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A Simplified Spectrophotometric Method for Routine Analysis of Saccharin in Commercial Noncaloric Sweeteners

PATRÍCIA LOS WEINERT, HELENA REDIGOLO PEZZA, JOSÉ EDUARDO DE OLIVEIRA, AND LEONARDO PEZZA*

Instituto de Química, UNESP, P.O. Box 355, 14801-970, Araraquara, SP, Brazil

A simple, rapid, and sensitive spectrophotometric method for routine analysis of saccharin in commercial noncaloric sweeteners is proposed. This method is based on the reaction of saccharin with tetrachloro-*p*-benzoquinone (*p*-chloranil) accelerated by hydrogen peroxide and conducted in an ethanol:acetone (4:1) medium, producing a violet-red compound ($\lambda_{max} = 550$ nm). Beer's law is obeyed in a concentration range of 2.05×10^{-4} to 3.00×10^{-3} M with an excellent correlation coefficient ($r = 0.9998$). The detection limit was 1.55×10^{-5} M, and the effect of interferences on the spectrophotometric measurements was evaluated. The proposed procedure was applied successfully to the determination of saccharin in noncaloric sweeteners. Recoveries were within 99.2−104.3% with standard deviations ranging from to 0.5−1.6%. Results of the proposed method compare very favorably with those given by the high-performance liquid chromatography method recommended by the Food and Drug Administration.

KEYWORDS: Saccharin; noncaloric sweeteners; spectrophotometric determination

INTRODUCTION

Saccharin (*o*-benzoic-sulfimide, $C_6H_4COSO_2NH$) and its salts of sodium are white, odorless, crystalline powders and, in diluted solution, are about 400−500 times more potent than sucrose (*1*). Saccharin has been widely used in medicine and in a variety of food products as a nonnutritive sweetener as a substitution for sucrose by diabetics and others needing to restrict their intake of carbohydrates. For this reason, its use in industrialized foods increased vastly. In 1972, the Food and Drug Administration (FDA) in the United States retracted saccharin's GRAS status because of its carcinogenic potential (*2*). In 1977, the FDA announced its intention to ban saccharin; however, Congress placed a moratorium on the ban, which was renewed several times until recently when the FDA, on the basis of new safety assessment studies, retracted its efforts to ban saccharin and removed all requirements of warning labels on saccharin-containing products. Nowadays, saccharin is approved in more than 90 countries all over the world. The acceptable daily intake (ADI) value, determined by the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives, is 0−5 mg/kg body weight for saccharin (*3*).

To fulfill the requirements of quality control, several analytical methods have been described to determine saccharin. Included among these are polarography (*4*, *5*), gravimetry (*6*), derivative ultraviolet spectrophotometry (*7*), high-performance liquid chromatography (HPLC) (*3*, *8*−*11*), potentiometry (*12*−*15*), and visible spectrophotometry (*16*−*20*). However, most of these methods are time-consuming or require expensive equipment and, consequently, are not ideal for routine analysis.

Absorption measurements find widespread application for the identification and determination of myriad inorganic and organic species. Molecular ultraviolet/visible absorption methods are perhaps the most widely used of all quantitative analysis techniques in chemical and clinical laboratories throughout the world (*21*), owing mainly to its simplicity, often demanding low-cost equipment. As already mentioned, spectrophotometric methods of saccharin determination have been reported including reaction with azure B (*16*), Nile-blue (*17*), astrazone pink FG (*18*), iodine (*19*), and phenothazine (*20*). These methods are often time-consuming and tedious or require drastic reaction conditions. For example, phenothiazine (*20*) requires the use of a copper(II) catalyst and heating at 70 °C for 50 min before extraction into xylene. The method using iodine requires derivation (*19*) before quantification of saccharin. This method is based on the bromination of saccharin to form N-bromo derivative, which reacts with potassium iodide to liberate iodine. The iodine liberated is then reacted with leucocrystal violet, and the crystal violet dye formed shows λ_{max} at 593 nm.

From the above considerations, the need for a simple, fast, low-cost, and selective method for determination of saccharin seems clear. In the search for such a method, our attention was attracted to a spot test for primary arylamines and secondary amines described by Feigl (*22*), which is based on the reaction of these compounds with tetrachloro-*p*-benzoquinone (*p*-chloranil) in dioxane medium. Saccharin contains an imide group and forms a violet-red compound on reaction with *p*-chloranil

* To whom correspondence should be addressed. Tel: +55-16-201-6665. Fax: +55-16-222-7932. E-mail: pezza@iq.unesp.br.

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in the presence of hydrogen peroxide, according to preliminary experiments carried out in this laboratory.

On the basis of this verification, a new spectrophotometric method for routine analysis of saccharin in commercial noncaloric sweeteners is proposed. This method for direct determination of saccharin in ethanol:acetone (4:1) mixture is based on the reaction of saccharin with *p*-chloranil, accelerated by hydrogen peroxide, yielding a violet-red compound ($\lambda_{max} = 550$ nm). This reaction, as well as its acceleration by H_2O_2, has never been described in the literature and has been observed on the first time in our laboratory. The proposed method is simple, rapid, sensitive, and inexpensive.

The results agreed fairly well with those given by the HPLC method recommended by the FDA (*11*). The influence of interfering substances normally found along with saccharin in noncaloric sweetener samples is also studied.

MATERIALS AND METHODS

Apparatus. All absorbance measurements were carried out on a Hewlett-Packard HP 8453 spectrophotometer equipped with 1.00 cm silica cells. Brand and Eppendorf micropipets were used to measure the smaller volumes in the experiments.

Reagents. All chemicals used were of analytical reagent grade. The sodium saccharin stock solution, $C_7H_4NNaO_3S \cdot 2H_2O$ (8.50×10^{-3} M), was prepared daily by dissolving 0.1028 g of sodium saccharin (Synth, São Paulo, Brazil) in 10 mL of absolute ethanol (Mallinckrodt, Xalostoc, Mexico) by stirring for 15 min and then diluting to 50 mL with the same solvent.

p-Chloranil (Sigma, St. Louis, MO) solution (2.00×10^{-2} M) was prepared daily by dissolving 0.1230 g in 25 mL of acetone (Mallinckrodt). H_2O_2 solution (7.10 M) was prepared from a concentrated H_2O_2 30% (Merck, Darmstadt, Germany) by convenient dilution in ethanol (Mallinckrodt) and standardized as described in the literature (*23*).

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

Liquid Sweeteners. Aliquots of 250 or 350 μL of each liquid sweetener sample were transferred into a 50 mL Erlenmeyer flask, 10 mL of chloroform:ethanol (2:1) was added, and the mixture was stirred for 2 min. In the sequence, 2.00 mL of cold chloroform was added and the solution was filtered through Whatman no. 41 paper and washed with the same solvent. The filtrate was evaporated to dryness, and the dry residue was dissolved in ethanol. Finally, the resulting solution was filtered, washed with ethanol, transferred to a volumetric flask of 10 mL, and diluted to the mark with the same solvent.

Solid Sweeteners. An accurately weighed aliquot of 1.000 g of solid sweetener was transferred to a 50 mL Erlenmeyer flask, 7 mL of ethanol was added and then stirred for 15 min, and the solution was filtered through Whatman no. 41 paper and washed with the same solvent. The filtrate was evaporated until ca. 4 mL, and the solution was cooled in an ice bath for 5 min. Finally, the resulting solution was filtered and transferred to a volumetric flask of 10 mL and diluted to the mark with the same solvent.

Construction of Calibration Curve and Analysis of Samples. A calibration curve was prepared as follows: aliquots of saccharin stock solution (comprising 2.05×10^{-4} to 3.00×10^{-3} M of saccharin concentration) were transferred into 10 mL volumetric flasks. In the sequence, 2.00 mL of *p*-chloranil solution (2.00×10^{-2} M) and 0.50 mL of H_2O_2 (7.10 M) were added. Finally, the volume was completed with ethanol, and the absorbance was measured at 550 nm against the reagent blank (prepared in a similar way, but omitting saccharin) after placing the solution for 10 min at room temperature (25 °C). Calibration graphs are prepared by plotting absorbance against saccharin concentration. For analysis of the samples, convenient aliquots of each sample were used and analyzed according to the procedure above-described.

Reference Method. For accuracy assessment of the results obtained by the proposed method, the samples were analyzed by the HPLC method recommended by the FDA (*11*). The HPLC system used consisted of a Shimadzu SPD-10A liquid chromatograph (Shimadzu Seisakusko Kyoto, Japan) equipped with a LC-10AS Shimadzu pump, variable UV−visible detector (SDR-10A, Shimadzu) set at 254 nm, and a Rheodyne 20 μL injector. A stainless steel Supelcosil LC-18 analytical column was used (250 mm × 4.6 mm i.d., Supelco, Bellefonte, PA) 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 the peak area was measured with an integrator (Shimadzu C-R6A Chromatopac recording integrator).

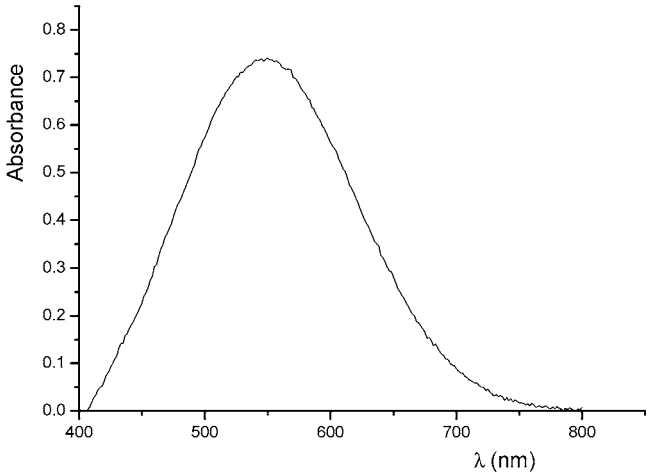

Figure 1. Absorption spectrum of the reaction product. Conditions: [Sodium saccharin] = 2.20×10^{-3} M, [*p*-chloranil] = 4.00×10^{-3} M, and [H_2O_2] = 0.35 M. Optical path = 1 cm. Measurements taken at 25 °C against the blank after the solution was placed for 10 min.

RESULTS AND DISCUSSION

The molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge transfer complexes and radical ions, which absorb radiation in the visible region of the spectrum (*24*). The spectrophotometric methods based on these interactions are usually simple and convenient because of the rapid formation of the complexes. Saccharin reacts with *p*-chloranil (π acceptor) in the presence of hydrogen peroxide resulting in the formation of an intense violet-red product, which exhibits an absorption maximum at 550 nm (**Figure 1**). The spontaneous formation of violet-red color from the yellow appearance of *p*-chloranil upon reaction with saccharin is sufficient evidence that a charge transfer complex has formed (*25*, *26*). This reaction is markedly accelerated by hydrogen peroxide; however, without hydrogen peroxide, the reaction is very slow (**Figure 2**). Probably, saccharin is oxidized by hydrogen peroxide to N-hydroxysaccharin, which donates an electron to *p*-chloranil to yield the intensely colored radical anion. Recently, N-hydroxysaccharin has been described as a novel carbon radical chain promoter (*27*).

The spectrophotometric properties of the colored product as well as the different experimental parameters affecting the color development and its stability were studied carefully. Such factors were changed individually while the others were kept constant. The factors include the effect of different organic solvents, the concentration of reagents (*p*-chloranil and H_2O_2), and the time to develop the color.

The influence of the solvents was studied using different organic solvents such as ethanol, methanol, and 1,4-dioxane. Ethanol, in the presence of H_2O_2, was found to be the best solvent to complete the volume of solutions, because it provides

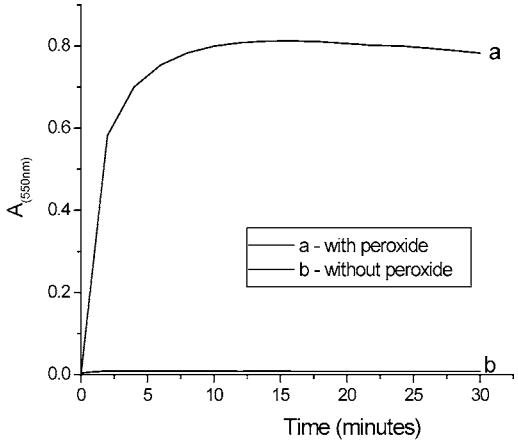

Figure 2. Absorbance changes at 550 nm as a function of time: (**a**) with H_2O_2 and (**b**) without H_2O_2. Conditions: [Sodium saccharin] = 2.54×10^{-3} M, [*p*-chloranil] = 4.00×10^{-3} M, and [H_2O_2] = 0.350 M. Optical path = 1 cm; T = 25 °C.

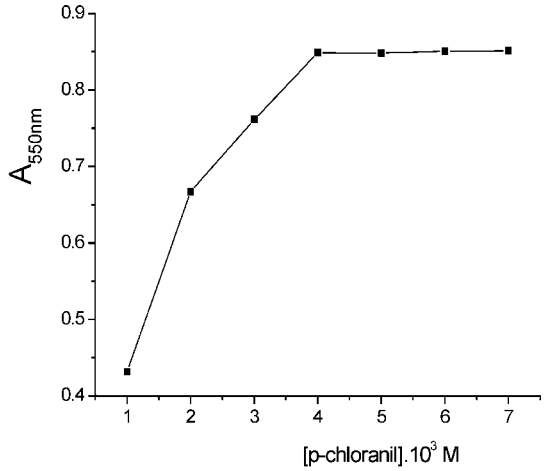

Figure 3. Effect of *p*-chloranil concentration on the proposed reaction for determination of saccharin. Conditions: [Sodium saccharin] = 2.54×10^{-3} M and [H_2O_2] = 0.35 M. The wavelength was set at 550 nm. Optical path = 1 cm. Measurements taken at 25 °C against the blank after the solution was placed for 10 min.

a rapid reaction time and also is less toxic than methanol or 1,4-dioxane.

Effect of *p*-Chloranil Concentration. The effect of *p*-chloranil concentration on the development of the reaction is shown in **Figure 3**; the result demonstrated that the $A_{550\ nm}$ increased with the increase of the *p*-chloranil concentrations, from 1.00×10^{-3} to 4.00×10^{-3} M, and then it remained unchanged when the *p*-chloranil concentration continually increased. Hence, the optimum concentration of *p*-chloranil was chosen as 4.00×10^{-3} M.

Effect of H_2O_2 Concentration. The effect of hydrogen peroxide concentration is shown in **Figure 4**, which demonstrates that the absorbance value increased in direct ratio with H_2O_2 concentration. $A_{550\ nm}$ maximum was reached when 0.35 M of H_2O_2 was added, and then, it remained constant. So this concentration was chosen as the optimum.

Effect of Reaction Time. With concentrations of reagents and solvent conditions selected, the optimum reaction times were determined by following the color development at ambient temperature (25.0 ± 0.3 °C) at different intervals. Complete color development was attained after 8 min, and the color remained stable for 20 min as shown in **Figure 2a**. This time

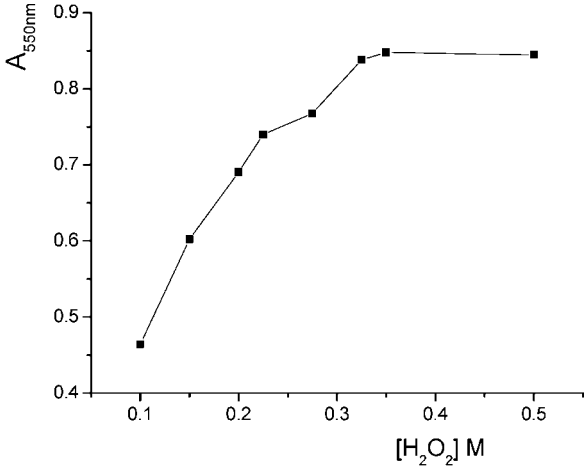

Figure 4. Effect of H_2O_2 concentration on the proposed reaction for determination of saccharin. Conditions: [Sodium saccharin] = 2.54×10^{-3} M and [*p*-chloranil] = 4.00×10^{-3} M. The wavelength was set at 550 nm. Optical path = 1 cm. Measurements taken at 25 °C against the blank after the solution was placed for 10 min.

is sufficient for analysis. An excess of reagents was required to reach equilibrium rapidly, thus minimizing the time required to attain maximum absorbance.

Analytical Characteristics. The developed analytical method was validated by evaluating linear dynamic range, precision, limit of detection (LOD), and limit of quantification (LOQ). Under the described experimental conditions, Beer's law was obeyed in the concentration range of 2.05×10^{-4} to 3.00×10^{-3} M with an excellent correlation coefficient (r = 0.9998). The regression equation was found to be $A = -0.0196 + 339.988C$, where A is the absorbance at 550 nm and C is the concentration of saccharin in molar. Assay precision was defined by determining intraday and interday variation, expressed as relative standard deviation (SD). The interday variation was evaluated over 3 days. The intraday precision and interday precision were studied for 10 replicate analyses of 1.30×10^{-3} M saccharin solution. The coefficients of variation were 0.8 and 1.4%, respectively. The LOD ($3.SD^{blank}$/slope of analytical curve) and LOQ ($10.SD^{blank}$/slope of analytical curve) were 1.55×10^{-5} and 5.15×10^{-5} M, respectively (*28*).

Effect of Interferences. The effects of the common excipients, which often accompany saccharin in noncaloric sweeteners commercialized in Brazil, were carefully examined. The excipients studied were lactose, fructose, sucrose, sucralose, benzoic acid, sorbitol, mannitol, starch, caffeine, citric acid, ascorbic acid, silicon dioxide, methyl- and *n*-propyl-*p*-hydroxybenzoate, and cyclamate. For this study, solutions containing saccharin and each one of the excipients taken separately in concentrations equal or 10 times greater than that of saccharin were shaken with ethanol in a magnetic mixer, diluted, and analyzed under the same conditions described in section of the construction of the calibration curve. No interferences were observed in the presence of the substances tested, except for cyclamate, which showed a strong interference in the absorbance value. Substances, such as aspartame and acesulfame K, also interfere in the proposed method. However, these substances are not found in noncaloric sweeteners commercialized in Brazil and in many other countries.

In applying the proposed method for preparations of noncaloric sweeteners, it is necessary to remove cyclamate from the mixture. This interference was quantitatively eliminated during sample preparation by precipitation of cyclamate in ethanol for

Table 1. Determination of Sodium Saccharin in Artificial Sweeteners by the Proposed Method and the HPLC Method (*11*)

		proposed method				HPLC method (*11*)	
samples	label values[a]	found[d]	recovery (%)[d]	*t* value (2.31)[e]	*F* value (6.94)[e]	found[d]	recovery (%)[d]
A[b]	83.0	82.8 ± 0.4	99.7 ± 0.4	1.6	4.0	82.4 ± 0.2	99.2 ± 0.3
B[b]	83.0	83.6 ± 0.7	100.7 ± 0.8	0.6	2.0	83.9 ± 0.5	101.1 ± 0.6
C[b]	60.0	59.5 ± 0.6	99.3 ± 1.0	0.8	4.0	60.0 ± 1.2	100.0 ± 1.3
D[b]	83.0	82.4 ± 1.3	99.2 ± 1.6	0.9	2.1	81.6 ± 0.9	98.3 ± 1.0
E[b]	83.0	84.3 ± 1.3	101.5 ± 1.6	0.1	2.1	84.4 ± 1.6	101.7 ± 2.0
F[c]	15.0	15.6 ± 0.2	104.3 ± 1.4	0.8	1.0	15.5 ± 0.2	103.6 ± 1.5

[a] Amount of sodium saccharin measured in mg/g for solid sample and in mg/mL for liquid samples. [b] Liquid samples. [c] Solid sample. [d] Average ± SD of five determinations per sample. [e] Theoretical values of *t* and *F* at 95% confidence level.

solid samples or ethanol:$CHCl_3$ (1:2) for liquid samples. This procedure is based on the low solubility of cyclamate in ethanol. In the liquid samples containing sorbitol in your composition, which increase the solubility of cyclamate in ethanol medium, the use of a mixture of ethanol:$CHCl_3$ (1:2) was necessary.

Analytical Application. The proposed method was applied to determine saccharin in commercial noncaloric sweeteners containing saccharin or saccharin in combination with cyclamate. The samples were analyzed by the proposed method and by the HPLC method (*11*) in replicate ($N = 5$). The results of the comparison of the proposed method with the HPLC method recommended by FDA (*11*) are shown in **Table 1**. For all samples assayed, the results obtained by FDA and proposed methods were compared statistically (*29*) by applying the *F* test and *t* test at a 95% confidence level. In all cases, the calculated *F* and *t* values did not exceed the theoretical values, indicating that there is no significant difference between either method concerning precision and accuracy in the determination of saccharin in noncaloric sweeteners. The average recoveries by the proposed method ranged from 99.2 to 104.3% and the SDs were smaller than 2.0% in all cases. In addition, this method can be applied for the saccharin determination in dietary foods and beverages containing saccharin or saccharin in combination with cyclamate.

In conclusion, the spectrophotometric method proposed is simple, sensitive, rapid, and low-cost, does not involve tedious manipulation steps, and gives precise and accurate results. Its usefulness for saccharin determination in commercial noncaloric sweeteners was demonstrated, suggesting its use as a reliable and advantageous alternative to most other previously reported methods for routine analysis of saccharin in these samples.

ABREVIATIONS USED

FDA, Food and Drug Administration; FAO, Food and Agriculture Organization; WHO, World Health Organization; ADI, acceptable daily intake.

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