ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 1249–1256

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

# Polarographic determination of EDTA in certain pharmaceutical dosage forms

F. Belal *, F.A. Aly, M.I. Walash, A.O. Mesbah

*Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt*

Received 28 August 1997; received in revised form 26 October 1997

**Abstract**

A highly sensitive polarographic method was developed for the determination of EDTA added as a preservative in certain pharmaceutical preparations. The method involved chelation with Eu(III) followed by polarographic measurement of the chelate formed. A well-defined cathodic wave was developed in Britton–Robinson buffers over the pH range 2–12. The wave was characterized as being quasi-reversible and diffusion controlled. The current–concentration relationship was found to be rectilinear over the ranges 8–160 and 2–120 μg $ml^{-1}$, using DCt and DPP modes, respectively, with limit of detection of 0.1 μg $ml^{-1}$ using the DDP technique. The mechanism of the electrode reaction was verified. The proposed method was applied for the determination of EDTA in certain pharmaceutical dosage forms, and the results obtained were in agreement with those obtained by a reference method. 

*Keywords:* EDTA; Polarography; Dosage forms; Pharmaceutical analysis; Europium chloride

## 1. Introduction

Ethylenediaminetetraacetic acid (EDTA) is widely used in pharmaceutical industry as an antioxidant, chelating agent for heavy metals, and preservative in many pharmaceutical formulations. EDTA is added to eye wash and ophthalmic solutions having bactericidal properties [1]. The British Pharmacopoeia [2] and United States Pharmacopoeia XXII [3] recommended classical titrimetric procedures for the quantitative determination of EDTA. Various methods have been reported for the determination of EDTA in different pharmaceutical preparations, including colorimetry [4,5], atomic absorption [6,7], HPLC [8–11], GC [12], capillary electrophoresis [13], polarography [14,15]. All the above-mentioned methods involve complicated procedures and require highly sophisticated instrumentation. A good guide to the work published for EDTA is found in the excellent review written by Sillanppa and Sihvonen [16].

This work describes a sensitive and rapid polarographic method for the determination of EDTA in dosage forms. The method is based on the fact that EDTA is polarographically inactive, but

* Corresponding author. Present address: Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh-11451, Saudi Arabia.

0731-7085/98/$ - see front matter 
*PII* S0731-7085(98)00039-9

upon chelation with Eu(III) in Britton–Robinson buffer (BRb) of pH 4.0, it is converted into a polarographically active chelate.

## 2. Experimental

### 2.1. Materials

Sodium EDTA, dihydrate was purchased from Aldrich. Preparations of pharmaceuticals containing EDTA as a preservative were obtained from commercial sources in the Egyptian market.

### 2.2. Reagents

Britton–Robinson buffers (BRb; 0.08 M) [17], covering the pH range 2–12, were used.

A stock solution ($5 \times 10^{-3}$ M) of EDTA in water was prepared, and further diluted with water to the appropriate concentration for the working solutions.

$EuCl_3$ (Aldrich), aqueous solution ($5 \times 10^{-3}$ M) was prepared.

### 2.3. Apparatus

The polarographic study and DPP measurements were made with a Polarecord E 506 Metrohm (Herisau, Switzerland). The drop time of 1 s was electronically controlled using a 663 VA stand manufactured by the same company. The polarograms were recorded using a potential scan rate of 10 mV $s^{-1}$. A three-electrode system composed of a dropping mercury electrode (DME), an Ag°/AgCl reference electrode and a graphite rod as an auxiliary electrode, was used. Phase-selective alternating current (ACt) polarograms were recorded using the same instrument. The superimposed alternating voltage has an amplitude of 15 mV at a frequency of 75 Hz, and the current was measured at a phase angle of 90°. The effect of mercury height was studied using a Sergent Welch Polarograph.

The cyclic voltammetry (CV) apparatus consists of a BAS CV-27 Voltammograph. A BAS X–Y recorder model PXYT, and a three-electrode system composed of a graphite working electrode, an Ag°/AgCl reference electrode and a platinum wire auxiliary electrode, was used. The voltammograms were recorded at scan rates of 100 and 200 mV $s^{-1}$.

### 2.4. Procedure

Aliquots containing suitable concentrations of EDTA over the working range were transferred into separate 25-ml standard flasks. To each flask, 2 ml of $5 \times 10^{-3}$ M of $EuCl_3$ was added. The reaction mixture was diluted to the mark with BRb solution, pH 4.0. The entire contents of the flask were transferred into the polarographic cell. Nitrogen gas was purged through the solution for

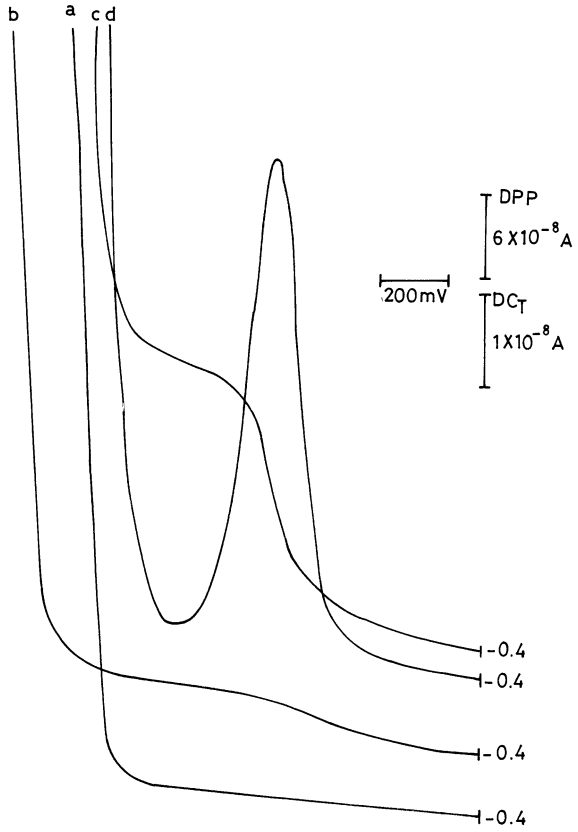


Fig. 1. Typical polarograms of Eu–EDTA chelate (corresponding to $3.96 \times 10^{-4}$ M of EDTA) in BRb, pH 4.0. (a) Polarogram of Eu(III) in BRb, pH 4.0; (b) polarogram of Eu(III) in BRb, pH 6.0; (c) DCt was of Eu–EDTA chelate at pH 4.0; (d) DPP wave of Eu–EDTA chelate at pH 4.0.

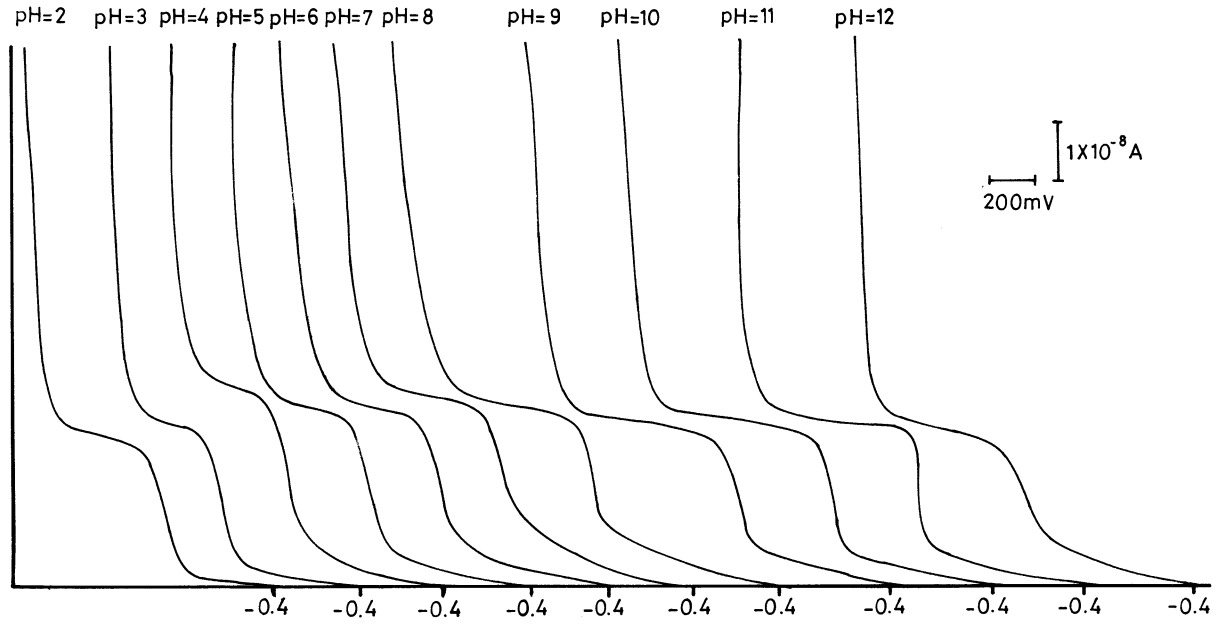


Fig. 2. Effect of pH on the development of the polarographic waves of Eu–EDTA chelate in BRb (corresponding to $3.96 \times 10^{-4}$ M of EDTA).

5 min. Polarograms were recorded in the potential range $-0.4$ to $-1.5$ vs. Ag°/AgCl electrode.

### 2.5. Procedure for pharmaceutical dosage forms

#### 2.5.1. Ophthalmic preparations

Mix the contents of five eye drops bottles. Transfer aliquots of the mixed solutions equivalent to 25 mg of EDTA into 100-ml volumetric flask. Mix with an equal volume of 1% ammonium nitrate solution. Leave for 5 min. and filter. Transfer 2 ml of the filtrate into a 25-ml standard flask and proceed as described above. The edetate content of Visin eye drops (tetrahydrozoline hydrochloride, 0.05%) and phenylephrine (phenylephrine, 10%) eye drops were calculated either from previously plotted calibration graphs or using the regression equations. Alternatively, the concentrations were determined from a comparison with the wave-height obtained from a standard solution prepared simultaneously.

#### 2.5.2. Procedure for ampoules

Cevarol ampoules (containing 20% ascorbic acid solution) were used.

Mix the contents of 10 ampoules. Transfer 2.5 ml of the mixed solutions into a 50-ml volumetric flask and complete to the mark with water. Heat the solution in a boiling water-bath for 1 h then cool. Transfer 2 ml of the solution into a 25-ml standard flask and proceed as described above. The edetate content of cevarol ampoules was calculated from previously plotted calibration graphs or using the regression equations.

## 3. Results and discussion

Europium and all lanthanides are characterised by the small size of their nucleui. Its ions in solution are highly solvated by a large number of solvent molecules [18]. This renders their reduction at the dropping mercury electrode (DME) a difficult and slow process. EDTA forms a stable complex with $Eu^{3+}$, with a stability constant of 17.35 [19]. Upon complexation with EDTA, the positive charge that europium carries is distributed over the bulky complex and this, presumably, facilitates its reduction resulting in well-defined waves. At the same time the excess free Eu(III) does not interfere in the assay.

The Eu–EDTA complex was reported to be reduced over the pH range 6–8, giving a cathodic wave with $E_{1/2}$ of $-1.22$ V [20]. In the present study, it was found that, at this pH value, the free europium(III) ions had a reduction wave but it was, however, ill-defined as shown in Fig. 1. At pH 4, however, a well-defined cathodic wave of the Eu–EDTA complex was produced. At that pH value, no reduction wave of the free Eu(III) appeared, as shown in Fig. 1. Therefore, BRb of pH 4.0 was utilized during the course of analysis.

Eu–EDTA chelate shows a well-defined cathodic wave over the entire pH range in BRb, as shown in Fig. 2. The $E_{1/2}$ of the reduction wave is a linear function of pH as shown in (Fig. 3), with two breaks at pH 6.2 and 10.6 corresponding to the p$K$ values of the amino groups in EDTA. These values are in accordance with the reported p$K$ values of EDTA [21], the latter, however, is slightly shifted. The relation between $E_{1/2}$ and pH is given by the following equations:

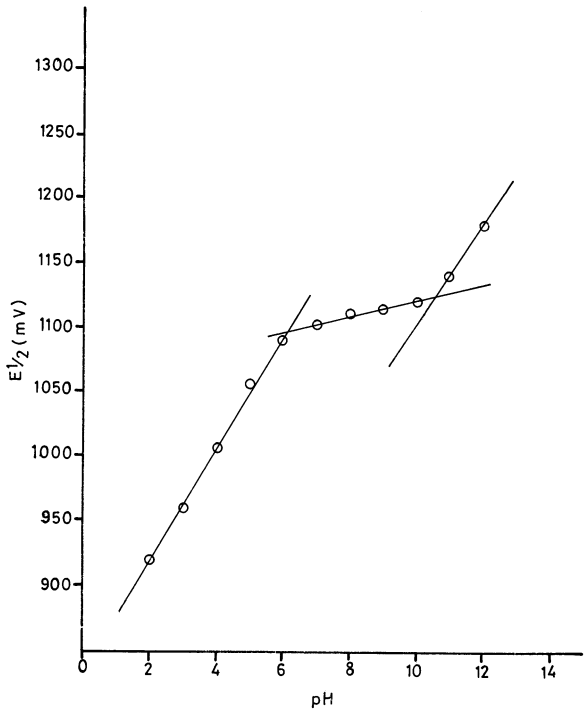


Fig. 3. Effect of pH on the $E_{1/2}$ of Eu–EDTA chelate (corresponding to $3.96 \times 10^{-4}$ M of EDTA).

for the pH range 2–6:

$$E_{1/2}\,(\mathrm{V}) = -0.832 - 0.044\,\mathrm{pH} \quad (R = 0.9969)$$

and for the pH range 7–12:

$$E_{1/2}\,(\mathrm{V}) = -1.056 - 0.065\,\mathrm{pH} \quad (R = 0.9827)$$

Logarithmic analysis of the reduction waves of the chelate resulted in straight lines. As the rate-determining step involves the transfer of one electron, the values of slope point out to the quasi-reversible character of the reduction process. Applying the treatment of Meites and Israel [22], the $\alpha n_a$ values were computed and are presented in Table 1.

The diffusion-current constant $I_d$ was calculated at 25°C and was found to be $0.87 \pm 0.02$.

### 3.1. Number of electrons involved in the electrode reaction

The number of electrons consumed during the reduction was determined by comparing the wave-height of the chelate with that obtained from an equimolar solution of cadmium(II) ions. The wave-height was found to be half that of the cadmium(II) ions, hence, it is concluded that only one electron is transferred during the reduction process.

$$[\mathrm{EuY}]^{1-} + \mathrm{e} \rightarrow [\mathrm{EuY}]^{2-}$$

The cyclic voltammetric CV measurements of Eu–EDTA chelates were performed at pH values of 4.0, 7.5 and 9.0 (Fig. 4). A cathodic peak was obtained using scan rates of 100 and 200 mV $s^{-1}$. The peak potentials display cathodic shift on increasing the scan rate by 60, 90 and 100 mV at pH 4.0, 7.5 and 9.0, respectively, thus revealing the quasi-reversible nature of the reduction process [23].

### 3.2. Study of the wave characteristics

The limiting current of the produced chelate was found to be a linear function of the square root of the height of mercury head ($h$). Plotting log $h$ vs. log wave-height ($W$) gave a straight line, the slope of which was about 0.5. Also, the wave-height and $E_{1/2}$ potentials were independent of the

Table 1
Effect of pH on the development of polarographic waves of Eu–EDTA chelate

| pH | ΔpH | $E_{1/2}$ (mV) | $\Delta E$ | $\Delta E_{1/2}/\Delta$pH | Half/peak width (mV) | $\alpha n_a$ |
|---|---|---|---|---|---|---|
| 2 | | −920 | | | 160 | 0.7 |
| | 1 | | −40 | −40 | | |
| 3 | | −960 | | | 160 | 0.62 |
| | 1 | | −50 | −50 | | |
| 4 | | −1010 | | | 160 | 0.81 |
| | 1 | | −50 | −50 | | |
| 5 | | −1060 | | | 165 | 0.7 |
| | 1 | | −30 | −30 | | |
| 6 | | −1090 | | | 170 | 0.7 |
| | 1 | | −10 | −10 | | |
| 7 | | −1100 | | | 170 | 0.7 |
| | 1 | | −10 | −10 | | |
| 8 | | −1110 | | | 175 | 0.8 |
| | 1 | | −5 | −5 | | |
| 9 | | −1115 | | | 180 | 0.65 |
| | 1 | | −5 | −5 | | |
| 10 | | −1120 | | | 190 | 0.7 |
| | 1 | | −20 | −20 | | |
| 11 | | −1140 | | | 190 | 0.8 |
| | 1 | | −40 | −40 | | |
| 12 | | −1180 | | | 200 | 0.7 |

buffer concentration over the range 0.015–0.07 M. These characteristics reveal a diffusion-controlled reduction process.

The alternating current behaviour of solution of the europium(III)–EDTA chelate was studied, using a phase-selective angle of 90°. In BRb of pH 4.0, 7.0 and 10.0 (Fig. 5) the summit potentials ($E_s$) were about 10, 90 and 70 mV more positive than the corresponding $E_{1/2}$ values, indicating that the wave is diffusion controlled and adsorption phenomenon has no role in the reduction process at pH 7 and 10, while at pH 4 the current drop indicates adsorption of the depolarizer on the dropping mercury surface.

The relation between the limiting current $i_d$ (μA), and the concentration (μg ml$^{-1}$) was found to be rectilinear over the concentration ranges of 8–160 and 2–120 μg ml$^{-1}$ in the DCt and DPP modes, respectively. Linear regression analysis of the above data gave the following equations.

$$C = 0.5 + 290\, i_d \quad (R = 0.999)$$

Table 2
Application of the proposed method and official method to the determination of EDTA in pure form

| No. | Proposed method, recovery (%) | | Official method [2] |
|---|---|---|---|
| | DCt | DPP | |
| 1 | 101.00 | 99.00 | |
| 2 | 101.37 | 101.50 | |
| 3 | 99.08 | 100.87 | |
| 4 | 99.78 | 99.68 | |
| 5 | 101.64 | 100.54 | |
| 6 | 98.81 | 99.15 | |
| 7 | 99.80 | 100.47 | |
| 8 | 100.30 | 99.55 | |
| | | 100.17 | |
| $\bar{x} \pm$ S.D. | 100.22 ± 1.0427 | 100.10 ± 0.827 | 99.72 ± 0.71 |
| $t$ | 2.037 (2.228) | 1.66 (2.201) | |
| $F$ | 2.11 (4.35) | 1.33 (4.07) | |

Each result is the average of three separate determinations. The figures in parentheses are the tabulated values of $t$ and $F$.

using the DCt mode; and

$C = 0.2 + 281\ i_d \quad (R = 0.999)$

using the DPP mode.

Where $C$ is the concentration in μg ml$^{-1}$, and $i_d$ is the current in μA.

### 3.3. Analytical applications

Polarograms of the Eu–EDTA chelate in BRb of pH 4.0 exhibit very well-defined waves that are proportional to the concentration over a convenient range. Both DCt and DPP results were statistically compared with those given with the official method (Table 2) and were found to be in good agreement [24]. The proposed method was successfully applied to the determination of EDTA in pharmaceutical preparations, and the results obtained are listed in Table 3. The results were compared with those given with a reference method [14] and were found to be in good agreement.

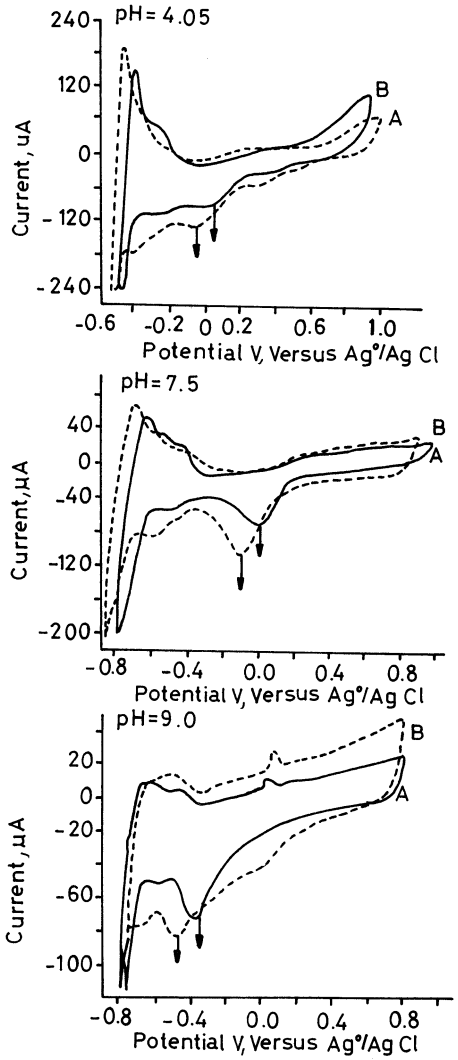


Fig. 4. Cyclic voltammograms of Eu–EDTA chelate in BRb of different pH values at scan rates of (A) 100 and (B) 200 mV s$^{-1}$.

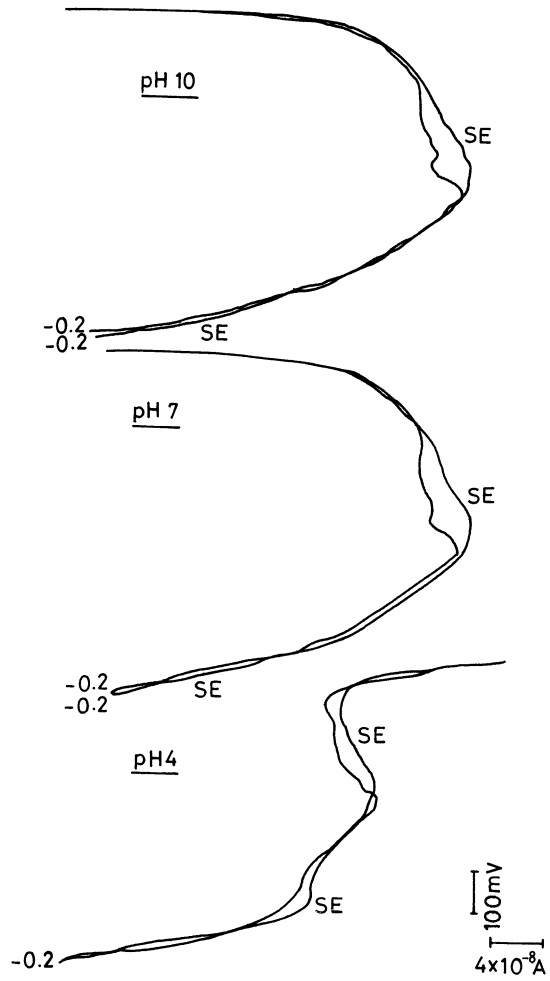


Fig. 5. Alternating current behaviour of Eu–EDTA chelate in BRb of different pH values. Superimposed alternating voltage, 15 mV; phase angle, 90° (SE, supporting electrolyte).

Table 3
Application of the proposed method to the determination of EDTA in pharmaceutical preparations

| Preparation | % Recovery | | |
|---|---|---|---|
| | DPP | DCt | Reference method [14] |
| (1) Visin eye[a] drops (EDTA, 0.1%) | 99.77 | 99.04 | |
| | 99.62 | 99.52 | |
| | 100.01 | 99.52 | |
| | 99.04 | 100.01 | |
| $\bar{x} \pm$ S.D. | $99.61 \pm 0.41$ | $99.52 \pm 0.39$ | $99.38 \pm 0.82$ |
| (2) Visin AC[b] drops (EDTA, 0.1%) | 99.04 | 99.14 | |
| | 99.72 | 99.77 | |
| | 99.52 | 99.62 | |
| | 100.01 | 99.68 | |
| $\bar{x} \pm$ S.D. | $99.57 \pm 0.40$ | $99.55 \pm 0.28$ | $99.40 \pm 0.85$ |
| (3) Phenylephrine[a] drops (EDTA, 0.1%) | 99.18 | 98.81 | |
| | 99.29 | 99.05 | |
| | 99.45 | 99.37 | |
| | 99.89 | 99.59 | |
| $\bar{x} \pm$ S.D. | $99.45 \pm 0.31$ | $99.20 \pm 0.34$ | $99.30 \pm 0.68$ |
| (4) Cevarol ampols[d] (EDTA, 0.1%) | 99.05 | 99.15 | |
| | 99.52 | 99.22 | |
| | 99.02 | 99.52 | |
| | 98.76 | 99.01 | |
| $\bar{x} \pm$ S.D. | $98.83 \pm 0.24$ | $98.97 \pm 0.31$ | $98.45 \pm 0.65$ |

Each result is the average of three separate determinations.
[a] Product of Pfizer, Cairo Egypt, S.A.E.
[b] Product of Pfizer, Cairo Egypt, S.A.E.
[c] Product of Misr Co., for Pharmaceutical Industry, Cairo, Egypt.
[d] Product of Memphis Chemical Co., Cairo, Egypt.

Comparing the proposed method with the other published polarographic methods [14,15], it is evident that the proposed method is more simple. The method involving the use of cadmium(II) or zinc(II) [14] is an indirect one, since the decrease in wave-height of the metal upon complexation with EDTA is measured. Consequently correction for the dilution effect must be done, and complicated calculations are necessary for every determination. In the method involving the use of bismuth(III) [15], EDTA had to be separated from the preparation by column chromatography before analysis.

Benzalkonium chloride (BAK), which is frequently included in ophthalmic preparations, must be eliminated before analysis because of its suppressive effect on the wave. BAK is reported to be precipitated from solutions using 2 M $HNO_3$ [3]. In this study, ammonium nitrate was attempted as an alternative precipitating agent instead of $HNO_3$ in order to keep the value of pH constant, and this gave satisfactory results.

On the other hand, ophthalmic preparations that contain polymeric compounds, especially, polyvinyl alcohol, could not be analysed for EDTA content by the proposed method. The polymer produced undesirable frothing when the polarographic cell contents were purged with nitrogen, and exhibited strong maximum suppressor effect. This maximum suppressor could not be diluted out due to the low level of EDTA present in the samples. Also trials to extract polyvinyl alcohol did not succeed due to the high solubility of the alcohol in aqueous solutions.

The proposed method is characterised by simplicity, satisfactory accuracy and sensitivity. It can be readily adopted for the determination of EDTA in quality control laboratories and for in-process control analysis.

## Acknowledgement

F. Belal would like to thank the Alexander von Hemboldt Foundation, Bonn, Germany for donating to him the instrument used in this work.

## References

[1] E.F. Reynold (Ed.), Martindale, The Extra Pharmacopoeia, 30th ed., The Pharmaceutical Press, London, 1993.
[2] The British Pharmacopoeia, vols. I and II, Her Majesty's, Stationary Office, London, 1990, p. 205.
[3] The United States Pharmacopoeia XXIII and National Formulary XVIII, The US Pharmaceutical Convention, Rockville, MD, 1995.
[4] E.E. Kaminski, D. Pacenti, J. Pharm. Sci. 63 (1974) 1133–1136.
[5] T. Hamano, Y. Mitsuhashi, N. Kojima, N. Aoki, M. Shibata, Y. Oji, Analyst 118 (1993) 909–912.
[6] R.J. Hurtubise, J. Pharm. Sci. 63 (1974) 1131–1133.
[7] E. Farina, P.M. Bevinino, Boll. Chim. Igien 41 (1990) 493.
[8] J. Bauer, D. Heathcote, S. Krogh, J. Chromatogr. 369 (1986) 422–425.
[9] A.S. Kord, I. Tumanova, W.L. Matier, J. Pharm. Biomed. Anal. 13 (1995) 575–580.
[10] E.L. Inman, R.L. Clemens, B.A. Olsen, J. Pharm. Biomed. Anal. 8 (1990) 513–520.
[11] G. Tran, C. Chen, R.B. Miller, J. Liq. Chromatog. Rel. Technol. 19 (1996) 1499.
[12] C. Retho, L. Diep, Z. Lebensm. Unters. Forsch. 188 (1989) 223.
[13] N.E. Ballou, G.R. Ducatte, C. Quang, V.T. Remcho, J. High Resolut. Chromatogr. 19 (1996) 183.
[14] C. Marvin, L.E. Habeger, J. Pharm. Sci. 69 (1980) 826–828.
[15] C. Schramm, S. Maier, O. Bobleter, P. Blankenhorn, GIT Fachz. Lab. 38 (1994) 184–186; Thr. Anal. Abstr. 56 (1994) 11E–106.
[16] M. Sillanppa, M.L. Sihvonen, Talanta 44 (1997) 1487.
[17] J. Heyrovsky, P. Zuman, Practical Polarography, Academic Press, London, 1968, pp. 179–180.
[18] D.F. Shriver, P.W. Atkins, G.H. Langford, in: Inorganic Chemistry, Textbook, Oxford University Press, Oxford, 1990, pp. 27–28.
[19] J. Kragten, in: Atlas of Metal-Ligand Equilibria in Aqueous Solutions, Ellis Horwood, Chichester, 1978, p. 278.
[20] J. Heyrovsky, J. Kuta, in: Principles of Polarography, Czechoslovak Academy of Science, Prague, 1965.
[21] G.H. Jeffery, J. Bassett, J. Mendham, R.C. Denney (Eds.), Vogel's Textbook of Quantitative Inorganic Analysis, 5th ed., Longman, Bath, 1989, p. 55.
[22] L. Meites, Y. Israel, J. Am. Chem. Soc. 83 (1961) 4903–4906.
[23] P. Delhay, in: New Instrumental Methods in Electrochemistry, Interscience, New York, 1953.
[24] R. Caulcutt, R. Boddy, Statistics for Analytical Chemisits, Chapman and Hall, London, 1983.