Ionska kromatografija

Definicija

- **Ion-chromatography** is a process that allows the separation of ions and polar molecules based on the charge properties of the molecules.

Tipi ionske kromatografije

- Ionska izmenjevalna kromatografija
- Ionska izključitvena kromatografija
- Ionska kromatografija ionskih parov

Ion Exchange Chromatography (IEC)

Ion-Exchange Chromatography

Ion-exchange chromatography is used for analysis of mixtures of inorganic salts, some organometallics, amino acids, proteins, and some other biological molecules.

Many of the above mentioned molecules have little or no UV absorptivity. These molecules can be detected using an electrical conductivity which is well suited for ion-exchange chromatography.

Mass spectrometer detection may require a mobile phase that is completely volatile. This is possible with ion-exchange buffers, but generally ion-pair reagents are not volatile enough to be used with a mass spectrometer.

Ionska izmenjevalna kromatografija

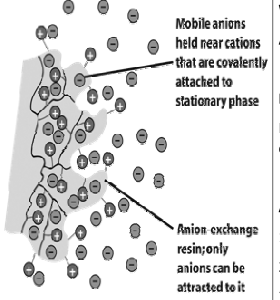
- Uporablja se za separacijo skoraj vseh nabitih molekul od majhnih anorganskih anionov ali kationov do večjih nabitih moleul kot so proteini, manjši nukleotidi in amino kisline.
- Uporablja se za čiščenje proteinov, analizo vode.

Principle

- Ion exchange chromatography retains analyte molecules based on ionic interactions.
- The stationary phase surface displays ionic functional groups (R-X) that interact with analyte ions of opposite charge.
- This type of chromatography is further subdivided into:

1. **cation exchange chromatography**
2. **anion exchange chromatography**.

Ion-Exchange Chromatography



What is exchanged?

Anion for anion and cation for cation

How are the ions exchanged?

Ions with higher affinity displace ions of lower affinity to the stat. phase

Affinity increases as:

1. Charge increases
2. Size of (solvatized) ions decreases
3. Polarizability increases

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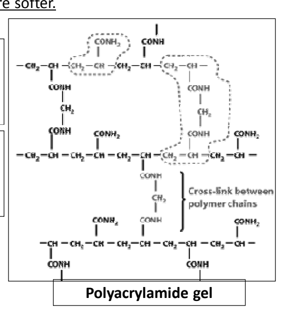
Stationary Phases in Ion-Exchange Chromatography

"**Resins**" or "**Gels**" that **carry** the ion-exchanger surface. Both are amorphous particles of organic material, but gels are softer.

Polystyrene resins:
made by co-polymerization of styrene and vinyl-bearing molecules

Cellulose and dextran gels:
Dextran, cross-linked to glycerin, is called (**Sephadex™**)



Polyacrylamide gel

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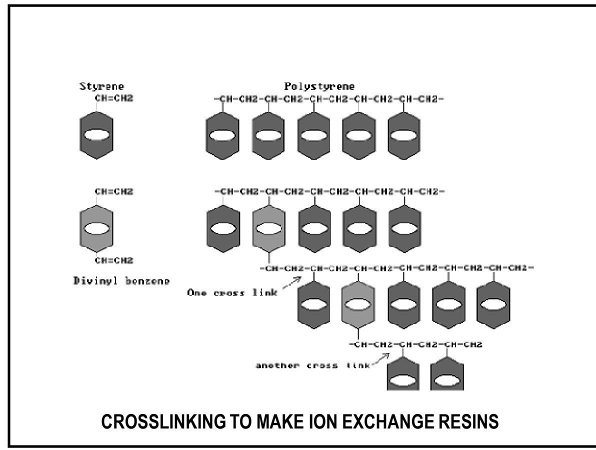
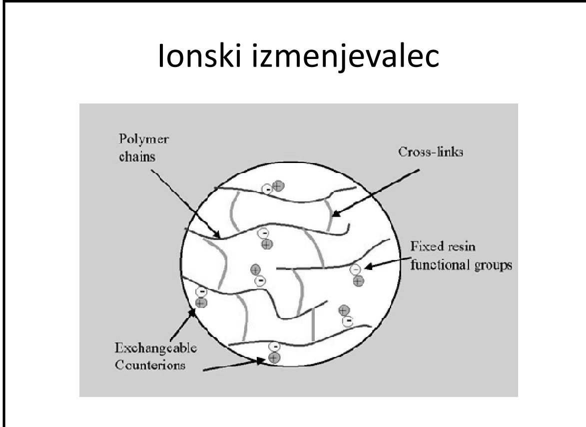
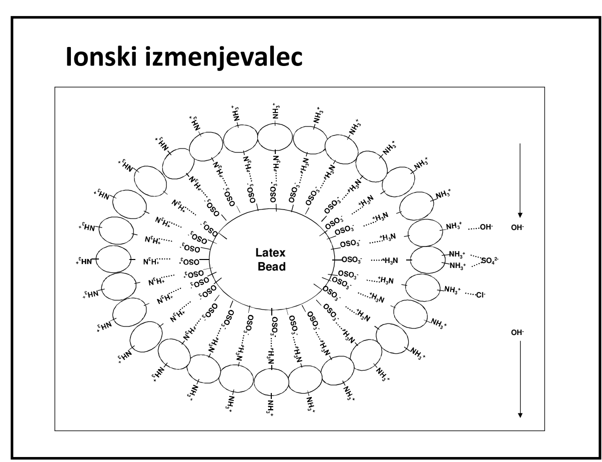


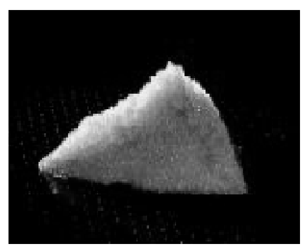
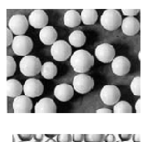
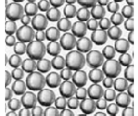
CROSSLINKING TO MAKE ION EXCHANGE RESINS

Ionski izmenjevalec



Ionski izmenjevalec



RESIN PARTICLE AND BEADS

Stationary Phases in Ion-Exchange Chromatography

- The benzene ring of the support can be modified to produce cation exchange resin containing sulfonate group (SO_3^-) or an anion exchange resin containing ammonium groups (NR_3^+). **Ion exchangers are classified to strongly or weakly acidic or basic.** SO_3^- is strong because it remains ionized even in strong acidic solutions. CO_2^- is weak because it becomes protonated at pH 4 and thus looses its ion exchange capacity. Strongly basic quaternary ammonium salts remain cationic at all pH values. Weakly basic tertiary anion exchangers are deprotonated in moderately basic solutions and lose their ability to bind anions.
- **The resin becomes more rigid and porous as cross linking increases**. Lightly cross linked resins permit rapid equilibration of solute between the inside and outside of the particle, however they swell in water which decreases the density of ion exchange sites and selectivity of the resin to different ions.
- **Cellulose and dextran which are polymers of glucose possess larger pore size and lower charge densities than polystyrene resins**. They are well suited to large molecules like proteins which may be irreversibly bound to resins because they are highly charged.

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Ion exchangers – Functional groups

Anion exchanger

- Aminoethyl (AE-)
- Diethylaminoethyl (DEAE-)
- Quaternary aminoethyl (QAE-)

Cation exchanger

- Carboxymethyl (CM-)
- Phospho
- Sulphopropyl (SP-)

Examples of Ion-Exchange Resins

Resin type	Chemical constitution	Usual form as purchased	Rohm & Haas	Dow Chemical	Selectivity	Thermal stability
Strongly acidic cation exchanger	Sulfonic acid groups attached to styrene and divinylbenzene copolymer	$Aryl—SO_3^-H^+$	Amberlite IR-120	Dowex 50W	$Ag^+ > Rb^+ > Cs^+ > K^+ > NH_4^+ > Na^+ > H^+ > Li^+$ $Zn^{2+} > Cu^{2+} > Ni^{2+} > Co^{2+}$	Good up to 150°C
Weakly acidic cation exchanger	Carboxylic acid groups attached to acrylic and divinylbenzene copolymer	$R—COO^-Na^+$	Amberlite IRC-50	—	$H^+ >> Ag^+ > K^+ > Na^+ > Li^+$ $H^+ >> Fe^{2+} > Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$	Good up to 100°C
Strongly basic anion exchanger	*Quaternary* ammonium groups attached to styrene and divinylbenzene copolymer	$Aryl—CH_2N(CH_3)_3^+Cl^-$	Amberlite IRA-400	Dowex 1	$I^- >$ phenolate$^- > HSO_4^- > ClO_3^- > NO_3^- > Br^- > CN^- > HSO_3^- > NO_2^- > Cl^- > CHO_3^- > IO_3^- > HCOO^- >$ acetate$^- > OH^- > F^-$	OH^- form fair up to 50°C; Cl^- and other forms good up to 150°C
Weakly basic anion exchanger available;	Polyalkylamine groups attached to styrene and divinylbenzene copolymer	$Aryl—NH(R)_2^+Cl^-$	Amberlite IR-45	Dowex 3	$Aryl—SO_3H >$ citric $> CrO_3 > H_2SO_4 >$ tartaric $>$ oxalic $> H_3PO_4 > H_3AsO_4 > HNO_3 > HI > HBr > HCl > HF > HCO_2H > CH_3CO_2H > H_2CO_3$	Extensive information not tentatively limited to 65°C

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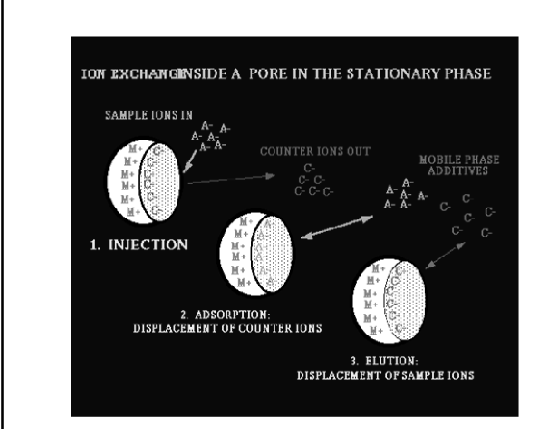

Cation exchange chromatography

- Cation exchange chromatography retains positively charged <u>cations</u> because the stationary phase displays a negatively charged functional group

$$R\text{-}X^{-}C^{+} + M\,B \rightleftharpoons R\text{-}X^{-}M^{+} + C^{+} + B^{-}$$

Cation exchange chromatography

---positively charged molecules are attracted to a negatively charged solid support. Commonly used cation exchange resins are S-resin, sulfate derivatives; and CM resins, carboxylate derived ions

S-cation exchanger   CM-cation exchanger

Anion exchange chromatography

- Anion exchange chromatography retains anions using positively charged functional group:

$$R\text{-}X^{+}A^{-} + M^{+}B^{-} \rightleftharpoons R\text{-}X^{+}B^{-} + M^{+} + A^{-}$$

Anion exchange chromatography

---negatively charged molecules is attracted to a positively charged solid support. Commonly used anion exchange resins are Q-resin, a Quaternary amine; and DEAE resin, DiEthylAminoEthane

Q-anion exchanger   DEAE-anion exchanger

Elution in Ion exchange chromatography

Gradient elution is a powerful technique in ion chromatography.

1. **Concentration gradients:**

Eluents used in **anion** exchange contain an **anionic** compound in **high** concentration which competes with the analyte (an anion of course) for sites on the resin. **Gradient elution is accomplished by increasing the concentration of the eluent anion during the run.**

2. **pH gradients:**

A fixed concentration of a weak acid (eluent A) is mixed with an increasing concentration of a strong base as NaOH (eluent B).

pH gradients are a type of concentration gradients as the purpose of increasing the pH during an anion exchange run is **to increase the concentration of the dissociated form of the weak acid eluent.**

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Ion-Exchange Chromatography

Different ions are retained differently on the exchange resin.

The relative strength of anions from weakest to strongest is:

$F^- < OH^- <$ acetate$^- < Cl^- < SCN^- < Br^- < CrO_4^- < NO_3^- < I^- <$ oxalate$^{2-} < SO_2^{2-} <$ citrate^{3-}

The relative strength of cations from weakest to strongest is:

$Li^+ < H^+ < Na^+ < NH_4^+ < K^+ < Ag^+ < Mg^{2+} < Zn^{2+} < Cu^{2+} < Ni^{2+} < Ca^{2+} < Ba^{2+}$

Ion-Exchange Chromatography

The Eluent Suppressor Column

Electrical conductivity detectors should be well suited for the detection of inorganic ions. However, in ion-exchange chromatography, mobile phases with high electrolyte concentration are needed to elute many analytes, and the conductivity of the mobile phase interferes with the detection of the analyte ions.

The suppressor column is added to the chromatography system immediately following the ion exchange column. The suppressor works by converting the ionic mobile phase to a molecular species that does not conduct.

Ion-Exchange Chromatography

The Eluent Suppressor Column

An example of how this works can be seen in the following equation using hydrochloric acid as the mobile phase buffer and the hydroxal ion on the exchange resin.

$$H^+(aq) + Cl^-(aq) + Resin^+OH^-(s) \rightarrow Resin^+Cl^-(s) + H_2O$$

Note: For cation exchange chromatography, an anion exchange resin is used in the suppressor column, otherwise the analytes would be affected by the column.

The elimination of the interference from the mobile phase significantly increases the sensitivity of conductivity detectors.

Ion-Exchange Chromatography

An example of an additional advantage of the suppressor columns is given below:

Separation of Anions such as Ca^+ Cl^-

In the suppressor column, this would be converted to H^+Cl^- Which has a higher conductivity-
increasing sensitivity

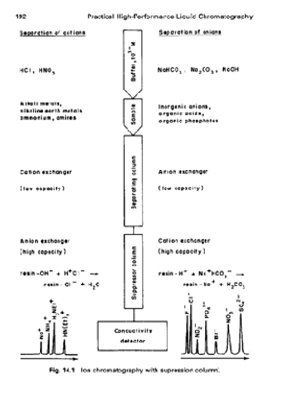
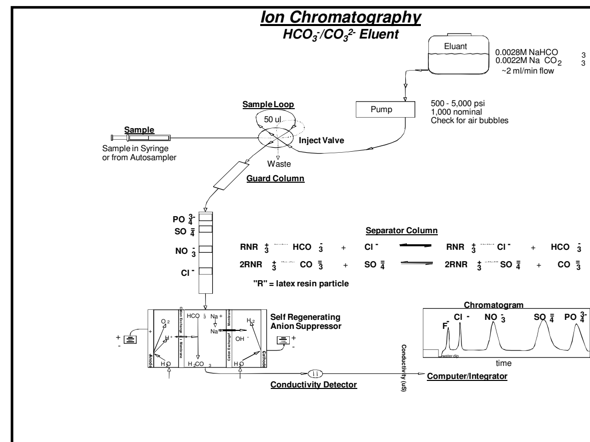
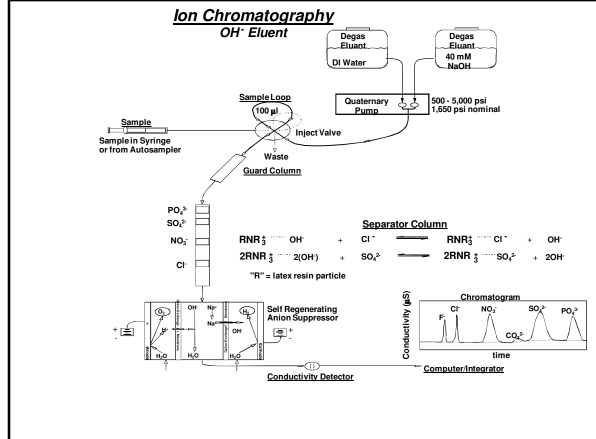


Fig. 14.1 Ion chromatography with suppression column.

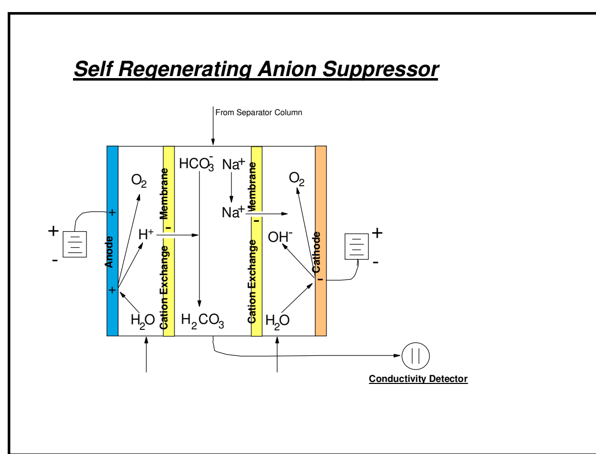


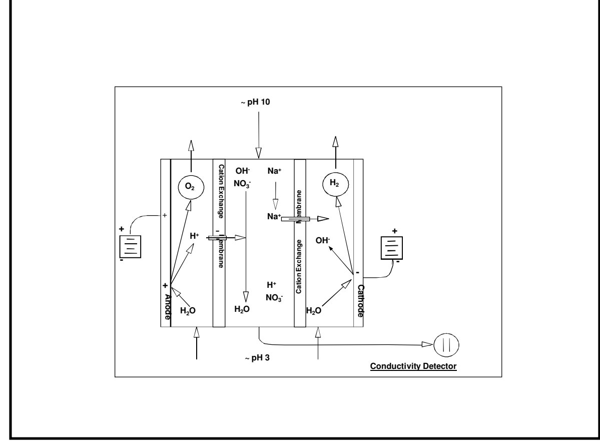


Detektorji

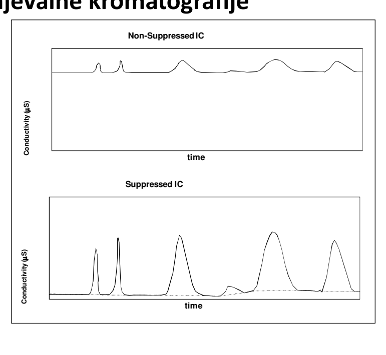
- Električna prevodnost
- UV/VIS spektrometer
- Elektrokemčni

Detektor na električno prevodnost ponavadi uporabljamo v kombinaciji s supresorsko kolono.



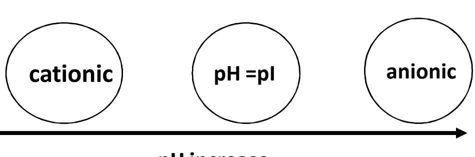


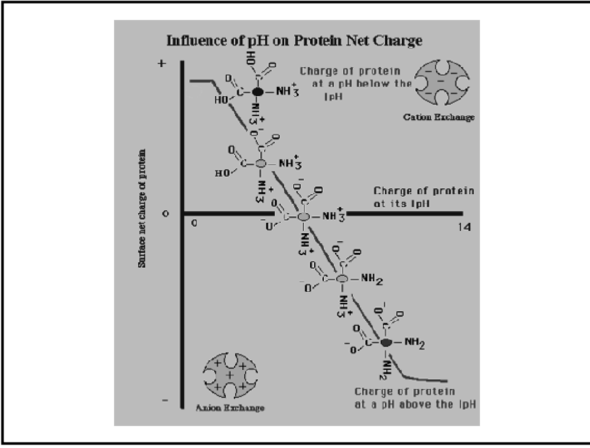
Primerjava supresirane in nesupresirane ionske izmenjevalne kromatografije



Effect of pH in the separation of proteins

- Proteins are charged molecules. At specific pH, it can exist in **anionic** (-), **cationic** (+) or **zwitterion** (no net charge) stage.



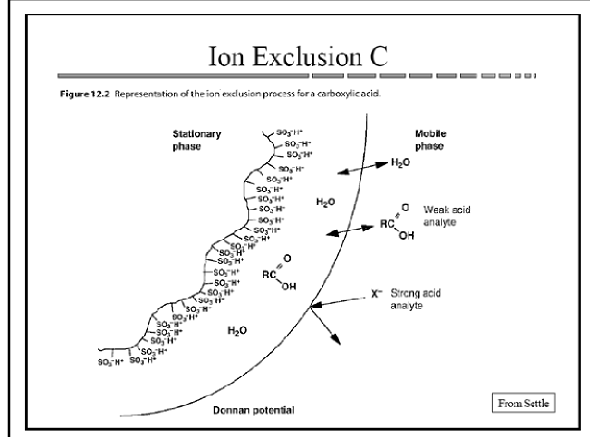


Uporaba ionske izmenjevalne kromatografije

- Določevanje anorganskih anionov
- Določevanje anorganskih kationov
- Določevanje biogenih aminov
- Določevanje organskih baz
- Določevanje sladkorjev

……

Ion Exclusion Chromatography



Ion Exclusion C. II

- Basis of separation
  - Electrical potential btw stationary phase with high density of ion exchange sites
  - And dilute mobile phase
- Donnan potential
  - Diffusion equalizes H^+ concentration, stationary phase has net negative charge
  - Neutral molecules can enter stationary phase
  - Charged molecules are repelled
  - Analytes are separated by their extent of ionization
- Used especially for carboxylic acids

Ion Pair Chromatography (IPC)

Ion-Pair Chromatography

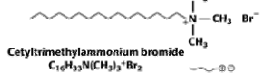
Stationary phase: common RP (no ion-exchange!)

Mobile phase: contains surfactant that is "loosely attached" to stationary phase and so gives ion-exchange surface.

Separation: counter-ions passing the column are attracted by surfactant and are exchanged.

Separation mechanism: partition + ion-exchange

Example: to separate a mixture of cations, an anionic surfactant is added to the mobile phase. The surfactant lodges in the stationary phase and effectively transforms it into an anion exchanger. When analyte cations pass through the column, they can associate with the stationary phase by electrostatic attraction to the surfactant anion.



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Ion-Pair Chromatography

In addition to the aqueous buffer and an organic solvent that is typical for reversed-phase, the mobile phase contains a counter ion that combines with the analyte ions to form an ion pair. This forms a neutral species that can be separated in the normal way by the reversed phase packing.

Advantages:

Better chromatography of large ions (vs. ion exchange).

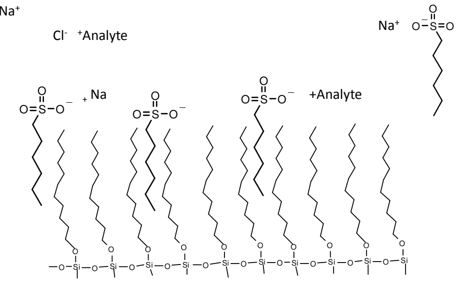
Can separate neutral and charged ions at the same time.

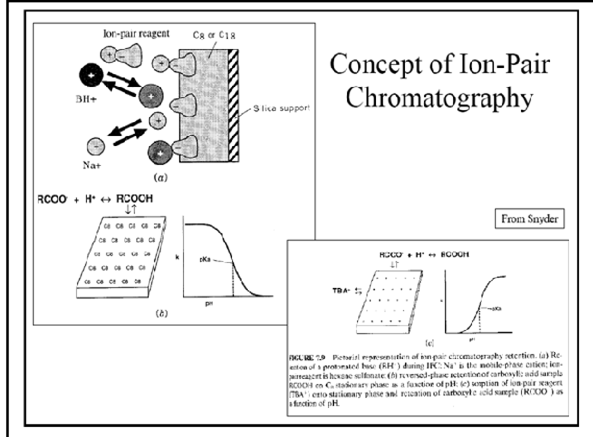
Analytes with very high or very low pK_a values which are resistant to separation based on pH adjustment can be separated.

Ion-Pair Chromatography

Illustration of Silica Support with C18 Bonded Phase

With the Addition of an Ion-Pair Reagent





Ion-Pair Chromatography

Depending on the concentration of the ion-pair reagent, chromatography can vary from mostly reverse phase (low concentration) to mostly ion-exchange.

Ion-pair reagents can also vary based on the hydrophobicity of the reagent, ie. C6-sulfonic acid, C8-sulfonic acid, C10-sulfonic acid. The longer the alkyl chain, the more hydrophobic the reagent is and the more strongly it is retained by the column.

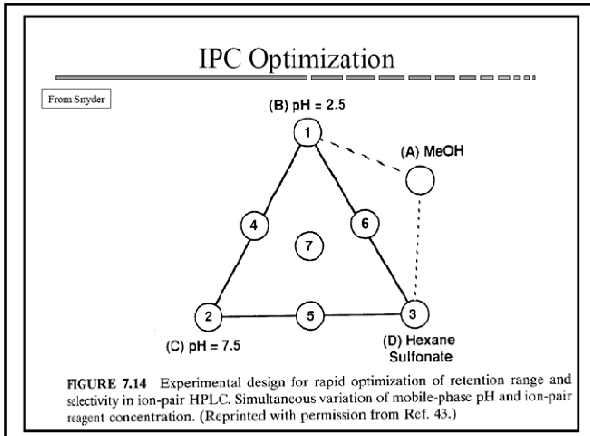
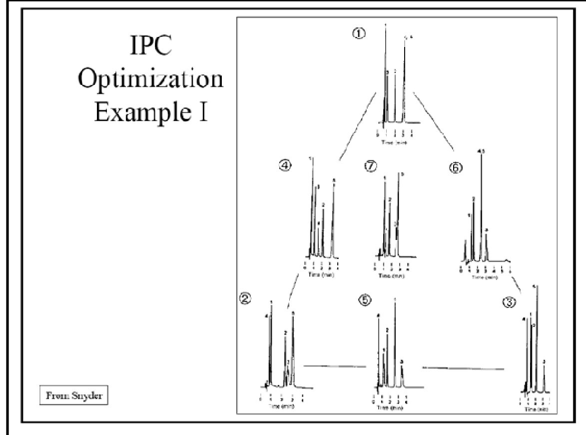


FIGURE 7.14 Experimental design for rapid optimization of retention range and selectivity in ion-pair HPLC. Simultaneous variation of mobile-phase pH and ion-pair reagent concentration. (Reprinted with permission from Ref. 43.)



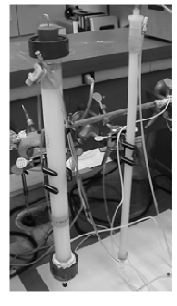
Ion-Pair Chromatography

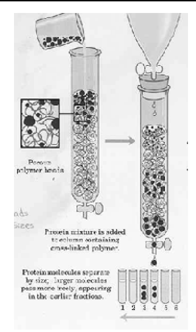
Because of the additional complexity, method development is much more difficult than under simple reversed-phase.

Solvent type, solvent strength, buffer type, buffer concentration, temperature, the type of ion-pair reagent, and reagent concentration are important factors in method development.

Interactions between these factors can also be complex. For example, the effect of temperature becomes a very critical factor and columns are almost always temperature controlled with ion-pair chromatography.

Size Exclusion Chromatography





SIZE EXCLUSION CHROMATOGRAPHY

Background

- Size exclusion chromatography is used primarily for analytical assays and semi-preparative purifications
- It is not commonly used for process scale work due to the low capacity of the size exclusion mode
- Tosoh Corporation (Toyo Soda) introduced its first SEC columns in 1973 and has continuously added new column types and instruments since then.

The technique can be applied in two distinct ways:

Group separations:
components of a sample are separated into two major groups according to size range

High resolution fractionation of biomolecules:
Components of a sample are separated according to differences in their molecular size

Introduction

Size-exclusion chromatography (SEC) is a chromatographic method in which molecules in solution are separated by their size, and in some cases molecular weight

It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers

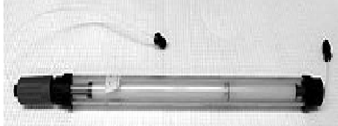


Fig 1. A size exclusion column

Theory background

gel filtration medium is packed into a column to form a packed bed

The medium is a porous matrix in the form of spherical particles that have been chosen for their chemical and physical stability, and inertness

The liquid inside the pores is the stationary phase and this liquid is in equilibrium with the liquid outside the particles called to as the mobile phase

Group separation

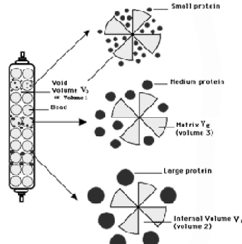
group separation mode to remove small molecules from a group of larger molecules and as a fast, simple solution for buffer exchange

Small molecules such as excess salt (desalting) or free labels are easily separated

is often used in protein purification schemes for desalting and buffer exchange

Molecules that are too large to enter into the pores of the beads are unretained by the column and are not separated.

Molecules that are very small in relation to the pore size all behave similarly and these small molecules are also not separated.



Medium sized molecules are separated based on how far they penetrate into the gel beads.

High resolution fractionation

- **Gel filtration is used in fractionation mode, uses porous particles to separate multiple components in a sample on the basis of differences in their size**
  - **Molecules that are smaller than the pore size can enter the particles and therefore have a longer path and longer transit time than larger molecules that cannot enter the particles**

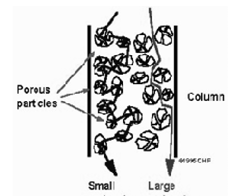


Fig 3. Schematic of a size-exclusion chromatography column

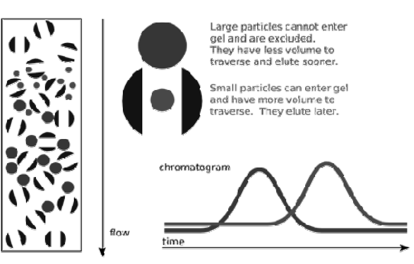


Fig 4. Schematic of a size-exclusion chromatography column

One of the earliest materials developed for size exclusion is still in use and is called Sephadex. Sephadex is made from dextran cross-linked (polymerized) with epichlorohydrin.

Structure of Sephadex

The gels can be manufactured with different amounts of cross-linking which will produce gels of different porosities and different ranges of molecular size separation.

3.2. Chromatographic Parameters in SEC

$$V_t = V_g + V_i + V_0$$

- V_t: column total volume
- V_g: volume occupied by solid matrix
- V_i: volume of the solvent held in the pores
- V_0: free volume outside particles

V_0: If no mixing or diffusion occurred, this is the volume of the solvent needed to transport components through the whole column that are too large to enter pores.

$V_0 + V_i$: Volume of the solvent needed to transport components that are small enough to intrude into pores easily

$$V_e = V_0 + KV_i$$

V_e is the **elution volume** of a substance, i.e. the volume of mobile phase that is required for elution of this compound. **At constant flow rate, it is proportional to retention time.**

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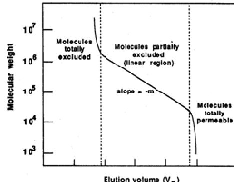
Large molecules elute after a volume of mobile phase equal to the void volume V_o has passed through the column. Small molecules will elute with a volume of mobile phase equal to the void volume and pore volume ($V_o + V_i$). Medium size molecules will elute between V_o and ($V_o + V_i$).



What is the "Size" do we talk about?

“molecular size” =“hydrodynamic volume”

i.e. the volume created by the **movement** of the molecule in **liquid** (by adhesion of solvent, so depending on the surface area):

orig. volume → *hydrodynamic volume* | *orig. volume* → *hydrodynamic volume*

Proteins tend to be **<u>globular</u> molecules**, while **DNA or polysaccharides** are more likely **<u>linear</u>**, the latter have a larger hydrodynamic volume and thus are <u>eluted earlier</u>.

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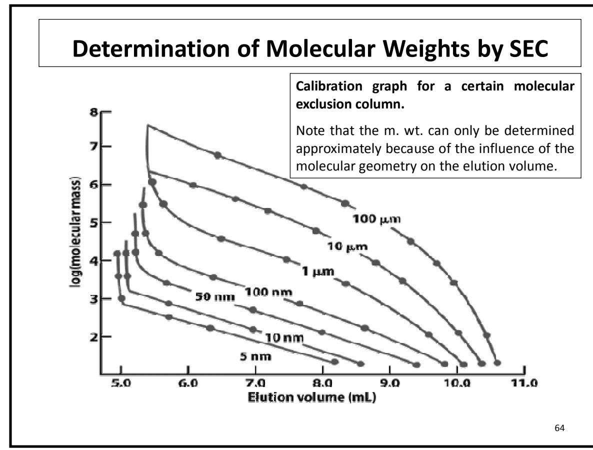
Proteins are often separated using size exclusion.

Molecular Shape has an effect on the relationship between the molecular weight and retention.

Fig. 6.5 — Relationship between solute geometry and calibration curve slope. Reproduced with permission from Ref. 6; copyright (1979) John Wiley & Sons, Inc.



Interactions between the stationary phase and the analytes should be avoided.

Cationic adsorption effects can be reduced by the addition of mobile phases with an ionic strength of 0.05-0.1 M. Low pH (around 3) will also help reduce some stationary phase interactions. Methanol can be added to the mobile phase to reduce hydrophobic retention, and ethylene glycol may help reduce the adsorption of proteins.

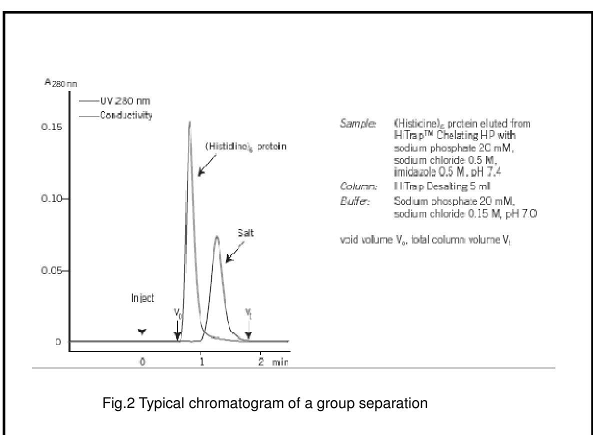


Fig.2 Typical chromatogram of a group separation

Mechanism of action

- samples that contain few components or partially purified by other chromatography techniques will give the best result
- Single buffer system, packed bed(chemically and physically stable and inert), pore size in stationary phase separates proteins according to their molecular weight
- Elution of Proteins: one buffer used for both loading and elution of the sample

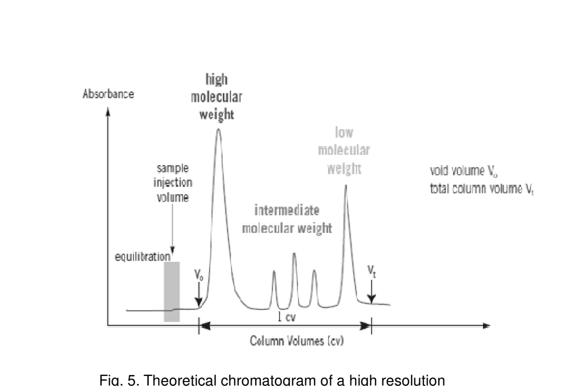


Fig. 5. Theoretical chromatogram of a high resolution fractionation (UV absorbance)

- Molecules larger than the pore size can not enter the pores and elute together as the first peak in the chromatogram
- Molecules that can enter the pores will have an average residence time in the particles that depends on the molecules size and shape
- Different molecules therefore have different total transit times through the column
- Molecules that are smaller than the pore size can enter all pores, and have the longest residence time on the column and elute together as the last peak in the chromatogram

Principle

- One requirement for SEC is that the analyte does not interact with the surface of the stationary phases
- Differences in elution time are based solely on the volume the analyte
- A small molecule that can penetrate every corner of the pore system of the stationary phase (where the entire pore volume and the interparticle volume ~80% of the column volume) and will elute late
- A very large molecule that cannot penetrate the pore system only the interparticle volume (~35% of the column volume) and will elute earlier when this volume of mobile phase has passed through the column
- The underlying principle of SEC is that particles of different sizes will elute (filter) through a stationary phase at different rates. Particles of the same size should elute together

Analysis

The collected fractions are often examined by spectroscopic techniques to determine the concentration of the particles eluted

Common spectroscopy detection techniques are refractive index (RI) and ultraviolet (UV)

For molecules, which can enter the beads, there is an inverse logarithmic relationship between the size of the molecule and the volume eluted from the column. Finally, can use a standard curve to estimate the molecular weight

Commercially avaiable columns

The typical column diameters are 7.5–8mm for analytical columns and 22–25mm for (semi)preparative columns; usual column lengths are 25, 30, 50, and 60 cm

The packings are based on either porous silica or semirigid (highly crosslinked) organic gels, in most cases copolymers of styrene and divinylbenzene

For example: TSKgel GFC columns for protein analysis (TSKgel SW-type columns are silica-based)

125Å pore size for analysis of small proteins and peptides
250Å pore size for most protein samples
450Å pore size for very large proteins and nucleic acids

Commercially available columns and properties:

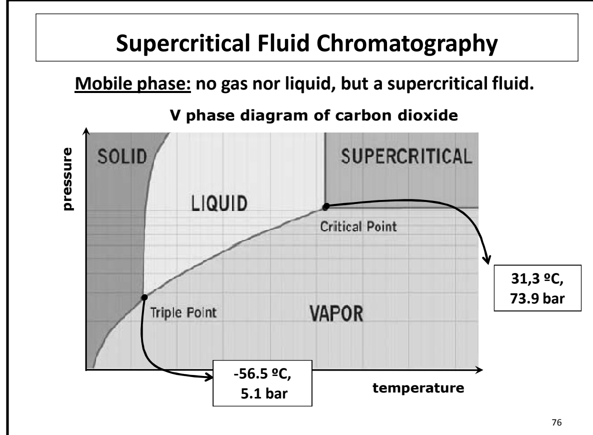
Product	pH stability	Particle size
Superdex Peptide	Long term: 1–14 Short term: 1–14	13–15 µm
Superdex 75	Long term: 3–12 Short term: 1–14	13–15 µm
Superdex 200	Long term: 3–12 Short term: 1–14	13–15 µm
Superdex 30 prep grade	Long term: 3–12 Short term: 1–14	22–44 µm
Superdex 75 prep grade	Long term: 3–12 Short term: 1–14	22–44 µm
Superdex 200 prep grade	Long term: 3–12 Short term: 1–14	22–44 µm

Superdex 200 - the molecular weight of the protein of interest is unknown
Superdex 200 or Superdex 200 prep grade - especially suitable for the separation of monoclonal antibodies from dimers and from contaminants of lower molecular weight

Advantages

- **Unlike ion exchange or affinity chrom. molecules do not bind to the medium so buffer composition does not directly affect resolution**
- **is well suited for biomolecules that may be sensitive to changes in pH, conc. of metal ions or co-factors and harsh environmental conditions**
- **conditions can be varied to suit the type of sample or the requirements for further purification, analysis or storage without altering the separation**
- **Can be used after any chrom. tech. bcz components of any elution buffer will not affect the final separation**

Supercritical Fluid Chromatography



Supercritical Fluids

- At temperatures and pressures above its critical temperature and pressure (**critical point**), a substance is called a **supercritical fluid**. The **critical temperature** is the temperature above which a distinct liquid phase cannot exist. The vapor pressure at its critical temperature is its **critical pressure**.
- Where supercritical fluids exist: The forces from the kinetic energy of the molecules exceeds the forces from condensing influence of the intermolecular forces, so no distinct liquid phase exists

SFC Mobile Phases

- Mobile phases should have critical parameters that are easily reached using chromatographic pumps and ovens common to currently used instrumentation.
- Advantages of supercritical fluids over carrier gasses and liquid mobile phases are in its solubility properties, physical properties, and detector compatibility.

Use of Supercritical Fluids

Properties (density) **between gas and liquid** ⇨ **Speed** and **resolution** better compared to liquid chromatography (faster diffusion inside the column cavity, thus faster equilibration)

- **Supercritical fluids**
  - …possess **lower surface tension** *than liquids* (i.e. they spread faster over stat. phase).
  - …**dissolve non-volatile substances** *unlike gases*
  - …evaporate upon pressure reduction after passing the column: **analytes** are in gaseous phase and thus easily detectable
- **mostly used: CO_2**
  - compatible with common detectors (FID, UV).
  - low critical temperature.
  - non-toxic.

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SFC Separations

- SFC is a hybrid of gas and liquid chromatography that combines some of the best features of each
- As in HPLC, variation of the mobile phase composition affects separation
- In SFC, mobile phase affinity for the analyte is a function of mobile phase density
- Density is controlled by controlling system pressure
- Highly polar samples are not easy to handle (high critical parameters & high reactivity)

SFC Advantages vs HPLC

- Supercritical fluids have low viscosities
  - faster analysis (5 to 10 X faster)
  - less pressure drop across the column
  - the use of open tubular columns is feasible
- Column lengths from 10 to 20 m are used
- Can be used with a wide range of sensitive detectors
- Resolving power is ~5X that of HPLC

Flow Rate and Gradient Elution in SFC

HETP
HPLC
SFC
flow rate u

- ✓ **best** (smallest) **plate height** at higher flow rate compared to HPLC
- ✓ **min. plate height** only **1/3** compared to HPLC

➢ ***What gradient?***
- in HPLC: usually solvent gradient
- in GC: usually temperature gradient
- In ion exchange: concentration or pH gradient
- ***in SFC: pressure gradient***

Density increases as **pressure increases**. The **denser** the mobile phase, the **bigger** its capacity for a solute, the **less distribution** into stat. phase, the **lower** the **retention.**

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SFC Advantages vs GC

- Can analyze non-volatile, polar, or adsorptive solutes without derivatization.
- Can analyze thermally labile compounds.
- Can analyze solutes of much higher molecular weight.

SFC Instrumentation

- Solvent delivery system
- Injector
- Column/Column Oven
- Restrictor
- Detector
- Data System

Solvent Delivery System

- Maintains precise mobile phase flow (1 to 10 µL/min {OT} or 1 to 10 mL/min {Packed}).
- Aids in the control of the system pressure (up to 60 Mpa).
- Moves mobile phase in the liquid state under pressure through the injector & into the column.

Injectors

- Typical HPLC design injectors for packed columns.
- Split/Splitless valve injector (0.01 to 0.05 µL injections) for open tubular columns.
- Timed - split injector (0.01 to 0.05 µL injections) for open tubular columns.

Detectors

- Most any detector used in GC or HPLC can be used.
- FID and UV detectors commonly used.
- Coupled Detectors
    - MS
    - FTIR

SFC Columns

- Open tubular (derived from GC)
    - Large # theoretical plates (~X500)
    - Easier to control pressure (low P drop)
- Packed (derived from HPLC)
    - Faster analysis
    - Higher flow rates
    - Higher sample capacity

Open Tubular Columns

- Smaller than GC capillary columns, typically 50 μm i.d., 10 to 20 m in length
- MP must be more stable due to extreme conditions of supercritical fluids

Packed Columns

- Similar to HPLC columns (10, 5, or 3 μm porous particles)
- Silica based chemically bonded phases
- Typically 10 cm long X 4.6 mm i.d

SFC and Retention

- Retention dependent on temperature, pressure, mobile phase density, and composition of the stationary and mobile phase.
- Complex interactions and not easily predictable.
- For supercritical fluids
  - solvating properties similar to liquids - viscosity closer to gases
- Solvating power α density

Temperature/Pressure Effects

- At lower P, > T, < solubility
- At higher P, > T, > solubility
  -> T, P_v of solute > solute solubility
  -< fluid density < solubilizing power
- > T, < solvent ρ
- >P, > solvent ρ

Supercritical CO_2 Density

P (MPa)	T (^oC)	ρ (g/cm^3)
7.3	40	0.22
7.3	80	0.14
7.3	120	0.12
40	40	0.96
40	80	0.82
40	120	0.70

Solvent Programming

- Programming is very useful in controlling solvent strength.
- Variations in P (density), T, and mobile phase composition.
- Density programming is most widely used (not simple relationship, T & P).
  -> density, > solubility, < retention
  - Combined T & P programming to control ρ and thereby solubility and diffusion

SFC Mobile Phases

- Generally non-polar compounds with low to moderate critical properties
  - CO_2, N_2O, ethane, pentane
- Normal phase type separations
  - non-polar mp and low polarity sp (substrate + amino, diol, or cyano groups)
- Elution = function of molecular mass & polarity

Carbon Dioxide: SFC Solvent

- Low T_c
  - operating T as low as 40°C
- Moderate P_c and ρ_c of 0.448g/cm3
  - reach high ρ with $P < 40$ MPa
- Safe to use
  - nontoxic, nonflammable, noncorrosive, inert
- Detector compatible
- Wide ρ range

Other SFC Solvents

- Nitrous Oxide - Similar in solvating and separations properties to CO_2
- Alkanes - less safe and not as detector compatible than CO_2
  - better solvent characteristics for non-polar solutes
- Halocarbons, xenon, etc. - specialty applications only
- More polar solvents for highly polar & high molecular weight compounds

Solvent Modifiers

- Add organic modifiers to > solvent strength
  - methanol
  - isopropanol
  - dichloromethane
  - THF
  - acetonitrile