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Separation of sugars by thin layer chromatography

PRINCIPLE

The technique of Thin-layer chromatography (TLC) was developed by Stahl (1964).
The movement of a compound carried in a solvent through a thin layer of adsorbent material on a suitable support depends on its relative affinity for the adsorbent (stationary phase), which is generally polar, as compared to the solvent (moving phase), which is generally nonpolar.
In adsorption TLC, if the sample is a mixture of compounds, it continuously gets fractionated as it is carried by the solvent moving through the stationary phase.
Generally, the more polar compounds are held back by the adsorbent while the less polar materials advance further.
In case of sugars, as in case of paper chromatography, speed of migration decreases in the following order:
Pentoses, hexoses, disaccharides, trisaccharides.

Advantages over paper chromatography

Variety of supporting media can be used as also mixtures.
Fluorescent dyes can be incorporated into the medium to assist in identification of spots.
Method is rapid and many separations can be accomplished in less than an hour.
Spots are compact and it is possible to detect compounds at a lower concentration than on paper.
Separated compounds can be detected by corrosive sprays and an elevated temperature which is not possible with paper.

Production of a thin layer

Rf values are affected by thickness of the layer below 200 microns and a depth of 250 microns is suitable for most separations.
Silica gel has been found to be useful for separating a variety of compounds.

Development

It is necessary to make sure that the atmosphere of the chamber is fully saturated; otherwise Rf will differ from tank to tank. This can be achieved by lining the wall of the tank with paper soaked in the solvent.
Development is done by ascending technique.

Location

Located as in paper chromatography, by spraying the plate with appropriate reagent.

MATERIALS

Chemicals

1. Silica gel G (e.g. Merck; contains Calcium sulphate which acts as a binding agent).
2. n-butanol
3. Glacial acetic acid
4. Aniline
5. Phthalic acid
6. Standard sugars (lactose, glucose, xylose, mannose, sucrose)
7. Acetone
8. Boric acid

Glassware and equipment

1. Thin layer plates of silica gel G

Prepare slurry of silica gel G in 0.1M boric acid and pour the plates 250 microns thick. Activate before use by heating at 105XCfor 30 mins.

2. Glass tank with lid.
3. Beakers
4. Capillary tubes
5. Hair dryer
6. Oven
7. Sprayer/atomizer

PREPARATION OF SOLUTIONS

1. **Solvent**

n-butanol:glacial acetic acid:water::60:30:10 (Stahl,1964)

OR

Ethyl acetate: isopropanol: water: pyridine: 26:14:7:2 (Plummer, 1971)

OR

n-butanol:pyridine:water::6:4:3

2. **Standard sugar solutions**

3% aqueous solution (e.g. rhamnose, lactose, ribose, xylose, glucosamine, fructose, glucose, sucrose).

3.Developing solution (Aniline-Phthalic acid)

1.5ml aniline and 1.6g phthalic acid in 100ml of 95% acetone.

Preparation of silica gel plates

Take 1 part silica gel G and 2 parts 0.1M boric acid (w/v)in a beaker. Mix rapidly using a glass rod to make a homogeneous paste and avoid formation of bubbles. Take 15ml of this paste on the far end of the plate.

Hold a half-plate at an angle of 30 and pull the slurry steadily towards the near end. This gives a layer of about 250 microns thickness.

METHOD

1. Carefully spot the sugars on the silica gel plate without making a hole in the adsorbent.
2. Place the plates in the chamber saturated with the solvent.
3. Develop the chromatogram until solvent front is 15cm from the origin.
4. Draw a line across the plate at this point and remove the plate when the solvent reaches this point.
4. Dry the plates in a stream of cold air.
5. Locate the sugars by spraying with the aniline hydrogen phthalate reagent.
6. Heat the plates at 105°C for 5minutes.
7. Note the colour of each sugar and calculate the Rf value.

$$Rf = \frac{\text{Distance traveled by the component from the base line}}{\text{Distance traveled by the solvent}}$$

PRECAUTIONS and some USEFUL HINTS

1. Line the tank with filter paper.
2. Equilibrate the tank with the developing solvent for about one hour.
3. Mix the solvent in a beaker to make sure it forms one phase and then pour into the solvent tank.
4. Marks for solvents should be at least 8mm apart (1cm is convenient).
5. Draw a line for the solvent front about 2cm from the top of the plate. This will allow for different plates to be run similarly and make comparisons easy.
6. For comparison purposes, the concentration of the sample should be the same.

EXPECTED RESULT

The sugar spots appear as yellow – brown coloured spots in the blue background of the plate.

REFERENCES

1. Plummer D.T. 1979. An Introduction to Practical Biochemistry. Tata McGraw-Hill Publishing Company Ltd.
2. Stahl E. (Ed.) 1965. Thin Layer Chromatography – A Laboratory Handbook. Academic Press Inc. Publishers.