PW N° 2: column chromatography of ink

Introduction

The term **chromatography** comes from two Greek words, "**chromate**" meaning color and the word "**graphon**" meaning to write.

Chromatography is a method for analyzing complex mixtures (such as ink) by separating them into the chemicals from which they are made. Chromatography is used to separate and identify all sorts of substances in police work. Drugs from narcotics to aspirin can be identified in urine and blood samples, often with the aid of chromatography.

PRINCIPLES

Column chromatography follows the same principles as TLC, with the following differences:

• The stationary phase is contained inside a column, rather than applied as a coating on a plate. The column is commonly made of glass, but some are made of metal or other materials.

• The sample to be separated is loaded from the top. The eluting solvent, or mobile phase, is also added from the top.

• The solvent flows down the column by gravity, carrying with it the components of the sample. By the same principles that apply in TLC, the components travel at different rates effecting the separation.

Materials and Products

Column or burette, cotton, distillated water, silica gel, black felt-tip pen, Pasteur pipette, saline solution (40 g/L) and ethanol, test tubes, TLC.

Procedure

1. Fill the column about one third with solvent (saline solution (40 g/L) and ethanol)
2. in a beaker, measure out the required amount of silica gel.
3. in a separate flask or beaker, measure solvent approximately one and a half times the volume of silica.
4. Add the silica to the solvent, a little at a time, while swirling. Use a Pasteur pipette or glass rod to mix the slurry.
5. Tap the column gently to encourage bubbles to rise and the silica to settle.
6. Rinse the inside of the column by pipetting solvent down the inside edge.
7. Drain the solvent until the solvent level is just even with the surface of the stationary phase

8. Add a 3 mm layer of sand on top of the silica gel to prevent it from moving during solvent Addition (facultative).

You are now ready to load your column with black ink.

9. Start by preparing your solution. Break a black felt-tip pen and remove the central ink sponge. Squeeze out a few drops of ink into a small beaker, or vial. You might need to add a bit of water to the sponge first.

10. Use a pipette to transfer your product mixture to the column;

11. Let the solvent run just slight below the surface of the silica gel.

12. First use 50 mL of saline solution (40 g/L) to elute the column and start collect the fractions in test tubes that are fitted in a rack.

13. Then increase the solvent polarity to ethanol and continue to elute the column until TLC shows no more products is coming down from the column.

14. Combine the fractions of the test tubes that show product spots.

15. Evaporate the solvents and transfer the product to a vial.

Questions

1. Write down carefully the different steps of column chromatography
2. Why is it important to prevent having air bubbles in the column
3. What's the role of cotton?
4. Why did the inks separate?
5. What might be some challenges with large scale chromatography in industry?
6. How do scientists use column chromatography in their investigations?
7. What are the colors of the collected solutions?