Talanta 84 (2011) 1169–1173

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Potentiometric detection of citrate in beverages using a graphite carbon electrode

Celso L. Araújo, Edmar I. Melo, Nívia M.M. Coelho *

Institute of Chemistry, University of Uberlândia, Av. João Naves de Ávila 2121, CEP 38400-902, Uberlândia, MG, Brazil

ARTICLE INFO

Article history:
Received 16 December 2010
Received in revised form 8 March 2011
Accepted 10 March 2011
Available online 17 March 2011

Keywords:
Citrate
Graphite carbon electrode
Potentiometry
Beverages

ABSTRACT

The development, evaluation and application of a simple and low-cost graphite carbon electrode for the direct determination of citrate in food samples are described here. The electrode exhibits a linear response with a slope of -29.0 ± 1.0 mV decade^{-1} in a concentration range of 0.07–7.0 mmol L^{-1} in 0.1 mol L^{-1} KCl/1.0 mmol L^{-1} phosphate buffer solution with a limit of detection of 3.0 μmol L^{-1}. The electrode is easily constructed at a relatively low cost and has a fast time response (within 120 s) with no significant changes in its performance characteristics. The performance of the graphite sensor was tested to determine citrate in beverage samples (juices and an isotonic drink), and the results were validated against a reference procedure. The proposed method is quick, inexpensive, selective and sensitive, and is based entirely on conventional instrumentation.

1. Introduction

Citric acid and its respective sodium, potassium and calcium salts are the most commonly used food preservatives, especially in beverages [1]. They are generally used for inhibiting yeast and mold growth, being also effective against a wide range of bacteria. These compounds are most active in foods with low pH values and essentially ineffective in foods at neutral pH [2]. As an additive, citric acid is used as a flavoring and preservative in food and beverages, especially fruit juices. Citrate salts of various metals are used to deliver minerals in a biologically available form in many dietary supplements. The joint FAO/WHO Expert Committee on Food Additives (JEFCA) has allocated to citric acid an acceptable daily intake (ADI) of "non-specified" category. This means that, on the basis of the available data (chemical, biochemical, toxicological and others), the total daily intake of a substance, arising from its use at the levels required to achieve the desired effect, does not represent a health hazard. However, legislation in some countries recommends that its use be strictly limited and states that it must be mentioned on the ingredients list. Therefore, to be able to enforce this legislation there is a need for an analytical tool capable of detecting and quantifying citric acid addition.

The reference methods available for the determination of citric acid in food products, recommend tedious methodologies involving large amounts of reagents incurring considerable costs [3].

Various analytical methods for the determination of citric acid in food products have been reported in literature, for instance: high performance liquid chromatography [4,5], gas chromatography [6], polarography [7], spectrometry [8–10] and capillary electrophoresis [11].

However, many of these methods are complicated and time consuming or require expensive equipment. Thus, there is a significant demand for simple, low-cost, sensitive and rapid alternative methods for determining citric acid in food products.

Several methods based on electrochemistry have been reported for determining citric acid. These include amperometric biosensors using either citrate lyase with pyruvate oxidase [12]. Potentiometric sensors have also been reported, based on modified carbon electrodes [13] and copper wire indicator electrodes for determining citrate as part of a flow injection system [14–16].

Potentiometric detection based on ion-selective electrodes has proved to be effective in food sample analysis, because these sensors offer important advantages including simplicity, fast response and limit of detection of 0.43 μmol L^{-1} for sorbate ion [17].

The use of potentiometric sensors for the determination of ascorbate in food products using a graphite carbon electrode, based on the steady-state response, has been reported in literature [18]. The mechanism of this electrode involves the process of adsorption and subsequent oxidation of ascorbate on the electrode surface. However, the use of a potentiometric sensor for detecting citrate, in particular the use of carbon-based electrodes, has received little attention.

Flow injection (FI) methods offer great potential for determining citric acid in different foods, drinks (e.g., fruit juices), pharmaceuticals, etc. They characteristically employ simple and cheap devices which are easy to perform and provide high quality results with good analytical features (sampling frequency, accuracy and precision) [14].

This article describes the development, evaluation and application of a simple and low-cost graphite carbon electrode coupled

* Corresponding author. Tel.: +55 34 3239 4385; fax: +55 34 3239 4385.
E-mail address: nmmcoelho@ufu.br (N.M.M. Coelho).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.03.027

with a flow system for determining citrate in food samples, in particular, beverages. The proposed procedure uses only readily available reagents and instrumentation and has the advantages of simplicity, sensitivity, fast response, stability and repeatability.

2. Experimental

2.1. Apparatus

All electrochemical experiments were performed with a potentiostat (model 760C, CH Instruments, Austin, USA). A pH meter (model PG 1800, Gehaka, São Paulo, Brazil) was used to adjust pH values. The surface of the electrode was examined using atomic force microscopy (Nanoscope III, Digital Instruments, Ottawa, Canada).

The flow injection analysis (FIA) manifold for determining citrate is schematically shown in Fig. 1. All solutions and the carrier were pumped using a peristaltic pump (Model Minipuls 3, Gilson, Villiers-le-Bel, France) and Tygon® pumping tubes. The connecting and mixing tubes were made of PTFE tubing with an i.d. of 0.5 mm. A proportional injection valve [19] with a sample loop volume of 65 μL and a flow through cell connected to a potentiostat (São Paulo, Brazil) were used, both being made in the laboratory. Samples (65 μL) were injected into a stream containing 0.1 mol L^{-1} KCl/1.0 mmol L^{-1} phosphate buffer solution with a flow rate of 1.5 mL min^{-1}. The response of the electrode was evaluated by monitoring the open circuit potential as a time function.

2.2. Chemicals and solutions

Standard citric acid solution (100 mmol L^{-1}) was prepared by weighting 0.01931 g of citric acid (Vetec, São Paulo, Brazil) and diluting it to the mark with 0.1 mol L^{-1} KCl/1.0 mmol L^{-1} phosphate buffer in a 100 mL volumetric flask. Working standard solutions were freshly prepared and diluted daily as appropriate directly before use. All other chemicals were of analytical grade and high purity deionized water (resistivity 18.2 MΩ cm) obtained from a Gehaka Master system (São Paulo, Brazil) was used throughout.

2.3. Electrodes

A graphite disk (28.26 mm^2) (99.9%, Alfa Aesar – Karlsruhe, Germany) and Ag/AgCl electrode with 3.0 mol L^{-1} KCl were used as the indicator and reference electrodes, respectively. The graphite electrode was sealed into the acrylic cell and the reference electrode was sealed into a plastic pipette tip. Electrical contact was established with a carbon bar. Prior to the experiments, the graphite electrode was polished mechanically on a cloth with an aqueous slurry of 0.3 μm alumina, ultrasonicated, washed with deionized water and dried with ultra pure N_2. The electrochemical cell used for potential measurements was:

Graphite electrode |Citrate, 0.1 mol L^{-1} KCl/1.0 mmol L^{-1} phosphate buffer solution ‖ 3.0 mol L^{-1} KCl, AgCl (s) |Ag.

2.4. Interference

The interference effect of organic acids on the potential response of the electrode to citric acid was studied by injecting 1.5 mmol L^{-1} standard solutions of citric acid containing 1.0 and 5.0 mmol L^{-1} of ascorbic and benzoic acid solutions, separately, into the flow system. Five replicate measurements were taken at each level of potential interference.

2.5. Samples

Samples of fruit juices and an isotonic drink were obtained on the local market. These samples were filtered to remove suspended solids, after which they were then diluted appropriately with 0.1 mol L^{-1} KCl/1.0 mmol L^{-1} phosphate buffer solution and 65 μL were injected into the flow injection system without prior pre-treatment. The samples were also analyzed using a standard titrimetric method [3] for reference purposes.

3. Results and discussion

The performance of the graphite carbon electrode was evaluated using the flow system (Fig. 1) and the potential difference was observed after injecting citric acid into the flow injection system. The precise nature of this mechanism is still not fully understood, however, the observed electrode response to citric acid solution may be attributed to the ion-exchange occurring between the electrode surface and citrate, as suggested by Chen and Yu [18] for the detection of ascorbate.

3.1. Evaluation of the experimental parameters

In order to find a compromise between sensitivity and sampling frequency, the effects of the flow rate, injection volume and pH were investigated. The analytical signal for consecutive injections of 1.0 mmol L^{-1} citric acid increased proportionally with the injected volume (33, 65 and 130 μL). Higher volumes were not used in this proposed method because, in this case, there is a long time period for the analytical signal to return to the baseline and to maintain the compromise with the analytical frequency. Sample volumes of 65 μL were used for further experiments. Increasing the flow rate from 1.0 mL min^{-1} to 3.0 mL min^{-1} increased the signal. Appropriate results were obtained with a flow rate of 1.5 mL min^{-1}, favoring the stability of the electrode and the generation of smaller amounts of waste.

The influence of the electrolyte pH on the electrode response was tested by varying the pH in the carrier solution. Solutions of citric acid were prepared at pH 7.0 (0.1 mol L^{-1} KCl/1.0 mmol L^{-1} phosphate buffer solution) and the pH of the solution stream (0.1 mol L^{-1} KCl) was varied between 3.0 and 8.4. The electrode response increased as the pH increased from 3.0 to 8.4. Such an effect may result from the graphite electrode suffering from H^+ interference because the surface of the graphite electrode creates an ion-exchange layer, which confers some degree of the selective response. Consequently, the adsorption of citrate on the electrode surface by ion-exchange decreased as the electrolyte pH decreased. The following equations show these processes of citric acid ionization:

$$H_3C_6H_5O_7 \leftrightarrow H^+ + H_2C_6H_5O_7^- \quad (pKa = 3.13)$$

$$H_2C_6H_5O_7^- \leftrightarrow H^+ + HC_6H_5O_7^{2-} \quad (pKa = 4.76)$$

$$HC_6H_5O_7^{2-} \leftrightarrow H^+ + C_6H_5O_7^{3-} \quad (pKa = 6.40)$$

The response mechanism of this electrode suggests that the electrode potential change resulted from the ion-exchange adsorption of citrate on the electrode surface. Although the nature of this mechanism is not fully understood, it is possible that the observed electrode response to citric acid solution is due to the ion-exchange occurring between the electrode surface and citrate. The results show that the electrode surface contains pores of varying size which may affect the ion-exchange adsorption of citrate onto the electrode surface. AFM quantitative analysis showed that the surface of the electrode, which was thoroughly washed with distilled water

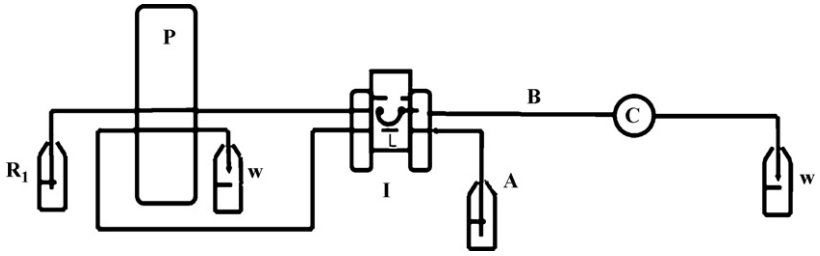

Fig. 1. Schematic diagram of the flow injection system used for determination of citric acid. R_1: carrier solution (0.1 mol L^{-1} KCl) and flow rate of 1.5 mL min^{-1}; P: peristaltic pump; W: waste; L: loop of 65 μL; I: injection valve; A: standard and sample solutions; B: dispersion coil 20 cm; C: potentiometric detector.

after use, contained hydrogen and oxygen elements which may facilitate citrate adsorption.

The performance of the electrode was tested considering the time required for the signal to return to baseline (response time). The electrode presented a fast time response (within 120 s) with no significant changes in its performance characteristics for a flow rate of 1.5 mL min^{-1}.

3.2. Interference

The effect of ascorbic and benzoic acids on the determination of citric acid was studied using a mixed solution method, where the solution contained a fixed concentration of citric acid in 0.1 mol L^{-1} KCl/1.0 mmol L^{-1} phosphate buffer solution and various concentrations of interfering ions. Solutions were prepared containing 1.0 mmol L^{-1} of citric acid and 1.0 and 5.0 mmol L^{-1} of possible interfering ions. The solutions containing the citric acid sample plus the potential interference ions were analyzed by the proposed method. The response was compared to that obtained from an uncontaminated citric acid solution. Ascorbic and benzoic acids, when present in concentrations of up to 5.0 and 1.0 mmol L^{-1} respectively, do not interfere with the response.

3.3. Analytical figures of merit

The response characteristics of the electrode were evaluated according to the method recommended by IUPAC [20]. The electrode has a linear response to citrate with a slope of -29.0 ± 1.0 mV $decade^{-1}$ in a concentration range of 0.07–7.0 mmol L^{-1} ($R^2 = 0.999$). The limit of detection based on the 3 r/s where r is the standard deviation of 10 measurements of the blank and s is the slope of the calibration graphs, was 3.0 μmol L^{-1}. Table 1 shows analytical figures of merit for the proposed method.

Under the optimized conditions the injection frequency is around 22 samples h^{-1}. The relative standard deviation of ten replicate determinations of 1.0 mmol L^{-1} of citric acid is 0.5%. The selected conditions were determined from the slope and the linearity of the calibration graph obtained within a reasonable analysis time.

Table 1
Figures of merit of the proposed system.

Figures	Graphite carbon electrode
Linear range (mmol L^{-1})	0.07–7.0
Limit of detection (μmol L^{-1})	3.0
Relative standard deviation (%)[a,b]	0.5
Slope (mV/log C)	-29.0 ± 1.0
Time response (s)[a]	<120

[a] For 1.0 mmol L^{-1} citric acid.
[b] $N = 10$. See Section 3.3 for details.

3.4. Samples

The citric acid content is the main component of the total acidity in beverages. Thus, obtaining information on the citric acid content is highly relevant. The focus of this research study was on the determination of the citric acid content in juices and an isotonic drink. Different types of juices (passion fruit, orange, pineapple and strawberry) were chosen in order to evaluate the suitability of this method for the analysis of samples with various compositions and colors. Fig. 2 shows typical signals for the standards and sample solutions of citric acid. After setting the optimized conditions, the systematic determination of citric acid in the isotonic drink and juice samples was carried out using the proposed method and a titrimetric method. The results (Table 2) ranged from 1.60 g L^{-1} to 4.40 g L^{-1} and 2.00 g L^{-1} to 4.90 g L^{-1} for the potentiometric and titrimetric methods, respectively. These values indicate the good accuracy of the method and thus the method is suitable for determining citric acid. The results obtained from the application of the two methods were not significantly different (according to the t-test at the 95% confident interval).

In the present study, the method proposed for determining citric acid in foods was described and compared according to the detection technique used. The most important details of the published procedures for citric acid determination, in terms of kind of sample, are presented in Table 3. Most of the methods were applied to the analysis of citric acid in foods (fruits, juices of fruits, soft drinks and candies). To compare the method studied in this article the limit of detection has been taken into account. The spectrophotometric methods provide the lowest limit of detection (about 3.6×10^{-3} mmol L^{-1}). However, spectrophotometric methods present more important interferences derived from the

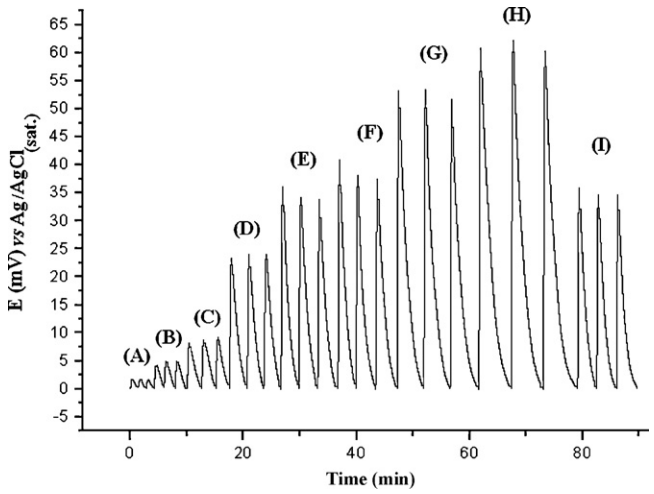

Fig. 2. Analytical signals for proposed sensor. (A) 0.03 mmol L^{-1}, (B) 0.07 mmol L^{-1}, (C) 0.1 mmol L^{-1}, (D) 0.3 mmol L^{-1}, (E) 0.7 mmol L^{-1}, (F) 1.0 mmol L^{-1}, (G) 3.0 mmol L^{-1}, (H)7.0 mmol L^{-1}, and (I) sample.

Table 2
Determination of citric acid in beverages using proposed method.

Sample[a]	Citric acid ($g\,L^{-1}$)	
	Potentiometric method using proposed electrode	Titrimetric method
Isotonic drink	2.70 ± 0.30	3.10 ± 0.30
Strawberry isotonic drink	4.10 ± 0.40	4.90 ± 0.50
Passion fruit juice	4.40 ± 0.30	4.90 ± 0.50
Orange juice	4.00 ± 0.30	4.70 ± 0.50
Pineapple juice	1.60 ± 0.40	2.00 ± 0.20
Green fruit juice	2.70 ± 0.40	2.70 ± 0.50

[a] $N = 3$, confidence level 95%.

Table 3
Comparison of methods for citric acid determination.

Sample	Detection technique	Detection limit ($mmol\,L^{-1}$)	Linear range ($mmol\,L^{-1}$)	Interferences	Refs.
Fruit, fruit juice, soft drinks	Amperometry	0.004	0.015-0.5	Malic acid	[12]
Soft drinks, drugs	Spectrophotometry	0.48	0.48–24.6	No data	[10]
Juices, drugs	Voltammetry	0.2	Until 2000	Oxalic acid and EDTA	[13]
Fruit juice	Conductimetry, spectrophotometry	5.2	Until 114.6	Sucrose and NaCl	[9]
Soft drinks and drugs	Chemilumunescence	No data	2.0×10^{-4}–0.1	Cu^{2+}, Mn^{2+}, Cr^{3+}, Co^{2+}, oxalate and tartrate	[21]
Fruit juices, soft drinks and candies	Flame atomic absorption spectrometry	3.6×10^{-3}	0.011–0.21	Tartaric acid	[22]
Fruits and fruit juices	Potentiometry	No data	1–10	Glucose, fructose, sucrose, ascorbic acid	[23]
Soft drinks, beers and drugs	Potentiometry	No data	No data	Chloride, nitrate ascorbate, glucose, fructose, sucrose	[24]
Juices and isotonic drink	Potentiometry	0.003	0.07–7.0	No data	This study

presence of coloured matter and/or turbidity in the samples that absorb light in the same region of the citric acid. For this reason, these methods often require tedious pre-treatments of the sample that will vary if is a solid or liquid sample and depend on the concentration of the possible interference substances. The electroanalytical methods provided limit of detection about 0.004–5.2 $mmol\,L^{-1}$. These methods present potentially interference substances that are substances formed by different anions of organic acids (ascorbic, malic, oxalic, lactic, tartaric, EDTA) and sugars, such as glucose, fructose and sucrose, customarily existent in the analyzed samples, produced negative peak signals.

Although electrochemical methods are subject to these typical interferences; in our work, the substances present in the samples are acceptably tolerated without producing significant errors because they are present in smaller concentrations in several orders of magnitude than the citric acid.

The procedure developed based on a graphite carbon sensor with potentiometric detection allowed for the determination of citrate at the level of $\mu mol\,L^{-1}$ in soft drinks, without the need for a specific sample preparation step.

4. Conclusions

The graphite carbon electrode described here represents a low cost sensor for potentiometric detection of citrate in beverages. It offers high sensitivity, selectivity and stability. The data obtained suggest that the electrode response to citric acid may result from the adsorption of citrate on the electrode surface by ion-exchange. The graphite carbon electrode was successfully applied for the assays of citric acid in samples of beverages, such as juices and isotonic drinks, and the results compare well with those of the standard method.

Acknowledgements

The authors are grateful for financial support from the Brazilian government agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and from the MG state government agency Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG). We thank Dr. J.M. Madurro for facilities in measurement techniques.

References

[1] A.P. Miles-Prouten, Fruit Proc. 94 (1994) 6.
[2] J.N. Sofos, Antimicrobial agents, in: J.A. Maga, A.T. Tu (Eds.), Food Additive Toxicology, Marcel Dekker, New York, 1995, p. 501.
[3] Association of Official Analytical Chemists (AOAC), Official Methods of Analysis, 15a ed., Washington, DC, 1990.
[4] H.J. Jeuring, A. Brands, P. Doorninck, Z. Lebensmitteluntersuchung und-Forsch. A 168 (1979) 185.
[5] H. Kelebek, S. Selli, A. Canbas, T. Cabaroglu, Microchem. J. 91 (2009) 187.
[6] K. Miyakoshi, M. Komoda, J. Am. Oil Chem. Soc. 54 (1977) 331.
[7] K. Hasebel, S. Hikama, H. Yoshida, Fresenius' J. Anal. Chem. 336 (1990) 232.
[8] S. Bureau, D. Ruiz, M. Reich, B. Gouble, D. Bertrand, J.M. Audergon, C.M.G.C. Renard, Food Chem. 115 (2009) 1133.
[9] K. Grudpan, P. Sritharathikhun, J. Jakmunee, Anal. Chim. Acta 363 (1998) 199.
[10] L.F. Capitán-Vallvey, E. Arroyo-Guerrero, M.D. Fernández-Ramos, F. Santoyo-González, Microchim. Acta 151 (2005) 93.
[11] R.G. Peres, E.P. Moraes, G.A. Micke, F.G. Tonin, M.F.M. Tavares, D.B. Rodriguez-Amaya, Food Control 20 (2009) 548.
[12] M.I. Prodromidis, S.M. Tzouwara-Karayanni, M.I. Karayannis, M.I. Prodromidis, P.M. Vadgama, Analyst 122 (1997) 1101.
[13] K.C. Honeychurch, L. Gilbert, J.P. Hart, Anal. Bioanal. Chem. 396 (2010) 3103.
[14] A. Moreno-Cid, M.C. Yebra, X. Santos, Talanta 63 (2004) 509.
[15] J.L.F.C. Lima, C. Delerue-Matos, M. Carmo, V.F. Vaz, J. Silva, Fresenius' J. Anal. Chem. 364 (1999) 266.
[16] Z.L. Chen, D.B. Hibbert, J. Chromatogr. A 766 (1997) 27.
[17] A.O. Santini, H.R. Pezza, J. Carloni-Filho, R. Sequinel, L. Pezza, Food Chem. 115 (2009) 1563.
[18] Z. Chen, J.C. Yu, Talanta 49 (1999) 661.

[19] H.F. Bergamin, B.F. Reis, A.O. Jacintho, E.A.G. Zagatto, Anal. Chim. Acta 117 (1980) 81.
[20] IUPAC Analytical Chemistry Division, Commission on Analytical Nomenclature Recommendations for nomenclature of ion selective electrodes, Pure Appl. Chem. 48 (1976) 127.
[21] T. Pérez-Ruiz, C. Martınez-Lozano, V. Tomás, O. Val, Analyst 120 (1995) 471.
[22] M.C. Yebra, R.M. Cespón, Fresenius' J. Anal. Chem. 365 (1999) 370.
[23] T. Fuse, F. Kusu, K. Takamura, J. Agric. Food Chem. 45 (1997) 2124.
[24] M.F.C. Ribeiro, C. Delerue-Matos, M. Goreti, F. Sales, M. Carmo, V.F. Vaz, Anal. Chim. Acta 471 (2002) 41.