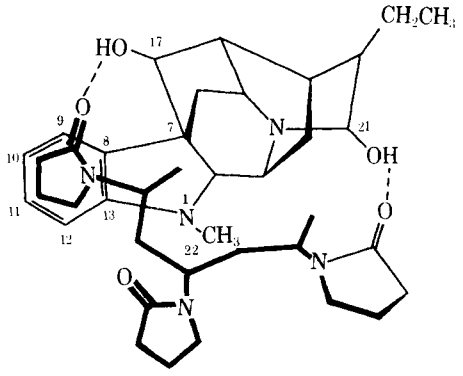

I

rolidone seemed to be influenced by hydrogen bond formation (Table I).

In addition to the influence in the signals of C-7, C-17, and C-21, there was a relatively large influence in the signals of aromatic carbons in ajmaline by polyvinylpyrrolidone; that is, the signals of C-8 and C-13 tended to shift upfield and those of C-9 and C-10 shifted downfield. The electron densities of C-8 and C-13 became higher due to the presence of polyvinylpyrrolidone, and those of C-9 and C-13 became lower.

Considering the relatively small changes of the aromatic carbons by diacetylation, it does not seem that these electron density changes were brought about indirectly through hydrogen bond formation. Furthermore, the upfield shift of C-13 and the downfield shift of C-10 do not seem to support the interaction with the nitrogen atom at position 1 in ajmaline. Accordingly, it is reasonable to consider that a complex was formed between the amide groups of the pyrrolidone ring in polyvinylpyrrolidone and the aromatic ring in ajmaline, as suggested previously (5). It is expected that the electron densities of C-8 and C-13 of the aromatic ring in ajmaline are affected and become higher because of the approach of the positively charged nitrogen atom of the amide group of the pyrrolidone rings of polyvinylpyrrolidone. Considering such mechanisms of complex formation, Laszlo (12) described it as a dipole–induced dipole complex in view of the nomenclature of these complexes.

From these results, an interaction model between polyvinylpyrrolidone and ajmaline is proposed (I). In the coprecipitate, ajmaline apparently was molecularly dispersed in solid polyvinylpyrrolidone through these interactions. As a result, the dissolution rate of ajmaline from the coprecipitate was enhanced markedly.

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Polarographic Determination of Edetate Disodium in Eyewash and Ophthalmic Decongestant Solutions

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Abstract □ The quantitative analysis of edetate disodium in nonprescription eyewash and ophthalmic solutions is described. The method involves differential pulse polarography using a dropping mercury electrode. A known concentration of cadmium or zinc is added to a buffer in a polarographic cell. The sample solution is incremented into the cell with a micropipet. The peak current decreases because the resulting chelate is not reducible at the potentials used. The quantity of edetate disodium in the sample then is determined graphically. Some contact lens cleaning and wetting solutions containing polymeric compounds are amenable to assay for edetate disodium if extraction, precipitation, centrifugation, or dilution steps minimize the maximum suppressor effect of the additives. These steps are very effective with cellulose ether compounds but are ineffective with polyvinyl alcohol.

Keyphrases □ Edetate disodium—polarographic determination in eyewash and ophthalmic decongestant solutions □ Polarography, differential pulse—analysis, edetate disodium in eyewash and ophthalmic decongestant solutions □ Ophthalmic preparations—polarographic determination of edetate disodium in eyewash and ophthalmic decongestant solutions

Edetate disodium (I) is added to eyewash and ophthalmic solutions containing bactericides such as benzalkonium chloride, chlorobutanol, and thimerosal to increase their bactericidal properties (1). Compendial methods (2–4) employ classical titrimetric procedures for the quantitative determination of I, but these methods are not suitable for the levels encountered in the drug preparations (0.01–0.25%).

A literature search revealed two methods used to determine I in pharmaceutical preparations. One employed colorimetric detection for ophthalmic solutions (5); the other used atomic absorption for an antibiotic preparation (6).

This paper proposes a sensitive, rapid, and quantitative polarographic method for determining I concentrations by stepwise addition of sample to the cell. The resulting

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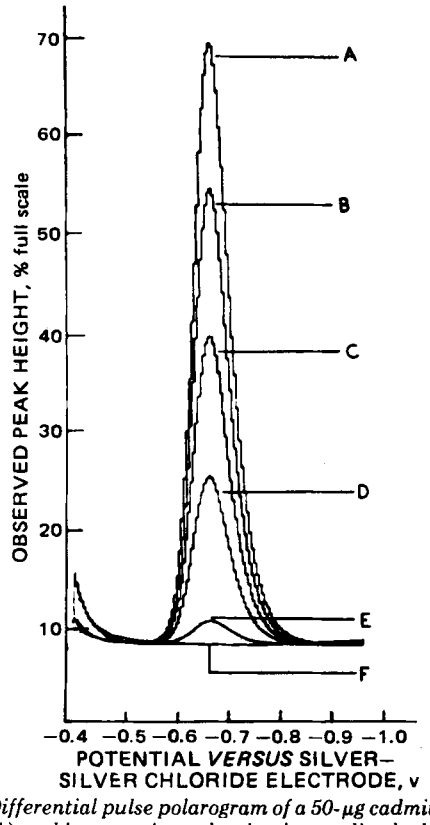

Figure 1—*Differential pulse polarogram of a 50-μg cadmium standard in the cell (A) and its stepwise reduction in amplitude due to the addition of 40-μl increments of a standard 0.1% edetate disodium solution (B–F) (5 μamp full scale).*

chelation causes a corresponding decrease in the peak amplitude of the free metal (cadmium or zinc) originally in the cell.

EXPERIMENTAL

Reagents and Chemicals—Analytical reagent grade chemicals were used.

Solution Preparation—An ammonium–ammonia buffer supporting electrolyte was prepared by diluting 2.0 g of ammonium nitrate and 1.75 ml of concentrated ammonia to 500 ml with water. A maximum suppressor was prepared by dissolving 100 mg of gelatin powder[1] in 100 ml of water. The mixture was warmed to promote dissolution. Gelatin solutions older than 72 hr were not used.

Metal standard solutions (1000 μg/ml) were made by dissolving cadmium wire[2] or zinc shot[2] (99.99%) in 100 ml of 20% HNO_3 and diluting to 1 liter with water. Edetate disodium[3] (99.0–101.0% pure) was assayed (3) and then employed in preparing standard solutions in water and in simulated commercial eye drop and eyewash preparations. Triple-distilled mercury[4] was washed with 10% HNO_3 in a mercury cleaning tower[5] and then pinholed to remove residual wash water.

Instrumentation—Experiments were performed with a commercial polarographic system[6] and an x–y recorder[7]. Adjustable micropipets[8] having volumes of 5–50, 50–250, and 200–1000 μl were used. Nitrogen was passed through a scrubbing tower[9] of vanadous chloride followed by a gas washing tower containing the supporting electrolyte.

[1] BBL, Cockeysville, Md.
[2] Ventron Corp., Beverly, Mass.
[3] Mallinckrodt, St. Louis, Mo.
[4] Bethlehem Apparatus Co., Hellertown, Pa.
[5] Thomas-John, Arthur H. Thomas Co., Philadelphia, Pa.
[6] Princeton Applied Research model 174 polarographic analyzer, model 315 automated electroanalysis controller, and model 303 static mercury drop electrode apparatus.
[7] Houston Instruments model 2000.
[8] Finnpipette, distributed by Markson Science Inc., Del Mar, Calif.
[9] Princeton Applied Research Corp. application note AN-108. The vanadous chloride oxygen scrubbing tower may not be necessary, depending on the nitrogen purity.

Table I—Linearity Data [a] Relating Volume of Sample Added to Adjusted Net Current

0.1% (w/v) edetate disodium added, μl	0	40	80	120	160
Adjusted net current, μamp	3.07	2.35	1.59	0.87	0.12

[a] Linear regression value at x intercept (9) = 166.7 μl, and coefficient of determination (9) = 0.999.

Instrument Parameters—A potential scan from −0.4 to −1.0 v (cadmium) or from −0.8 to −1.5 v (zinc) at a rate of 5 mv/sec was used. The differential pulse mode was employed at a modulation amplitude of 50 mv and a current range of 5 μamp full scale. A dropping mercury electrode with a drop time of 1 sec also was used.

Assay—To the polarographic cell were added 10.0 ml of buffer, 250 μl of gelatin solution, and 50 μl of cadmium or zinc standard solution. Oxygen was removed from the solution by purging with nitrogen for 5 min, and the solution then was scanned to obtain the metal peak. A micropipet was used to add sample to the cell. If the sample solution contained 0.1% I and was complexed with cadmium, 40-μl aliquots were added to the cell. For the sample solutions containing 0.01% I, 400-μl increments were added to the cell. The cell contents were purged for 1 min after each sample addition. Chelation resulted in an ~20% decrease in the observed current for the polarographic peak (Fig. 1). The sample additions were continued until there was no observed metal peak (Fig. 1).

The cadmium–I complex was not readily observed in the potential scan range of 0 to −1.5 v using the differential pulse mode. A broad, low-amplitude wave was observed at ~−1.1 v, which increased in a nonlinear fashion with the addition of I to the cell. Cadmium metal was reduced at −0.67 v.

The zinc–I complex did not exhibit a reduction wave in the potential scan range of 0 to −1.5 v, while zinc metal was reduced at −1.25 v. These potentials were peak maxima *versus* a silver–silver chloride reference electrode. The data of Meites (7) on cadmium and zinc in ammonia–ammonium chloride and I supporting electrolytes agree with the experimental observations.

Calculations—The following formula was used to calculate the adjusted net current after each sample addition:

$$I_A = I_O\left(\frac{V_2}{V_1}\right) \qquad \text{(Eq. 1)}$$

where I_A is the adjusted net current in microamps, I_O is the observed current in microamps, V_1 is the original cell volume in milliliters, and V_2 is the total cell volume in milliliters after each sample addition.

The I_A value is the observed current adjusted to the original cell volume, assuming a linear decrease in response due to dilution.

The sample volume added (microliters) was plotted on the x axis *versus* the adjusted net current on the y axis (Table I).

The x intercept represents the volume of the sample in microliters equivalent to the metal originally present in the cell. The following formula was used to calculate I in the sample:

$$\text{percent sample (w/v)} = V_{std}C_{std}\left(\frac{D}{A}\right)\left(\frac{0.1}{V_{sam}}\right) \qquad \text{(Eq. 2)}$$

where percent sample (w/v) is the percent edetate disodium in the sample calculated on a weight to volume basis, V_{std} is the volume of the metal standard solution in microliters, C_{std} is the concentration of the metal standard solution in micrograms per microliter, D is the molecular weight of edetate disodium, A is the atomic weight of the standard metal (cadmium or zinc), V_{sam} is the end-point volume of the sample solution in microliters, and 0.1 is a factor converting microliters to milliliters, micrograms to grams, and grams per milliliter to percent. The molecular weight of edetate disodium is 372.24, and the atomic weights of cadmium and zinc are 112.40 and 65.37, respectively.

DISCUSSION

The graph of the polarographic peak heights, corrected for dilution, is linear with respect to the sample volume (titrant) added. The most convenient method for calculating sample concentration is to perform a linear regression for the best line fit of the data points (adjusted net current, microamperes *versus* microliters of sample added). A coefficient of determination calculation then is performed to see how well the data fit the linear regression (Table I). Edetate disodium forms 1:1 complexes with many metal ions, but the degree of complex formation is strongly influenced by pH. Since the cadmium and zinc chelates are stable in basic

Table II—Assay Results for Edetate Disodium in Various Solutions Using Cadmium

Type of Product	Amount Declared, %	Amount Found, %	Percent of Declared	Range, %
Standard edetate disodium in water	0.0126	0.0130[a]	103.2	1.6
	0.1040	0.1025[b]	98.6	3.7
	0.5044	0.5033[c]	99.8	4.0
Eye drops[d]	0.1	0.1013[a]	101.3	5.2
Eye drops[e]	0.1	0.1000[a]	100.0	2.5
Eye drops[f]	0.1	0.0994[a]	99.4	3.3
Eye drops[g]	0.1000	0.1028[a]	102.8	3.1
Eyewash[h]	0.10	0.1102[a]	110.2	3.0
Eyewash[g]	0.1000	0.1032[a]	103.2	1.0
Contact lens[i] cleaning solution	0.2	0.1864[a]	93.2[j]	1.1
Contact lens[k] soaking solution	0.01	0.0097[a]	97.0[l]	0.0

[a] Average of two determinations. [b] Average of four determinations. [c] Average of three determinations. [d] Clear Eyes, Abbott Laboratories. [e] Visine, Pfizer. [f] Murine Plus, Abbott Laboratories. [g] Simulated preparation, Minneapolis District, Food and Drug Administration. [h] Lavoptik, Lavoptik Co. [i] Softmate, Barnes-Hind Pharmaceuticals. [j] Ten-milliliter sample extracted with 10 ml of water-washed chloroform and then centrifuged for 20 min at 1500 rpm. [k] Contique, Alcon Laboratories. [l] Five-milliliter sample diluted with 15 ml of water, extracted, centrifuged for 20 min at 1500 rpm, and then allowed to stand until adequately cleared.

or weakly acidic solutions (8), the pH 9.95 buffer used in this assay satisfies a major condition for quantitation in that the uncomplexed metal-ion concentration decreases linearly throughout the titration.

Sample eyewash and eye drop solutions have a pH of ~6–7, so their effect is minimal upon addition to the buffer in the polarographic cell. Some eye drop preparations contain methylcellulose, a viscosity-increasing agent. Since these solutions tend to wet the surface of the plastic micropipet tips, care must be taken to ensure complete removal of the entire sample aliquot from the micropipet tip.

Contact lens cleaning and wetting solutions comprise a large class of samples. These solutions commonly contain I but also may contain polymeric compounds such as methylcellulose and other closely related cellulose ethers, polyvinyl alcohol, and tyloxapol in relatively significant concentrations. From a polarographic analysis viewpoint, these polymers exhibit a strong maximum suppressor effect, resulting in an unusable polarogram.

Table III—Assay Results of Edetate Disodium in Various Solutions Using Zinc

Type of Product	Amount Declared, %	Amount Found, %	Percent of Declared	Range, %
Standard edetate disodium in water	0.0126	0.0134[a]	106.3	0.8
	0.1040	0.1067[b]	102.6	0.7
	0.5044	0.5182[a]	102.7	1.0
Eye drops[c]	0.1	0.1000[a]	100.0	0.6
Eye drops[d]	0.1	0.1039[a]	103.9	2.8
Eye drops[e]	0.1	0.0999[a]	99.9	3.4
Eye drops[f]	0.1000	0.1024[a]	102.4	2.6
Eyewash[g]	0.10	0.1092[a]	109.2	4.5
Eyewash[f]	0.1000	0.1034[a]	103.4	1.3
Contact lens[h] cleaning solution	0.2	0.1808[a]	90.4[i]	4.4
Contact lens[j] soaking solution	0.01	0.0105[a]	105.0[k]	4.0

[a] Average of two determinations. [b] Average of three determinations. [c] Clear Eyes, Abbott Laboratories. [d] Visine, Pfizer. [e] Murine Plus, Abbott Laboratories. [f] Simulated preparation, Minneapolis District, Food and Drug Administration. [g] Lavoptik, Lavoptik Co. [h] Softmate, Barnes-Hind Pharmaceuticals. [i] Ten-milliliter sample extracted with 10 ml of water-washed chloroform and then centrifuged for 20 min at 1500 rpm. [j] Contique, Alcon Laboratories. [k] Five-milliliter sample diluted with 15 ml of water, extracted, centrifuged for 20 min at 1500 rpm, and then allowed to stand until adequately cleared.

The cellulose ether compounds may be precipitated from solution by an extraction procedure using water-washed chloroform. Any remaining suspended material may be removed completely by centrifugation and standing. A preliminary 10-fold sample dilution with water also may reduce cellulose interference, providing the original concentration of I is ≥0.1%. In many instances, this treatment is sufficient for successful sample analysis if polyvinyl alcohol is absent. Polyvinyl alcohol presents several problems: the maximum suppressor effect cannot be diluted out due to the low levels of I present in the samples, extraction of the polymer is compromised due to the freely soluble nature of the alcohol in aqueous solutions, and the alcohol causes undesirable frothing when the polarographic cell contents are purged with nitrogen.

If lens cleaning solutions contain no polyvinyl alcohol, this method can determine the edetate disodium concentration in these samples.

The results in Tables II and III demonstrate the validity of analyzing commercial eyewash, eye drop, and some contact lens cleaning solutions for I. The assay values (Tables II and III) for edetate disodium were generally higher when standard zinc solution was used; however, cadmium exhibited a more reproducible baseline upon addition of certain sample matrixes such as lens cleaning solutions. Based on examination of the tabular data and the experience gained using both cadmium and zinc, cadmium is recommended as the preferred standard.

An important aspect of this assay is the need for accurate dispensing of edetate disodium sample and standard solutions using adjustable micropipets. Since these volumes are <1 ml, the micropipets should be calibrated.

This procedure can be readily used to analyze I in powder and injectable forms as presented in the USP and "Food Chemicals Codex."

Edetate disodium also is widely used in the food industry as a preservative, sequestrant, and stabilizer. It is feasible that I can be extracted from food into a water phase and subjected to further cleanup and that the concentration then can be determined as specified in this method.

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