CHEMISTRY 5 (INSTRUMENTAL)

AGRICULTURAL BIOTECHNOLOGY, LEVEL 2

By

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2021.05.19

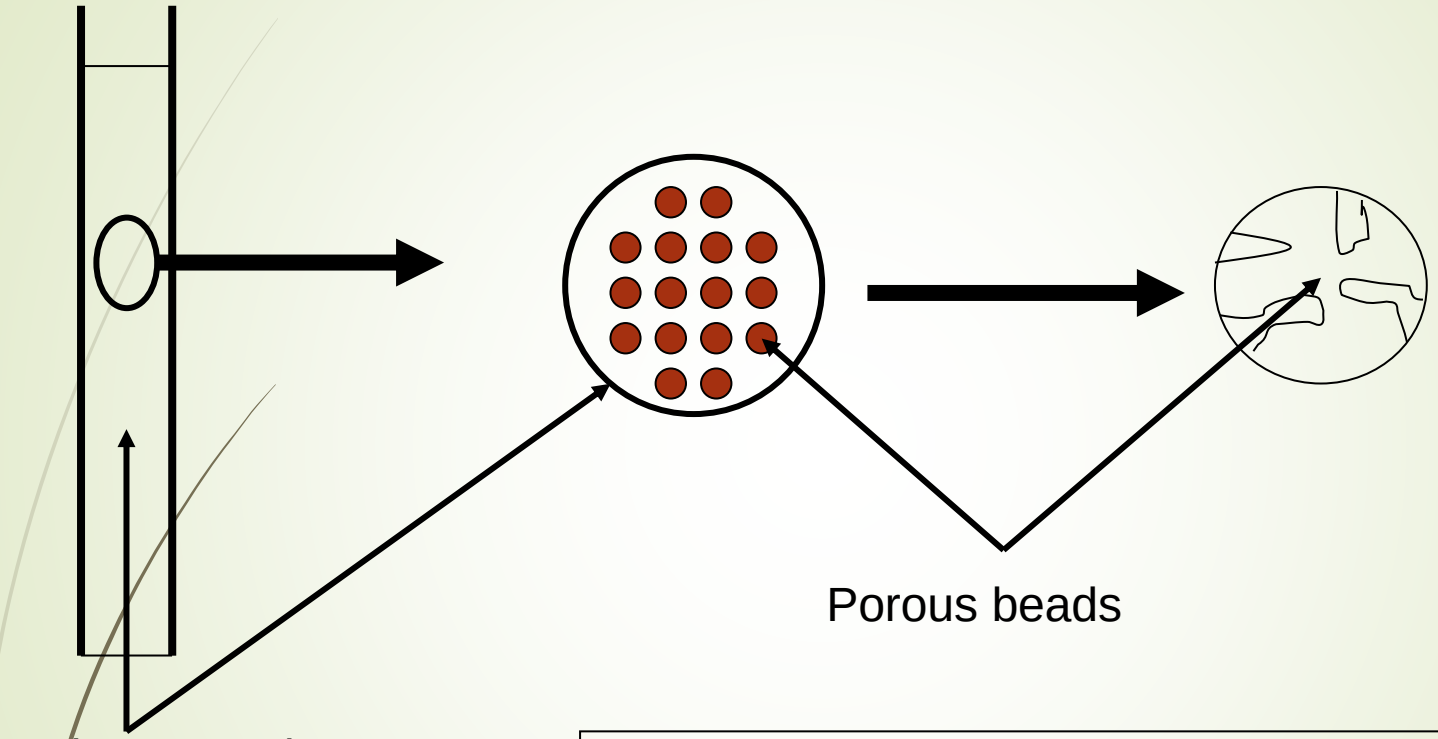
Gel Filtration

Gel permeation chromatography

Size exclusion chromatography

Separation of molecules on the basis of size (and shape)

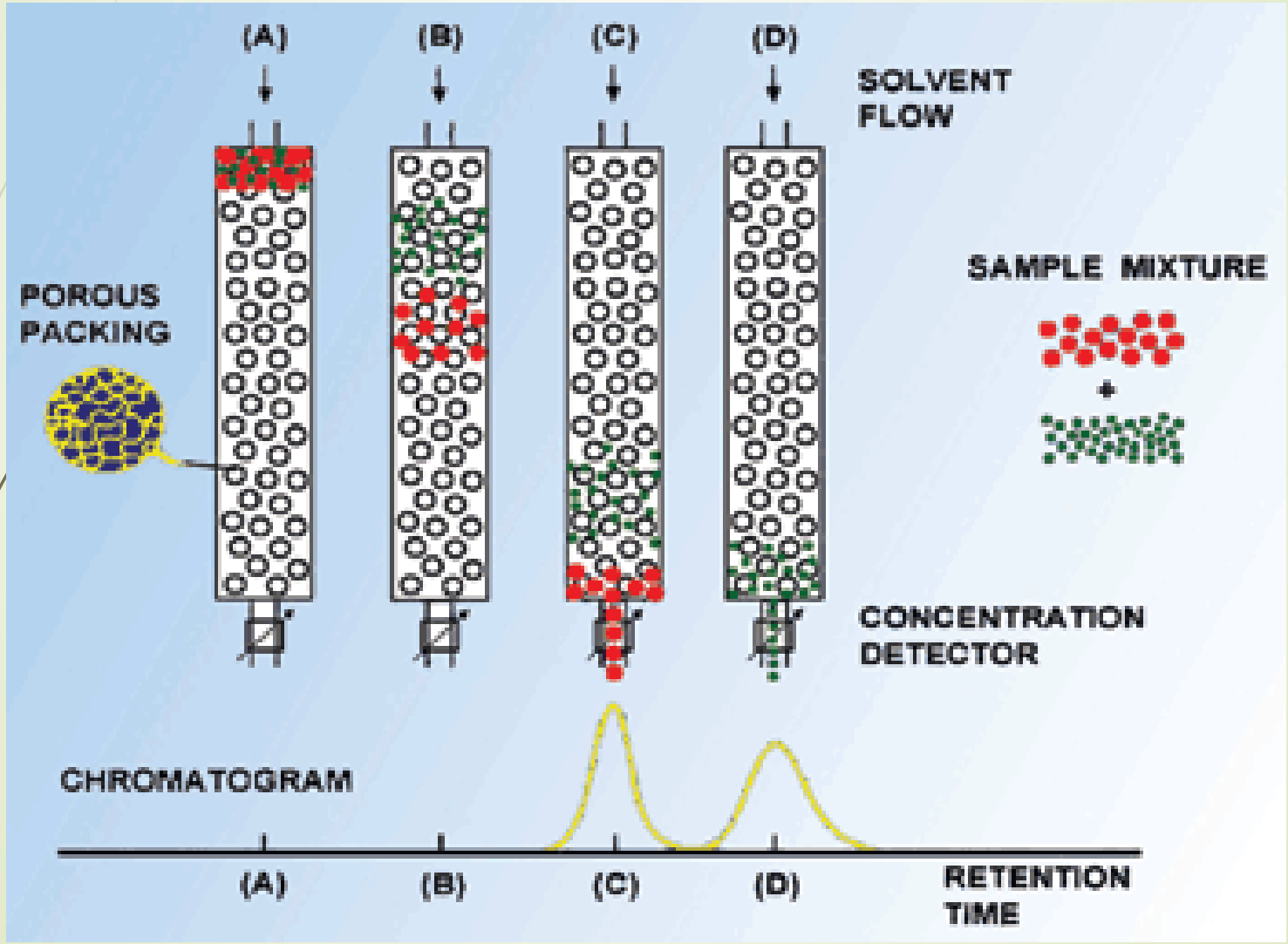
Theory



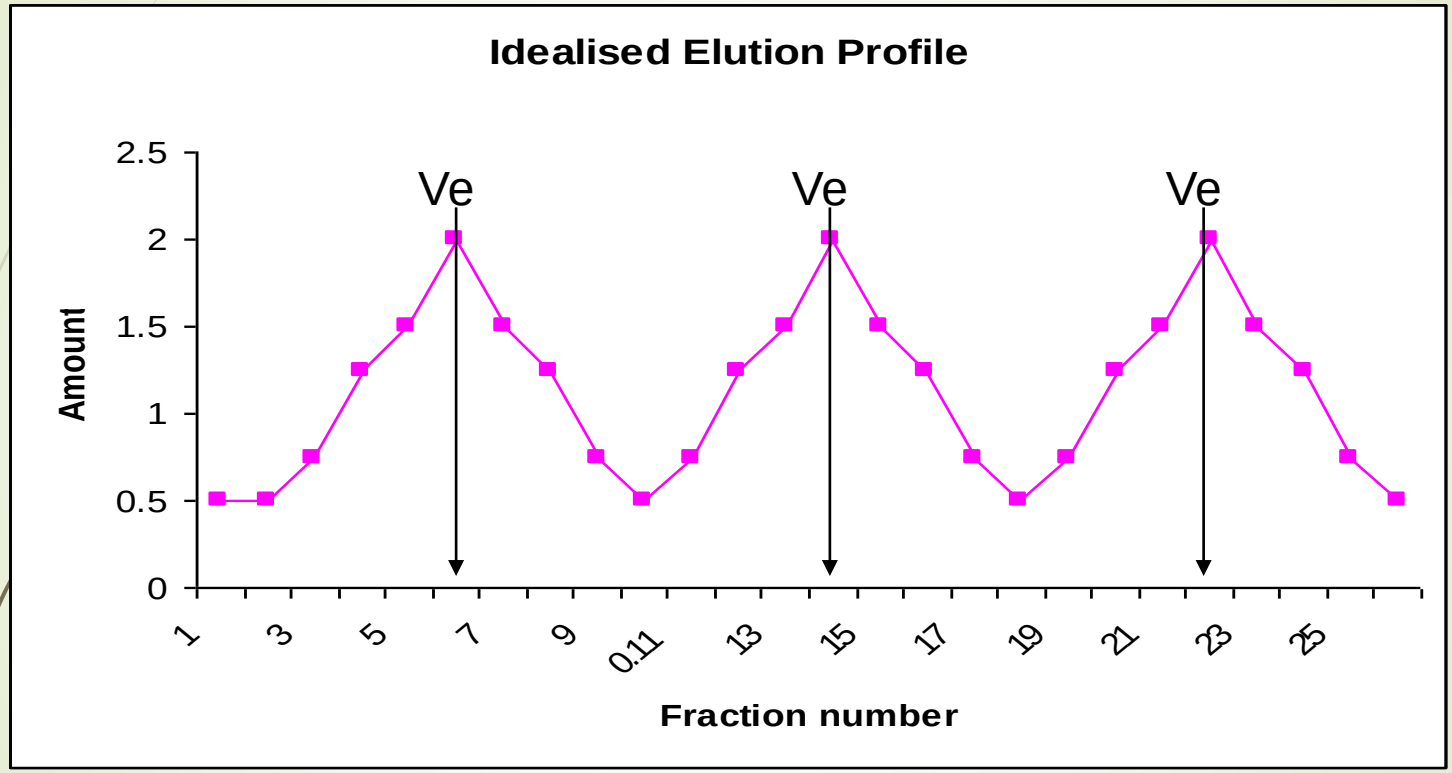
Large molecules are “excluded” from the pores and travel through the column fastest

Small molecules are “included” – can diffuse into the pores and elute later

Theory

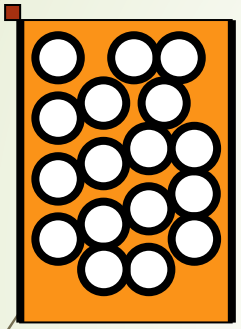

(A)
(B)
(C)
(D)
SOLVENT FLOW
POROUS PACKING
SAMPLE MIXTURE
CONCENTRATION DETECTOR
CHROMATOGRAM
(A)
(B)
(C)
(D)
RETENTION TIME

Elution Profile

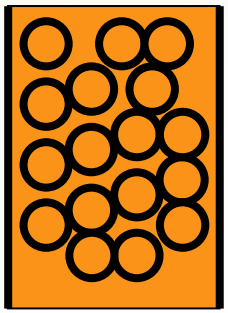


Ve = Elution volume (volume of solvent between injection and elution). Dictated by proportion of porous matrix available to molecules (Kd).

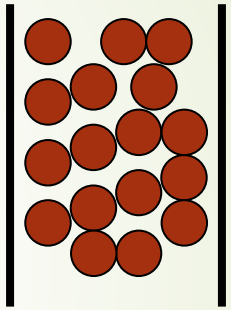
Column Parameters



Vo = void volume



Vt = total volume



Vs= volume of solvent held in the pores. This is normally approximated to

Vt-Vo = volume of beads

Vo = Elution volume of a large “totally excluded” molecule such as blue dextran

Vt = Physical volume of column

Calculation of Ve

For a molecule that can partially enter the pores:

$$Ve = Vo + Kd\ (Vs)$$

or $Ve = Vo + Kav\ (Vt-Vo)$

Kav = proportion of pores available to the molecule.

Totally "exclude" Kav = 0 and Ve = Vo

Totally "included" Kav = 1 and Ve = Vt

Behaviour of Molecule on any Column

$$K_{av} = \frac{V_e - V_o}{V_t - V_o}$$

Thank you