(Son of) Flash Chromatography

Almost all science teachers are familiar with chromatography in one form or another. Though they differ in many respects, all forms of chromatography work by exploiting different interactions between the dyes and the two components of the chromatography setup.

The stationary phase - this is a solid. As the name implies it does not move and the fluids pass through it. (In ordinary paper chromatography, the paper is the stationary phase.)

The mobile phase - this is the fluid that moves through the stationary phase (like the water through filter paper).

Paper chromatography is a familiar technique in schools. A mixture of dyes (often an ink) is placed on some filter paper and a solvent is allowed to run up or along the paper carrying the different dyes different distances. Column chromatography works on the same principle. The dye mixture is washed down a column filled with a permeable solid. The different dyes move through the column at different speeds, depending on their size and interactions with the stationary phase, and so come out of the bottom at different times. The advantage of this is that you can get samples of your separated dyes to do further work on.

The problem, particularly in schools, is that column chromatography is slow - taking several hours. It can be speeded up by applying pressure to force the liquid through - a technique called *Flash Chromatography* [1]. Previous versions of this use a glass tube filled with a starch slurry. This is then linked by rubber tubing to a syringe to apply the pressure.

Unfortunately, this method is rather fiddly, as the stirring of the slurry is critical and the tube tends to pop off when pressure is applied.

In this new method, the syringe itself is used as the column and the stationary phase is packed dry, making the whole process much simpler.

The Method

Preparing the column

a) Insert a loose plug of mineral wool into the syringe (Figure 1) and tamp down with a stirring rod.
b) Use a spatula to put your medium into the barrel of the syringe, compressing with the plunger and adding more if need be.

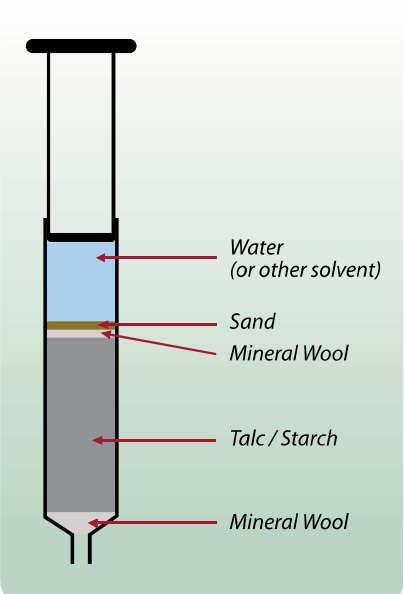

Figure 1 *- Setup of syringe.*

c) Insert another plug of mineral wool and tamp it down - quite firmly.

Running the Column

a) Take a 1 cm^3 Pasteur pipette and use it to pick up about 0.5 cm^3 of the dye mixture. Carefully dot this over the mineral wool in the barrel of the syringe (try not to get any on the sides).
b) Put a layer of silver sand on the top, a few mm thick, then carefully add solvent, trying not to disturb the sand too much so you keep the solvent clean.
c) Insert the plunger and apply pressure.
d) When you see the first hint of colour, move the syringe so it is over the first test tube and keep pressing. (Be careful, in some dye mixtures the first colour is literally one or two drops.
e) As you see the colour changing, move to the next tube and keep the pressure on.
f) If your syringe runs dry, pull out the plunger, use a glass rod to push back the plug that will probably have been pulled up by the suction, put more solvent in, replace the plunger and carry on.

Hints and Tips

Syringe size - The best separation seems to come using a narrow bore such as a 1 cm^3 syringe but they are very fiddly to pack. Generally, 5 cm^3 or 10 cm^3 syringes are the best. (It is a good use for old syringes with the markings worn off).

Packing - It is quite possible to run the column dry - i.e. without running solvent through first. Running some solvent through first does help with the packing but it is by no means essential.

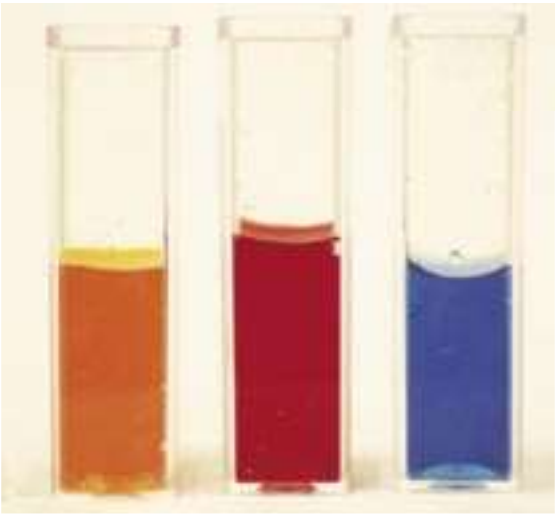

Figure 2 *- Dyes in black food colouring.*

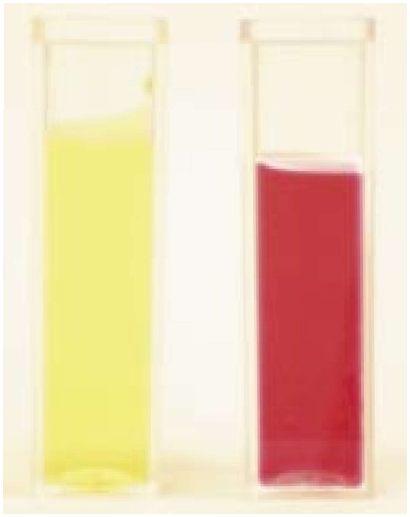

Figure 3 *- Dyes in a purple Skittle®.*

Pressure - Maintaining the pressure can be a bit tiring but it seems not to cause problems if you release the pressure for a bit, to rest or change hands. Alternatively, if you use a 5 cm^3 syringe, you can place it in the jaws of a clamp and use that to apply the pressure.

Stationary Phase - We have had success with starch and, especially, talc (using simple cornflour and talcum powder). Work is still ongoing to investigate other materials. Different materials will interact with dyes in different ways so colours will sometimes come out in a different order.

Eluent volume - when all the solvent has gone into the column you can add more solvent by simply pulling out the plunger (carefully), re filling the barrel of the syringe and carrying on. If the plug of talc/ starch gets pulled up the tube, or it breaks and part of it does, pushing back down and carrying on seems to cause few problems.

Mobile Phase - Most dye mixtures can be separated using water or propanone but other solvents may work just as effectively. If you find a colour remains stuck in the column, if you change the solvent you will probably be able to elute it.

Technique - there is usually overlap between the dyes so you will need to watch carefully to make sure you are getting pure samples of the individual dyes. A good way is to have an array of tubes and allow the same volume to fall into each one (5 or 10 drops, say) before moving on.

Experiments (sources of dyes, colourings and pigments)

1) Food colouring - You will need to select your food colouring carefully. Green and black food colours seem to be the only ones containing more than one dye (and not all of those) so check first.

Figure 2 shows the dyes in Dr Oetker black food colouring* separated by this method using talc as the stationary phase in a 1 cm^3 syringe and water as the solvent.

The yellow dye is only one or two drops so care is needed when the colour first comes through.

2) Sweets - the colours can be extracted by putting two in a test tube with just enough water to cover them, though better results are obtained by using more sweets and evaporating some of the water to concentrate the colours. Many sweets don't have different colouring dyes, so try the brighter ones like Skittles® and M&Ms®. Figure 3 shows a purple Skittle ® using talc and water - you can see the red and yellow have come out well. In addition, a blue dye remained in the column.

3) Plant Extracts

It is possible, with care to separate out at least some of the different leaf pigments. Grass is a good source of chlorophylls and is certainly the most readily available. Grind up a few grams of chopped grass with some silver sand and extract with the minimum quantity of propanone. Load it onto the 'column' and elute using propanone (or another solvent).

Figure 4 shows sample results, using propanone. As it is a polar solvent, the constituents come out in order of polarity. The yellow tube on the left contains chlorophyll b, then comes the green chlorophyll a and then the yellow on the right is a mixture of xanthophylls and β-carotene.

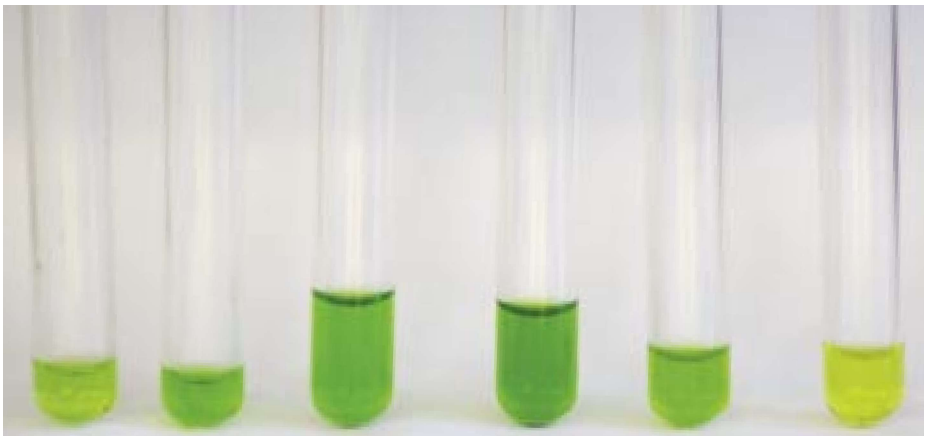

Figure 4 *- Photosynthetic pigments from grass.*

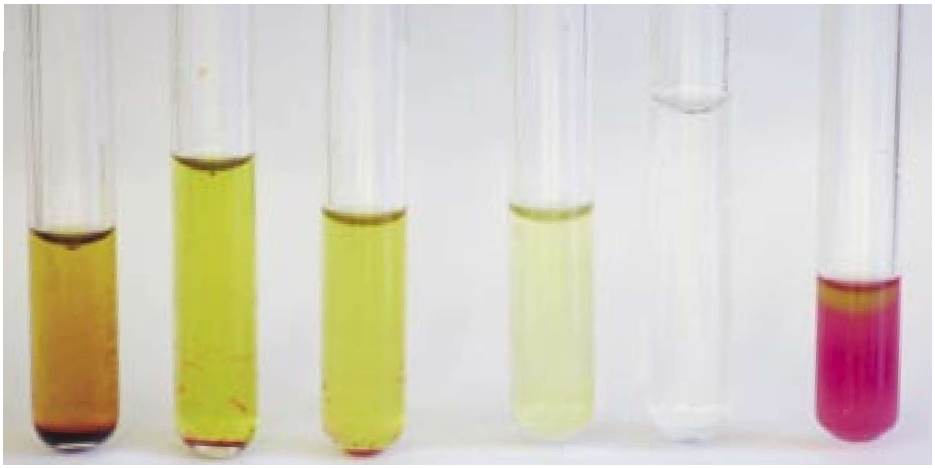

Figure 5 *- Anthocyanins from dried cotinus leaves.*

If you choose a red-leafed plant, it is possible to separate out the pigments that give it the red colour as well. These need extracting in acidified methanol or ethanol (1 cm^3 $HCl_{(c)}$ in 100 cm^3 alcohol).

Figure 5 shows *Cotinus* (a common ornamental shrub) leaves extracted in propanone and then eluted with cyclohexane (any other non-polar solvent should work). The results below were from some dried leaves so the chlorophylls are much less evident than is the case with fresh leaves. One of the red colours (an anthocyanin) comes through first with the chlorophylls next. The bright pink colour (another anthocyanin) remains resolutely in the talc medium until you add a more polar solvent, in this case ethanol, to extract it.

Conclusion

While this technique does not give as good separation as a proper column, the availability of the materials, the simplicity of the preparations and the speed of the procedure make it an excellent introduction to the more complex and time-consuming methods that are in widespread use. In particular, it is a valuable method of introducing chromatography as a purification technique, rather than a simple, though valuable, analytical tool.

* We have recently learned that the Dr Oetker range has been changed and the black colour no longer contains this mixture of dyes. At the time of going to press, we are still exploring suitable alternatives.

Reference

[1] Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. Still, W. C.; Kahn, M.; Mitra, A. J. Org. Chem. 1978, **43**, 2923-2925.

Health & Safety

Advanced Higher Students and SSERC

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- If using e-mail, initial contact should be established by a teacher or technician. Alternatively, the student may e-mail SSERC directly, provided that a member of staff is cc'd into the initial communication. The teacher or technician will thereafter be cc'd or, if preferred, bcc'd into all correspondence, or can act as an intermediary between SSERC and the student.
- If using the telephone, again initial contact should be made by a teacher or technician. Students should not contact SSERC using their personal mobile phones. Should they do so, SSERC staff will ask to speak to a teacher or technician. A member of school staff should be present during subsequent phone calls.
- Students should never contact SSERC about chemical disposal, spillage or immediate remedial measures. This should always be done through a member of staff.

We trust that the reasons for these measures are obvious and that having to comply with the protocols will not dissuade anyone from seeking help.