Answer Sheet

1. Setting up your TLC – practical considerations:

a) Ink would travel up the TLC sheet with the mobile phase. Hence, distinguishing the spots belonging to the ink and the analyte would be impossible. Graphite, (the "lead" in pencils) does not travel up the TLC sheet.

b) Capillary action would be uneven if the TLC sheet touched the sides of a jar. Consequently, one side of the TLC sheet would reach the solvent front faster than the other.

c) To be able to calculate R_f values accurately.

d) A new solvent front line would need to be drawn where the solvent was when the TLC sheet was removed from the jar. Then, the R_f value would be calculated according to the new distance between the baseline and the solvent front. Note: If the solvent reached the very top of the TLC sheet, the experiment would have to be repeated.

2. Experiment 5a – Red and pink

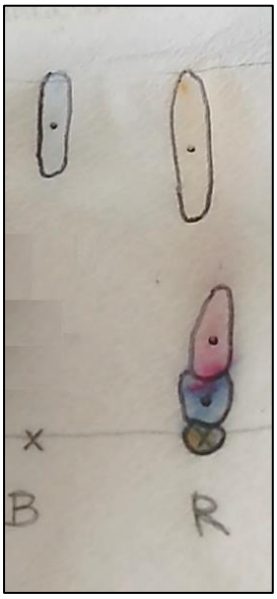
The pink dye contains only one component (pink spot) while the red dye contains three components (orange, red and blue spots). It is possible that both dyes contain in fact another component as there is an orange-yellow spot visible on the baseline for both of them (Figure 1).

R_f (Pink) = 4.3/5.0 = 0.86
R_f (Red – Orange spot) = 3.5/5.0 = 0.70 (can be higher)
R_f (Red – Red spot) = 1.3/5.0 = 0.26
R_f (Red – Blue spot) = 0.5/5.0 = 0.10

Note: The R_f values you calculated based on your TLC sheet may differ slightly from the above values. In particular, the orange spot sometimes travels quite far up and does not streak so the R_f value can be higher than in this example. If your values for the red and blue spots differ by more than 0.04, try to repeat the experiment.

Figure 1. TLC sheet containing pink ("P") and red ("R") dye.

3. Experiment 5b – Red and blue



The blue dye contains only one component (top blue spot, analyte labelled as "B") (Figure 2). Its R_f value is, however, much higher than the R_f value of the blue dye present in the commercial red dye. Therefore, the blue spot in the blue dye is <u>not</u> the same chemical as the one responsible for the blue spot in the red dye.

R_f (Blue) = 4.5/5.0 = 0.90
R_f (Red – Orange spot) = 4.0/5.0 = 0.80
R_f (Red – Red spot) = 1.2/5.0 = 0.24
R_f (Red – Blue spot) = 0.4/5.0 = 0.08

Figure 2. TLC sheet containing blue ("B") and red ("R") dye.

4. Experiment 5c – Blue + yellow = green

a) Spot "1" contains the mixture of blue and yellow dyes because both components are of similar intensity (remember that you mixed them in a 1:1 ratio). Spot "2" only contains a very small and faint blue spot near the solvent front (Figure 3).

   Note: Your experiment may have spots "1" and "2" switched.

b) A mixture due to the very faint blue spot that was observed near the solvent front.

c) Both components of the green dye are the same as in commercial yellow and blue dyes because they have the same colour and R_f value as the yellow ("Y") and blue ("B") spots.

d) The difference in R_f values is due to the oily nature of the yellow dye. It caused smearing for the blue dye present in spot "1" and decreased its R_f value. No smearing occurred in spot "B".

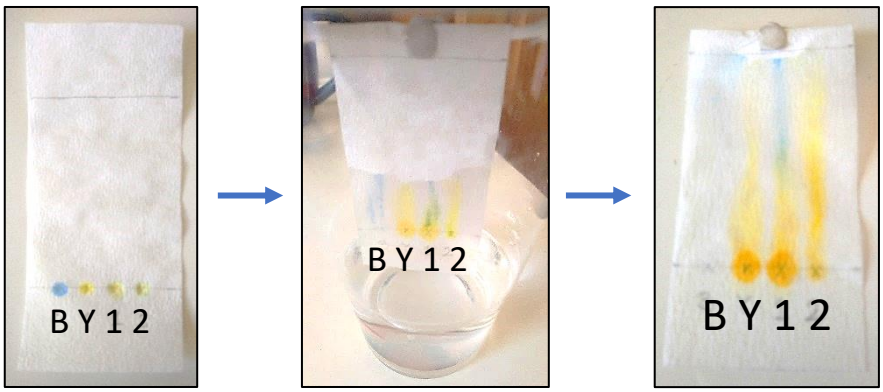


Figure 3. TLC sheet containing blue dye ("B"), yellow dye ("Y"), a mixture of blue and yellow dye ("1") and green dye ("2").

5. Experiment 5d – Red or pink?

The colour of the beetroot juice spot is the same as the pink dye, but slightly paler than the red spot in the red dye (Figure 4). The R_f values are as follows:

R_f (Pink) = 4.3/5.0 = 0.86
R_f (Beetroot) = 3.7/5.0 = 0.74
R_f (Red – Red spot) = 1.3/5.0 = 0.26

Solution to the research question: It is clear that beetroot juice is not present in the red dye, however, it is difficult to conclude from the above values whether it is present in the pink dye. The R_f values are not dissimilar enough and could be well within the error considering this experiment was done at home, and that beetroot juice also contains other components that may have caused smearing (see Experiment 5c) and hence a lower R_f value in spot "J".

Figure 4. TLC sheet containing pink dye ("P"), beetroot juice ("J") and red dye ("R").

6. Experiment 5e – Which natural source?

Solution to the research question: Sweetcorn is not used as a commercial dye.

a) You can check the ingredients of each dye on its label. The summary is in Table 1.

Table 1. Natural sources in commercial dyes.

Natural source	Commercial dye colour
Turmeric (curcumin)	Yellow, Green
Beetroot	Pink
Paprika extract	Red
Blue spirulina	Blue, Green
Green spirulina	Green
Anthocyanin pigments	Red

b) Vegetable carbon is present in black commercial dyes.

Optional Advanced Experiments

7. Experiment 6a – (In)solubility of curcumin

a) Curcumin is insoluble in water, hence remains on the baseline. As the mobile phase reaches the baseline, only compounds partially or fully soluble in it will move.

b) For example, ethanol, isopropanol, ethyl acetate, chloroform, dimethyl sulfoxide.

c) 50 mL of a 1:1 isopropanol:water solution contains 25 mL of isopropanol. 25 mL of isopropanol is in 25/7 x 10 = 35.7 mL of 70% solution of isopropanol in water. To make 50 mL of a 1:1 solution, take 35.7 mL of the 70% solution and add 14.3 mL of water.

d) Changing the mobile phase to a solvent in which curcumin would be more soluble would make it travel further up the TLC sheet. For example, ethyl acetate, higher percentage of isopropanol in water, or even pure isopropanol might achieve that.

e) As the mobile phase travels up the TLC sheet and reaches the baseline, it will be more miscible with the commercial yellow dye spot that contains some proportion of water than with the completely oily turmeric powder spot. Hence, curcumin in the commercial dye spot will travel faster.

Solution to the research question:
When we spotted the yellow and green commercial dyes as well as the turmeric powder dissolved in 2:1:1 oil:isopropanol:water and ran the TLC sheet in 1:1 isopropanol:water as the eluent, we obtained three very similar traces (Figure 5). Hence, it can be concluded that both the green dye as well as turmeric powder contain curcumin.

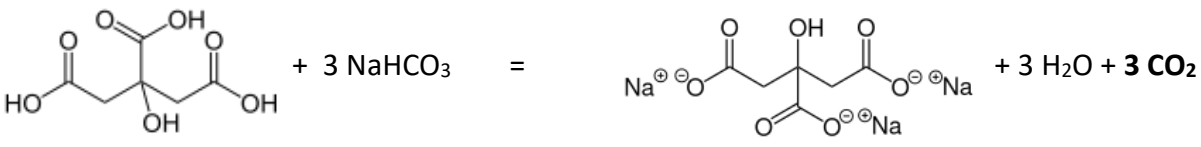
Figure 5. TLC sheet containing yellow dye ("Y"), green dye ("G") and turmeric powder dissolved in 2:1:1 oil:isopropanol:water ("T"). Eluent: 1:1

8. Experiment 6b – Anthocyanins – red, blue or purple?

Solution to the research question: It was the red spot that changed colour to blue on addition of sodium bicarbonate. Therefore, the red spot in the red dye corresponds to anthocyanin pigment.

a) The colour of the pigment in acidic media is red but in basic environment it is blue.

b) The gas responsible for the effervescence is carbon dioxide (CO_2). It is produced by the neutralisation reaction of citric acid with sodium bicarbonate:



Citric acid Sodium bicarbonate Trisodium citrate

c) Examples of naturally occurring pH indicators: purple cabbage, beetroot, turmeric
Examples of synthetic pH indicators: phenolphthalein, bromothymol blue

9. Experiment 6c – Monitoring the progress of a reaction

Solution to the research question: The TLC on the left in Figure 18 in the main protocol shows a reaction at its beginning (intense starting material spot, faint product spot) while the one on the right shows a nearly completed reaction (very faint starting material spot, intense product spot).

a) A reaction that is at its half point would have the starting material ("SM") and product ("P") spots equally intense in the reaction mixture ("RXN") spot (Figure 6).

b) In a reaction that produces only one product there is no risk: the spot that is not the starting material must be the product.

However, if a reaction produces by-products, hence multiple spots on the TLC plate, it may be difficult/impossible to distinguish them from the desired product spot. R_f values may help to differentiate certain functional groups based on their polarity (e.g. alcohols are more polar than esters and would have a lower R_f value), but for structurally similar compounds this may not be possible.

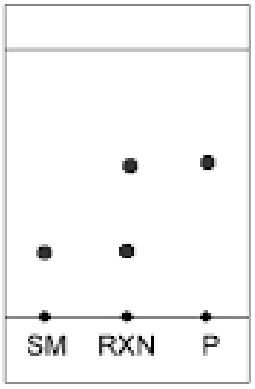


Figure 6. TLC plate of a reaction that is 50% complete.

Generally, if a reaction produces multiple products, we would:

- monitor the disappearance of the starting material until it has all reacted or there is no change in the intensity of the starting material spot over time,
- stop the reaction,
- separate the multiple products (e.g. by distillation, sublimation, recrystallisation or column chromatography – see bonus material),
- identify the products (using e.g. infrared spectroscopy, nuclear magnetic resonance spectroscopy, UV-vis spectroscopy, X-ray crystallography or mass spectrometry).

Bonus Material

10. Experiment 7a – When TLC is not good enough

a) 1) Red:Blue = 1:1 2) Red:Blue = 2:1 3) Red:Blue = 9:1 4) Red:Blue:Yellow = 2:1:1

b) For example, UV-vis spectroscopy, nuclear magnetic resonance spectroscopy, gas chromatography, high-performance liquid chromatography.

c) UV-vis spectroscopy is a quantitative technique: the relative absorbance of the red and blue dye would be reflected in the spectrum. The spectrum would contain three traces (each corresponding to solution "1", "2" or "3") and within each trace, the

relative absorbance (or ratio) of red and blue dye can be calculated at their respective λ_{max}. The largest ratio would be for the solution containing red:blue in 9:1, then the 2:1, then the 1:1.

Note: Given that all three solutions "1", "2" and "3" contain different number of drops of each dye, the absolute absorbances of the three traces would differ.

d) The values you extracted may vary slightly to those in Table 2. If the λ_{max} value is different by more than 8 nm, or the absorbance by more than 0.1, have a look at the spectra again.

Table 2. λ_{max} and absorbance values for red, yellow and blue dye.

	Red dye	Yellow dye	Blue dye
λ_{max}	519 nm	428 nm	628 nm
A	1.52	1.12	1.14

e) We need to use the Beer-Lambert Law: $A = \varepsilon.c.l$
- Path length of the cell (l) = 1 cm
- $\varepsilon = 1.54 \times 10^6$ $M^{-1}cm^{-1}$ for phycocyanin = blue dye
  $\varepsilon = 26\,900$ $M^{-1}cm^{-1}$ for anthocyanin = red dye
  $\varepsilon = 55\,000$ $M^{-1}cm^{-1}$ for curcumin = yellow dye

➢ Therefore: concentration (c) = $A/(\varepsilon \cdot l)$
- Blue dye: $c = 1.14/(1.54 \times 10^6 \text{ M}^{-1}\text{cm}^{-1} \cdot 1 \text{ cm}) = 7.40 \times 10^{-7}$ M
- Red dye: $c = 1.52/(26\,900 \text{ M}^{-1}\text{cm}^{-1} \cdot 1 \text{ cm}) = 5.65 \times 10^{-5}$ M
- Yellow dye: $c = 1.12/(55\,000 \text{ M}^{-1}\text{cm}^{-1} \cdot 1 \text{ cm}) = 2.04 \times 10^{-5}$ M

f) An extract of the M&M dye could be placed into a UV spectrometer and a UV trace recorded. We can then:
- identify all λ_{max} values (this equals to the number of dyes present),
- assign the λ_{max} values to the correct dyes (colours),
- find the ε value for each dye present in the extract,
- calculate the concentration of each dye by using the Beer-Lambert Law.

g) The spectrum would look somewhat like a superimposition of each of the three individual traces (Figure 7). The absorbance at each of the three λ_{max} values would be determined by the Beer-Lambert Law:
- absorbance of the red dye = 3.20
- concentration = $3.20/(26\,900 \text{ M}^{-1}\text{cm}^{-1} \cdot 1 \text{ cm})$
  $= 1.19 \times 10^{-4}$ M
- therefore, the concentration of the yellow dye: 1.19×10^{-4} M / 4 = 2.97×10^{-5} M; its absorbance is then: $A = 2.97 \times 10^{-5} \text{ M} \cdot 55\,000 \text{ M}^{-1}\text{cm}^{-1} \cdot 1 \text{ cm} = \underline{1.64}$
- The concentration of the blue dye = 1.19×10^{-4} M / 80 = 1.49×10^{-6} M; its absorbance is then: $A = 1.49 \times 10^{-6} \cdot 1.54 \times 10^6 \text{ M}^{-1}\text{cm}^{-1} \cdot 1 \text{ cm} = \underline{2.29}$

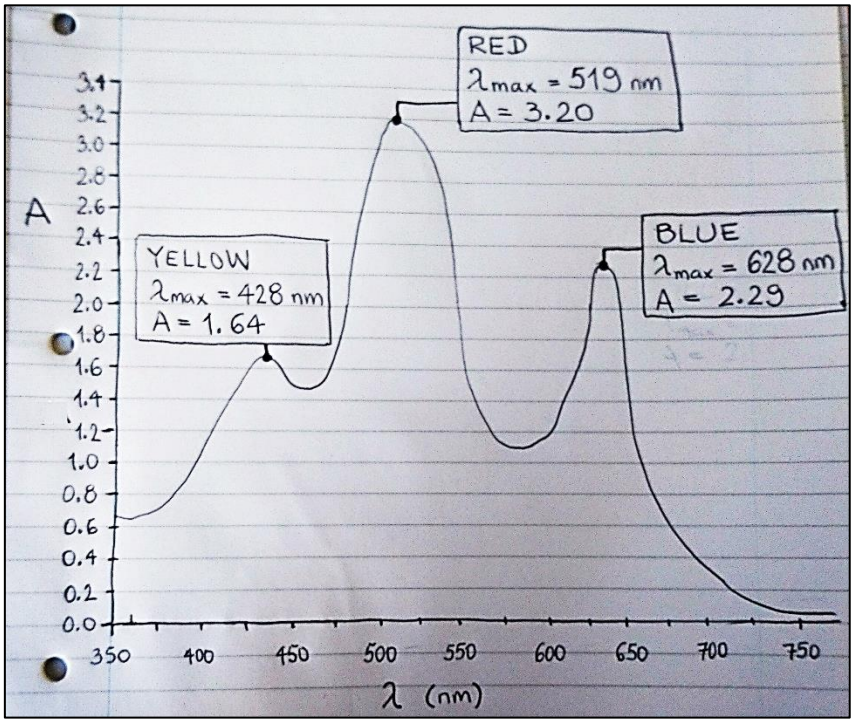


Figure 7. Example of a plausible UV-vis spectrum.

11. Experiment 7b – Column chromatography

a) ○ Both contain a stationary phase and a mobile phase.
○ They serve to separate and/or determine the number of components of a mixture.
○ Separation occurs based on the interactions of the analyte with stationary and mobile phases.
○ However, while TLC operates on a small scale, column chromatography is performed on a larger scale and allows isolation of compounds. While TLC usually only takes a few minutes, column chromatography may take several hours.

b) Method:

1. As a column, an evenly narrow and tall transparent bottle works best. Cut off the bottom part and pierce a hole into the bottle lid with a needle. Cover the bottle lid hole with a sticky adhesive (e.g. white tack). Make sure you remove the sticky adhesive once you start eluting the mobile phase. Also, make sure the column stands straight in its stand (you may need to secure it with Sellotape).
2. Load the column with cellulose stationary phase.
3. Add the mobile phase and run it through the column in order to compact the cellulose and to remove air bubbles.
4. Deposit carefully and evenly the red dye (10 mL) as a thin layer on top of the cellulose layer without disturbing it.
5. Add cotton wool pads on top to prevent the solvent disturbing the red dye.
6. Run the mobile phase (water) through the column, watch the separation occur.
7. Collect the first, fastest moving component (orange layer). Keep eluting.
8. Collect the second, slower component (red layer) into a new cup. Keep eluting.
9. Collect the third, slowest component (blue/purple layer) into a new cup. This completes the process.