Laboratory Protocol

TLC Analysis of Food Dyes

In this experiment, you will:

1. **Analyse** several readily available food dyes by **thin-layer chromatography (TLC).**
2. **Learn** the background theory of TLC and apply it to several scenarios.
3. **Compare** the conditions at home to what you would expect in a chemical laboratory.
4. Bonus material: two advanced laboratory techniques commonly used to identify and/or separate mixtures.

Figure 1. Materials needed:


Water

Sodium Bicarbonate


Fresh or Pickled beetroot


Sainsbury's Food Dyes (Pink, Red, Blue, Yellow, Green)

M&Ms

Figure 2. Equipment needed:


Teaspoon


Jar or Cup with a lid

Sticky tack or Sellotape


4 shot glasses

Coffee machine filter papers


Long Wooden skewer or Cotton bud


Pencil


Ruler


Scissors


Calculator


Needle with an eye (CAUTION!)


Marker pen

Optional:


Turmeric powder + oil

Materials and equipment are given as a suggestion. However, if these are not something you can access, we strongly encourage you to use any materials you have available that you think would work in a similar way!

We would love to know what materials you end up using and how you think they affected your experiment! Share this with us on Instagram or Twitter using the handle @kclchemoutreach and the #openaccesslabs. Alternatively, you can let us know by sending us an email to chemistry-outreach@kcl.ac.uk!

Open Access Labs

Open Access Labs are part of the Outreach programme at King's College London. Their aim is to make science, in particular chemistry, available to students who are interested in pursuing a career in STEM. In the last three years, several events have been organised and a number of young prospective scientists were able to experience a real chemical lab. They made aspirin, looked at chemistry of water, changed states of matter or used a range of analytical methods such as infrared spectroscopy, thin-layer chromatography and proton NMR spectroscopy, that are normally only covered in their textbooks.

The Open access labs Outreach programme is supported by RSC and is available for pupils from non-selective schools. At King's, we create a diverse and inclusive culture within our department. We have an equal split of male and female undergraduate students and more than 70% of our students are BME. We establish close collaborations with local schools and form lasting relationships with students to support them on their academic journeys.

In response to COVID-19 making our UG teaching inaccessible, we have created online content that will enable you to perform an Open Access Lab day remotely. This resource has been developed by Dr Filip Sebest in collaboration with Dr Helen Coulshed. We hope you enjoy it as much as we have enjoyed creating it!

Dr Filip Sebest Chemistry Teaching Technician	**Dr Helen Coulshed** Lecturer in Chemical Education

1. Learning Outcomes

By the end of this practical, you should be able to:

1. Describe the principle of thin-layer chromatography.
2. Design and perform thin-layer chromatography experiments.
3. Calculate retardation factor (R_f) values.
4. Realise that chemistry is all around us!
5. *Advanced experiments*: Understand more complex aspects of thin-layer chromatography.
6. *Bonus material*: a) Learn the theory behind UV spectroscopy and column chromatography.
   b) Perform simple calculations using the Beer-Lambert Law.

Transferable skills you will practice during this experiment:

- Manual dexterity
- Critical thinking
- Numeracy skills
- Safe working
- Data analysis
- Time management
- Decision making
- Observation skills
- Following instructions
- Experiment design

2. Before you start

Scientific Method:

1. Read the Introduction and Background Theory as well as the Method sections.
2. Think about how you would approach each experiment.
3. If you are unsure about something, try reading the Introduction and Background Theory and/or the Method section again. Online resources are also provided.
4. Watch our instructional video which demonstrates the key techniques that you will be using during the experiment.

 media.kcl.ac.uk/media/1_r60ic59q

3. Introduction and Background Theory

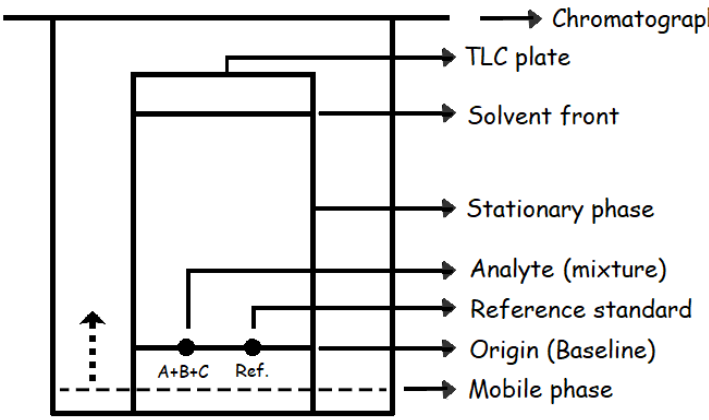
Thin-layer chromatography (TLC) is a chromatographic technique used to separate non-volatile mixtures. It is often used to monitor the progress of a reaction, to identify compounds present in a given mixture, and to determine the purity of a substance. TLC is performed on a thin layer of adsorbent material, usually silicon dioxide (silica), aluminium oxide (alumina) or cellulose. This layer of adsorbent is known as the **stationary phase**.

After the sample, also known as the **analyte**, has been applied to the stationary phase, a solvent or solvent mixture is drawn up the plate via **capillary action** (a phenomenon associated with surface tension whereby liquids can travel horizontally or vertically against the force of gravity). This solvent or solvent mixture is known as the **mobile phase** or **eluent**.

Because the mobile phase has different properties from the stationary phase, different analytes ascend the TLC plate at different rates and separation is achieved. To quantify the results, the distance travelled by an analyte from the **origin** is divided by the total distance travelled by the mobile phase from the origin to the **solvent front**. This ratio is called the **retardation factor, R_f**. (This is sometimes wrongly written as retention factor).

In general, a substance whose structure resembles the stationary phase will have a low R_f value, while one that has a similar structure to the mobile phase will have a high R_f value. Retardation factors are characteristic, but will change depending on the exact condition of the mobile and stationary phase. For this reason, chemists usually apply a sample of a known compound to the plate as well – known as the **reference standard**.

An example of a typical TLC setup and a calculation of retardation factor values is shown in Figures 3 and 4, respectively.



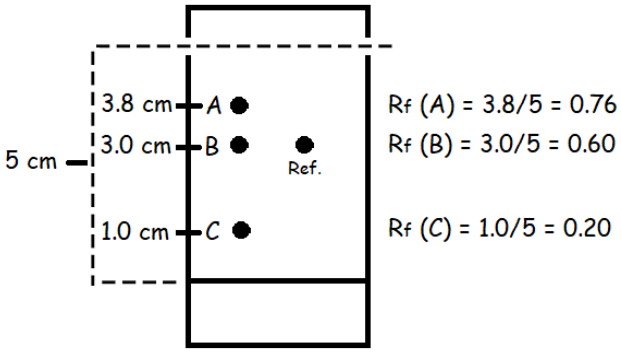


Figure 3. Labelled TLC diagram. **Figure 4.** How to calculate retardation factor (R_f).

4. Method

Learning how to perform a TLC experiment successfully may take some practice, but once you have mastered the process it is not difficult to repeat it. The step-by-step procedure below should help you with grasping the practical aspects of it in a relatively short time.

Prepare your TLC plate

a) Using a ruler and scissors, cut an 8x4 cm rectangular sheet of paper from coffee machine filter paper. (Note: size can vary based on the experiment). Please ensure you cut the paper as straight as possible and with 90° angles in the corners.

b) Using a ruler and a pencil, draw two straight lines at 2 cm (baseline) and 7 cm (solvent front) from the bottom of the sheet, respectively.

c) Using a pencil, draw two small crosses on the baseline approximately 1 cm from either edge of the sheet.

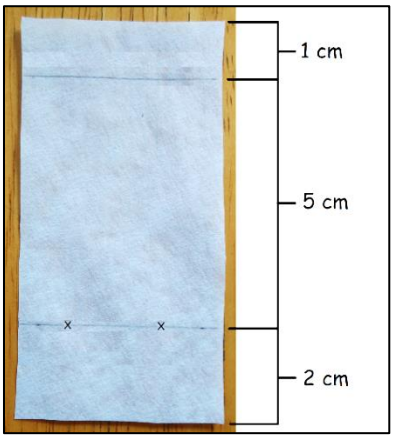


Figure 5. Preparing a TLC plate.

Prepare your chromatography tank

Note: In the laboratory, the TLC plate would consist of a layer of silica coated on a rigid sheet of aluminium foil which would allow it to stand evenly and stably in the chromatography tank. However, our simple paper sheet is too soft for that.

d) Attach your sheet of paper to a cotton bud stick using a piece of white tack or Sellotape (Figure 6, picture #1).

e) Fit/wedge it firmly in the top part of a jar or a cup (Figure 6, picture #2). The cup should be at least 10 cm tall and 5 cm wide so that the sheet does not touch the sides,

and the cotton bud should not fall into the cup. Note: any stick or support is fine if the cotton bud does not fit well into your cup. The sheet of paper should hang freely inside.

f) On the cup, mark the depth up to which the sheet of paper is hanging (Figure 6, picture No. 2). Remove the cotton bud stick and paper, then fill the cup with water < 0.5 cm above your mark.

Figure 6. Setting up a chromatography tank.

Spot your analytes

g) Label your "X" marks on the baseline with a descriptive letter underneath it, e.g. "B" for "blue" (Figure 7).

h) To deposit an analyte on a TLC plate in the lab we would use a small capillary tube. A good substitute available at home is a needle with a small eye (**CAUTION:** Please be extra careful when working with a needle!). Hold the needle on the sharp end side ~1 cm above the sharp end so that you do not stab yourself. Submerge the needle eye into a solution and slowly make a small spot with it by touching the "X" mark on the baseline with the needle eye approaching mostly horizontally (a ~20° angle works best) (Figure 7).

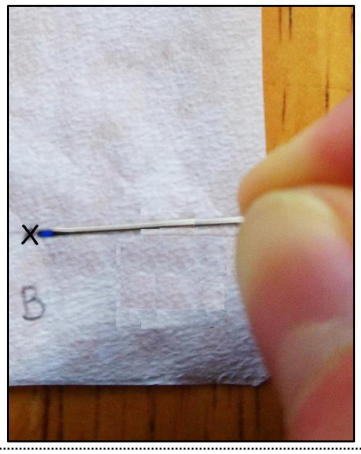


Figure 7. Spotting analyte on a TLC plate.

i) Repeat this process until you are happy with the size of the spot. The key is not to make the spot too large or diffuse as this might result in poor separation, overlap with other analytes or streaking across the paper sheet. On the other hand, a too small or a faint spot may not give the desired intensity and it may be tricky to see clearly the components of a mixture if they are present in low quantities. It may take a few attempts to strike the right balance. Examples of a spot too small (left), spot too large (centre) and an optimal spot (right) are shown in Figure 8.

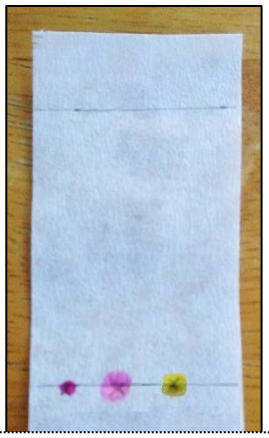


Figure 8. Different sizes of analyte spots.

j) Once you have deposited your first analyte, wash the needle with water and deposit the next one. Repeat until you have deposited all analytes (most often 2–3). Note: If you need to deposit more than two analytes, make sure you draw the

appropriate number of crosses on the baseline and space them out evenly (refer to part c of the Method section). Remember: there should be ~1 cm between each spot. For more than four spots, the paper sheet needs to be wider than 4 cm.

Separation by TLC

Figure 9. TLC sheet in a chromatography tank.

k) Holding the cotton bud stick with both hands, slowly wedge your TLC sheet with deposited analytes into the cup containing water, making sure it is lowered into the water parallel to the surface to achieve a straight mobile phase line. Only ~0.5 cm of the TLC sheet should be submerged in water. Double check the cotton bud stick is firmly wedged in and that the TLC sheet is hanging as straight down as possible, then cover the cup with a lid (Figure 9). If you are unsure how to do this you can watch our instructional video: media.kcl.ac.uk/media/1_r60ic59q. The mobile phase will travel up the sheet via capillary action – this process will slow down as the mobile phase reaches the upper parts of the TLC sheet

l) Watch how the different analytes travel up the TLC sheet (Figure 10, picture #1). Any mixtures should begin to separate and it is vital not to disturb the capillary process by moving the plate or the cup. Let the mobile phase reach the solvent front (but not further), at which point carefully remove the TLC sheet from the cup and place it on a piece of dry paper or kitchen roll (Figure 10, picture #2).

m) Once the TLC sheet has dried out, circle all differently coloured spots on the sheet and put a dot in the centre of each spot with a ruler and a pencil (Figure 10, picture No. 3). This can be used to calculate retardation factors (R_f values).

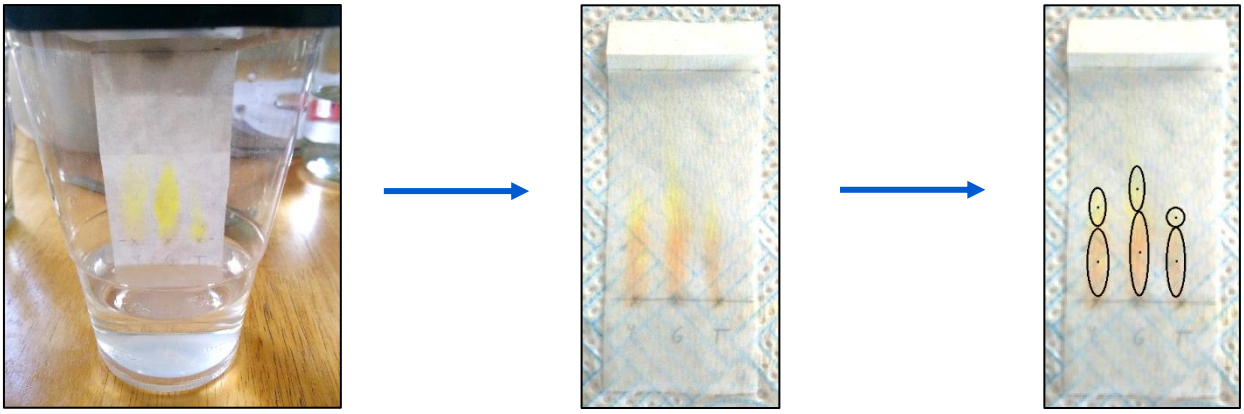


Figure 10. Separation by TLC.

5. Experiments

a) Red and pink

Scenario: You are a chemist and would like to know how many coloured compounds are in red and pink dyes. How you would go about this task? Send us your ideas to @kclchemoutreach #openaccesslabs

WHAT TO DO?

- Review the instructions in the Method (Section 4).
- Draw two crosses on the baseline of your TLC sheet and mark them "R" (red) and "P" (pink).
- Deposit your red and pink dye (Figure 7) on the correct cross and attach the TLC sheet to a cotton bud stick.
- Put the TLC sheet into your cup/glass, add the lid and run the mobile phase (water) up the TLC sheet to the solvent front.
- Remove the TLC sheet from the tank, let it dry and circle all differently coloured spots.
- Find their centre with a ruler and calculate the R_f value for each spot.

Note: You can find pictures and check your progress and analysis of this and the following experiments in our answer document which you can download from our outreach page.

b) Red and blue

In the first experiment we discovered that one of the components of the commercial red dye is a blue dye! How can we test whether the blue component of the red dye is the same chemical as is present in the commercial blue dye?

WHAT TO DO?

- Review Experiment 1.
- Plot both the red dye (label as "R") and the blue dye (label as "B") on a TLC sheet.
- To conclude from a TLC sheet that two chemicals are likely the same, **both** the colour and the R_f value of the spots need to be the same. Please be aware that even if two spots do have the same colour and R_f value, it does not exclusively mean they are the same compound (it could be a coincidence).

c) Blue + yellow = green

Pour 10 drops of the commercial green dye into a shot glass. Into another shot glass pour 5 drops of blue and yellow dye each. Mix the contents of the second shot glass thoroughly.

Ask someone to label one shot glass "1" and the other "2" and randomly shuffle them so that you do not know which one is which.

Research question: What is the source of the green dye? Is it a combination of the yellow and blue commercial dyes?

WHAT TO DO?

We need to spot 4 different analytes: the contents of the two shot glasses as well as two commercial dyes – yellow and blue. Note: commercial yellow and green dyes can be oily – make sure you do not make the spots too large or intense as 'streaking' (smearing) will occur on your TLC plate. Diluting them with water by 50% may also help to alleviate this issue.

d) Red or pink?

Apart from its outstanding nutritional value, beetroot is also commonly used as a dye.

Research question: Is beetroot juice part of the red or pink commercial dye, or both?

WHAT TO DO?

Spot three analytes: beetroot juice and the red and pink commercial dyes. **Make sure your beetroot juice spot is sufficiently intense on the baseline (but not too diffuse).**

Compare the colours and R_f values of the spots that you see after running the experiment and justify your conclusions.

e) Which natural source?

Beetroot is not the only natural source of commercial dyes. In fact, there are many more!

Research question: Which of the natural materials below are NOT used as a commercial dye?

Turmeric (Curcumin)

Beetroot

Paprika extract

Blue-green algae (spirulina)

Blue spirulina
(Phycocyanin pigment)

and

Green spirulina
(Arthrospira)

Vegetable carbon

Sweetcorn

Anthocyanin pigments

6. Advanced Experiments

a) (In)solubility of curcumin

Figure 11. Turmeric powder dispersed in water.

During Experiment 5c, the yellow commercial dye had an intense orange/yellow spot that remained on the baseline of the TLC sheet (see Figure 12, picture #1, spot "Y" below). Looking at the dye ingredients, it can be seen that the yellow commercial dye contains curcumin.

Research Question: Using the yellow dye, confirm that turmeric powder also contains curcumin (see Exercise 5e).

Note: Curcumin is practically insoluble in water (Figure 11).

- Even though curcumin is insoluble in water, it is soluble in "fatty" or organic solvents. The commercial yellow and green dyes therefore contain a higher proportion of vegetable oil compared with other colours to ensure curcumin would be soluble.
- However, using vegetable oil as the mobile phase is impractical:
  - it is very dense,
  - it travels up the TLC sheet very slowly,
  - it is coloured which may interfere with the colours of the dyes under study.

Theory of solubility: Generally, more polar compounds travel faster in polar mobile phases (water, alcohols, ethyl acetate) while non-polar compounds travel faster in non-polar mobile phases (e.g. petroleum ether, hexane). When we find a polar mobile phase that curcumin is soluble in, it will move up from the baseline. The more polar the mobile phase the further curcumin will move. **Note: The mobile phase always needs to be transparent and homogeneous (in the same phase), a mixture of water and oil would not work.**

Curcumin is soluble in several commonly available solvents: ethanol (spirits), acetone/ethyl acetate (nail polish remover) and isopropanol (rubbing alcohol).

If we create a mobile phase that is a 1:1 isopropanol:water (these two solvents are fully miscible and transparent), we will obtain a TLC sheet that is different to the one we saw in Experiment 5c (Figure 12, spot "Y" – yellow dye).

- Unlike the TLC sheet in Experiment 5c (Figure 12, picture No. 1), there is not an intense spot being left on the baseline. Instead, the yellow-orange curcumin dye has moved up the TLC sheet (Figure 12, picture #2)! Note: Isopropanol is only available in certain pharmacies, so the results have been included.

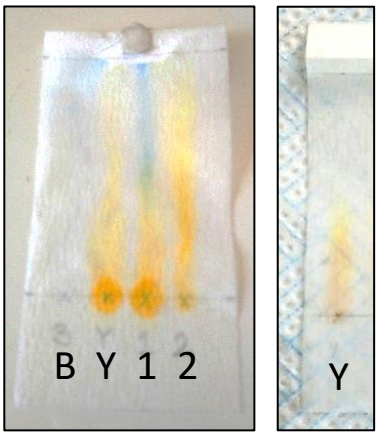


Figure 12. Yellow dye in water (left) and 1:1 isopropanol:water (right) as the eluent.

WHAT TO DO?

- Dissolve a small amount of turmeric powder (Figure 13) in vegetable oil.
- If we were to now spot it on a TLC sheet next to the commercial yellow dye and run the 1:1 isopropanol:water mobile phase up the sheet, the curcumin in the turmeric powder spot (Figure 14, spot "T" = turmeric powder) will not travel as far up as the curcumin in the commercial yellow dye spot (Figure 14, spot "Y" = yellow dye) – notice the height of the **orange** spot – despite being the same chemical. How is this possible?

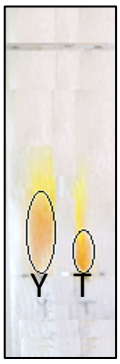


Figure 14. Yellow dye (Y) and turmeric powder dissolved in oil (T) in 1:1 isopropanol:water eluent.

Figure 13. Amount of turmeric powder to be dissolved in oil.

Solution:
Mix the dissolved turmeric powder in vegetable oil with an equal volume of 1:1 isopropanol:water solvent. The solution, and therefore the spot, now more closely resembles the mobile phase and will be considerably more miscible with it, and hence will travel faster. Now the relative height of the two orange spots is comparable (Figure 15).

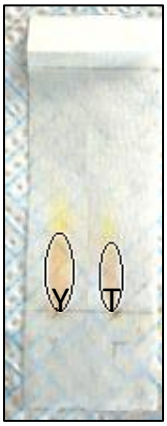


Figure 15. Yellow dye (Y) and turmeric powder dissolved in oil + 1:1 isopropanol:water (T) in 1:1 isopropanol:water eluent.

b) Anthocyanins – red, blue or purple?

Paprika extract (oleoresin) is typically yellow-reddish orange in colour. Oleoresin is insoluble in water and is presumably responsible for the orange spot on the baseline in Figure 16. This phenomenon is analogous to what we have just discussed in the Curcumin experiment 6a.

Another component of the commercial red dye is a group of chemicals known as anthocyanins. Anthocyanins are water-soluble pigments that, depending on their pH, may appear red, purple, blue or black (a chemical that changes its colour based on the pH is called an **indicator**). For example, in neutral pH, anthocyanins appear purple (Figure 17). Food plants naturally rich in anthocyanins include the blueberry, raspberry, black rice, and black soybean.

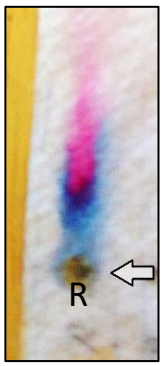


Figure 16. Red dye (R) in water eluent (zoomed to the bottom half of the TLC sheet).

Figure 17. Foods that naturally contain anthocyanins.

Research question: Which of the two components of the commercial red dye, the red or the blue spot, is the anthocyanin pigment? HINT: Is sodium bicarbonate acidic or basic in aqueous solution?

WHAT TO DO?

- The commercial red dye contains an acidifier (citric acid). Sodium bicarbonate, on the other hand, is slightly basic in aqueous solution (pH = 8–9). If we were to basify the commercial red dye with sodium bicarbonate, one of the spots should change colour.
- Into a shot glass pour approximately 10 drops of the red dye, add two pinches of sodium bicarbonate and mix thoroughly (you may observe effervescence). Then, run a typical TLC experiment with water as the mobile phase and two analytes: the commercial red dye without any sodium bicarbonate and the one with.
- If you see no colour change for either of the two spots, add two more pinches of sodium bicarbonate to your shot glass, stir, and repeat the TLC experiment. Continue with this procedure until you see a colour change for one of the spots.

c) In the laboratory – Monitoring the progress of a reaction

Probably the most common use of thin-layer chromatography in the laboratory is for monitoring the progress of a reaction. Typically, we would spot three analytes: the starting material (SM), the reaction mixture (RXN) and the product (P) (Figure 18).

Note: In cases when product is not available (e.g. we are synthesising a novel compound or it is the first time we are making a certain compound in our laboratory), we would only spot the starting material and the reaction mixture.

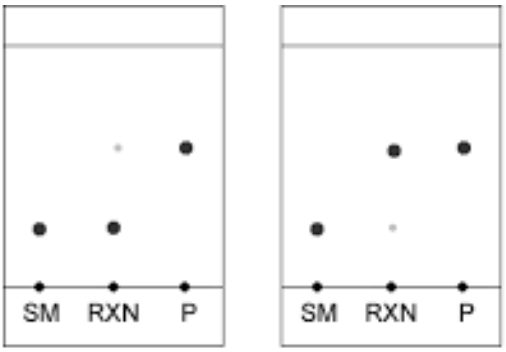


Figure 18. Monitoring the progress of a reaction by thin-layer chromatography.

Research question: What stage of a reaction does each of the two TLC plates in Figure 18 represent?

7. Bonus material

a) When TLC is not good enough

Sometimes we are not able to use TLC to fully extract all the information we need. For example, a recurring issue with TLC is that it is not a quantitative technique. Whilst the intensity of the spots can be somewhat indicative of the concentration at which a certain component is present in a mixture, it does not give an absolute value.

Research question: Can you distinguish the following 4 solutions using TLC?

1) 10 drops of red dye + 10 drops of blue dye
2) 12 drops of red dye + 6 drops of blue dye
3) 18 drops of red dye + 2 drops of blue dye
4) 10 drops of red dye + 5 drops of blue dye + 5 drops of yellow dye

WHAT TO DO?

Ask someone to label four shot glasses containing the above solutions "A", "B", "C" and "D" and randomly shuffle them so that you do not know which one is which. Spot each solution on the same TLC sheet and perform a TLC experiment with water as the eluent (Figure 19).

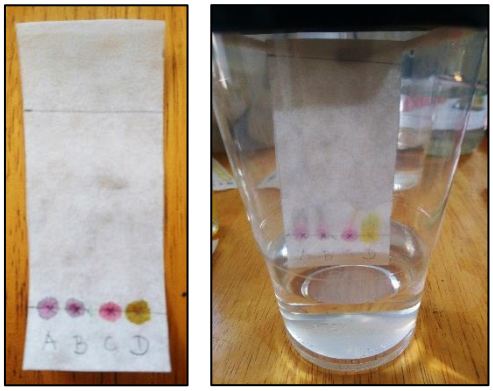


Figure 19. TLC sheet with solutions "A", "B", "C" and "D".

Solution:

You were probably able to identify solutions 3 and 4; however, solutions 1 and 2 were most likely too similar to be distinguished.

In a typical undergraduate experiment, UV spectroscopy is used to investigate the above mixtures of red, blue and yellow dyes and to quantify the individual components in each mixture. It is then used to study the dyes used for the preparation of a brown M&M candy.

The next section contains some essential background theory related to UV-vis spectroscopy and how it can be used to calculate the concentration of a component in a mixture.

UV-vis Spectroscopy & The Beer-Lambert Law:

Ultra-violet (UV) and visible radiation are both part of the electromagnetic spectrum. UV radiation has wavelengths in the range of 10 nm to 400 nm but cannot be seen by the human eye, while visible (vis) radiation has wavelengths in the range of 400 nm to 700 nm and is visible to us. Different types of bonds absorb different wavelengths of light – this is useful as it can help to determine the identity of a substance.

The wavelengths of light that are not absorbed are transmitted/reflected. It is these wavelengths that give rise to the colour of a substance: **the colour seen is complementary to the colour absorbed**. For example, if a substance appears blue, it absorbs strongly from the red/orange region of visible light (Figure 20).

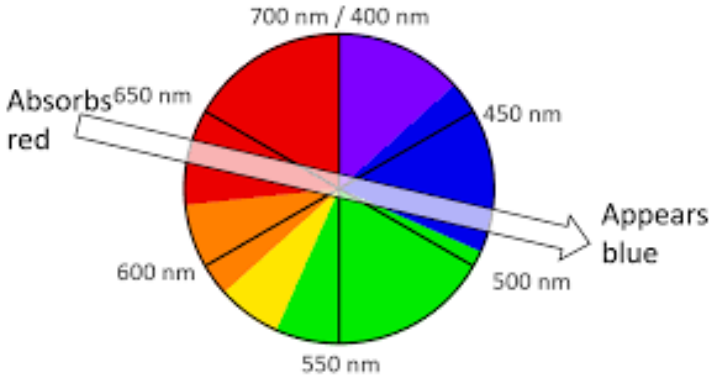


Figure 20. UV-vis colour wheel.

In UV-vis spectroscopy, a sample is placed in the instrument and light is shone through it; the sample will absorb some of that light. The intensity of incident light upon the sample (I_0) is known, and the intensity of light transmitted through the sample (I_t) is measured by the detector. The $\log_{10}$ of the ratio of the intensity of incident to transmitted light is termed the "absorbance (A)", i.e., $A = \log_{10} (I_0 / I_t)$.

When making measurements with a UV-vis spectrometer, the wavelength of light you wish to use is found by scanning the sample and finding the wavelength of maximum absorption, λ_{max}. This wavelength is then selected, and the spectrophotometer is adjusted to give a zero absorbance reading for the solvent or “blank”. The sample is then placed into the optical cell in the light path and the absorbance, A, is measured. The absorbance can be related to the concentration of the absorbing compound by the Beer-Lambert law, which states that:

$A = \varepsilon.c.l$

ε is the molar extinction coefficient of the compound, c is the molar concentration of the compound, and l is the pathlength of the cell in cm.

The molar extinction coefficient is the absorbance of a 1 M solution of a compound in a 1 cm cell. It is a constant for each compound at the λ_{max} for that compound. It can be seen from the Beer-Lambert Law that **the absorbance is directly proportional to the concentration of the solution at a given wavelength**. This means that a plot of A against c is a straight line passing through the origin.

Below are the UV-vis spectra for red, yellow and blue dyes, respectively (Figure 21).

Standard Dye Sheet

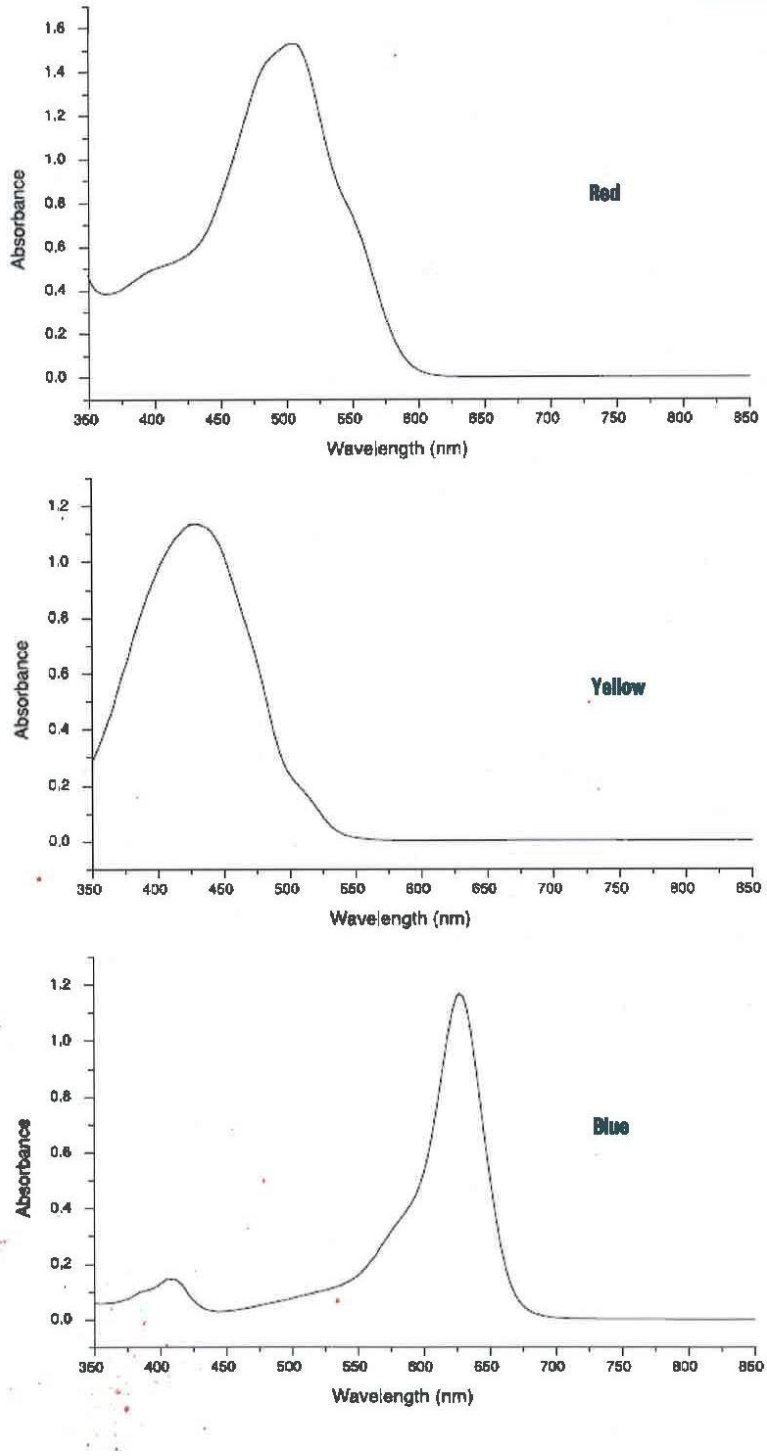


Figure 21. UV-vis spectra of red, yellow and blue dye.

Research question: What colours are present in the brown M&M candy?

Figure 22. Extracting the brown dye from brown M&M candies.

WHAT TO DO?

- Into a shot glass pour 10 mL of water and add 8–10 brown M&M candies trying to maximise their surface area that is submerged in water (Figure 22). Let it stand for 2 min, then carefully flip some of the M&Ms to extract as much of the brown dye as possible.
- Spot the brown solution onto a TLC sheet making sure it is concentrated enough. On this occasion, the spot may need to be quite diffuse (Figure 23).
- Run the TLC sheet in water as the eluent and try to identify which colours does brown M&M dye separate into (Figure 23).

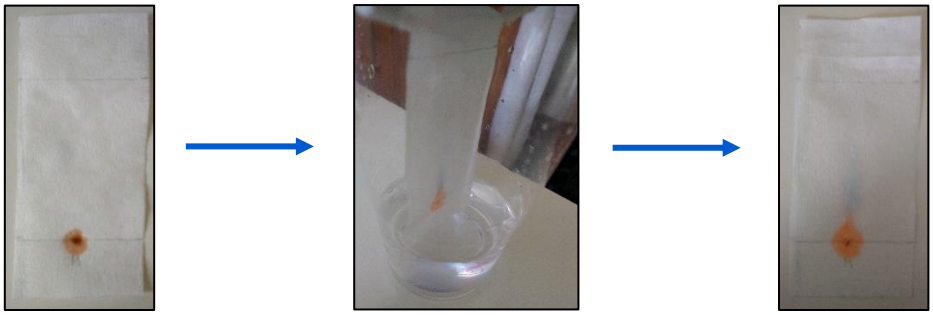


Figure 23. TLC experiment using dye from a brown M&M candy.

b) Column chromatography – bread and butter of an organic chemist

One of the most commonly used purification techniques, particularly in synthetic organic chemistry, is called column chromatography (Figure 24). It not only allows us to identify components of a complex mixture, but also to separate and isolate them. These components are then further studied using spectroscopic and spectrometric techniques and their structure is often determined.

Figure 24. Example of column chromatography.

How does column chromatography work?

The principle is in fact very similar to TLC, except that this method is performed in 3D and "upside down" (Figure 25). In practice, it often looks analogous to the diagram below:

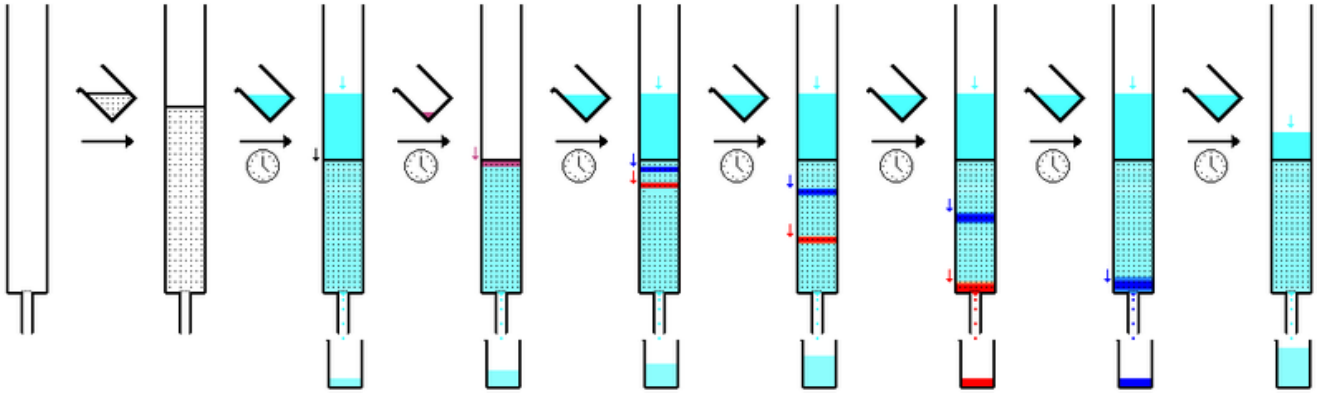


Figure 25. Stages of a column chromatography experiment.

Method:

1) Load the column with a stationary phase (most often silica).
2) Add the mobile phase and run it through the column in order to compact the silica layer and to remove air bubbles.
3) Carefully and evenly deposit your mixture as a thin layer on top of the silica layer without disturbing it (purple layer in Figure 25).
4) Add a thin layer of sand on top to protect the mixture from being disturbed by the solvent.
5) Run the mobile phase (eluting solvent) through the column (separation occurs).
6) Collect the first, faster moving component (red layer). Keep eluting.
7) Collect the second, slower moving component (blue layer). This completes the process.

If the mixture separates into more than two components, the principle remains the same and each component must be collected separately.

Research question: Can you think of a way to mimic the column chromatography process at home, to separate the components of the commercial red dye?

Send us your ideas to @kclchemoutreach #openaccesslabs

WHAT TO DO?

Column: An evenly narrow and tall transparent bottle works best. Cut off the bottom part and pierce a hole into the bottle lid. 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 (Figure 26).
Stationary phase: Cellulose powder is fairly accessible and non-toxic (unlike silica).
Mobile phase: Water.
Mixture: Commercial red dye. Deposit 10 mL evenly on top using a small teaspoon or straw.
To cover the mixture deposited on cellulose: Sand or cotton wool.

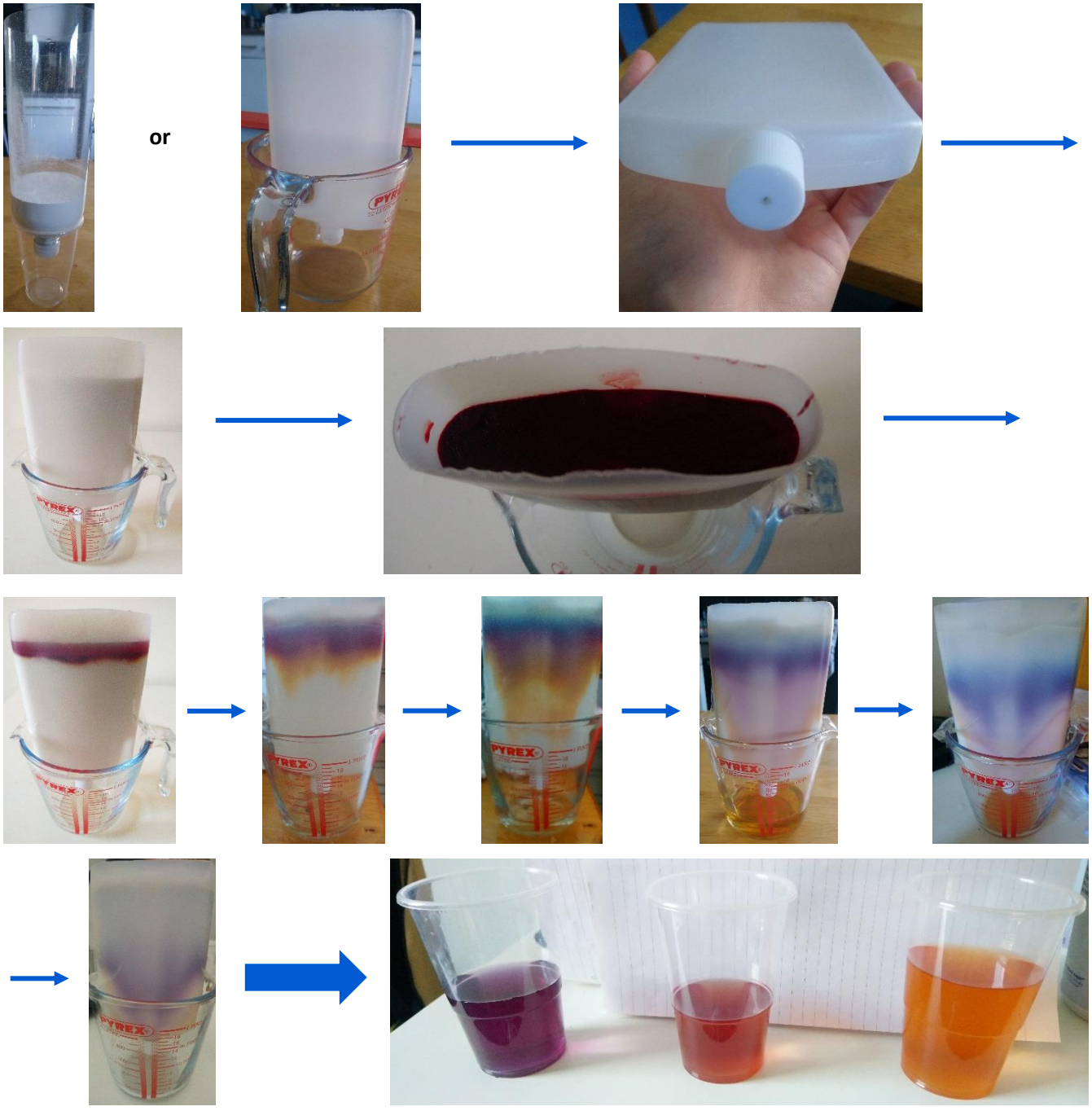


Figure 26. Column chromatography at home.