EXPERIMENT 7 DETECTION AND DETERMINATION OF SYNTHETIC COLOURS

Structure

7.1 INTRODUCTION

Only eight synthetic coal-tar dyes are permitted under PFA for addition to food products. PFA also specifies the foods to which such colours should not be added as well as the maximum permissible limits (0.2 g per kg) of the colours in the foods in which the colours are permitted.

Among the food additives, synthetic colours are viewed with extreme caution due to their potential toxicity to the human system. Therefore, identification and quantification of synthetic food colours in foods is very important.

Objectives

After studying and performing this experiment, you should be able to:

- identify coal-tar dyes added to foods; and
- determine the concentration of the colour(s) in the food product.

7.2 EXPERIMENT

7.2.1 Principle

Synthetic acidic colour(s) is dyed on to wool in acidic medium and extracted (stripped) from the wool into aqueous alkaline medium. The extracted colour(s) is developed (separated) by paper chromatography along with standard dyes using a suitable solvent system. Comparing their Rf values with that of standard colours identifies the sample colours. Quantification of the colours is done by spectrophotometry.

7.2.2 Requirements

Instruments and Apparatus

Chemical balance

Spectrophotometer with 1 cm quarts cells

Measuring cylinders – 25 ml, 50 ml

Volumetric flasks – 50 ml, 100 ml

Conical flasks – 100 ml, 250 ml

Beakers – 100 ml

Wool

Chromatographic chamber

Thin layer chromatogrphic plates

Chromatography column – glass column with tapered end (2.1 × 45 cm) filled with alumina

Glass capillaries

Chemicals

Butanol: Acetic acid: Water (BAW) solvent: 20: 5: 12 (v/v/v)

Liquor ammonia

Acetic acid

Sodium citrate

pH paper strips

Alumina (acidic aluminium oxide)

Standard reference colours

White knitting wool: Successively boiled in dilute ammonia, washed in water, boiled in dilute acetic acid, again washed in water and dried.

Whatman No. 1 and No.3 Chromatographic paper, 20 × 20 cm

7.2.3 Procedure

I. Detection

1. *Wool dyeing:* Take 50 ml or 50 g of the sample, add enough distilled water and prepare a free-flowing solution. Add 4 to5 pieces of 5 cm long woollen thread to the solution and acidify the solution with acetic acid (few ml, check with pH paper) and boil for 10 to 20 minutes. Take out the pieces of wool from the solution and wash in water. Transfer the woollen pieces to a beaker and strip (extract) the colour by boiling with dilute ammonia (1 part of liquor ammonia + 50 parts of distilled water) and remove the wool. Make the extracted solution acidic with acetic acid. Immerse fresh small pieces of wool in the extract and boil for 10 min. If the wool is not dyed then report absence of added artificial colouring matter. If the wool is dyed, it indicates the presence of a coal-tar dye. Wash the wool with water and again strip the colour in boiling ammonia solution, filter; evaporate to a small volume in a beaker for chromatography. The above method is not suited for basic dyes.

   For basic dyes reverse the method i.e. dye the wool first in dilute ammonia and then strip in acetic acid. At present, all the permitted water-soluble coal-tar dyes are acidic and hence an indication of the

presence of basic dye at this stage indicates presence of unpermitted colour.

2. *Paper chromatography:* Draw a horizontal line 2.5 cm from base of the filter paper (Whatman No.1). Spot the extracted colour solution on the line along with standard colour (dye) solutions and develop the chromatogram using one of the most effective solvent system viz. "BAW" – Butanol : Acetic acid : Water (20:5:12). When solvent front runs to a height of about 15 cm, remove the chromatogram and dry. Compare the sample Rf with the standard Rf, and identify the colour.

$$\text{Rf value} = \frac{\text{Distance moved by the solute (colour)}}{\text{Distance moved by the mobile phase (solvent)}}$$

Standard colour spot(s) corresponding to the sample colour(s) = -------- name of the standard colour(s)

II. Quantification

Samples containing single colour

Weigh about 5-10 g sample. Add about 25 ml water and mix well. Pass the solution through a column containing acidic aluminium oxide. Wash the column with water to remove sugars and natural colours. Elute the adsorbed colour with 1% ammonia. Transfer the eluate to a volumetric flask (25 ml) and make up to volume with 0.1 N HCl. Determine the absorbance of the dye solution at the absorption maxima (λ max).

Samples containing mixture of colours

Elute the mixture of colours by column chromatography and make up to a known volume (5 to 10 ml). Streak an aliquot of 0.5 ml on Whatman No. 3 paper and develop the chromatogram using the solvent system. Dry the paper and cut out the individual colour bands and elute with 0.1 N HCl. Make up to a known volume and determine the absorbance of each of the dye solutions at their absorption maxima.

7.2.4 Observations

a) Sample containing single colour

Weight of the sample	= W = ------ g
Volume of column eluate made up	= V = ------ ml
Absorbance of the solution	= A ----------
$E^{1\%}_{1cm}$ of the dye at λ max	= E ----------

b) Sample containing mixture of colours

Weight of the sample	= W_1 = ------ g
Volume of column eluate made up	= V_1 = ------- ml
Volume of the eluate streaked on chromatographic paper	= V_2 = ----- ml
Volume of made up HCl extract of each colour band	= V_3 = ----- ml
Absorbance of the made up HCl extracts	= A1, A2, A3 ---- An
$E^{1\%}_{1cm}$ of the dye	= E1, E2, E3 ----- En

7.2.5 Calculations

a) Dye content of the product (%) = $\dfrac{\text{Absorbance} \times \text{Volume of eluate made up} \times 100}{E^{1\%}_{1cm} \text{ of the dye} \times 100 \times \text{Wt. of sample}}$

$$= \frac{A \times V}{E \times W}$$

$$\text{Therefore, dye content of the product (ppm)} = \frac{A \times V}{E \times W} \times 10{,}000$$

b) Calculate the content of each dye separately. Here, calculation for the dye having absorbance = A1 and $E^{1\%}_{1cm}$ = E1 is given as an example.

$$\text{Content of the dye in the product (ppm)} = \frac{A \times V_3 \times V_1 \times 10{,}000}{E \times V_2 \times W_1}$$

$E^{1\%}_{1cm}$ for standard permitted food colours are given below.

$E^{1\%}_{1cm}$ = Extinction (Absorbance) of 1 % solution of a dye at its absorption maxima when measured in a 1 cm cell.

Colour	λ_{max} (nm)	$E^{1\%}_{1cm}$
Tartrazine	426	527
Sunset Yellow FCF	480	551
Ponceau 4R	505	431
Carmoisine	515	545
Erythrosine	526	1154
Indigo carmine	610	489
Fast Green FCF	625	1560
Brilliant Blue FCF	629	1637

7.2.6 Results

The quantity of added colour is expressed in parts per million (ppm) or mg/kg.

7.3 PRECAUTIONS

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.

While handling the dye solutions care should be taken to prevent it from getting them into the mouth because some of them may be unpermitted and harmful.

The spectrophotometer is a very costly instrument. Use it only after carefully understanding its operation. Similarly, the silica cells used are very costly and brittle. Handle them with utmost care. The silica cells should be wiped only with soft tissue to prevent scratching the transparent glass surface.