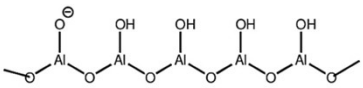
Chromatography

Using chapter 13 in Pearson and 19 in Lucarelli, answer the following questions

- Define chromatography and describe its purpose
- Describe the major components of chromatography
- Describe the principle concept of chromatography by referring to IMFs interaction between the particles in the sample and those in the stationary the mobile phases
- Compare different types of chromatography (paper chromatography, TLC, GC, HPLC) in terms of the stationary and mobile phases, mechanism (how it works), data collected, uses and limitations (SHE)
- Use knowledge of the polarity of the components of a mixture to be separated to select a chromatographic method for separation.
- Calculate retention factors for components in TLC and

1. *In thin-layer chromatography, alumina is often used as the stationary phase.*



Identify whether this would be a polar or non-polar solvent.

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2. *Explain why a polar component of a sample being separated by chromatography would not move far from the origin if alumina was used as the stationary phase. Discuss intermolecular forces in your answer.*

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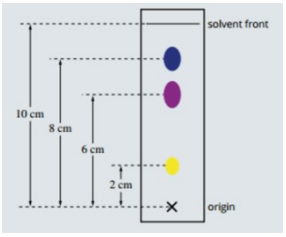
3. *Calculate the Rf value of the blue purple and yellow dots. Show your working.*



4. *A solvent of hexane was used as the mobile phase in column chromatography. Draw the structure of hexane.*

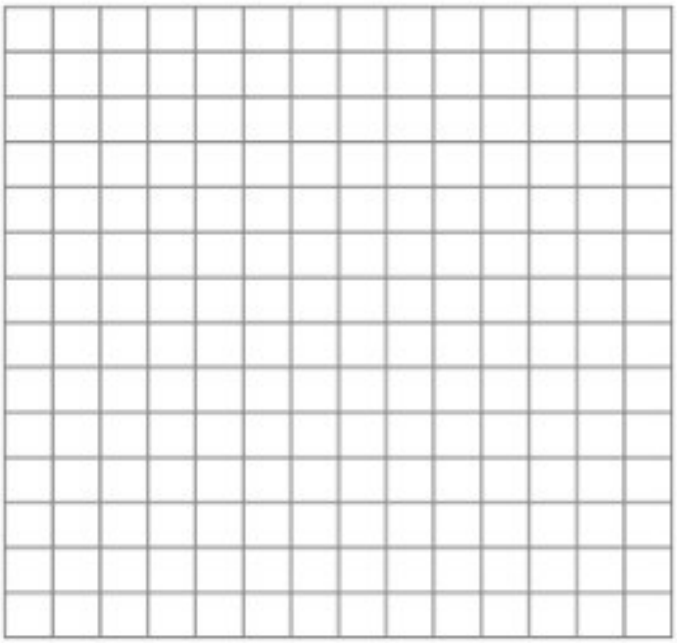
5. *Deduce whether hexane is a polar or non-polar solvent and hence what kind of components of a sample (polar or non-polar) would interact most with this mobile phase.*

6. *Explain, using ideas about intermolecular forces, why you think this interaction is more likely to occur.*

7. *One of the key differences between HPLC and Column is that HPLC uses particles that are 10-20 times smaller than those used in column chromatography. Why is this better?*

8. *Procymidone is a pesticide that can be used to prevent disease in oranges. Tests have been performed by HPLC to determine the concentration for this pesticide in a sample of orange juice. The chromatograms of a series of standards with accurately known concentrations of procymidone were obtained under the same conditions as the sample. The results from the chromatograms of the samples and the standards are shown in the following table. Determine the concentration of procymidone in the sample of orange juice.*

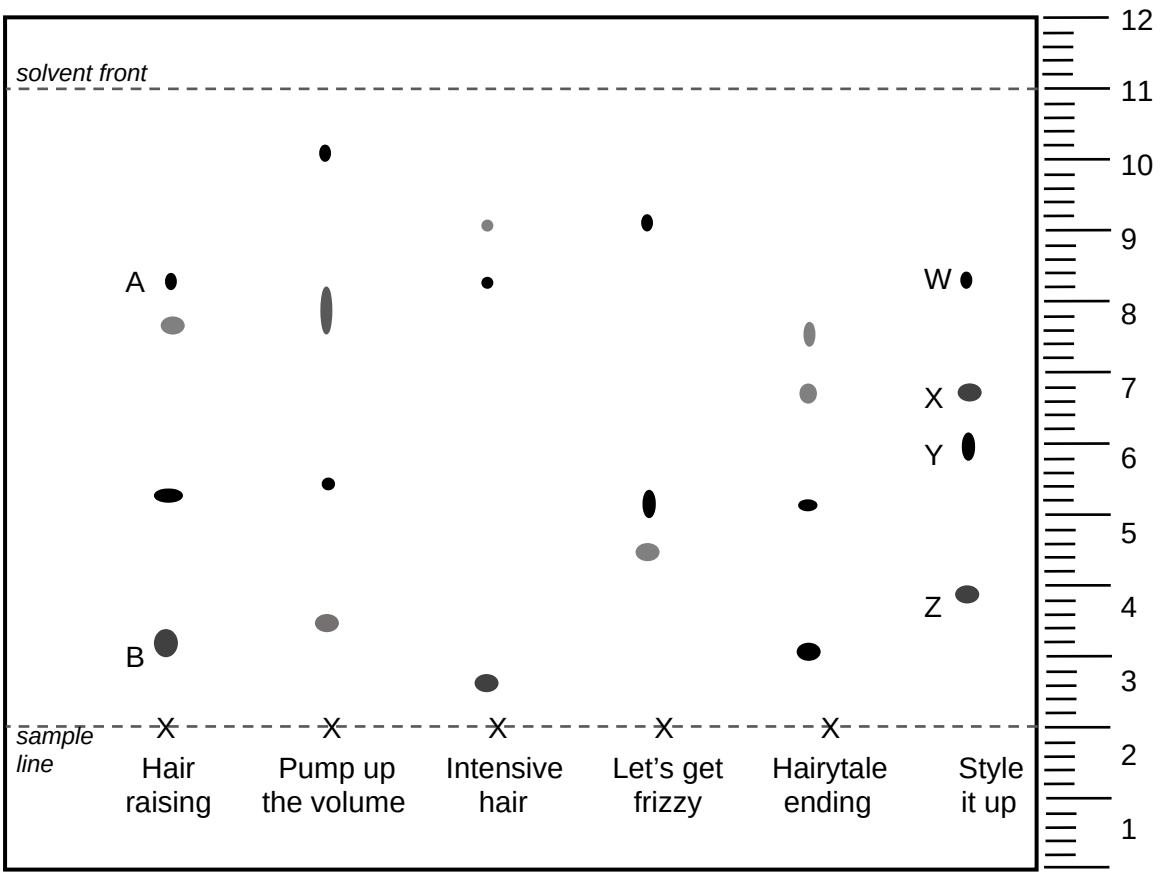
	Peak AREA	*Concentration (mg kg^{-1})*
Standard 1	*10*	*0.5*
Standard 2	*20*	*1.0*
Standard 3	*30*	*1.5*
Standard 4	*40*	*2.0*
Standard 5	*50*	*2.5*
Sample	*15*	*?*



The following questions are past semester exam questions:

Analysis of various hair products such as dyes, sprays and serums can be of great benefit to forensic investigators. Since hair samples are often found at crime scenes, identification of the hair products which are coating the hair can provide valuable information to investigators.

One common and effective method used for the analysis of hair products is thin layer chromatography (TLC). The TLC plate below shows the analysis of six (6) different popular brands of hair spray.



The plate, which is the stationary phase, is made of glass coated with silica. A small amount of each hair spray was spotted onto the sample line. The plate was then placed into a solution which acted as the mobile phase.

a) Briefly describe how the technique of TLC is able to separate the various components of a sample. Your answer should make reference to the role of both the stationary and mobile phases.

(4 marks)

For the TLC plate shown on the previous page, the scientists found that using a mobile phase composed of tetrachloromethane and cyclohexane (mixed in a 90:10 ratio) achieved optimal separation of components.

(b) Draw full structural diagrams for two structural isomers of cyclohexane. Your diagrams should indicate all bonds and atoms.

(2 marks)

Consider components A and B, which are labelled on the TLC plate, as well as the other information provided regarding the stationary and mobile phases.

(c) Which component, A or B, is likely to be the most polar? Justify your answer.

(3 marks)

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_____

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A hair sample from a crime scene was analysed by TLC and found to be coated in the hairspray 'Style it up'.

(d) Calculate the retention factor (R_f) values for the X and Y components (labelled on the TLC plate) found in the hairspray 'Style it up'.

(2 marks)

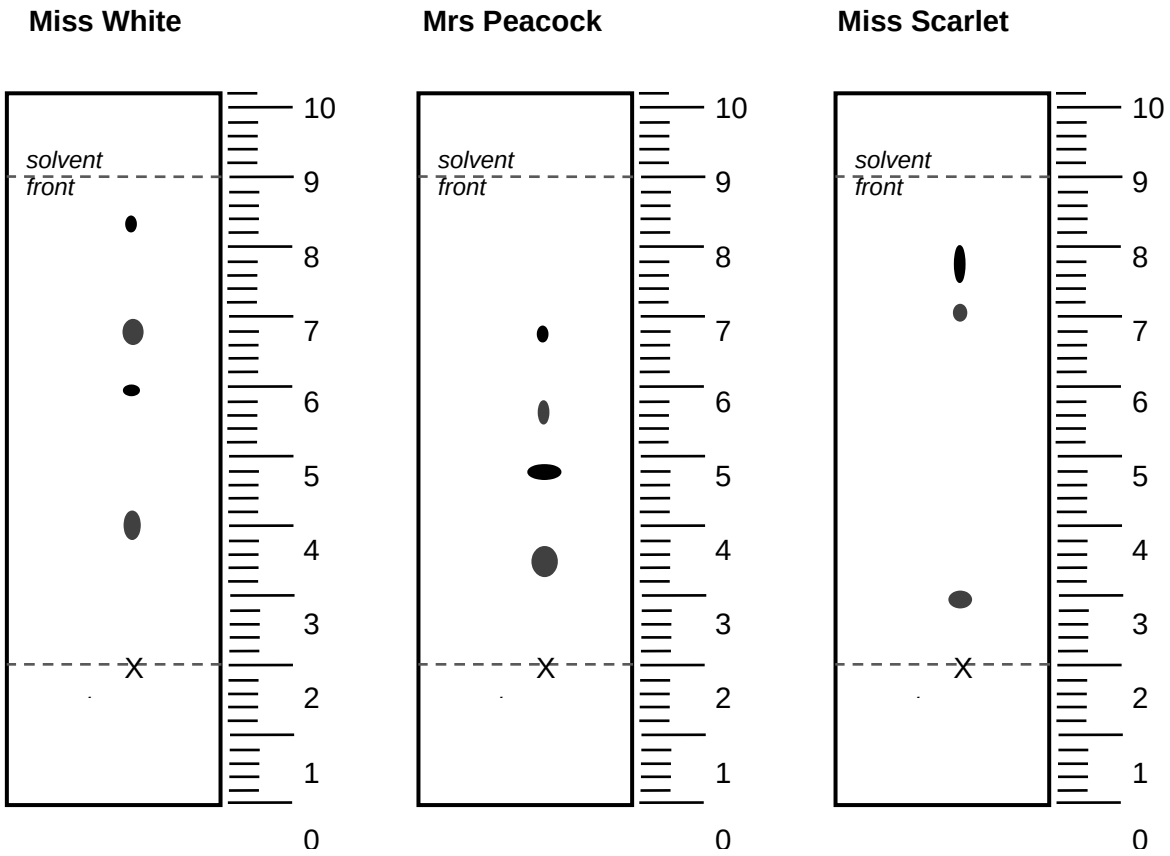
$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

	R_f
X	

Y	

Hair samples were then taken from three (3) suspects who had been arrested. The coatings on their hair samples were analysed by TLC. You may assume this analysis was performed under conditions identical to the original plate.

The results of the suspects' TLC analyses are shown below.



Based on the data provided by these TLC analyses;

(e) Which suspect is most likely to have been at the scene of the crime?(1 mark)

Thin layer chromatography (TLC) can be used to analyse the various natural sweeteners found in food and drink.

(a) *Explain how TLC is able to separate the components of a substance. Your answer should refer to the role of both the stationary and mobile phases and the effect of component polarity on the distance that the components travel along the TLC plate.*

(5 marks)

Thin layer chromatography (TLC) can be used to analyse the various natural sweeteners found in food and drink. The following table gives some data regarding the R_f values (see formula below) of different natural sweeteners.

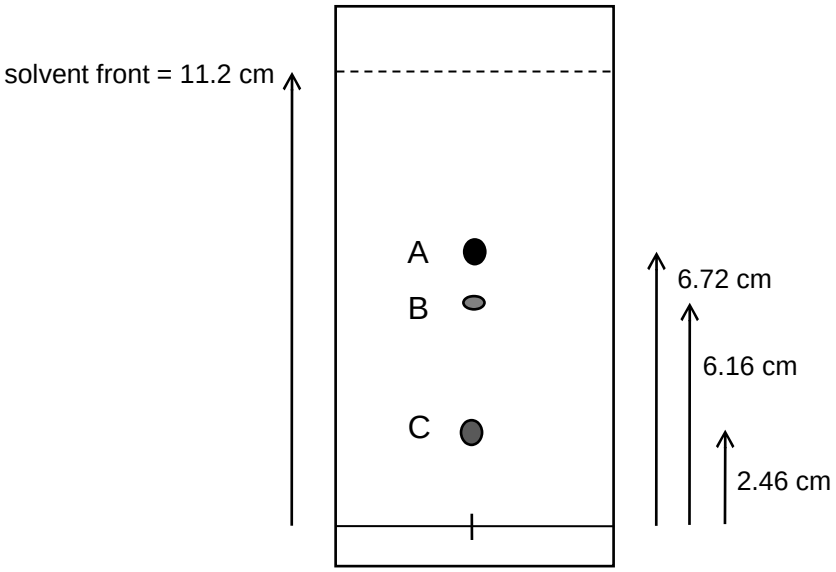
$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

Sweetener	**R_f**
Glucose	0.60
Fructose	0.60
Sucrose	0.55
Maltose	0.50
Maltotriose	0.41
Maltotetraose	0.30
Maltopentaose	0.22
Maltohexaose	0.16

(b) *Compare the polarity of maltose and glucose. Briefly justify your answer.*

(2 marks)

A small amount of food was analysed by TLC under the same conditions used to produce the data above. This plate is shown below.

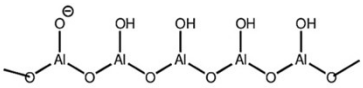


(c) *Identify which sweetener component B is most likely to be in this food sample. Justify your answer using R_f values?*

(2 marks)

ANSWERS:

1. *In thin-layer chromatography, alumina is often used as the stationary phase.*



Identify whether this would be a polar or non-polar solvent.
POLAR

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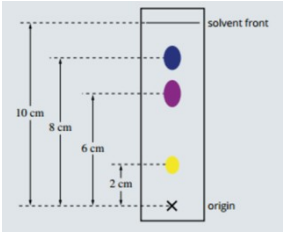
2. *Explain why a polar component of a sample being separated by chromatography would not move far from the origin if alumina was used as the stationary phase. Discuss intermolecular forces in your answer.*

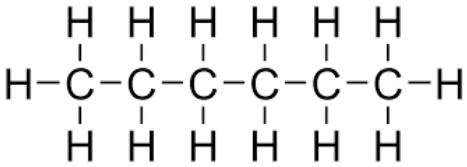
Since the stationary phase is polar, any polar component in the sample is likely to interact strongly with the stationary phase through intermolecular forces such as dipole-dipole or hydrogen bonding. This strong interaction would prevent the component from moving up through the stationary phase.

3. *Calculate the Rf value of the blue purple and yellow dots. Show your working.*



Yellow 2/10 = 0.2
Purple 6/10 = 0.6
Blue 8/10 = 0.8

4. *A solvent of hexane was used as the mobile phase in column chromatography. Draw the structure of hexane.*



5. *Deduce whether hexane is a polar or non-polar solvent and hence what kind of components of a sample (polar or non-polar) would interact most with this mobile phase.*

Hexane is non-polar

Other non-polar components are likely to interact most with hexane through dispersion forces.

6. *Explain, using ideas about intermolecular forces, why you think this interaction is more likely to occur.*

Polar substances are more likely to interact with other polar substances, non-polar substances are more likely to interact with other non-polar substances due to the similarity in intermolecular forces. For example in non-polar substances, dispersion forces are likely to be exhibited.

7. *One of the key differences between HPLC and Column is that HPLC uses particles that are 10-20 times smaller than those used in column chromatography. Why is this better?*

It allows for more frequent adsorption and desorption of the components giving much better separation.

8. *Procymidone is a pesticide that can be used to prevent disease in oranges. Tests have been performed by HPLC to determine the concentration for this pesticide in a sample of orange juice. The chromatograms of a series of standards with accurately known concentrations of procymidone were obtained under the same conditions as the sample. The results from the chromatograms of the samples and the standards are shown in the following table. Determine the concentration of procymidone in the sample of orange juice.*

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Standard 4	*40*	*2.0*
Standard 5	*50*	*2.5*
Sample	*15*	*?*

0.75 mg kg^{-1}

The following questions are past semester exam questions:

Analysis of various hair products such as dyes, sprays and serums can be of great benefit to forensic investigators. Since hair samples are often found at crime scenes, identification of the hair products which are coating the hair can provide valuable information to investigators.

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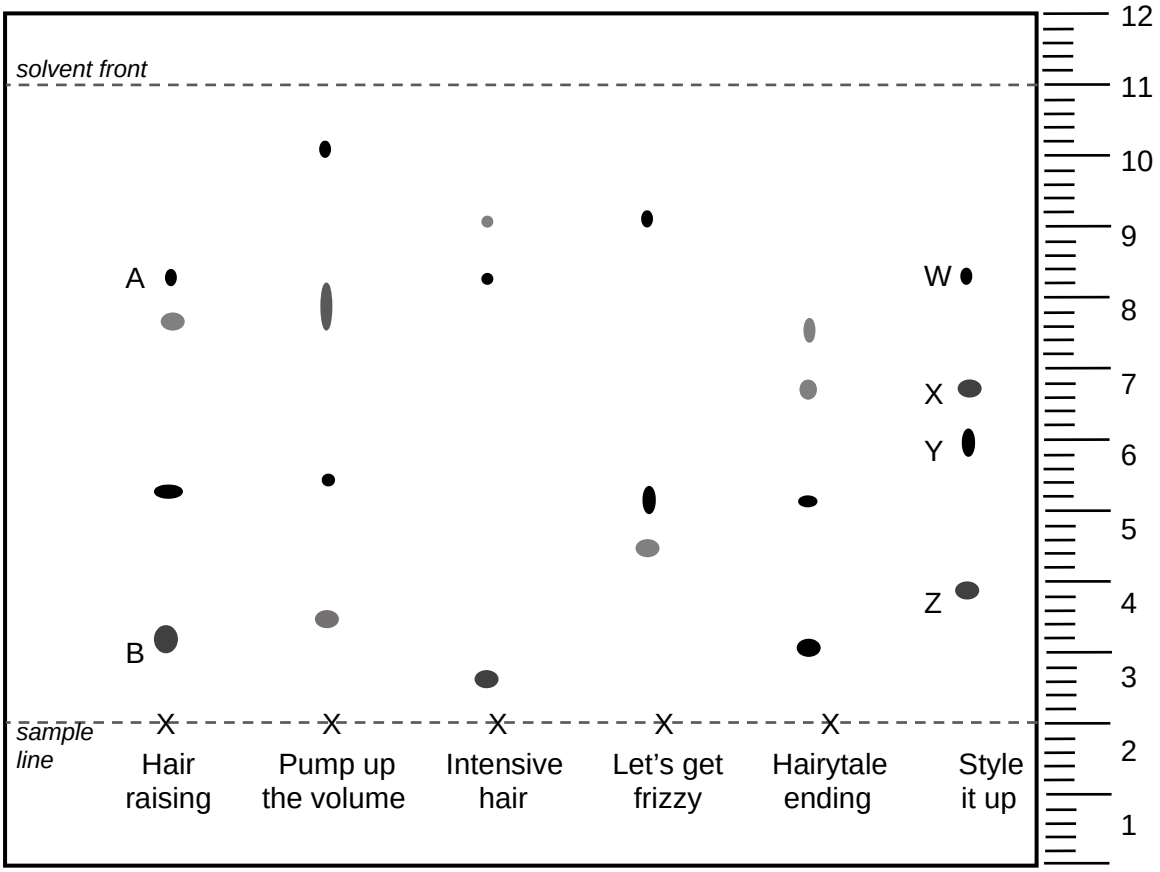


The plate, which is the stationary phase, is made of glass coated with silica. A small amount of each hair spray was spotted onto the sample line. The plate was then placed into a solution which acted as the mobile phase.

a) *Briefly describe how the technique of TLC is able to separate the various components of a sample. Your answer should make reference to the role of both the stationary and mobile phases.*

(4 marks)

- **Mobile phase moves upwards across stationary phase, carrying components of sample with it**
- **Components interact to varying degrees with stationary and mobile phases based on their polarity**
- **Components that adhere more strongly to the stationary phase move more slowly, whilst those dissolving to a greater degree in the mobile phase move more quickly**
- **Components therefore move at different rates up the plate and are thus separated**

For the TLC plate shown on the previous page, the scientists found that using a mobile phase composed of tetrachloromethane and cyclohexane (mixed in a 90:10 ratio) achieved optimal separation of components.

(b) *Draw full structural diagrams for two structural isomers of cyclohexane. Your diagrams should indicate all bonds and atoms.*

(2 marks)

Any two of the following compounds • **Methylcyclopentane** • **Dimethylcyclobutane** • **Hexene** • **Methylpentene** • **Dimethylbutane**	

Consider components A and B, which are labelled on the TLC plate, as well as the other information provided regarding the stationary and mobile phases.

(c) *Which component, A or B, is likely to be the most polar? Justify your answer.*

(3 marks)

- **B**
- **(Since the mobile phase is non-polar) the non-polar components will be more soluble in the mobile phase and move upwards more quickly**
- **Polar components will therefore move more slowly up the plate**

A hair sample from a crime scene was analysed by TLC and found to be coated in the hairspray 'Style it up'.

(d) *Calculate the retention factor (R_f) values for the X and Y components (labelled on the TLC plate) found in the hairspray 'Style it up'.*

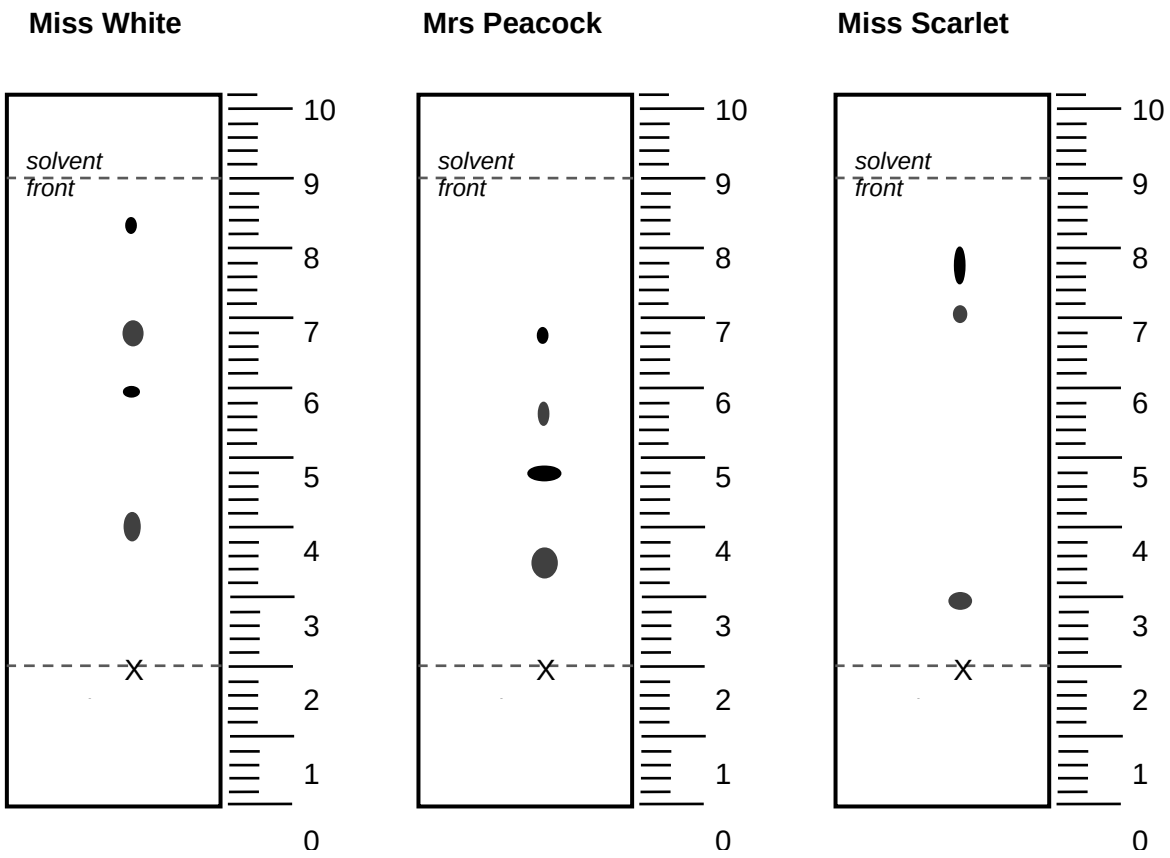
(2 marks)

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

	R_f
X	**accept between 0.51 - 0.53**
Y	**accept between 0.42 - 0.45**

Hair samples were then taken from three (3) suspects who had been arrested. The coatings on their hair samples were analysed by TLC. You may assume this analysis was performed under conditions identical to the original plate.

The results of the suspects' TLC analyses are shown below.



Based on the data provided by these TLC analyses;

(e) Which suspect is most likely to have been at the scene of the crime?(1 mark)

- **Miss White**

Thin layer chromatography (TLC) can be used to analyse the various natural sweeteners found in food and drink.

(a) Explain how TLC is able to separate the components of a substance. Your answer should refer to the role of both the stationary and mobile phases and the effect of component polarity on the distance that the components travel along the TLC plate.

(5 marks)

- **A sample of a mixture is added to the TLC plate embedded with polar SP and the plate is dipped into a non-polar MP solvent.**
- **Less/non-polar components will be more soluble/desorb/interaction in the mobile phase**
- **=> travel a larger distance**

- **(More) Polar components will adhere/interact more strongly to the stationary phase.**

- **=> travel a smaller distance**

Thin layer chromatography (TLC) can be used to analyse the various natural sweeteners found in food and drink. The following table gives some data regarding the R_f values (see formula below) of different natural sweeteners.

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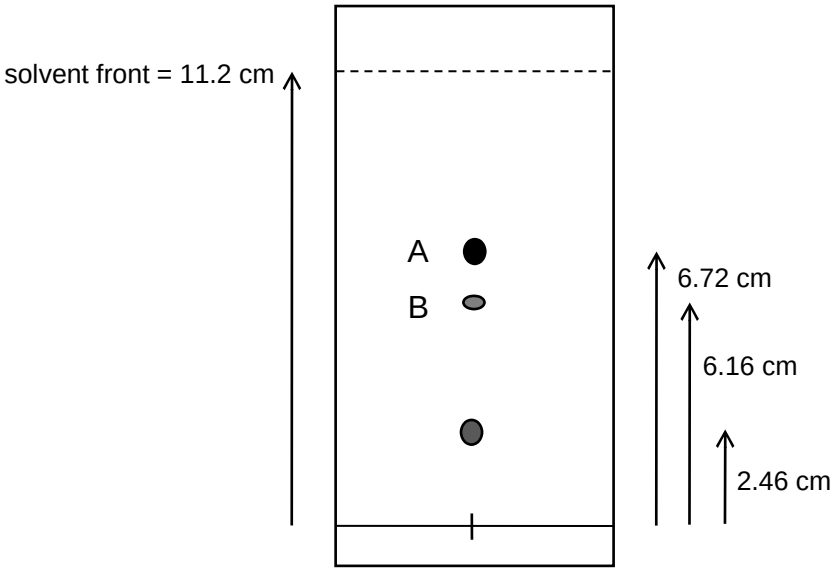
(b) Compare the polarity of maltose and glucose. Briefly justify your answer.

(2 marks)

Maltose is more polar than glucose / glucose is less polar than maltose.

Larger Rf value = travel larger distance along the TLC plate

A small amount of food was analysed by TLC under the same conditions used to produce the data above. This plate is shown below.



C

(c) *Identify which sweetener component B is most likely to be in this food sample. Justify your answer using R_f values?*

(2 marks)

Sucrose

Rf value = 0.55