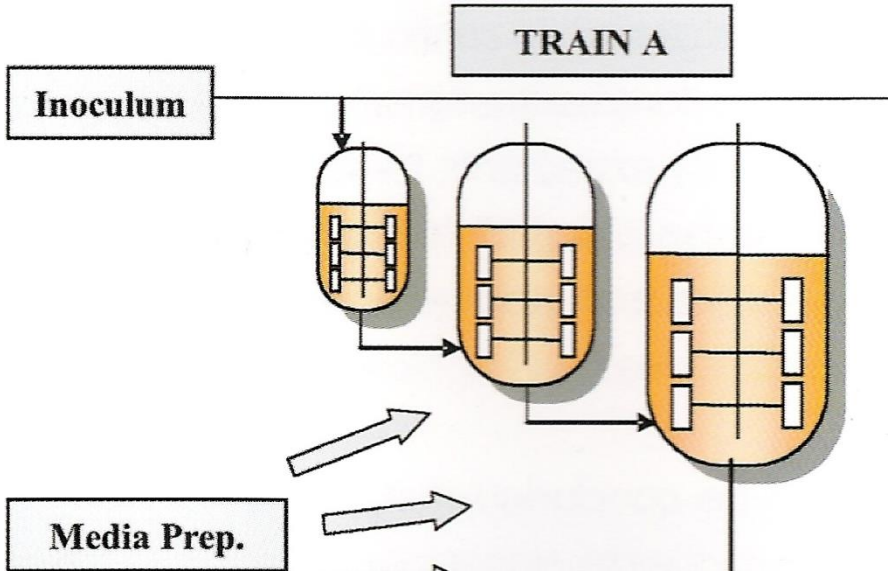
Introduction to preparative chromatography techniques and membrane separation technologies

Edit Székely

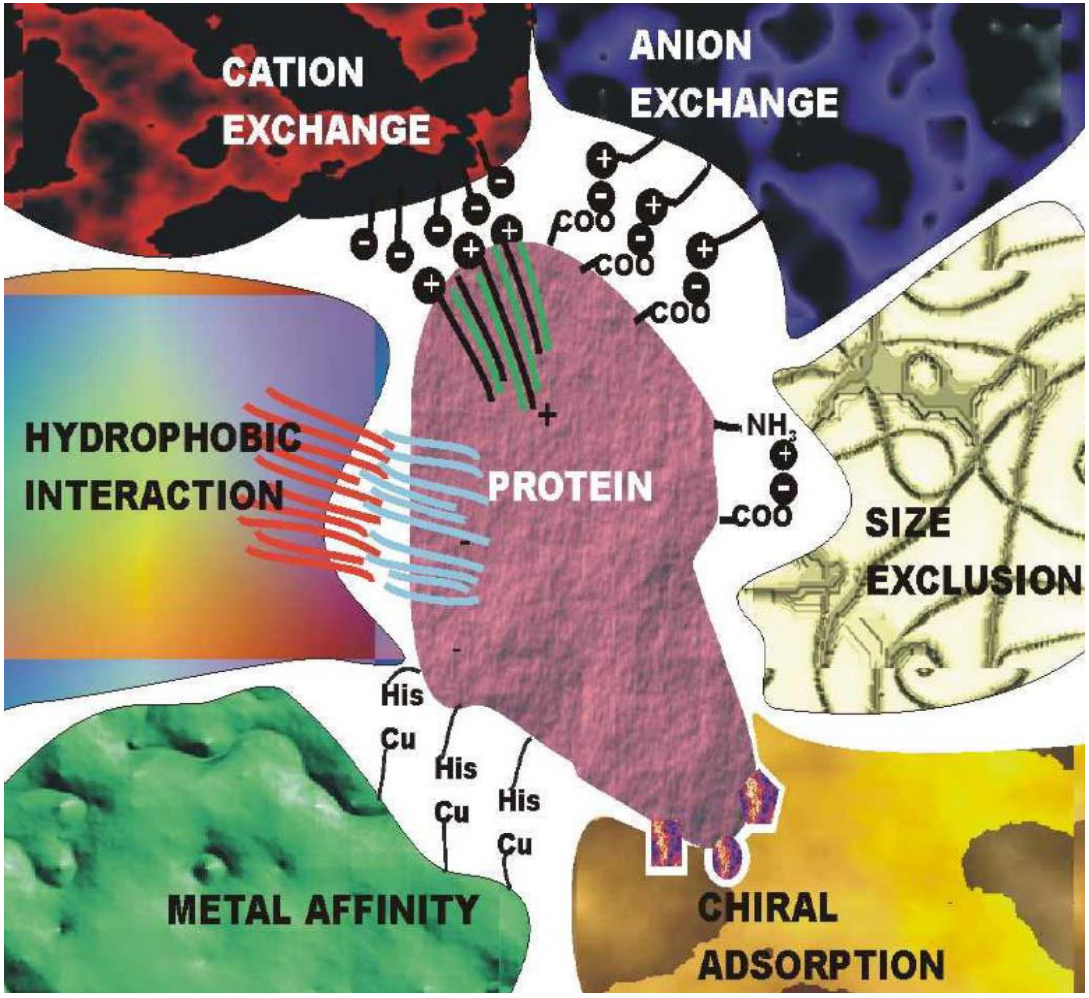
Modern separation technologies course, 2019, BME

The lecture is related to the downstream processing of proteins in biopharma

- What are the unwanted constituents of the fermentation broth?
  - sugars
  - salts
  - tensids
  - DNS, RNS
  - Other then target proteins
  - Broken cells
  - viruses
  - …

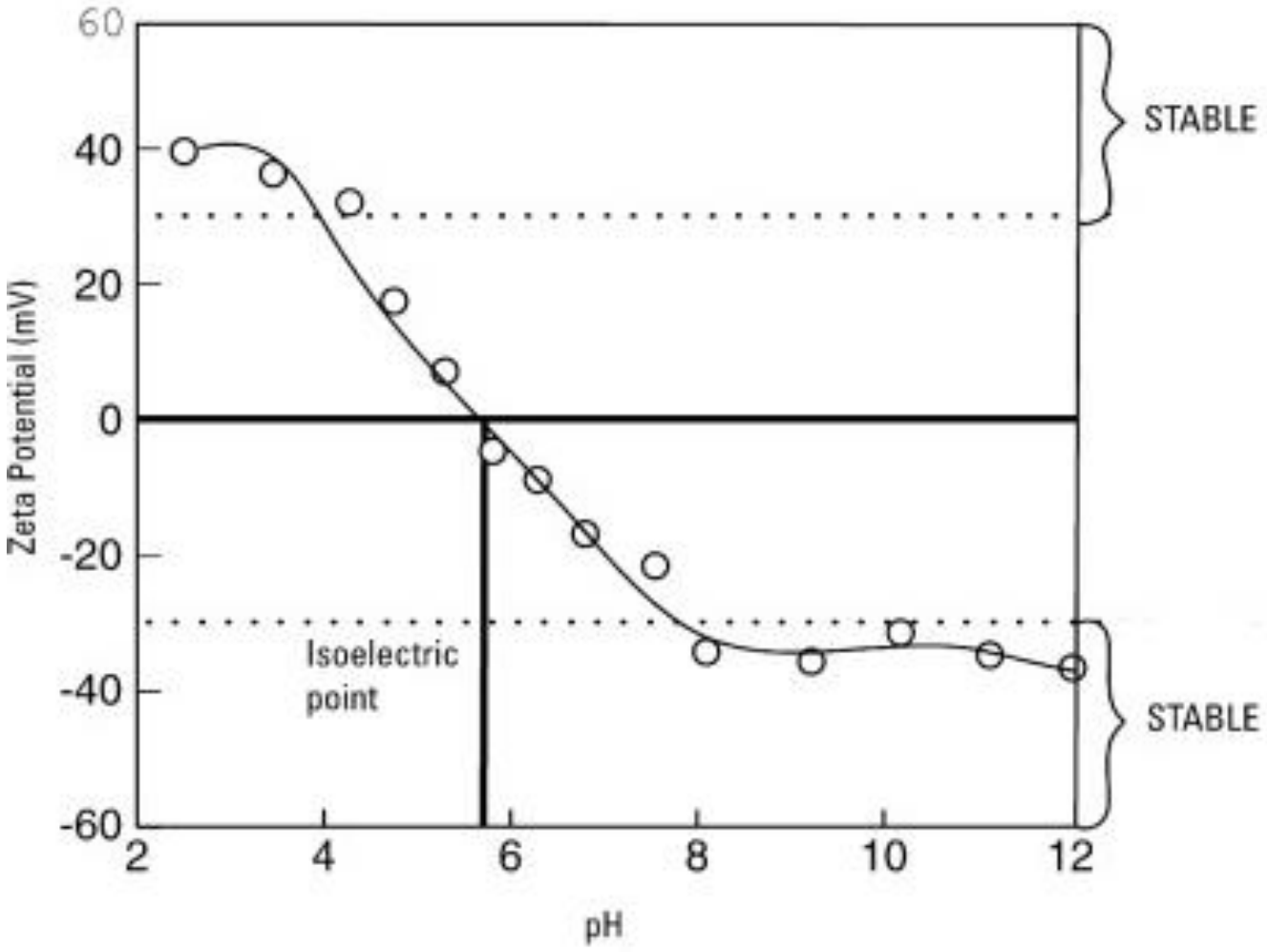


How can we „catch” a protein?



Isoelectric point (pI)

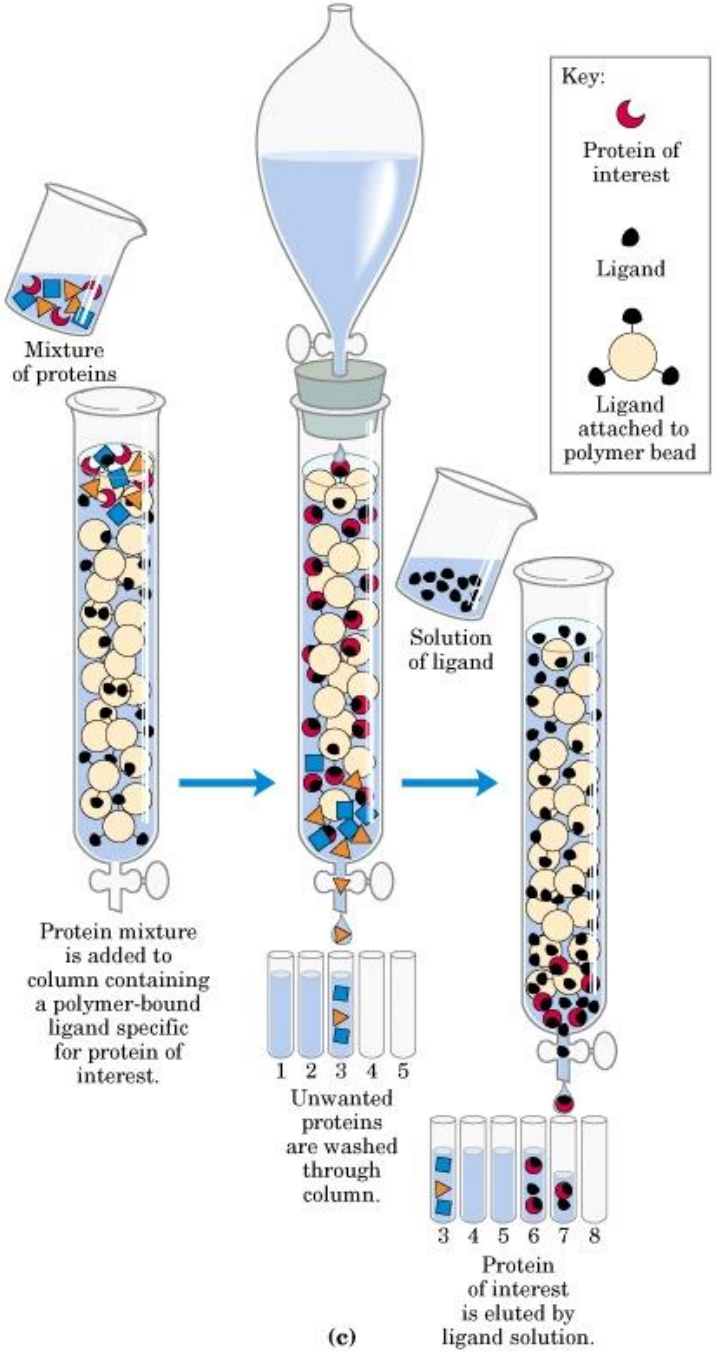
It is a pH value when the netto charge of the protein is 0. At pH=pI of our protein its solubility in water is the minimal.



Preparative column chromatography
e.g. affinity chromatography

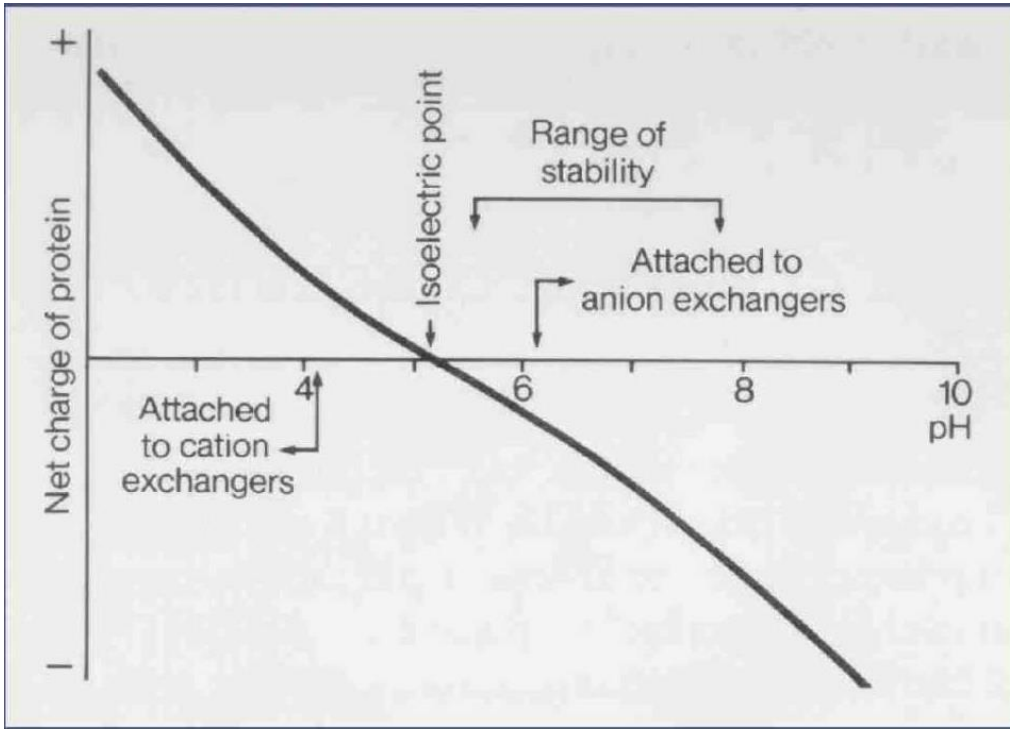
Steps:

1. Mixture is fed to the column. Typically the pH is close to the pI of the target protein.
2. Non selectively bonded components are washed off at the same pH as in step 1.
3. Changing the conditions to elude the target protein (e.g. changing the pH to 3-4, adding ligand to the solution, adding stronger binding components to the solution, or more than one of these)
4. Regeneration of the column (typically pH 1.5)
5. Washing and equilibration of the column for the next affinity step.

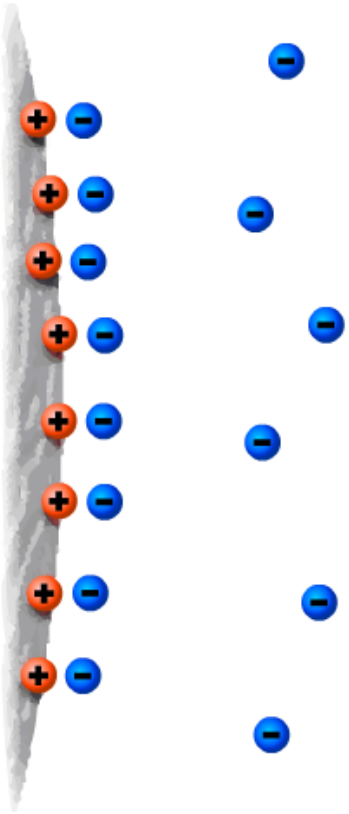


IEX: ion exchange chromatography

- Colun filled with an ionexchange resin
- Anion exchange: the resin is positively charged; the protein must be negatively charged; pH>pI.
- Cation exchange: the resin is negatively charged; the protein must be positively charged; pH<pI.
- The size of the protein also counts.

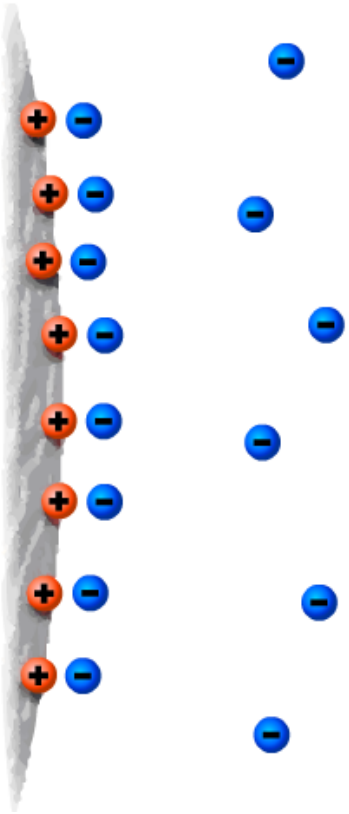


IEX: ion exchange chromatography
e.g. anion exchanger resin

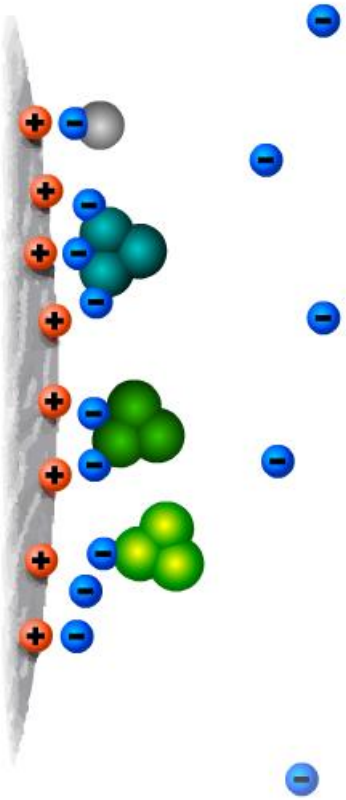


1. equilibration

IEX: ion exchange chromatography
e.g. anion exchanger resin

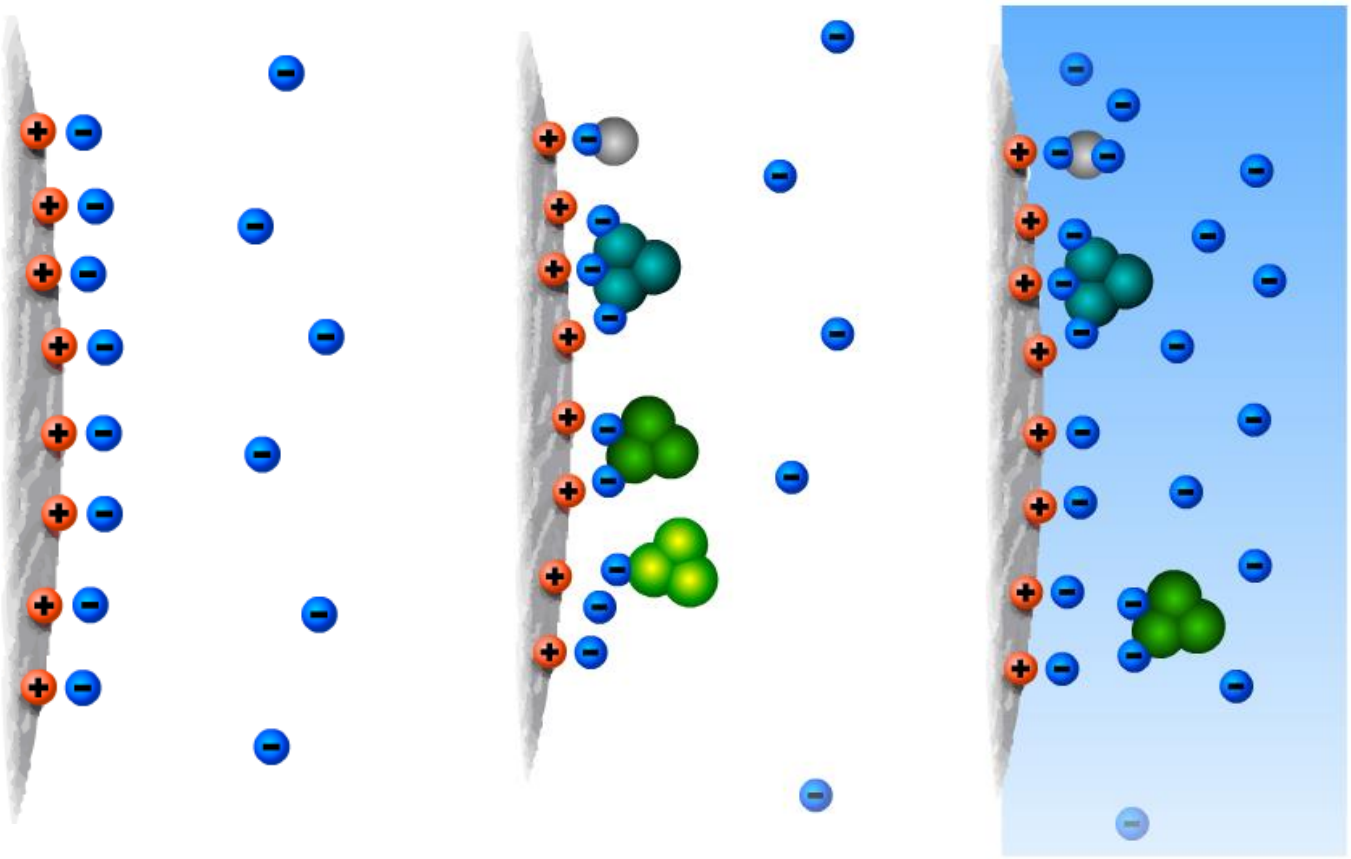


1. equilibration,
2. washing



2. Loading of the sample
pH>pI

IEX: ion exchange chromatography
e.g. anion exchanger resin

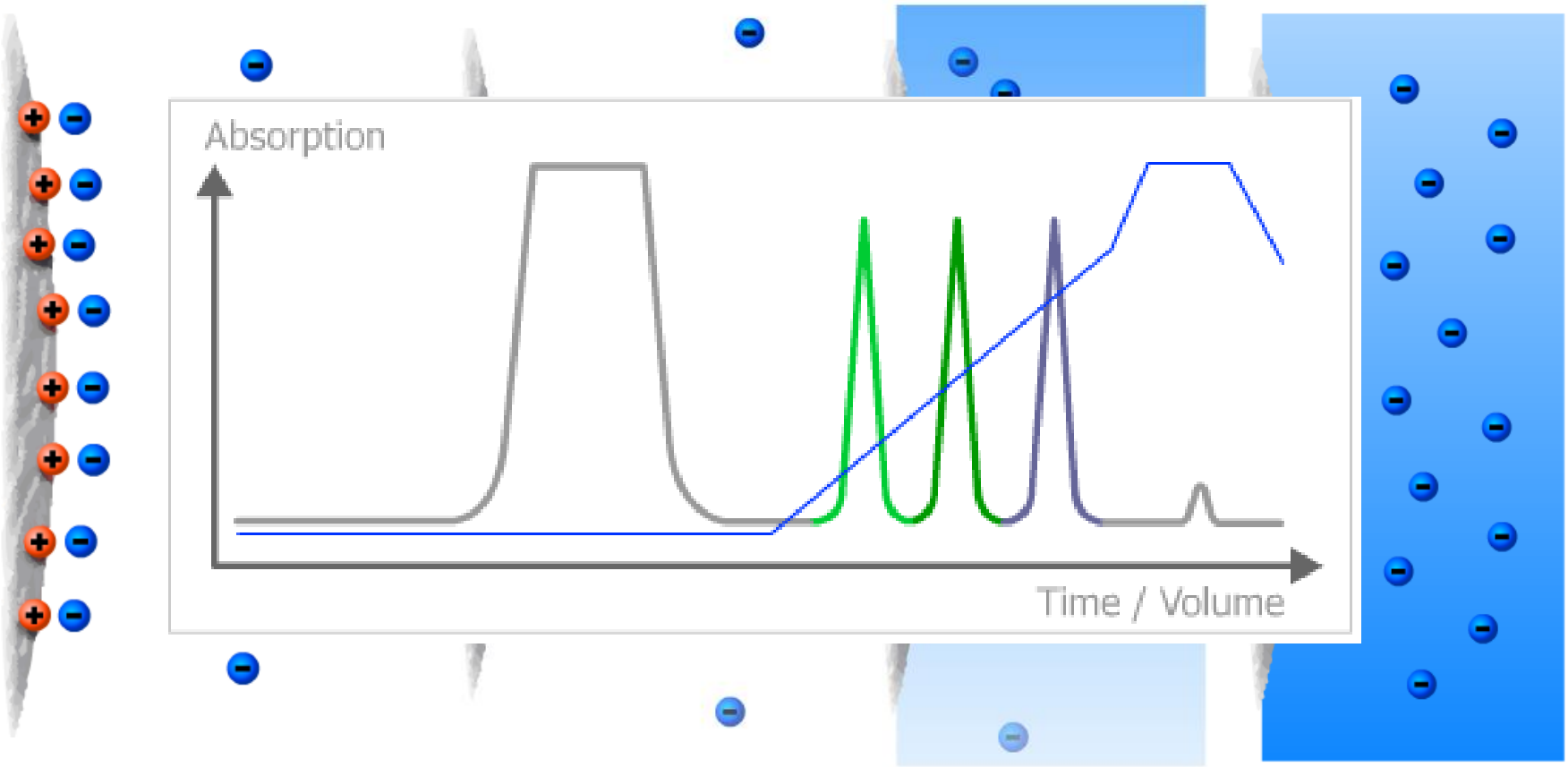


1. equilibration,
2. washing

2. Loading of the sample pH>pI

3. pH shift, elution pH>>pI

IEX: ion exchange chromatography
e.g. anion exchanger resin



1. equilibration,
2. washing

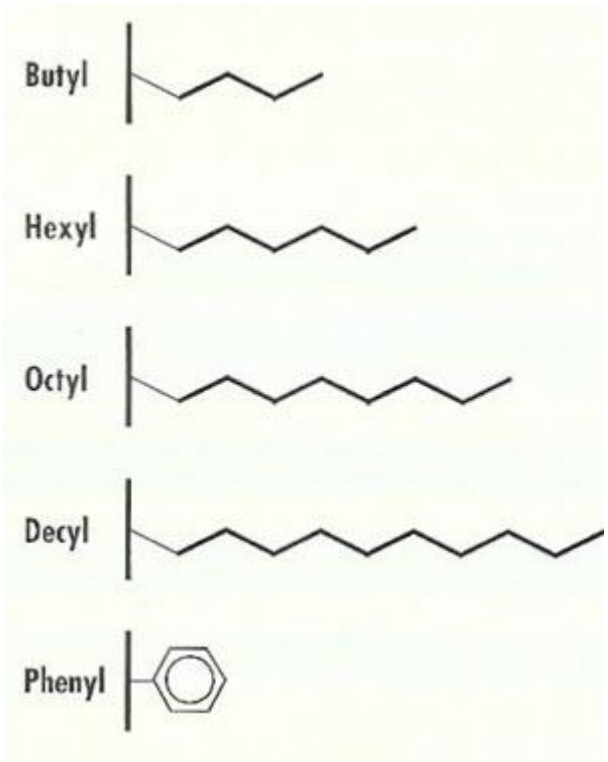
2. Loading of the sample pH>pI

3. pH shift, elution pH>>pI

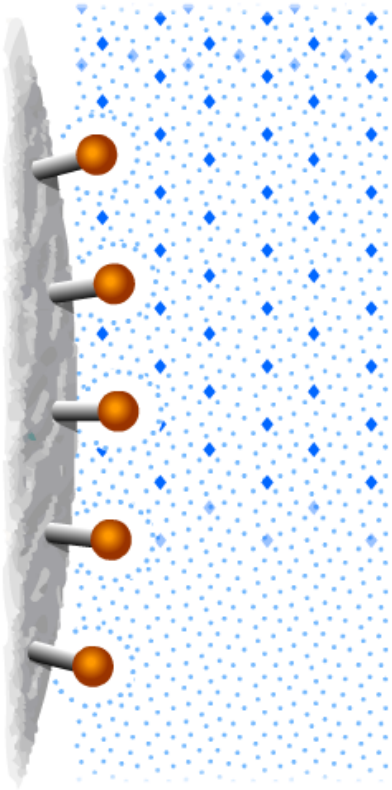
4. Washing and regeneration

HIC = hydrophobic interaction chromatography

- The HIC resin is apolar, protein adsorption is based on apolar interaction and salting out effects.
- High initial salt concentration, high ionic strength at the beginning, salting out effect; decreasing salt concentration for the elution.
- Typically after the IEX in the technology, since there for the elution we needed high ionic strength.

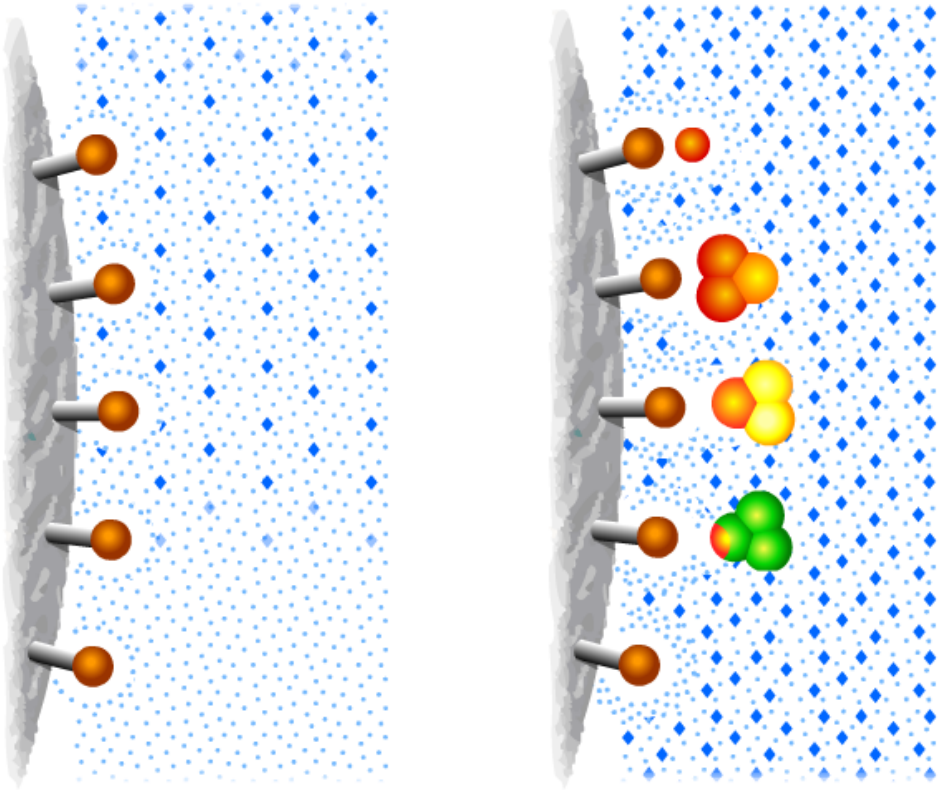


HIC = hydrophobic interaction chromatography



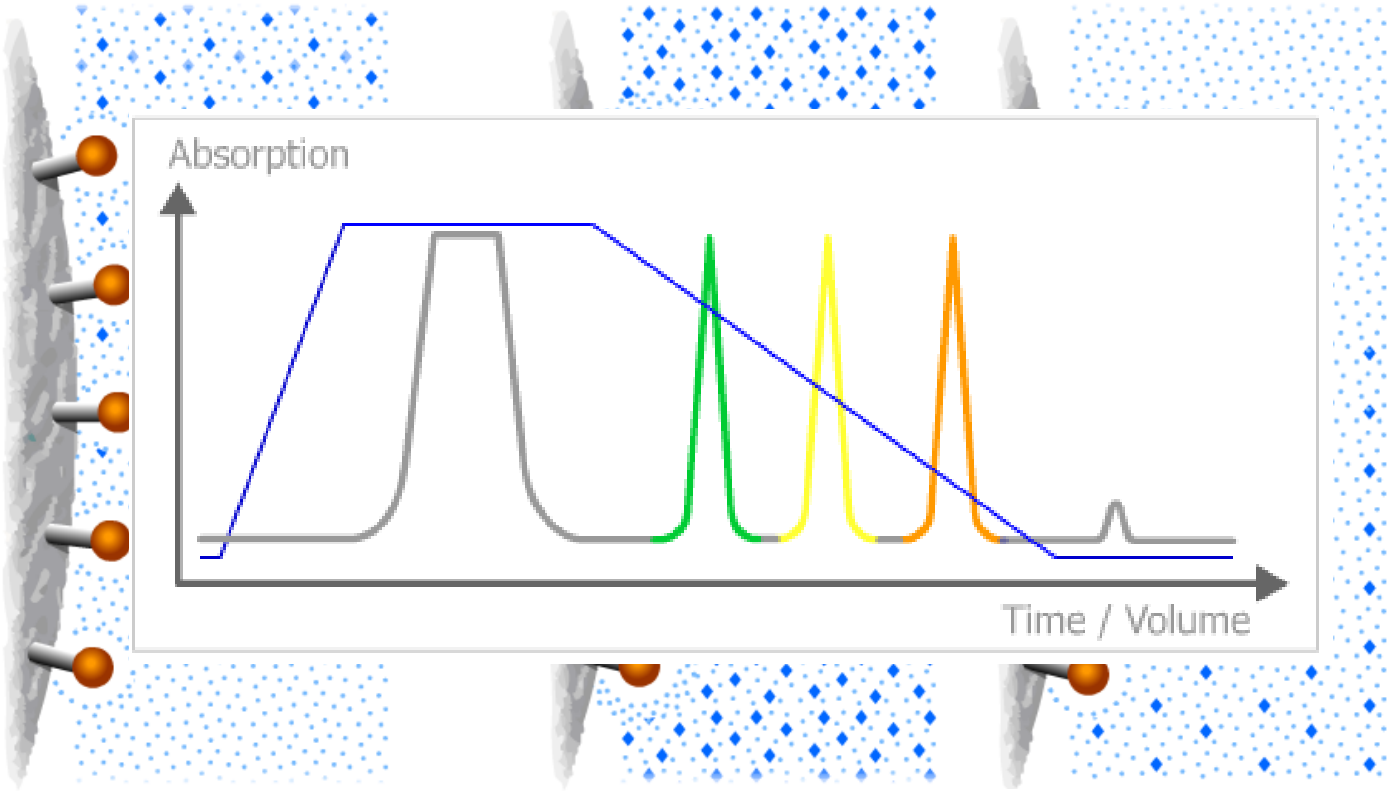
1. Washing, equilibration

HIC = hydrophobic interaction chromatography



1. Washing, equilibration
2. Loading the sample, adsorption

HIC = hydrophobic interaction chromatography



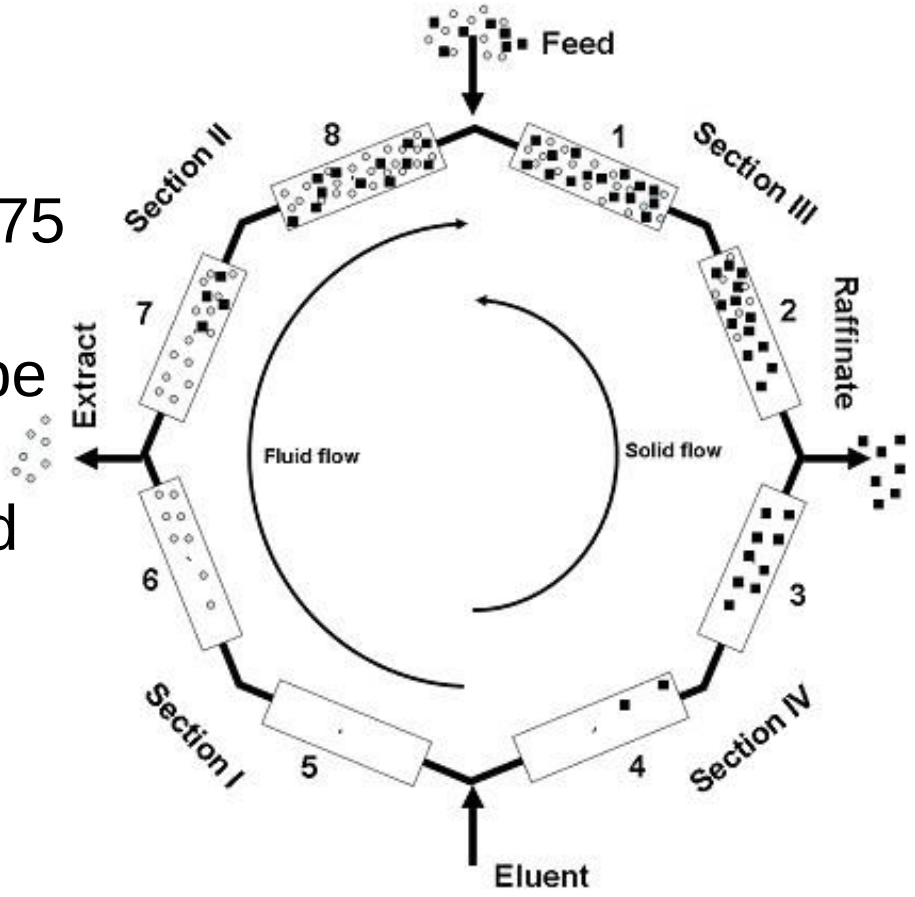
1. Washing, equilibration
2. Loading the sample, adsorption
3. Elution with ionic gradient

Preparative column chromatography

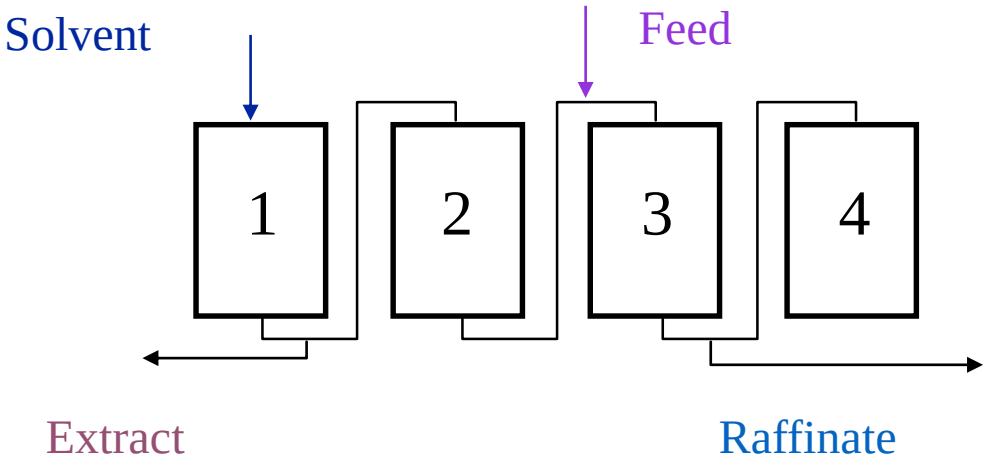
- ✓ High selectivity, fine tuning
- ✓ Well known, established techniques from lab to process scales
- ✓ Flexible, variable
- x Batch operation (collection of samples, storage, integration into the technological scheme)
- x High resin costs (especially affinity resins) so the low productivity of the batch operation makes it expensive
- x High solvent costs and consumption (environmental issue)
- x Resin stability problems (pH sensitivity)
- x Highly trained staff needed, low automation

Simulated moving bed chromatography (SMB)

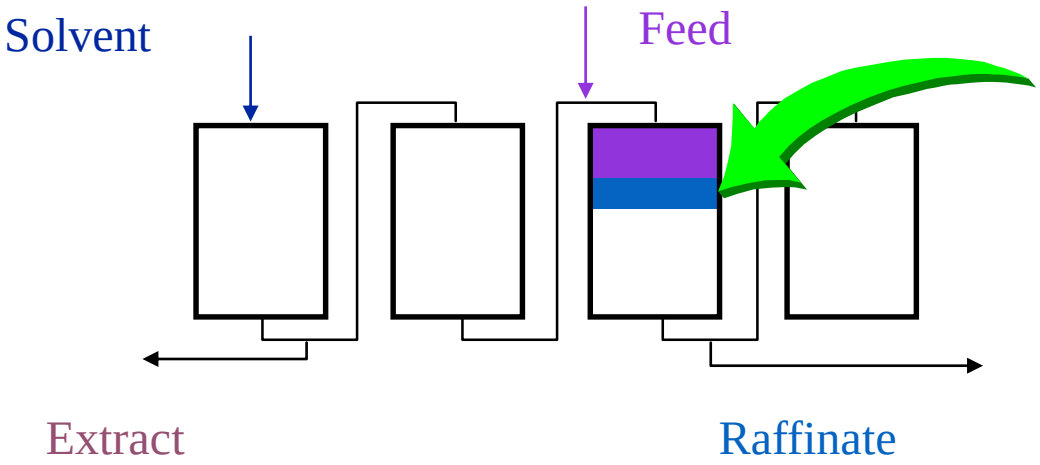
- Continuous preparative chromatography, scaled up already (min 7 plants with 10-75 Mt/year capacity).
- Diameter of the column may be large (e.g. 1000 mm!) low to medium pressures are applied (0.2 to 3-4 MPa)
- Realtively high selectivity is needed.
- Typical technology for 2 products (enantiomers, sugar isomers, cis-trans isomers etc.)



Simplified SMB - 1

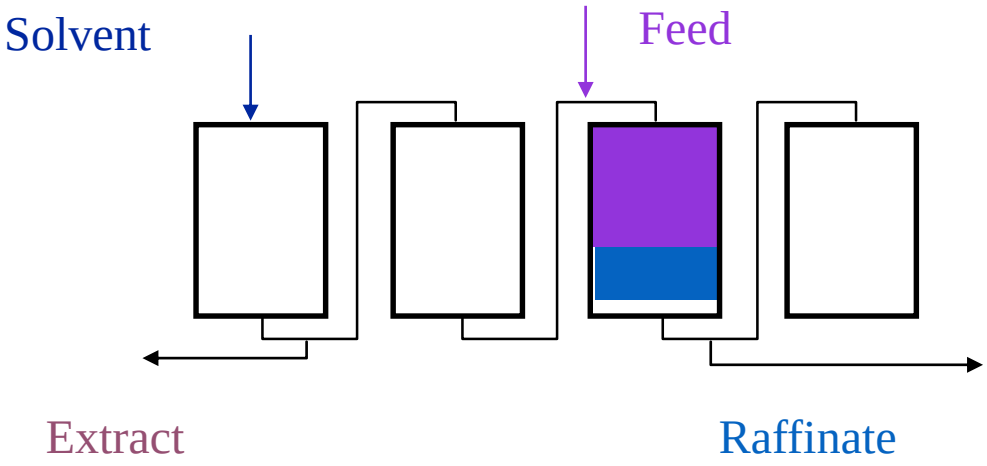


The system is started.....

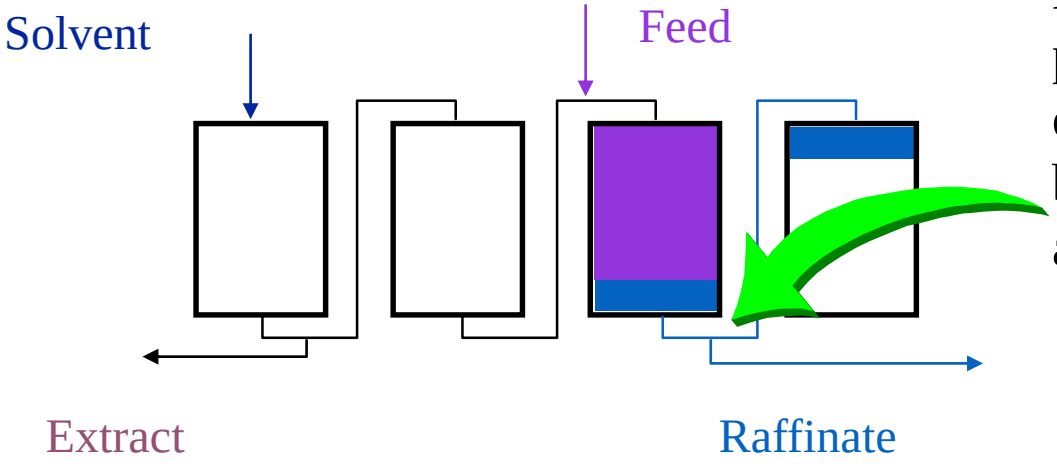


A frontal elution separation occurs in Section 3.

Simplified SMB - 2

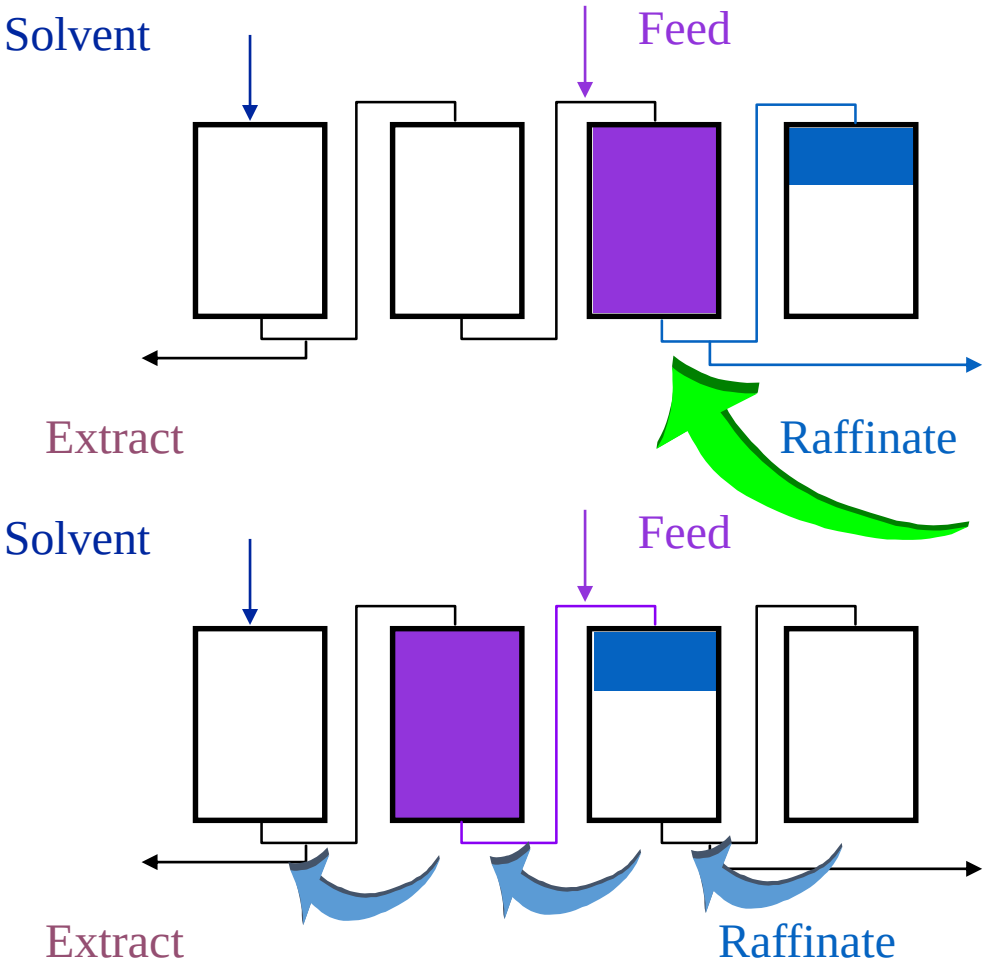


The separation continues.....



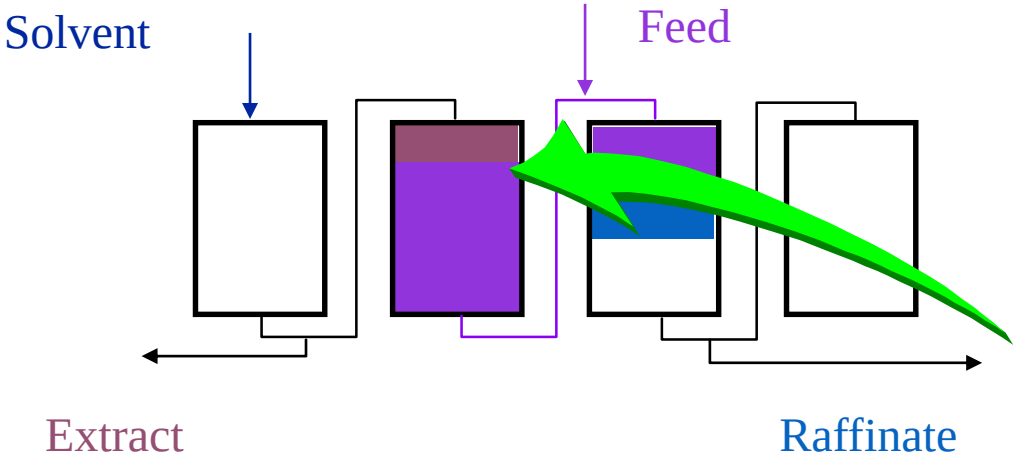
Eventually the front of pure product 1 reaches the outlet. It is distributed between the final Section and the product port

Simplified SMB - 3

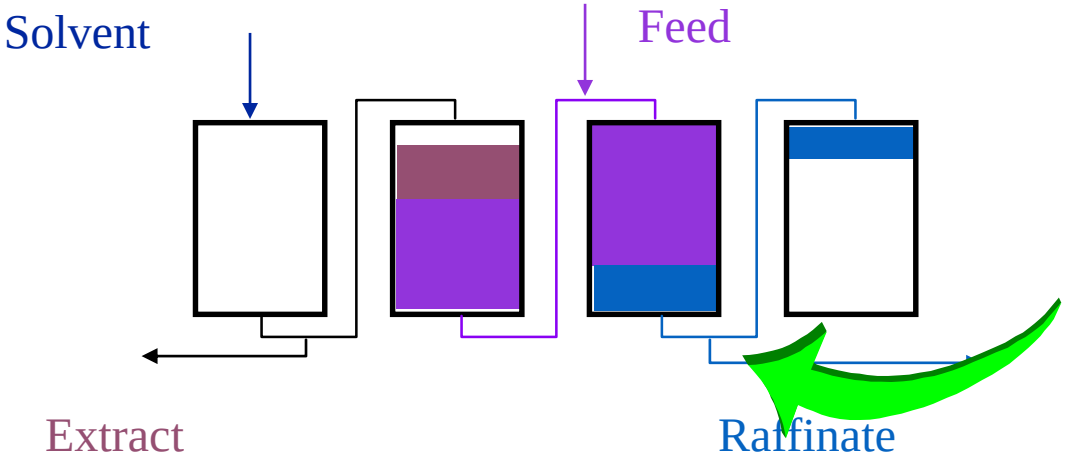


Finally, the mixed product reaches the outlet. To avoid collecting impure material, it is necessary to move the columns 1 position upstream.

Simplified SMB - 4

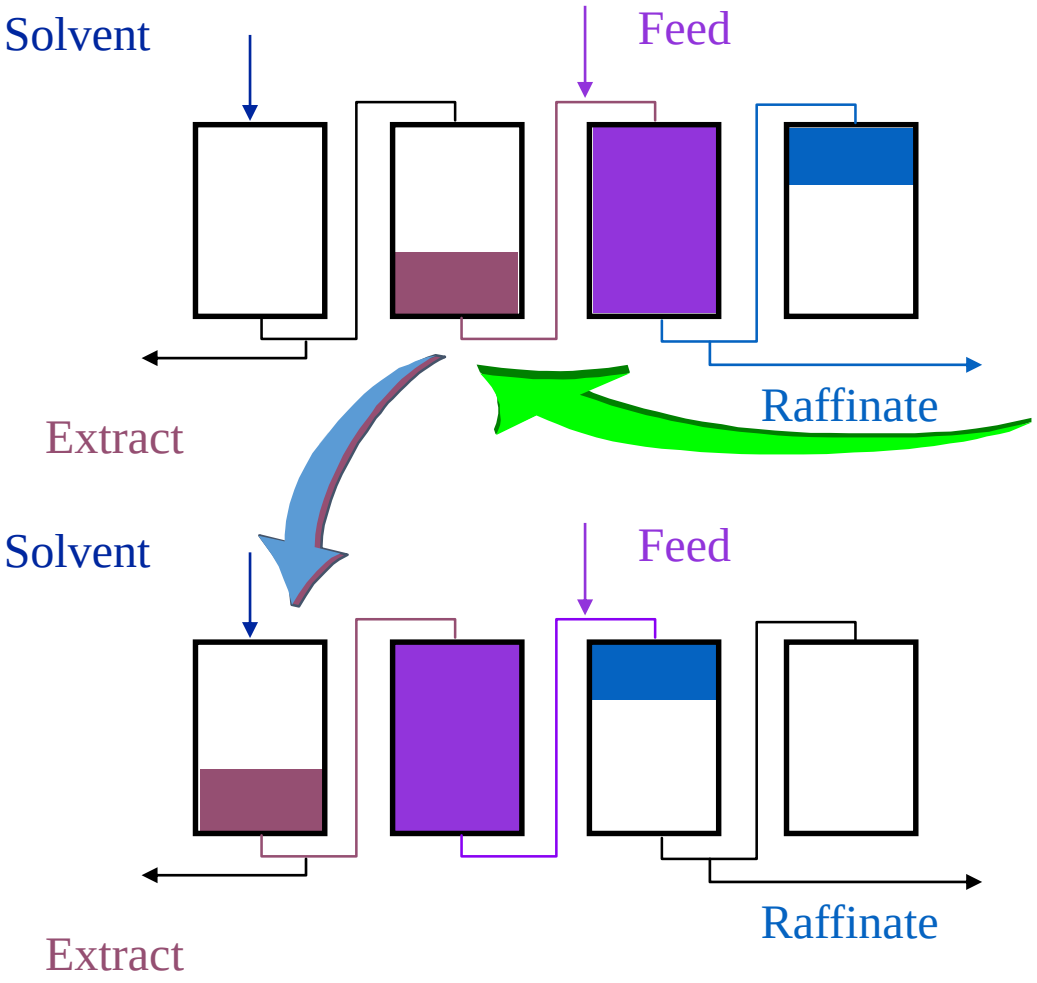


The frontal separation continues; at the same time, the slow moving product starts to separate from the tail of the mixed product band in Section 2



Eventually the fast moving product again reaches the outlet and more pure product is collected.

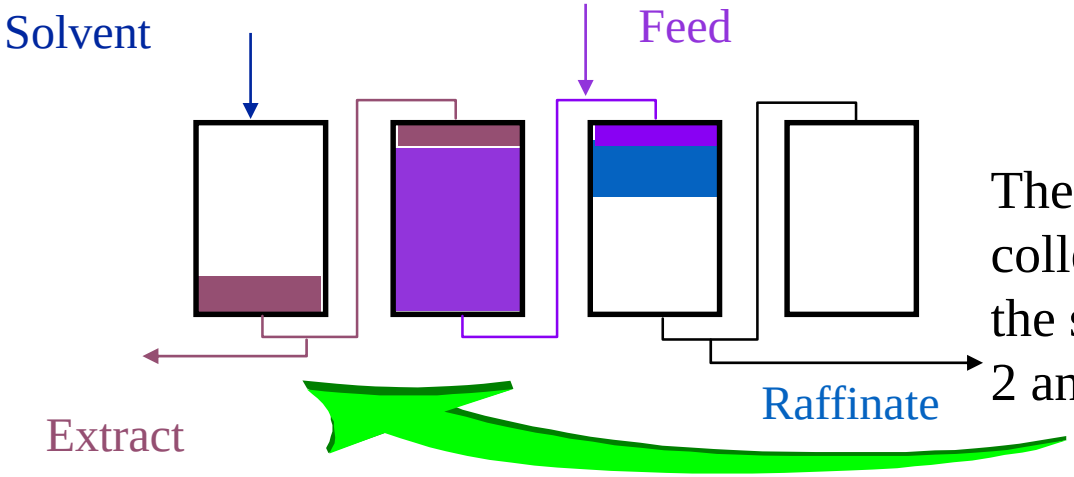
Simplified SMB - 5



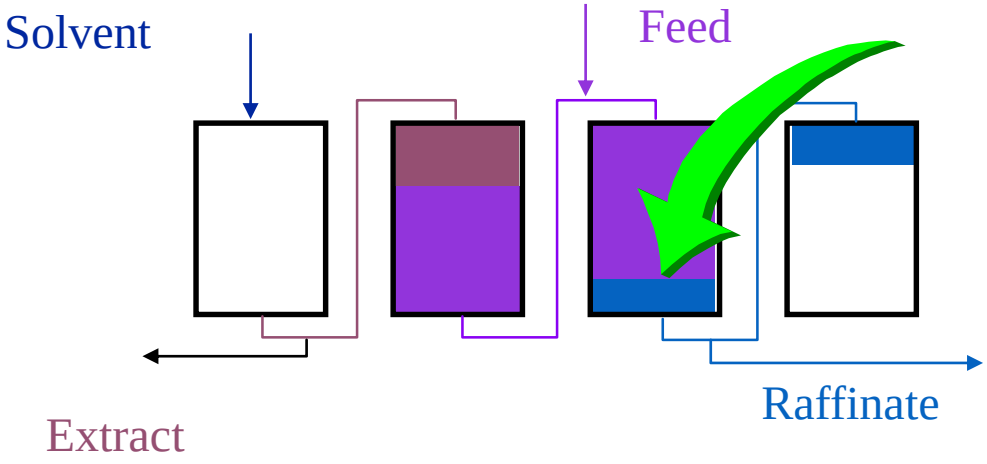
When the mixed band reaches the end of Section 3 its tail has left Section 2 (if the separation has been correctly designed) and only pure product 2 remains in Section 2.

To avoid collecting impure raffinate, the columns are moved once more. Now, the pure component 2 is in Section 1.

Simplified SMB - 6

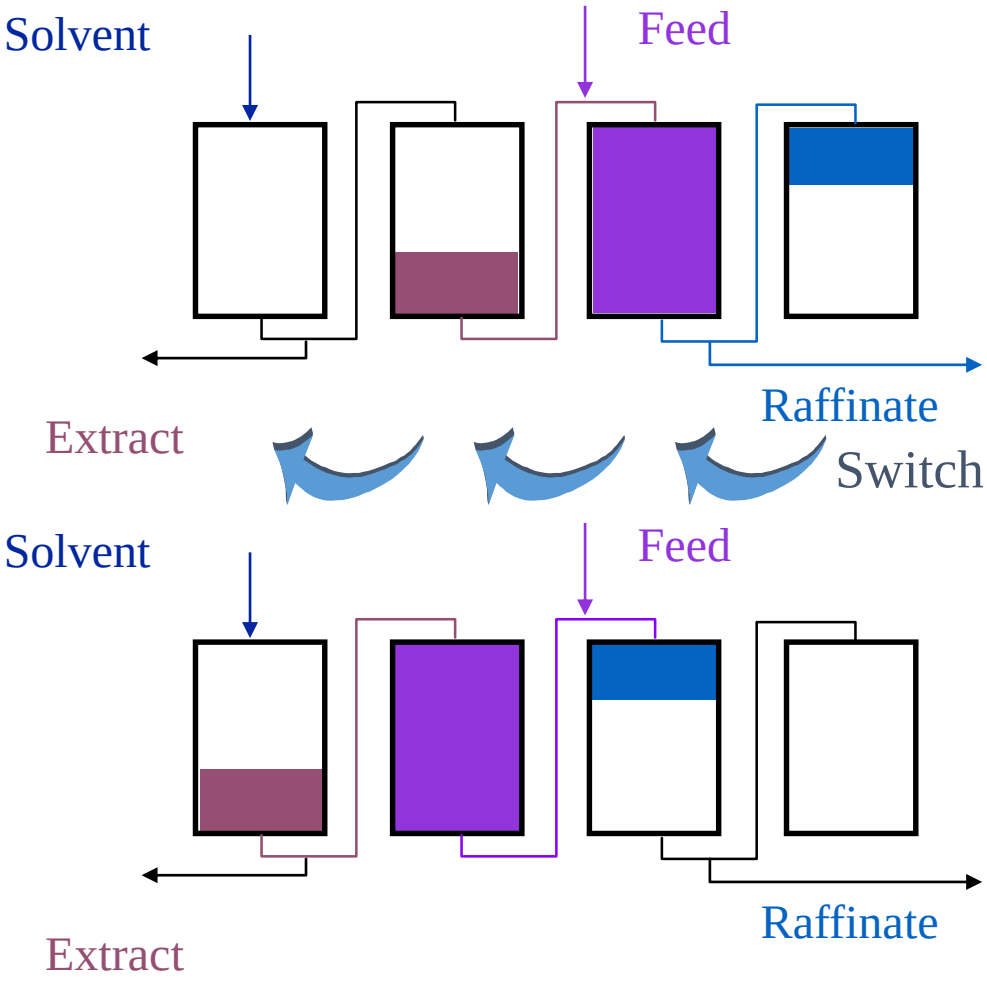


The second component is now collected at the Extract port while the separation continues in Sections 2 and 3.



The faster component reaches the Raffinate port and is again collected; note that the outlet concentrations are neither constant nor concurrent.

Simplified SMB - 7



Eventually, the mixed zone reaches the raffinate port and the columns are again switched.

This simplified system is now in a steady state mode and will continue to cycle.

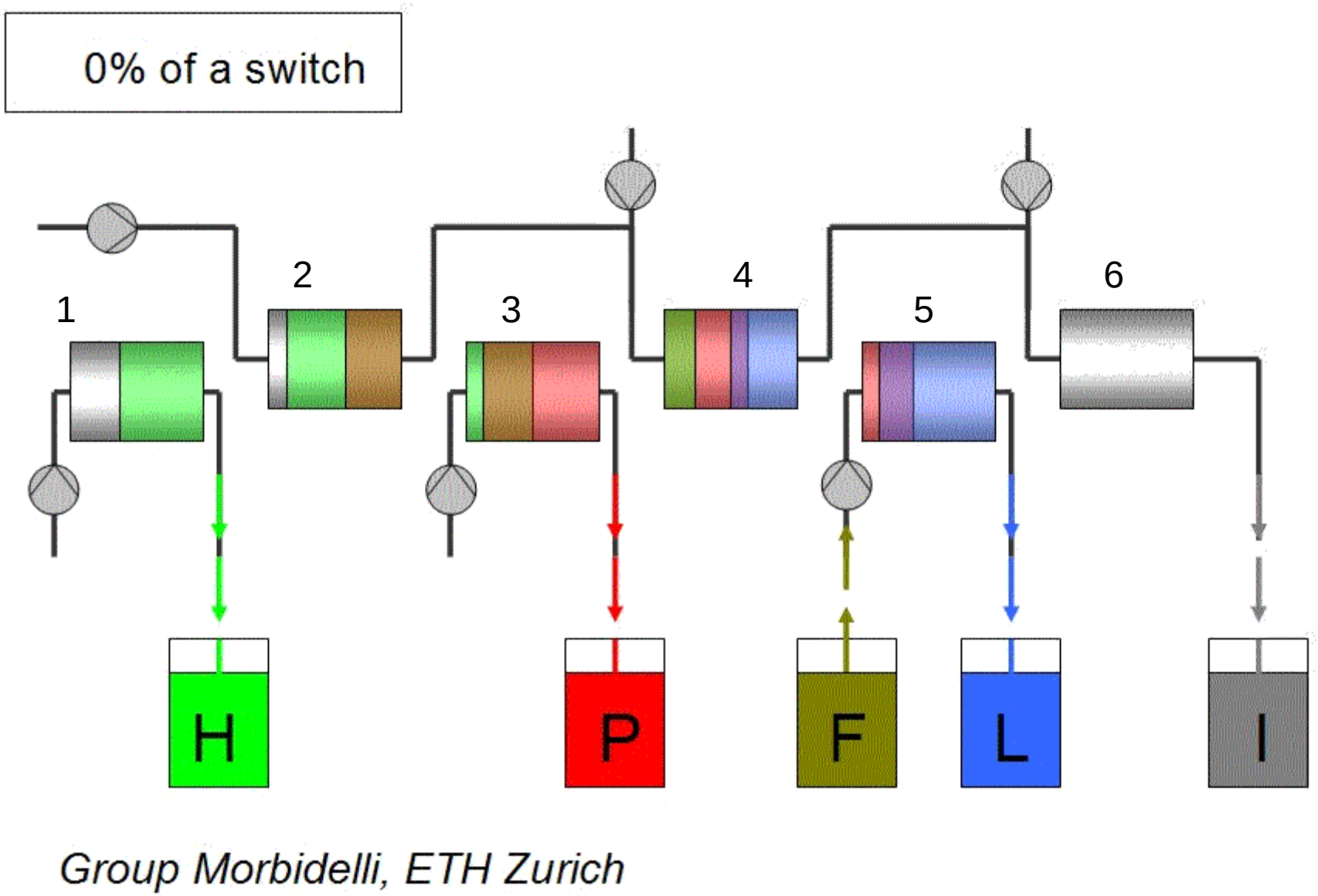
SMB Optimization

- Independent variables:
  - Flow rates
    - Recycle, Desorbent, Raffinate, Extract, Feed
  - Period (switching time)
- Procedure:
  - Educated guess based on batch experiments
  - Get the system bound, manipulate the flow rates to maximize throughput at required purity

Aerojet Fine Chemicals
80 cm diameter SMB columns

MCSGP= Multicolumn Countercurrent Solvent Gradient Purification Process

- Similar background as at SMB, but applicable for more than 2 products
- Solvent gradient is introduced additionally to the concept of switching the columns



6-column continuous MCSGP process for the three-fraction solvent gradient purifications. H: strongy adsorbing impurities, P: target protein, F: feed, L: weakly adsorbing impurities, I: very weakly adsorbing impurities.

MCSGP= Multicolumn Countercurrent Solvent Gradient Purification Process

- Lower solvent consumption than in batch operation (instead of 80 l/g just 10 l/g for IgG for example)
- Fully automated

ChromaCon Co.

Membrane techniques

Introduction

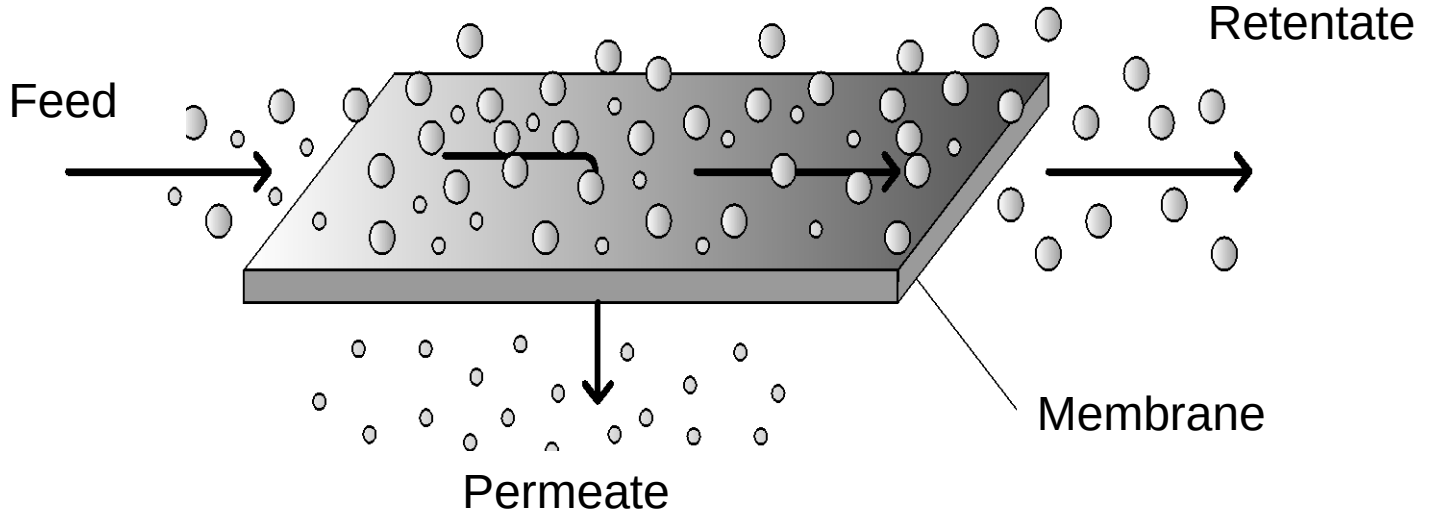
Separation technologies studied earlier are based on

- Affinity → extraction, absorption
- Density → centrifugal separation, sedimentation
- Vapor pressure, vapor-liquid equilibrium → distillation, rectification
- Size→ filtration

- Membrane separation technologies: all these gradients can be found. In general the driving force is the **chemical potential gradient**
- Membrane separation technologies: **non-equilibrium technology**
- Multi-disciplinar technology: wide variety of applications, and difficult to compare with the convenient technologies

Basic phenomena in membrane sperations

- Membrane (latin origin), meaning: skin or shell
- **Permselective** barrier between two liquids – semi-permeable layer
- Permeate: flow that transported through the membrane
- Retentate: all the components retained



Schemtaic drawing of membrane separation (cross-flow filtration)

The membrane

- Membrane: **semi-permeable layer**
- Selective layer, permeable only for given components (mainly water)
- Membranes from Nature: plant and animal origin (bladder of pig, filled with wine, and cooled in a well. First observation of diffusion.)
- First artificial membrane: in 1918,by **Richárd Zsigmondy**
- Applications in wide variety of industrial fields: food and beverage industry, health care, chemical indutry, wastewater treatment etc.

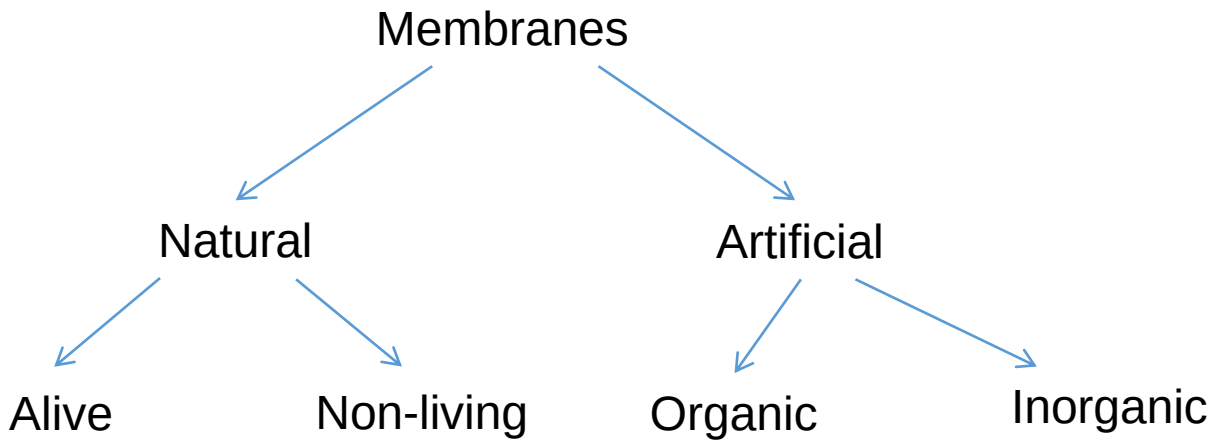
Classification of membranes

Fluid

Three liquid layered on each other and limited miscibility between the upper and middle phases, and lower and middle phases can be observed. (Liquid-liquid extraction)

Solid

Solid membranes can be classified as:

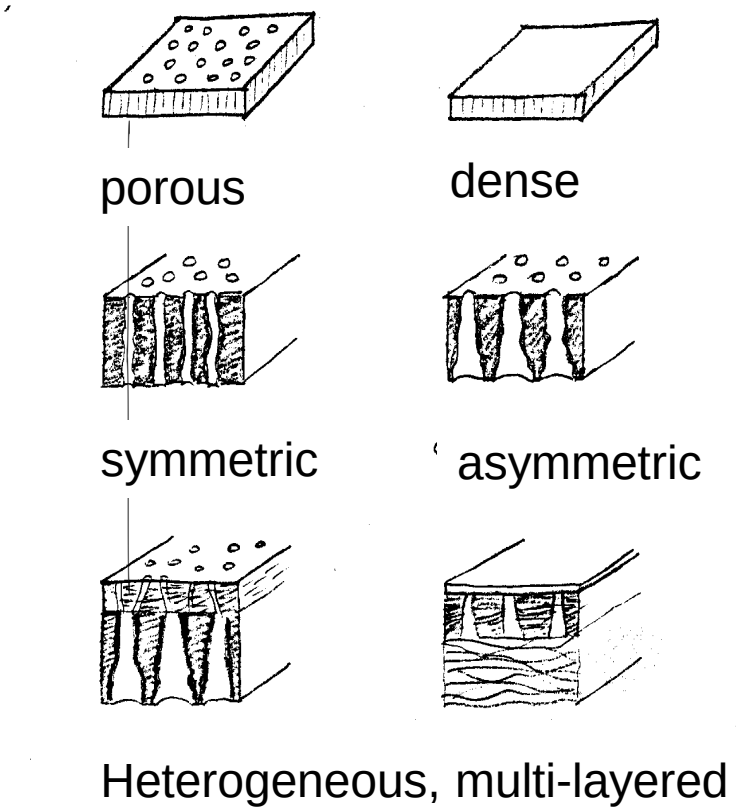


From now on, just the solid membranes are discussed.

Classification of solid membranes based on structure

- Porous

(separation is based on pore diameter)



- Non-porous, dense

(separation based on solubility and diffusivity)

- Porous

- Dense active layer (0.1-0.5 μm)
- Porous support layer (50-150 μm)

- Anisotropic = asymmetric and porous
- Isotropic = symmetric and porous
- Composite = porous and dense layers on each other from different material

Principles, definitions

- **Flux**: $J_V = \frac{1}{A} \cdot \frac{dV}{dt} \left[\frac{l}{m^2 \cdot h}\right]$ $J_m = \frac{1}{A} \cdot \frac{dm}{dt} \left[\frac{kg}{m^2 \cdot h}\right]$ or component *i*

Molar flux $J_{n,i} = \frac{1}{A} \cdot \frac{dn_i}{dt} \left[\frac{mol}{m^2 \cdot h}\right]$

- **Transmembrane pressure:** pressure difference between feed side (average pressure) and permeate side

$$TMP = \frac{p_f + p_r}{2} - p_p$$

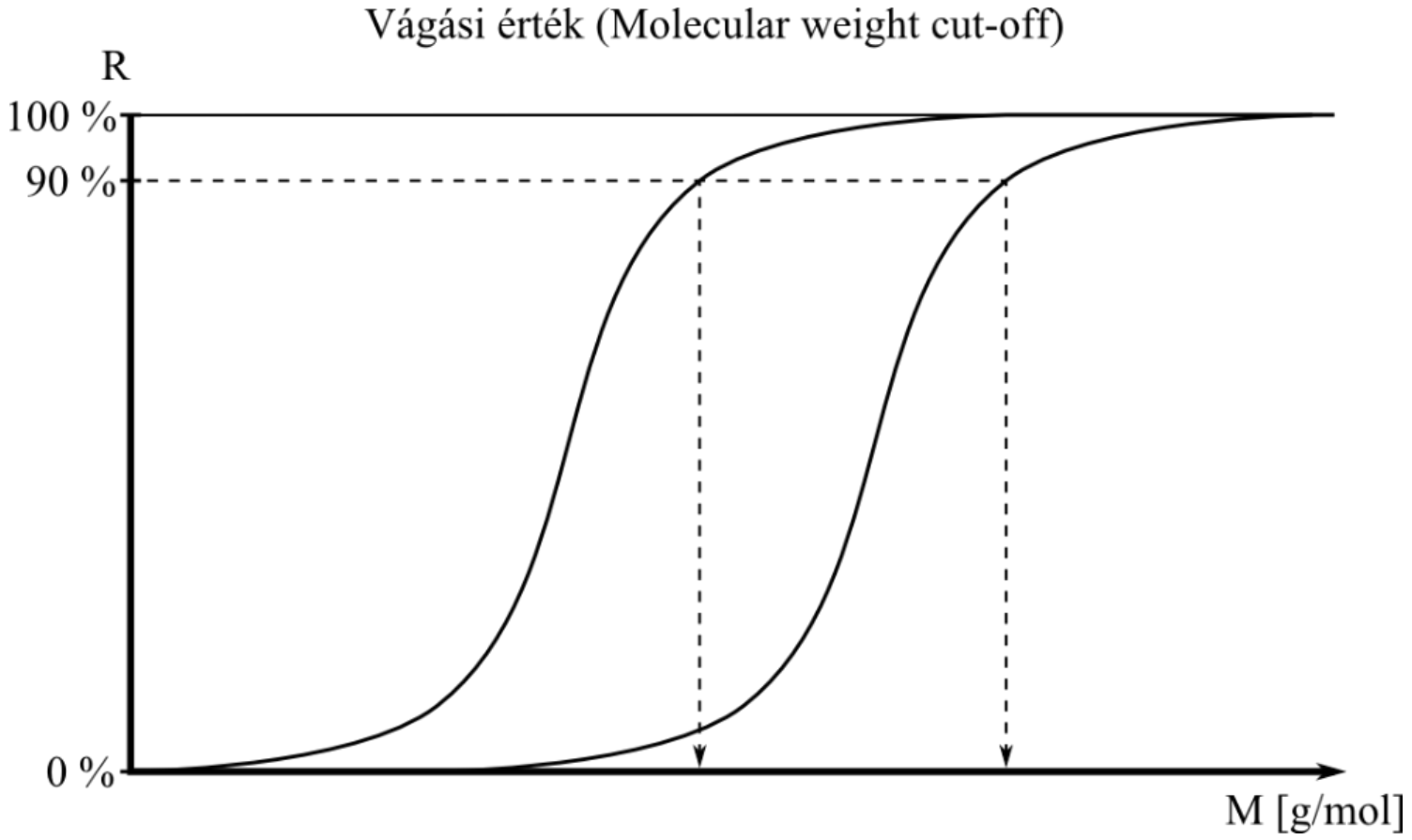
- **Rejection (or retention)**: how much percent of a given component remained in the retentate:

$$R = \frac{c_F - c_R}{c_F}(\cdot 100\%) = \left(1 - \frac{c_P}{c_F}\right) \cdot 100\%$$

where c_p the concentration of the permeate (mol/m^3), c_f the concentration of the feed (mol/m^3)

Principles, definitions

- **Molecular Weight Cut-Off:** is the molecular weight that is rejected by the membrane at 90%.



Principles, definitions

- **Volume reduction factor** $\mathrm{VRF} = \frac{V_f}{V_r}$ where V_f is the volume of feed (m^3), V_r is the volume of retentate in the same unit (m^3).
- **Pure water permeability**

$$J_V = \frac{L_p \cdot \Delta p}{\eta} = \frac{\Delta p}{\eta \cdot R_m}$$

where J_V is the volumetric flux of the solvent (m^3/m^2s^{-1}), L_p is hydraulic permeability of the membrane (m), η (Pa·s) the dynamic viscosity of the pure solvent, Δp (Pa) transmembrane pressure and R_m (1/m) is the membrane resistance

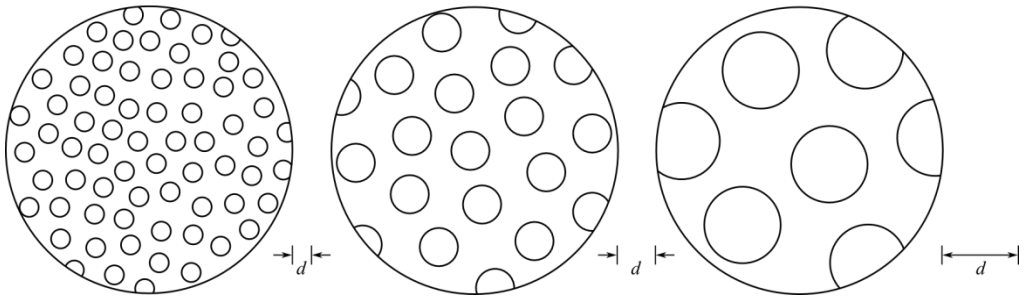
- **Limiting flux**: is a flux, that can not be exceeded in spite of the increase of transmembrane pressure

Principles, definitions

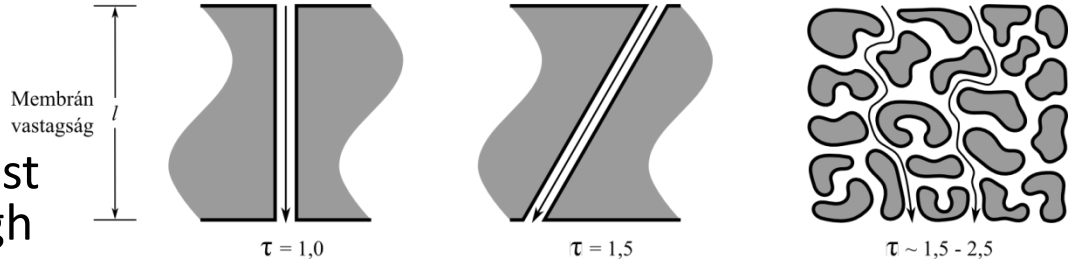
Characterization of porous membranes

- Porosity (ε)
  - = volume of sum of pores/ volume of membrane; average porosity: 0,3-0,7
  - = surface of sum of pores/ surface of membrane
- Tortuosity (τ)=average lenght of pores/thickness of membrane
- Pore-diameter (d):
  - MF: the diameter of the biggest molecule that can pass through the membrane
  - UF: average value of an interval

Membrane with same porosity and different pore-diameter



Cross sections of membranes with different tortuosity



[5]

Mass balance to continuous separation:

- *F=P+R*

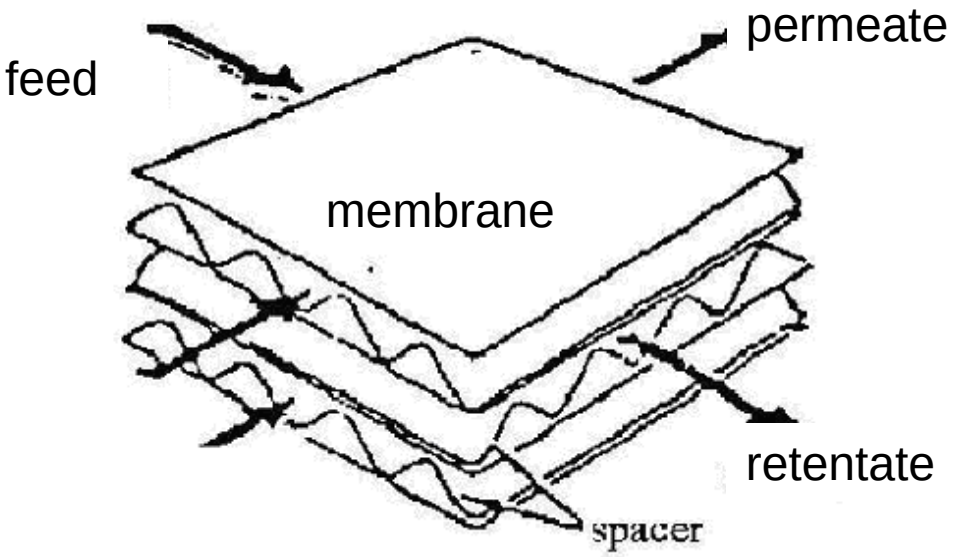
where F: feed flow [kg/s]
P: permeate flow [kg/s]
R: retentate flow [kg/s]

- (In case of batch separation mass can be used instead of mass flow.)
- **Component balance related to component *i*:**

$$F \cdot c_{F,i} = P \cdot c_{P,i} + R \cdot c_{R,i}$$

Where c is concentration in the same unit.

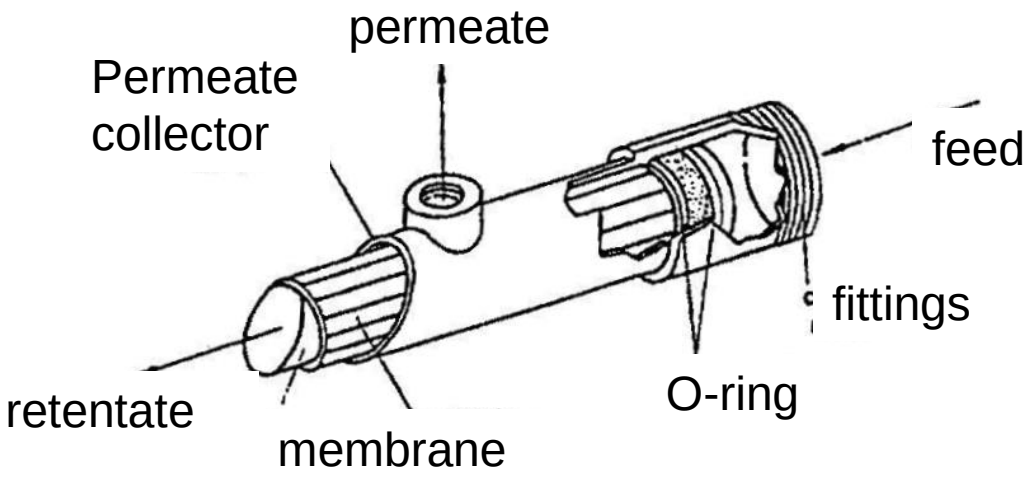
Membrane modules 1.



- **Plate and frame system**
  - Similar structure to plate heat exchanger
  - Membranes are separated by spacers
  - Feed and permeate flow in channels with a height of 0.5....1 mm
  - Velocity of the conductive flow can reach 2 m/s

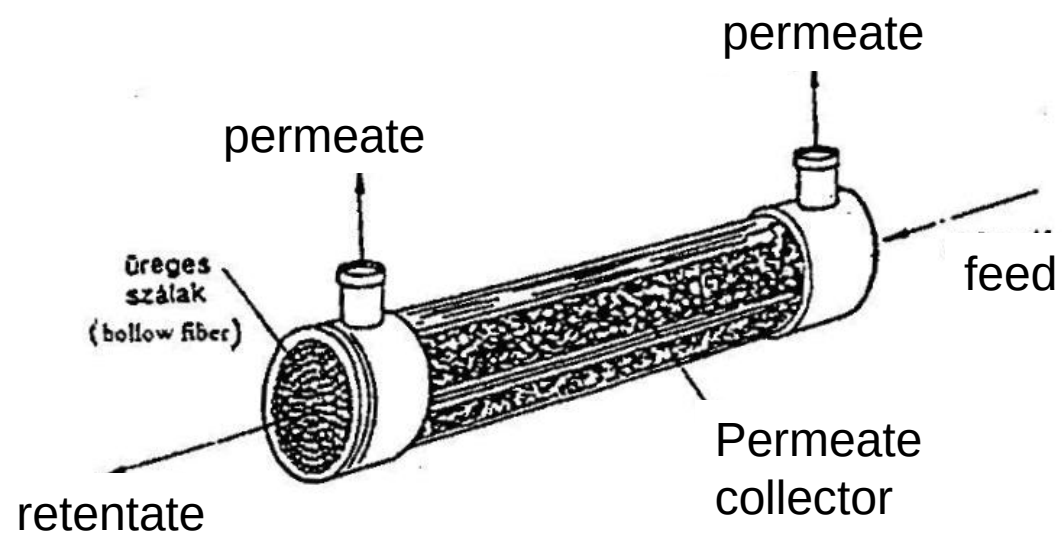
  Disadvantage: expensive, pumping cost is high, ratio of area to volume is low

Membrane modules 2.



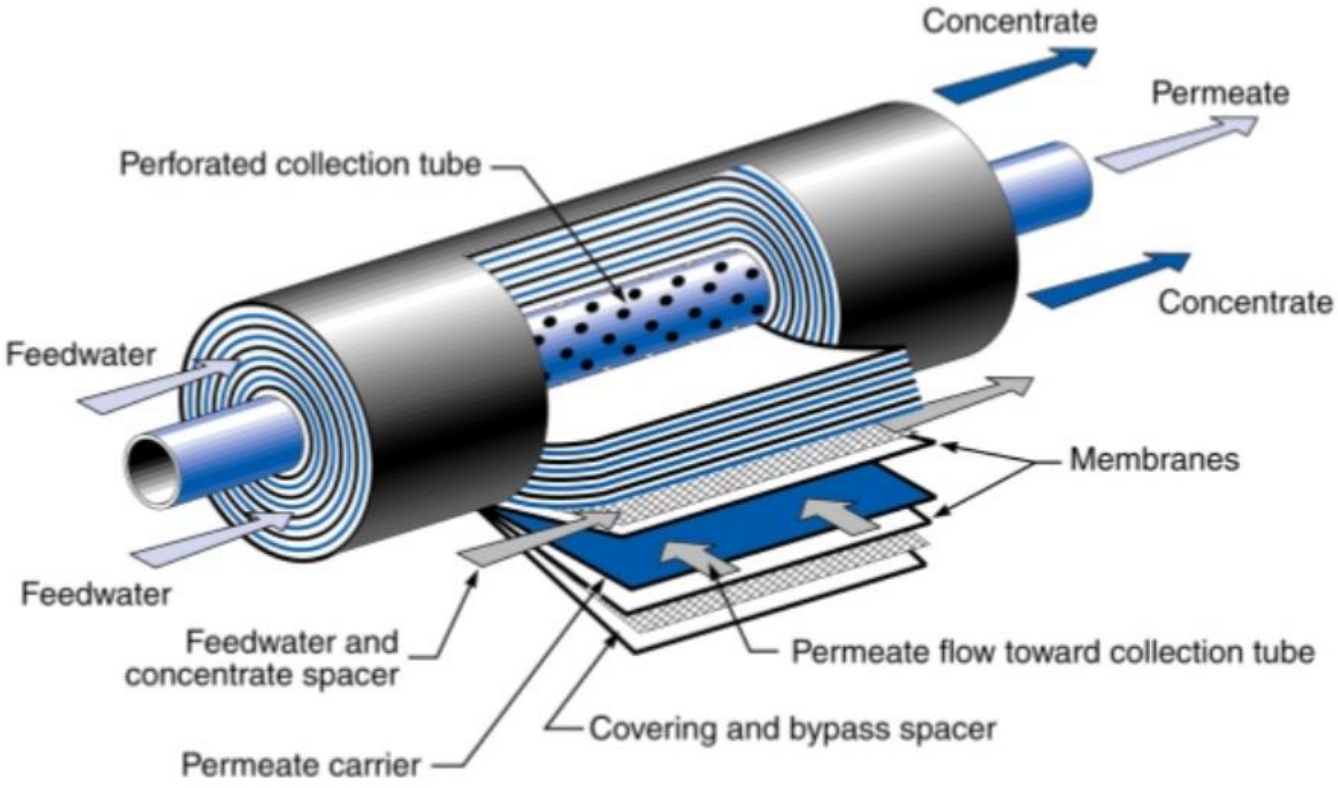
- Tubular system
  - Inside diameter of tubes 10...25 mm.
  - Turbulent flow, conductive velocity is in the range of 2...6 m/s
  - Ratio of area to volume is relatively low
  - Application: for the concnetration of suspensions

Membrane modules 3.



- Hollow-fiber system
  - Similar to shell-and-tube heat exchanger in structure
  - Inside diameter of tubes 0.5...4 mm.
  - Mechanical stability by the capillaries thickness
  - Thickness of fibers 120...180 μm.

Membrane modules 4.



- Spiral wound system
  - Structure: similar to a sandwich (membrane, spacer, permeate collector) are screwed or reeled around a perforated pipe
  - „Bag filter” reeled up
  - Ratio of area to volume is high

Expections to membranes

- In case of composite membranes: very thin active layer
- High permeability and selectivity
- Stable and long-life membrane modules
- Resistancy to mechanical and chemical stress
- Huge area in small volume
- No concnetration polarisatin or controllable
- Easy cleaning
- Cheap (when investing, or during service)

Membrane technologies advantages, disadvantages

- Switched to continuous
- Low energy cost
- Easily combined (hybrid processes)
- Moderate conditions
- Easy scale-up
- Variable
- No additional component is required to the separation

- Fouling
- Concentration-polarisation
- Short life-time (max. 3yrs)
- Selectivity←→flux
- Linear scale up (doubled sized, double investment cost)

Intensively growing field

Classification of membrane technologies 1.

MEMBRANE FILTRATION

Driving force: p, T, c, ϕ, all together **μ difference**

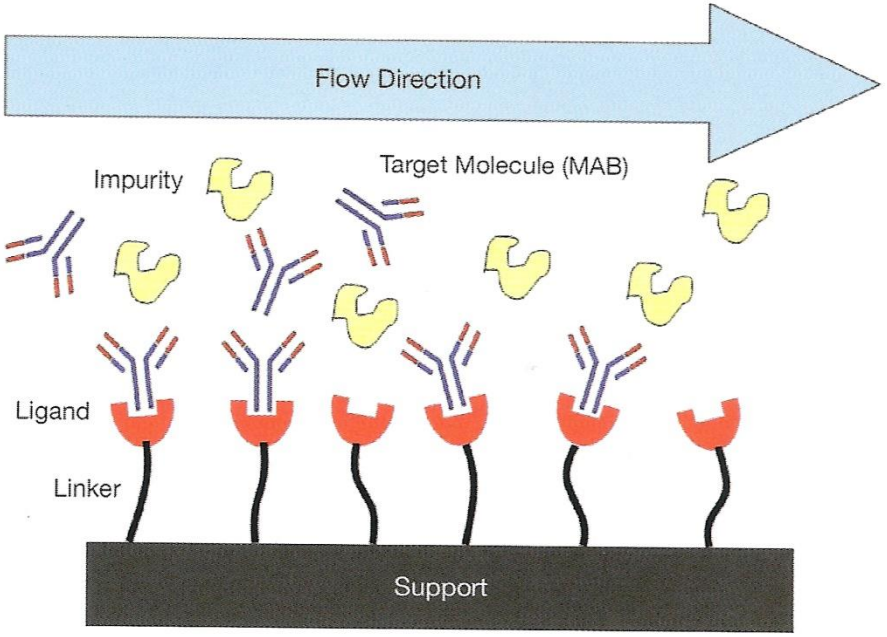
Processes	Type of membrane, pore size (representative values)	Main riving force *(Pressure and size difference)*	Applications, retained particles (size) (representative values)
Microfiltration MF	microporous **0.1-1μm =** **=** 100-1000nm	Transmembrane pressure difference 10-500 kPa	Starch, pigments bacteria, funghi, (100 000-10^6 Da)
Ultrafiltration UF	microporous **0.05-0.5 μm = =** 5-500nm	Transmembrane pressure difference 0.1-1 MPa	Macromolecules, colloids, virus, proteins (1 000-100 000 Da)
Nanofiltration NF	nanoporous **0.001-0.01μm = =** 1-10 nm	Transmembrane pressure difference 0.6-4 MPa	Sugars, di-valent ions (100-1 000 Da)
Reverse osmoses RO	Dense: no pores	Transmembrane pressure difference 2-10 MPa	Monovalent ions, drinking water from seawater) (10-100 Da)

Classification of membrane technologies 2.

Processes	Type of membrane, pore size (representative values)	Driving force	Applications
Dialysis	microporous **0.01-0.1 μm**	concentration gradient	Separation of salts and small-sized molecules from macromolecules
Electrodialysis ED	cation- and anion exchange membrane	Electric potential gradient	Desalination
Steam permeation GP	homogenous polymer membrane	Vapour pressure- and concentration gradient	Separation of steam components
Gas separation GS	homogenous polymer membrane	pressure- and concentration gradient	Separation of gas mixtures
Pervaporation PV	homogenous polymer membrane	Vapour pressure- and temperature gradient	Separation of azeotropic mixtures
Membrane distillation MD	hydrofobic porous membrane	Vapour pressure gradient	Desailnation
Processes based on liquid membranes	liquid membrane	Concentration gradient	Selective separation of metal ions

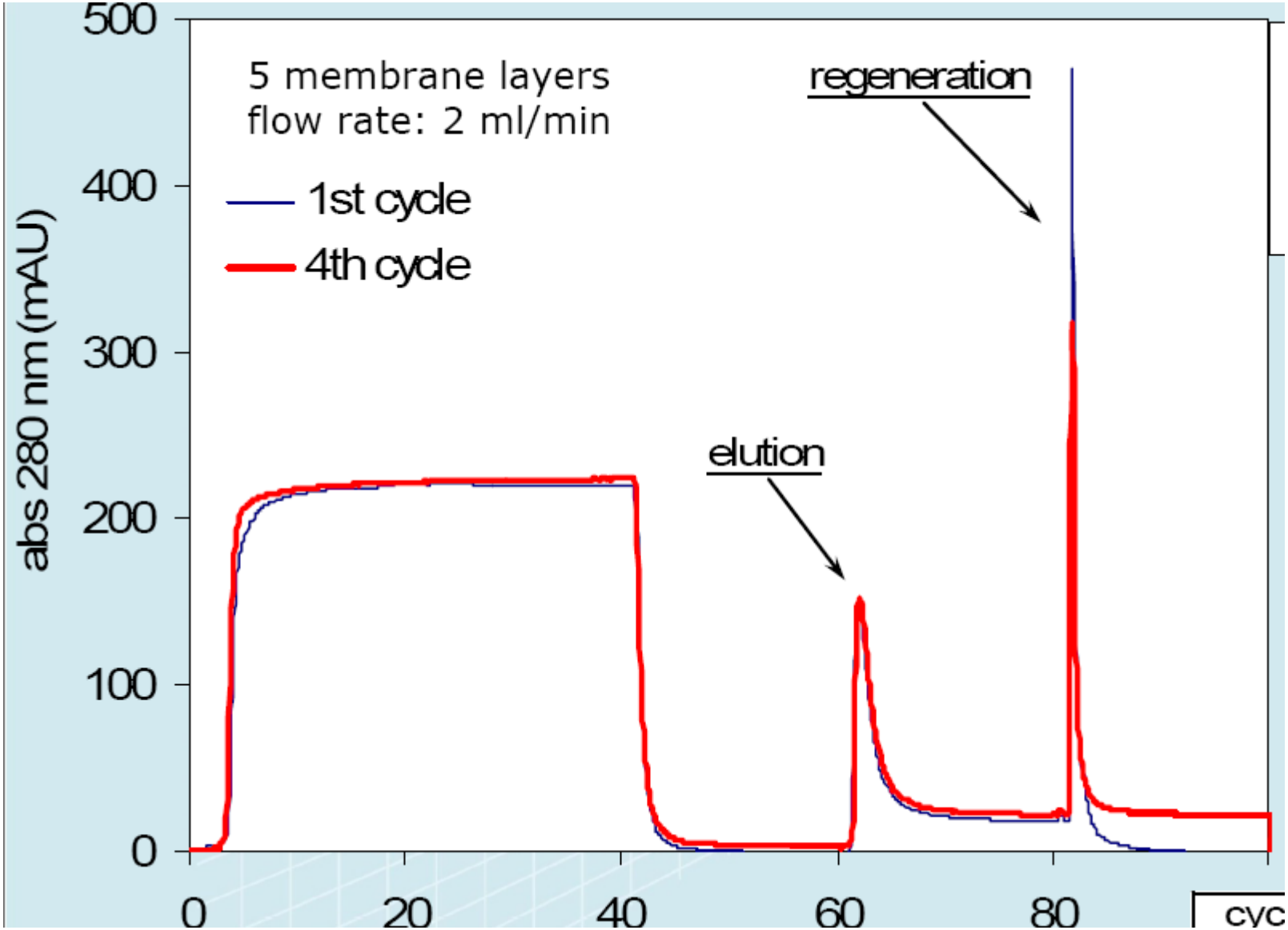
Affinity membranes

- Differ from the previous membrane techniques, it is an adsorption process, like the batch chromatography



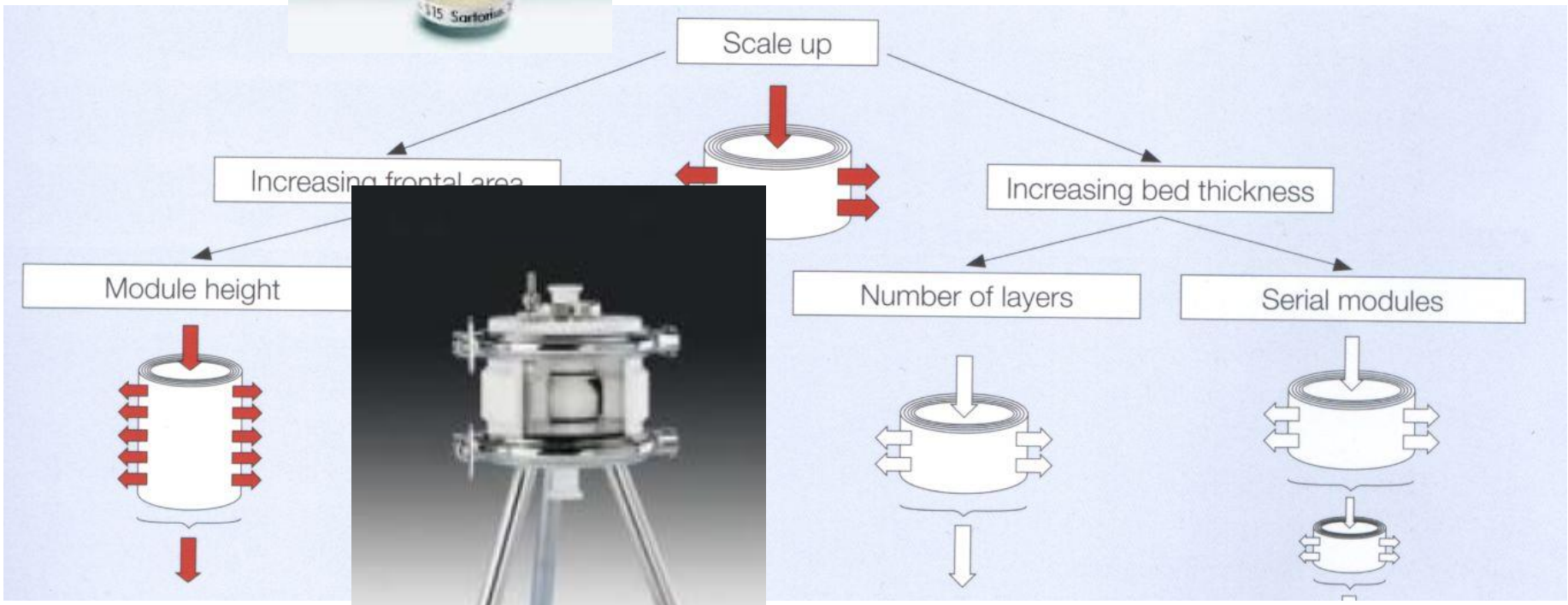
II. Affinitás membránok

- Különbözik az ismert membrán műveletektől, működési elve hasonló az affinitás kromatográfiáéhoz
- Az elválasztás ciklikus üzemben történik
- Előnye a „tetszőlegesen” növelhető felület több membránréteg és párhuzamosan kapcsolt modulok segítségével



Equilibration buffer: pH 7.4,
Eluation buffer: 0.1 M acetic acid pH 2.7, regeneration: 0.6 M NaOH

Affinity membranes – scale up vs. number up



Don’t forget

- Start to work on your own task: contact your supervisor!
- Next week: biomass valorization lecture

Thank you for your attention!