

Q1.

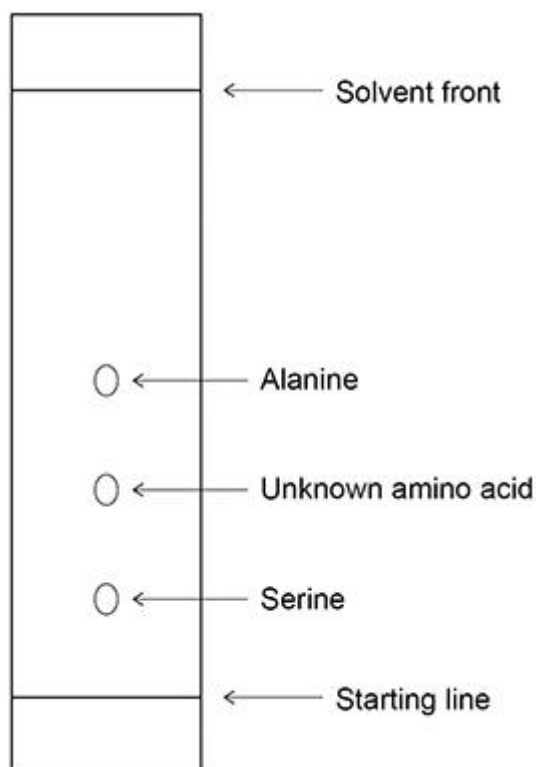
The protein fibroin can be broken down into amino acids using an enzyme.

- (a) A student uses thin-layer chromatography (TLC) to identify these amino acids.

The student identifies two of the amino acids as alanine and serine.

Use the figure below to calculate the R_f value of the unknown amino acid. Show your working.

Use your R_f value and the table below to identify the unknown amino acid.



Amino acid	R_f value
tyrosine	0.25
glycine	0.34
valine	0.64
leucine	0.73

R_f value _____

Identity _____

(2)

- (b) The amino acids cannot be seen as they move during the experiment.

State how the amino acids can be made visible at the end of the experiment.

(1)

- (c) State why each amino acid has a different R_f value.

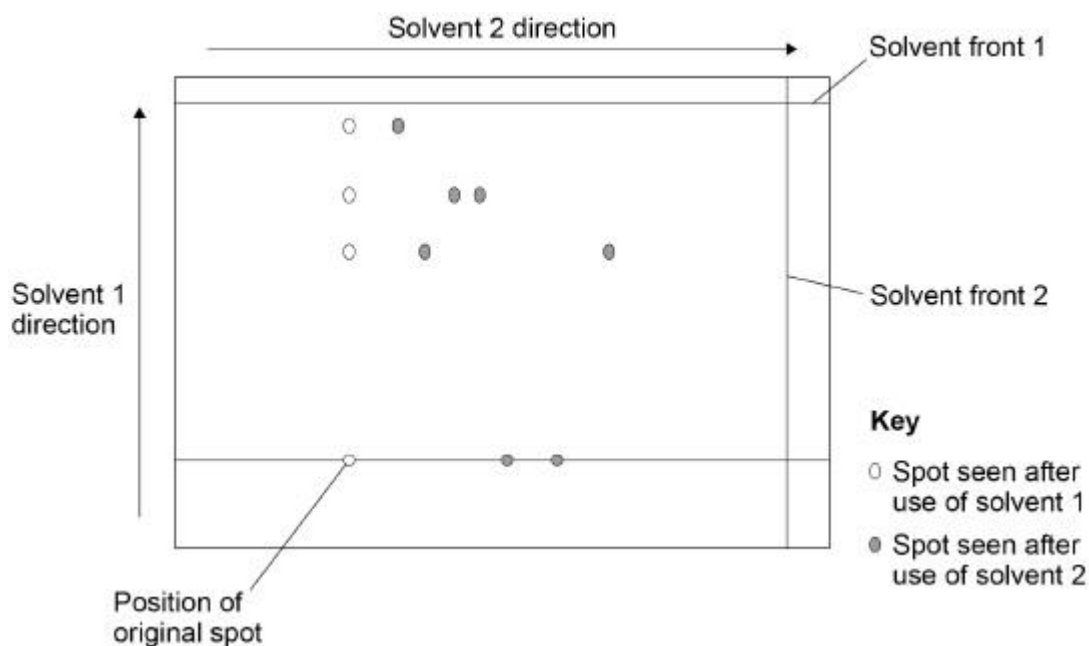
(1)

(Total 4 marks)

Q2.

This question is about thin-layer chromatography (TLC).

- A protein was hydrolysed to form a mixture of amino acids.
- A spot of this mixture was added to a TLC plate and the plate placed vertically in a small volume of solvent 1.
- When the solvent front reached nearly to the top of the plate, the plate was removed and allowed to dry.
- The plate was turned anticlockwise through 90° and placed vertically in a small volume of solvent 2.
- When the solvent front reached nearly to the top of the plate, the plate was again removed and allowed to dry.
- The diagram shows the final TLC plate.



- (a) Suggest a suitable reagent for the hydrolysis of a protein.

(1)

- (b) Suggest how the positions of the amino acids on the TLC plate were located.

(1)

- (c) Deduce the minimum number of amino acids present in the original mixture.

(1)

- (d) Suggest why it was necessary to use two different solvents.

(1)

(Total 4 marks)

Q3.

This question is about nitrobenzenes.

- (a) Nitrobenzene reacts when heated with a mixture of concentrated nitric acid and concentrated sulfuric acid to form a mixture of three isomeric dinitrobenzenes.

Write an equation for the reaction of concentrated nitric acid with concentrated sulfuric acid to form the species that reacts with nitrobenzene.

(1)

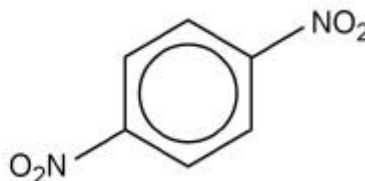
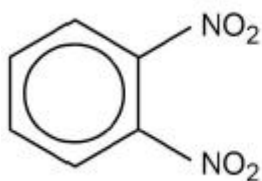
- (b) Name and outline a mechanism for the reaction of this species with nitrobenzene to form 1,3-dinitrobenzene.

Name of mechanism

Mechanism

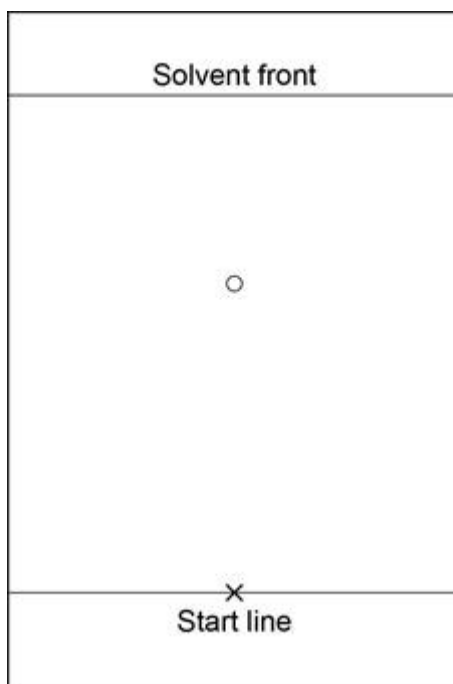
(4)

- (c) The dinitrobenzenes shown were investigated by thin layer chromatography (TLC).



In an experiment, carried out in a fume cupboard, a concentrated solution of pure 1,4-dinitrobenzene was spotted on a TLC plate coated with a solid that contains polar bonds. Hexane was used as the solvent in a beaker with a lid.

The start line, drawn in pencil, the final position of the spot and the final solvent front are shown on the chromatogram in the diagram below



Use the chromatogram in the diagram above to deduce the R_f value of 1,4-dinitrobenzene in this experiment.

Tick (✓) **one** box.

- | | | |
|----------|------|--------------------------|
| A | 0.41 | <input type="checkbox"/> |
| B | 0.46 | <input type="checkbox"/> |
| C | 0.52 | <input type="checkbox"/> |
| D | 0.62 | <input type="checkbox"/> |

(1)

- (d) State in general terms what determines the distance travelled by a spot in TLC.

(1)

- (e) To obtain the chromatogram, the TLC plate was held by the edges and placed in the solvent in the beaker in the fume cupboard. The lid was then replaced on the beaker.

Give one other practical requirement when placing the plate in the beaker.

(1)

- (f) A second TLC experiment was carried out using 1,2-dinitrobenzene and 1,4-dinitrobenzene. An identical plate to that in part (c) was used under the same conditions with the same solvent. In this experiment, the R_f value of 1,4-dinitrobenzene was found to be greater than that of 1,2-dinitrobenzene.

Deduce the relative polarities of the 1,2-dinitrobenzene and 1,4-dinitrobenzene and explain why 1,4-dinitrobenzene has the greater R_f value.

Relative polarities

Explanation

(2)

- (g) A third TLC experiment was carried out using 1,2-dinitrobenzene. An identical plate to that in part (c) was used under the same conditions, but the solvent used contained a mixture of hexane and ethyl ethanoate.

A student stated that the R_f value of 1,2-dinitrobenzene in this third experiment would be greater than that of 1,2-dinitrobenzene in the experiment in part (f)

Is the student correct? Justify your answer.

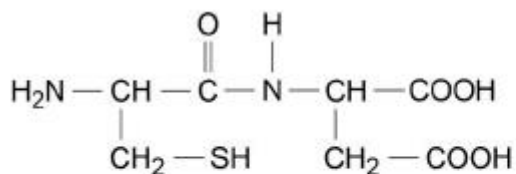
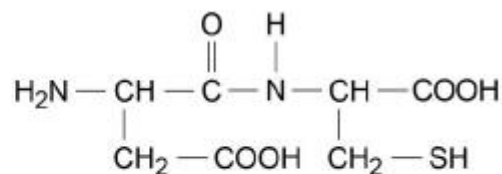
(2)

(Total 12 marks)

Q4.

Chromatography is used to identify amino acid sequences in compounds.

The dipeptide cysteine-aspartic acid (cys-asp), **J**, and the dipeptide aspartic acid-cysteine (asp-cys), **K**, are shown.

**J** (cys-asp)**K** (asp-cys)

- (a) A mixture of the two dipeptides **J** and **K** is analysed by gas chromatography followed by mass spectrometry (GC-MS).

Explain why **J** and **K** can be separated by gas chromatography and why mass spectrometry using electrospray ionisation does **not** enable you to identify them.

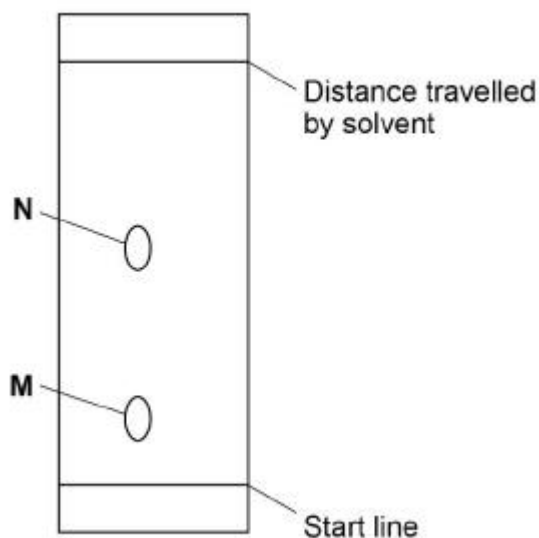
Gas chromatography explanation

Mass spectrometry explanation

(4)

- (b) A tripeptide, **L**, is partially hydrolysed with concentrated hydrochloric acid to produce two dipeptides and the amino acids alanine (ala), lysine (lys) and serine (ser).

The two dipeptides are separated by chromatography. The diagram below shows the chromatogram.



The table below contains the R_f values of some dipeptides.

Dipeptide	ala-lys	ala-ser	lys-ser	lys-ala	ser-ala	ser-lys
R_f value	0.55	0.85	0.10	0.20	0.15	0.45

Use the chromatogram in the diagram above and the R_f values in the table to identify the two dipeptides present in spots **M** and **N**.

Use your answers to deduce the order of the amino acids in the tripeptide **L**.

Dipeptide responsible for spot **M**

Dipeptide responsible for spot **N**

Order of amino acids in tripeptide **L**

(3)

(Total 7 marks)

Q5.

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**.
- (a) Parts of the procedure are in bold text.

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

(4)

- (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)

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

(2)