# Partition Chromatography

By-

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# Principle

- Like other forms of chromatography, partition chromatography is based on differences in retention factor, k, and distribution coefficients, Kd, of the analytes using liquid stationary and mobile phases.
- It can be subdivided into:
  - liquid–liquid chromatography, in which the liquid stationary phase is attached to a supporting matrix by purely physical means, and
  - bonded-phase liquid chromatography, in which the stationary phase is covalently attached to the matrix.
- An example of liquid–liquid chromatography is one in which a water stationary phase is supported by a cellulose, starch or silica matrix, all of which have the ability to physically bind as much as 50% (w/v) water and remain free-flowing powders.
- The advantages of this form of chromatography are that it is cheap, has a high capacity and has broad selectivity.
- Its disadvantage is that the elution process may gradually remove the stationary phase, thereby altering the chromatographic conditions.
- This problem is overcome by the use of bonded phases and this explains their more widespread use. Most bonded phases use silica as the matrix, which is derivatised to immobilise the stationary phase by reaction with an organochlorosilane.

# Normal-phase liquid chromatography

- In this form of partition chromatography, the stationary phase is polar and the mobile phase relatively non-polar.
- The most popular stationary phase is an alkylamine bonded to silica.
- The mobile phase is generally an organic solvent such as hexane, heptane, dichloromethane or ethyl acetate.
- These solvents form an elutropic series based on their polarity.
- Such a series in order of increasing polarity is as follows:

$$n\text{-hexane} < \text{cyclohexane} < \text{trichloromethane} < \text{dichloromethane} < \text{tetrahydrofuran} < \text{acetonitrile} < \text{ethanol} < \text{methanol} < \text{ethanoic acid} < \text{water}$$

- The order of elution of analytes is such that the least polar is eluted first and the most polar last.
- Indeed, polar analytes generally require gradient elution with a mobile phase of increasing polarity, generally achieved by the use of methanol or dioxane.
- The main applications of normal-phase liquid chromatography are its use to separate analytes that have low water solubility and those that are not amenable to reversedphase liquid chromatography.

# Reversed-phase liquid chromatography

- In this form of liquid chromatography, which has many similarities with hydrophobic interaction chromatography, the stationary phase is non-polar and the mobile phase relatively polar, hence the name reversed-phase.
- By far the most commonly used type is the bonded-phase form, in which alkylsilane groups are chemically attached to silica. Butyl (C4), octyl (C8) and octadecyl (C18) silane groups are most commonly used.
- The mobile phase is commonly water or aqueous buffers, methanol, acetonitrile or tetrahydrofuran, or mixtures of them.
- The organic solvent is referred to as an organic modifier.
- Reversed-phase liquid chromatography differs from most other forms of chromatography in that the stationary phase is essentially inert and only non-polar (hydrophobic) interactions are possible with analytes.
- Reversed-phase HPLC is probably the most widely used form of chromatography mainly because of its flexibility and high resolution.
- It is widely used to analyse drugs and their metabolites, insecticide and pesticide residues, and amino acids, peptides and proteins.

# Ion-pair reversed-phase liquid chromatography

- Although the separation of some highly polar analytes, such as amino acids, peptides, organic acids and the catecholamines, is not possible by reversed-phase chromatography, it is sometimes possible to achieve such separations by one of two approaches:
- **Ion suppression:** The ionisation of the analytes is suppressed by using a mobile phase with an appropriately high or low pH thus giving the molecules greater hydrophobic character.
- For weak acid analytes, for example, an acidified mobile phase would be used.
- **Ion-pairing:** A counter ion that has a charge opposite to that of the analytes to be separated is added to the mobile phase so that the resulting ion-pair has sufficient hydrophobic, lipophilic character to be retained by the non-polar stationary phase of a reversed-phase system.
- Octyl- and octadecylsilane-bonded phases are used most commonly in conjunction with a water/methanol or water/acetonitrile mobile phase.
- One of the advantages of ionpair reversed-phase chromatography is that if the sample to be resolved contains a mixture of non-ionic and ionic analytes, the two groups can be separated simultaneously because the ion-pair reagent does not affect the chromatography of the non-ionic species.

# Chiral chromatography

- This form of chromatography allows mixtures of enantiomers (mirror image forms, denoted either as D or L or as S or R) to be resolved.
- One of these techniques is based on the fact that diastereoisomers, which are optical isomers that do not have an object–image relationship, have different physical properties even though they contain identical functional groups.
- They can therefore be separated by conventional chromatographic techniques, most commonly reversed-phase chromatography.