography. Spectrophotometric and colorimetric methods include the estimation of chlorpromazine in biological fluids (4) by measuring the red color produced by concentrated sulfuric acid. Colorimetric determination of chlorpromazine in tablets containing methampyrone [sodium (antipyrinylmethylamino)methanesulfonate] was reported (5).

A method using acid indicator dyes was suggested (6) for the de-

termination of chlorpromazine hydrochloride; the reaction product with bromcresol purple was isolated and its structure was elucidated with elemental, IR, and UV analyses. Chlorpromazine was determined in biological specimens by UV spectrophotometry and corroborated by TLC (7). A spectrophotometric method was developed for the routine assay of conjugated and unconjugated chlorpromazine metabolites in human urine (8).

Among the many available titrimetric and gravimetric methods for the determination of chlorpromazine hydrochloride is an attempt (9) to determine the nitrogenous base gravimetrically by precipitation with silicotungstic acid in acid solution. The oxidation of the phenothiazine nucleus with standard bromate (10) was used to determine chlorpromazine, and the excess bromate was estimated.

A thermometric titration procedure for chlorpromazine hydrochloride using standard sodium hydroxide solution as the titrant was suggested (11). The neutralization of the protonated nitrogen with base resulted in an enthalpogram with a sharp end-point. The phenothiazine nitrogen, being not sufficiently basic in aqueous solution, failed to exhibit an end-point when titrated *versus* standard hydrochloric acid solution.

Amperometric titration of chlorpromazine (12) was accomplished using a molybdenum anode and a graphite, platinum, or tungsten cathode. Chlorpromazine, in tablets, was determined by diphasic titration against sodium lauryl sulfate, using methyl yellow as the indicator (13). It was stated that this method is of particular value for determining phenothiazine derivatives in the presence of other organic bases. A photometric titration procedure was also described (14) for determining this compound in several dosage forms, using ceric sulfate as titrant.

Methods involving titration in nonaqueous solvents were reviewed (15). Chlorpromazine hydrochloride, in pure form as well as in various dosage forms, was titrated (16), using acetone as the solvent and methyl red as the indicator. The titrant was a standard perchloric acid solution in dioxane. Alternatively, the nitrogenous base was liberated by aqueous potassium hydroxide solution and extracted by hexane, acetone was added, and the solution was titrated as described.

Chlorpromazine and some other phenothiazine derivatives were titrated in a mixture of ethylene dichloride and glacial acetic acid against aceteous perchloric acid solution, using tropaeolin OO as the indicator (17). It was also shown (18) that neutral or almost neutral solvents, such as acetone and methylcyanide, were more satisfactory than acid solvents, such as glacial acetic acid, in determining chlorpromazine hydrochloride in pharmaceutical preparations.

The most widely used method (19) for the determination of the hydrochlorides of nitrogenous bases cannot be applied to the determination of chlorpromazine hydrochloride due to the development of an intense red color which interferes with the color change of crystal violet indicator. Therefore, the purpose of the present investigation was to develop a nonaqueous titrimetric procedure applicable to the determination of chlorpromazine hydrochloride, in pure form as well as in different dosage forms, in which the end-point detection can be accomplished visually with crystal violet and glacial acetic acid, the most widely used indicator and solvent in nonaqueous titration.

EXPERIMENTAL

Apparatus and Materials—The following were used: a suitable titrimeter[1] equipped with a combination electrode[2], a magnetic stirrer, and a microburet of 10-ml capacity graduated to 0.02 ml.

Solutions—The following were used: a 0.1 *N* perchloric acid solution in glacial acetic acid, 5 and 6% (w/v) solutions of mercuric acetate in glacial acetic acid, a 0.2% (w/v) solution of crystal violet indicator in glacial acetic acid, and a saturated solution of methyl orange in acetone.

Acetous perchloric acid solution was standardized against potassium acid phthalate[3], previously dried at 110° for 2 hr, using glacial acetic acid as the solvent. The end-point was determined potentiometrically.

[1] Pye model 79.
[2] Pye Catalog No. 401 E07, series No. 546022.
[3] Rhone-Poulenc, France.

Table I—Determination of Chlorpromazine Hydrochloride in Pure Form, Tablets, and Injections Using the Proposed Visual Nonaqueous Titration Method

Sample	Weight, mg	Weight Found, mg	Recovery, %
Pure compound			
1	309.7	311.3	100.52
2	296.9	298.4	100.50
3	176.8	178.1	100.73
4	167.1	166.9	99.88
5	142.1	142.6	100.35
Mean percent recovery (P' = 0.05)			100.39 ± 0.39
Tablets (25 mg)			
1	300.0	298.4	99.47
2	252.7	251.0	99.33
3	207.7	206.6	99.47
Tablets (10 mg)			
4	155.5	154.1	99.09
5	103.5	102.9	99.42
6	103.2	103.3	100.09
Mean percent recovery (P' = 0.05)			99.48 ± 0.35
Injections (50 mg/2 ml)			
1	375.0	373.0	99.47
2	250.0	246.9	98.76
Injections (25 mg/5 ml)			
3	125.0	124.5	99.60
4	100.0	99.5	99.50
Mean percent recovery (P' = 0.05)			99.33 ± 0.61

For comparative purposes, chlorpromazine hydrochloride[3] was assayed by the official procedures of the USP XVIII and the BP 1963 and 1968.

Determination of Chlorpromazine Hydrochloride by Proposed Visual Procedure—An amount of chlorpromazine hydrochloride, as specified in Table I, was accurately weighed and dissolved in 50 ml of glacial acetic acid contained in a 150-ml titration beaker. Ten milliliters of 5% (w/v) mercuric acetate solution, 1.0 g of powdered ascorbic acid, and 2 drops of crystal violet indicator solution were added.

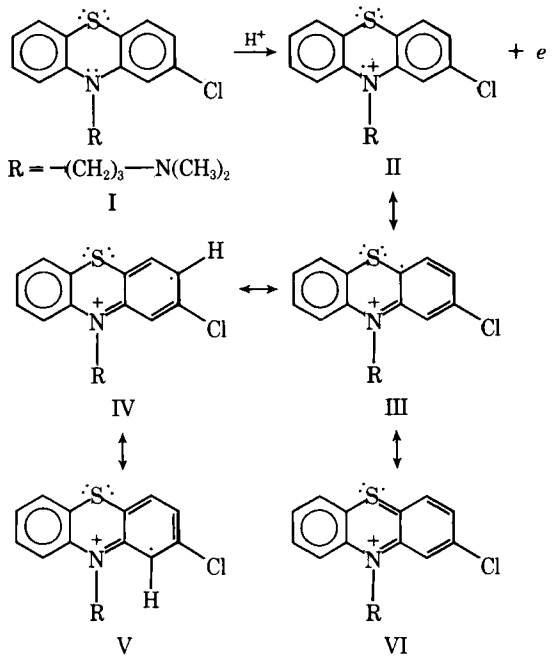
The solution, magnetically stirred, was titrated potentiometrically against 0.1 *N* acetous perchloric acid solution. The end-point in the titration was determined from the inflection in the titration curve, obtained by plotting the volume of titrant in milliliters against the millivolt readings. The proper color change of the indicator coinciding with the potentiometric end-point was noted. Such a color change was found to be from violet to blue. The titration was also performed as described but with 50 ml of a glacial acetic acid–dioxane (1:1) mixture as the solvent.

Analysis of Tablets—Twenty tablets were weighed and powdered. An amount of powdered tablets equivalent to a weight of chlorpromazine hydrochloride as shown in Table I was accurately weighed and placed in a 150-ml titration beaker containing 50 ml of glacial acetic acid. Then 10 ml of mercuric acetate solution, 1.0 g of ascorbic acid powder, and 3 drops of crystal violet indicator solution were added. The solution was stirred magnetically for several minutes and titrated to a blue color change of the indicator.

Analysis of Injections—A volume of the mixed contents of ampuls equivalent to an amount of chlorpromazine hydrochloride as indicated in Table I was pipetted, exactly, into a 150-ml titration beaker. The solution was evaporated on a water bath to a small volume (3–5 ml), and about 6.0 ml of acetic anhydride was added for each milliliter of solution remaining in the titration beaker. Then the solution was heated on a hot plate until it began to boil. The solution was cooled, and 10 ml each of glacial acetic acid and mercuric acetate solution, 1.0 g of ascorbic acid powder, and 2 drops of crystal violet indicator solution were added. It was then titrated visually with 0.1 *N* acetous perchloric acid as described.

RESULTS

Chlorpromazine hydrochloride, being a phenothiazine derivative, is sensitive to oxidation. Therefore, in glacial acetic acid medium, the oxidizing effect of perchloric acid is catalyzed by mercuric acetate (20), giving a red-colored oxidation product that makes visual end-point detection with crystal violet indicator impossible.



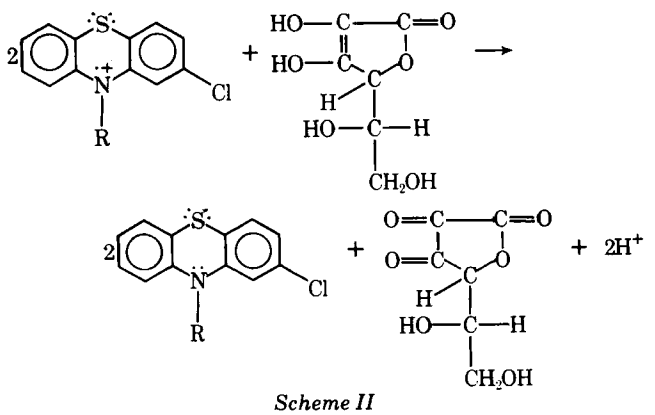
Scheme I

For this reason, the official compendia such as USP XVIII and BP 1963 employ potentiometric, rather than visual, end-point detection in the nonaqueous titration of chlorpromazine hydrochloride in glacial acetic acid solvent. BP 1968 replaces glacial acetic acid with acetone and uses a saturated solution of methyl orange in acetone as the indicator.

The formation of intensely colored compounds in acid solution of phenothiazine was explained by Michaelis *et al.* (21). Using methylene blue, a phenothazine derivative, as an example, these investigators proposed a free radical formation which depended on a state of equivalent resonance. Their explanation is satisfactory for the color developed by this compound, because it possesses two auxochrome amino groups in the 3- and 7-positions which are capable of participating in the development of a semiquinoid free radical structure and hence facilitate color production.

This explanation is not satisfactory, however, for phenothiazine derivatives stripped of all auxochrome groups. This objection was later resolved by the same authors (22) by pointing out that colored semiquinone radicals of phenothiazine as they exist in acid solution are stabilized by resonance involving the two bridge atoms, nitrogen and sulfur. They stated that such colored semiquinone free radicals can be obtained in a high concentration and in a quite stable condition in 80% acetic acid.

Chatten and Harris (23) reported the titration behavior of a number of phenothiazine derivatives, including chlorpromazine, in nonaqueous media and concluded that the reaction between chlorpromazine and perchloric acid in nitromethane solvent produced a distinctly colored compound, ranging from an initial pink to a final rose-red color. These authors attributed the formation and stabili-



Scheme II

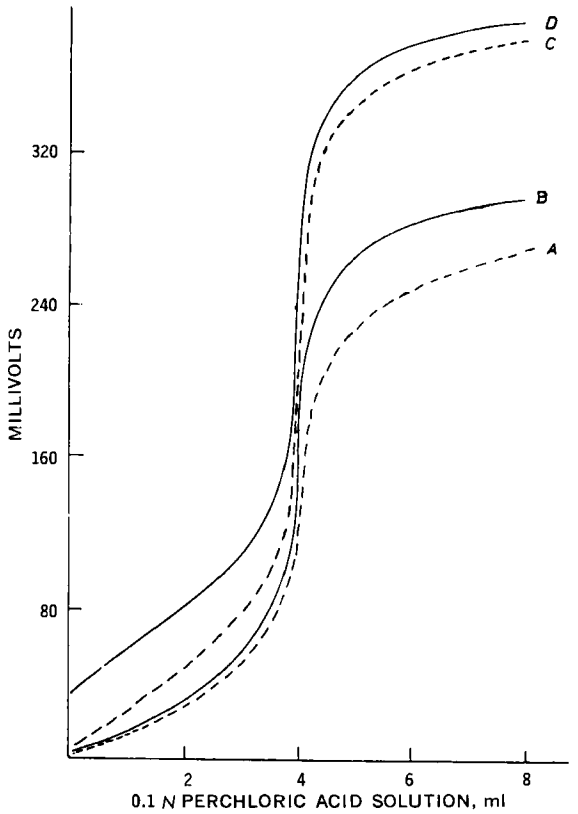


Figure 1—*Potentiometric titration of chlorpromazine hydrochloride. Key: A, USP XVIII and BP 1963 procedures in glacial acetic acid; B, proposed procedure in glacial acetic acid and using ascorbic acid; C, USP XVIII and BP 1963 procedures in glacial acetic acid–dioxane mixture; and D, proposed procedure in glacial acetic acid–dioxane mixture and using ascorbic acid.*

zation of the colored compound to complex formation between the resonating nitromethane and the titrated phenothiazine derivative, and they indicated that such a complex could result from ionic attraction of the oppositely charged centers in the phenothiazine compound and the carbanion-acinitromethane resonate.

Thus, the formation and stabilization of the semiquinone free radical, which is responsible for the red color production, may be due to the possible formation of many resonating forms. Scheme I illustrates the mechanism suggested for such a resonance. In acetic acid medium, chlorpromazine (I) is oxidized by the action of perchloric acid, whereby it loses one electron to give the semiquinone free radical (II). This structure is in resonance with Structures III, IV, and V, which arise from II by resonance involving one of the two benzene rings of the phenothiazine structure. When resonance involves the other benzene ring, resonating structures analogous to III, IV, and V would be possible from Structure II.

In addition, more resonating structures are expected to be formed *via* another route involving the two bridge atoms, nitrogen and sulfur. Such a route is represented by Structures II, III, and VI when resonance involves one of the benzene rings. However, resonance involving the other benzene ring through this route can be represented by Structure II, the structure corresponding to III, and Structure VI.

The formation of a red-colored solution during the titration of chlorpromazine hydrochloride with perchloric acid solution was prevented by the addition of finely powdered ascorbic acid to the contents of the titration beaker before beginning the titration. The absence of color from chlorpromazine solution throughout titration enabled accurate and sharp visual end-point detection, which is impossible in the USP XVIII and BP 1963 nonaqueous titration procedures.

The immediate disappearance of the red color and the subsequent prevention of such a color formation during the titration are due to reduction with ascorbic acid. The semiquinone free radical

Table II—Determination of Chlorpromazine Hydrochloride According to USP XVIII, BP 1963, and BP 1968 Methods

Sample	USP XVIII and BP 1963		BP 1968	
	Weight, mg	Recovery, %	Weight, mg	Recovery, %
1	608.1	100.10	291.0	100.15
2	299.1	101.39	172.7	99.50
3	199.9	100.00	89.7	101.65
4	142.1	101.33	79.1	101.18
5	141.6	100.69	58.2	100.39
	Mean percent recovery ($P' = 0.05$)	100.70 ± 0.81	Mean percent recovery ($P' = 0.05$)	100.57 ± 1.05

is reduced to chlorpromazine, whereby dehydroascorbic acid is formed as illustrated by Scheme II. Ascorbic acid and its oxidation product, dehydroascorbic acid, being neutral in glacial acetic acid, do not interfere with the determination of chlorpromazine hydrochloride.

Typical titration curves for the determination of chlorpromazine hydrochloride according to the proposed method and the USP XVIII and BP 1963 procedures in glacial acetic acid and in a glacial acetic acid–dioxane solvent system are illustrated in Fig. 1. Curve B, which represents the titration of the nitrogenous base hydrochloride in glacial acetic acid according to the proposed method, indicates that a sharper end-point detection is obtained when compared with the USP XVIII and BP 1963 procedures (curve A). The relative magnitudes in the breaks of the two curves are 540 and 340 mv/ml of titrant, respectively.

The visual end-point detection was also found to be sharp when the proposed method was applied. This difference in the sharpness of end-points may be attributed to the fact that in the official procedures a mixture of chlorpromazine and the semiquinone free radical, rather than a single compound, is titrated; in the proposed method, the formation of such a free radical is prevented. The semiquinone free radical is expected to be a weaker base than chlorpromazine in view of the presence of a positive charge, which decreases the electron density within the semiquinone free radical structure. Titration in the glacial acetic acid–dioxane (1:1) solvent system yielded an even sharper end-point detection.

Chlorpromazine contains two distinctly different types of nitrogen atoms, a tertiary amino and a heterocyclic nitrogen. The resonance within the heterocyclic ring system of chlorpromazine is such that the electron density around the heterocyclic nitrogen atom is incapable of reacting with protons. The tertiary nitrogen atom, being a strong base, is capable of holding a proton when titrated with perchloric acid solution. Therefore, in the solvent systems employed in this study, chlorpromazine behaves as a monoacidic base and gives only one inflection point when titrated potentiometrically.

The scope of the proposed visual titration method for control purposes was demonstrated by its application to samples of chlorpromazine hydrochloride in pure form and in synthetic dosage forms such as tablets and injections. Commonly used excipients, insoluble fillers, diluents, and lubricants such as lactose, starch, and talc do not interfere with the determination. An aliquot of powder mass of tablets is titrated directly, without preliminary extraction of the active constituent, after sufficient time is allowed for its solubility. The water content of injections is removed by evaporation to a small bulk, and the last traces are allowed to react with a sufficient amount of acetic anhydride.

The results of the titration of chlorpromazine hydrochloride, according to the proposed visual nonaqueous titration method, are shown in Table I. The mean percent recoveries ($P' = 0.05$) of the pure form, tablets, and injections are 100.39 ± 0.39, 99.48 ± 0.35, and 99.33 ± 0.61, respectively.

Table II lists the recovery data obtained when chlorpromazine hydrochloride is determined by the potentiometric nonaqueous titration method of USP XVIII and BP 1963 and by the BP 1968 procedure. The mean percent recoveries ($P' = 0.05$) are 100.70 ± 0.81 and 100.57 ± 1.05, respectively. Statistical analysis of the two sets of results given in this table indicated that the difference between the two mean percentage recoveries is statistically insignificant and that the two methods are equally precise and almost equally accurate. Statistical comparison of the data obtained using the proposed visual method and the potentiometric USP XVIII and BP 1963 procedure showed that the two methods are equally precise. However, the proposed method is more accurate.

With the proposed method, the end-point detection is accomplished visually using crystal violet indicator; such detection is not possible with the official methods. The proposed method also has the advantage of being applicable to chlorpromazine hydrochloride in the pure state as well as in dosage forms. The official compendia employ different assay methods for different dosage forms. Glacial acetic acid and crystal violet indicator, the most widely used solvent and indicator in nonaqueous titration, are also employed. The proposed method is useful and attractive to the pharmaceutical control chemist for routine analysis.

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[x] To whom inquiries should be directed.