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School of Maths & Science
Science Practical

Chromatographic Separation of Dyes

◆ Aim

Use and explain the principles of operation of TLC and Column chromatography to separate a simple solution of dye. Compare the results of the two techniques.

◆ Introduction

Liquid Solid Chromatography is a method where the liquid phase is the mobile phase used to elute the compound of interest from the column or separate a compound by moving it up a solid support. The solid phase is a stationary phase, usually paper. This may have a high or low retention of the compound of interest, either holding on to it or allowing it to elute quickly off the plate. This is known as absorbance, where a weak absorbance means the substance is eluted quickly, or a strong absorbance where the sample takes a long time to elute. This provides a means of separation as a compound may consist of a number of substances which elute at different rates due to differing partition coefficients. In LSC, the stationary phase is usually polar and the mobile phase is non-polar.

◆ Safety

Control Measures

- The wearing of a laboratory coat and safety glasses at all times will be sufficient to take into account most hazards and significant risks.
- All waste is to be placed into a labelled container immediately after use.
- You are reminded of the need of good laboratory practice in order to maintain a safe working environment.

Hazards

- Butanol
- Ethanol
- 2M ammonia
- Food dye can stain

◆ Procedure

Use the chromatography paper provided. Measure 10cm x 10cm and cut this out from the sheet provided.

- To prepare the sample, measure about 15mm up from the base draw a light pencil line across the base, touching the paper as little as possible.
- Divide the line into three and mark the line lightly with a pencil at each point.

- Carefully apply one drop of green ink at the first point. Leave a 1.5 cm gap and apply one drop of blue ink and then leave a 1.5 cm gap and apply one drop of yellow ink.

To make up the mobile phase:

- A solution of butanol, ethanol and 2M ammonia is required at the ratios of 60:20:20 v/v.

- However only 25 ml of this solution is required so you are required to calculate your quantities accordingly. Divide each volume of solution by four and use this quantity to make up your solution.

- Pour the solution into the tank and place the lid on the tank to prevent evaporation and loss of the solvent

To begin the chromatographic run:

- Pour the solvent into the tank and carefully slide in the plate containing the ink drops.

- Run the chromatogram for 1 hour. Watch the initial flow of the solvent across the origin and note how the pigments immediately begin to separate

- Remove the plate and mark the solvent front carefully by making a tiny mark on the paper where the solvent stops.

Measure the Rf values of each pigment, you should be able to see that the separated green ink gives spots of blue and yellow ink at the same Rf values as the standard inks.

◆ Method

In your own words, describe the method you used.

◆ Results

Calculate the Rf values for each colour dye and record them in the space below

The green dye has separated into two spots measure the Rf for each.

		Rf value
Green	Yellow	
	Blue	
Yellow		
Blue		
Solvent front		

◆ Conclusion and discussion

Discuss your results and include any possible sources of error, what are the advantages and disadvantages of this technique.