Chloramine-T as Titrimetric Reagent in Potentiometric Determination of Isoniazid, Phenelzine, and Dihydralazine

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Abstract □ A simple, rapid, and accurate direct potentiometric method was developed for the determination of isoniazid, phenelzine sulfate, and dihydralazine sulfate using chloramine-T as the titrimetric reagent. The oxidations were done at high temperatures in acidic medium. Quantitative recoveries are reported.

Keyphrases □ Chloramine-T—titrimetric reagent for potentiometric determination of isoniazid, phenelzine, and dihydralazine □ Potentiometric titration—isoniazid, phenelzine, and dihydralazine, chloramine-T as titrimetric reagent □ Hydrazine drugs—potentiometric determination of isoniazid, phenelzine, and dihydralazine, chloramine-T as titrimetric reagent

Hydrazine drugs have important therapeutic applications (1). Various oxidometric methods for their determination have been developed involving direct or indirect analysis. Iodometric and bromometric methods are used by the USP (2) and BP (3), respectively, for the assay of isoniazid. Hydralazine may be determined with the iodometric titration procedure (4) or with a standard iodate solution in hydrochloric acid medium (5). When an irreversible indicator (*p*-ethoxycrysoidine) is employed, the titration with iodate to the discharge of color can be used for isoniazid (6). The determination of isoniazid by the sodium nitrite method (7) and by cerate oxidometry using the residual titration procedure (8) was also discussed. Phenelzine can be determined iodometrically (2, 3) as well as with the bromine method (9). The bromometric assay of dihydralazine was reported (10). Potassium dichromate as the titrant in the presence of potassium bromide can also be used for determining dihydralazine (10).

Samek (11) noted that hydrazine can be titrated potentiometrically with chloramine-T in acid, as well as in neutral solution, in the presence of potassium bromide. Berka and Zyka (12) described the potentiometric titration of isoniazid using chloramine-T as titrant in the presence of added bromide. The behavior of chloramine-T as an oxidizing reagent and the methods of standardizing its solutions have been critically examined (13). Bishop and Jennings (14) studied the potentiometric titration of hydrazine with chloramine-T in the presence of added halides under varying conditions of acidity, but the results were unsatisfactory: the potentials at the end-point required more than 30 min to become steady (in chloride media), the end-points were not reproducible (in bromide media), the potentials were unsteady before the end-point, and the conditions for titration were rather critical (in iodide media). These investigators concluded that potentiometric titration with chloramine-T cannot be recommended for the accurate determination of hydrazine under any conditions. Nair and Nair (15) later described a back-titration procedure with chloramine-T as the oxidant for the determination of isoniazid. An excess of chloramine-T was employed and, after a suitable time, was determined by the well-known residual iodometric titration. When the reported (12) method for isoniazid was followed in this laboratory, low results and insufficient precision (93.39 ± 1.63% based on eight analyses) were obtained.

At room temperature the hydrazine–chloramine-T reaction is slow. In previous papers the effect of raising the temperature on the rate of reaction was not investigated. In the present work, it was established that hydrazine and some hydrazine derivatives react instantly and quantitatively with chloramine-T in acidic medium at about 60°, permitting simple, rapid, precise, and accurate direct potentiometric titrations. The proposed method is suitable for determining the antitubercular isoniazid USP and BP (isonicotinic acid hydrazide), the antidepressant phenelzine sulfate USP and BP (phenethylhydrazine sulfate), and the antihypertensive dihydralazine sulfate (1,4-dihydrazinophthalazine sulfate). However, with other hydrazine drugs, *e.g.*, nialamide BP and isocarboxazid BP, the proposed method was unsatisfactory due to secondary oxidative processes under the experimental conditions.

EXPERIMENTAL

Apparatus—Titrations were performed potentiometrically with an automatic titrator[1] equipped with a platinum electrode (a Pt flag) and calomel electrode[2]. The titration vessel is equipped with a hot plate magnetic stirrer.

Reagents and Solutions—The drug samples were the highest grades of commercially available materials and were used as received. Analysis by the USP and BP assays for isoniazid indicated a purity of >98%. Analysis by the USP and BP methods for phenelzine sulfate indicated a purity of >97 and 98%, respectively.

Chloramine-T (0.05 M*)*—Dissolve 7 g of chloramine-T (pure) in 500 ml of water and store in brown glass.

Hydrazine Sulfate—Weigh accurately about 350 mg of hydrazine sulfate (highest purity) and dissolve in 50 ml of water in a 100-ml volumetric flask and dilute to volume with 8 *N* sulfuric acid.

Standardization of 0.05 M *Chloramine-T Solution*—Pipet an

[1] Metrohm, Combi 3D.
[2] Metrohm, EA 404.

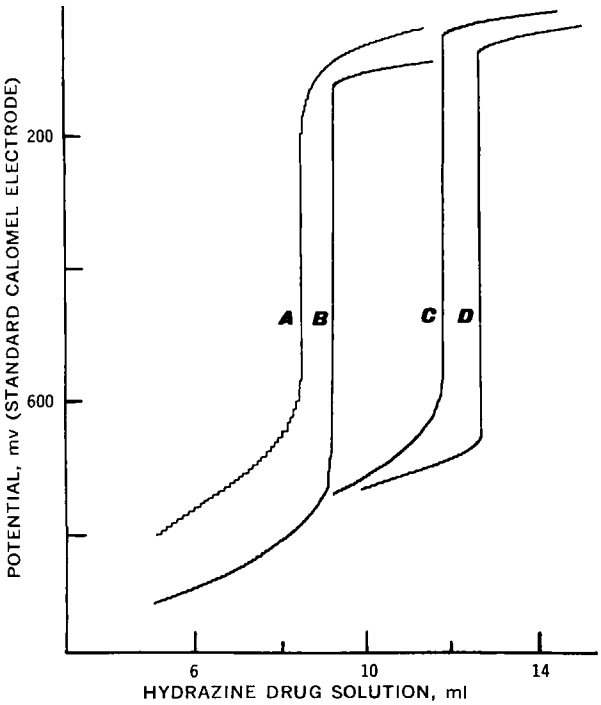


Figure 1—*Automatically recorded typical titration curves with chloramine-T as the oxidant (10 ml 0.05* M). *Key: A, standardization of chloramine-T with hydrazine sulfate (3.818 mg/ml); B, isoniazid (3.702 mg/ml); C, phenelzine sulfate (4.942 mg/ml); and D, dihydralazine sulfate (2.268 mg/ml).*

accurately measured aliquot (10–15 ml) of 0.05 *M* (0.1 *N*) chloramine-T into a 150-ml beaker and add about 30 ml of water. Adjust the temperature of the solution to 60 ± 3° with the hot plate magnetic stirrer and titrate potentiometrically against hydrazine sulfate solution using a platinum–calomel electrode system.

Procedure: Assay of Isoniazid, Phenelzine Sulfate, and Dihydralazine Sulfate—Weigh accurately about 300 mg of isoniazid or dihydralazine sulfate and about 500 mg of phenelzine sulfate. Place in a 100-ml volumetric flask and dissolve in, and dilute to volume with, 4 *N* sulfuric acid. Titrate an accurate volume (10–15 ml) of standardized chloramine-T with hydrazine drug solution, proceeding as for standardization of chloramine-T. From the volume of chloramine-T used, the normality of chloramine-T, and the factor for the hydrazine derivative, calculate the content of hydrazine derivative in the volume required for the titration. Each milliliter of 0.05 *M* chloramine-T is equivalent to 3.428 mg of isoniazid, 5.857 mg of phenelzine sulfate, and 2.883 mg of dihydralazine sulfate.

TLC Study—The solution from the chloramine-T–dihydralazine reaction, stopped at the end-point, was partially neutralized (pH 2.5) with 2 *N* NaOH and gently evaporated to dryness on a water bath. The residue was extracted with hot methanol, and the methanol-soluble extract was spotted on 10 × 20-cm silica gel plates[3] (0.25 mm) previously buffered by ascending development with a pH 7.6 McIlvaine buffer and removal of the solvent by heating for 2 hr at 70°. The mobile phase consisted of a mixture of ether and acetic acid (80:4). The location of migrating spots was found by UV light.

RESULTS AND DISCUSSION

Table I shows the results of the chloramine-T titration of three hydrazine drugs with potentiometric end-point detection. The recoveries by the proposed method were satisfactory, and a sharp fall in potential at the end-point was noted in the titration curves (about 500 mv) (Fig. 1).

[3] F_{254}, E. Merck, Darmstadt, Germany.

Table I—Results of Potentiometric Titrations with Chloramine-T

Drug	Theoretical Milligrams Consumed, Range	Found, % of Theoretical	Equivalents of Chloramine-T per Mole of Drug
Isoniazid	33.46–51.69	100.05 ± 0.44[a]	4
Phenelzine sulfate	57.30–88.22	99.53 ± 0.31	4
Dihydralazine sulfate	28.03–42.36	100.13 ± 0.29	10

[a] Standard deviation based on eight analyses.

In strong acid solution of chloramine-T, the free acid (RNHCl) and dichloramine ($RNCl_2$) predominate (11) and may react directly with hydrazine or the hydrazine part of isoniazid and phenelzine according to Scheme I:

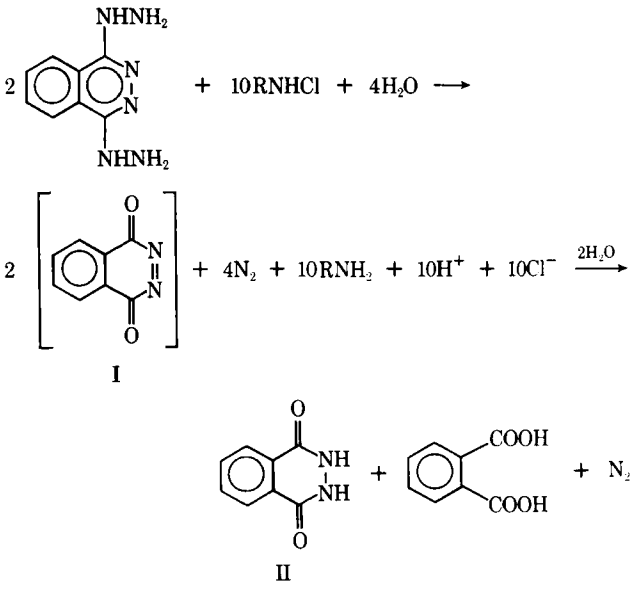
$$2RNHCl + N_2H_4 \longrightarrow 2RNH_2 + 2Cl^- + 2H^+ + N_2$$
$$RNCl_2 + N_2H_4 \longrightarrow RNH_2 + 2Cl^- + 2H^+ + N_2$$

Scheme I: R = p-Toluenesulfonyl

The hydrolytic products, isonicotinic acid or phenethyl alcohol, are not affected by chloramine-T under the present conditions. Four equivalents of the oxidant are consumed per mole of the reductant hydrazine derivative. However, in the case of dihydralazine, 10 equivalents of the oxidant are consumed per mole of drug, and this result is compatible with the proposed Scheme II. Following this route, 1 mole of 2,3-dihydrophthalazine-1,4-dione (II) and 1 mole of phthalic acid are produced.

Accordingly, the methanol-soluble extract of the chloramine-T–dihydralazine reaction yielded three spots in the TLC system described. The spots corresponding to *p*-toluenesulfonamide (R_f 0.92), II (R_f 0.57), and phthalic acid (R_f 0.065) were located by comparison with authentic samples treated similarly. On the other hand, studies of the oxidation of II with 1 mole of lead tetraacetate (14) provided evidence for the presence, in roughly equivalent amounts, of phthalic anhydride and unchanged II. The initial product of oxidation (I) is too unstable to be isolated (14). By taking advantage of its extraordinary reactivity as a dienophile, I was identified (14, 15) with butadiene as a Diels–Alder adduct.

Since the oxidation potential of chloramine-T is increased by lowering the pH, oxidations were carried out in a strong acid medium. Potentiometric analysis was performed with a reverse procedure. An accurately measured volume of standard chloramine-T solution, heated to about 60°, was titrated with hydrazine drug so-



Scheme II

lution in 4 *N* sulfuric acid. With increasing sulfuric acid concentration, from 4 to 16 *N*, no effect on the titration was observed; the rate of reaction decreased progressively toward the end-point as the sulfuric acid concentration was decreased from 4 to 0.5 *N*.

Hydrazine sulfate has been mentioned as a standard substance by several investigators (16); it is available in a highly purified form and its solutions are stable even if heated (17). The precision and accuracy of its potentiometric titration with chloramine-T under the proposed conditions suggested that it is suitable as a valuable primary standard substance in the standardization of chloramine-T solution. The possibility of adapting chloramine-T to the titrimetric analysis should not be overlooked. The stability of 0.05 *M* chloramine-T solution, stored in brown glass, was examined over 2 months and there was no detectable change in titer, in agreement with previous papers (11, 18).

The present method appears to offer a convenient alternative to the existing and more expensive oxidometric methods for the determination of the drugs listed in Table I.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 19, 1973, from the *Istituto di Chimica Farmaceutica dell'Università di Firenze, Via G. Capponi 9, 50121 Firenze, Italy.*

Accepted for publication April 4, 1974.

The authors thank Dr. P. Lacrimini for his assistance.

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Spectrophotofluorometric Analysis of Procainamide and Sulfadiazine in Presence of Primary Aliphatic Amines Based on Reaction with Fluorescamine

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Abstract □ The potential of 4-phenylspiro[furan-2(3*H*),1′-phthalan]-3,3′-dione (fluorescamine) for use as a reagent for determination of drugs containing the primary aromatic amino substituent in the presence of drugs containing primary aliphatic amino substituents was evaluated. Procainamide and sulfadiazine were used as test drugs in the evaluation. The selective determination is based on a difference in the extent of reaction of aromatic and aliphatic amines with fluorescamine at pH 5.5, as well as small differences in spectral characteristics of the two groups. Use of the reagent for microdetermination of aromatic primary amines was compared with that of the Bratton–Marshall and 9-chloroacridine techniques.

Keyphrases □ Procainamide—spectrophotofluorometric analysis with fluorescamine in presence of primary aliphatic amines □ Sulfadiazine—spectrophotofluorometric analysis with fluorescamine in presence of primary aliphatic amines □ Fluorescamine—reagent in spectrophotofluorometric analysis of procainamide and sulfadiazine in presence of primary aliphatic amines □ Spectrophotofluorometry—analysis, procainamide and sulfadiazine using fluorescamine in presence of primary aliphatic amines

Recently, Weigele *et al.* (1, 2) reported the synthesis and characterization of 4-phenylspiro[furan-2(3*H*),1′-phthalan]-3,3′-dione (fluorescamine) as a reagent for the rapid preparation of highly fluorescent derivatives of primary amines. Udenfriend and coworkers (3, 4) have since shown the applicability of this reagent to the analysis of proteins, peptides, and amino acids in various biological systems. The reaction upon which the technique is based proceeds with a half-time in the range of milliseconds, giving fluorescent derivatives that can be determined in subnanomole per milliliter concentrations and that are stable for at least 24 hr.

Because of the rapid, quantitative nature of this reaction and the apparent stability of the reaction products, it was decided to evaluate fluorescamine as a reagent for use in drug microanalysis. This report presents its use as a reagent for the selective spectrophotofluorometric analysis of drugs containing the primary aromatic amino substituent (with procainamide and sulfadiazine as representative drugs) in the presence of primary aliphatic amines. In addition, the technique is compared with the Bratton–Marshall (5) and 9-chloroacridine (6) analyses for drugs with primary aromatic amino substituents.