Separation

Pure substances	Contain only ONE thing - have fixed melting and boiling points
Mixtures	Contain MORE THAN ONE thing, do not have exact properties, formed by a physical change so they can be easily separated

Purification as part of drug synthesis

Synthesis	Purification	Character-isation
Weigh up starting materials	Extraction	NMR
Set up a reaction	Recrystallisation	IR
Monitor reaction	Distillation	MS
Work up reaction to start purification	Chromatography	RF
	Solid-phase extraction	Melting point

Separation techniques

1. Distil-lation	Used to separate the components of a LIQUID mixture by vapourising, condensing vapours and then collecting the liquid condensate. **Separation is a result of different boiling points**
2. Filtration	Physically separated solids from liquids Especially used for insoluble solids in liquids
3. Centri-fugation	Uses centrifugal force that spins the samples fast Separated even fine solid matter form liquids

Separation techniques (cont)

4. Recrystal-lisation	1. Dissolve impure crude material in minimum volume of solvent 2. Filter any insoluble material 3. Allow solution to cool slowly 4. Crystals will form

Polarity

Definition of solubility

> Ability of a solvent to dissolve in a solute, depending on the nature of the forces acting between the solute and solvent
> - **LIKE DISSOLVES LIKE**

> e.g. **Ionic** compounds are more soluble in **polar** solvents
> **Covalent** compounds are more soluble in **non-polar** solvents

Solvent mixtures

Immiscible: if the mixtures are too different, they will NOT mix, and will form separate layers or 'phases'

Partially miscible: mixtures are not too different but not too similar, they may mix at determinate proportions depending on the compos-itions

Miscible: if they are similar, they mix in EVERY proportion, forming one layer or 'phase'

Liquid-liquid Separation

What is liquid-liquid separation?

> A method in which 2 molecules initially present in one sample can be separated by giving them the choice of different immiscible solvents

- Very common when isolating or purifying a product
- Can be used to extract natural products
- Normally done in workup stage after a reaction

Criteria of organic solvent for liquid-liquid extraction

> SHOULD readily dissolve the substance to be extracted
> SHOULD NOT react with the substance to be extracted
> SHOULD NOT react with or be miscible with water (usual second solvent)
> SHOULD have a low boiling point so it can be easily removed from the products

Common extraction solvents are dichloromethane or ethyl acetate

Chromatography

What is chromatography?

> Physical method of separation where the components to be separated are distributed between 2 phases: the stationary and mobile phase

Common types:
- TLC (thin layer)
- Column
- HPLC (high performance liquid)
- GC (gas)
- SEC (size exclusion)
- Ion exchange
- Chiral

Chromatography - definitions

Analyte	Substance being separated (or analysed)
Mobile phase	Phase that moves in a definite direction - consists of sample being separated and the solvent that moves the sample through the column
Stationary phase	The substance fixed in place for the chromatography procedure
Eluent	Solvent **entering** column
Eluate	Solvent **leaving** column
Elution	Process of passing liquid through chromatography

DIFFERENT METHODS & RETENTION OF ANALYTES

1. Adsorption chroma-tography	Separation is based on differences between the **adsorption affinities** of the sample analytes for the surface of a solid-stationary phase Based on **non-colavent** interactions **Used for organic molecules**

DIFFERENT METHODS & RETENTION OF ANALYTES (cont)

2. Partition chroma-tography	Separation based on differences between the **solubility** of the sample analytes in the mobile and stationary phases → stationary phase = immobilised liquid Based on **non-covalent** interactions **Used for organic polar molecules**
3. Exclusion chroma-tography	Separation based on **exclusion effects** e.g. differences in size and shape **used for proteins and nucleic acids**
4. Gel electr-ophoresis	Specialised example of size-exclusion chromatography which uses agarose/polyacrylamide and passes electr-icity through Separates based on **size and shape** **Used for proteins and nucleic acids** Visualised by using UV and stains

DIFFERENT METHODS & RETENTION OF ANALYTES (cont)

5. Ion exchange chromatography	Separation based on **differences in ion exchange affinities** → e.g. differences in charge size and type Based on **ionic forces** **Used for cations, anions, proteins, peptides, amino acids and nucleic acids** - Cation exchangers = negative stationary phase Anion exchangers = positive stationary phase
6. Thin layer chromatography	Separation based on **Kx values** Polar stationary phase = thin layer of silica spread over a glass plate Stationary phase is placed in the mobile phase Analyte moves up the plate and components are spread based on their Kx values **Lower Rf = compound is MORE polar** **Higher Rf = compound is LESS polar** **Increased eluent polarity can increase the Rf** More polar = stronger interactions in the **normal** phase Less polar = stronger interactions in the **reverse** phase
7. Flash column chromatography	Used for large amounts of material (5-25mg) when TLC is not effective

INSTRUMENTAL CHROMATOGRAPHY

1. High performance liquid chromatography	Analytical use: identiry multiple analytes in complex mixtures Preparative use: purification
2. Gas chromatography	Separated by their **volatility** Forces the analyte through a column of the stationary phase by the gas mobile phase at a high pressure and temperature

Quantitative analysis

Single point calibration	**Multiple point calibration**	**Internal standard**
1. A solution containing a known concentration of the compound to be measured in the sample is injected → relation concentration obtained =AUC	1. Several solutions containing different concentrations of compound to be measured are injected → AUC obtained	1. One solution containing a known concentration of the compound to be measured AND a known concentration of internal standards are injected to find a Response factor
2. The sample with unknown concentration is injected → new AUC is compared with previous to find concentration in the sample	2. The sample with unknown concentration is injected → new AUC is compared with previous to find concentration in the sample	2. The sample and the same known concentration of internal standard are injected to find the AUC and Response factor

Separation in pharmacy

Drugs in the clinic must be pure:

1. Impurities can be harmful and cause side effects
2. Impurities can alter the ability to formulate a drug correctly
3. Impurities can also affect the stability and shelf-life of the drug

Drugs in development MUST be pure:

This is to ensure that it is the drug that brings about a therapeutic effect and not any impurities

- Before any new compound is tested, it is purified and characterised

Separation is also part of the quality control and monitoring process

Importance of ionisation

Some functional groups can be charged, and this depends on:

- whether the molecule is an acid or a base
- the pH of the molecule

Physiological pH = 7.4

we need to know what groups are charged at this pH as it will help us determine:

- types of drug-target binding interactions
- solubility (uptake and distribution)
- potential salt forms

e.g.1 - Carboxylic acids	e.g. 2 - Aliphatic amines
Acids react with water:	Bases react with water:
HA + H2O ⇌ H3O+ + A-	B + H2O ⇌ BH + OH-
pKa of carboxylic acids ~ <5	pKa of aliphatic amines ~ >8

These functional groups are almost ALWAYS ionised at physiological pH

pKa & pH = measures of dissociation

If the pKa = pH, then the functional group is 50% ionised

Partitioning

Partition	Distribution of a solute between 2 solvents
	Compound is present in BOTH phases according to its relative solubility in both
	Solute distributes itself between the 2 liquids in accordance with its **partition coefficient**

Partitioning (cont)

Partition coefficient	Ratio of the concentration of the solute in one liquid OVEr the concentration in the other
	Dynamic equilibrium exists between the 2 liquids, temperature dependent

Partitioning in pharmacy

Relative hydrophobic/hydrophilic properties of a drug are crucial as it influences:

- solubility
- adsorption
- distribution
- metabolism
- excretion

Drug too polar? It will not cross the cell membranes across the gut wall

Drug too lipophilic? Drug will be poorly absorbed, so will likely be taken up into the fat tissue and not circulated

LogP

$$P = \frac{\text{Concentration of drug in octanol}}{\text{Concentration of drug in water}}$$

LogP: used as a measure of hydrophobicity of a drug

Hydrophobic/hydrophilic character is measured using partition

- Hydrophobic molecules prefer the **octanol layer** → high P
- Hydrophilic molecules prefer the **water layer** → low P

LogP measures only unionised forms of the drug between octanol and water

LogD is sued to represent relative distributions of all species, charged or uncharged

Distribution Coefficient (Kx)

$$K_x = \frac{C(stat)}{C(mob)}$$

C(stat) = conc of X in **stationary** phase

C(mob) = conc of X in **mobile** phase

Each compound will have a different Kx

Chromatographic separations can be altered by changing the nature of the stationary and/or mobile phase(s)

Solid-phase extraction

Used in sample preparation to remove matrix interferences such as proteins

Concentrates sample of interest

Solid phase in SPE is similar to the stationary phase used in chromatography

Active substances can be retained or unretained

SPE vs. Chromatography

- Column is much smaller for SPE
- Specialised columns are more common
- Analytes are typically strongly retained on the SPE column, then impurities are washed away

↳ In chromatography, both the analyte and impurities are passed through at different rates

SPE is used for sample preparation, then chromatographic methods follow

Stationary phases

Normal phase	Reverse phase
Stationary phase = polar Mobile phase = non-polar	Stationary phase = non-polar Mobile phase = polar
Components elute in order of **increasing** polarity	Components elute in order of **decreasing** polarity
Used in TLC and flash column	Used in HPLC
More polar compounds are retained stronger More polar solvents increase elution	**Less** polar compounds are retained stronger Less polar compounds increase elution (e.g. methanol, ethanol)
Solvents = the complete range but rarely greater than 20% for very polar solvents	Solvents = very polar solvents (e.g. water, methanol)

A strong solvent for the normal phase is a weak solvent for the stationary phase

Component retention

For TLC, retention is expressed in terms of Rf

For column, retention is expressed in terms of column volumes (CV)

The relationship between Rf and CV are reciprocal

Lower Rf = greater CV → Low RF if preferred as it increases analyte contact time and improves chances of component separation or resolution

To separate adjacent compounds, a large ΔCV is desired

Order of elution

TLC and HPLC depend on **polarity**

- Normal phase: polar retained more, non-polar elutes first
- Reverse phase: non-polar retained more, polar elites first

Gas chromatography depends on **volatility or molecular weight**

- Highly volatile or low MW elutes first

Size exclusion depends on **molecular size**

- Low size retained more, largest size elutes first

Ion exchange depends on **charge**

- Complementary charges are retained more