Bio Factsheet

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How to identify foods: Food Tests and Chromatography

Food tests and chromatography are techniques used for the recognition of biologically important chemical compounds (exam questions on these techniques are common). The use of these techniques is not limited only to the food industry. Detectives can use chromatography to analyze fibres found at a crime scene and when used in conjunction with sniffer dogs, gas chromatography is a common way to identify explosives in airports, it has also been used in schools to perform drug screening.

Why do we need two methods for identifying foods?
Food tests are less precise, they basically identify foods into the category of proteins, lipids, starch, cellulose, reducing sugars or non-reducing sugars, whereas paper chromatography can identify specific molecules, for example, finding out which amino acids or monosaccharides are contained in a mixture.

Food Tests
It is worth learning the food tests as it can mean easy marks in an exam, it is pure recall. In an exam question always quote the reagent used, method, starting colour and end colour for a positive result.

Table 1. Food tests

Biological Molecule	Reagent/s used to test	Method	Starting Colour	End Colour (positive result)
Reducing Sugar (for example glucose, fructose ,maltose.	Benedict's solution (10% $CuSO_4$)	Add 2cm³ Benedict's solution an equal amount of the test sample, heat over a water bath.	Blue	Orange/Red precipitate.
Non-reducing Sugar (sucrose)	Benedict's solution (10% $CuSO_4$), HCl, $NaHCO_3$	1. Boil test sample with HCl (to break the glycosidic bonds). 2. Neutralise with $NaHCO_3$/alkali (otherwise the HCl would react with the Benedict's solution). 3. Add Benedict's solution to the test sample, heat over a water bath.	Blue	Orange/Red precipitate
Starch	Iodine solution (KI)	Add a few drops of Iodine solution to the test sample.	Yellow/brown	Blue-black
Lipids	The Emulsion Test - ethanol (C_2H_5OH) and water	1. Shake some of the test sample with about 4cm³ of ethanol. 2. Decant the liquid into a test tube of water, leaving any undissolved substances behind.	Clear	Formation of a cloudy white emulsion.
Proteins	Biuret reagent (alkaline $CuSO_4$)	Add a few drops of Biuret reagent to the test sample	Blue	Lilac/purple precipitate
Cellulose	Schultze's solution	Add a few drops of Schultze's solution to the test sample	Yellow	Purple

Three tips on food tests with sugars
1. To distinguish between reducing and non-reducing sugars, first test a sample for reducing sugars, to see if there are any present, if no positive result is achieved test for non-reducing sugars.
2. When testing for a reducing sugar it is not possible to distinguish between glucose and fructose, paper chromatography would need to be used.
3. When testing for a non-reducing sugar the solution will be neutralised when it stops fizzing.

Chromatography

There are many different types of chromatography:

1. **Paper chromatography** is the simplest, but does not always give very clean separation. It can be used for separating amino acids, anions, RNA fingerprinting, separating and testing histamines and antibiotics.
2. **Thin layer chromatography (TLC)** uses a thin layer of cellulose or silica coated onto a plastic or glass sheet. This is more expensive, but gives much better and more reliable separation this can be used for detecting pesticide or insecticide residues in food. It can also be used in forensics to analyze the dye composition of fibres.
3. **Column chromatography** uses a glass column filled with cellulose slurry. Large samples can be pumped through the column and the separated fractions can be collected for further experiments, so this is preparative chromatography as opposed to analytical chromatography.
4. **High performance liquid chromatography (HPLC)** is an improved form of column chromatography that delivers excellent separation very quickly. It can be to used test water samples to look for pollution by analysing metal ions and organic compounds in solutions. It uses liquids which may incorporate hydrophilic, insoluble molecules.
5. **Gas Chromatography** is used in airports to detect bombs and is used is forensics in many different ways. It is used to analyze fibres on a person's body and also analyze blood found at a crime scene. In gas chromatography helium is used to move a gaseous mixture through a column of absorbent material.
6. **Electrophoresis** uses an electric current to separate molecules on the basis of charge. It can also be used to separate on the basis of molecular size, and as such is used in DNA sequencing.

Paper Chromatography

On what principle does paper chromatography work?

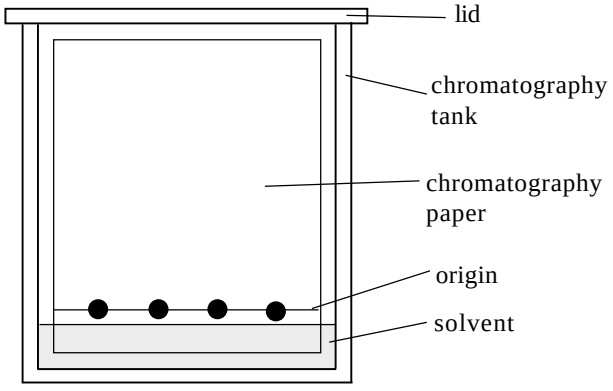
The principle is based on the solubility of chemicals (called 'solutes') i.e. how fast they dissolve in a solution. A solvent can then separate the solutes for example, if a solute is very soluble it will dissolve quickly in the solvent and as the solvent travels up the medium (paper) by capillary action it will separate out first on the paper before the solvent dissolves out the next solute. The result of this is seeing spots on the paper which represent separate solutes.

What types of biochemical molecules can be tested for?

1. Coloured molecules for example, ink, chlorophyll and fruit juice, these do not have to be stained as they are already coloured.
2. Colourless molecules for example, an amino acid or monosaccharide mixture. These have to be stained for example, ninhydrin is used to stain amino acids.

Method

A Chromatography tank should be set up as below:



1. Pour some solvent into a chromatography tank and seal it, this is so that the atmosphere is saturated with solvent vapour. Different solvents are suitable for different tasks, but they are usually mixtures of water with organic liquids such as ethanol or propanone.

2. Place a drop of the mixture to be separated onto a sheet of chromatography paper near one end, this is the origin of the chromatogram. The spot should be small but concentrated, this is achieved by spotting and drying, spotting and drying and so on, drying the spot stops it spreading. Repeat for any other mixtures. Label the spots with pencil, as ink may dissolve.

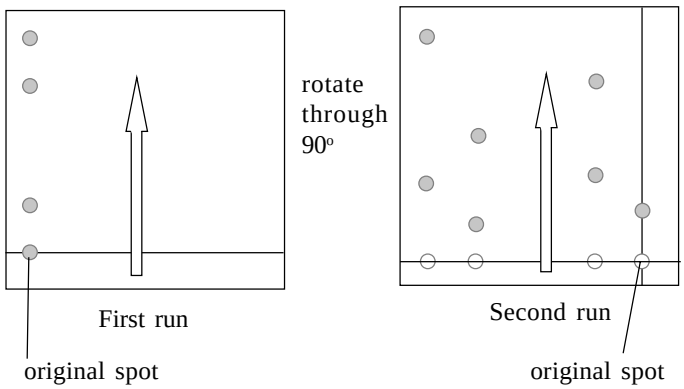
3. Place the chromatography sheet into the tank so that the origin is just above the level of solvent, and leave for several hours. The solvent will rise up the paper by capillary action carrying the contents of the mixture with it. Any solutes dissolved in the solvent will be partitioned between the organic solvent (the moving phase) and the water, which is held by the paper (the stationary phase). The more soluble a solute is in the solvent the further up the paper it will move.

4. When the solvent has nearly reached the top of the paper, the paper is removed and the position of the solvent front marked. The chromatogram may need to be developed to make the spots visible for example, some amino acids stain purple with ninhydrin.

What to do if the separation of molecules is poor

Sometimes chromatography with a single solvent is not enough to separate all the constituents of a mixture, the spots may appear as a smear. If this happens separation can be improved by two-way chromatography.

The chromatography paper is turned through 90° and run a second time in a different second solvent. Solutes that didn't separate in one solvent will separate in another because they have different solubilities. The diagram below demonstrated this:



Analysis of the Chromatogram

Methods for analysing a chromatogram that can be used are:

1. Run known substance/s alongside unknown and spot positions compared to the known substances.
2. Work out how far the substance has moved compared with the solvent, this is called R_f value (hint: it is always less than 1). The formula for R_f value is:

$$R_f = \frac{\text{Distance moved by the spot}}{\text{Distance moved by the solvent front}}$$

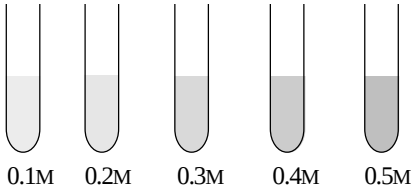
These methods are possible as a particular substance will travel the same distance up the paper when a known solvent is used under standard conditions.

However, these two methods can only be used to identify substances. Another method must be adopted to quantify the substances, i.e. how to work out how much is there of each amino acid or monosaccharide in the sample.

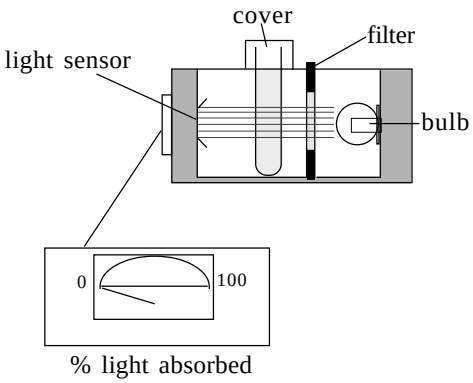
A colorimeter is used to detect the shade of the colour – the darker the colour, the more the amino acid or sugar there is i.e. the colorimeter measures concentration of samples by measuring the colour concentration within them.

Method

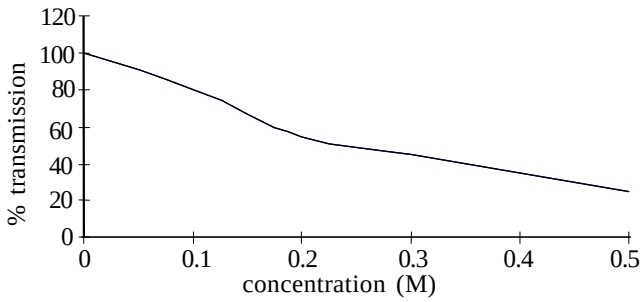
1. Obtain a range of known concentrations of for example, amino acids and stain them.



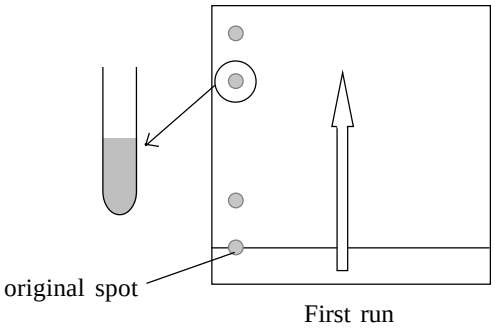
2. Place each concentration into a cuvette (a flat sided test tube), then place in the colorimeter and read off the percentage transmission.



3. Plot concentration against percentage transmission on a graph called a calibration curve.



4. Elute the spot i.e. cut it out and dissolve into a set amount of solvent, place it in a cuvette.

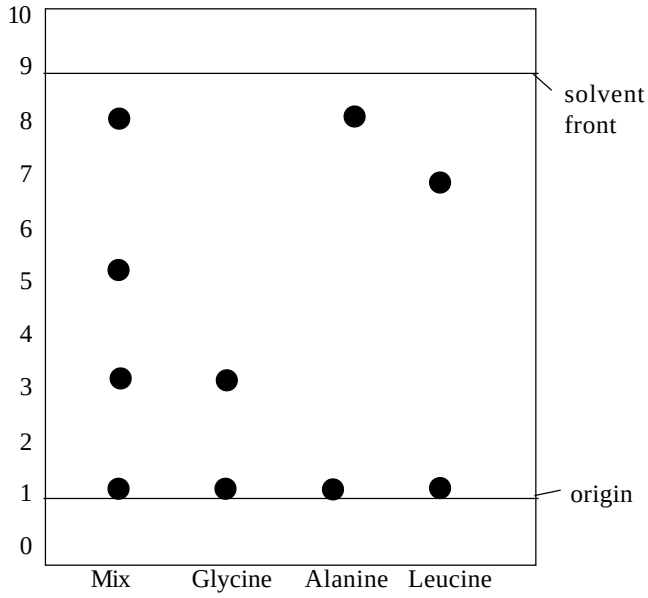


First run

5. Put this into the colorimeter and read off the percentage transmission for example if the percentage transmission was 60% this value can now be read off the calibration curve to determine the concentration.

Typical Exam Question

The amino acids in a sample of fruit juice were separated by paper chromatography. The pattern obtained is shown below:



1. Which component is the most soluble?
2. How many amino acids are found in the mixture?
3. What amino acids are not found in the mixture?
4. If the mixture contained 4 amino acids but only 3 were seen on the chromatogram, what does it mean?
5. What is the R_f value of Leucine?
6. If the mixture was a protein, how would you separate it out into its amino acids?
7. How would you work out the amount of glycine in the mixture (no details needed)?

Answers

1. The one furthest away/Alanine.
2. Three.
3. Leucine.
4. One amino acid is insoluble in that solvent.
5. 6/8 = 0.75.
6. Hydrolyse with protease or acid and then run chromatography.
7. Use a colorimeter.

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