ChemFactsheet

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Chromatography 1 : Paper and Thin-layer

What is chromatography?

Chromatography is an analytical technique used to *separate* the components of a mixture between a mobile phase and a stationary phase.

> Chromatography, literally meaning "colour writing", was invented in 1903 by the Russian botanist Mikhail Semyonovich Tsvet, who used it for separating coloured leaf pigments

In addition, chromatography may *identify* the components but, in other cases, the separated components are passed on to other analytical methods (e.g. mass spectrometry) for identification.

Chromatography can separate chemicals which are very closely related chemically and physically. Thus mixtures of plant pigments, amino acids, dyes, and sugars that may not be separated by techniques such as fractional distillation or fractional crystallisation (especially when in very small quantities) can be readily separated and often identified by chromatography.

Chromatography mechanisms

The sample to be separated is carried by a ***mobile phase*** *(MP;* a liquid or a gas*)* through a ***stationary phase*** *(SP;* a solid or a liquid supported on a solid*).*

In general terms, components with a high affinity for the MP and a low affinity for the SP move through the system more quickly. However, components with a low affinity for the MP and a high affinity for the SP move through the system more slowly. If suitable SP and MP are chosen a mixture of even similar substances, such as amino acids, can be separated as they travel at different rates through the SP. This is because every component of the mixture is affected differently by the two phases. The separated mixture is called a **chromatogram**.

(a) **Partition chromatography** is the term applied when the SP is a liquid.

e.g. **Paper chromatography**: The SP is liquid water which is adsorbed on the cellulose fibres of the chromatography paper. The –O-H groups of the cellulose and of the water molecules form hydrogen bonds. The MP is another liquid (e.g. butan-1-ol) or mixture of liquids which moves through the paper by capillary action.

Separation is possible because the mixture to be separated will contain some substances that are more soluble in the water of the SP than in the liquid MP. These substances will tend to dissolve in the stationary water phase and not travel so far in a given time. Substances in the mixture which are more soluble in liquid MP than in the adsorbed water will tend to remain in the MP and hence travel further in a given time.

(b) **Adsorption chromatography** is the term applied when the SP is a solid.

e.g. **Thin Layer Chromatography, TLC**: The SP is a thin layer of solid such as SiO_2 or Al_2O_3 on a glass, metal or rigid plastic sheet for support.

Separation is possible because the mixture to be separated will contain some substances that are relatively strongly bonded when they adsorb on to the surface of the thin layer. These substances will tend to not travel so far in a given time. They are said to have a lower Rf values (see later). Conversely, substances in the mixture which are relatively weakly bonded when they adsorb on to the surface of the thin layer will tend to travel much further in a given time. They are said to have a higher Rf values (see later).

Types of chromatography

There are many different types but the following table summarises the most common ones. Others are essentially variations on these, often developed for a specialised application. e.g. the SP may be chiral to allow the separation of chiral components.

Type of chromatography	Type of mobile phase	Type of stationary phase	Nature of the distribution process
Paper chromatography	Liquid	Liquid	Partition
Thin-layer chromatography	Liquid	Solid	Adsorption
Column chromatography	Liquid	Solid	Adsorption
Gas chromatography (GC)	Gas	Solid	Adsorption
High pressure liquid chromatography (HPLC)	Liquid	Liquid	Partition
Gas liquid chromatography (GLC)	Solid	Liquid	Partition

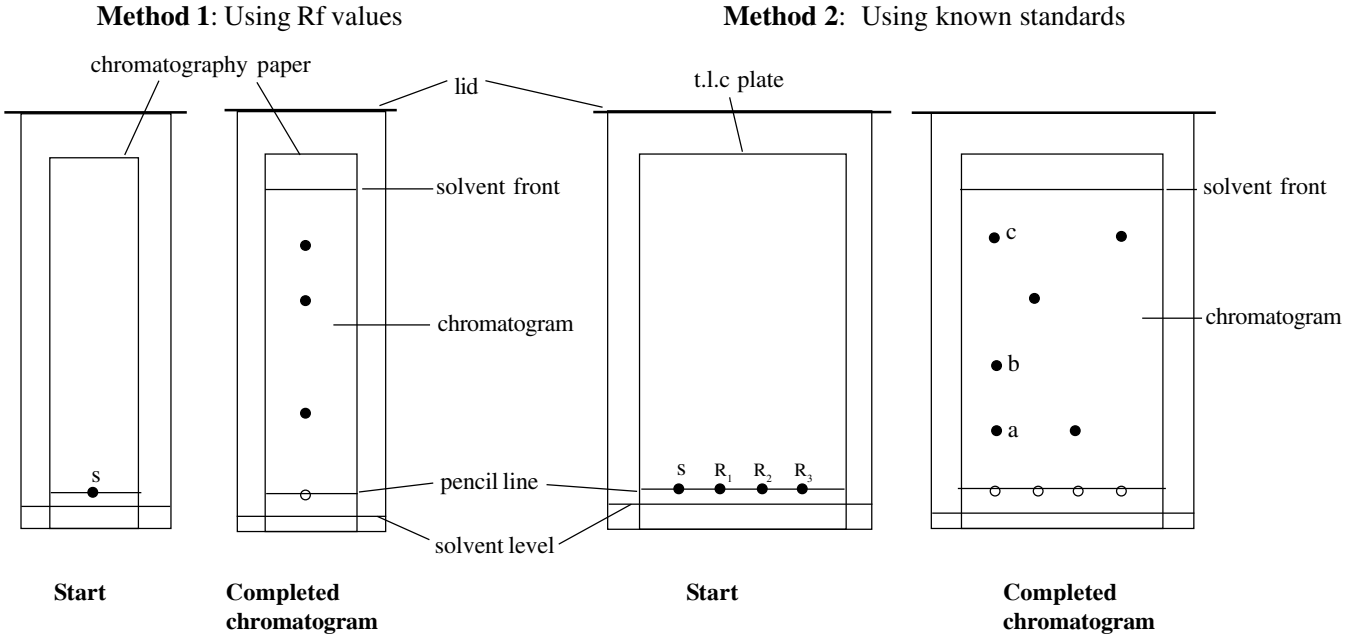
R_f values (retardation factor)

This is a quantitative measure of the amount of movement of a component ("spot") of a mixture during paper chromatography or TLC.

Definition. $R_f = \frac{\text{Distance moved by "spot" (centre)}}{\text{Distance moved by solvent front (MP)}}$

R_f values are less than 1 unless the component had negligible affinity for the stationary phase compared to the mobile phase. R_f values have no units.

Paper and thin-layer chromatography experimental



1. The solvent is poured into the chromatography tank to a height of about 1 cm.

2. A pencil line is drawn about 2 cm from the bottom of the paper or very carefully on the tlc plate.

3. The sample (S) is applied to the line using a fine pipette to produce a small concentrated spot. (Possible components (R_1, R_2, R_3), can be similarly applied along side the sample (S)).

4. The prepared paper/plate is carefully lowered into the tank and covered with a lid to produce a closed system.

5. The covered tank is left until the solvent front is close to the top of the paper/plate
.
6. The paper/plate is removed and the solvent allowed to evaporate until the paper/plate is dry.

7. If the spots are invisible the chromatogram is "developed" to produce visible spots; e.g. by spraying with a **locating agent** such as ninhydrin for amino acids and then warming or using UV light for organic molecules with conjugated systems.

8a. Method 1: The R_f values of the spots on the chromatogram are measured and compared with data R_f values of known substances using the same solvent to identify the components.

8b. Method 2: Identification may be immediate by direct comparison. Thus the sample 's' has three components 'a' which is R_2, 'c' which is R_3 and 'b' an unknown. 's' does not contain R_1.

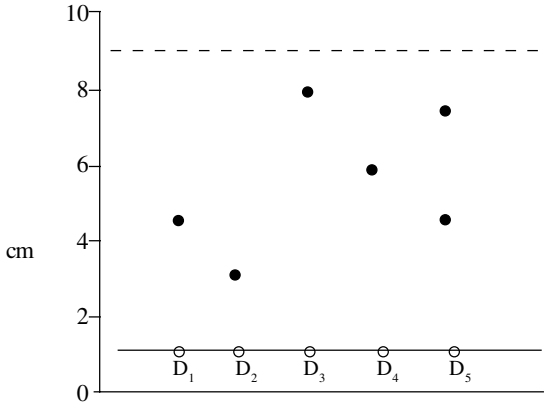
Note: Method 2 is better since method 1 requires the data R_f values to be determined under identical conditions (e.g. solvent, paper/plate) as the experiment R_f values.

Note: In theory if the solvent front is allowed to reach the top of the paper/sheet no further movement of the spots will occur as the system has reached equilibrium. However should the lid not completely seal the system the spots will continue to rise beyond their R_f values and the experiment is ruined.

Parctice Questions

1. Glutathione is a *tripeptide* antioxidant, protecting cells from toxins such as free radicals. When completely hydrolysed, the mixture of produced will consist of three amino acids. Describe, using diagrams, how paper chromatography could be used to confirm this statement.

2. Paper chromatography was used to investigate some permitted food dyes (labelled D1 - D5). The resulting chromatogram is shown on the right.



   (a) Explain the meaning of the terms *Rf value, stationary phase, mobile phase* and *solvent front*.
   (b) State, giving a reason, whether D_5 is a single substance or a mixture.
   (c) Calculate the value of R_f value of the spot from D_3.
   (d) What would be the Rf value of spot from D3 if the experiment has been stopped when the solvent front had travelled half the distance shown?
   (e) Why is it important to allow the solvent front to reach close to the top of the paper before stopping the experiment?

3. A drop of green dye is placed 2 cm from the bottom of a strip of filter paper. The filter paper is suspended in a graduated cylinder with 1 cm of the paper immersed in a water–alcohol solvent. After 30 minutes, the green spot is no longer present and there are blue and yellow spots.
   (a) Describe how the R_f value of the yellow spot could be determined.
   (b) Account for the difference in the R_f values of the blue and yellow dyes.
   (c) What is the significance of an Rf value of 1.0?

4. A tripeptide consists of three amino acids joined in a linear sequence by two peptide bonds. Isoleucine-proline-proline (IPP) is a tripeptide found in milk – a lactotripeptide.
   (a) If IPP is ***partially*** hydrolysed and the hydrolysate separated by ascending paper chromatography how many spots will be produced after suitable treatment to make all spots visible?
   (b) The Rf values of Isoleucine and proline using butan-1-ol, ethanoic acid and water (4:1:1) as the mobile phase are 0.72 and 0.43 respectively. Which amino acid has the greater affinity for the mobile phase?
   (c) Explain which of the two amino acids is the more polar?
   (d) State and explain two precautions relating to the health of the experimenter when carrying out this chromatography experiment.

Answers

1. Diagram as Method 1 start. Key points: 1. Pencil line. 2. Solvent below sample. 3. Cover the tank (beaker). (1, 2, and 3 are seen in a well-drawn and labeled diagram.) 4. Leave until solvent front is close to the top. 5. Remove paper and dry. 6. Spray with ninhydrin (and heat) to locate three spots.

2. (a) *Rf* = Distance travelled by the component / Distance travelled by the solvent front. Stationary phase - the water in the fibres of the paper. Mobile phase - the solvent that moves up the paper. Solvent front – the position of the solvent on the paper.
   (b) Mixture since more than one spot is produced.
   (c) *Rf* value = 7/8 = 0.875.
   (d) 0.875 – the ratio of distance moved by spot to distance moved by solvent remains constant.
   (e) The greater the distance measured the smaller the % error.

3. (a) Measure the distances travelled by the yellow spot (centre) and solvent front from the origin. Divide the distance moved by the spot by the distance moved by the solvent front.
   (b) Each dye has different attractions/affinities for the paper and the solvent (so they move different distances from the origin).
   (c) There is negligible attraction/affinity between the dye and paper (compared with that of dye and solvent).

4. (a) 5. (IPP, IP, PP, I and P).
   (b) Isoleucine – highest Rf.
   (c) Proline – more soluble in the polar water SP.
   (d) Keep solvent away from naked flames as organic compounds are often flammable. Do the experiment in a fume cupboard / well ventilated lab. as organic vapours are noxious. Also, ninhydrin is carcinogenic.

The Police, F.B.I. and other organisations use chromatography when trying to solve crimes. For example, it can be used to determine the presence of cocaine in urine, alcohol in blood, PCB's in fish, and lead in water. Chromatography is used by many different people in many different ways.

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