Table I—Determination of D-Dopa in Levodopa Standard Preparations

| Standard Preparations | D-Dopa Found[a] mg | % |
|---|---|---|
| Levodopa, 10.000 mg, + D-dopa, 0.500 mg | 0.492 | 98.32 |
| Levodopa, 10.000 mg, + D-dopa, 0.200 mg | 0.195 | 97.42 |
| Levodopa, 10.000 mg, + D-dopa, 0.100 mg | 0.095 | 95.60 |
| Levodopa, 10.000 mg, + D-dopa, 0.050 mg | 0.048 | 96.66 |
| Levodopa, 10.000 mg, + D-dopa, 0.025 mg | 0.027 | 103.02 |
| | Mean ± *SD* | 98.20 ± 1.28 |
| Levodopa, 10.000 mg | 0.000 | 0.000 |
| D-Dopa, 10.000 mg | 9.998 | 99.980 |

[a] Values represent average of three determinations.

dopa samples. This method is based on the property of the L-amino acid oxidase (EC 1.4.3.2) enzyme to convert selectively and quantitatively levodopa into 3,4-dihydroxyphenylpyruvic acid. According to Anton and Sayre (4), the latter does not interfere in the subsequent fluorometric assay of the unchanged D-dopa.

## EXPERIMENTAL

In a test tube (26 × 110 mm) fitted with a cap and equipped with tubes for the bubbling of oxygen (1 bubble/sec), 10.0 mg of standard levodopa[1], various amounts of standard D-dopa[1], 10.0 mg of pure L-amino acid oxidase[1], 30.0 μg of flavine-adenine dinucleotide[1] in a total volume of 10.0 ml of 0.1 *M* phosphate buffer (pH 6.5) were mixed. The mixture was incubated at 37° for 3 hr in the dark and subsequently centrifuged at 40,000×*g* for 20 min at 4°. The supernate was diluted 1:200 with 0.1 *M* phosphate buffer (pH 6.5), and D-dopa was determined fluorometrically[2] according to Anton and Sayre (4). The excitation and emission wavelengths were 330 and 380 nm (uncorrected data), respectively.

[1] Sigma.

[2] A model MPF-2A spectrophotofluorometer (Hitachi, Ltd.) was used.

## RESULTS AND DISCUSSION

From the results reported in Table I, the validity of the method is confirmed by incubating separately pure samples of levodopa and D-dopa with amino acid oxidase enzyme. Levodopa is converted to 3,4-dihydroxyphenylpyruvic acid quantitatively while D-dopa is recovered unchanged. The dilution of the supernate is performed to obtain minimal interferences and D-dopa concentrations lower than 2 μg/ml where the response to the fluorometric assay is linear. The sensitivity limit for the determination of D-dopa in the presence of levodopa is 0.25%.

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# Determination of Disodium Edetate Dihydrate in Streptomycin by Atomic Absorption Spectrophotometry

**R. J. HURTUBISE**

**Abstract** □ An atomic absorption method was developed for determining disodium edetate dihydrate (I) in the antibiotic streptomycin. It is free of phosphate interference and has a reproducibility of ±1.3 μg/g and a limit of detection near 4 μg/g. Quantitation involves the formation of a nickel–I complex, release of the complexed nickel by pH adjustment, and the determination of nickel by atomic absorption. The amount of nickel determined is directly proportional to the amount of I.

**Keyphrases** □ Streptomycin—determination of disodium edetate dihydrate, atomic absorption spectroscopy □ Disodium edetate dihydrate—determination in streptomycin, atomic absorption spectroscopy □ Edetate disodium dihydrate—determination in streptomycin, atomic absorption spectroscopy

In the production of pharmaceuticals, small amounts of disodium edetate dihydrate (I) may be used to remove alkaline earth impurities and to improve the efficiency of production processes. To control low levels of I in pharmaceuticals, rapid, accurate, and precise analytical methods are important. A search of the chemical literature yielded one method for I in pharmaceuticals (1). Other methods have been developed for I in waste water, urine, and protein solutions (2–6). No methodology was reported

**Table I**—Percentage Recovery of Disodium Edetate Dihydrate in Streptomycin Sulfate[a]

| Sample | Present, μg/g | Added, μg/g | Found, μg/g | Recovery, % |
|---|---|---|---|---|
| 1 | 60 | 14 | 74 | 100 |
| 1 | 60 | 35 | 91 | 89 |
| 2 | 25 | 36 | 60 | 97 |
| 3 | 17 | 49 | 63 | 94 |
| 3 | 17 | 49 | 64 | 96 |
| 5 | 9.8 | 50 | 58 | 96 |
| 6 | 27 | 50 | 75 | 96 |
| 4 | 17 | 71 | 82 | 92 |
| 4 | 17 | 71 | 82 | 92 |
| 1 | 60 | 71 | 127 | 94 |
| 1 | 60 | 106 | 161 | 95 |
| 7 | 7.2 | 144 | 144 | 95 |
| 3 | 17 | 196 | 193 | 90 |
| 3 | 17 | 196 | 192 | 89 |
| | | | Average | 94 |

[a] Replicate samples of 14.0 ± 0.2 g were used.

for determining I in the antibiotic streptomycin in the presence of triphosphate. One method[1] for I in boiler water can tolerate triphosphate up to 12 μg/ml; however, higher levels of triphosphate result from initial steps in the purification of streptomycin.

The method developed, which proved free of phosphate interference, involves the formation of a nickel–I complex. The amount of nickel reacting is directly proportional to the amount of I present in streptomycin. Excess nickel is removed by precipitation with dimethylglyoxime; the nickel complexed by I is released by pH adjustment and is then determined by atomic absorption. Darbey (7) used a similar approach for colorimetric determination of I.

## EXPERIMENTAL

**Apparatus**[2]—A single element nickel hollow-cathode lamp[3] was used for the determinations. The following instrument settings were used for all measurements: resonance line, 2324 Å; slit 2; lamp current, 40 mamp; air flow, 21.2 liters/min; and acetylene flow, 6.22 liters/min.

**Reagents**—For the nickel solution, dissolve 13.3 g of reagent grade nickel sulfate hexahydrate in distilled water and dilute to 1 liter with distilled water. For the dimethylglyoxime solution, dissolve 3.75 g of reagent grade dimethylglyoxime in 3-A alcohol and dilute to 250 ml with 3-A alcohol.

The following reagents were also used: 3-A alcohol–5% (v/v) methanol in ethanol and analytical grade I, ammonium hydroxide, hydrochloric acid, and sodium hydroxide.

**Procedure for Standards**—Standards of 8.0, 4.0, 2.0, and 1.0 μg/ml of I were prepared in distilled water with a few drops of 1 *N* NaOH added so the final volume was 100 ml and the pH was 6.0–6.5. A 100-ml distilled water blank was prepared by adding a few drops of 1 *N* HCl so the pH was 6.0–6.5. Fifteen milliliters of nickel solution was added to the standards and blank and after 10 min, 5 ml of concentrated ammonium hydroxide was added. After 5 min and occasional swirling, 15 ml of dimethylglyoxime reagent was added to all solutions. After 5 min, nickel dimethylglyoxime was filtered off and about 60 ml of each filtrate was collected. Fifty milliliters of each filtrate was pipeted into 125-ml erlenmeyer flasks and 2.5 ml of concentrated hydrochloric acid was added to each flask. The absorbance of the standards was determined, and then absorbance *versus* total micrograms of I was plotted on linear graph paper.

[1] ASTM Method D 3113-72T.

[2] A Perkin-Elmer 403 atomic absorption spectrophotometer was used as described in the Perkin-Elmer instruction manual.

[3] P-E Intensitron Lamp.

**Table II**—Reproducibility of Disodium Edetate Dihydrate in Streptomycin Sulfate[a]

| Sample | | Micrograms per Gram |
|---|---|---|
| 2 | | 24 |
| | | 25 |
| | | 24 |
| | | 27 |
| | | 25 |
| | | 27 |
| | Average | 25 |
| Standard deviation = 1.4 | | |
| 95% confidence limit = 25 ± 1.5 μg/g | | |
| 5 | | 9.5 |
| | | 9.5 |
| | | 8.5 |
| | | 11 |
| | | 9.5 |
| | | 11 |
| | Average | 9.8 |
| Standard deviation = 0.98 | | |
| 95% confidence limit = 9.8 ± 1.0 μg/g | | |
| 6 | | 27 |
| | | 28 |
| | | 25 |
| | | 27 |
| | | 25 |
| | | 27 |
| | Average | 27 |
| Standard deviation = 1.3 | | |
| 95% confidence limit = 27 ± 1.4 μg/g | | |

[a] Replicate samples of 14.0 ± 0.2 g were used.

**Procedure for Sample**—Solutions of accurately weighed 14-g samples of streptomycin were prepared in distilled water with a few drops of 1 *N* NaOH added so the final volume was 100 ml and the pH was 6.0–6.5. The streptomycin solution was treated exactly as the standards beginning with: "Fifteen milliliters of nickel solution was added. . . ." The micrograms of I were obtained from the calibration curve and the micrograms per gram of I was calculated.

## RESULTS AND DISCUSSION

The results in Table I show that the average percent recovery, 94, is somewhat low over a concentration range of 14–196 μg/g I added to streptomycin. Darbey (7) showed that ferric, calcium, and magnesium ions in water solution do not interfere in the complex formation of nickelous ion with I at 40 μg/ml and subsequent precipitation of excess nickelous ion with dimethylglyoxime. Ferric, calcium, and magnesium ions are present at micrograms per gram levels in streptomycin; with the method developed, I is about 3.0 μg/ml in the test solution. At such low concentrations of I, a negative bias could be introduced into the results if ferric ion partially complexed I in favor of nickelous ion.

In water solution at pH 6, ferric ion has a conditional formation

**Table III**—Effect of Sodium Triphosphate on Determination of Disodium Edetate Dihydrate in Streptomycin Sulfate[a]

| Sample | Calculated Phosphate Added, μg/g | I, μg/g |
|---|---|---|
| 8 | 0 | 7.4 |
| | 500 | 7.4 |
| | 1000 | 7.4 |
| | 5000 | 7.4 |
| 9 | 0 | 16 |
| | 500 | 15 |
| | 1000 | 14 |
| | 5000 | 15 |

[a] Replicate samples of 15.0 ± 0.1 g were used.

**Table IV**—Effect of Sodium Dihydrogen Phosphate Monohydrate on Determination of Disodium Edetate in Streptomycin Sulfate[a]

| Sample | Calculated Phosphate Added, μg/g | I, μg/g |
|---|---|---|
| 8 | 0 | 7.4 |
| | 500 | 7.4 |
| | 1000 | 8.4 |
| | 5000 | 7.4 |
| 9 | 0 | 16 |
| | 500 | 15 |
| | 1000 | 15 |
| | 5000 | 14 |

[a] Replicate samples of 15.0 ± 0.1 g were used.

constant of $10^{14.6}$ while nickelous ion has a conditional formation constant of $10^{13.8}$ (8). Ferric ion, with the greater conditional formation constant, probably would compete effectively with nickelous ion for I in a streptomycin solution. However, because the ferric-ion concentration is so low and nickelous ion is present in excess in the test solution, the reaction between nickelous ion and I should be favored over the reaction of ferric ion and I. Thus, the magnitude of error introduced by ferric ion should be small. Calcium and magnesium ions would not be expected to compete strongly for I, having conditional formation constants of $10^{5.9}$ and $10^{3.9}$, respectively, at pH 6.0 (8). Other factors that might contribute to the low percentage recovery are adsorption of I-complexed nickel on the precipitated nickel dimethylglyoximate and the complex composition of streptomycin solutions causing matrix effects. A systematic investigation of interferences in solution and interferences in the flame was not undertaken.

Table II indicates that within a concentration range of 10–24 μg/g I, an average reproducibility of ±1.3 μg/g I can be obtained at the 95% confidence level. The results also show that good precision is obtainable at the relatively low concentrations of I, *e.g.*, 9.5 μg/g. The limit of detection is near 4 μg/g I.

Tables III and IV show the effect of sodium triphosphate and sodium dihydrogen phosphate monohydrate on the determination of I. The results indicate that there is no interference from phosphate species. This conclusion is contrary to that of Darbey (7), who suggested that phosphates would give high results with his colorimetric method because these anions complex nickel.

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# Spectrophotometric Titration of Edetic Acid in Ophthalmic Solutions


**E. E. KAMINSKI[x] and DANIEL M. PACENTI**



**Abstract** □ The procedure described here is applicable for determining edetic acid (ethylenediaminetetraacetic acid) at the 0.05–0.2% level in ophthalmic solutions. This method employs a spectrophotometric titration, using a magnesium-ion solution as titrant and arsenazo I as the indicator. The chemistry involved is an adaptation of a procedure to determine water hardness using edetic acid as titrant. The apparatus employed is an automated system utilizing a probe colorimeter to monitor the titration. Good precision was demonstrated with relative standard deviations of less than 1%. The method was also shown to be applicable to commercially available ophthalmic solutions, either decongestants or hard contact lens cleaning solutions.



**Keyphrases** □ Edetic acid—spectrophotometric titration in ophthalmic solutions □ Preservatives—spectrophotometric titration of edetic acid in ophthalmic solutions □ Titration, spectrophotometric—determination of edetic acid in ophthalmic solutions


Edetic acid (ethylenediaminetetraacetic acid) is widely knwn for its chelating properties (1). It is also used as a preservative system in pharmaceutical preparations such as ophthalmic solutions (2–5). It became necessary to determine the edetic acid content in such solutions where the label claim concentration was 0.1% (1 mg/ml).

The classical colorimetric determination of the ferric iron–edetic acid complex (6) suffered from fading color and poor sensitivity with these ophthalmic solutions. Fritz *et al.* (7) reported a procedure for determining water hardness by an edetic acid titration using arsenazo I as an indicator; they showed spectrophotometric titration curves for magnesium and calcium. They also determined formation constants for these metals with arsenazo I. Based on these data, it seemed feasible that edetic acid could be titrated with magnesium ion using arsenazo I as the indicator.

To automate the method, a spectrophotometric ti-