Activity One: Interaction between molecules

1. Chromatography

In chemistry, chromatography is used as a separation, identification and purification technique. Analytical chemists use chromatography extensively to identify and quantify individual compounds from mixtures *e.g.* inks, dyes, colouring agents in food, and in most of the chemicals synthesised in a laboratory, biochemical compounds, biological material etc.

2. What is required in order to perform a chromatographic separation?

Three components are required to perform a chromatographic separation: the **analytes** (usually a mixture of multiple compounds), a **mobile phase** (solvent) and a **stationary phase** (a material that facilitates separation upon interaction with the analytes).

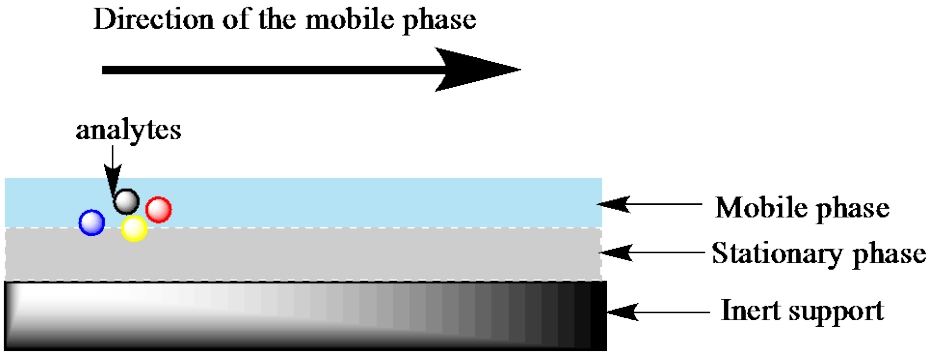

Figure 1. The three components required for chromatography: analytes, mobile phase, stationary phase on an inert support.

3. How does it work?

Separation of the analytes occurs upon interaction with both the stationary and mobile phases. Within a mixture, each molecule/analyte has a unique pattern of interaction with the stationary and mobile phase. At a molecular level, different types of forces generated by functional groups (e.g. -OH –> alcohol, $-NH_2$ –> amine) are found on analytes, solvents and stationary phases, which create that unique pattern of interaction. Ultimately, this can be observed as the analytes are constantly oscillating between two states: either flowing with the mobile phase or being adsorbed in the stationary phase.

This balance, between moving with the solvent or attaching to the stationary phase, is the basis of chromatography and is specific to each analyte. The stationary phase is responsible for the retention of the movement of molecules, which ultimately causes each molecule to have a different net rate of migration. The retention factor (R_f) of an analyte is the distance travelled by the analyte divided by the distance travelled by solvent in the same time. The R_f value is characteristic to each molecule and is dependent on both the solvent system and the stationary phase used.

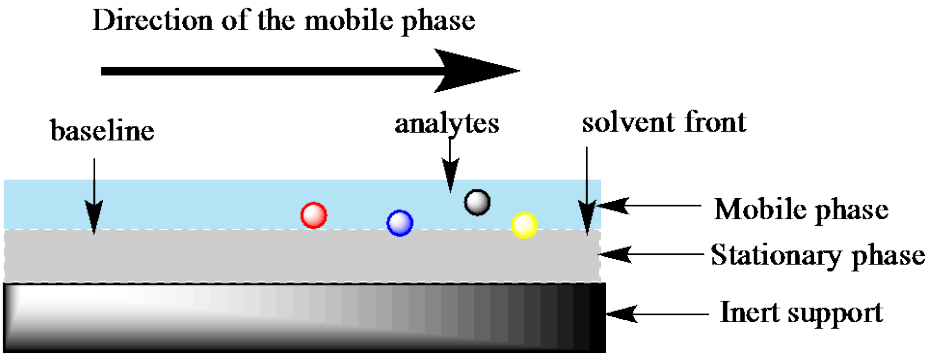

Figure 2. The analytes travel from the baseline, along with the solvent, on the surface of the stationary phase. The retention factor represents the distance from the baseline travelled by the analytes divided by distance from the baseline to the solvent front.

4. What types of interaction occur between analyte, mobile phase and stationary phase?

The interactions are specific to each molecule and they depend on intermolecular forces. Here are the main intermolecular forces:

Weak forces:
These weak intermolecular interactions are also known as Van der Waals interactions. There are three types of Van der Waals forces:

a) *Keesom forces* can occur between two permanent dipole molecules. It is also known as dipole-dipole interaction.

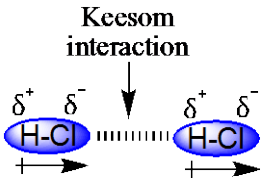

Figure 3. Permanent dipole interacting with another permanent dipole through Keesom forces

b) *Debye forces* occur between a permanent dipole molecule and an induced dipole one. The permanently polarized electron cloud of one permanent dipole molecule is inducing a temporary dipole in a non-polar molecule thus generating the Debye forces.

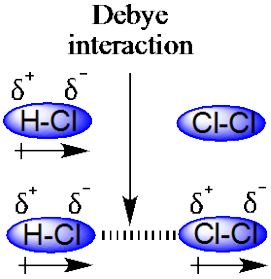

Figure 4. A permanent dipole (top left) induces a dipole in a non-polar molecule (top-right) generating a Debye interaction (dashed line).

c) *London dispersion* forces occur between two induced dipole atoms. The attraction force arises upon spontaneous polarisation of one atom followed by induced polarisation of the neighbouring atom. The attraction increases with the size of the atoms due to increase polarizability of large atoms with large electron clouds.

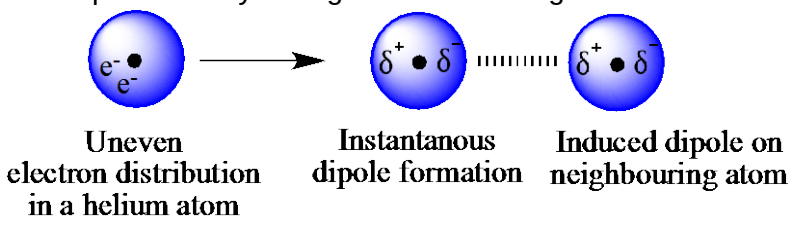

Figure 5. London dispersion forces (dashed line) are generated upon uneven distribution of electrons in atoms (left sphere), which leads to a sudden dipole formation (centre sphere). This dipole then induces a dipole (right sphere) in an adjacent molecule.

Strong forces:

Electrostatic interaction between ionic compounds *e.g.* polystyrene sulphonate (ion exchange stationary phase) can bind cations such as magnesium or calcium tightly.

Hydrogen bonding

Hydrogen bonds are formed between the electropositive hydrogen atoms attached to an electronegative atom (e.g. oxygen, fluorine, nitrogen) of a molecule with an electronegative atom of a different molecule. This type of bonding is stronger than Van der Waals (see 'weak forces' above) but less strong than ionic and covalent bonding. It generally occurs in H, N and O atom containing molecules such as water, proteins and DNA etc.

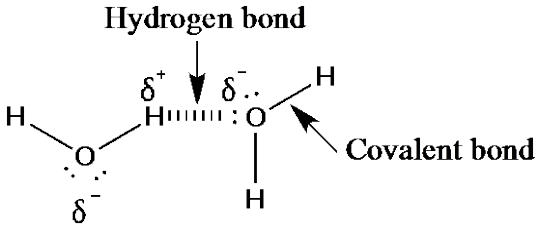

Figure 6. Hydrogen bonding occurring between two water molecules.

Therefore all types of molecules are capable of interacting with each other regardless of them being polar, non-polar, able of hydrogen bonding or not.*

5. Types of stationary phase and their influence on retention factor

* You can read more about these forces here:
https://jahschem.wikispaces.com/intermolecular+forces and here:
http://www.bbc.co.uk/bitesize/higher/chemistry/energy/bsp/revision/2/

The stationary phase is there to **adsorb** molecules dissolved in the mobile phase, to slow their movement and separate them. (see definition of **adsorption** in the glossary and compare it to **absorption**)
There are three main types of stationary phases:

1. Polar: cellulose (paper), silica gel, alumina. These types are used in **normal phase** chromatography, which consists of a polar stationary phase and a range of non-polar and polar solvents as mobile phases.

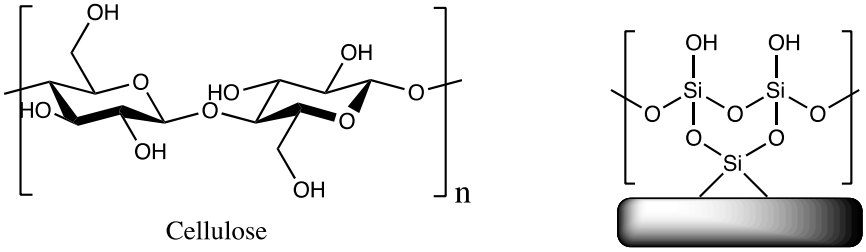

Figure 7. Molecular structure of cellulose (left) and molecular structure of silica gel (right)

2. Non-polar: C-18 silica. Silica functionalised with C-18 fatty chains. This is used in reverse-phase chromatography, which uses a non-polar stationary phase and polar solvents.

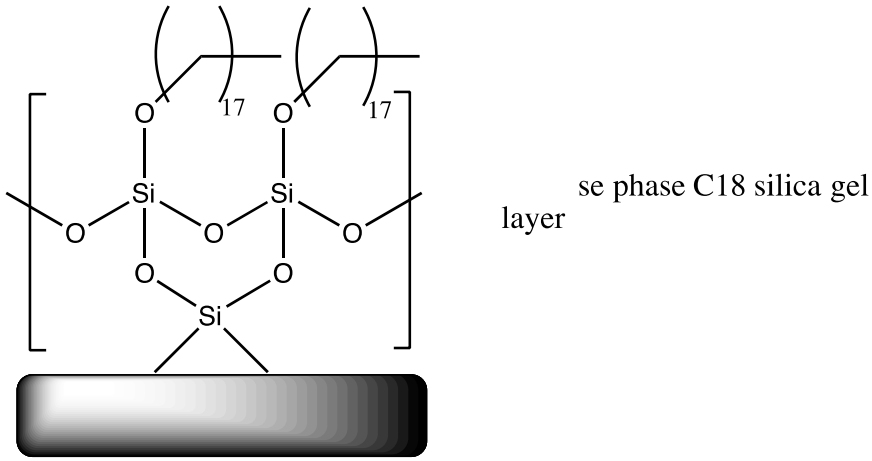

Figure 8. Molecular structure of C18 functionalised silica with applications in reverse-phase chromatography

3. Ionic: Ion exchange chromatography is usually used to separate molecules based on their charge at different pH values. There are two main types of stationary phases: acidic (*e.g.* sodium polystyrene sulfonate, a cation exchange resin) and basic (*e.g.* polystyrene trimethyl ammonium chloride, an anion exchange resin).

Figure 9. Sodium polystyrene sulfonate ion exchange resin

6. Solvents and influence on retention factor

The mobile phase has a great influence on the R_f and therefore on the separation of molecules. Generally, polar solvents dissolve polar compounds and non-polar solvents dissolve non-polar molecules. Therefore, a polar analyte will travel further in a polar solvent compared to a non-polar solvent, assuming that the stationary phase is polar. Similarly, a non-polar analyte will travel further in a non-polar solvent compared to a polar solvent if the stationary phase is non-polar.
For example, a polar molecule will adsorb on a silica stationary phase and due to H-bonds being formed, will not travel a considerable amount unless the solvent is sufficiently polar. This is due to the fact that silica is a polar stationary phase with lots of polar OH groups on the surface ready to hydrogen bond to the polar analyte and slow its progress up the plate.

Questions:

1. Discuss the types of interactions between a mixture of these molecules: sodium acetate, methanol and paraffin.
2. Arrange the solvents in order of increasing polarity: water, trichloromethane (chloroform), tetrachloromethane (carbon tetrachloride)