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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ACKNOWLEDGMENTS AND ADDRESSES

Received February 1, 1974, from the *Quality Control Department, Pfizer Inc., Terre Haute, IN 47808*

Accepted for publication March 25, 1974.

The author acknowledges the technical assistance of E. W. Fagg and the helpful comments of J. A. Means.

Spectrophotometric Titration of Edetic Acid in Ophthalmic Solutions

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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-

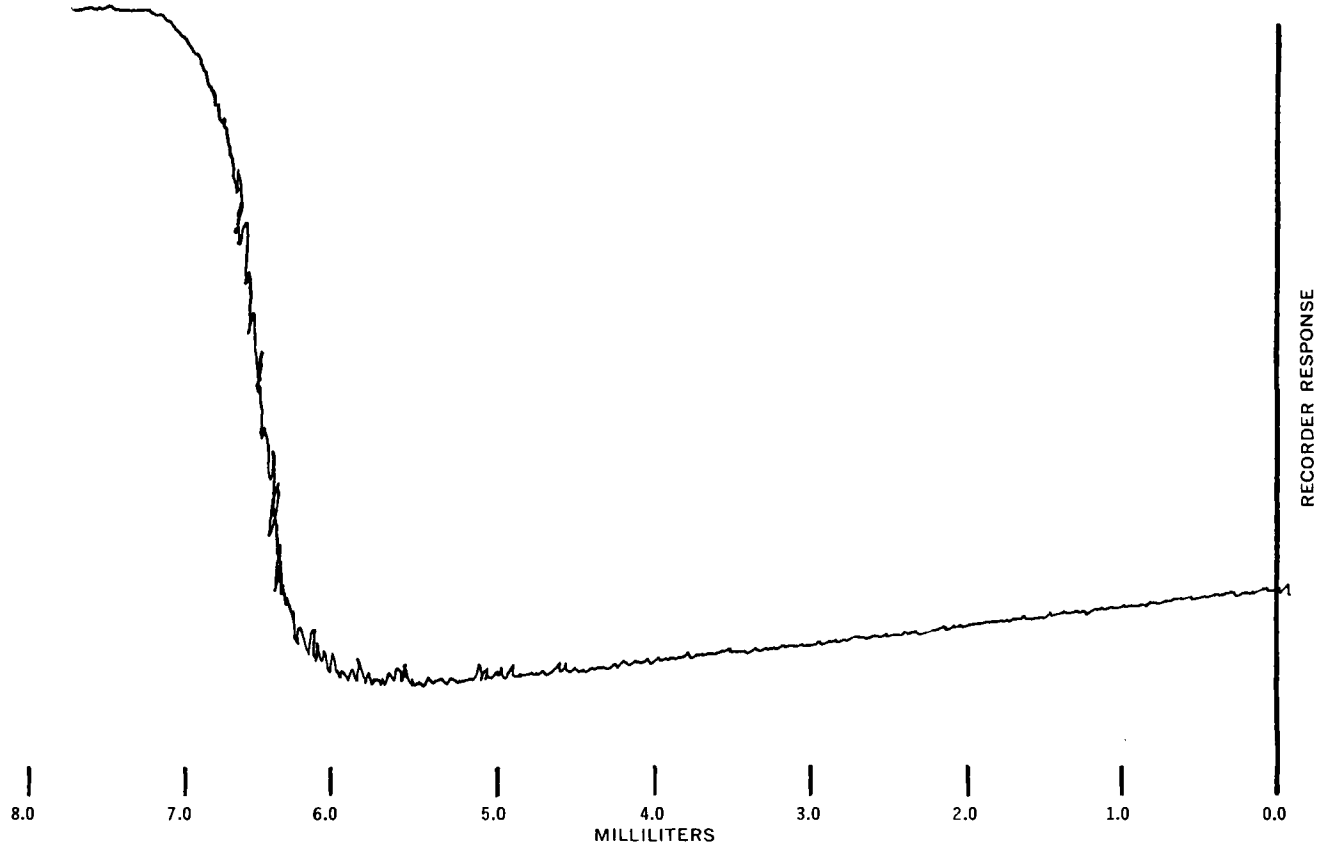

Figure 1—*Spectrophotometric titration curve of Solution A.*

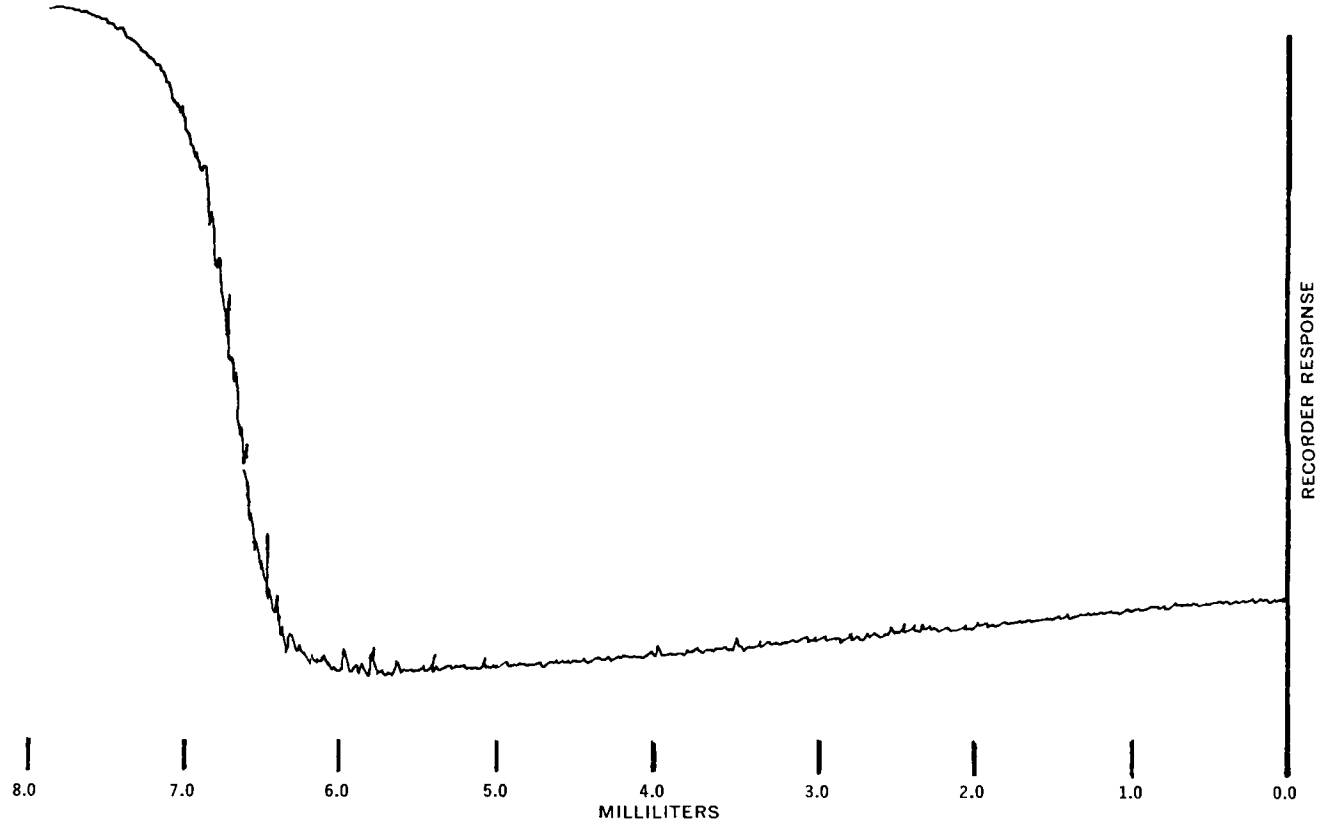

Figure 2—*Spectrophotometric titration curve of Solution B.*

Table I—Precision Data

	Solution A, Edetic Acid Found[a], mg/ml	Solution B, Edetic Acid Found[a], mg/ml
First day	0.968	0.959
	0.963	0.933
	0.975	0.943
Second day	0.964	0.938
	0.972	0.939
	0.968	0.946
Third day	0.961	0.939
	0.961	0.936
	0.975	0.943
Mean (mg/ml) ($\bar{x}$)	0.967	0.941
Standard deviation[b] (mg/ml)	±0.006	±0.007
Relative standard deviation	0.59%	0.76%

[a] $\text{mg/ml found} = \dfrac{\text{ml titrant} \times M \text{ titrant} \times 372.24 \text{ mEq/ml}}{\text{sample volume}}$

[b] $\text{Standard deviation} = \left[\dfrac{1}{n-1}\sum(x_i - \bar{x})^2\right]^{1/2}$

where $n = 9$, $\bar{x}$ = the mean, and x_i = each individual result.

Table II—Standard Addition–Recovery Experiments

	Edetic Acid Added, mg	Edetic Acid Found, mg	Recovery[a], %
Solution A	0.916	0.878	95.9
	1.373	1.365	99.4
	1.831	1.788	97.7
Solution B	0.916	0.986	107.6
	1.373	1.422	103.6
	1.831	1.874	102.3

[a] Percent recovered = [(mg found − mg initial)/mg added] × 100.

tration procedure was desired. A probe colorimeter filled the requirements for a sensitive, accurate, and rapid flowthrough measurement system. The system is further automated by using an automatic delivery buret and a recorder to trace the titration curve.

EXPERIMENTAL

Equipment—A colorimeter[1] with a stainless steel probe tip is used with an automatic constant-rate 10-ml buret[2]. The titration curve is plotted using a variable range recorder[3].

Reagents—A 1-mg/ml solution of arsenazo I trihydrate indicator[4] is prepared by dissolving 100 mg of indicator and 1.0 g of tromethamine (the buffer) in 10 ml of 2-propanol and diluting with water in a 100-ml volumetric flask.

Prepare pH 10 buffer by dissolving 13.1 g of tromethamine in about 40 ml of water, adding 4.0 ml of concentrated hydrochloric acid, and then adding 30 ml of concentrated ammonium hydroxide. Dilute the resulting solution to 100 ml with water, mix well, and then further dilute 1 to 10 with water.

The standard magnesium-ion solution (0.002 *M*) is prepared by transferring about 122 mg of magnesium metal ribbon to a 1000-ml volumetric flask and adding 75 ml of water before completely dissolving the magnesium with the slow addition of 3 *N* hydrochloric acid. Dilute the solution to volume with deionized water and then further dilute 200 to 500 ml with deionized water. Calculate the molarity based on the exact weight of magnesium ribbon.

Procedure—Pipet 5.0 ml of sample (containing approximately 0.1% edetic acid) into a 50-ml Griffin low-form beaker containing a magnetic stirring bar. Then add 10.0 ml of pH 10 buffer, 10.0 ml of deionized water, and 0.5 ml of indicator. The pH of the solution should now be 10.

Set the filter on the colorimeter at 570 nm and the range on the recorder at 125 mv. Insert the probe tip port and delivery tube into the beaker and, while stirring, set the pen on the recorder paper between 70 and 90 by adjusting the "absorbance zero" knob. It is important to ensure that the probe tip port is below the solution level and free of air bubbles or the noise level of the recorder tracing will become excessive.

Place the standard magnesium-ion solution in the automatic buret, open the delivery tube stopcock, and titrate to the end-point. In an identical manner, prepare and titrate a reagent blank solution.

[1] Brinkman PC/1000.
[2] Sargent-Welch.
[3] Sargent model SR.
[4] Aldrich Chemical Co.

Table III—Ingredients of Ophthalmic Solutions

Solution	Edetic Acid Concentration	Other Preservatives	Vasoconstrictor
A	0.1%	Benzalkonium chloride, 0.01%	Naphazoline hydrochloride, 0.012%
B	0.1%	Benzalkonium chloride, 0.1%	—
C	0.05%	Benzalkonium chloride, 0.01%	Phenylephrine hydrochloride, 0.2%
D	0.1%	Benzalkonium chloride, 0.01%	Tetrahydrozoline hydrochloride, 0.05%
E	0.05%	Thimerosal, 0.002%	—
F	0.2%	Benzalkonium chloride, 0.01% Chlorobutanol, 0.4%	—

RESULTS AND DISCUSSION

The titration curves for the titration of two ophthalmic solutions (A and B) are shown in Figs. 1 and 2. The end-point breaks are at least 30 scale units in each case, thus facilitating the location of the inflection point. The noise level was not a detrimental factor.

One variable investigated was the effect of indicator concentration on the titration curve. Accordingly, various amounts of indicator were used in titrating solutions *via* the recommended procedure. The shapes of the curves and the end-point determination were not affected when the volume of indicator was varied from 0.20 to 0.75 ml (40–150% of the recommended concentration).

The precision of this method was determined by performing three separate assays per day for 3 days on each ophthalmic solution (Table I).

Standard addition–recovery experiments were performed to insure the lack of interference in the procedure (Table II). The additional edetic acid was added by pipeting 1.0, 1.5, and 2.0 ml of a standard solution to respective sample solutions and titrating as before. Since a graduated 5.0-ml pipet was used for the trans-

Table IV—Titration Results Using Edetic Acid

Solution	Label Claim, mg/ml	Found, mg/ml	Label Claim, %
A	1.0	0.967[a]	96.7
B	1.0	0.941[a]	94.1
C	0.50	0.505[b]	101.0
D	1.0	1.000[b]	100.0
E	0.5	0.429[c]	85.7
F	2.0	2.042[b]	102.1

[a] Average of nine determinations. [b] Average of five determinations. [c] Average of three determinations.

fer, these recoveries are considered adequate to indicate the lack of any significant interferences.

An additional way to indicate lack of interference and also to demonstrate the applicability of the method is to analyze other ophthalmic solutions[5]. Decongestant ophthalmic drugs contain preservatives, vasoconstrictors, and other ingredients such as buffers and cleaning agents. Hard contact lens cleaning solutions do not contain the vasoconstrictors (8).

The common vasoconstrictor drugs available for over-the-counter use in ophthalmic preparations include phenylephrine, naphazoline, and tetrahydrozoline. The common preservatives are chlorobutanol, benzalkonium chloride, thimerosal, and edetic acid. Table III lists the combinations present in Solutions A–F, and Table IV lists the results for titration of these solutions by the proposed method. There were no problems with the titrations, and curves with end-point breaks similar to Figs. 1 and 2 were obtained. The low result for Solution E might be due to the more viscous nature of this solution, but sampling in triplicate did produce identical results.

CONCLUSION

This spectrophotometric titration procedure for determining edetic acid in ophthalmic solutions is simple and accurate. A complete analysis can be accomplished in 15 min using the automated equipment described herein. The precision and accuracy of the method have been quantitatively demonstrated.

[5] The commerical solutions used in this work were: A, Clear Eyes; B, Lensine; C, Degest; D, Visine; E, Adapt; and F, Soquette.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 18, 1973, from *Abbott Laboratories, North Chicago, IL 60064*

Accepted for publication March 4, 1974.

The authors thank Dave Williamson and Tom Eng for their assistance.

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Titrations of Barbiturates and Sulfa Drugs in 3-Methyl-2-oxazolidone

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Abstract □ 3-Methyl-2-oxazolidone was evaluated as a solvent for the titration of selected barbiturates and sulfa drugs. Its high dielectric constant and wide liquid range contribute to its outstanding solvent properties. Tetrabutylammonium hydroxide was used as the titrant. End-points were determined potentiometrically using a glass–calomel electrode system. Data evaluation was performed by suitable computer programs, and relative acid strengths were determined.

Keyphrases □ 3-Methyl-2-oxazolidone—evaluated as solvent for titration of barbiturates and sulfa drugs, determination of relative acid strengths □ Barbiturates—potentiometric titration using 3-methyl-2-oxazolidone as solvent, relative acid strengths determined □ Sulfa drugs—potentiometric titration using 3-methyl-2-oxazolidone as solvent, relative acid strengths determined

Information is available concerning the physical properties, preparation, and pharmacological usefulness of highly substituted 2-oxazolidones. Since most of these compounds are solids at room temperature, they have little usefulness as analytical solvents. However, a few *N*-alkyl-substituted derivatives of the parent compound are liquids and have a wide liquid range. Little is known regarding 2-oxazolidones as possible solvents for acid–base titrations (1). Some liquid *N*-substituted 2-oxazolidones were suggested as promising solvents (2).

In this study, 3-methyl-2-oxazolidone was chosen due to its high dielectric constant and relatively low melting point. When pure, the compound is odorless and colorless. Some of its important physical properties at 25° (2) are: mp, 15.9°; bp at 1 mm, 74–75°; dielectric constant at 1 MHz, 77.5; viscosity, 2.450 cps; density, 1.1702 g/ml; and refractive index, 1.4522. A series of important weak acids of pharmaceutical interest was used to determine the suitability of 3-methyl-2-oxazolidone as a solvent for such analyses.

EXPERIMENTAL

Apparatus—A digital pH/mv meter[1] equipped with an electrode switch[2] was used for potential measurements. Glass indicator electrodes[3] were used in conjunction with porous ceramic junction calomel reference electrodes[4]. The saturated aqueous potassium chloride solutions of the calomel electrodes were replaced with a saturated solution of potassium chloride in ethanol. The electrodes were soaked for several days in 3-methyl-2-oxazolidone prior to use. To stabilize potential readings, any one pair of electrodes was resoaked in 3-methyl-2-oxazolidone before reuse.

The titrant was dispensed from a 5-ml microburet[5] graduated in 0.01-ml divisions, which meets National Bureau of Standards Specification NNN-B-789. The storage vessel was fitted with a

[1] Orion model 601.
[2] Orion model 605.
[3] Sargent 30050-15c.
[4] Sargent 30080-15c.
[5] Kimax model 17110 Class A.