

Polarographic and Spectrophotometric Determination of Citrate in Commercial Orange and Lemon Drinks

A. Sánchez Pérez, J. Hernández Mendez & J. A. Gómez Báez

Departamento de Química Analítica, Nutrición y Bromatología,
Universidad de Salamanca, Salamanca, Spain

(Received 13 January 1988; revised version received and
accepted 19 May 1988)

ABSTRACT

A polarographic method for the direct determination of citrate is developed and applied to the analysis of citric acid and citrate contents in orange and lemon soft drinks. The method is based on the anodic oxidation of a dropping copper amalgam electrode (DCAE) in the presence of citrate in perchlorate medium, pH 6.8–8.3, by measuring the first derivative polarographic peak obtained at -0.15 V (vs SCE). The method is compared with the spectrophotometric one based on the formation of coloured species when citric acid and citrate are treated with metol and potassium dichromate.

INTRODUCTION

The determination of citric acid and citrate in commercial fruit juices is a common analytical problem. Spanish Food Legislation (BOE, 1981) regulates the maximum amount of citric acid permissible in soft drinks; French legislation (Lecoq, 1965) also fixes the amounts of this acid in commercial fruit drinks as a measure of their quality. Titration with sodium hydroxide is the method most commonly employed to determine the acidity of canned fruits mainly due to citric acid when only this acid is added to commercial fruit juices (Noronha *et al.*, 1982). Different authors have determined citric acid in fruit juice and in orange and lemon drinks (Pearson, 1976; Royo *et al.*, 1976; Park *et al.*, 1983). Recently, the content of citric acid and citrates in orange and grapefruit juices has been evaluated by HPLC and

isotachophoresis techniques (Tschoepe & Ritz, 1980; Shaw *et al.*, 1983). Enzymic procedures for the analysis of citric acid and other substances in fruit juices have been reviewed (Henninger, 1984).

Several polarographic (Elving & Van Atta, 1954; Mohay *et al.*, 1970; Tur'yan & Saksin, 1970; Prasad, 1977; Banica & Carlea, 1979) and many spectrophotometric methods (Ettinger *et al.*, 1952; Nekshorocheff & Wajzer, 1953; Pro & Nelson, 1956; Hartford, 1962; Matulis & Guyon, 1964; Lee *et al.*, 1968; Ma & Nazimowitz, 1969; Roman *et al.*, 1981) have been suggested for the determination of citric acid; some of them are based on the conversion of citric acid into pentabromoacetone, which, as is well known, is the principal step in other gravimetric and volumetric methods proposed for the determination of citric acid and the citrate anion (Williams, 1979).

In a previous work (Sánchez Pérez *et al.*, 1984), we proposed a direct polarographic method for the determination of citrate based on the oxidation of a dropping copper amalgam electrode (DCAE) in the presence of this anion (pH = 7.5) followed by measurement of the peak intensity of the corresponding first derivative polarographic curve; this method proved to be sufficiently precise and selective. In the present work this method is applied to the analysis of citric acid and citrate in commercial orange and lemon beverages. The results obtained are compared with those obtained with a spectrophotometric method (Rama *et al.*, 1980) based on the formation of coloured species, $\lambda_{\max} = 545$ nm, when citric acid is treated with metol and potassium dichromate at pH = 3.3, set up by us for the analysis of citric acid in these samples.

EXPERIMENTAL

Reagents

Solutions of cupric nitrate at concentrations close to 0.020M normalized gravimetrically by reduction to metallic copper. Solution (0.100M) of citric acid normalized by potentiometric titration with sodium hydroxide. Solution of potassium dichromate (0.010M) prepared by direct weighing. Other reagents employed were: sodium perchlorate, perchloric acid, sodium hydroxide, potassium biphthalate, potassium nitrate, metol (*p*-methylaminophenol sulfate), distilled mercury for polarography and nitrogen at a purity of 99.8%. All reagents used were of analytical grade.

Apparatus

—Amel model 564 polarograph with three electrodes: working (DCAE), reference (SCE) and platinum counterelectrode.

- Metrohm E-510 potentiometer with a combined glass and reference electrode for pH measurement.
- Metrohm E-211 coulometer as a constant intensity source for carrying out electrodeposition and forming the amalgam.
- Beckman DB-GT spectrophotometer.
- Kerry pulsation model 250 ultrasonic cuvette; 50 kHz vibration frequency.

Electrodes

- Working electrode: A dropping copper amalgam electrode (DCAE) was employed; its preparation was described in an earlier work (Hernández Méndez *et al.*, 1975). In all cases, a Cu(Hg) concentration of 2×10^{-2} atom-g/litre was employed which is sufficiently high for the oxidation of copper in the presence of citrate not to be controlled by diffusion of this metal through the mercury.
- Reference electrode: Saturated calomel electrode (SCE) Radiometer K 401.
- Counterelectrode: Commercial platinum, electrode Ingold Pt-805,N.S.

Procedures

The following section describes the procedures proposed for each of the methods employed. The modifications made to them, such as previous dilution of samples, are detailed in the sections corresponding to each of the studies carried out.

Polarography

Five millilitres of 1M sodium perchlorate were added to an aliquot of 5–10 ml of commercial fruit juice previously centrifuged; pH was adjusted in the $7.0 < \text{pH} < 8.3$ range, and volume was brought up to 50.0 ml with distilled water. This solution was transferred to the polarographic cell, temperature was fixed at 25°C and N₂ bubbled through until all the dissolved O₂ had been removed. The derivative polarogram was recorded applying an anodic sweep to the DCAE in the -0.400 to $+0.400$ V range. The height of the derivative curve corresponding to the polarographic wave produced at -0.15 V was measured.

The polarographic calibration graph used had the following equation: $i_p (\mu\text{A}) = 0.0623 + 7.656 C$ (mM), where i_p is the intensity of the derivative curve. This equation can be used in the 0.2–10 mM range.

Spectrophotometry

An aliquot of 5 to 10 ml of commercial fruit juice previously centrifuged was diluted to 50.0 ml with distilled water. Fifteen millilitres of potassium biphthalate-HCl buffer (pH = 3.3), 2 ml of a freshly prepared aqueous solution of 0.2% metol and 3 ml of a solution of 0.010M $K_2Cr_2O_7$ were added successively to a 1.0 ml aliquot of the latter diluted sample solution. Volume was brought up to 25.0 ml with distilled water and absorbance was measured at 545 nm against the corresponding blank prepared in the same way.

The spectrophotometric calibration graph employed had the following equation: $A = -0.0046 + 2.012 C$ (mM), in the 0.04–0.3 mM range.

RESULTS AND DISCUSSION

Previous treatment of the fruit juices

Samples of commercial orange and lemon drinks were subjected to centrifugation until the solids in suspension were completely eliminated. Following this, and in order to avoid errors in the pipetting of the different aliquots, the samples were sonicated in a cuvette until all bubbles due to the carbonic acid in them had disappeared. Determination of citric acid and citrates was performed on the same day as the above treatments in order to avoid deterioration of the fruit juices.

Precision of the polarographic method

Ten solutions were prepared from 5.0 ml of commercial orange and lemon juice and the polarographic procedure described was applied. The citric acid and citrate content was determined by the corresponding calibration. Relative standard deviations of 2.5% and 2.0% for orange and lemon drinks were obtained, respectively.

Precision of the spectrophotometric method

In order to compare the validity of the results obtained with the polarographic and spectrophotometric methods, the precision of the comparison spectrophotometric method was determined.

Both types of fruit drink were previously diluted: (1 + 4) in the case of orange drinks and (1 + 9) in the case of the lemon drinks. The spectrophotometric method was applied to these solutions. The content in citric acid and citrate anion was determined by the corresponding calibration.

TABLE 1

Polarographic Determination of Citrate in Commercial Orange Drinks—Recovery of Citric Acid added to Orange Drink Samples

<i>Sample</i>	<i>Citric acid added (mg)</i>	<i>Citric acid found (mg)</i>	<i>Recovery (%)</i>
1	9.60	9.53	99.3
	19.21	19.07	99.3
2	9.60	10.06	104.8
	19.21	19.07	99.3
3	9.60	10.06	104.8
	19.21	18.61	96.9
Mean recovery			100.7

Relative standard deviations of 1.3% and 1.7% for the orange and lemon drinks were obtained.

Accuracy of the polarographic method

The accuracy of the polarographic method was determined from the recovery percentages obtained upon addition of precisely known amounts of citric acid to three samples of commercial orange drinks and three of lemon drinks. Polarograms corresponding to solutions obtained from 5.0 ml of commercial drinks and solutions also containing 0.5 and 1.0 ml of 0.1M citric acid were obtained following the procedure described and the amount of citric acid recovered was compared with the amount added (Tables 1 and 2). Recoveries ranging between 96.9% and 104.8% were obtained, with a mean of 100.7%.

TABLE 2

Polarographic Determination of Citrate in Commercial Lemon Drinks—Recovery of Citric Acid added to Samples of Lemon Drink

<i>Sample</i>	<i>Citric acid added (mg)</i>	<i>Citric acid found (mg)</i>	<i>Recovery (%)</i>
1	9.60	9.53	99.3
	19.21	19.07	99.3
2	9.60	9.53	99.3
	19.21	19.59	102.0
3	9.60	10.06	104.8
	19.21	19.07	99.3
Mean recovery			100.7

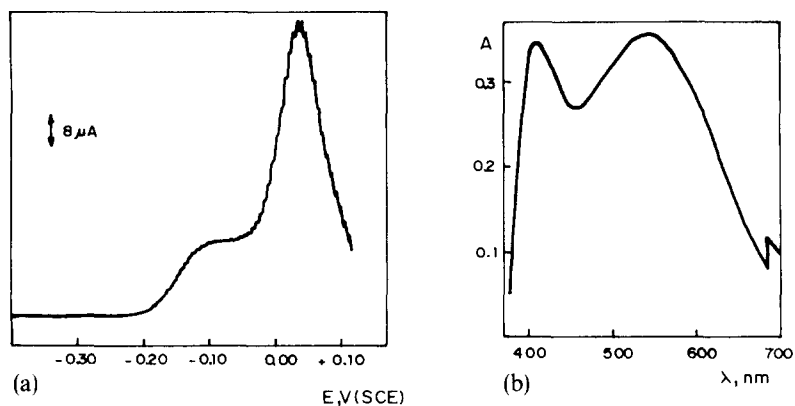


Fig. 1. Commercial orange drinks: (a) first derivative polarographic curve; (b) absorption spectrum of a sample.

Selectivity

The interference caused in the polarographic determination of citrate by other organic anions and amino acids found jointly with citric acid and citrates in natural and synthetic products was studied: lactic, ascorbic, benzoic, malic, tartaric, sorbic and acetic acids do not interfere at a ratio of 1:1. Likewise, the amino acids glycine, alanine, asparagine, aspartic acid and glutamic acid do not interfere in the same ratio.

The spectrophotometric method is specific and the results obtained with it are in good agreement with those obtained with the pentabromoacetone colorimetric method. Constituents such as malic acid, phosphoric acid, glucose, succinic acid, oxalic acid and acetic acid usually present in fruit products do not interfere up to 25 micromoles but tartaric acid interferes

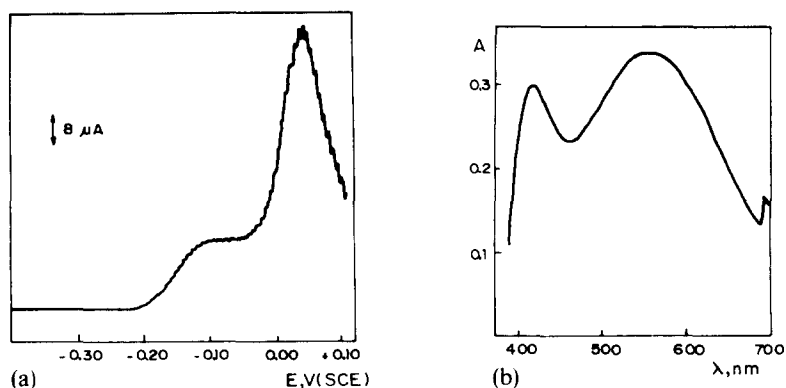


Fig. 2. Commercial lemon drinks: (a) first derivative polarographic curve; (b) absorption spectrum of a sample.

when present at concentrations of more than 10 micromoles (Rama *et al.*, 1980).

Determination of citric acid and citrate content

The polarographic and spectrophotometric methods described were applied to the analysis of citric acid and citrate contents in commercial orange and lemon fruit drinks. The solutions studied were prepared in the same way as the procedures indicated for each of the methods. The experiments were conducted in duplicate for each method, expressing the results in grams of citric acid/100 ml of fruit drink.

Figures 1 and 2 show the first derivative polarographic curves and the absorption spectra obtained for one orange drink and another of lemon of those assayed. The values of citric acid content determined by both methods are shown in Tables 3 and 4.

Statistical comparison of results

A statistical study was carried out from the results obtained in the previous section. To do so, the 'F' (Snedecor) and 't' (Student) criteria

TABLE 3
Determination of Citrate in Commercial Orange Drinks—Comparison of Methods

Sample	Polarography ^a		Spectrophotometry ^b		Difference (%)
	i_p (μ A)	Citric acid (g/100 ml)	A	Citric acid (g/100 ml)	
1	21.6	0.272	0.220	0.268	1.49
2	19.2	0.242	0.195	0.238	1.68
3	17.2	0.433	0.355	0.429	0.93
4	18.0	0.453	0.370	0.447	1.34
5	24.0	0.604	0.250	0.602	0.33
6	19.2	0.483	0.405	0.489	1.24
7	21.2	0.267	0.215	0.262	1.91
8	40.4	0.508	0.415	0.501	1.40
9	32.0	0.805	0.335	0.799	0.75
10	16.0	0.403	0.165	0.405	0.50
Mean values		0.447		0.444	1.16

^a Dilutions carried out in the polarographic method were the following: Samples 1, 2, 7 and 8 (1 + 4); samples 3, 4, 5, 6, 9 and 10 (1 + 9).

^b Dilutions carried out in the spectrophotometric method were as follows: Samples 1, 2, 3, 4, 6, 7, 8 and 10 (1 + 124); samples 5 and 9 (1 + 249).

TABLE 4
Determination of Citrate in Commercial Lemon Drinks—Comparison of Methods

Sample	Polarography ^a		Spectrophotometry ^b		Difference (%)
	i_p (μA)	Citric acid (g/100 ml)	A	Citric acid (g/100 ml)	
1	16.4	0.413	0.340	0.411	0.49
2	15.2	0.383	0.320	0.387	1.04
3	30.4	0.764	0.320	0.774	1.31
4	23.6	0.594	0.240	0.584	1.71
5	32.8	0.825	0.340	0.822	0.36
6	28.8	0.724	0.300	0.727	0.41
7	26.4	0.332	0.280	0.340	2.41
8	12.0	0.151	0.255	0.155	2.65
9	27.2	0.684	0.280	0.679	0.74
Mean values		0.487		0.488	1.24

^a Dilutions carried out in the polarographic method were as follows: Samples 1, 2, 3, 4, 5, 6 and 9 (1 + 9); samples 7 and 8 (1 + 4).

^b Dilutions carried out in the spectrophotometric method were as follows: Sample 8 (2 + 123); samples 1, 2 and 7 (1 + 124) and samples 3, 4, 6 and 9 (1 + 249).

TABLE 5
Statistical Comparison of Results

Statistical criterion	Type of fruit drink	
	Orange	Lemon
<i>F</i> -Test		
Tabulated values ^a F_{tab}	3.18	3.18
Experimental values F_{exp}	3.15	1.56
<i>t</i> -Test		
Determinations in single samples		
Tabulated values t_{tab}	2.101	2.101
Experimental values t_{exp}	1.344	0.442
Determinations in different samples		
Tabulated values t_{tab}	2.262	2.306
Experimental values t_{exp}	1.845	0.789

^a 95% confidence level.

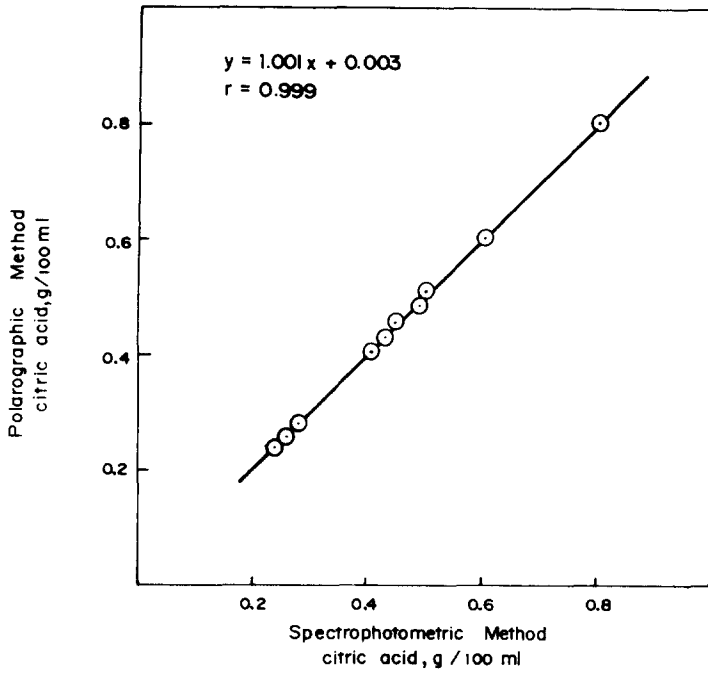


Fig. 3. Relationship between the citric acid contents in orange fruit drinks determined by polarography and by spectrophotometry.

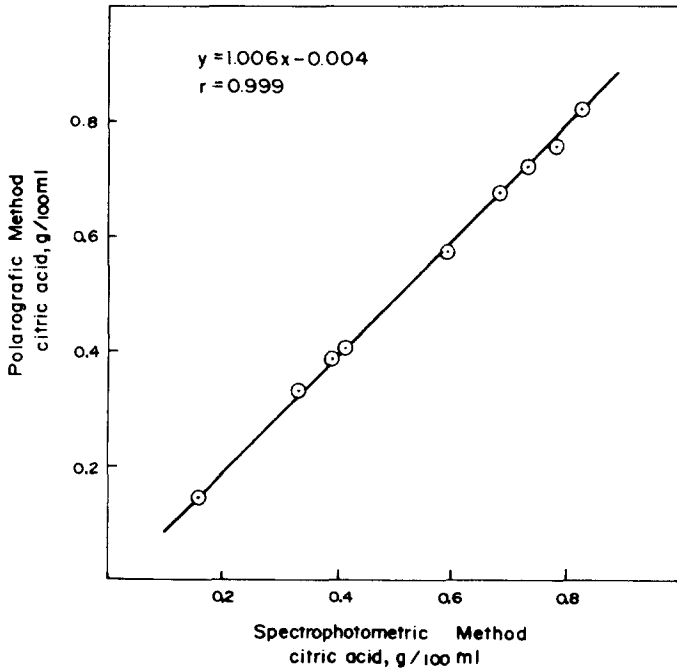


Fig. 4. Relationship between the citric acid contents in lemon fruit drinks determined by polarography and by spectrophotometry.

(Eckschlager, 1969) were used, for a confidence level of 95%; the results are summarized in Table 5. It may be concluded that there are no statistically significant differences between the two methods employed for the commercial orange and lemon drinks.

Figures 3 and 4 show the results obtained by the polarographic method, expressed in grams of citric acid/100 ml of fruit drinks, compared with those determined by the spectrophotometric method. The values of the slopes of the regression straight lines are close to unity and the ordinates at the origin close to zero, which shows that either of the two methods is valid for determining the citric acid and citrate contents in commercial orange and lemon drinks. Moreover, the regression coefficients are close to unity, implying a highly significant correlation between the two methods for both kinds of fruit drink.

REFERENCES

- Banica, F. G. & Carlea, L. (1979). Polarographic determination of citric acid using the kinetic wave of Ti(IV) ($C_6H_6O_7^{2-}$). *Rev. Chim.*, **30**, 802-4.
- Boletín Oficial del Estado (Spain) (BOE)*, (1981). 27th March and 4th June.
- Eckschlager, K. (1969). *Errors, Measurements and Results in Chemical Analysis*. ed. R. A. Chalmers, Van Nostrand, London, pp. 111-20.
- Elving, P. J. & Van Atta, R. E. (1954). Polarographic determination of citric acid. Polarography of pentabromoacetone. *Anal. Chem.*, **26**(1), 295-8.
- Ettinger, R. H., Goldbaum, L. R. & Smith, L. H. (1952). A simplified photometric method for the determination of citric acid in biological fluids. *J. Biol. Chem.*, **199**, 531-6.
- Hartford, C. G. (1962). Rapid spectrophotometric method for the determination of itaconic, citric, aconitic and fumaric acids. *Anal. Chem.*, **34**(1), 426-8.
- Henninger, G. (1984). Enzymic procedures in the analysis of fruit juices. *Fluss. Obst.*, **53**(1), 111-40.
- Hernández Méndez, J., Sánchez Pérez, A. & Lucena Conde, F. (1975). Polarographic determination of glycine with a dropping copper amalgam electrode. *J. Electroanal. Chem. Interfacial Electrochem.*, **66**, 53-65.
- Lee, K. S., Lee, D. W. & Hwang, J. Y. (1968). Spectrophotometric determination of some organic acids with the ferric 5-nitrosalicylate complex. *Anal. Chem.*, **40**(2), 2049-52.
- Lecoq, R. (1965). *Manuel d'analyses alimentaires et d'expertises usuelles*. Vol. 2. Doin, Paris, 1130-4.
- Ma, T. S. & Nazimowitz, W. L. (1969). Colorimetric methods for determining organic functional groups. I. Microdetermination of certain oxygen functions by means of ceric sulfate. *Mikrochim. Acta*, **2**, 345-51.
- Matulis, R. M. & Guyon, J. C. (1964). Spectrophotometric determination of alpha-hydroxy carboxylic acids. Application to citric acid. *Anal. Chem.*, **36**(1), 118-20.
- Mohay, J., Szepesvary, E. & Pungor, E. (1970). Determination of the composition of magnesium citricum. Polarographic investigation of citric acid. *Acta Pharm. Hung.*, **40**, 205-10.

- Nekshorocheff, J. & Wajzer, J. (1953). Colorimetric microdetermination of citric and *cis*-aconitic acids. *Bull. Soc. Chim. Biol.*, **35**, 695–6.
- Noronha da Silveira, M. I., Batista, M. T. & Baeta, M. L. (1982). Contribução para o estudo dos alimentos industrializados. I. Estudo bromatológico dos sumos de frutos enlatados. *Anal. Bromatol.*, **33**(1), 91–110.
- Noronha da Silveira, M. I., Batista, M. T. & Baeta, M. L. (1982). Contribução para o estudo dos alimentos industrializados. II. Vitamina C e conservantes. *Anal. Bromatol.*, **33**(1), 111–26.
- Park, G. L., Byers, J. L., Pritz, C. M., Nelson, D. B., Navarro, J. L., Smolensky, D. C. & Vandercook, C. E. (1983). Characteristics of California navel orange juice and pulp wash. *J. Food Sci.*, **48**, 627–32.
- Pearson, D. (1976). *The Chemical Analysis of Foods*. ed. C. Livingstone, Longman, London, p. 181.
- Prasad, R. (1977). Determination of citrate as its iron(III) complex by differential pulse polarography. *Analyst*, **102**, 601–6.
- Pro, M. J. & Nelson, R. A. (1956). Determination of distinctive added organic material in whisky. VI. Spectrophotometric determination of citric acid. *J. Assoc. Off. Anal. Chem.*, **39**, 952–7.
- Rama, R., Siraj, P. & Prakasa, C. S. (1980). Spectrophotometric determination of citric acid with metol and chromium(VI). *Fresenius' Z. Anal. Chem.*, **303**, 411.
- Roman Ceba, M., Muñoz Leyva, J. A. & Vinagre Jara, F. (1981). Determinación espectrofotométrica de aniones mediante reextracción por intercambio de ligandos. II. Determinación de trazas de citrato usando indol-2-carboxilato férrico en alcohol isoamílico. *An. Quim.*, **77B**, 94–7.
- Royo Iranzo, J., Aranda, A. & Peris Toran, M. J. (1976). Composición y calidad de los zumos comerciales de naranja. II. Zumos y néctares de naranja embotellados, vendidos en los países citricolas de la cuenca del Mediterráneo. *Rev. Agroquím. Tecnol. Aliment.*, **16**(2), 241–51.
- Sánchez Pérez, A., Hernández Méndez, J. & Gómez Bárez, J. A. (1984). Determinación polarográfica de citrato con electrodo de gotas de amalgama de cobre. *Stud. Chem.*, **9**, 269–81.
- Shaw, P. E., Buslig, B. S. & Wilson, C. W. (1983). Total citrate content of orange and grapefruit juices. *J. Agric. Food Chem.*, **31**, 182–4.
- Tschoepe, W. & Ritz, E. (1980). *Proceedings of the 2nd International Symposium on Isotachopheresis, Eindhoven*, p. 63.
- Tur'yan, Ya. I. & Saksin, E. V. (1970). Polarographic catalytic current of the citrate complex of Ti(IV): Determination of citric acid. *Zh. Anal. Kim.*, **25**(5), 998–1003.
- Williams, W. J. (1979). *Handbook of Anions Determination*. Butterworths, London, pp. 62–7.