

## PW N°1: Identification Of carbohydrates Present in Foods By Thin Layer Chromatography

**1. Purpose:** in this practical work, we are interested in the identification of sugars present in milk using thin layer chromatography (TLC). Chromatography is a chemical analysis method that consists of separating and identifying the constituents of a mixture. It is based on the difference in affinity of a component between a mobile phase and a stationary phase.

### 2. Operating procedure

#### a. Material

- The stationary phase: chromatography paper (silica gel on aluminum). You must not touch the white side on which the deposits will be made.
- The mobile phase: eluent, migration solvent (a mixture of 3 volumes of acetone, 1 volume of methanol, and 1 volume of ethanoic acid).
- Solutions of glucose (1), sucrose (2), fructose (3), lactose (4) at 10 g/L.
- Foods: whole milk, fruit juice, etc.
- Capillaries for the deposits.
- Revealing reagent: 50 mL of potassium permanganate (20 g/L) mixed at the time of use with 50 mL of anhydrous sodium carbonate (40 g/L).
- Hair dryer.

#### b. Manipulation

##### Preparation of the stationary phase on the chromatography plate

- Very lightly trace a line for the deposits 1 cm from the lower edge of the plate.
- Trace the solvent front line 1 cm from the upper edge of the plate.
- Gently mark the spotting positions using a pencil, at 0.5 cm from the lateral edges and with equal spacing between spots.
- The labels indicating the nature of the deposits are written in pencil on the upper part of the plate, beyond the solvent front.

##### Preparation of the chromatographic vessel

A container usually made of glass, closed with a tight lid.

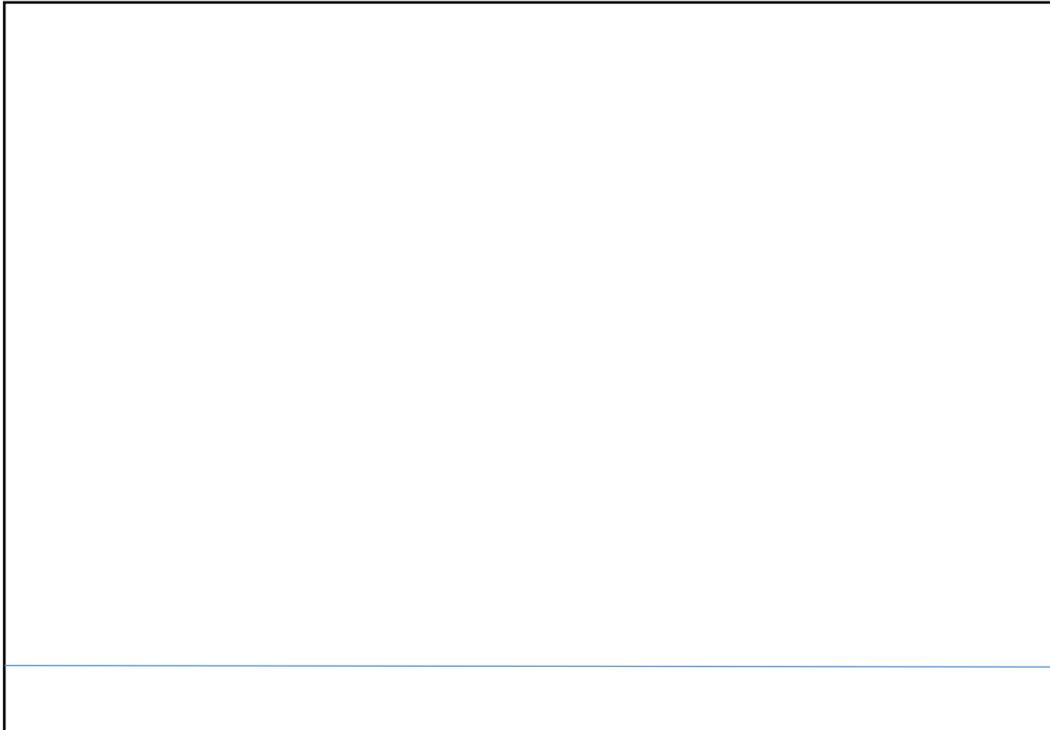
- Introduce the eluent or the mixture of solvents into the tank.
- Adjust the level to about 0.5 cm from the bottom of the tank.
- Line the inside of the tank with filter paper impregnated with eluent.
- Close the container and allow it to saturate with solvent vapor.

##### Chromatography set-up

- Using a capillary tube, apply a drop of each sugar solution, whole milk, and fruit juice three times at the same spot, so that each deposit is sufficiently concentrated.
- Allow the solvent to evaporate completely between each application to concentrate the deposits.
- Place the plate in the tank, ensuring the deposits are above the solvent level.
- Close the tank. Let it migrate; monitor the migration until the solvent reaches the upper front line, as migration times may vary.
- Take out the plate, dry it with a hairdryer until the smell of acetic acid completely disappears.
- Immerse the plate in the freshly mixed developer, covering the silica gel for about 10 seconds, then dry again.

Name : \_\_\_\_\_ First Name : \_\_\_\_\_ Group : \_\_\_\_\_ Date : \_\_\_\_\_

**Report of PW N°2: Thin Layer Chromatography (TLC) of Carbohydrates**



1. Sketch the chromatogram obtained after development.
2. Identify the carbohydrate(s) present in whole milk and fruit juice.
3. What is the role of the eluent?

4. Calculate the frontal ratio  $R = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent at the migration front}}$