

Amines, amino acids and condensation polymers in action

Chemistry grades 11-12 (A Level · IB · Class 11-12) · Lab

Name: _____ Date: _____ Class: _____

Colourless amino acids are hard to tell apart by eye. In this lab you separate them by paper chromatography, develop the spots with ninhydrin and use R_f values to identify the amino acids in an unknown mixture.

Level: Chemistry grades 11-12 (A Level · IB · Class 11-12) · **Curriculum:** A Level 3.3.11-3.3.13

Aim

To separate and identify the amino acids in an unknown mixture by paper chromatography and R_f values.

Equipment

- Chromatography paper, 20 cm × 12 cm
- Glass tank or large beaker with a lid
- Solvent: butan-1-ol, ethanoic acid and water mixed 4 : 1 : 1 by volume
- 0.1 mol/dm³ solutions of glycine, alanine, valine and leucine
- Unknown solution X
- Capillary tubes, pencil, ruler
- Ninhydrin spray (0.2 % in propanone) and a hot air dryer
- Disposable gloves and a fume cupboard

Safety

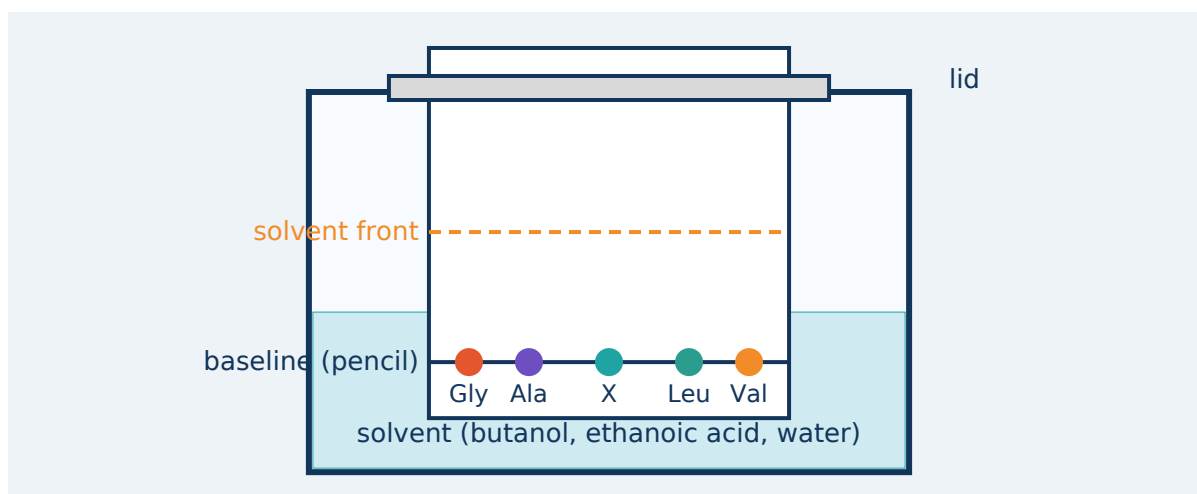
Safety

Wear gloves and eye protection. Butan-1-ol, ethanoic acid and propanone are flammable and irritant, so keep away from flames. Spray ninhydrin only in a fume cupboard; it stains skin. Wash hands after the lab.

Method

1. Draw a pencil baseline 2.0 cm from the bottom of the paper and mark five points along it.
2. Use a clean capillary for each solution and place a small spot at each point, drying between additions to keep spots small.

3. Pour solvent into the tank to a depth of 1.0 cm, below the baseline, and put the lid on for 5 minutes.
4. Hang the paper in the tank and replace the lid. Let the solvent rise until it is 2.0 cm from the top.
5. Remove the paper, mark the solvent front at once in pencil and dry it in a fume cupboard.
6. Spray with ninhydrin, warm gently until purple spots appear and mark their centres.
7. Measure the distance from the baseline to each spot centre and to the solvent front.



Ascending paper chromatography of amino acid solutions: three known samples, one unknown X and a mixture.

Results

The solvent front moved 12.0 cm from the baseline. Sample measurements:

Sample	Distance moved by spot / cm	Rf = distance ÷ 12.0
Glycine	3.1	0.26
Alanine	4.6	0.38
Valine	6.6	0.55
Leucine	8.8	0.73
Unknown X	4.6 and 8.8	to be calculated

Analysis questions

Question 1 Calculate Rf for X

Calculate the two Rf values for unknown X and compare them with the table.

Question 2 Identify X

Identify the amino acids in X and justify your decision.

Question 3 Pencil and ninhydrin

Explain why the baseline is drawn in pencil and why the paper is sprayed with ninhydrin.

Question 4 Solvent level

Explain why the solvent level must be below the baseline at the start.

Question 5 Why R_f varies

State two factors that change the R_f of an amino acid and one way to reduce their effect.

Question 6 A close pair

Serine runs 3.3 cm in the same system. Calculate its R_f and say whether it could be confused with glycine.

Question 7 Improve the lab

Suggest two improvements so that the identification of X is more reliable.