Assay of Methenamine Mandelate and Its Pharmaceutical Dosage Forms by Direct Cerimetric Titration

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Avoidance of bisulfate ion and control of acidity permit the direct cerimetric titration of the mandelic acid moiety of methenamine mandelate to a sharp instrumental or indicator end point. Separation and titration procedures for the drug in its dosage forms are described. The procedures compare favorably in speed and selectivity with the U.S.P. assays. The scope and limitations of the analytical reaction are defined.

METHENAMINE mandelate[1] and its tablet and oral suspension dosage forms are recognized in U.S.P. XVII (1) in the urinary antibacterial category. The drug is unique among official salts in that its biological activity derives from both ions. The U.S.P. monograph provides 96% minimum assay values for methenamine mandelate by a procedure selective for methenamine and for mandelic acid by titration with alkali. The net reaction in the U.S.P. mandelic acid assay may be represented:

$$(CH_2)_6N_4H^+ + OH^- = (CH_2)_6N_4 + HOH$$

where the actual acid titrated is the conjugate acid of methenamine; thus the mandelic acid content is inferred rather than determined.

Titration of methenamine mandelate preparations with alkali does not discriminate between methenamine conjugate acid and other organic acids. Difficulty in distinguishing mandelic acid from other organic acids generated during exaggerated stability studies of experimental methenamine mandelate formulations led to the study of conditions for the selective titration of mandelic acid reported here.

Mandelic acid is quantitatively and selectively oxidized by ceric salts according to the equation:

$$C_6H_5CHOHCO_2H + 2Ce(IV) \rightarrow C_6H_5CHO + CO_2 + 2H^+ + 2Ce(III)$$

Helmstaedter (2) applied the Verma and Paul (3) cerimetric titration of mandelic acid to methenamine mandelate. The procedure requires heating mandelic acid with excess ceric sulfate in sulfuric acid solution at reflux and back-titration with a standard reductant. Under these severe conditions the ceric reagent functions as a powerful and general oxidant and formaldehyde liberated from methamine is oxidized also (2). Mathur and Rao (4) reported a residual titration procedure at room temperature with ceric sulfate using visible light catalysis. Chafetz (5) found that ceric oxidation is general for arylglycolic acids and described procedures for their quantitative estimation by ultraviolet spectrometry of the carbonyl reaction product. Because spectrophotometry requires use of a reference standard and the residual titration methods are time consuming and are lacking in selectivity, conditions for the direct titration of mandelic acid in methenamine mandelate and its pharmaceutical dosage forms were investigated.

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[1] Marketed as Mandelamine by Warner-Chilcott Laboratories, Division of Warner-Lambert Pharmaceutical Co., Inc.

Kinetic studies of the Ce(IV) oxidation of mandelic acid (6, 7) showed specific retardation of the reaction rate by bisulfate and hydrogen ions. Using ceric ammonium nitrate in dilute nitric acid, it was found that the oxidation rate of mandelic acid was sufficiently rapid to afford direct titration to an instrumental or color end point. This is the first report to our knowledge of a direct titration of mandelic acid with ceric ion in aqueous system.

EXPERIMENTAL

Titrant.—An approximate 0.05 *N* solution (2.45%) of ceric ammonium nitrate in *N* nitric acid was employed. The titrant was filtered through paper and standardized against primary standard grade ferrous ethylenediammonium sulfate (G. F. Smith Co.), using about 400 mg. of the ferrous salt, accurately weighed, in 100 ml. of distilled water. One drop of nitroferroin indicator solution was used, prepared by dissolving 150 mg. of 5-nitro-1,10-phenanthroline in 15 ml. of freshly made 1.4% aqueous ferrous sulfate. The color change, pink to colorless, coincided with the potentiometric end point in mandelic acid titrations.

Other Reagents and Supplies.—Chemicals used in the study were the best available commercial grades, used without further purification. These included mandelic acid, benzilic acid, phenylacetic acid, atropine sulfate, homatropine hydrobromide, and methenamine mandelate. Methenamine mandelate dosage forms described in this report were samples of regular production lots of Warner-Chilcott Laboratories. Dowex 1-X2 (50–100 mesh), a strong-base anion exchange resin, and a 5% potassium nitrate solution in 0.05 *N* nitric acid were employed in the tablet assay procedures.

Potentiometric titrations were carried out using various commercial platinum billet indicator and saturated calomel reference electrodes with a Sargent model D automatic titrator (used at the "slow" speed) or a Beckman zeromatic pH meter.

Preliminary Observations.—Oxidation of mandelic acid with 0.1 *N* ceric sulfate (U.S.P. XVII, p. 1082) was found to be too slow to afford direct titration at room temperature, even when the benzaldehyde is extracted into a hydrocarbon solvent phase, an expedient suggested by previous work (5). Direct titration proceeded smoothly using a nitric acid system and a sample solution about neutral in pH. Use of *N* nitric acid for the sample as well as titrant led to a severe fall off in reaction rate. Induction of the oxidation can be readily discerned by the increasingly strong odor of benzaldehyde which develops during the titration, a much more selective identity test for mandelic acid than that given in U.S.P.

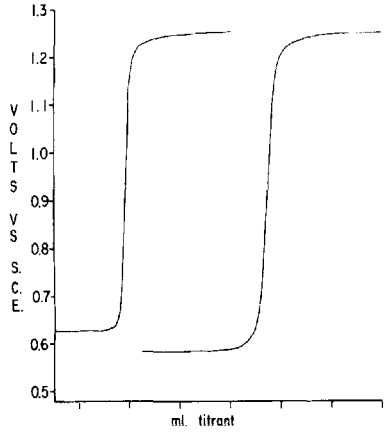

Fig. 1.—Typical titration curves using the recording titrator for methenamine mandelate (left) and mandelic acid by direct cerimetric titration.

Procedure for Mandelic Acid or Methenamine Mandelate U.S.P.—Dissolve approximately 45 mg. mandelic acid or 90 mg. methenamine mandelate, accurately weighed, in 25–50 ml. of water, and titrate the magnetically stirred solution with standard 0.05 *N* ceric ammonium nitrate in *N* nitric acid to the redox indicator or potentiometric end point. The milliequivalent weight of mandelic acid is 76.08 mg.; of methenamine mandelate, 146.17 mg.

$$\text{mg. } C_8H_8O_3 \text{ or } C_6H_{12}N_4 \cdot C_8H_8O_3 = \text{ml.} \times N \times \text{meq. wt.}$$

Assay of Methenamine Mandelate Oral Suspension U.S.P.—Transfer an accurately weighed sample of methenamine mandelate oral suspension, equivalent to about 275 mg. of methenamine mandelate, to a 250-ml. separator. Add 35 ml. of heptane and exactly 100.0 ml. of distilled water. Shake vigorously and allow the layers to separate. Filter about 50 ml. of the aqueous phase through paper, and transfer exactly 25.0 ml. to a 100-ml. beaker. Add 1 drop of nitroferroin indicator solution, and titrate with standard 0.05 *N* ceric reagent from a 25-ml. buret.

$$\text{methenamine mandelate, mg./5 ml.} = \text{ml.} \times N \times 146.17 \text{ mg.} \times 100/25 \times 5 \times \text{density/Gm. sample}$$

Assay of Methenamine Mandelate Tablets U.S.P.—Determine the average weight of 10 tablets and pulverize them. Transfer a portion of the powdered tablets, equivalent to about 1000 mg. of methenamine mandelate, accurately weighed, to a 100-ml. volumetric flask. Add about 60 ml. of distilled water, and shake the mixture mechanically for 10 min. Dilute to the mark with water, mix, and filter through paper. Transfer exactly 5.0 ml. of filtrate to a 5–10 mm. i.d. ion exchange column containing about 1 Gm. of ion exchange resin[2] in the hydroxide cycle. (Prepare the column by equilibrating the resin with about 5 ml. of *N* sodium hydroxide for 10 min., then transferring it to the column and washing with distilled water until the effluent is neutral.) Allow the liquid to drain to the top of the resin bed, then wash the column with about 10 ml. of water, allowing the effluent to drain at the rate of 1–2 ml./min. Stop the flow when the wash level reaches the top of the resin bed, place a 150-ml. beaker as receiving vessel, and elute the mandelic acid with about 50 ml. of 5% potassium nitrate in 0.05 *N* nitric acid at a rate of about 3 ml./min. After collecting about 50 ml., add 1 drop of nitroferroin indicator, and titrate with 0.05 *N* ceric titrant.

$$\text{methenamine mandelate, mg./tablet} = \text{ml.} \times N \times 146.17 \times 100/5 \times \text{mg. av. tablet wt./mg. sample wt.}$$

It may be noted that the procedure may readily be adapted to single unit assay.

Titration of Related Compounds.—Benzilic acid, phenylacetic acid, homatropine hydrobromide, and atropine sulfate were titrated as described above for mandelic acid, using approximately 0.3 mmole of each compound. The ester alkaloids were titrated before and after alkaline saponification.

[2] Marketed as Dowex 1-X2 by the Dow Chemical Co., Midland, Mich.

RESULTS AND DISCUSSION

Potentiometric Data.—Typical titration curves obtained on mandelic acid and methenamine mandelate using an automatic recording titrator are shown in Fig. 1. The oxidation potential of the reaction, Ce(IV) + e = Ce(III), was calculated as +1.464 v. from the titration curve. Half-cell potentials for irreversible oxidation of mandelic acid, methenamine mandelate, and nitroferroin were calculated to be +0.868, +0.832, and +1.044 v., respectively.

Assay of Mandelic Acid and Methenamine Mandelate.—Titrations proceeded smoothly with mandelic acid or its methenamine salt, with no apparent interference from the methenamine. Data found are presented in Table I.

Methenamine Mandelate Oral Suspension.—Samples of suspension in which the vegetable oil vehicle has become rancid cannot be accurately assayed by the U.S.P. method, for titration with alkali does not discriminate between methenamine conjugate acid and fatty acids. The cerimetric method could be successfully applied to assay of methenamine mandelate in rancid vegetable oil without separation of the drug using the potentiometric

Table I.—Cerimetric Titration of Methenamine Mandelate[a]

Trial	mg. Weighed	mg. Found	% Found
1	94.1	93.30	99.2
2	99.9	99.79	99.9
3	109.8	110.30	100.5
4	112.0	111.0	99.1
5	115.5	115.99	100.4
6	100.6	100.76	100.2

[a] Average recovery is 99.9% with relative standard deviation of 0.6%.

Table II.—Replicate Assays of Methenamine Mandelate Oral Suspension

Trial	*1*	*2*	*3*	*4*	*5*	*6*
mg./5 ml. (250 mg. declared)	254.5	257.0	255.0	257.5	256.5	255.0

method by the simple expedient of immersing the electrode tips in the aqueous layer; however, use of the color indicator was preferred because of its simplicity. Observation of the indicator color change in one formulation with a pink color was difficult, for it required discerning a change from pink to colorless in the aqueous layer in the presence of a pink organic layer. Typical results obtained by the extraction procedure using the redox indicator are presented in Table II.

Methenamine Mandelate Tablets.—The ion exchange resin isolation procedure prescribed was employed to circumvent slight interference from sucrose, used as an excipient in the tablet coating. The sugar was slowly oxidized by the titrant. Triplicate assays of methenamine mandelate tablets declaring 500 mg. provided results of 491, 490, and 493 mg./tablet, using a composite sample.

Titration of Related Compounds.—Ceric oxidation of arylglycolic acids appears to be a general reaction (5) which may be represented:

$$\mathrm{Ar{-}\underset{\displaystyle R}{\underset{|}{C}}HOHCO_2H + 2Ce(IV) \rightarrow ArCOR + CO_2 + 2H^+ + 2Ce\,(III)}$$

In this reaction, Ar = aromatic or heteroaromatic, and R = H, an aromatic or alicyclic group. Wholly aliphatic glycolic acid derivatives do not oxidize with the same stoichiometry (6).

Benzilic acid, where Ar = R = C_6H_5—, can be titrated as readily as mandelic acid. Duplicate titrations of benzilic acid provided values 100.1% of theory.

Phenylacetic acid, $C_6H_5CH_2CO_2H$, which may be considered in this discussion a desoxymandelic acid, does not consume titrant. Atropine, the tropine ester of tropic acid, $C_6H_5CH(CH_2OH)CO_2H$, homologous to mandelic acid, did not consume titrant before or after saponification.

The titration could be applied to homatropine hydrobromide, the mandelic acid homolog of atropine, after saponification, but the only indication of reaction in the intact ester was a slight benzaldehyde odor. The saponified ester titrated readily to a value 100.3% of theory.

Interferences.—Although oxidations with ceric salts are selective for mandelic acid and other arylglycolic acids, they are by no means specific. The products obtained depend on reaction conditions employed. Although no interference by methenamine was encountered in the titrations described here, heating methenamine mandelate with excess ceric sulfate results in oxidation of the formaldehyde liberated from methenamine to formic acid (2) as well as oxidation of the mandelic acid to benzaldehyde. In general, some type of separation procedure is necessary for determination of methenamine mandelate in the presence of reducing agents or in colored solutions where the indicator method of end point detection is used.

SUMMARY AND CONCLUSIONS

Mandelic acid is quantitatively and selectively oxidized by ceric salts to benzaldehyde, carbon dioxide, and hydrogen ion. Although residual titration procedures employing excess oxidant have been described, no direct titration has been reported previously. The specific rate retarding effect of bisulfate ion, present in the conventional ceric sulfate titrant, is eliminated by using ceric ammonium nitrate in nitric acid as titrant. This affords a sufficiently rapid reaction rate to permit direct titration to a sharp indicator or instrumental end point, obviates one standard solution, and saves time. The benzaldehyde odor produced during the titration serves as a confirmatory identity test for mandelic acid.

Application of the titration in conjunction with appropriate separation procedures is described for assay of methenamine mandelate and its official preparations. The proposed assays compare favorably with the U.S.P. procedures.

The scope and limitations of the reaction are defined.

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