CHAPTER 33 CHROMATOGRAPHY

1 A peptide is hydrolysed to form a solution containing a mixture of amino acids. This mixture is then analysed by silica gel thin-layer chromatography (TLC) using a toxic solvent. The individual amino acids are identified from their R_f values.

Part of the practical procedure is given below.

1. **Wearing plastic gloves to hold a TLC plate**, draw a pencil line 1.5 cm from the bottom of the plate.
2. Use a capillary tube to apply a very small drop of the solution of amino acids to the mid-point of the pencil line.
3. Allow the spot to dry completely.
4. In the developing tank, add the developing solvent to **a depth of not more than 1 cm**.
5. Place your TLC plate in the developing tank.
6. Allow the developing solvent to rise up the plate **to the top**.
7. Remove the plate and quickly mark the position of the solvent front with a pencil.
8. Allow the plate to dry **in a fume cupboard**.

Parts of the procedure are in bold text.

(a) For each of these parts, consider whether it is essential and justify your answer.

[4 marks]

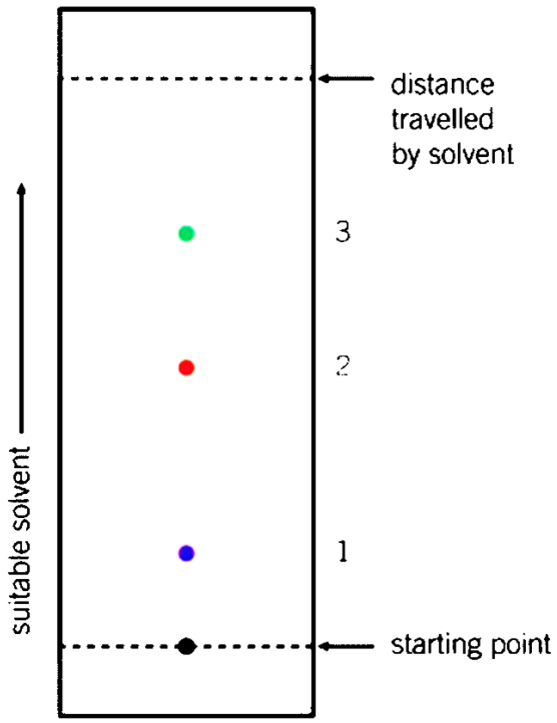
(b) Outline the steps needed to locate the positions of the amino acids on the TLC plate and to determine their R_f values.

[4 marks]

(c) Explain why different amino acids have different R_f values.

[2 marks]

2 Figure 1 shows a chromatogram used to separate some amino acids by paper chromatography, using solvent X —a mixture of ethanoic acid, butan-1 -ol and water.



(a) Identify the amino acids using the table below. R_f values of some amino acids using solvent X:

Amino Acid	R_f Value
alanine	0.38
arginine	0.16
glycine	0.26
leucine	0.73
tyrosine	0.50
valine	0.60

1 _____

2 _____

3 _____

(3 marks)

(b) Why is it essential to know the solvent used in the process?

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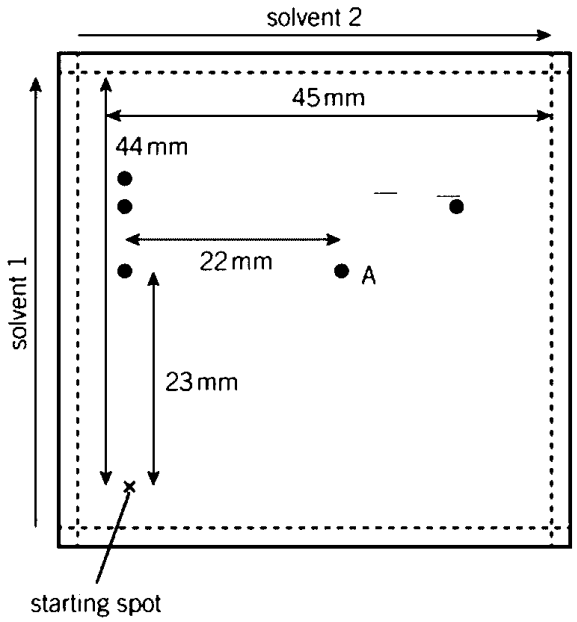
(1 mark)

3 Two-way paper chromatography was used to separate a mixture.
The results are shown below.



(a) Describe briefly the method of doing this.

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(4 marks)

(b) Why does two-way chromatography make identification of the components of the mixture more certain?

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(1 mark)

(c) Find the R_f values of A:

(i) after the first run in solvent 1

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(1 mark)

(ii) after the second run in solvent 2.

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(1 mark)

4 A bottle was discovered labelled propan-2-ol. The chemist showed, using infrared spectroscopy, that the propan-2-ol was contaminated with propanone.

The chemist separated the two compounds using column chromatography. The column contained silica gel, a polar stationary phase.

The contaminated propan-2-ol was dissolved in hexane and poured into the column. Pure hexane was added slowly to the top of the column. Samples of the eluent (the solution leaving the bottom of the column) were collected.

- Suggest the chemical process that would cause a sample of propan-2-ol to become contaminated with propanone.
- State how the infrared spectrum showed the presence of propanone.
- Suggest why propanone was present in samples of the eluent collected first (those with shorter retention times), whereas samples containing propan-2-ol were collected later.

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(4 marks)