Biomedical Admissions Test (BMAT)

Section 2: Chemistry

Topic C8: Separation Techniques

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Chemical processes

The first group of separation techniques are **chemical processes** which separate elements from a compound via a **chemical reaction**.

Displacement

A more reactive metal is able to **displace** a less reactive metal from a compound. This means that a metal in solution can be extracted to solid form.

For example, magnesium is more reactive than copper. This means that when magnesium is added to copper sulfate, the dissolved copper is **reduced** to solid copper metal. This metal can then be removed from the mixture using **filtration**.

Electrolysis

- **Electrolysis** uses electricity to separate ionic compounds. This requires the ions to be able to move, which can be achieved by ionic compounds being in solution or molten.
- **Negative ions** (anions) are attracted to the positive electrode (**anode**) where **oxidation** occurs.
- **Positive ions** (cations) are attracted to the negative electrode (**cathode**) where **reduction** occurs.

For **molten electrolytes**, both the positive and negative ions are brought out of solution. A key example of this is Sodium Chloride (NaCl). Sodium (the anion) is found at the cathode and Chloride ions (the cation) is found at the anode.

Physical Processes

Other separation techniques separate compounds or elements from a mixture based on differences in a **physical property** such as boiling point.

Decanting

This is the simplest way to separate a solid from a solution or liquid. Solids will settle at the bottom of a vessel. **Decanting** is pouring away the liquid without moving the solid.

Filtration

An insoluble solid can be separated from a liquid using **filtration**. This works because the liquid is able to pass through filter paper but the solid particles cannot.

The **filtrate** is the liquid in the collection vessel and the **residue** is the solid found on the filter paper.

Separating Funnel

Two immiscible (unable to mix) liquids can be separated using a **separating funnel** as they form two layers in the funnel. Opening the tap allows the lower layer to be poured out. The funnel narrows before the tap, allowing precision when closing the tap when the final drops of the lower layer have been released.

Distillation

A solvent can be separated from a solution using **distillation** due to their differing boiling points. The solution is raised above the boiling point of the solvent which becomes a vapour. This then cools in the liebig condenser and collects in a second container.

Fractional Distillation

Miscible liquids can be separated using **fractional distillation**. This requires a column with a temperature gradient with higher temperatures towards the bottom of it.

- ➔ The mixture is heated and when it reaches the boiling point of the liquid with the lowest boiling point. At this point, the component boils off and is condensed and collected.
- ➔ The remaining liquids are heated to a higher temperature and the next component is distilled off.

Evaporation and Crystallisation

Evaporation and crystallisation separates a solute from a solvent to obtain the **solute**. The solution is heated in a crucible until the moments crystals appear on the edge of the vessel - the **crystallising point**. The solution is left to evaporate, leaving the solute in the vessel.

Paper Chromatography

Chromatography is a practical technique that is used to separate mixtures into their components so that the individual substances making up a sample can be identified. Separation by chromatography produces a **chromatogram**.

Chromatography consists of two phases, a **mobile phase** and a **stationary phase** which will move over this mobile phase.

- ➔ The mobile phase is always a liquid or a gas. This is because the particles need to be able to move in this phase. Solid particles are fixed and cannot move and therefore the mobile phase cannot be a solid.

- ➔ The stationary phase will most usually be a solid because the particles are fixed and will not move. In some cases, a thick, viscous liquid can be used as the stationary phase, so long as its particles do not move.
- ➔ The mobile phase will move over the stationary phase.

The stationary phase is paper.

Method:

1) Draw a line in pencil 1cm from the bottom of the paper. This is the **baseline**
2) Put a small spot of the mixture to be separated onto this line.
3) Place the paper into a beaker into a beaker with a small volume of **solvent**. The solvent is the mobile phase. The solvent level must be below the level of the pencil line.
4) As the solvent moves up the paper it will dissolve the sample spot and carry this up the paper with it. This demonstrates the mobile phase (the solvent) moving over the stationary phase (the paper).
5) Do not let the solvent reach the top edge of the paper. When the solvent comes close to reaching the top edge of the paper, remove the paper from the solvent and allow the paper to dry. However before the solvent is evaporated, mark how far the solvent has travelled up the plate. This line is known as the solvent front as is necessary to calculate **Rf values**, which will be explained later.
6) Lots of spots will be seen on the paper in different distances away from the baseline. Each spot represents one substance in the mixture. The spots may need to be located with a suitable **locating agent** such as iodine or be shined under a UV lamp.

The reason why the spots are all seen at different distances from the baseline is due to the chemicals spending **different amounts of time in the stationary and mobile phases**.

- ➔ The chemicals that have travelled up the paper have spent more time in the mobile phase than in the stationary phase as they have been carried further.

The amount of time each chemical spends in the stationary phase and mobile phase is determined by the **amino acid constitution** of that chemical.

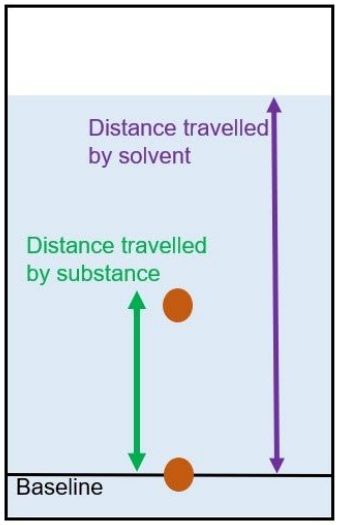
- ➔ Different amino acids will form different bonds with the solvent and the paper and so the amino acid constitution of the chemical will determine how soluble it is in the solvent and also how attracted it is to the paper.

For example if a chemical contains many amino acids with an NH_2 group and the solvent being used is H_2O, many hydrogen bonds will be formed between the NH_2 group and the H_2O and so the chemical will have a high solubility in the solvent.

- ➔ If it is only weakly attracted to the paper but with a high solubility in the solvent, the chemical will be carried further up the paper away from the baseline due to spending more time in the mobile phase than in the stationary phase.

The position that the spots are seen at can be used to identify what chemical is represented by each spot. To do this, the Retention Factor, Rf, value needs to be found. This is a ratio of the distance moved by the spot and the distance moved by the solvent:

$$Rf = \frac{Distance\ moved\ by\ spot}{Distance\ moved\ by\ solvent}$$



Compare the Rf values of each spot in your unknown sample to standard reference samples (SRMs). You can also run a pure, known sample alongside your unknown mixture. If the Rf values match and the spots are in the same place, it is likely that the substances are the same.

The Rf value is always the same for a particular substance using the same stationary and mobile phase.

The lower the Rf value, the less distance the substance has travelled from the baseline in the solvent in comparison to the solvent front.

Centrifugation

Mixtures can be separated based upon differences in particle mass using **centrifugation**. This is mostly used to separate solids from liquids or solutions. In medicine, this is used to separate the components of blood.

Containers are spun at a high speed in a circle to encourage settling as the heavier particles move to the tube's end, forming a pellet. The remaining liquid is known as the **supernatant** which can be further separated through centrifugation at a higher speed.

Choosing a method

The following table explains the methods of separation used in various situations.

Component	Technique used
Liquid from solid-liquid mixture	Distillation
Solid from solid-liquid mixture	Decantation, filtration, crystallisation or centrifugation
Immiscible liquid-liquid mixture	Separating funnels
Gas-gas mixture	Centrifugation or cyrogenic distillation

More complex mixtures require different techniques one-by-one to obtain the desired substance.

Testing purity

Chromatography can be used to assess purity of substances. This is because Rf values for the same combination of stationary phase and solvent are always constant. If multiple, different Rf values are found, this suggests an impurity.

Evaporation can be used to find dissolved impurities as any residue left when a solution is evaporated to dryness would be a dissolved impurity.

Distillation can be used to find impurities as if a liquid boils off at a higher temperature than expected, this suggests an impurity.

An impurity would also affect the **freezing point** as an impure liquid will freeze at a lower temperature than expected.

Pure samples melt and boil at a specific defined temperature whilst impure samples melt and boil at a range of temperatures.