CHAPTER II

MATERIALS AND METHODS

MATERIALS :-

1. Chemical reagents
2. Organic solvents
3. Apparatus

1. Chemical reagents

1.1 Benzoic acid, B.P. , Powder

1.2 Sulfuric acid, A.R.

1.3 Ferric chloride, Lab. Grade

1.4 Hydrochloric acid, A.R.

1.5 Lead acetate, A.R.

1.6 Resorcinol, Lab. Grade

1.7 Saccharin sodium

1.8 Sodium carbonate, anhydrous, A.R.

1.9 Sodium hydroxide, A.R.

1.10 Sodium sulfate, anhydrous, A.R.

2. Organic solvents

2.1 Carbon tetrachoride, Lab. Grade

2.2 Chloroform, Lab Grade

2.3 Diethyl ether, A.R.

2.4 Ethanol, Lab. Grade

3. Apparatus

3.1 Analytical balance, Mettler

3.2 Büchi melting point apparatus

3.3 Unicam AR 25 Linear Recorder

3.4 Unicam SP 1800 Ultraviolet Spectrophotometer

METHODS

1. Preparation of stock reagents for detection and determination

2. Preparation of standard saccharin from commercial saccharin sodium

3. Preparation of standard saccharin solution

4. Preparation of sample for analysis

5. Method of analysis

1. Preparation of stock reagents for detection and determination

1.1 Benzoic acid solution, 0.1 per cent

Dissolve 100 mg of benzoic acid in 10 ml of ethanol and dilute to volume in 100 ml volumetric flask with distilled water.

1.2 Ferric chloride test solution

Dissolve 10 g of ferric chloride in distilled water and adjust to 100 ml.

1.3 Hydrochloric acid solution, 1 : 4

Dilute 200 ml of hydrochloric acid with distilled water in 1,000 ml graduated beaker and make up to volume.

1.4 Lead acetate solution, 5 per cent

Dissolve 10 g of lead acetate in distilled water and adjust to 200 ml.

1.5 Sodium carbonate solution, 1 per cent

Dissolve 10 g of sodium carbonate in distilled water and adjust to 1,000 ml.

1.6 Sodium hydroxide solution, 0.5 N

Dissolve 10 g of sodium hydroxide in distilled water and make up to 500 ml.

1.7 Sodium hydroxide solution, 10 per cent

Dissolve 100 g of sodium hydroxide in distilled water and adjust to 1,000 ml.

2. Preparation of standard saccharin from commercial saccharin sodium

Commercial saccharin sodium (2 g) was dissolved in 100 ml of 0.5 N sodium hydroxide solution, then extract with chloroform (2 x 50 ml). Collect the aqueous (upper) layer. Hydrochloric acid solution (1 : 4) was added to the aqueous solution until the solution was acid (tested with universal indicator paper), some

part of saccharin in solution was precipitated because of the insolubility of saccharin in water. The precipitated saccharin was filtered through a Büchner funnel under reduced pressure and washed 2-3 times with each of 10 ml distilled water, then dried in a vacuum desiccator. The mother liquor (after saccharin precipitated was filtered) was extracted with carbon tetrachloride (2 x 50 ml) to remove impurity. Keep the aqueous (upper) layer. Then extract with diethyl ether (3 x 50 ml), the ethereal extracts were combined and washed with 10 ml distilled water and was evaporated on a water bath in the hood. Dry the residue in a vacuum desiccator. The saccharin obtained was dissolved in sufficient quantity of 0.5 N sodium hydroxide solution so that it was just completely dissolved, and was repurified using the same procedure as above.

The repurified saccharin was checked for purity as follows :

2.1 Melting point determination

Melting point of saccharin is 228.8°-229.7°C (Stecher, 1968).

Determine the melting point of the repurified saccharin using Büchi Melting Point Apparatus. It has melting point of 229°C.

2.2 Saccharin spectra

Both saccharin and saccharin sodium, in 1 per cent sodium carbonate solution give characteristic double absorption maxima at 229 nm and 235 nm (Hussein, Jacin, Rodriguez, 1976) which are shown in Figure 5.

Spectra of repurified saccharin is shown in Figure 6.

3. Preparation of standard saccharin solution

3.1 Standard saccharin stock solution, 100 mg/100 ml

Dissolve 100 mg of the repurified saccharin in 1 per cent sodium carbonate solution and adjust to 100 ml.

3.2 Standard saccharin solution, 2 mg/100 ml

Accurately transfer 2 ml of saccharin stock solution into a 100 ml volumetric flask and dilute to volume with 1 per cent sodium carbonate solution.

4. Preparation of samples for analysis

4.1 Soft Drinks and Carbonated Beverages

Decarbonated the beverage by repeat shaking and pouring from one beaker to another. Transfer 50-100 ml of sample to a 250 ml separatory funnel, and dilute with distilled water to make a total volume of 100 ml. Add 5 ml of 0.5 N sodium hydroxide solution, extract with chloroform (2 x 50 ml) to remove flavorants. Collect the upper (aqueous) layer. Then proceed as in "General

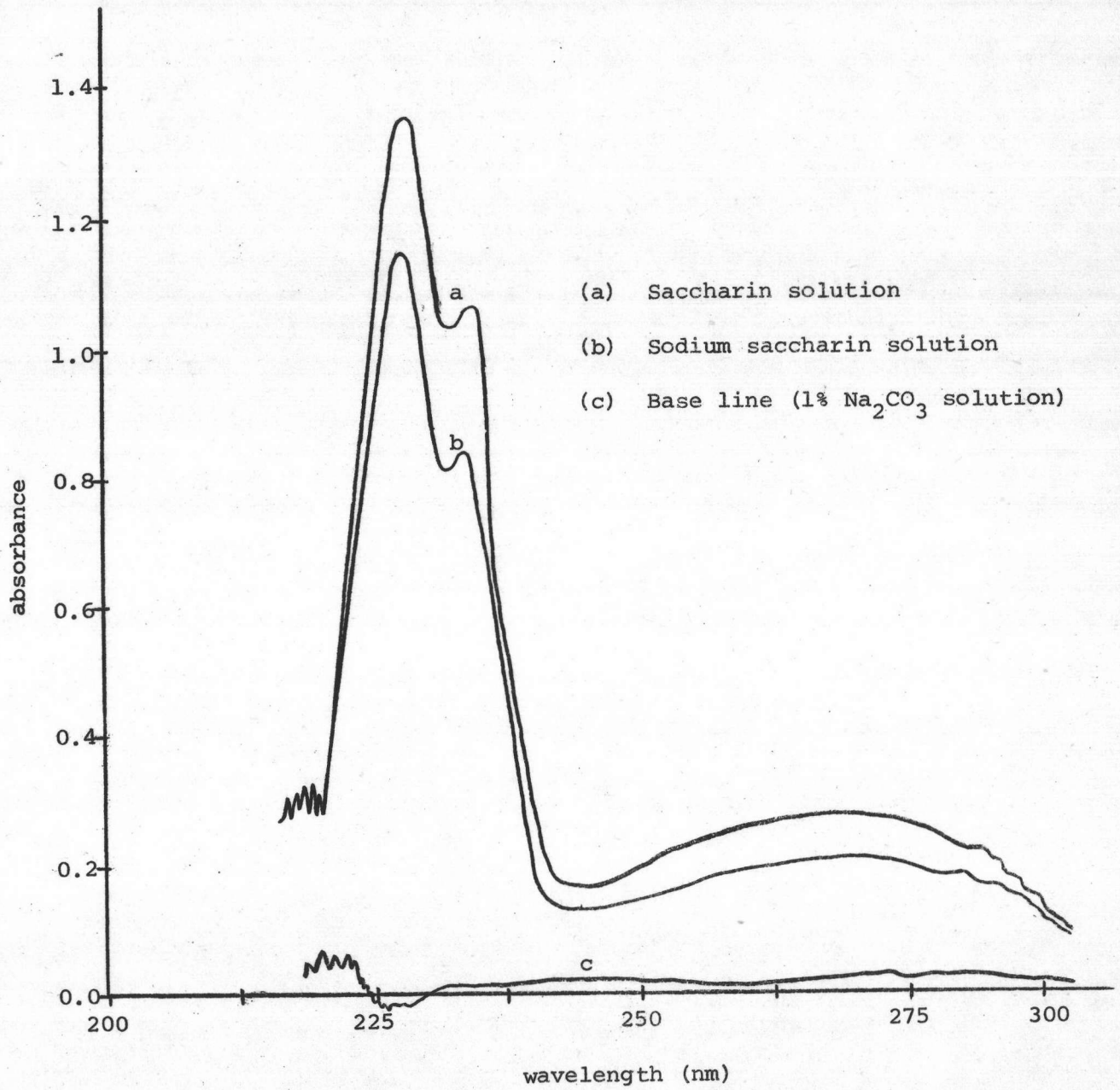


Figure 4 Spectra of saccharin and saccharin sodium in 1% Na_2CO_3 solution, concentration of each is 0.03 mg/ml (Reference is 1% Na_2CO_3 and path length is 10 mm.)
(After Hussein, Jacin, Rodriguez, 1976)

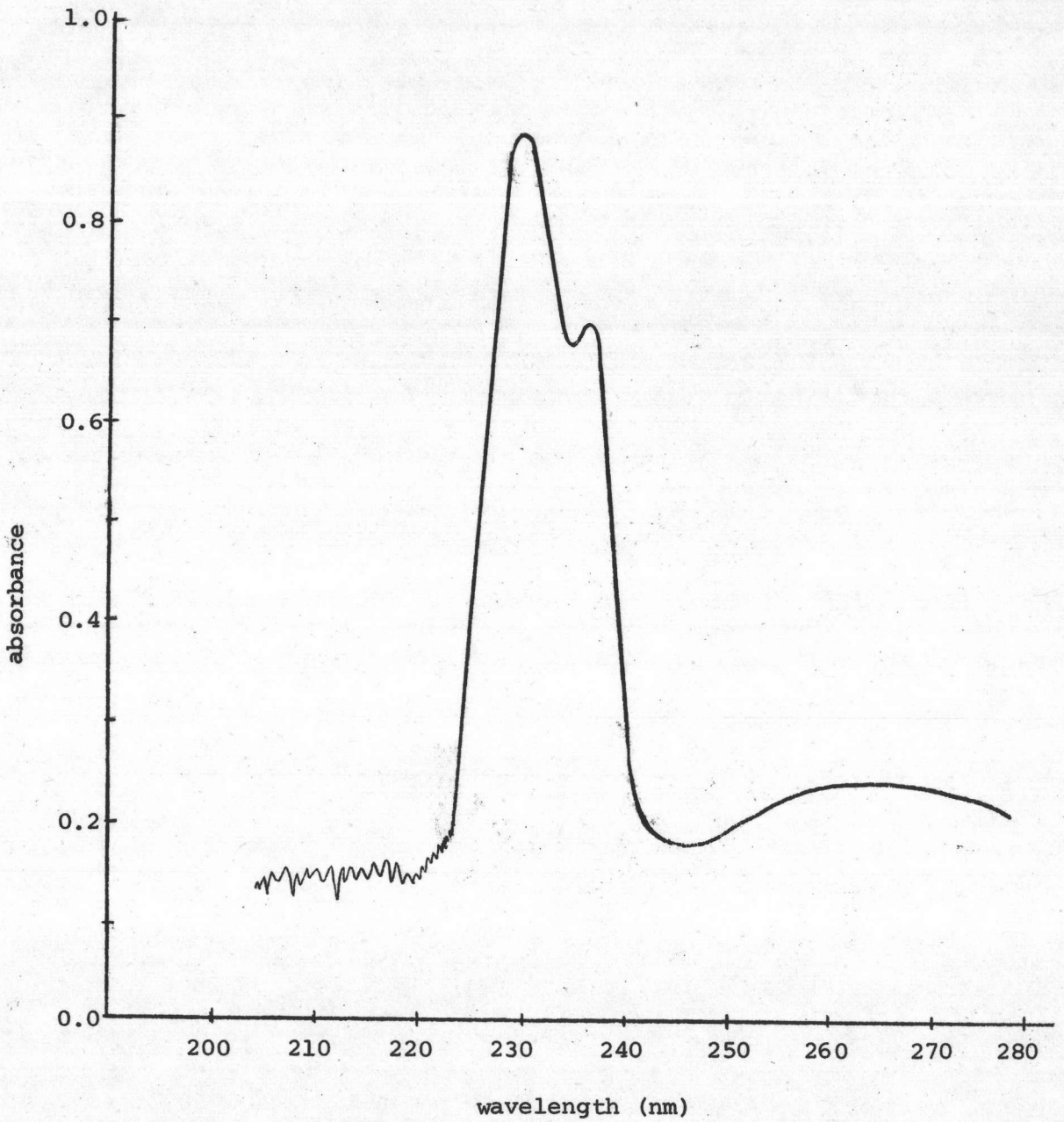


Figure 5 Spectra of repurified saccharin in 1% Na_2CO_3 solution, concentration 0.02 mg/ml. (Reference is 1% Na_2CO_3 and path length is 10 mm.)

Procedure", beginning with "Add 15 ml of hydrochloric acid (1 : 4) to the aqueous solution, ... in the hood until dry."

4.2 Concentrate Syrups

Measure 100 ml of concentrate syrup into a 500 ml volumetric flask, dilute to volume with distilled water. Take an aliquot (50 ml) for analysis. Add distilled water to make 100 ml, add 5 ml of 0.5 N sodium hydroxide solution, extract with chloroform (2 x 50 ml). Collect the aqueous (upper) layer and proceed as in "General Procedure", beginning with "Add 15 ml of hydrochloric (1 : 4) to the aqueous solution, ... in the hood until dry."

4.3 Fruit juice and samples which the color interferes in the ethereal extract

Transfer 50 ml of sample to a 100 ml volumetric flask. Add 5 per cent neutral lead acetate solution in slight excess (10 ml). Dilute to volume with distilled water. Mix, let stand for 1 hour and filter. To the filtrate add 5 ml of 0.5 N sodium hydroxide solution, extract with chloroform (2 x 50 ml). Collect the aqueous (upper) layer, then proceed as in "General Procedure", beginning with "Add 15 ml of hydrochloric acid (1 : 4) to the aqueous solution, ... in the hood until dry."

"General Procedure"

Transfer an aliquot, as specified, of prepared sample and standard solution (containing 1-3 mg of saccharin) to a 250 ml

separatory funnel. Add distilled water to make a total volume of 100 ml, and 5 ml 0.5 N sodium hydroxide solution, extract with chloroform (2 x 50 ml) to remove flavorants. Collect the aqueous (upper) layer. Add 15 ml of hydrochloric acid (1 : 4) to the aqueous solution, extract the mixture with carbon tetrachloride (3 x 50 ml) to remove preservatives. Keep the aqueous (upper) layer, then extract with diethyl ether (3 x 50 ml). Collect the ethereal extracts and wash with two portions of 10 ml each of distilled water. Evaporate the extract on water bath in the hood until dry.

Duplicate determinations are performed for each sample. Test for the presence of saccharin in the residue. If the results were positive, determine the quantity of saccharin in those samples.

5. Method of analysis

5.1 Detection of saccharin in beverages

5.2 Determination of saccharin in beverages

5.1 Detection of saccharin in beverages

5.1.1 Organoleptic test (AOAC 1975)

Prepared sample as in paragraph 4., proceeded as in "General Procedure" until dried residue was obtained. Taste for sweetness. The presence of 20 mg of saccharin per liter of original sample can usually be detected by its sweet taste.

5.1.2 Conversion to salicylic acid (BP 1973)

The residue obtained from "General Procedure" was mixed with 5 ml of 10 per cent w/v sodium hydroxide solution, evaporated to dryness, and gently fused the residue over a small flame until ammonia was no longer evolved. Allowed to cool, dissolved in 20 ml distilled water, neutralized the solution with hydrochloric acid. On the addition of a few drops of ferric chloride test solution, a violet color was produced by means of the presence of saccharin (this method was so called "false saccharin").

Reaction

Because of conversion of saccharin to salicylic acid which has a carboxyl group ortho position to a hydroxyl group so gives violet color on the addition of ferric chloride test solution (Veibel, 1972).

According to Noller (1965), explained that ortho hydroxyl group to carboxyl group formed chelate complex with iron and gave violet color.

Salicylic acid

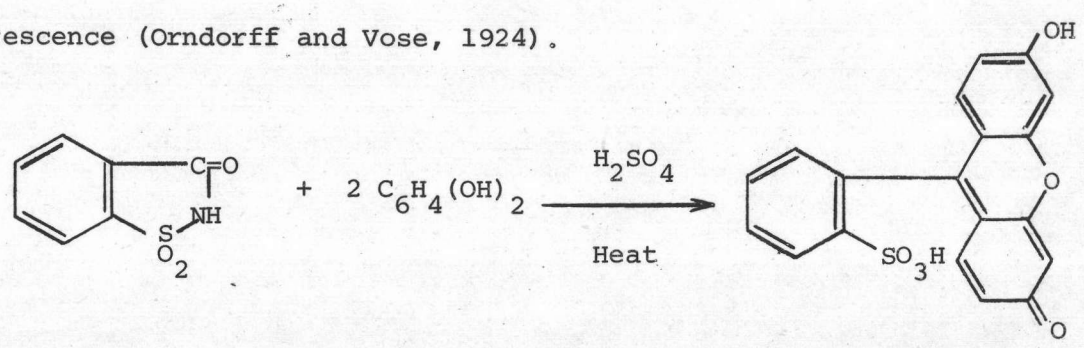
Complex (violet color)

5.1.3 Fluorescent test (BP 1973)

Mixed the residue (collected from "General Procedure") with resorcinol approximately about two times of the residue, added 10 drops of sulfuric acid and heated over small flame until it assumed a dark green color. Allowed to cool, added 10 ml of distilled water and an excess of 10 per cent w/v sodium hydroxide solution, a fluorescent, green liquid was produced in the presence of saccharin.

Reaction

Saccharin condensed with resorcinol in strong sulfuric acid at elevated temperatures, it gives rise to sulfo-fluorescein. This product, in alkaline solution gives an intensely green fluorescence (Orndorff and Vose, 1924).



Saccharin Resorcinol Sulfofluorescein

5.2 Determination of saccharin in beverages

The samples and standard saccharin solution (containing 1-3 mg of saccharin, were processed according to the "General Procedure" until dried residue was obtained. Added 20 ml of 1 per

cent sodium carbonate solution to the residue in the beaker and placed it on the water bath for 5 min, clumps of residue, if any, were broken with glass rod. The solution was cooled and transferred to a 100 ml volumetric flask, made up to volume with 1 per cent sodium carbonate solution. (If filtration were necessary, wash the filter thoroughly with 1 per cent sodium carbonate solution into the volumetric flask before diluting the contents to volume.)

A standard containing approximately equal amount of saccharin to that of the sample was proceeded along with the sample.

The sample and standard saccharin solution were scanned from 220 nm - 300 nm on a recording spectrophotometer against 1 per cent sodium carbonate solution as blank,

The absorbance of saccharin in standard and sample solutions were determined at 235 nm, and 244 nm for correction.

The saccharin concentration in the samples were calculated as follow :

$$\text{Net absorbance, } A = A_{235} - A_{244}$$

$$\left[\text{Saccharin}\right] = (A_x/A_s)\, C_s\, (V_t/V_x)\, (100/W)$$

where C_s = Concentration of standard solution, mg/100 ml, in this experiment = 2 mg/100 ml

V_t = Total volume of sample solution

V_x = Volume of aliquot of sample taken for analysis

A_x = Net absorbance of sample solution

A_s = Net absorbance of standard solution

W = Weight of original sample in milligram

In case of liquid sample, replaced W by "volume of original sample x 1000

Let V_o = Volume of original sample

The final concentration of saccharin will be in per cent (w/v)

$$\text{Then } \left[\text{Saccharin}\right] = (A_x/A_s)\ 2(V_t/V_x)\ (1/10V_o)$$