SPECTROPHOTOMETRIC DETERMINATION OF SACCHARIN BY COUPLING WITH DIAZOTISED 4-NITROANILINE[+]

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Abstract

A spectrophotometric method is described for the determination of saccharin. The Intended reacts with diazotised 4-nitroaniline in alkaline solution to form a stable brown coloured azo dye. The absorbance is measured at 427 nm.

Beer's law is obeyed over the concentration range of 10 – 200 µg of saccharin in a final volume of 25 ml, i.e. , (0.4 – 8 ppm) with a molar absorptivity of 2.75×10^4 1. mol^{-1}, cm^{-1} and Sandell sensitivity index is 0.0088 µg. cm^{-2} , a relative error of – 0.541 to + 1.851 % and a relative standard deviation of a ± 0.35 to ± 2.2 %, depending on the concentration level. Further, the method requires neither temperature control nor solvent extraction.

المستخلص

يتضمن هذا البحث طريقة لتقدير السكرين، تعتمد الطريقة على مفاعلة السكرين مع ٤-نايتروأنيلين المؤزوت في الوسط القاعدي لتكوين صبغة آزوية ذات لون قهوائي مستقرة وتعطي أعلى إمتصاص عند ٤٢٧ نانوميتر، وكانت حدود قانون بير ٠,٤ – ٨ جزء / مليون جزء وكانت الامتصاصية المولارية ٢,٧٥ × ١٠[٤] لتر. مول[-١].سم[-١] ودلالة ساندل ٠,٠٠٨٨ مايكروغرام. سم[-٢] والخطأ النسبي بين – ٠,٥٤١ و + ١,٨٥١ % والانحراف القياسي النسبي بين ± ٠,٣٥ و ± ٢,٢ % اعتماداً على مستوى التركيز، فضلا عن ذلك فإن الطريقة لا تحتاج إلى ضبط درجة الحرارة ولا إلى عملية الاستخلاص بالمذيب.

Introduction

Saccharin was discovered by accident in the late 1800s by scientists at Johns Hopkins University. Though it's widely used to sweeten a multitude of commercial food products, including soft drinks, alcoholic beverages, canned goods, backed goods, candies and various deserts, as well as in the home [1,2].

Saccharin is a white crystalline powder having a taste about 500 times sweeter than can sugar [3] and 300 times sweeter than sucrose [4], used as a calorie-free sweeteners. It is also Also called ***benzosulfimide***. Saccharin sold as an artificial sweetener is usually the sodium salt of saccharin, which has the chemical formula ***$C_7H_4NNaO_3S.2H_2O$*** [3]

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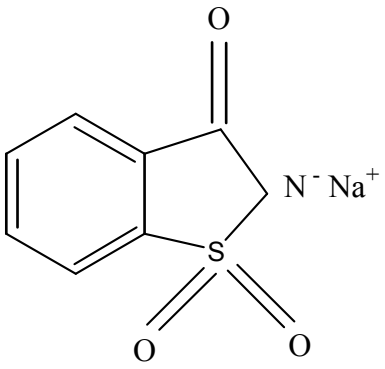


M.Wt = 241.20 gm/mole

Saccharine was an important discovery, especially for diabetes. Saccharin is not digested by the body and goes directly through the human digestive system. Therefore it does not affect blood insulin levels, and has effectively no food energy[5].

Various methods have been reported for the determination of Saccharin. These includes Chromatographic [6], Electrochemical [7] and spectrophotometric methods [8].

In the present investigation, 4-nitroaniline as a diazotising reagent has been used in the spectrophotometric determination of Saccharin.

Experimental

Apparatus

Spectrophotometric measurements are carried out on a SP 800 Ultraviolet Spectrophotometric (UNICAM, England).

Absorption spectra are carried out using Shimadzu UV-Visible Recording Spectrophotometric UV-160, with 1 cm matched silica cells.

The pH-measurements are performed using WTW pH 560,8120 Weilheim.

Reagents

Stock standard Saccharin (10 mg ml^{-1}): A 1.3167 g amount of pure saccharin sodium was dissolved in 100 ml distilled water.

Working saccharin solution (100 $\mu g.ml^{-1}$): A 1-ml volume of saccharin stock solution was diluted to 100 ml with distilled water.

Sodium nitrite solution (1%): Prepared by dissolving 1.0 g of sodium nitrite in 100 ml distilled water in a volumetric flask. This solution was kept in a brown bottle and is stable for, at least, one week.

Diazotised 4-nitroanilin(25mM): A 0.691 g of 4-nitroaniline is dissolved in about 50 ml of distilled water, containing 2 ml glacial acetic acid, then 6.6 ml of concentrated HCl is added and the solution is heated. The mixture is transferred to a 200 ml volumetric flask and is cooled to $\approx 5^{\circ}C$. A 34.5 ml of 1% $NaNO_2$ is added and the mixture is stirred occasionally for 5 min and the volume is completed to 200 ml

with additional (≈5°C) cooled distilled water. This solution is stored in darkness over ice and used after 15 min. This reagent solution when kept in the refrigerator (≈5°C) is stable for 3 days at least [9].

Sodium hydroxide solution (40%): This solution is prepared by dissolving 40 g of sodium hydroxide in distilled water. Then the volume is complemented to 100 ml in a volumetric flask with distilled water and transferring it to a plastic bottle.

Recommended Procedure

Into a series of 25ml volumetric flasks , increasing volumes of saccharin working solution (100 μg.ml^{-1}) is transferred followed by 5 ml of 25 mM diazotized 4-nitroaniline. The mixtures are shaken. After 25 min, 6 ml of 40% sodium hydroxide solution is added and the volume is made to the mark with distilled water. The absorbances are measured at 427 nm against the corresponding reagent blank, using 1-cm cells.

Results and Discussion

For the following experiments, 100 μg of saccharin were taken in a final volume of 25 ml.

Absorption Spectra

An intense brown coloured azo dye is formed as a result of mixing dilute aqueous solution of saccharin and diazodised 4-nitroaniline in the presence of sodium hydroxide. The intense azo dye formed shows maximum absorption at 427 nm (Fig. 1), in contrast to the reagent blank.

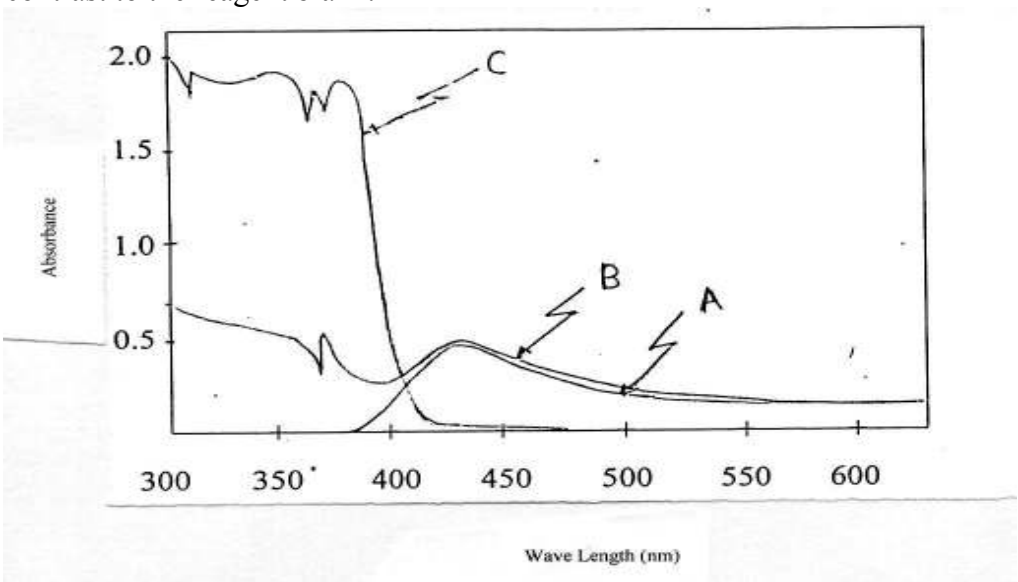


Fig. 1.: Absorption spectra of 100 μg saccharin / 25 ml treated according to the recommended procedure, and measured against (A) blank (B) distilled water and (C) blank measured against distilled water.

Choice of Diazotized Reagent

In order to achieve the optimum spectrophotometric conditions, five diazotised reagents are tested. These reagents are diazotised sulphanilic acid, diazotised

anthranilic acid, diazotised 2,4-dinitroaniline, diazotised 4-aminobenzoic acid and diazotised 4-nitroaniline. Diazotised 4-nitroaniline, has been selected since it satisfies certain requirements (intensity of the azo dye formed and rapid coupling rate).

Amount of Diazotised Reagent

A 5-25 mM diazotised 4-nitroaniline reagent solution in different volumes has been examined for optimal recording of absorbance. The results are given in Table 1.

Table 1. Effect of the amount of diazotised 4-nitroaniline on absorbance

mM of diazotised 4-nitroaniline	Type of Absorbance	Absorbance / ml of diazotised reagent					
		1	3	5	7	10	15
5	A	0.061	0.081	0.100	O.108	0.110	0.123
	B	0.027	0.028	0.030	0.050	0.048	0.051
10	A	0.120	0.125	0.130	0.137	0.135	0.140
	B	0.026	0.030	0.028	0.022	0.030	0.029
15	A	0.180	0.199	0.220	0.250	0.298	0.310
	B	0.016	0.023	0.027	0.031	0.035	0.040
20	A	0.355	0.347	0.365	0.392	0.410	0.412
	B	0.022	0.015	0.023	0.018	0.019	0.018
25	A	0.417	0.449	0.456	0.452	0.451	0.456
	B	0.018	0.022	0.019	0.017	0.021	0.020

A: Absorbance of azo dye against blank.
B: Absorbance of blank against distilled water.

From the results, 5 ml of 25 mM diazotised 4-nitroaniline solution has been selected for the subsequent experiments. This amount of reagent gives higher sensitivity with low absorbance of blank at λ max = 427 nm.

Effect of Base

The preliminary experiments have shown that saccharin can give coloured dye with diazotised 4-nitroaniline only in strongly basic medium. Table 2 shows the results.

Table 2. Effect of base on absorbance

ml of 40% base	Type of absorbance	Absorbance/ ml of base				
		2	4	6	8	10
KOH	A	0.380	0.410	0.4198	0.420	0.422
	B	0.022	0.018	0.027	0.030	0.04
NaOH	A	0.445	0.450	0.453	0.452	0.456
	B	0.021	0.017	0.018	0.020	0.022

A: Absorbance of azo dye against blank.
B: Absorbance of blank against distilled water.

From the results, 6 ml of 40% NaOH (final pH $\approx$ 14) is selected for the subsequent experiments.

Effect of Time

The effect of standing time (before adding the base) on the production of the azo dye has been investigated. The results are shown in Table 3.

Table 3. Effect of time on absorbance

Standing time*,min	0	5	10	15	20	25	30	35
Absorbance	0.125	0.222	0.339	0.415	0.449	0.456	0.452	0.455

* Before adding the base.

From the data given in the above table, 25 min standing time (before adding the base) is selected for the subsequent work.

Order of Addition of Reagents

Different orders for the addition of the base (6ml of 40% NaOH) have been examined. The experimental data reveal that the presence of a base is essential for developing reaction. If the base is added to saccharin before diazotised 4-nitroaniline the azo dye formation diminishes completely because the base will compete with saccharin for diazotised 4-nitroaniline and the later is converted to the corresponding diazonium hydroxide ($Ar–N=N–O^-$)with inactive towards coupling [10].

Effect of Surfactants

Different amounts (1-5 ml of 10^{-3} M) of different surfactants (Sodium dodecyl sulphate (SDS), Cetylpyridinium chloride(CPC), Tween 80) in different orders have been tested and none has been found successful.

Effect of Time on Colour Development

Under the conditions optimized above, the brown azo dye develops immediately and it's intensity does not change experimentally for at least 1 hour. The stability of the coloured azo dye is sufficient for a large number of measurements to be performed at the same time.

Calibration Graph

Employing the conditions described under recommended procedure , calibration graph (correlation coefficient r = 0.9999) for the determination of microgram amounts of saccharine was obtained with slope of (0.0045) and intercept of (+ 0.00013). Beer's law is obeyed over the concentration range 0.4-8 μg. ml^{-1} of saccharin (Fig 2) with a molar absorptivity of 2.75×10^4 1.mol^{-1}. cm^{-1} and Sandell sensitivity of 0.0088 μg. cm^{-2}.

$$y = 0.0045x + 0.00013$$
$$R = 0.9999$$

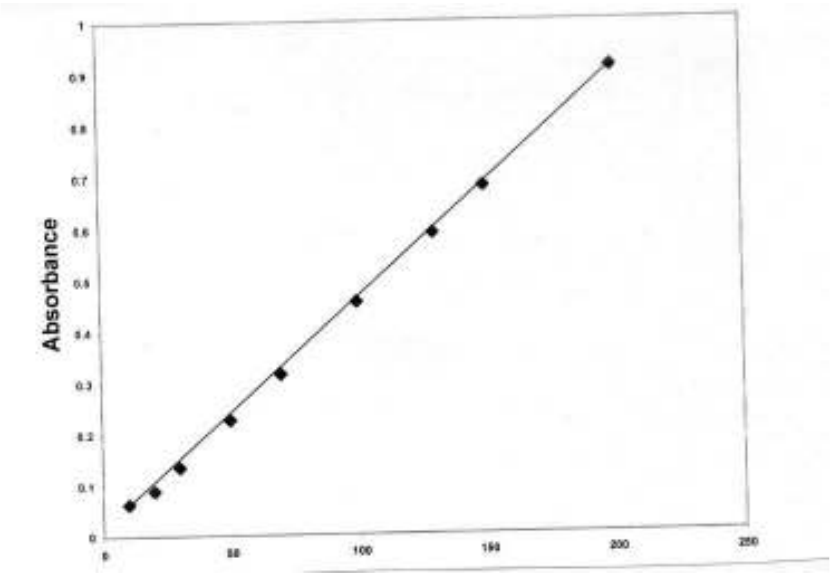


Fig 2.: Calibration graph of saccharin determination

Accuracy and Precision

To check the accuracy and precision of the method, saccharin was determined at three concentrations. The results shown in Table 4 indicate that the present method is accurate and precise.

Table 4. Accuracy and precision of the proposed method

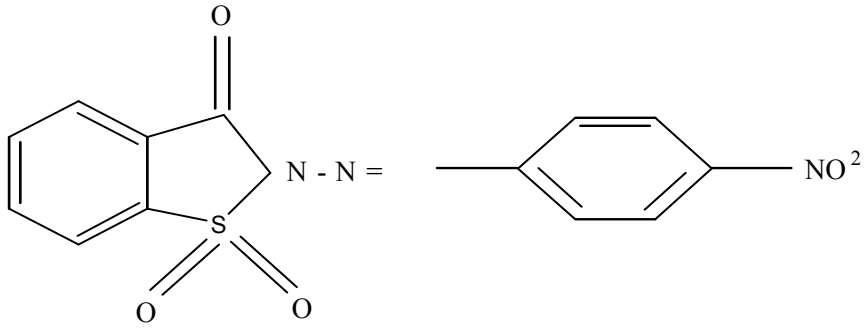
Amount of saccharin taken/μg.ml^{-1}	Relative error*, %	Relative standard deviation*, %
10	+ 1.851	± 2.2
50	– 0.541	± 1.8
100	0	± 0.35

* from five determinations.

The Nature of Reaction Product

To estimate the empirical formula of the azo dye species formed under the present conditions as described in the recommended procedure Job's method of continuous variations method and More-ratio method are used. The results obtained show that a 1:1 product has been formed between saccharin sodium and diazotized 4-

nitroaniline at 427 nm. Therefore, the structure of the dye formed may be written as follows:



Brown Azo Dye

The conditional stability constant at the dye in an aqueous solution, under the established experimental conditions, is 0.454×10^{7} Molar $^{-1}$.

Interferences

The effect of a variety of compounds was studied. The results are shown in Table 5. It can be concluded that a simple and sensitive spectrophotometric method for the determination of trace amounts of saccharin in aqueous solution needless of temperature control and solvent extraction step has been developed. It is based on the reaction of saccharin with diazotised 4-nitroaniline in basic medium aqueous solution.

Table 5. Effects of 5-fold excess of foreign compounds on the determination of 10 μg. ml $^{-1}$ of saccharin

Foreign Compound	Error for saccharin determination %
∝ – Amino Succinic acid (aspartame)	+ 0.8
γ – Amide, ∝ – Amino Succinic acid (aspargine)	+ 2.3
Benzoic acid	– 1.7
Citric acid	+ 2.8
4 – Hydroxy Benzoic acid	– 2.3
Malic acid	+ 1.8
Magnesium chloride	– 2.5
Sodium Cyclamate	+ 1.9
Sodium hydrogen carbonate	– 1.1
Starch	– 3.1
Vitamin – C	+ 1.6

Conclusion

A simple, rapid and sensitive spectrophotometric method has been developed for the determination of trace amounts of saccharin in aqueous solution, based on the reaction of saccharin with diazotised 4-nitroaniline reagent in basic medium. The proposed method does not require either temperature control or a solvent extraction step.

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