CHAPTER SIX

Spectrophotometric Determination Of Saccharin In Food And Pharmaceutical Products
Spectrophotometric Determination Of Saccharin In Food And Pharmaceutical Products

Summary

A simple and highly sensitive method is described for the spectrophotometric determination of saccharin. The method is based on the bromination of saccharin to form N-bromoderivative, which reacts with potassium iodide to liberate iodine. The liberated iodine is then reacted with leucocystal violet and the crystal violet dye formed shows maximum absorbance at 593 nm. The colour system obeys Beer's law in the concentration range of 0.05 - 0.5 µg ml⁻¹ of saccharin and has apparent molar absorptivity of $2.93 \times 10^5$ l mol⁻¹ cm⁻¹. The method is free from interferences of common diverse ions and many of the ingredients commonly found in food products and has been satisfactorily applied to the determination of saccharin in food and pharmaceuticals.

Introduction

Saccharin (o-benzoic sulfamide, C₇H₆COSO₂NH) and its salts are white crystalline powders, odourless and in diluted solution are about 400 - 500 times sweeter than sucrose (1). Due to its characteristics properties, it is one of the most consumed artificial sweetener in some countries. It has been widely used in medicine and in variety of food products as a non-nutritive sweetner (2). It is used primarily in disease such as diabetes, where it is harmful for the patient to use sugar and persons dieting to lose weight often use saccharin in place of sugar (3 - 4). In order to avoid excessive use, several countries have set tolerance limit (5). Saccharin is very sweet in dilute solution but is bitter in concentrated solution (3).

The artificial sweetener saccharin is a weak bladder carcinogen and causes risk to human and animal (4 - 8). When saccharin is given in diet it causes cancer of the urinary tract, when it is inserted in bladder as an implant saccharin, it causes bladder cancer (9). Recent studies suggested that high doses of saccharin and sodium saccharin produces cytotoxicity of the urothelium and consequently regeneration hyperplasia (10). It enhanced cell proliferation and promote tumor (11). Due to its possible carcinogenic effects its use in food products is prohibited. So, due to medical and legal aspects the determination of saccharin and other non-fattening artificial sweeteners in dietary products has an economical and social relevance (4).

Due to its wide use in food products such as canned fruit juices, vegetables, cookies, other backery products, flavouring extracts, gelatin, pudding, frozen desserts, imitation jams, jellies, marmalades and salad dressing etc. (12) and beverages, literature contains numerous methods for the determination of saccharin in various food products, beverages and pharmaceuticals. Various techniques such as gas chromatography (13-15), thin layer chromatography (16-20), high performance liquid chromatography (21-24), gas liquid chromatography (25 -26), polarography (27-28), fluorimetry (29), ultraviolet spectrophotometry (30-32), infrared spectrophotometry (33), gravimetry (34-35), molecular emission cavity analysis (36), ion selective electrode (37), flow injection analysis (38), volumetric (39), potentiometric (40-41) etc. are being used for its determination.

A number of methods have also been reported for the spectrophotometric determination of saccharin (5, 28, 42-49). The common reagents reported include azure B (42), tris-(1:10 phenanthroline)-iron II chelate (43), methylene blue (44), chlorophenothiazine (45), phenothiazine (5), azure C (46), ferroin (47), phenol-sulphuric acid (28, 48) and starch-CdI₂ (49). Nevertheless most of these methods are time consuming and laborious. While others either need costly equipment or have limitations in sensitivity, selectivity and instability of colour.

Here a simple and non expensive manual method is proposed for the determination of saccharin. The method is based on the bromination of saccharin to form N-bromoderivatives.
which on reaction with potassium iodide liberates iodine. The liberated iodine selectively oxidises leucocystal violet and the crystal violet dye so formed shows maximum absorbance at 593 nm. Various analytical parameters were investigated for optimum sensitivity. The method is free from the interference of a number of substances commonly found in food products and has been applied to the determination of saccharin in food and pharmaceuticals.

Experimental

Apparatus - A Systronics UV-VIS Spectrophotometer 108 with matched silica cells was used for all spectral measurements. A Systronics pH meter model 331 was used for pH measurements.

Reagents - All chemicals used were of AnalR grade and double distilled water was used throughout the experiment.

Saccharin - A stock solution containing 1mg/ml of saccharin was prepared by dissolving 100 mg of saccharin in 100 ml of double distilled water and standardized volumetrically (39).

Bromine water - A saturated solution of bromine in water was prepared. The solution was prepared daily.

Formic acid - A 50% aqueous solution.

Potassium iodide (E. Merck, Germany) - 0.1% aqueous solution.

Leucocystal violet (LCV) (Eastman Kodak Co., New York) - To a 1 l volumetric flask 200 ml water, 3 ml 85% phosphoric acid and 200 mg of leucocystal violet were added and shaken gently until the dye gets dissolved. The content of the flask was then diluted to 1l with water. This solution was stable for several months when kept in dark.

Sodium hydroxide - 1 M aqueous solution.

Procedure

Preparation of Calibration Curve - An aliquot of working standard solution containing 1.25-12.5 µg of saccharin was taken in 25 ml calibrated tubes. To it 0.5 ml of bromine water was added and the mixture was shaken gently for 2 min. The excess of bromine was removed by dropwise addition of formic acid (30) after which 1 ml of potassium iodide was added. The yellow solution obtained was shaken gently for few seconds. Then 1 ml of LCV solution and 4-5 drops of 1 M sodium hydroxide were added. The solution was kept in a thermostat maintained at 55°C for 5 min. The solution was cooled diluted to 25 ml with deionised water and kept for 10 min for full colour development and measured at 593 nm against a reagent blank.

Determination of Saccharin in Soft Drinks - Samples of different soft drinks were decarbonated by repeated shaking and pouring from one beaker to another. 10 ml of the solution was transferred into a 60 ml separating funnel. 1 ml of 10% sulphuric acid was added
and the mixture was equilibrated with 2 x 6 ml of diethyl ether. The lower aqueous layer was discarded. The upper ether layer was equilibrated with 2 x 2 ml of 2% sodium hydrogen carbonate solution. The ether layer was discarded and aqueous layer was acidified with 2 ml of 5% hydrochloric acid and then extracted with 2 x 5 ml of diethyl ether into a conical flask. All of the ether was evaporated from the extract on a hot water bath. The saccharin residue was dissolved in 10 ml of water and transferred completely into a calibrated flask and made up to 25 ml with water (42). Aliquots were then analysed as described above. Results are shown in Table 1.

**Determination of Saccharin in Jam, Condensed Milk and Ice-Creams** - 1 gm of each sample such as jam, condensed milk and icecreams were dissolved in 10 ml of water and deproteinised by adding 25% lead acetate (51) and acidified with 2 ml of 5% hydrochloric acid then extracted with 3 x 6 ml of diethyl ether. The etheral solution was then washed with 5 ml of water. The ether layer was separated and evaporated on a hot water bath. Residue was dissolved in 10 ml of water and transferred completely into a calibrated flask and the volume was made up to 25 ml (51) and aliquots were then analysed as described above. Results are given in Table 1.

**Determination of Saccharin in Pharmaceuticals** - All drug samples tested were purchased from the local pharmacy. A saccharin tablet was ground, into a fine powder, weighed and stirred for 2-3 min with 50 ml of deionised water. 1 ml of 5% EDTA was added and the solution was filtered through Whatman no. 40 filter paper. The insoluble mass was washed with three successive 5 ml portions of water and the filtrate plus washing were diluted to volume in a 250 ml calibrated flask (42). A known volume was further diluted depending upon the saccharin content and the colour of the sample. Aliquots were analysed by recommended procedure. Results are shown in Table 2.

**Determination of Saccharin in Cosmetic Samples** - Various samples of tooth paste (1 gm of each) were dissolved in 25 ml of hot water. The solution was cooled and centrifuged at 1850 g for 5 min. The supernatant solution was acidified with 10% hydrochloric acid and saccharin was extracted into 3 x 6 ml of ethylacetate. The combined extract was evaporated to dryness and the residue was dissolved in 25 ml of water (52). Aliquots were then analysed as described above. The results are shown in Table 1.

**Results and Discussion**

**Spectral characteristics** - The absorption spectra of crystal violet dye formed in the proposed reaction shows maximum absorbance at 593 nm. The reagent blank gave negligible absorbance at this wavelength (fig 1).
Adherence to Beer's law, Molar absorptivity and Sandell's sensitivity - The colour system obeys Beer's law over the concentration range of 1.25-12.5 µg of saccharin per 25 ml of final solution (0.05-0.5 ppm) at 593 nm (fig 2). The molar absorptivity and Sandell's sensitivity were found to be $2.93 \times 10^5$ l mol$^{-1}$ cm$^{-1}$ and 0.0006 µg cm$^{-2}$ respectively.

Effect of reagent concentration - It was found that under optimum condition 0.5 ml of bromine water, 1 ml of 0.1% potassium iodine, 1 ml of LCV and 4-5 drops of 1 M sodium hydroxide were required for full colour development. A few drops of formic acid was sufficient for the removal of excess of bromine. It was also found that if the proposed reagent concentration is reduced or increased the absorbance value decreases (fig 3).

Effect of time - It was found that about 2 min were sufficient for bromination and iodine was liberated on addition of potassium iodide immediately. Full colour development required about 15 min. It was found that once developed the colour was stable for several days (fig 4).

Effect of temperature - 65°C-65°C temperature was found to be most suitable for colour reaction. The temperature was obtained by keeping the solution in a thermostat maintained at about 55°C. At higher and lower temperature the colour gradually faded (fig 4).

Effect of pH - pH of 4.5-5.5 was found to be best for obtaining constant and maximum absorbance values. Increase of pH above 5.5 severely affected the stability and sensitivity of the dye. Colour development did not take place below pH 4.5.

Reproducibility - Reproducibility of the method was found by analysing 10 µg per 25 ml of the dye over the period of seven days. Standard deviation and relative standard deviation were found to be ±0.011 and 1.83% respectively.

Interference studies - The validity of the method was assessed by investigating the effect of common foreign species added to a solution containing 10 µg per 25 ml of saccharin. Table 3, shows that saccharin can be selectively determined in the presence of many substances that are likely to be present in the soft drinks and the saccharin tablets. Most of the common ions did not interfere in the procedure.

Colour Reaction

The colour reaction involves the following steps (Scheme A) —

1. Formation of N-bromoderivatives with bromine water (49).
2. Liberation of iodine by the reaction of N-bromoderivative with potassium iodide in acetic medium.
3. Liberated iodine oxidises the leucocrystal violet to form crystal violet dye.
Application

The method has been satisfactorily applied to the determination of saccharin in food products, pharmaceuticals and cosmetic products. Table 1-2. The results of pharmaceuticals analyses obtained by the proposed method also agreed with the claimed value on the labels in all instances. Table 2.

To check the validity of the method known amount of saccharin was added to various saccharin free samples such as icing sugar, fresh apple, oranges and grapes juices and then determined by the proposed as well as reported method (28, 48) Table 4. The recoveries of saccharin added to different samples were found to be 95.8-98.4% which is in agreement with the results obtained by the established methods indicating the accuracy of the proposed method.

Conclusion

It can be concluded that the proposed method is simple, rapid, accurate and reproducible and can be further automated for the routine measurements. The method has been compared favourably with most of the other methods and found to be highly sensitive and selective. Table 5.
Scheme A - Colour Reaction of Saccharin.

(1) Saccharin + Br₂ → N-bromodervative

SO₂

CO

N

(2) NBr

SO₂

CO

N

KI

I₂

Iodine

N

(3) Leucocrystal Violet + I₂ → Crystal Violet dye

\( \lambda_{\text{max}} 593 \ \text{nm} \)
WAVELENGTH, nm
A: CONCENTRATION OF SACCHARIN: 10 µg/25 ml
B: CONCENTRATION OF SACCHARIN: 5 µg/25 ml
C: REAGENT BLANK

fig 1. - ABSORPTION SPECTRA OF THE DYE AND REAGENT BLANK

fig 2. - CALIBRATION CURVE FOR DETERMINATION OF SACCHARIN
fig 3. - EFFECT OF AMOUNT OF BROMINE WATER AND POTASSIUM IODIDE ON COLOUR REACTION

fig 4. - EFFECT OF TEMPERATURE AND TIME ON COLOUR REACTION
CONCENTRATION OF SACCHARIN: 10 µg/25 ml

fig 5. - EFFECT OF pH ON COLOUR REACTION
### Table 1. Determination of saccharin in food and cosmetic samples.

<table>
<thead>
<tr>
<th>Sample mass/volume</th>
<th>Saccharin found (μg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
</tr>
<tr>
<td>Soft drinks³</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4.94(±0.020)</td>
</tr>
<tr>
<td>B</td>
<td>5.41(±0.025)</td>
</tr>
<tr>
<td>C</td>
<td>3.07(±0.021)</td>
</tr>
<tr>
<td>Ice-creams²</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6.56(±0.017)</td>
</tr>
<tr>
<td>B</td>
<td>6.61(±0.020)</td>
</tr>
<tr>
<td>C</td>
<td>6.09(±0.023)</td>
</tr>
<tr>
<td>Condensed milk³</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.05(±0.035)</td>
</tr>
<tr>
<td>Jam²</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.77(±0.020)</td>
</tr>
<tr>
<td>Tooth paste³</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>8.64(±0.011)</td>
</tr>
<tr>
<td>B</td>
<td>8.12(±0.020)</td>
</tr>
<tr>
<td>C</td>
<td>8.75(±0.020)</td>
</tr>
</tbody>
</table>

P = Proposed method  
R = Reported method  
*Mean of three replicate analysis.  
²²³a = 10 ml of sample was treated as described in the procedure section and then 1 ml of aliquot was analysed.  
²²⁵b, c, d, e = 1 gm of sample was treated as described in the procedure section and then 1 ml of aliquot was analysed.

### Table 2. Determination of saccharin in pharmaceuticals.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Saccharin mg/tablet³</th>
<th>Claimed value mg/tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>R</td>
</tr>
<tr>
<td>Tablet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>11.80 (1.66%)</td>
<td>11.60</td>
</tr>
<tr>
<td>B</td>
<td>51.20 (0.58%)</td>
<td>51.00</td>
</tr>
<tr>
<td>C</td>
<td>12.40 (0.83%)</td>
<td>12.30</td>
</tr>
</tbody>
</table>

³a = Mean of three replicate analysis.  
³P = Proposed method, R = Reported method²²⁵
Table - 3. Effect of foreign species.

Concentration of saccharin = 10 µg / 25 ml (0.4 ppm)

<table>
<thead>
<tr>
<th>Foreign species</th>
<th>Tolerance limit* (µg/ml, ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>80</td>
</tr>
<tr>
<td>Citric acid</td>
<td>500</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>600</td>
</tr>
<tr>
<td>Gelatin</td>
<td>750</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>850</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>1000</td>
</tr>
<tr>
<td>Malic acid</td>
<td>1500</td>
</tr>
<tr>
<td>Sucrose</td>
<td>3000</td>
</tr>
<tr>
<td>Glucose</td>
<td>4000</td>
</tr>
<tr>
<td>Co²⁺, Cu²⁺</td>
<td>400</td>
</tr>
<tr>
<td>Cr³⁺, Al³⁺, Zn²⁺, Be²⁺, Fe³⁺</td>
<td>850</td>
</tr>
<tr>
<td>PO₄³⁻, Acetate, SO₄²⁻</td>
<td>2500</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>4000</td>
</tr>
</tbody>
</table>

*The amount causing ±2% error in absorbance value.
Table - 4. Recovery of saccharin in saccharin free food product and fresh fruit juices.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Saccharin added</th>
<th>Total saccharin found* (µg)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>R²#</td>
<td>P</td>
</tr>
<tr>
<td>Icing sugar⁻</td>
<td>10.00</td>
<td>9.73 (±0.020)</td>
<td>9.53</td>
</tr>
<tr>
<td>Apple juiceᵇ</td>
<td>10.00</td>
<td>9.58 (±0.030)</td>
<td>9.52</td>
</tr>
<tr>
<td>Orange juiceᶜ</td>
<td>10.00</td>
<td>9.68 (±0.011)</td>
<td>9.60</td>
</tr>
<tr>
<td>Grapes juiceᵈ</td>
<td>10.00</td>
<td>9.73 (±0.017)</td>
<td>9.65</td>
</tr>
</tbody>
</table>

P = Proposed method, R = Reported method
Size of sample: a = 1 gm, b, c, d = 10 ml.
* Mean of three replicate analysis.

Table - 5. Comparison with spectrophotometric methods.

<table>
<thead>
<tr>
<th>Method/Reagent/Ref.</th>
<th>λ.max (nm)</th>
<th>Beer's law range/ detection limit (ppm)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol-Sulphuric acid⁻</td>
<td>575</td>
<td>0.4</td>
<td>Long waiting time (2h), corrosive reagent used less sensitive.</td>
</tr>
<tr>
<td>Azure Bᵇ</td>
<td>685</td>
<td>2-68</td>
<td>Less sensitive.</td>
</tr>
<tr>
<td>Phenothiazine⁵</td>
<td>510</td>
<td>20-400</td>
<td>Less sensitive and selective.</td>
</tr>
<tr>
<td>Starch-CdI₂⁰</td>
<td>600</td>
<td>0.4-1.6</td>
<td>Less sensitive.</td>
</tr>
<tr>
<td>LCV (Proposed method)</td>
<td>593</td>
<td>0.05-0.5</td>
<td>Sensitive, free from most of the interferants.</td>
</tr>
</tbody>
</table>
References:

44. Bellagy Y.A.; Rida S.M. and Issa A., Pharmazine, 1974, 29, 64.
51. Whittle E.G., Analyst, 1944, 69, 45.