MOLECULAR (SIZE) EXCLUSION CHROMATOGRAPHY

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Principle

- This chromatographic technique for the separation of molecules on the basis of their molecular size and shape exploits the molecular sieve properties of a variety of porous materials.
- The terms **exclusion** or **permeation chromatography** or **gel filtration** describe all molecular separation processes using molecular sieves.
- The general principle of exclusion chromatography is quite simple.
- A column of microparticulate cross-linked copolymers generally of either styrene or divinylbenzene and with a narrow range of pore sizes is in equilibrium with a suitable mobile phase for the analytes to be separated.
- Large analytes that are completely excluded from the pores will pass through the interstitial spaces between the particles and will appear first in the eluate.
- Smaller analytes will be distributed between the mobile phase inside and outside the particles and will therefore pass through the column at a slower rate, hence appearing last in the eluate.

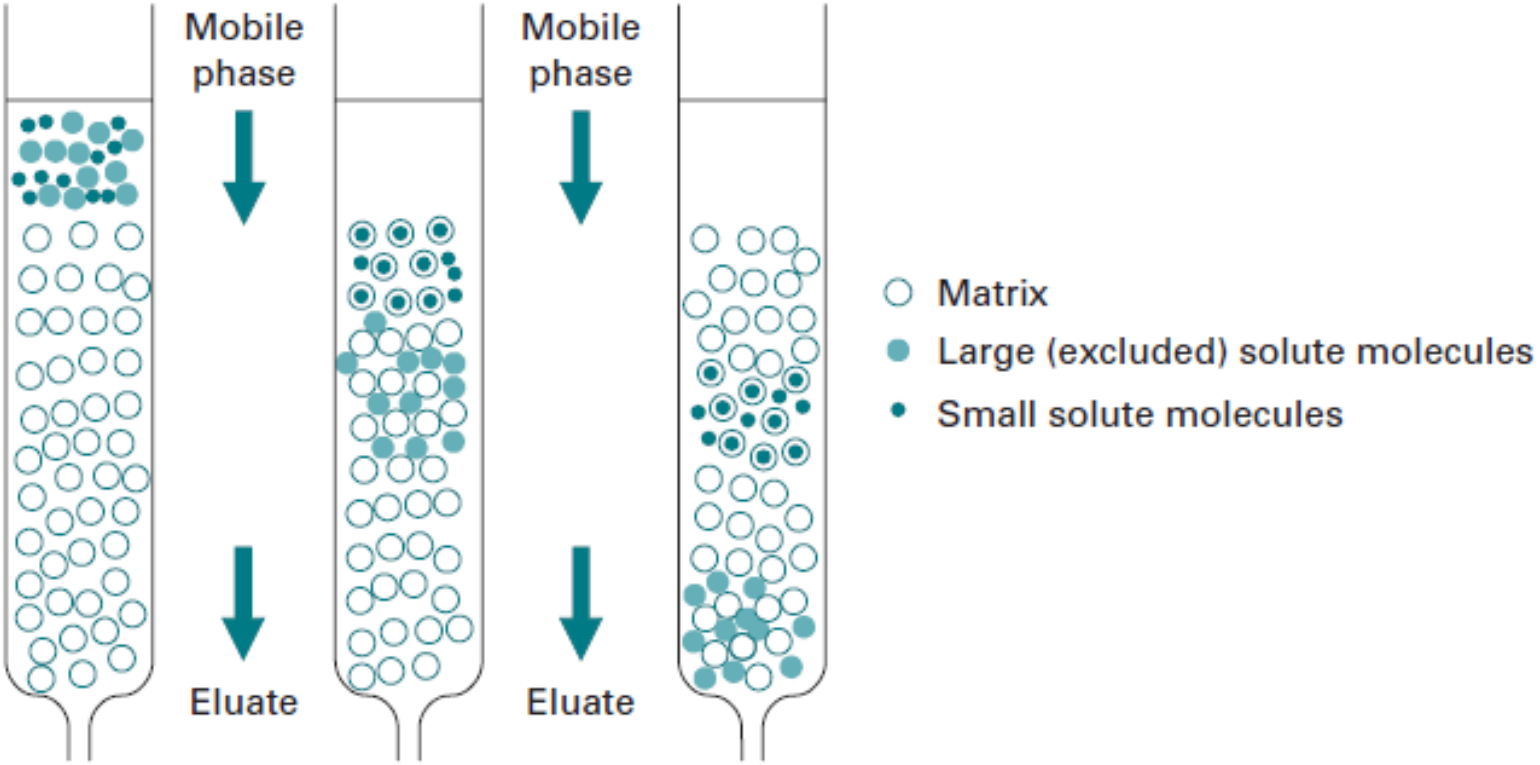

Fig. 11.9 Separation of different size molecules by exclusion chromatography. Large excluded molecules are eluted first in the void volume.

...Principle

- The mobile phase trapped by a particle is available to an analyte to an extent that is dependent upon the porosity of the particle and the size of the analyte molecule.
- Thus, the distribution of an analyte in a column of cross-linked particles is determined solely by the total volume of mobile phase, both inside and outside the particles, that is available to it.
- For a given type of particle, the distribution coefficient, Kd, of a particular analyte between the inner and outer mobile phase is a function of its molecular size.
- If the analyte is large and completely excluded from the mobile phase within the particle, Kd = 0, whereas, if the analyte is sufficiently small to gain complete access to the inner mobile phase, Kd = 1.
- Due to variation in pore size between individual particles, there is some inner mobile phase that will be available and some that will not be available to analytes of intermediate size; hence Kd values vary between 0 and 1.
- It is this complete variation of Kd between these two limits that makes it possible to separate analytes within a narrow molecular size range on a given particle type.

...Principle

- For two analytes of different relative molecular mass and Kd values, K'd and K''d, the difference in their elution volumes, V_S, can be shown to be:

$$V_S = (K'_d - K''_d)V_i$$

- Where Vi is the inner volume within the particle available to a compound whose Kd = 1.
- Tt is advisable to reduce the sample volume below the value of V_S because the ratio between sample volume and inside particle volume affects both the sharpness of the separation and the degree of dilution of the sample.

Materials

- The stationary phases for exclusion separations are generally based on silica, polymethacrylate or polyvinyl acetate or chloride or on cross-linked dextran or agarose.
- All are available in a range of pore sizes.
- They are generally used where the eluent is an organic system.

Applications

Purification

- The main application of exclusion chromatography is in the purification of biological macromolecules by facilitating their separation from larger and smaller molecules.
- Viruses, enzymes, hormones, antibodies, nucleic acids and polysaccharides have all been separated and purified by use of appropriate gels or glass granules.

Relative molecular mass determination

- The construction of a calibration curve, with proteins of a similar shape and known Mr, enables the Mr values of other proteins, even in crude preparations, to be estimated.

...Applications

Solution concentration

- Solutions of high Mr substances can be concentrated by the addition of dry Sephadex G-25 (coarse).
- The swelling gel absorbs water and low Mr substances, whereas the high Mr substances remain in solution.

Desalting

- By use of a column of, for example, Sephadex G-25, solutions of high Mr compounds may be desalted, i.e. removed from contaminants such as salts, detergents, lipids and chaotropic agents.
- The high Mr compounds move with the void volume, whereas the low Mr compounds are distributed between the mobile and stationary phases and hence move slowly.
- This method of desalting is faster and more efficient than dialysis.