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Food Chemistry 83 (2003) 297–301

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Potentiometric determination of saccharin in commercial artificial sweeteners using a silver electrode

João Carloni Filho, Alberto Oppermann Santini, Ana Lúcia M. Nasser, Helena Redigolo Pezza, José Eduardo de Oliveira, Cristo Bladimiros Melios, Leonardo Pezza*

Instituto de Química, UNESP, C.P. 355, CEP 14801-970, Araraquara, SP, Brazil

Received 1 October 2002; received in revised form 16 February 2003; accepted 16 February 2003

Abstract

A simple, precise, rapid and low-cost potentiometric method for saccharin determination in commercial artificial sweeteners is proposed. Saccharin present in several samples of artificial sweeteners is potentiometrically titrated with silver nitrate solution using a silver wire as the indicator electrode, coupled to a titroprocessor. The best pH range was from 3.0 to 3.5 and the detection limit of sodium saccharin was 2.5 mg/ml. Substances normally found along with saccharin in several commercial artificial sweeteners such as maltodextrin, glucose, sucrose, fructose, aspartame, cyclamate, caffeine, sorbitol, lactose, nitrate, methyl- and *n*-propyl-*p*-hydroxybenzoate, benzoic, citric and ascorbic acids do not interfere even in significant amounts (e.g. 20 excess relative to saccharin). Chloride ion interferes when present in concentrations larger than 10 mg l^{-1}; this interference is eliminated with previous extraction of the sweetener from the aqueous medium with ethyl acetate. The results obtained by applying the proposed method compared very favorably with those given by the HPLC method recommended by the FDA.

Keywords: Potentiometric determination; Silver electrode; Saccharin; Artificial sweeteners

1. Introduction

Saccharin (*o*-benzoic sulfimide, $C_6H_4COSO_2NH$) was discovered accidentally by Remsen and Fahlberg at Johns Hopkins University in 1879 during an academic study of the oxidation of *o*-toluenesulphonamides (Townshend, 1995). Afterwards it was discovered that saccharin was slowly absorbed and not metabolized by the human organism being consequently an appropriate artificial sweetener for diabetics. Saccharin is 300–500 times sweeter than sucrose. For these reasons its use in industrialized foods increased vastly; in 1907 the consumption mark of 2500 t/year had already been reached.

In 1972, the Food and Drug Administration (FDA) in the USA removed the saccharin from the safe additive's list because of its carcinogenic potential (Fatibello-Filho, Vieira, Gouveia, Calafatti, & Guaritá-Santos, 1996). In 1977 the FDA prohibited saccharin use in dietary products and drugs, annulling that prohibition in 1991. Nowadays saccharin is approved in more than 90 countries all over the world and is widely used in many pharmaceutical and dietary products despite controversy over its safety (The European Parliament and The Council of The European Union, Directive 94/35/EC, 1994 & Directive 96/83/EC, 1996).

Several analytical methods for determining saccharin are described in the literature, such as high-performance liquid chromatography (HPLC) (Argoudelis, 1984; Kantasubrata & Imamkhasani, 1991; Yano et al., 1992), visible spectrophotometry (Gowda, Gowda, & Rangappa, 1984; Ramappa & Nayak, 1983; Sastry, Srinivas, Rama, Prasad, & Krishnamacharyulu, 1995; Vianna-Soares & Martins, 1995), fluorimetry (Wengi, 1996), polarography (AOAC, 1995, chapter 47; Hannisdal & Schroeder, 1993; Holak & Krinitz, 1980), potentiometry (Alfaya, Alfaya, Gushikem, Rath, & Reyes, 2000; Fatibello-Filho & Aniceto, 1997; Fatibello-Filho & Guaritá-

* Corresponding author. Fax: +55-16-222-7932.
E-mail address: pezza@iq.unesp.br (L. Pezza).

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doi:10.1016/S0308-8146(03)00123-7

Santos, 1993; Negash, Moges, & Chandravanshi, 1997), flow injection analysis (Fatibello-Filho, Nóbrega & Guaritá-Santos, 1994; Yebra, Gallego, & Valcárcel, 1995) and gravimetry (Fatibello-Filho, Teixeira, & Guaritá-Santos, 1993). However, most of these methods are time consuming, involve risks of environmental nature or require expensive equipment and consequently are not suitable for routine analysis. Moreover, certain problems, mainly those concerning interference, reproducibility, stability of the measurements and specifically problems relating to electroanalytical techniques gave rise to non-linear response and short lifetimes of the electrodes. These problems were poorly solved, justifying the search for improved methods.

Saccharin forms a silver salt of low solubility, whose precipitation is quantitative, according to a previous study of Parikh and Mukherji (Parikh & Mukherji, 1960) and confirmed by recent experiments, carried out in this laboratory. Based on this verification, a new potentiometric method for the saccharin determination in commercial artificial sweeteners is proposed. Saccharin was potentiometrically titrated with $AgNO_3$ solution and the remaining Ag^+ was monitored by a silver electrode. The employed titroprocessor allows the automatic evaluation of the equivalence point with good precision (i.e., less than 3% in volume, among replicates, for samples containing 18–83 mg/ml or mg/g of saccharin) and accuracy. The results obtained by the potentiometric method were compared with those obtained by HPLC method recommended by the US FDA (1993). The proposed method does not present the inconveniences above mentioned and has proved suitable for the routine quality control of saccharin in artificial sweeteners.

2. Experimental

2.1. Apparatus

2.1.1. Potentiometry

Potentiometric measurements were carried out using a Metrohm model 670 Titroprocessor with ±0.1 mV precision (Metrohm Ltd., Herisau, Switzerland). The indicator electrode used was a silver wire of 1 mm diameter and 15 mm length. Before carrying out each experiment the silver electrode was immersed in a dilute HNO_3 solution, washed with deionized water and dried with absorbent paper.

The reference electrode was a Metrohm Ag|AgCl double junction, model 6.0726.100 electrode containing 0.010 mol l^{-1} NaCl and 0.490 mol l^{-1} $NaNO_3$ in the inner chamber and 0.500 mol l^{-1} $NaNO_3$ in the outer chamber. A thermostated titration cell (25.0±0.1)°C was employed.

Volume measurements (±0.001 ml) were performed with a Metrohm model 665 automatic burette.

2.1.2. High-performance liquid chromatography

The HPLC system used consisted of a Shimadzu SPD-10A liquid chromatograph (Shimadzu Seisakusho, Kyoto, Japan) equipped with a LC-10 AS Shimadzu pump, variable UV-visible detector (SRD-10 A, Shimadzu) set at 254 nm and a Rheodyne 20 µl injector (Rheodyne Inc., Berkeley, CA, USA). A stainless steel Supelcosil LC-18 analytical column was used (150×4.6 mm i.d., Supelco, Bellefonte, PA, USA) with 5 µm particle size packing material. The mobile phase consisted of a mixture of 20%(v/v) reagent grade glacial acetic acid in water, buffered to pH 3.0 with saturated sodium acetate solution. Before injection the samples were filtered through a Millex unit (Millex-HV, 0.45 µm, Millipore). Chromatograms were recorded and peak heights measured with an integrator (Shimadzu C-R6A Chromatopac recording integrator).

2.2. Reagents and solutions

High purity deionized water (resistivity 18.2 MΩ cm) obtained by using a Milli-Q plus system (Millipore Corp., Bedford, MA, USA) was used throughout. All solutions were prepared with analytical reagent grade chemicals, obtained from E. Merck, Darmstadt, Germany.

The saccharin stock solution (200 ml) was prepared by dissolving 9.6476 g of sodium saccharinate in water. This solution is stable for at least 3 months (Fatibello-Filho, Nóbrega, & Guaritá-Santos, 1994) when stored at 5 °C. The reference solutions were prepared by suitable dilutions of the stock solution with water. The silver nitrate solutions (8.05×10^{-2} mol l^{-1} or 1.00×10^{-2} mol l^{-1}) were prepared, standardized and stored according to recommendations of the literature (Skoog, West, & Holler, 1996). The ionic strength of the reference sodium saccharinate and silver nitrate solutions were adjusted to 0.500 mol l^{-1} by adding appropriate volumes of a 5.128 mol l^{-1} stock $NaNO_3$ solution, using a Metrohm mod. 665 automatic burette. The pH of these solutions was adjusted to 3.0 by adding dilute HNO_3 solution dispensed from a Metrohm mod. 665 automatic burette. The pH values were measured by using a combination electrode (glass–Ag|AgCl; Metrohm mod. EA-125) connected to a Metrohm model 692 instrument. For the analyses of commercial liquid and powder samples, the approximate content of both sodium saccharinate and sodium cyclamate were taken into account for ionic strength adjustment with $NaNO_3$.

2.3. Sample preparation for potentiometric determinations

Samples of liquid and powder sweeteners were purchased from a local food store in Araraquara (SP), Brazil.

2.3.1. Liquid sweeteners

A volume of 15.000 ml of each liquid sweetener sample was diluted to 50 ml in a volumetric flask. The ionic strength (I) of each sample was adjusted to 0.500 mol l^{-1} with $NaNO_3$ and the pH to 3.0 with nitric acid. An aliquot containing 15.000 ml of $AgNO_3$ 8.05×10^{-2} mol l^{-1} (pH = 3.0 and I adjusted to 0.500 mol l^{-1} with $NaNO_3$) was transferred to a thermostated glass cell (25.0±0.1 °C) and directly titrated with a sample of sweetener prepared as above described.

2.3.2. Solid sweeteners

Accurately weighed amounts of 4.5–5.0 g of solid sweetener samples were dissolved in 15 ml of water, homogenized in an ultrasonic bath and transferred to a 25 ml volumetric flask. The ionic strength (I) of samples was adjusted to 0.500 mol l^{-1} with $NaNO_3$ and the pH to 3.0 with nitric acid, before volume completion. An aliquot containing 15.000 ml of $AgNO_3$ 1.00×10^{-2} mol l^{-1} (pH = 3.0 and I adjusted to 0.500 mol l^{-1} with $NaNO_3$) was transferred to a thermostated glass cell (25.0±0.1 °C) and directly titrated with a sample of sweetener prepared as above described.

2.3.3. Liquid sweeteners with chloride addition

An aliquot of 25 ml of a certain liquid sweetener sample with added chloride concentration 20 times larger than that of saccharin was transferred to a 125 ml separatory funnel, 1.0 ml of concentrated nitric acid was added and mixed well. The resulting solution was extracted twice with 25 ml portions of ethyl acetate (shaking time: at least 1 min for each extraction). The aqueous phase was discarded and the combined organic extract was evaporated in a rotary evaporator under reduced pressure to dryness. To the resulting residue sufficient 0.1 mol l^{-1} NaOH solution was added to dissolve it. The pH of the resulting solution was adjusted to 3.0 with HNO_3 and it was quantitatively transferred to a 50 ml volumetric flask. The volume was completed with deionized water, after previous adjustment to pH 3.0 and 0.500 mol l^{-1} ionic strength with HNO_3 and $NaNO_3$ respectively. Finally, the resulting solution was treated as previously described.

3. Results and discussion

3.1. Lifetime of the electrode

The potential of the silver electrode relative to the silver-silver chloride electrode in 1.00×10^{-1} mol l^{-1} $AgNO_3$ was continually monitored, no variation larger than ±0.2 mV was observed. The silver electrode was submitted to continuous operation for about one year without noticeable change in its electrochemical behavior.

3.2. pH Effect

The influence of pH on the determination of 8.05×10^{-2} mol l^{-1} saccharin using 8.05×10^{-2} mol l^{-1} silver nitrate was studied over the pH range 2.0–6.0. For pH values below 2.0 a significant decrease concerning the potentiometric jump was observed. This is probably due to the change of saccharinate anion (p*K*a = 1.6) (Albert & Serjeant, 1984) into its protonated form. For pH values within the 2.0–2.9 and 3.6–6.0 ranges, the results for saccharin analyses show errors larger than 5% relative to the expected values, making clearly apparent that interference took place. For pH values above 4.0 an increase in concentration of deprotonated forms of organic acids usually found in artificial sweeteners preparations (benzoic, ascorbic and citric) is observed, which interfere with the saccharin analysis probably by formation of insoluble silver carboxylates. The best results were reached for pH values within the 3.0–3.5 range, where even a 0.28 mol l^{-1} solution of citric acid did not yield a precipitate with an equimolar solution of silver nitrate, at I = 0.500 M ($NaNO_3$). As metallic electrodes, like ion-selective sensors, provide activity values rather than concentrations for individual ions (Pezza, Molina, Moraes, Melios, & Tognolli, 1996), the ionic strength was kept constant at 0.500 mol l^{-1} (adjusting with $NaNO_3$) during the entire course of the titrations.

3.3. Potentiometric titration

Fig. 1(a) shows a typical titration curve. In all the experiments the best results were obtained when the analyte was used as titrant. The time required for the analysis by potentiometric method was 15–25 min per sample.

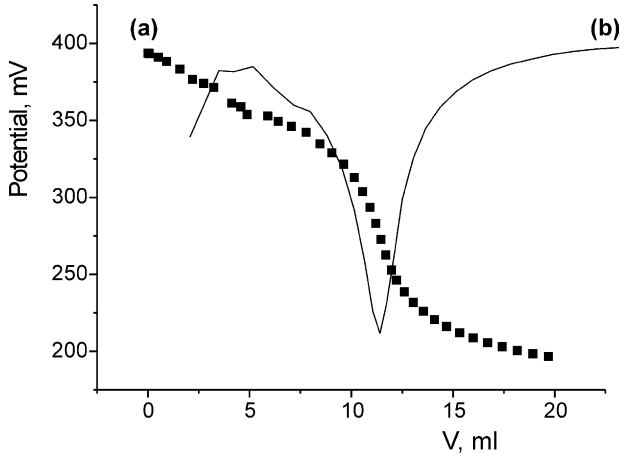

Fig. 1. (a) Typical titration curve of 15.000 ml 8.05×10^{-2} mol l^{-1} silver nitrate with a artificial sweetener (Sample A) both at ionic strength 0.500 mol l^{-1}, pH 3.0 and 25.0±0.1 °C; (b) first derivative plot generated by the titroprocessor (Metrohm, model 670).

Fig. 1(b) shows the first derivative of the titration curve generated by the internal algorithm of the titroprocessor. The evaluation of the titration curve by the titroprocessor is fully automatic yielding an accurate end point.

The detection limit of the method determined as described by Fatibello-Filho and Guaritá-Santos (1993), was 2.5 mg/ml.

3.4. Effect of interferences

The potential interference of most of the common components found along with saccharin in commercial samples of several artificial sweeteners such as maltodextrin, sucrose, glucose, fructose, aspartame, cyclamate, caffeine, sorbitol, lactose, methyl- and *n*-propyl-*p*-hydroxybenzoate, benzoic, citric and ascorbic acids, nitrate and chloride in the proposed method were investigated in concentrations (mg ml^{-1} or mg g^{-1}) at least 20 times higher than that of saccharin. For all tested substances/materials (except chloride) no difference larger than ±3% relative to the expected saccharin value was found. Chloride presents strong interference in concentrations larger than 10 mg l^{-1}. However, the interference due to this ion could be eliminated with previous extraction of the sweetener from the aqueous medium with ethyl acetate. In the samples analysed in this work (artificial sweeteners) the chloride ion is normally present in very small amounts (i.e., 0.2–0.5 mg l^{-1}) and does not interfere with the method. Moreover, to the best of our knowledge, other Ag^+ precipitating anions (Br^-, I^-, SCN^-, etc.) are not found in commercial sweeteners and hence the proposed method can be used for the determination of saccharin in commercial samples of artificial sweeteners.

Table 1
Recovery of saccharin from various commercial artificial sweeteners

Samples	Saccharin (mg/ml)		Recovery[a] (%)
	Added	Found[a]	
A (liquid)	10.1	9.9±0.6	98.0±0.4
	15.2	15.1±0.3	99.3±0.2
	19.3	19.4±0.4	100.5±0.2
B (liquid)	10.1	10.3±0.3	101.9±0.3
	15.2	15.6±0.5	102.6±0.4
	19.3	19.4±0.2	100.5±0.3
C (powder)	10.1	10.2±0.2	100.9±0.4
	15.2	15.3±0.3	100.6±0.2
	19.3	19.2±0.2	99.5±0.3
D (liquid)	10.1	9.8±0.3	97.0±0.5
	15.2	15.1±0.5	99.3±0.4
	19.3	19.4±0.1	100.5±0.3
F (powder)	10.1	9.9±0.2	98.0±0.3
	15.2	15.4±0.3	101.3±0.4
	19.3	19.2±0.3	99.5±0.2
H (liquid)	10.1	10.3±0.4	101.9±0.2
	15.2	15.1±0.2	99.3±0.4
	19.3	19.0±0.3	98.4±0.5

[a] Average±standard deviation of five determinations.

3.5. Addition and recovery study

In this study 10.1, 15.2 and 19.3 mg/ml of saccharin reference solutions were added in six commercial artificial sweeteners (Samples A–D, F and H).

The results presented in Table 1 show recoveries ranging from 97.0 to 102.6% of saccharin for these commercial products; the SDs were within 0.2–0.5. These results point out the accuracy and precision of the method and the absence of significant matrix effects on the potentiometric measurements.

3.6. Analytical application

A potentiometric titration was employed for determination of saccharin in commercial samples of artificial sweeteners. The results of potentiometric determinations of saccharin in commercial artificial sweeteners, along with those obtained by applying a HPLC procedure to the same samples are given in Table 2. The adopted HPLC method was that recommended by the FDA (1993). Comparison of the results comprised in Table 2 shows good agreement with the label values and also with the HPLC method thereby reflecting the utility of the proposed method.

Table 2
Saccharin determination in commercial artificial sweeteners

Samples	Label values[a]	Saccharin found (mg/ml or mg/g)[a,b]	
		Potentiometric method	HPLC
A (liquid)	83.0	84.7±0.3	83.2±0.6
B (liquid)	83.0[c]	82.9±0.7	81.6±0.9
C (powder)	19.0–23.0[c]	23.7±0.6	24.5±0.8
D (liquid)	60.0	61.5±1.8	60.0±1.2
E (powder)	19.0–23.0[c]	21.8±0.6	23.1±0.4
F (powder)	16.0–18.0[c]	17.6±0.5	18.4±0.6
G (liquid)	50.0	51.1±1.1	50.2±0.8
H (liquid)	60.0	61.7±0.9	62.9±1.2
X[d] (liquid)	60.0	59.8±0.7	61.3±0.6
Y[e] (liquid)	83.0	84.1±0.5	82.3±0.8

[a] Samples C, E and F powders are measured in milligrams per gram; all other samples are in milligrams per milliliter.

[b] Average±SD of five determinations per sample.

[c] Value obtained from the manufacturer.

[d] Sample X = H (liquid) + Cl^- (added in a concentration 20 times higher than that of saccharin); previous extraction with ethyl acetate was required.

[e] Sample Y = B (liquid) + Cl^- (added in a concentration 20 times higher than that of saccharin); previous extraction with ethyl acetate was required.

4. Conclusion

The potentiometric method proposed in this paper is simple, precise, rapid and low-cost. Its usefulness for saccharin determination in commercial artificial sweeteners was demonstrated, suggesting its use as a reliable and advantageous alternative to most other previously reported methods in the routine control of saccharin in these samples.

Acknowledgements

The authors thank FAPESP (Grant 97/01953-7), FUNDUNESP (Grant 080/98-DFP), CAPES and CNPq Foundations, Brazil, for financial support. A.O.S. is indebted to FAPESP for a fellowship (97/12267-7).

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