Other types of liquid chromatography

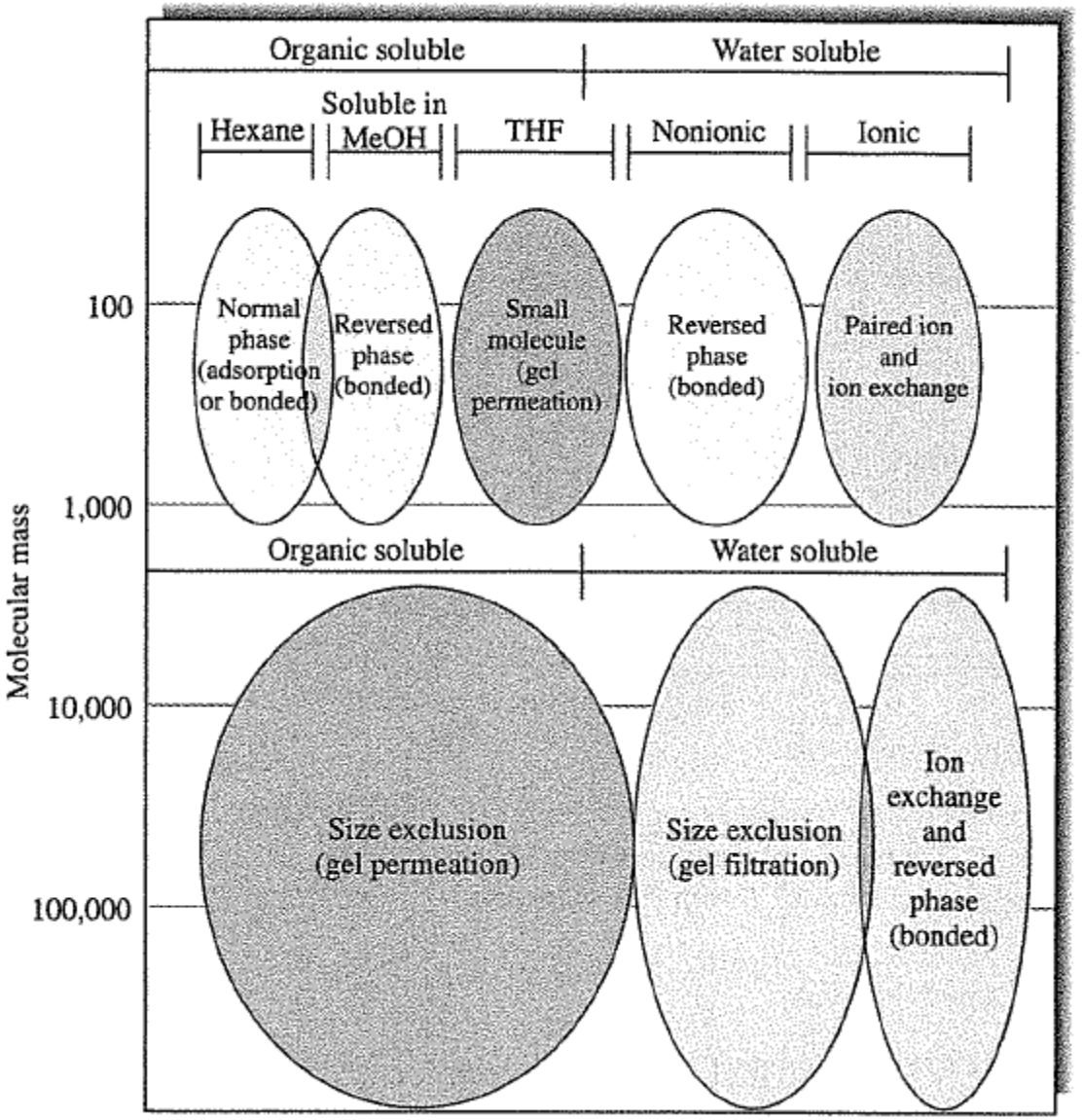


FIGURE 28-1 Selection of LC modes. Methods can be chosen based on solubility and molecular mass. In most cases for non-ionic small molecules ($\mathcal{M} < 2000$), reversed-phase methods are suitable. Techniques toward the bottom of the diagram are best suited for species of high molecular mass ($\mathcal{M} > 2000$). (Adapted from *High Performance Liquid Chromatography*, 2nd ed., S. Lindsay and J. Barnes, eds., New York: Wiley, 1992. With permission.)

Objectives:

After this discussion you should be able to:

- Define IEC
- Basic mechanism
- Relationship between net charge and isoelectric point (pI)
- Relationship between net charge and pH
- Stationary phase material
- Experimental flow
- Effects of pH, salt concentration, flow rate etc
- Detectors
- Few areas of applications

Ion exchange chromatography (IEC)

https://youtu.be/q3fMqgT1do8

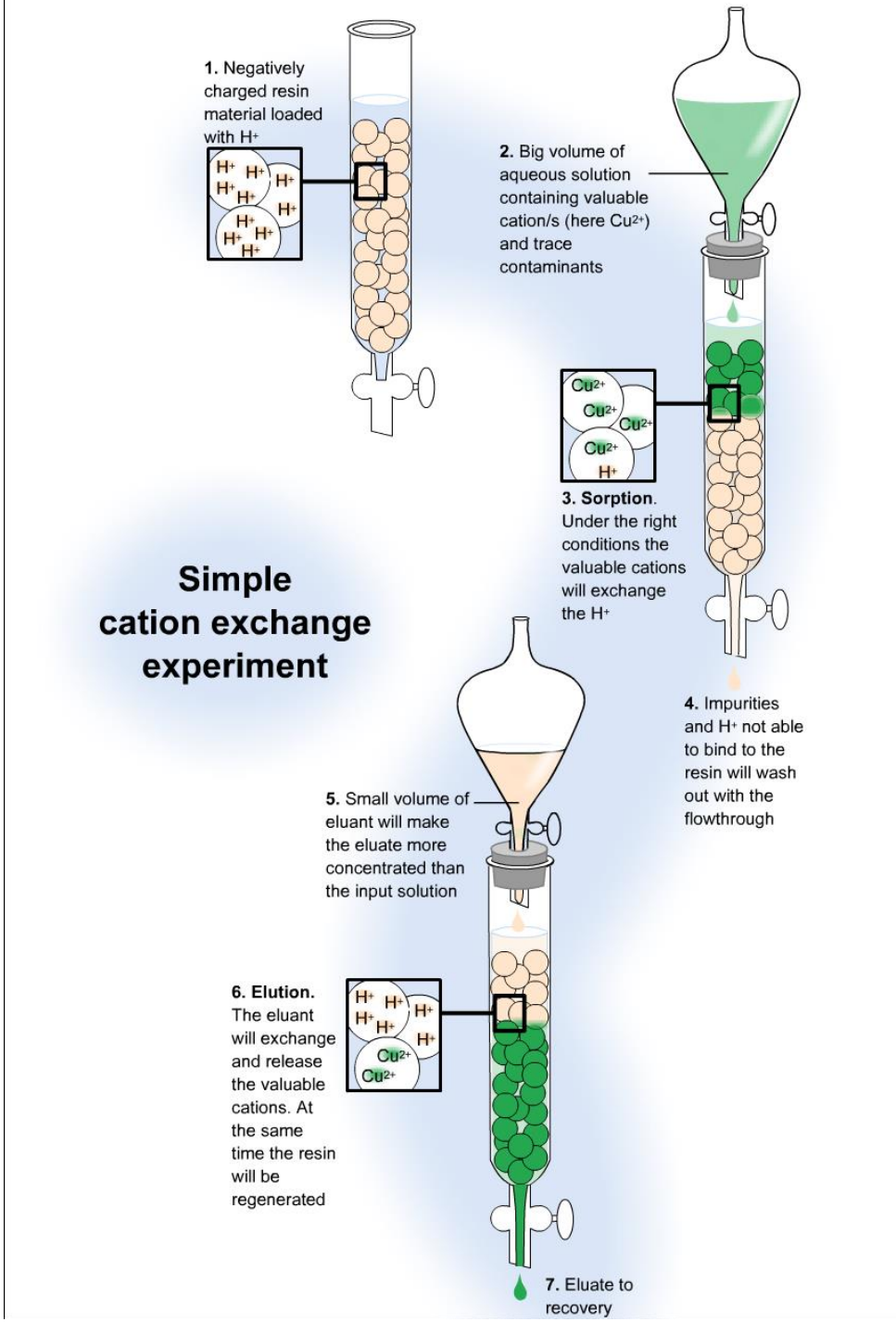
History of IEC

- Since the industrial revolution in Europe-IEC was used to reduce the hardness of water
- **1850** – J.T. Way and H.S. Thompson successfully extracted ammonia and released calcium from clay samples through the use of carbonate and ammonium sulfate. This is the very first instance of ion methods being used in scientific processes.
- **1942** – J. Schubert, G.E. Boyd and A.W. Adamson demonstrated the aptitude of ion exchange for adsorption of trace amounts of fission materials. This led to the development of the modern version of ion chromatography, by choosing adsorbents which fastened onto uranium elements (Manhattan project)

Ref.:

What is IEC?

- Separation of ionizable molecules based on their total charge
- Total charge molecule can be change by altering pH or salt concentration, depending upon the characteristics of the analyte.



Reference:
http://wiki.biomine.skelleftea.se/wiki/images/d/d3/Simple_cation_exchange_with_description_071111.png

Mechanism

$$f = \frac{q_1 q_2}{\varepsilon r^2}$$

f= Coulombic force of interaction between two ions

q_1, q_2= charges on both ions

ε = dielectric constant of the medium

r = distance between two ions

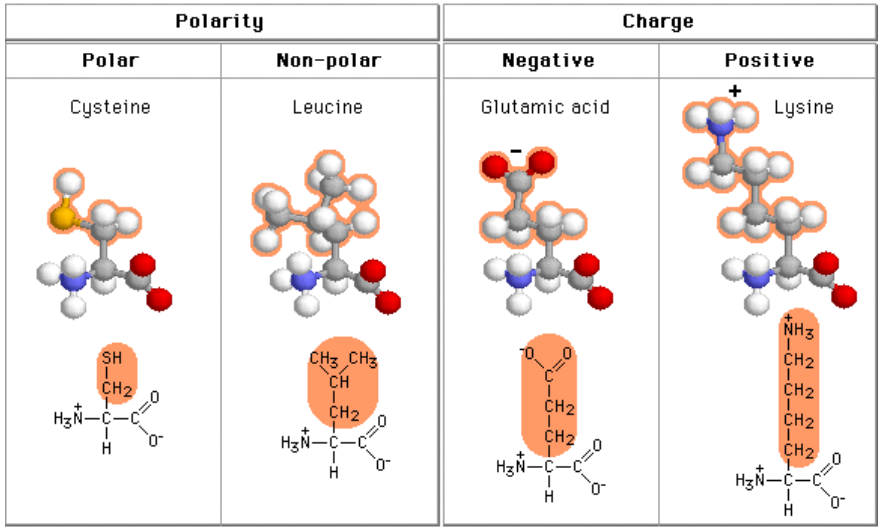
- Force of attraction with monovalent stationary phase and mono/multivalent analyte is as follows:

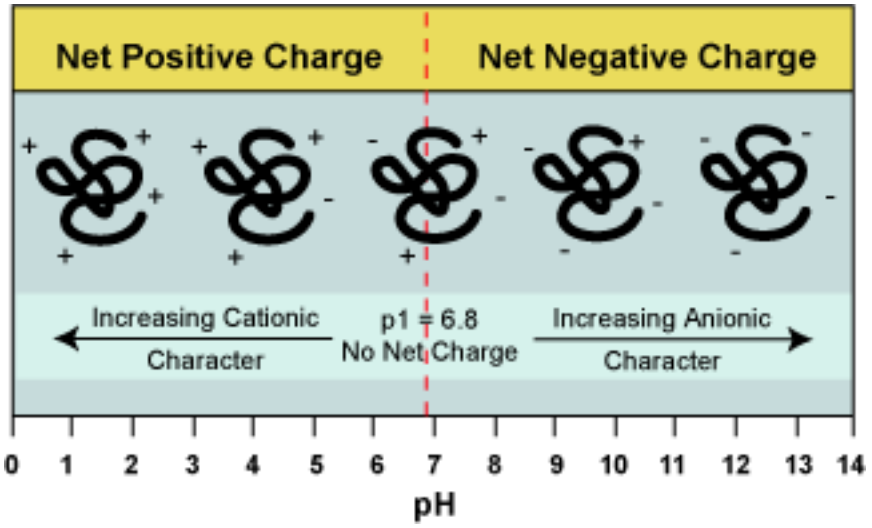
  Trivalent>divalent (sulfate)>monovalent(chlorine)

- Charge density on the surface ion rather than its actual charge
- Hydration or solvation

Net charge and isoelectric point (pI)

- Separation of charged molecules based on **“net charge”**
  - E.g., proteins, peptides, amino acids or nucleotides
- **Net positive charge / net negative charge / no charge**
- **Isoelectric point:** pH at which a molecule has no net charge

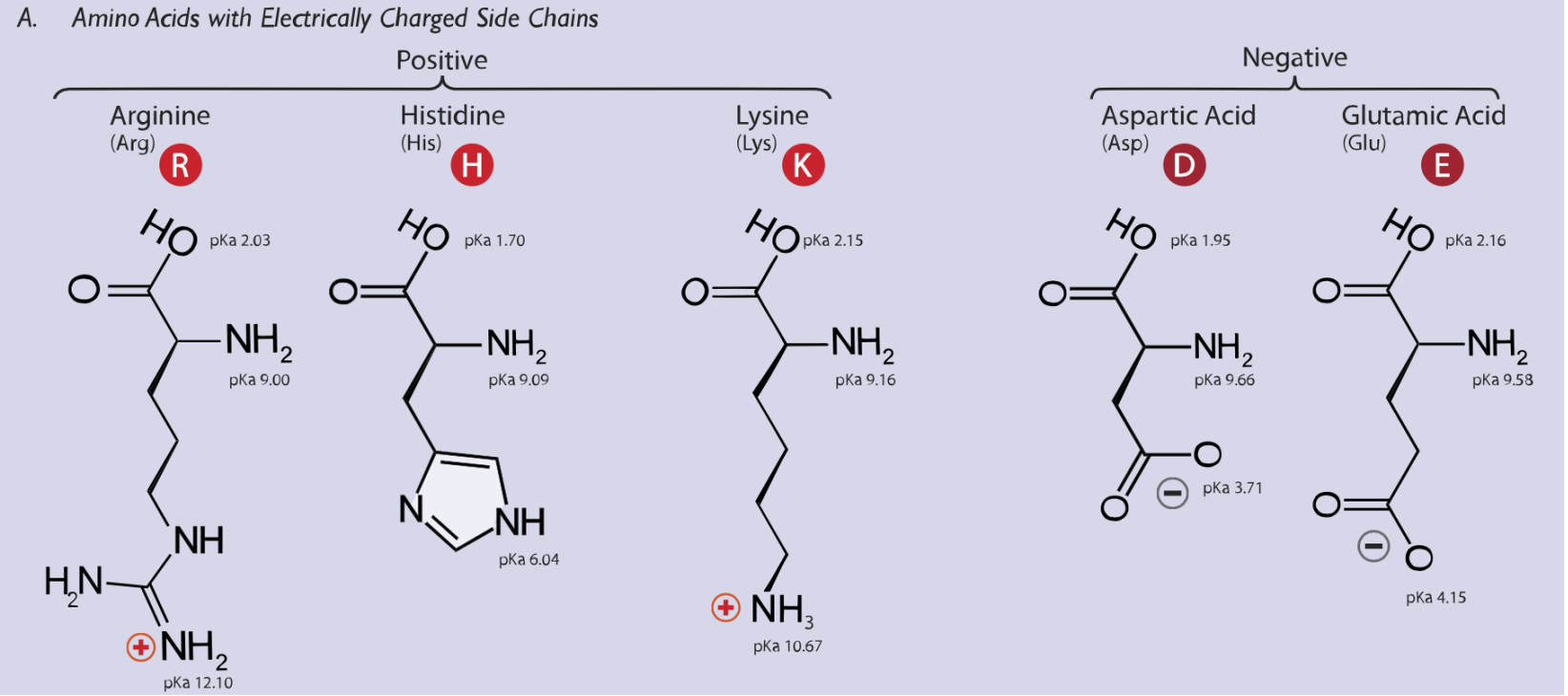




Ref.: (1) www.phschool.com (2) www.intranet.tdmu.edu.te.ua

Charge of the amino acid side chains

- Two are negative charged: **aspartic acid** (Asp, D) and **glutamic acid** (Glu, E) (**acidic** side chains),
- and three are positive charged: **lysine** (Lys, K), **arginine** (Arg, R) and **histidine** (His, H) (**basic** side chains).



Net charge and pH

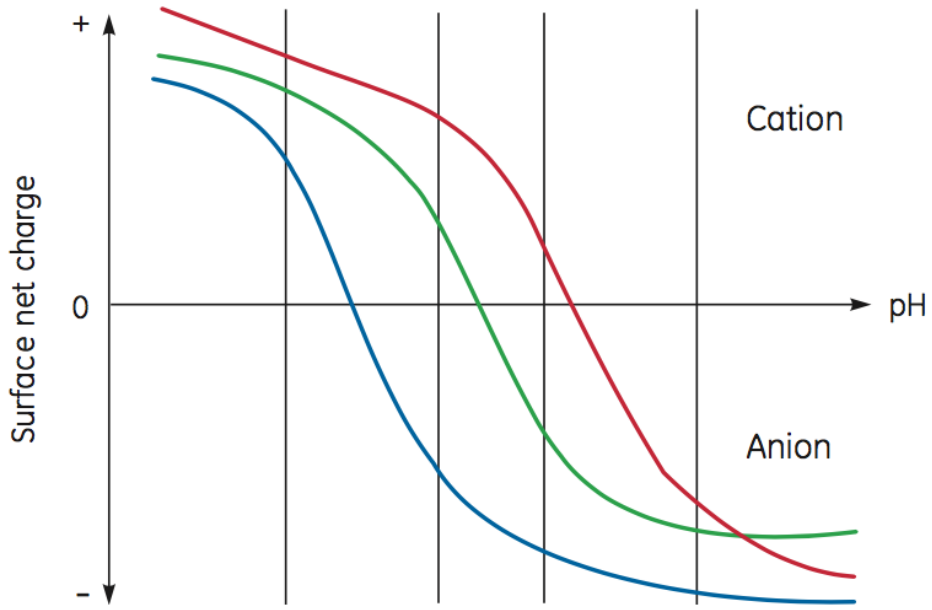
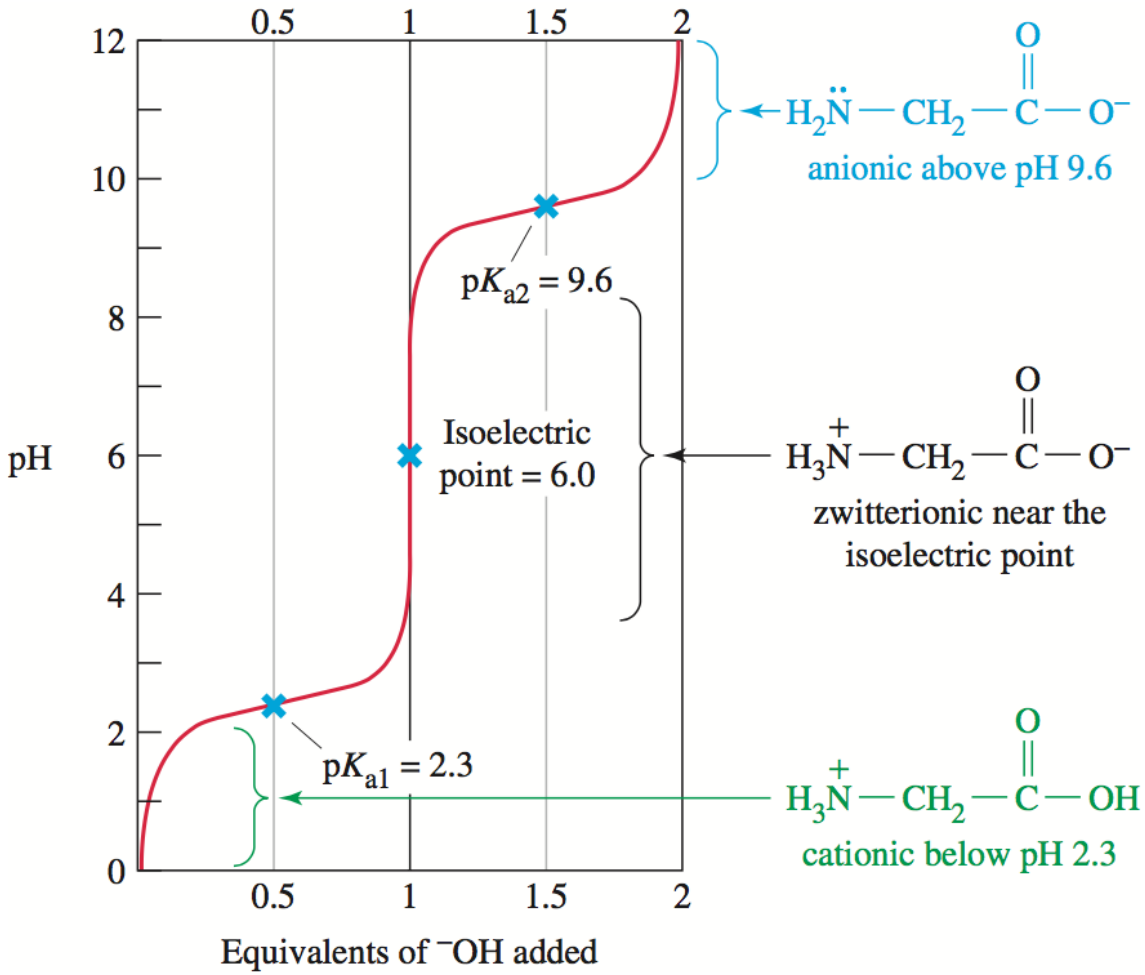


Fig.: Theoretical protein titration curves: how net surface charge varies with pH?

- overall charge,
- charge density,
- surface charge distribution
- net surface charge is highly pH dependent
- amphoteric nature of protein

pKa and pI (isoelectric point)



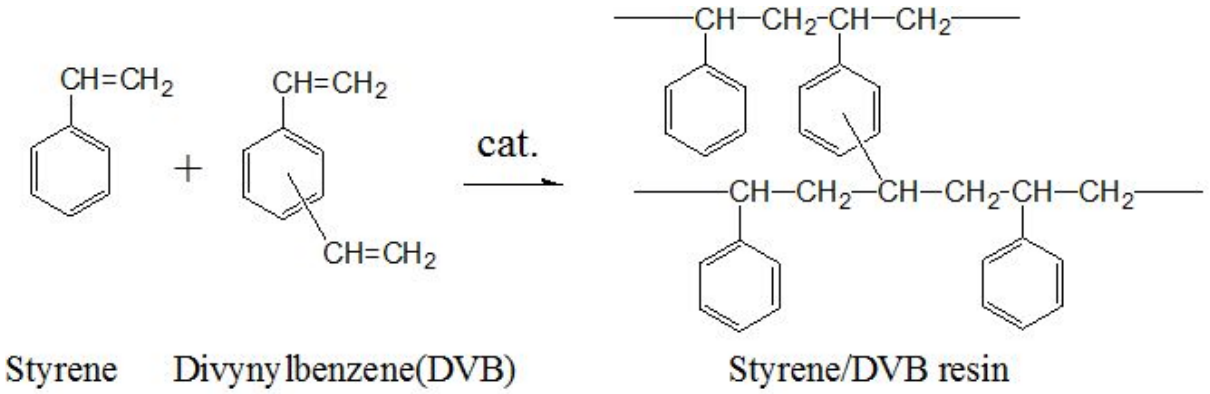
- Henderson-Hasselbach equation:
- $pH = pK_a + \log[A^-]/[HA]$

A titration curve for glycine.

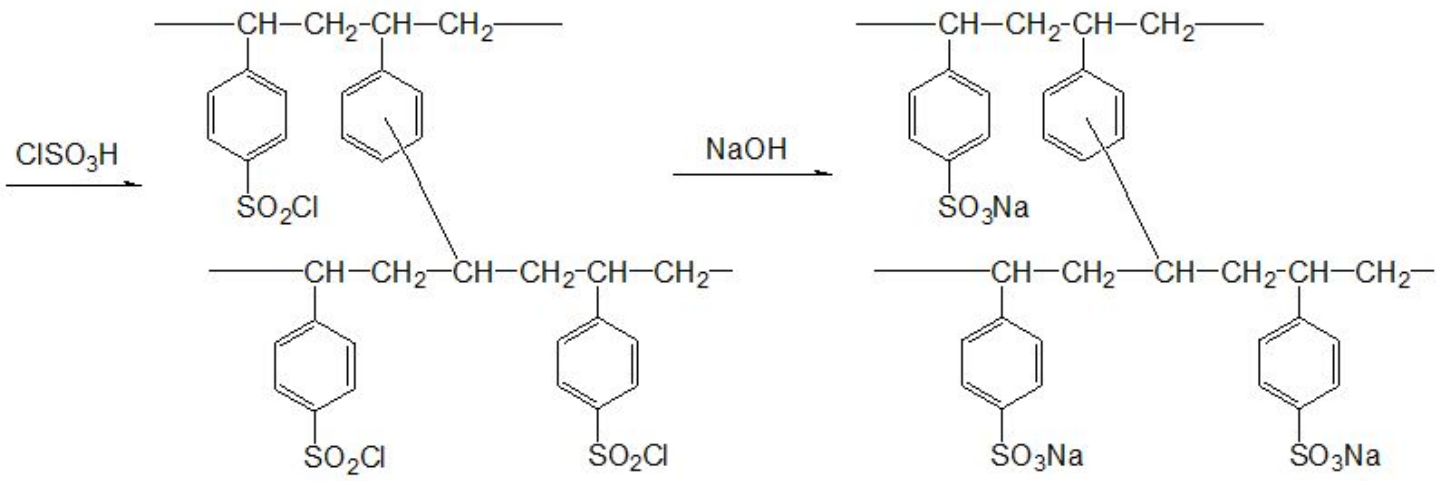
cationic below pH 2.3; zwitterionic between pH 2.3 and 9.6; anionic above pH 9.6. The isoelectric pH is 6.0

Ref: http://web.pdx.edu/~wamserc/C336S09/Wade_Ch24.pdf

Resin: structure and synthesis



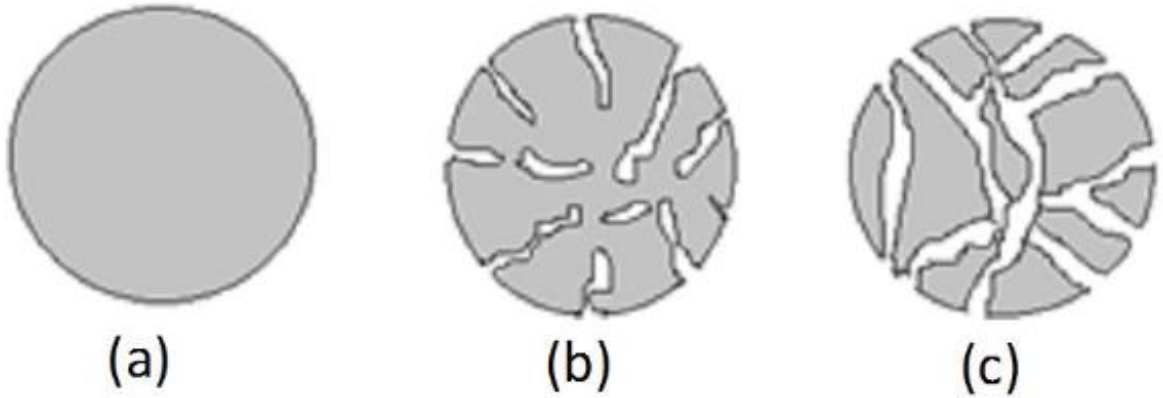
Styrene Divynylbenzene(DVB) Styrene/DVB resin



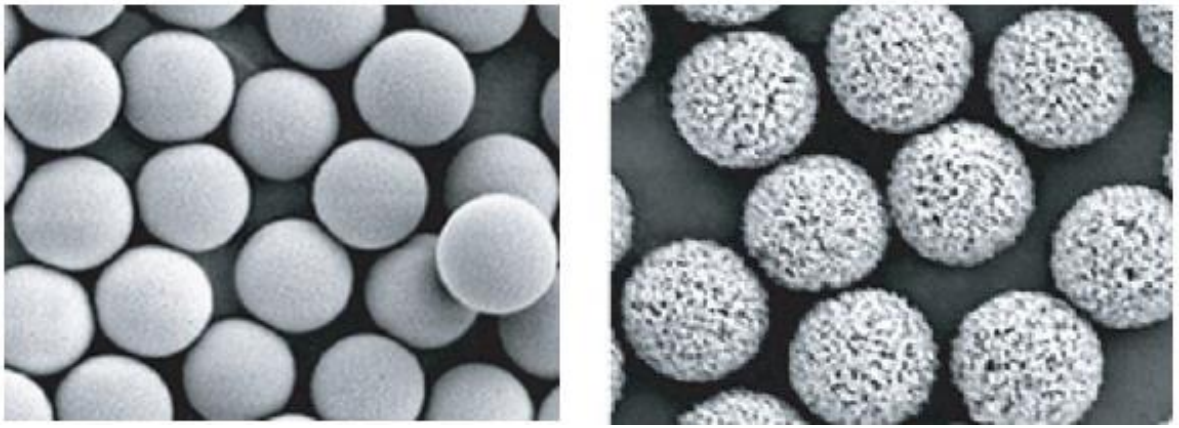
Chlorosulfonated resin Ion-exchange resin

Ref.: Nitrogen Isotope Separation by Ion Exchange Chromatography By Xingcheng Ding and Xunyue Liu DOI: 10.5772/51311

Porosity: increases interactions



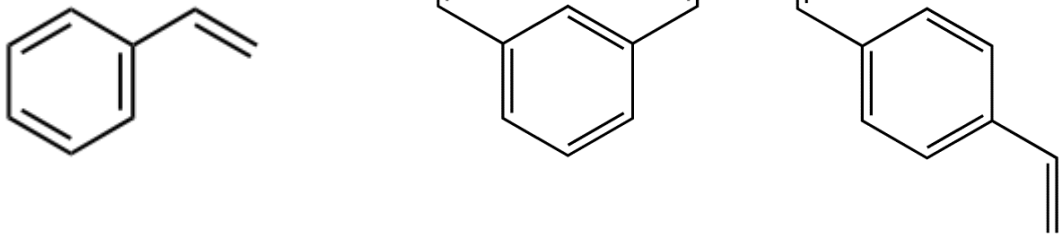
Schematic presentation of different matrix types (a) non-porous beads (b) microporous beads (c) macroporous beads



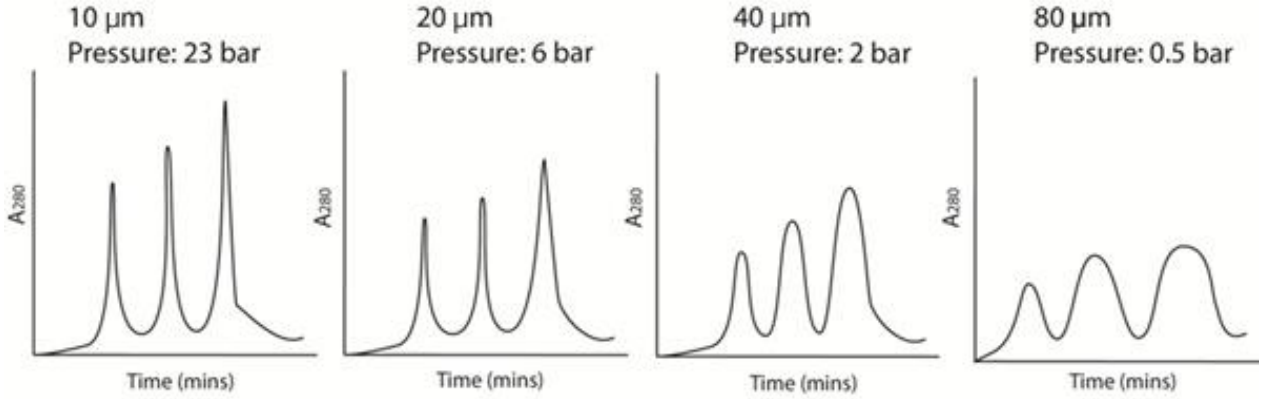
http://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications

- The structural polymeric backbone of the resin is **styrene cross-linked with 2 to 8% divinylbenzene.**

- The amount of cross-linkage determines both the pore **size of the media** and the **capacity of the resin**. (The pore size can be significant when separating proteins but is of little significance when doing inorganic separations.)



Particle Size of resins



Smaller particles:

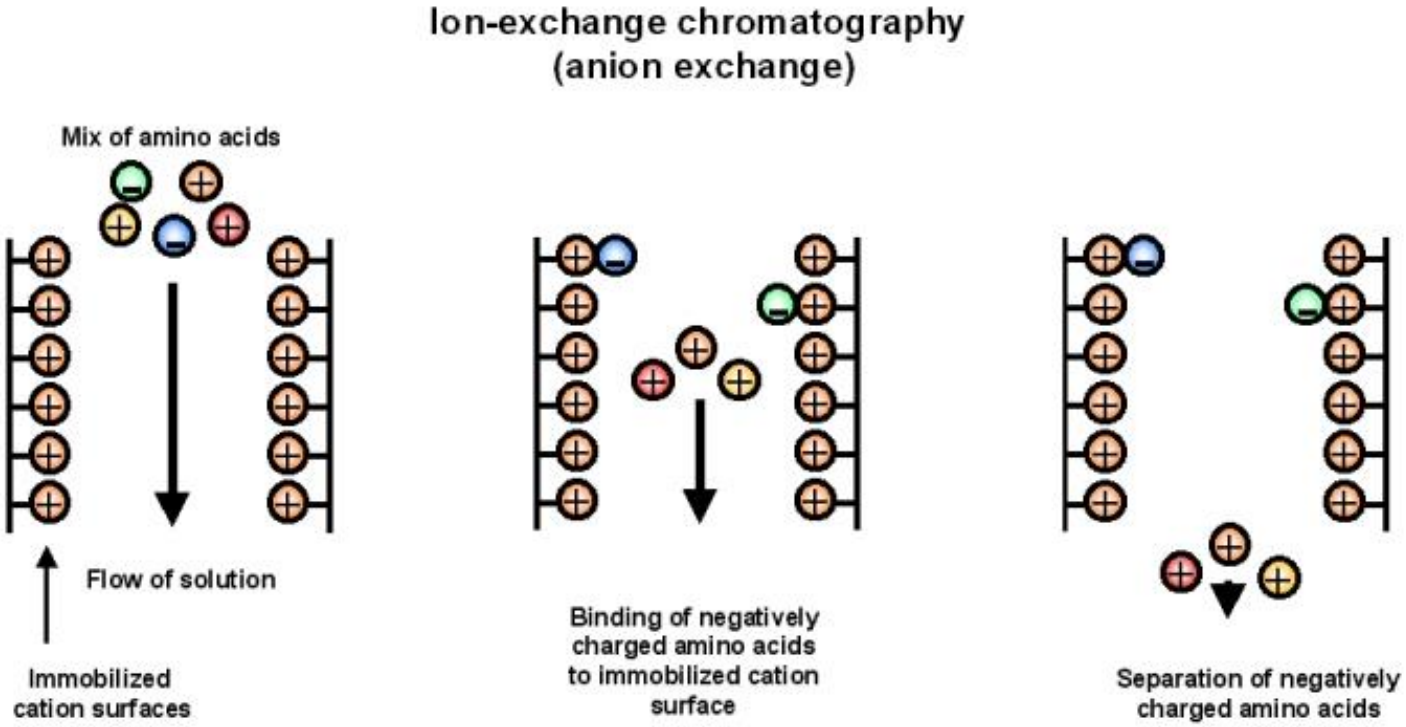
- Higher resolution with lower flaw rate
- Best choice for analytical and small-scale work
- Avoid viscous sample, e.g., Glycerol containing sample

Larger particles:

- Permit higher flow rates but yield lower resolution
- Best choice for preparative work and viscous sample

Stationary phases: cationic and anionic exchange

- Most popular method for the separation or purification of charged molecules.
- In cation exchange chromatography, positively charged molecules are attracted to a negatively charged solid support.
- Conversely, in anion exchange chromatography, negatively charged molecules are attracted to a positively charged solid support, as below.



http://chemwiki.ucdavis.edu/Analytical_Chemistry/Instrumental_Analysis/Chromatography/Liquid_Chromatography/Ion_Exchange_Chromatography

The experiment

To **optimize binding** of all charged molecules, the mobile phase is generally of low to medium salt concentration.

The **adsorption** of the molecules to the solid support is driven by the **ionic interaction** between the oppositely charged ionic groups in the sample molecule and in the functional ligand on the support.

The **strength of the interaction** is determined by the **number and location of the charges** on the molecule and on the functional group.

By increasing the salt concentration (generally by using a linear **salt gradient**), the molecules with the **weakest ionic** interactions start to elute from the column first.

Molecules that have a **stronger ionic interaction** require a **higher salt concentration** and elute later in the gradient.

The **binding capacities** of ion exchange resins are generally quite high.

Examples of stationary phases: Anion exchange resin

- Spectra/Gel Anion Exchange Resins are strong ion exchange resins.
- Use a trimethylbenzylammonium group as the exchange site.
- They are supplied in the chloride form

Anion Exchange Resin Characteristics

Type:	strong base anion exchanger
Active Group:	trimethylbenzylammonium
% divinylbenzene:	2%, 4%, or 8%
Supplied Ionic Form:	Cl^-
Moisture Content:	43% to 48%
Volume Change:	Cl^- to OH^- is +20%
pH Range:	0 to 14
Selectivity:	Cl^-/OH^- is about 25
Order of Selectivity:	I > NO3 > Br > Cl > acetate > OH > F

http://www.spectrumlabs.com/chrom/IonExchange.html

Examples of stationary phases: Cation exchange resin

- Spectra/Gel Cation Exchange Resins are strong acid ion exchange resins.
- Contains sulfonic acid active group as the exchange site.
- supplied with a hydrogen ion occupying the exchange site.

Cation Exchange Resin Characteristics:

Type:	strong acid cation exchanger
Active Group:	sulfonic acid
% divinylbenzene:	2%, 4%, or 8%
Supplied Ionic Form:	H^+
Moisture Content:	51% to 54%
Volume Change:	Na^+ to H^+ is +8%
pH Range:	0 to 14
Selectivity:	Na+/H+ is about 1.5
Order of Selectivity:	$Ba^{++} > Rb^{++} > Ca^{++} > Mg^{++} > Be^{++} > Ag^+ > Cs^+ > Rb^+ > K^+ > NH4^+ > Na^+ > H^+ > Li^+$

http://www.spectrumlabs.com/chrom/IonExchange.html

Mobile phase pH

As a rule, the pH of the mobile phase buffer must be between the pI (isoelectric point) or pKa (acid dissociation constant) of the charged molecule and the pKa of the charged group on the solid support.

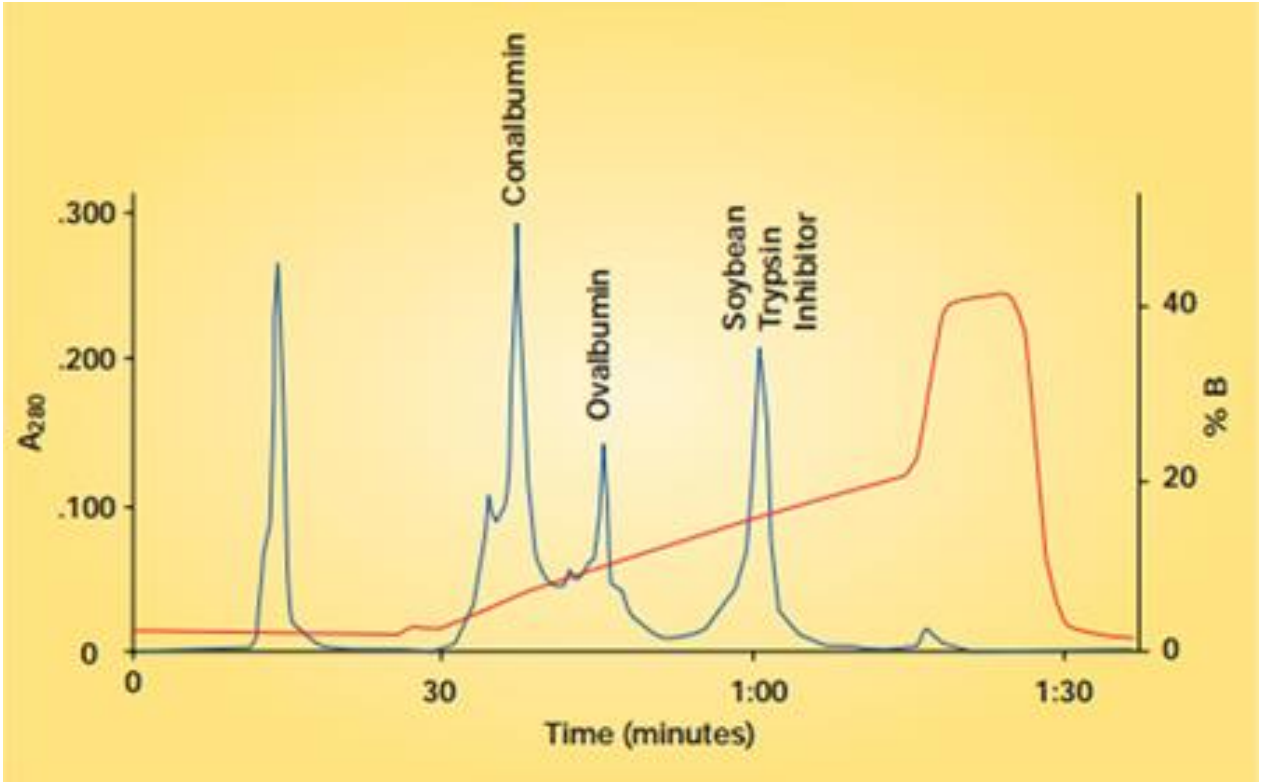
In anion exchange chromatography a molecule with a pI or pKa of 6.3 may be run in a mobile phase buffer at e.g. pH 7.5 when the pKa of the solid support is 9.8.

Trimethyl benzyl ammonium: pKa = 9.8

In cation exchange chromatography, using a functional group on the solid support with a pKa of -2, a sample molecule with a pI of pKa of 10.8 may be run in a mobile phase buffer of e.g. pH 7.0.

Sulfonic acid: pKa = -2.0

Salt Gradients



[Elution of proteins (**blue trace**) with an increasing salt gradient (**red trace**)}

A gradient of linearly increasing salt concentration is then applied to elute the sample components from the column.

An alternative to using a linear gradient is to use a step gradient. Requires easy setup

Varying the pH

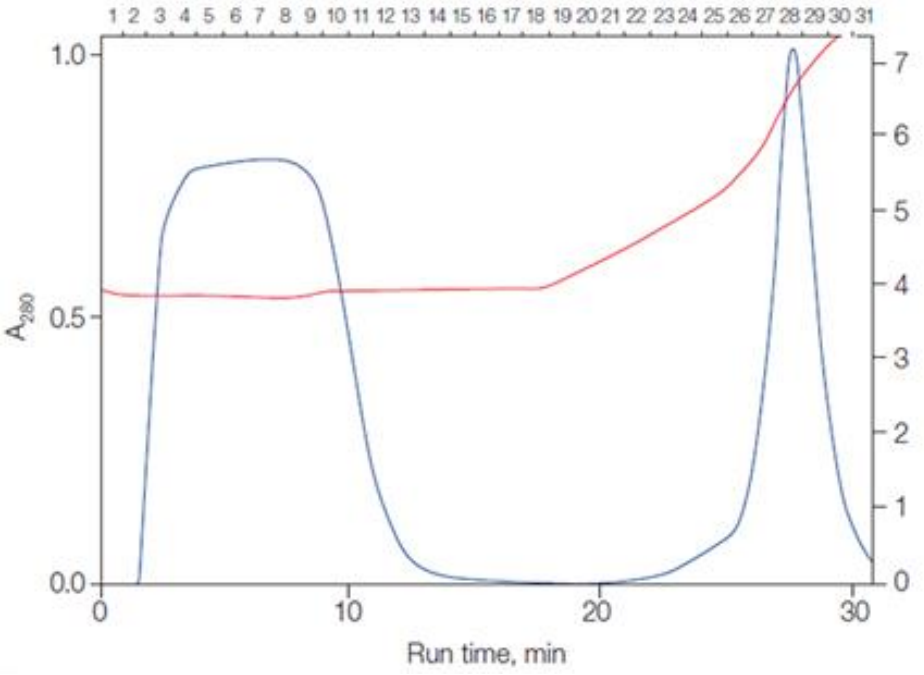
In anion exchange chromatography, lowering the pH of the mobile phase buffer will cause the molecule to become more protonated and hence more positively charged.

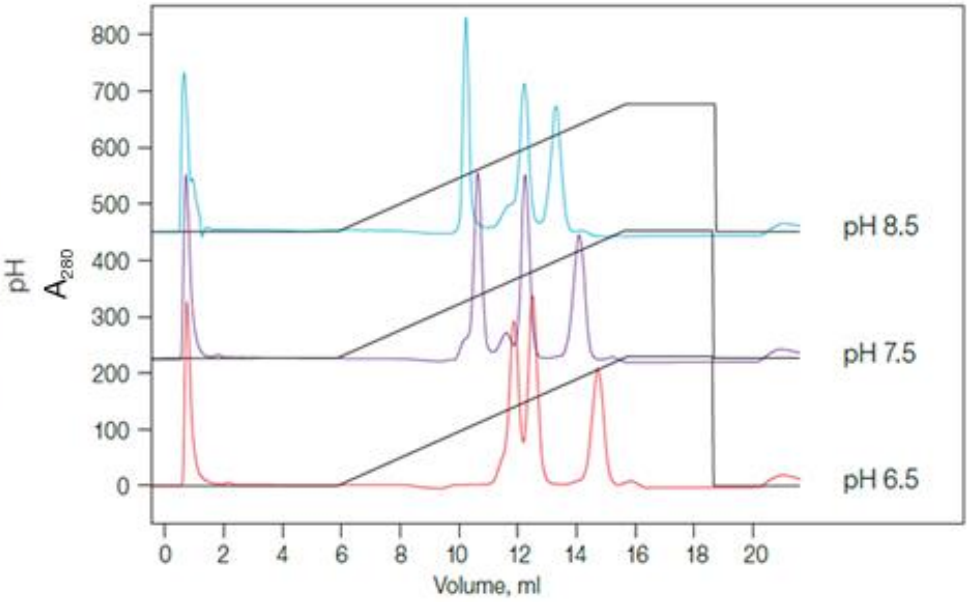
The result is that the molecule no longer can form a ionic interaction with the positively charged solid support which causes the molecule to elute from the column.

In cation exchange chromatography, raising the pH of the mobile phase buffer will cause the molecule to become less protonated and hence less positively charged.

The result is that the molecule no longer can form a ionic interaction with the negatively charged solid support, which ultimately results in the molecule to elute from the column.

pH gradient

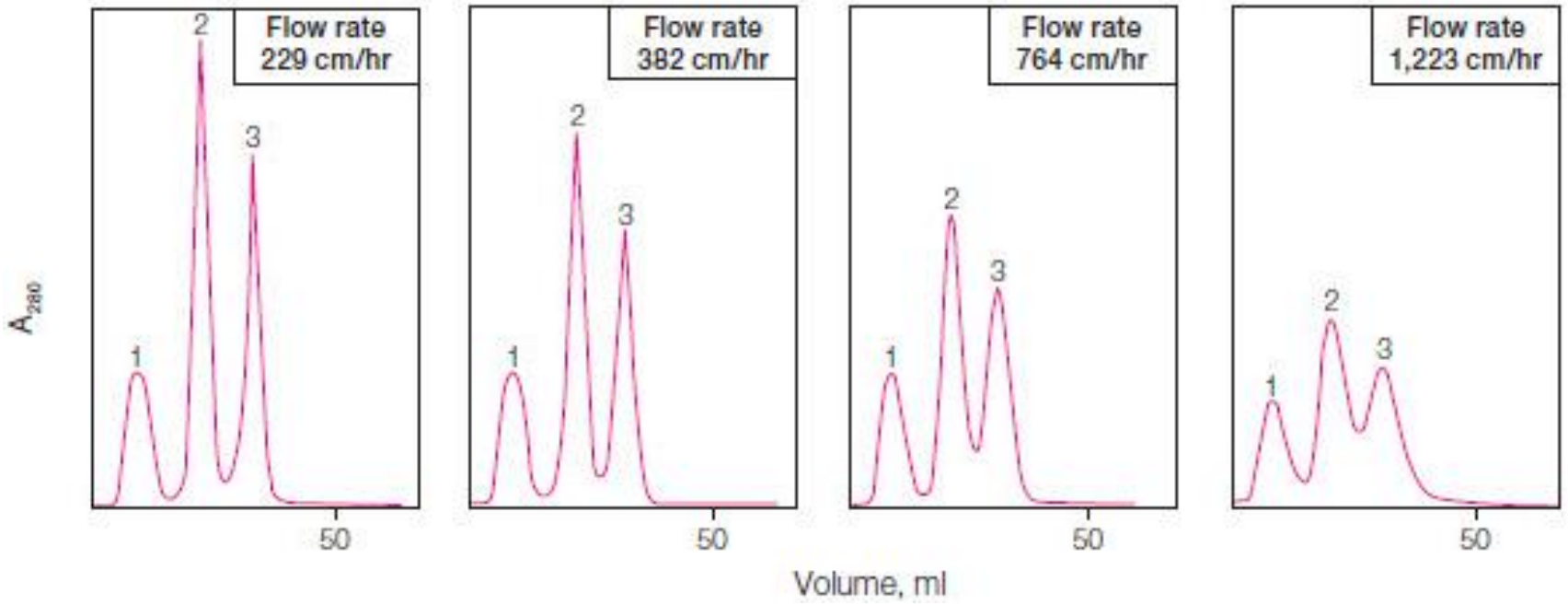

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
1.0
0.5
0.0
A280
0
10
20
30
Run time, min
7
6
5
4
3
2
1
0
pH


800
700
600
500
400
300
200
100
0
A280
0
2
4
6
8
10
12
14
16
18
20
Volume, ml
pH 8.5
pH 7.5
pH 6.5

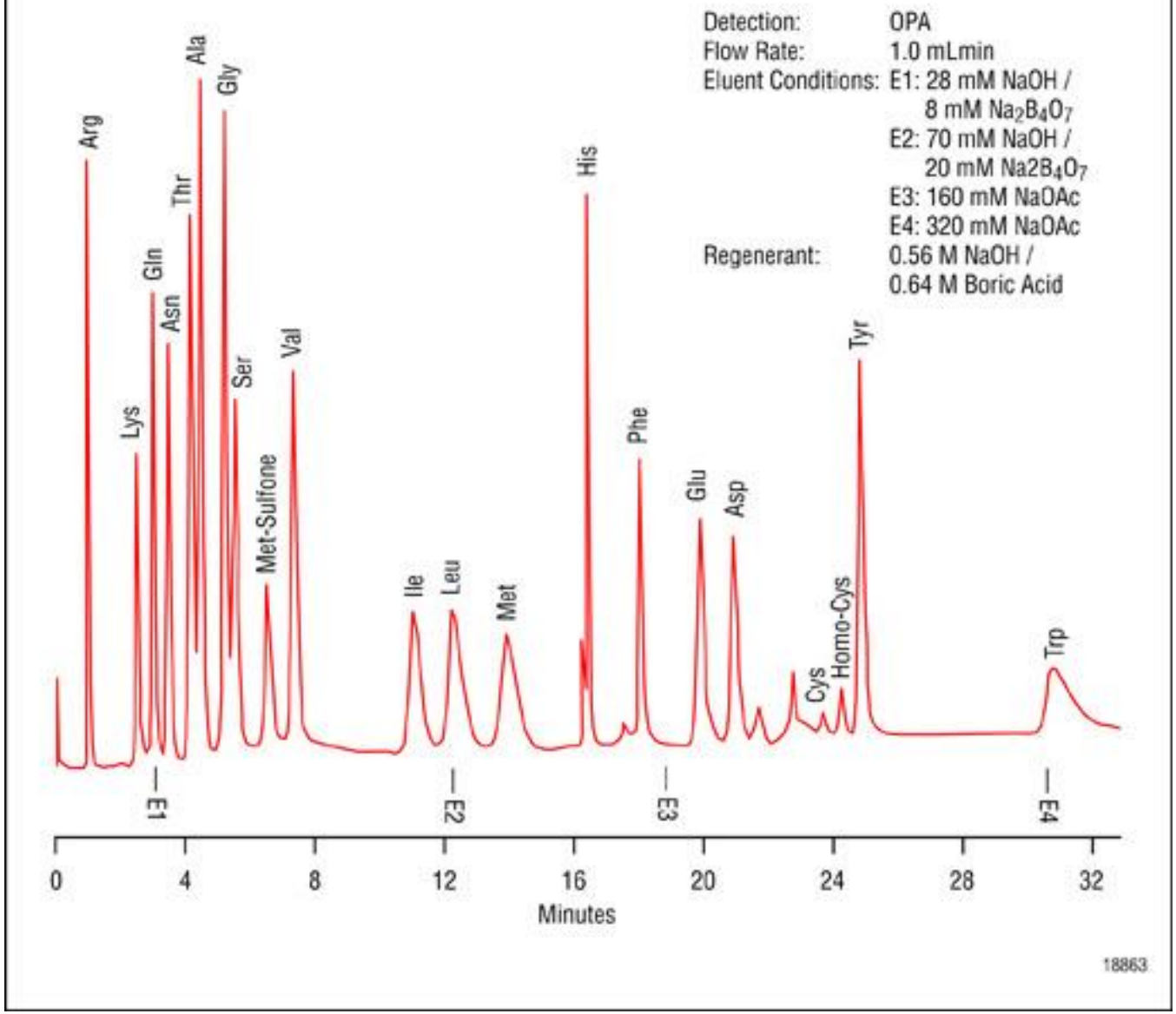
Flow rate

- Flow rate determines the amount of time in which proteins can interact with the column resin, which is called the **residence time** of a particular column at a given flow rate

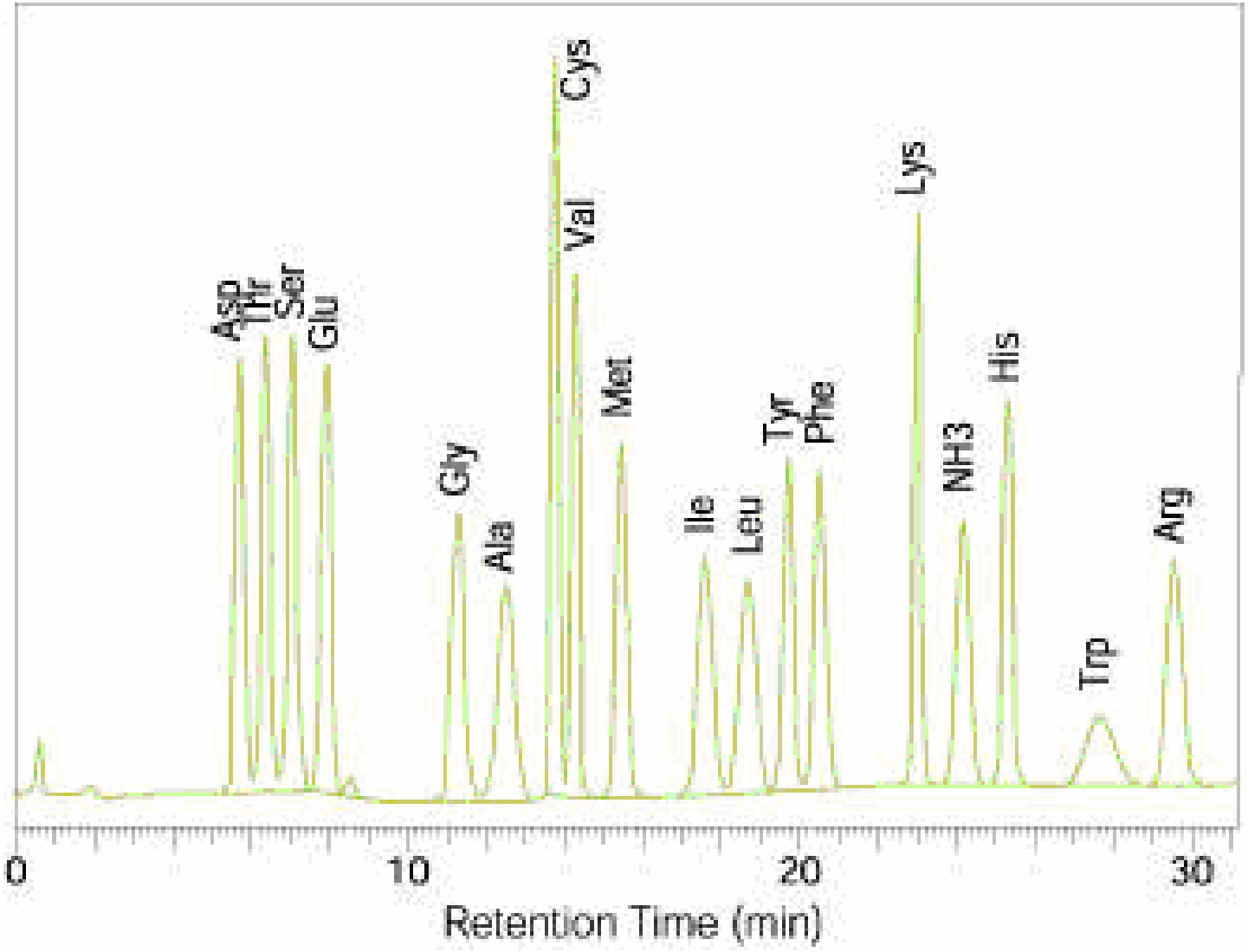
- Flow rate affects both resolution and capacity



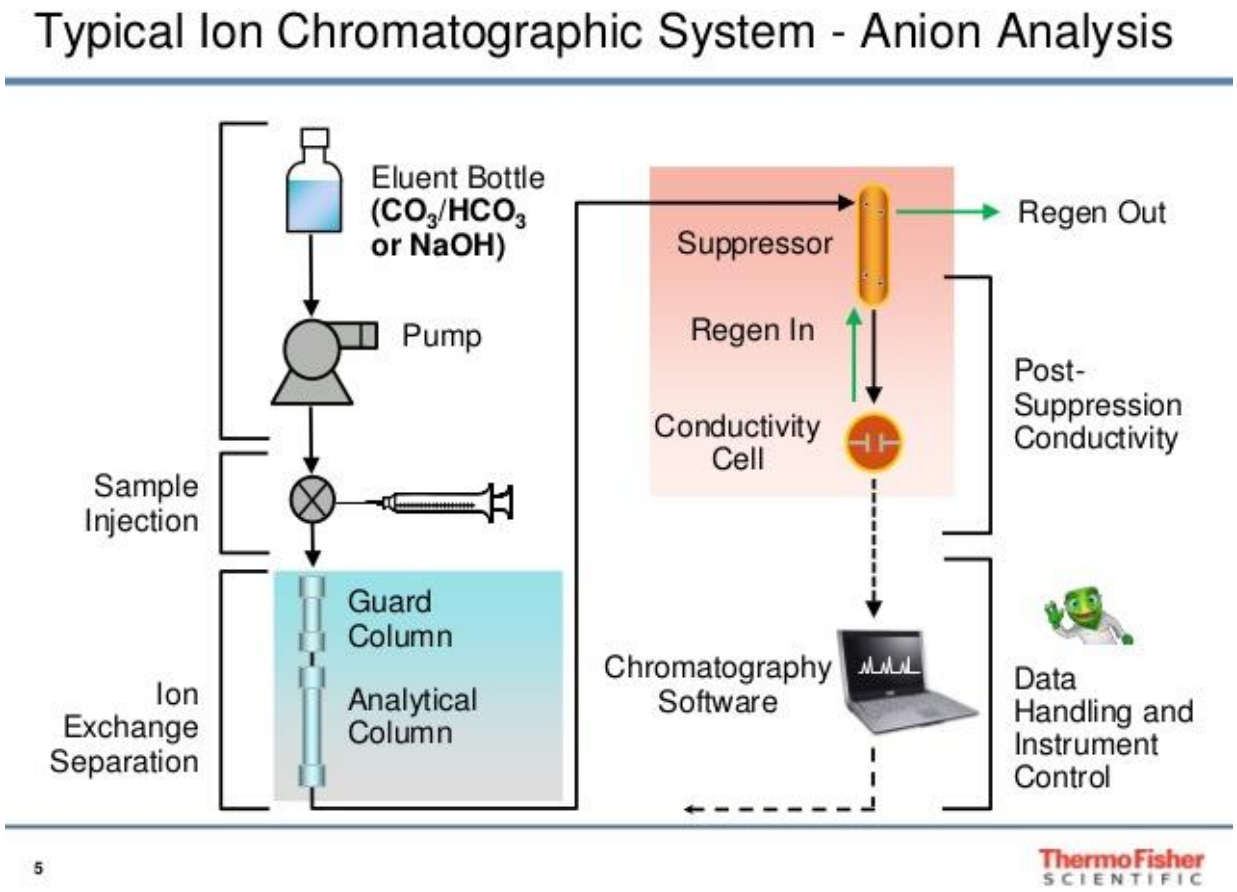
Amino Acids by Anion Exchange Using The AminoPac® PA1 Column



Amino acids by cation exchange chromatography



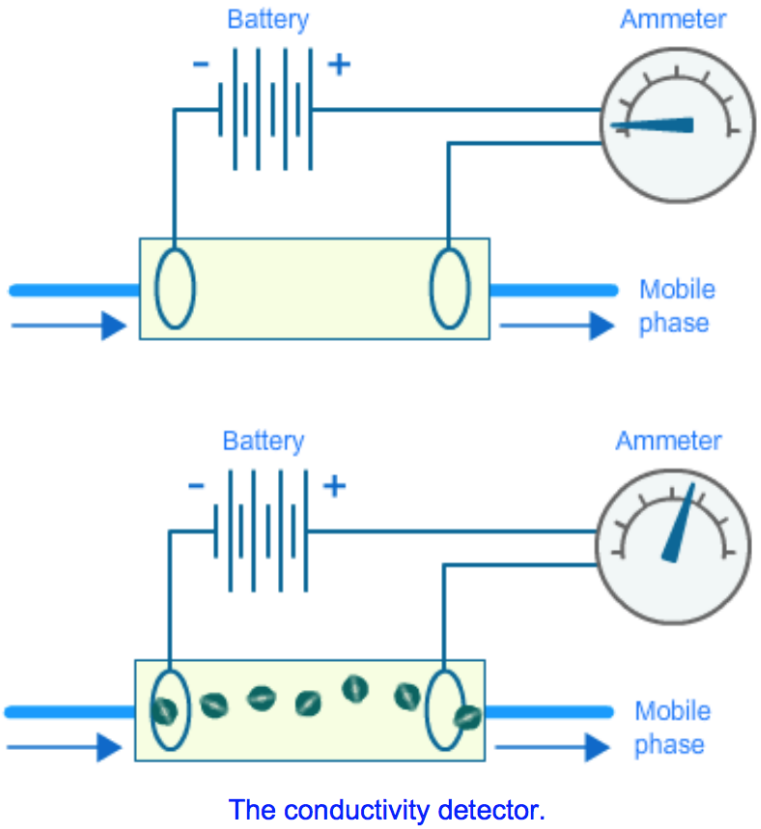
IEC instrumentation



Detectors

- Electrochemical Detection
  - Conductivity
  - Amperometry
  - Coulometry
  - Voltammetry
- Optical Detection
  - UV-Vis
  - Fluorescence
  - Refractive Index Others
- Mass Spectroscopy

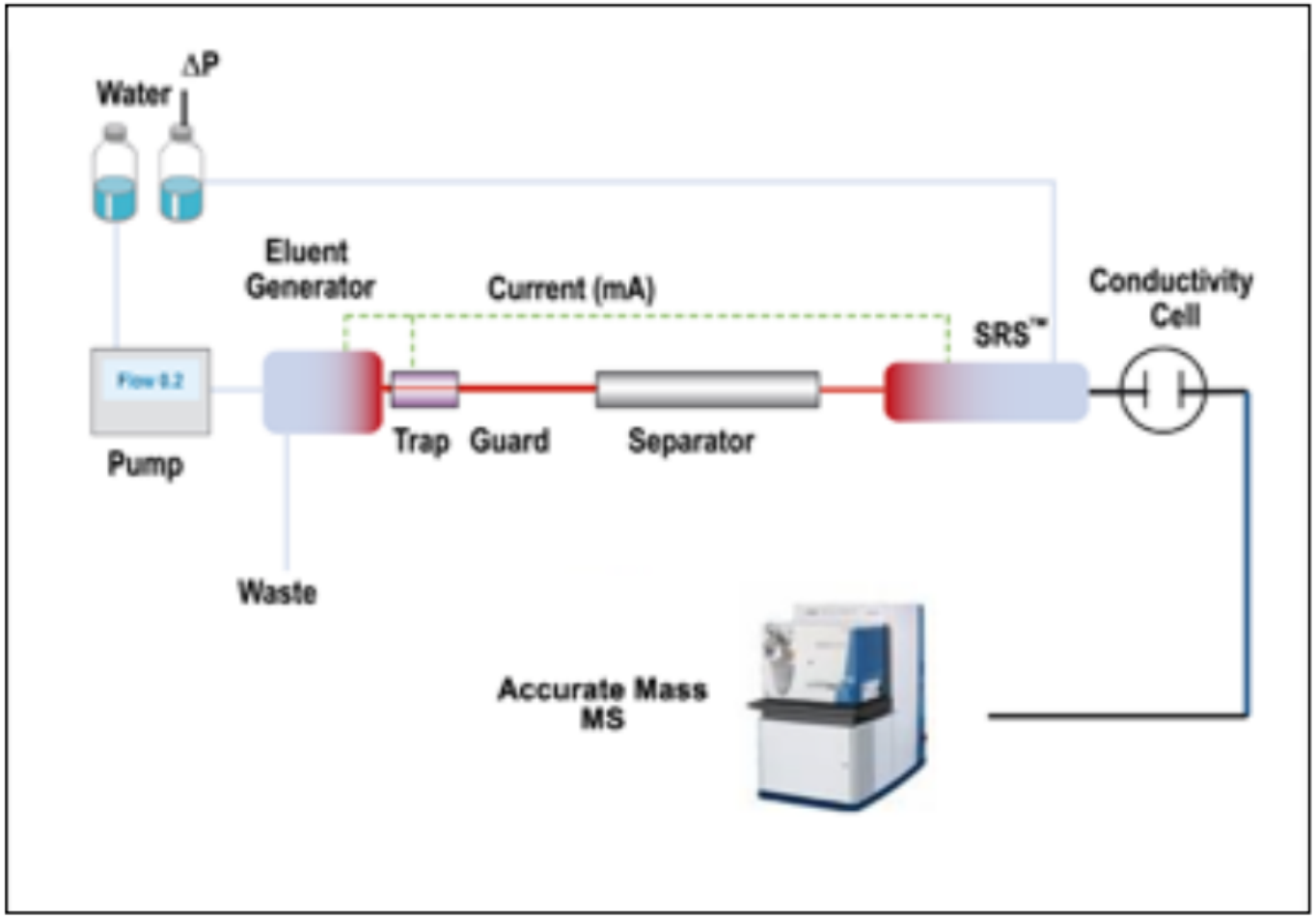
Conductivity: Most common



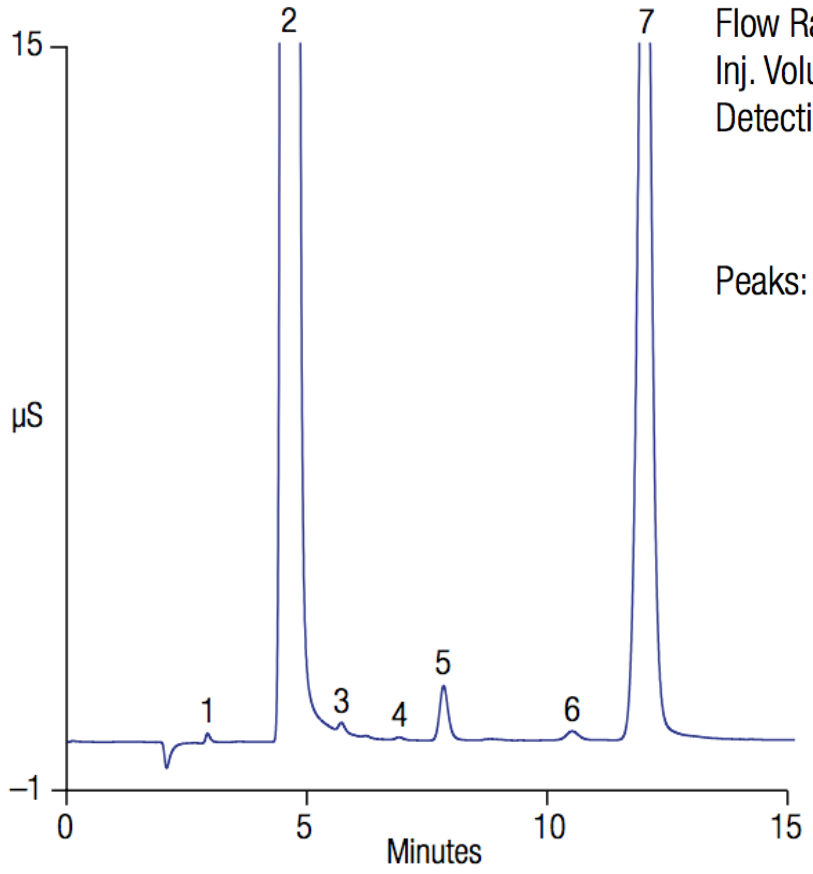
The conductivity detector.

- Conductivity is measured by a detection system consisting of two electrodes to which an alternating potential is applied. The corresponding current is proportional to the conductivity of the ionic solution in which the cell is dipped

Mass spectrometer



Determination of anions in drinking water



Separation of anions in municipal drinking water sample on the Dionex IonPac AS22 column using the Dionex ICS-900 system.

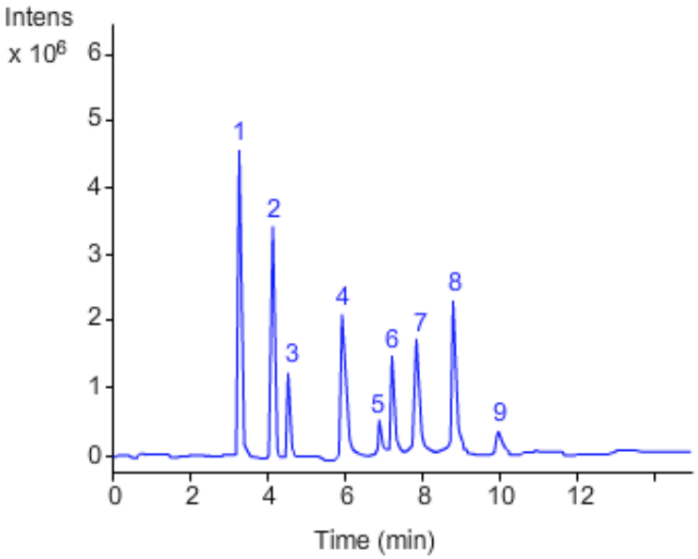
Column:	Dionex IonPac AG22/AS22, 4 mm
Eluent:	4.5 mM Sodium Carbonate 1.4 mM Sodium Bicarbonate
Column Temp.:	Ambient
Flow Rate:	1.2 mL/min
Inj. Volume:	25 µL
Detection:	Conductivity, Suppressed Conductivity Thermo Scientific™ Dionex™ AMMS™ 300 Anion MicroMembrane suppressor, 50 mM Sulfuric Acid

Peaks:		
1. Fluoride	0.19	mg/L
2. Chloride	98.1	
3. Nitrite	0.54	
4. Bromide	1.22	
5. Nitrate	2.43	
6. Phosphate	3.12	
7. Sulfate	48.2	

- Fluoride: bone disease, nitrite and nitrate: birth defects

Agrochemistry

Mono-chlorophenols (MCPs) and di-chlorophenols (DCPs) are used as disinfectant agents and as the base for different pesticides; however, due to new environmental regulations their use has been restricted.



IC-MS trace of a river water sample preconcentrated by SPE for gradient elution.

Sample: 1= 2-CP; 2= 4-CP; 3= 3-CP; 4= 2,6-DCP; 5= 2,3-DCP; 6= 2,5-DCP; 7= 2,4-DCP; 8= 3,4-DCP; 9= 3,5-DCP

Column: anion exchange column 250mm×4.0mm.

Eluent system: 0–4.5 min, 20 mM KOH; 4.5–10.0 min, 20–40 mM KOH (linear gradient); 10.0–12.0 min, 40 mM KOH.

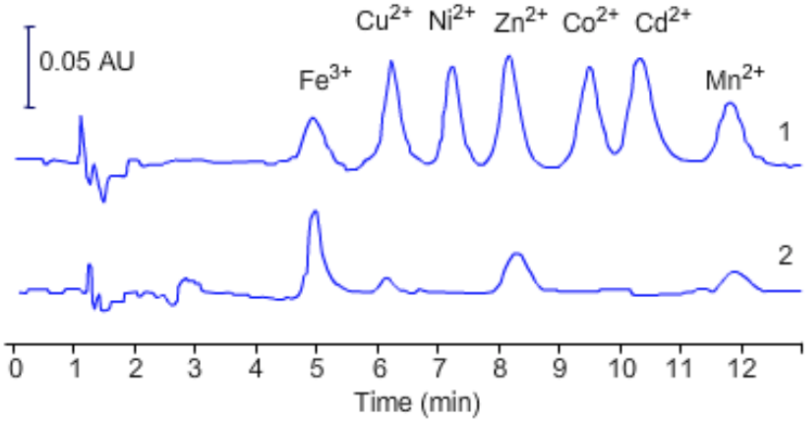
Eluent flow rate: 1.0 mL/min

Ref: Micong Jin, Yiwen Yang. "Simultaneous determination of nine trace mono- and di- chlorophenols in water by ion chromatography atmospheric pressure chemical ionization mass spectrometry" Analytica Chimica Acta 566 (2006) 193–199

clinical chemistry

Clinical Chemistry

The determination of metal ions in physiological fluids is of considerable diagnostic interest in clinical chemistry.



Separation of heavy metal ions with spectrophotometric detection (530nm) after post column derivatization. (1) standard solution, (2) serum sample.

Column: ethylvinylbenzene functionalized with ammonium and sulfonate functional groups 250mm×4.6mm, 9µm.

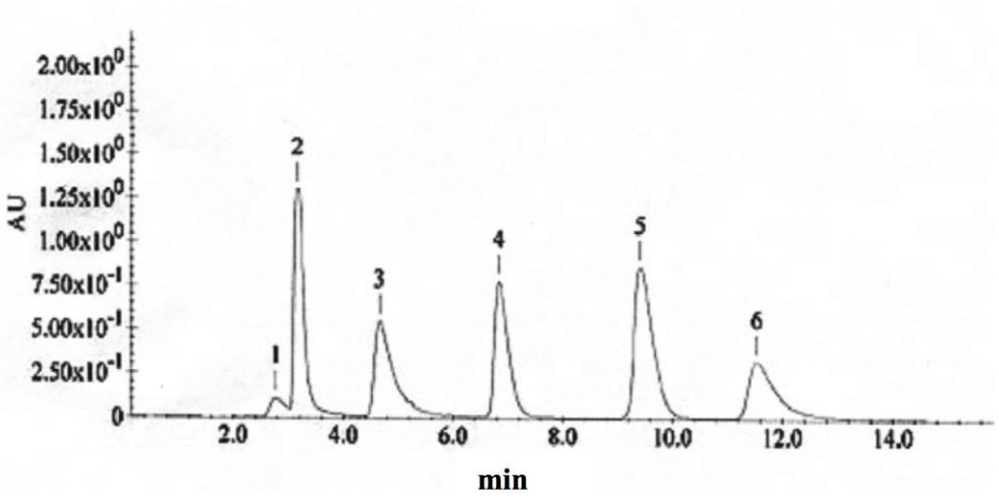
Eluent system: 1.4 mM pyridine-2,6-dicarboxylicacid + 13.2 mM potassium hydroxide + 1.1 mM potassium hydroxide + 14.8 mM formic acid (pH = 4.2 ± 0.1)

Detection: postcolumn reagent 0.5mM (4-(2-pyridylazo) resorcinol) + 1.0 M 2-dimethylaminoethanol + 0.5 M ammonium hydroxide + 0.3 M sodium bicarbonate (pH = 10.4 ± 0.2)

Eluent flow rate: 0.3mL/min

Ref: Anna Błażewicz, Grażyna Orlicz-Szczęsna, Andrzej Prystupa, Piotr Szczęsny. "Use of ion chromatography for the determination of selected metals in blood serum of patients with type 2 diabetes" Journal of Trace Elements in Medicine and Biology 24 (2010) 14–19

Transition metals ions



1: Pb2+, 2: Cu2+, 3: Cd2+, 4: Co2+,5: Zn2+, 6: Ni2+

Detector: UV-vis

Light absorbing complexes

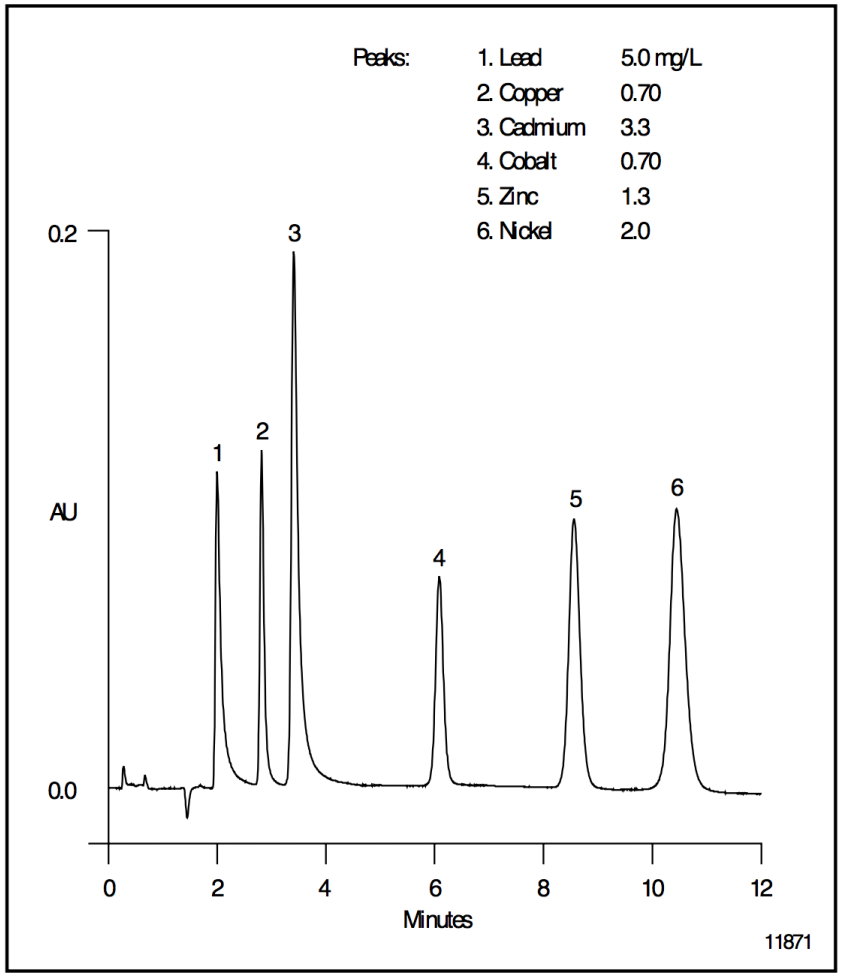


Figure 2 *Separation of transition metals using oxalic acid with the IonPac CS5A, method B conditions.*

Ref.: Theromofisher