Activity Two: Techniques used to perform chromatography

Chromatography is widely used in academic and industrial research, forensic laboratories and pharmaceutical companies. The method is very flexible and therefore various techniques emerge. The most popular techniques include: thin layer chromatography, paper chromatography, column chromatography, high pressure liquid chromatography (HPLC, LCMS) and gas chromatography. These can be analytical and/or purification methods and divided into two categories depending on the polarity of the stationary phase.

Normal Phase Chromatography (Polar Stationary Phase)

Paper Chromatography

Paper chromatography is a very cheap technique, mostly used for educational purposes, to observe the composition of coloured mixtures. It uses cellulose as a polar stationary phase to separate mixtures and hence polar compounds (capable of hydrogen bonding) that interact more strongly with cellulose and therefore are retarded, which means that they move more slowly than non-polar compounds.

Thin layer chromatography (TLC)

TLC is based on the same principle as paper chromatography but instead of cellulose, the stationary phase is a thin layer of silica or alumina deposited on a glass, aluminium or plastic plate. Both silica and alumina are polar media (acidic and basic respectively) therefore polar compounds are retarded more than non-polar compounds. Similarly to cellulose, silica and alumina can show hydrogen bonding to molecules containing H, N and O atoms. The mobile phase can move faster and more evenly as opposed to cellulose thus creating a more consistent separation of mixtures. Another advantage is that the TLC plates can be stored after analysis and do not bend or rip as easily as paper.

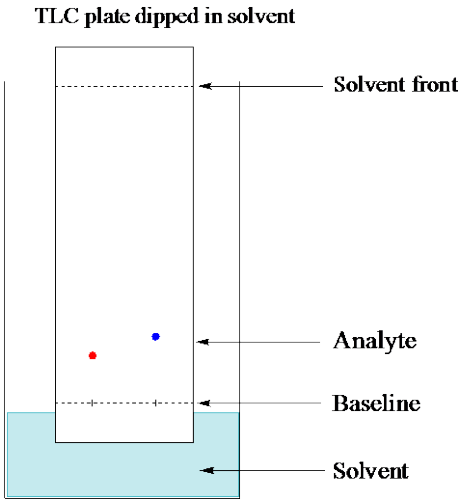

Figure 1. Thin layer chromatography diagram. Analysis of TLC involves measuring the R_f of each analyte.

You can find more examples of TLCs here:
(https://jahschem.wikispaces.com/thin+layer+chromatography)

Many molecules are invisible to the human eye therefore, in order to see them, either a stain is used (such as iodine, $KMnO_4$) or an UV lamp for molecules containing conjugated system. TLC plates are often made by mixing silica with a fluorescent material, zinc sulphide (ZnS), which enables the plate to glow green under UV light except where the analyte absorbs UV light in which case a dark spot is observed. Another example of a fluorescent material is quinine. This is found in tonic water and will glow blue when subjected to UV light.

Here you can find more stains that could be employed to colour the compounds.
(https://orgprepdaily.wordpress.com/2006/09/27/tlc-staining-solutions/)

Column Chromatography

This is a purification method widely used through academic and industrial research laboratories to separate and purify individual compounds from crude mixtures. This technique can purify up to a few grams of crude mixtures. It uses silica gel, a fine white powder that upon mixing with solvents can be packed tightly in a column.

Figure 2. Column chromatography - you can see the different colour bands representing different compounds being separated.

Analytes are loaded on top of the column and flowed through using solvents. Column chromatography uses gravity at normal atmospheric pressure to elute purified components. The separation occurs due to the polar OH groups on silica surface, polar compounds travel down the column more slowly than non-polar ones, as the hydrogen bonding interaction they make with the silica gel surface slows them down. Compressed air or inert gas can also be used to speed up the process by pushing solvent through. This technique is called flash column chromatography due to the fact that it's considerably faster.

Reverse-Phase Chromatography (Non-Polar Stationary Phase)

Liquid Chromatography (LC), High performance Liquid Chromatography (HPLC) and Ultra-High Performance Liquid Chromatography (UHPLC)

Reverse-phase columns pack silica functionalised with non-polar fatty chains. This makes the stationary phase non-polar and thus polar compounds elute faster than non-polar compounds, which have weak association with the fatty chains.

Reverse-phase chromatography is generally used in conjunction with a high-pressure pump. These systems are called Liquid Chromatography (LC) systems and are extensively used for purification of more complicated compounds: proteins, oligonucleotides and lipophilic compounds.
High Performance Liquid Chromatography systems are the most popular as they combine the flexibility of interchanging tightly packed columns for either analytical or purification applications with the reproducibility of the results due to automation.
HPLC systems can employ thin analytical columns or much thicker preparative columns that can purify 50 milligrams or more of compound on each load. (cf. column chromatography can purify hundreds of milligrams up to a few grams)

The difference between LC, HPLC and UHPLC is the pressure that the system is operating on. LC systems use up to 100 bar/ 1450 psi of pressure to flow solvent through the column, HPLC up to 400 bar/ 5800psi and UHPLC up to 1000 bar/ 14500psi; compare this to tyre pressures on your bike (3.4 bar/ 50 psi) or a normal family car (2 bar/ 30 psi). This high pressure is required to push the liquid through a very tightly packed C18 silica column. The finer the particle size of the stationary phase, the tighter the packing and the higher the pressure required to push the solvent through the column. If you are interested in why finer, tighter packed columns separate compounds much better you could study the theoretical plates theory here:

https://en.wikipedia.org/wiki/Theoretical_plate .

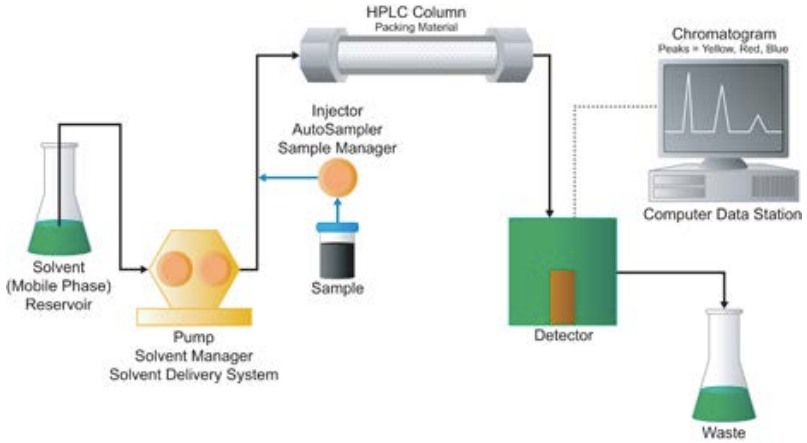

Figure 3. Liquid chromatography system. A pump will generate solvent flow through the pipes, connections, HPLC tube/column and detector (UV lamp and/or mass spectrometer) at high pressure. The sample is injected and flowed through the column thus separating individual compounds out of a mixture. These compounds are then identified by the detector and therefore discarded or collected. Diagram taken from http://www.waters.com/waters/en_GB/How-

Does-High-Performance-Liquid-Chromatography-Work%3F/nav.htm?cid=10049055&locale=en_GB

Column types:

The most widespread HPLC columns are the reverse-phase C18 functionalised silica. These allow good separation of a range of chemical and biochemical compounds. Similar to TLC, each analyte has a characteristic called retention time (r.t.) measured in minutes. This is the time required for a compound to travel through the HPLC column with a specific solvent and flow rate.

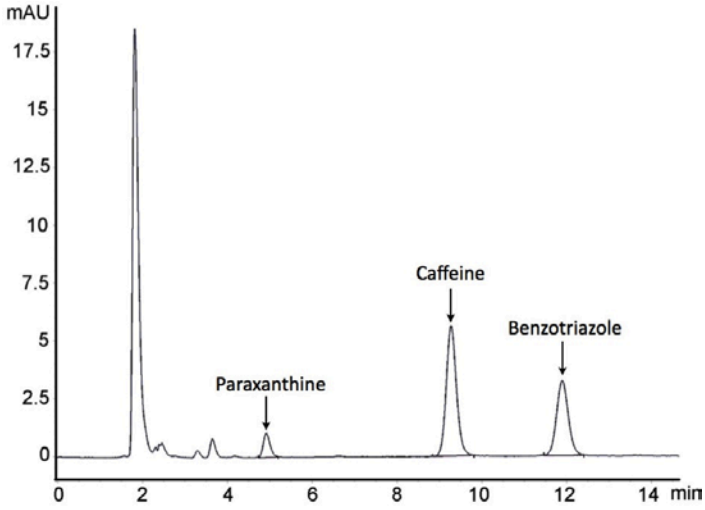

Figure 4. Chromatogram of a mixture containing 12 compounds. The peaks are measured in a.u. –absorbance units (or milli absorbance units in this case) . Each peak (compound) is associated with a r.t. – retention time. Diagram taken from Nature Neuroscience (http://www.nature.com/neuro/journal/v17/n2/images/nn.3623-SF1.jpg)

Other types of columns are available, for example chiral columns are used for easy separation of enantiomers (for more details about enantiomers and chirality please visit *Topic 1: Chirality and Why it is Important*).

Gas Chromatography:

Gas chromatography is similar to liquid chromatography but uses an inert gas (He, Ar, N_2) as the mobile phase that flows through a capillary hollow column which is heated up in an oven. This technique is used to determine the composition of a volatile mixture. For example, it can be used to determine the concentration of alcohol in a range of beverages.

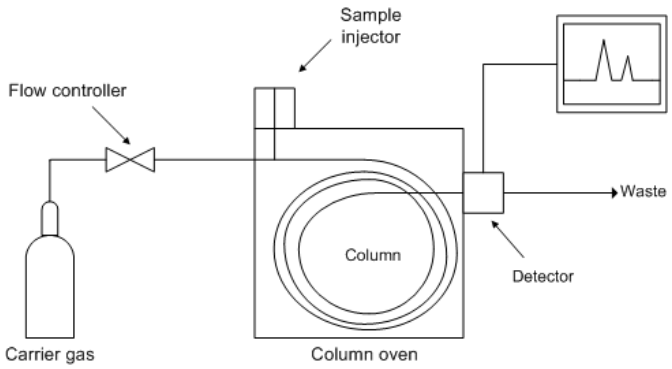

Figure 5. Gas chromatography system diagram. The system is coupled to a gas cylinder that pushes carrier gas through the pipes and connections, through the heated column and detector. Analytes are injected and due to high heat, instantly vaporise and are separated by interacting with the column walls. Digram taken from http://jahschem.wikispaces.com/file/view/350px-Gas_chromatograph.jpg/375340992/350px-Gas_chromatograph.jpg

Questions:

What is difference between adsorption and absorption? Discuss which is better at adsorbing water: plain A4 paper or tissue paper.
Which one would you use for paper chromatography and why? Think about diffusion through the material and how this might affect the experiment. Try it experimentally to convince yourself. (see experimental section)