Therefore, it is concluded that the reaction between isoniazid and II under the experimental conditions of the assay method likely proceeds as depicted in Scheme I.

The present method can be modified to make it applicable to the determination of *free* isoniazid in biological fluids since none of the most important metabolites of isoniazid that have been identified (acetylisoniazid, isonicotinic acid, isonicotinylglycine, pyruvic acid isonicotinylhydrazine, and α-oxoglutaric acid isonicotinylhydrazine) (12) possesses a free amino function in the molecule. Further studies are in progress, and the results will be reported at a later date.

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Potentiometric Titration of Antithyroid Drugs with Mercuric Acetate Solution

SERGIO PINZAUTI▲, VITTORIO DAL PIAZ, and ENZO LA PORTA

Abstract □ A simple and accurate potentiometric titration procedure for the analysis of five antithyroid drugs is described. The drugs dissolved in aqueous solution at particular pH values are titrated with 0.01 *M* mercuric acetate. The end-point is determined potentiometrically by the use of three different indicating electrodes (mercury drop, amalgamated gold, and silver). Quantitative recoveries are reported.

Keyphrases □ Thyroid inhibitors—analysis, potentiometric titration with mercuric acetate □ Methimazole—analysis, potentiometric titration with mercuric acetate □ Thiobarbituric acid—analysis, potentiometric titration with mercuric acetate □ Thiouracil—analysis, potentiometric titration with mercuric acetate □ Methylthiouracil—analysis, potentiometric titration with mercuric acetate □ Propylthiouracil—analysis, potentiometric titration with mercuric acetate □ Potentiometric titrimetry—analysis of five thyroid inhibitors using mercuric acetate □ Mercuric acetate—used in potentiometric titration of five thyroid inhibitors

Methimazole (1-methylimidazole-2-thiol), 2-thiobarbituric acid, 2-thiouracil, 6-methyl-2-thiouracil, and 6-propyl-2-thiouracil are commonly employed in treatment of hyperthyroidism. Many types of titrimetric procedures for their determination have been proposed (1–15). The mercurimetric method of Abbot (4) was the basis in the BP monographs (16) for the determination of 6-methyl-2-thiouracil and 6-propyl-2-thiouracil. Accordingly, thiouracil (0.35 g.) is dissolved in an excess of sodium hydroxide and the resulting disodium salt is titrated in a buffered acetate system with mercuric acetate (0.05 *M*); the end-point is determined by the use of diphenylcarbazone as the indicator. Although satisfactory results are attainable by this method, the indicator change is not sharp and the procedure is not suitable for other antithyroid thiols, *e.g.*, methimazole.

In the present study, a simple, rapid, and accurate direct potentiometric titration is reported for the determination of methimazole USP, thiouracil, methylthiouracil BP, propylthiouracil USP and BP, and 2-thiobarbituric acid. The use of three different indicating electrodes is described.

EXPERIMENTAL

Apparatus—Titrations were performed potentiometrically with a pH meter[1] equipped with a mercury drop electrode (17) and mercurous sulfate electrode[2]. An amalgamated gold electrode (a gold flag dipped into metallic mercury for 20 sec.) or a silver electrode (a silver coil) was also employed as indicating electrodes. The mercury drop and amalgamated gold electrodes can be used for many titrations if rinsed with water between titrations.

Reagents and Solutions—Pure drug samples (listed in Table I) were obtained from commercial sources. Analysis by the USP assay for methimazole indicated a purity of better than 98%. Analysis by the BP assay for methylthiouracil and propylthiouracil indicated a purity of better than 98%.

Borax (0.025 M)—Dissolve 9.535 g. of $Na_2B_4O_7 \cdot 10H_2O$ (reagent grade) in sufficient water to make 1000 ml.

Phosphate Buffer (pH 7.2)—Dissolve 17.337 g. of $Na_2HPO_4 \cdot 12H_2O$ (reagent grade) and 2.485 g. of KH_2PO_4 (reagent grade) in sufficient water to make 1000 ml.

Acetate Buffer (pH 4.6)—Dissolve 3.28 g. of CH_3COONa (reagent

[1] Metrohm model E 148c.
[2] Metrohm EA 406.

Table I—Results of Potentiometric Titration of Antithyroid Drugs with Mercuric Acetate and Three Different Indicating Electrodes

Drug	Weighed Range, mg.	Proposed Method, % Recovery with Mercury Drop Electrode	Proposed Method, % Recovery with Amalgamated Gold Electrode	Proposed Method, % Recovery with Silver Electrode	BP Method, % Recovery
Methimazole	20.20–57.05	99.90 ± 0.22[a]	99.78 ± 0.27[a]	99.69 ± 0.15[a]	—
Thiouracil	21.06–55.34	99.67 ± 0.36	99.58 ± 0.24	99.71 ± 0.38	—
Methylthiouracil	23.20–62.15	99.83 ± 0.38	99.70 ± 0.16	99.59 ± 0.33	99.26 ± 0.25[a]
Propylthiouracil	20.90–56.50	100.06 ± 0.33	100.03 ± 0.25	100.37 ± 0.18	99.20 ± 0.16
2-Thiobarbituric acid	24.84–67.56	100.14 ± 0.29	100.36 ± 0.32	99.93 ± 0.40	—

[a] Standard deviation based on six analyses.

grade) in 200 ml. of water; add 2.3 ml. of glacial acetic acid and sufficient water to make 400 ml.

Mercuric Acetate (0.01 M)—Dissolve 800 mg. of mercuric acetate (reagent grade) in 250 ml. of water, and add 0.3 ml. of glacial acetic acid to prevent hydrolysis. Titrate the solution potentiometrically with 0.01 *M* ethylenediaminetetraacetic acid in pH 4.6 acetate buffer, using a mercury drop–mercurous sulfate electrode system.

Procedure—*Assay of Methimazole*—About 20–60 mg. of methimazole, accurately weighed, was dissolved in 50 ml. of 0.025 *M* borax. The solution, magnetically stirred, was titrated potentiometrically with 0.01 *M* mercuric acetate. The end-point in the titration was determined from the inflection in the curve obtained by plotting milliliters of titrant *versus* millivolt readings. Each milliliter of 0.01 *M* mercuric acetate is equivalent to 2.283 mg. of methimazole.

Assay of Methylthiouracil and Propylthiouracil—About 20–60 mg. of the drug, accurately weighed, was dissolved in 50 ml. of pH 7.2 phosphate buffer, with warming to complete solution. After cooling to room temperature, titration was performed in the same way (as for methimazole). Each milliliter of 0.01 *M* mercuric acetate is equivalent to 2.844 mg. of methylthiouracil or 3.405 mg. of propylthiouracil.

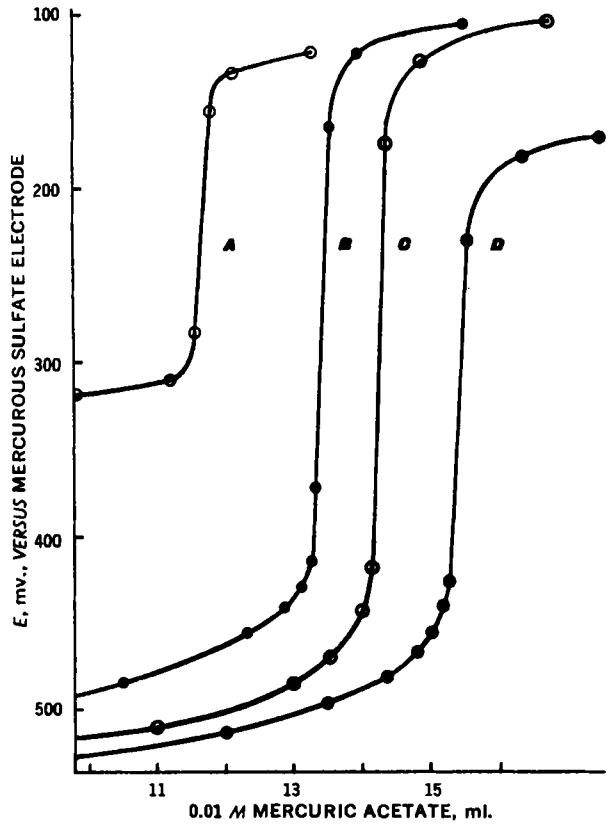

Figure 1—*Typical titration curves for antithyroid drugs. Key: A, 2-thiobarbituric acid, 33.82 mg. (silver electrode); B, methylthiouracil, 38.25 mg. (silver electrode); C, propylthiouracil, 48.50 mg. (amalgamated gold electrode); and D, methimazole, 35.46 mg. (mercury drop electrode).*

Assay of Thiouracil—About 20–60 mg. of the drug, accurately weighed, was dissolved in 50 ml. of pH 4.6 acetate buffer, with warming to complete solution. After cooling to room temperature, titration was performed as already described. Each milliliter of 0.01 *M* mercuric acetate is equivalent to 2.563 mg. of thiouracil.

Assay of 2-Thiobarbituric Acid—About 20–60 mg. of the drug, accurately weighed, was dissolved in 50 ml. of water. The solution was adjusted to pH 1 with nitric acid (20% w/w), and titration was performed as already described. Each milliliter of 0.01 *M* mercuric acetate is equivalent to 2.883 mg. of 2-thiobarbituric acid.

RESULTS AND DISCUSSION

Table I shows the results of mercurimetric determination of five antithyroid agents with potentiometric end-point detection. The recoveries by the proposed method are satisfactory, and only small amounts of drug are required for analysis. A mercury drop electrode, an amalgamated gold electrode, and a silver electrode were used with success as pHg indicators for the potentiometric titration. The silver electrode behaves as reversible with respect to the anion RS^-.

For the analysis of methylthiouracil, propylthiouracil, and methimazole, the largest change in potential at the end-point [about 200 mv./0.1 ml. Hg^{+2} (0.01 *M*)] was observed using the amalgamated gold electrode (Fig. 1). However, the sharp rise produced with the mercury drop and silver electrodes was also sufficiently steep (about 160 mv./0.1 ml. of titrant). In the titration of thiouracil, a lower rise in the potential was noted with each electrode used (about 90 mv./0.1 ml. of titrant). In the assay of 2-thiobarbituric acid, the best results were obtained with the silver electrode (about 160 mv./0.2 ml. of titrant), but the inflections observed with the mercury drop or amalgamated gold electrode (about 100 mv./0.2 ml. of titrant) were also analytically useful.

Antithyroid thiols react with mercuric acetate to form very slightly soluble sulfides. Two moles of the sulfido form (RS^-) of the thiol reacts with 1 mole of the mercuric titrant. The quantitative presence of the sulfido form is pH dependent (20). In preliminary experiments for methimazole in the pH 4–6.8 region, no detectable end-point was apparent. The best results for the assay of methimazole were attained at pH 8.2–9.4, using borax dilute solutions. Methylthiouracil and propylthiouracil gave the most satisfactory titraton curves at pH 7.2–8 (phosphate buffer). The greater sensitivity in end-point detection was noted for 2-thiouracil at pH 4.6 and for 2-thiobarbituric acid in the pH range of 1–2.2.

The potential of the mercury electrode depends on pH by formation of mercuric oxide (18) (the electrode reaction may be written as: $Hg + 2OH^- \leftrightharpoons HgO + H_2O + 2e^-$). In the case of methylthiouracil, propylthiouracil, and methimazole, the solubility products of the precipitate sulfides are evidently lower than HgO ($pSP_{HgO} = 25.5$) (19), permitting sharp breaks in the titration curves under alkaline conditions.

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Determination of Dopa in Pharmaceutical Dosage Forms Based on Oxidation at Tubular Carbon Electrode

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Abstract □ A method for the determination of dopa [3-(3,4-dihydroxyphenyl)alanine] in pharmaceutical dosage forms based on electrochemical oxidation at the tubular carbon electrode is presented. A comparison with a colorimetric method shows this new method to be considerably faster and simpler without a significant loss in precision or accuracy. Between 25 and 30 samples may be determined each hour, for which the variance due to the method results in a standard deviation no greater than ±0.9%.

Keyphrases □ Dopa—analysis in dosage forms, electrochemical oxidation at tubular carbon electrode □ Electrochemical analysis—dopa in dosage forms, tubular carbon electrode

The problems associated with the fluorometric or colorimetric determination of levodopa [(−)-3-(3,4-dihydroxyphenyl)-L-alanine] in pharmaceutical dosage forms were recently summarized (1). Although highly sensitive and specific, these methods are inconvenient due to lengthy procedures involving separation and other manipulative steps. A new colorimetric method by Maggi and Cometti (1), designed specifically for the determination of levodopa in dosage forms, eliminates separatory steps but requires a number of manipulations including a precisely controlled reaction for color formation. A possible alternative to the colorimetric approach may be an electrochemical method similar to the one presented for ascorbic acid (2).

The method for ascorbic acid is based on continuous analysis in flowing streams by oxidation of the drug at the tubular carbon electrode (3). This electrochemical method has advantages over other commonly employed methods of being extremely fast and simple without any significant loss in accuracy or precision. Studies (4) of the electrochemical oxidation of catecholamines, similar in structure to levodopa, suggested that the drug may be assayed in this manner. Thus, an investigation of the oxidation of levodopa at the tubular carbon electrode was undertaken with the idea of developing an analytical method. In this paper a method for the determination of dopa in pharmaceutical dosage forms is presented and compared to the colorimetric method of Maggi and Cometti (1). In addition, the utility of the new electrochemical method in continuously monitoring the dissolution of solid dosage forms is demonstrated.

EXPERIMENTAL

Instrumentation—The electrode assembly, flow system, pump, and polarography system were the same as reported earlier (2). The tablet dissolution apparatus was described by Levy and Hayes (5).

Chemicals—All chemicals were of the highest quality commercially available. Standard solutions of dopa were prepared using the powder[1].

Procedures—Current–voltage curves were determined using a tubular carbon electrode (TCE) which had previously been cleaned with ethyl acetate. Volume flow rates between 4 and 10 ml./min. were commonly employed and were controlled to ±1%. The voltage was scanned anodically from 0 v. *versus* the saturated calomel electrode (SCE) at 0.20 v./min. Standard buffers (6) were used to determine the effect of pH on the oxidation half-wave potential of the compounds studied.

Solid dosage forms were assayed by dissolving weighed quantities in sufficient 0.1 *N* HCl to give approximately 10^{-4} *M* dopa solutions. The sample solutions were pumped through a pledget of glass wool to the TCE (with the potential set at 0.90 v. *versus* the SCE) and the limiting currents were recorded. A calibration plot of the limiting current *versus* concentration was determined daily, and intermittent standards were run to check and adjust the calibration.

The accuracy of the TCE method was evaluated by comparison with a colorimetric method (1). Three tablets were weighed and powdered, and weighed aliquots were taken for analysis; the capsules were emptied and weighed, and similar aliquots were taken. The sample aliquots were dissolved in 1 ml. of 1 *N* HCl and diluted to 1 l. with water; then each aliquot was assayed by the colorimetric method three times. These same aliquots were then

[1] Nutritional Biochemical Corp., Cleveland, Ohio.