Print version

The Introduction of Ion Chromatography

CEE 772

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1, Introduction

What is IC? What are their names of each part?

2, Principle

How does anion or cation be determined?

3, Process

How does IC work?

4, Application

Where can we use IC?

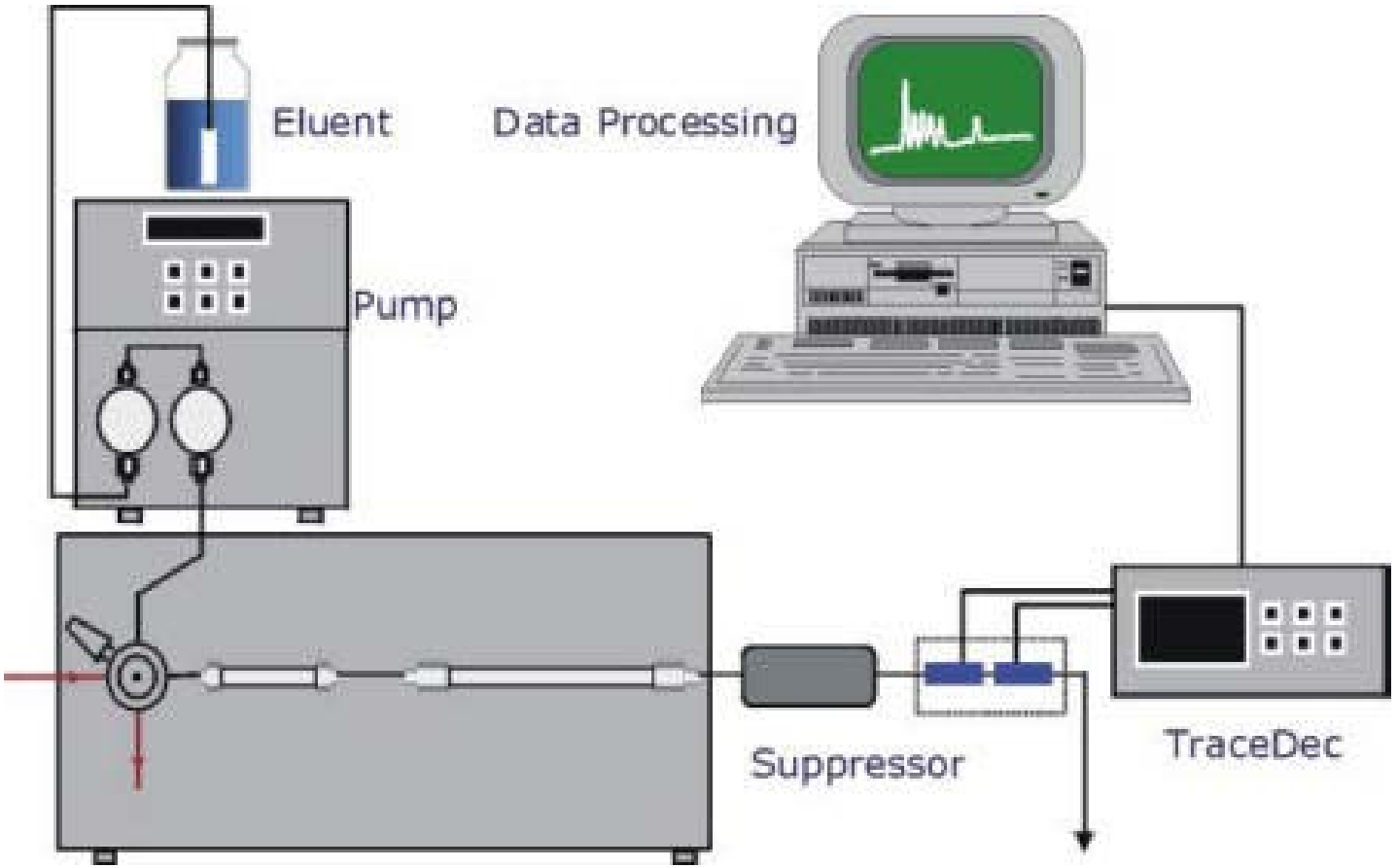
5, Operation Steps

How should I operate this machine properly?

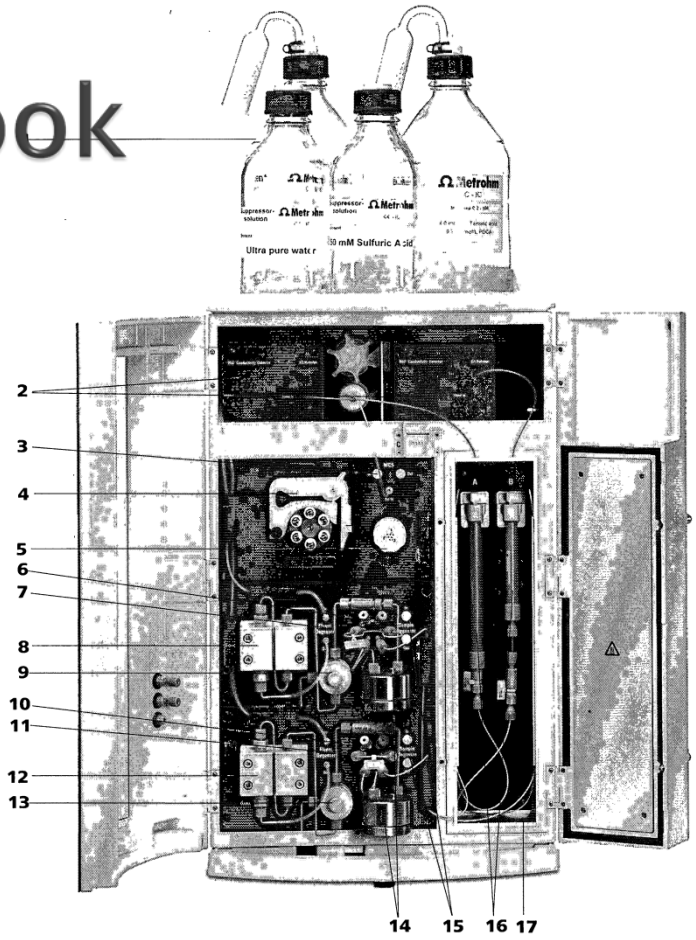
Introduction

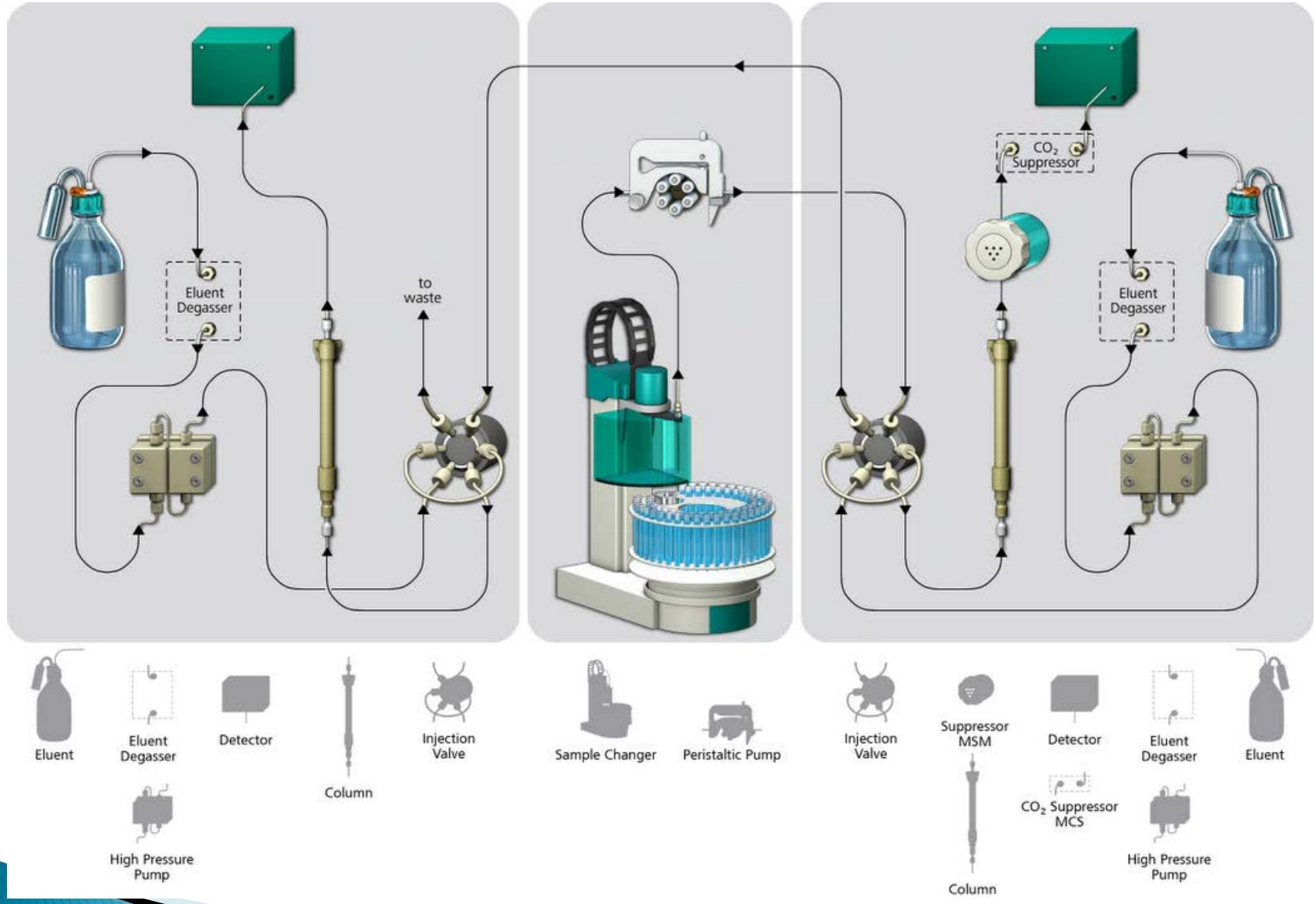
- ion chromatography is a process that allows the separation of ions and polar molecules based on their affinity to the ion exchanger. It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids. The solution to be injected is usually called a **sample**, and the individually separated components are called **analytes**. The media that carries ions are called **elutes**.

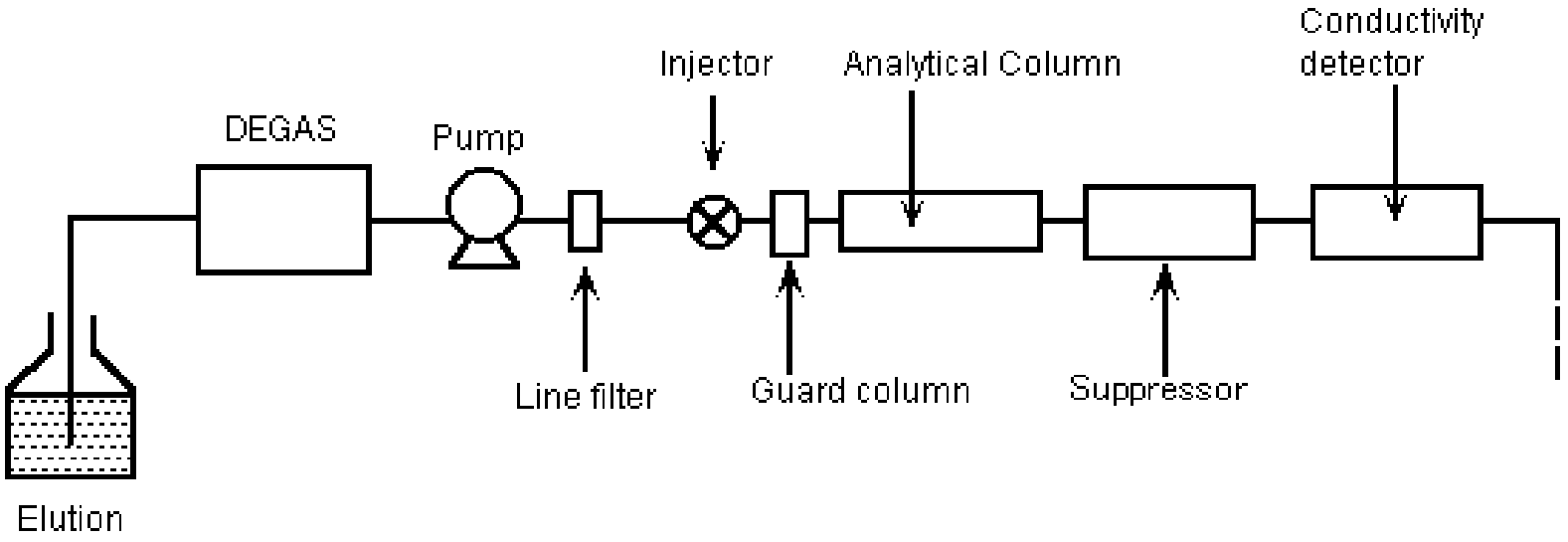

850 Professional IC
Metrohm
872 Extension Module
Metrohm
858 Professional Sample Processor


Eluent
Data Processing
Pump
Suppressor
TraceDec

A quick look at the IC




Eluent Degasser
to waste
CO_2 Suppressor
Eluent Degasser
Eluent
Eluent Degasser
Detector
Column
Injection Valve
High Pressure Pump
Sample Changer
Peristaltic Pump
Injection Valve
Suppressor MSM
Detector
Eluent Degasser
Eluent
CO_2 Suppressor MCS
High Pressure Pump
Column


Conductivity
detector
Injector
Analytical Column
DEGAS
Pump
Line filter
Guard column
Suppressor
Elution

Principle

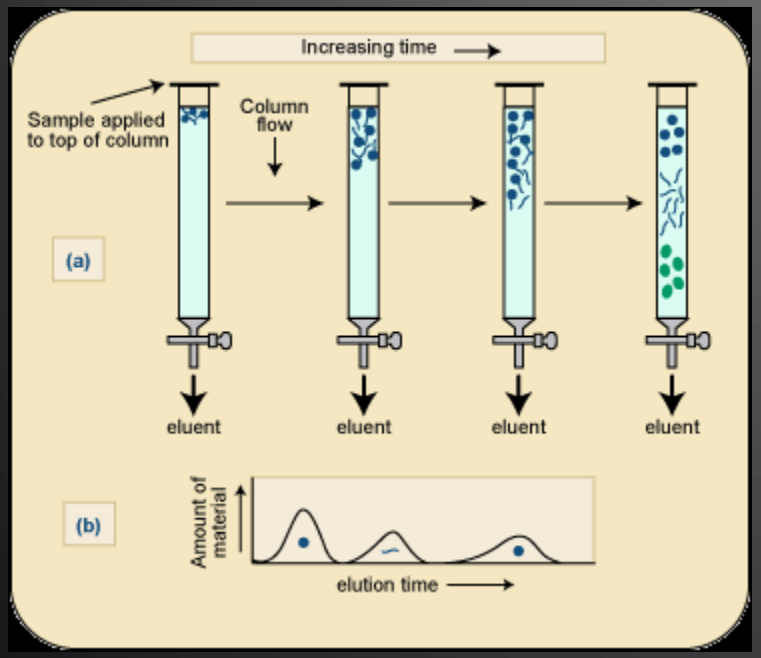
- Ion-exchange chromatography retains analyte molecules on the column based on coulombic (ionic) interactions. The stationary phase surface displays ionic functional groups (R-X) that interact with analyte ions of opposite charge. The ionic compound consisting of the cationic species M^+ and the anionic species B^- can be retained by the stationary phase.

- Cation exchange chromatography retains positively charged cations because the stationary phase displays a negatively charged functional group:
- $R\text{-}X^- C^+ + M^+ B^- \rightleftarrows R\text{-}X^- M^+ + C^+ + B^-$
- Anion exchange chromatography retains anions using positively charged functional group:

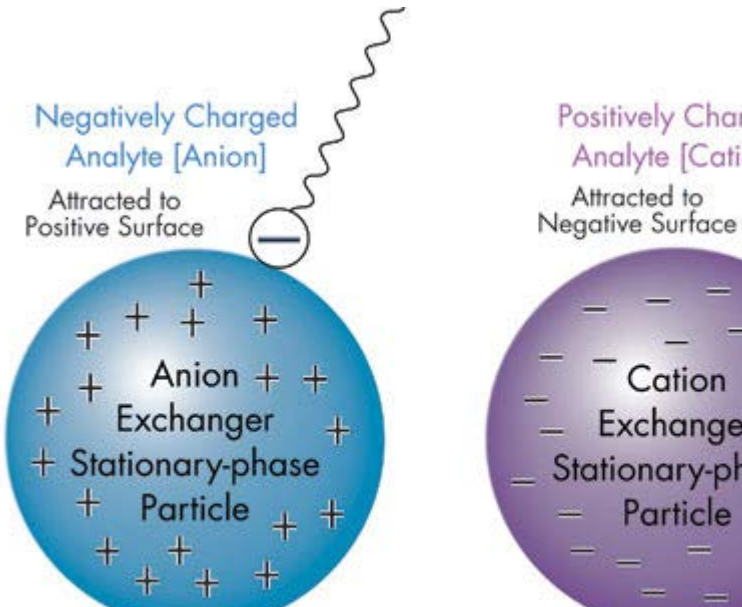
$$R\text{-}X^+ A^- + M^+ B^- \rightleftarrows R\text{-}X^+ B^- + M^+ + A^-$$

- Note that the ion strength of either C+ or A− in the mobile phase can be adjusted to shift the equilibrium position and thus retention time.
- The ion chromatogram shows a typical chromatogram obtained with an anion exchange column.

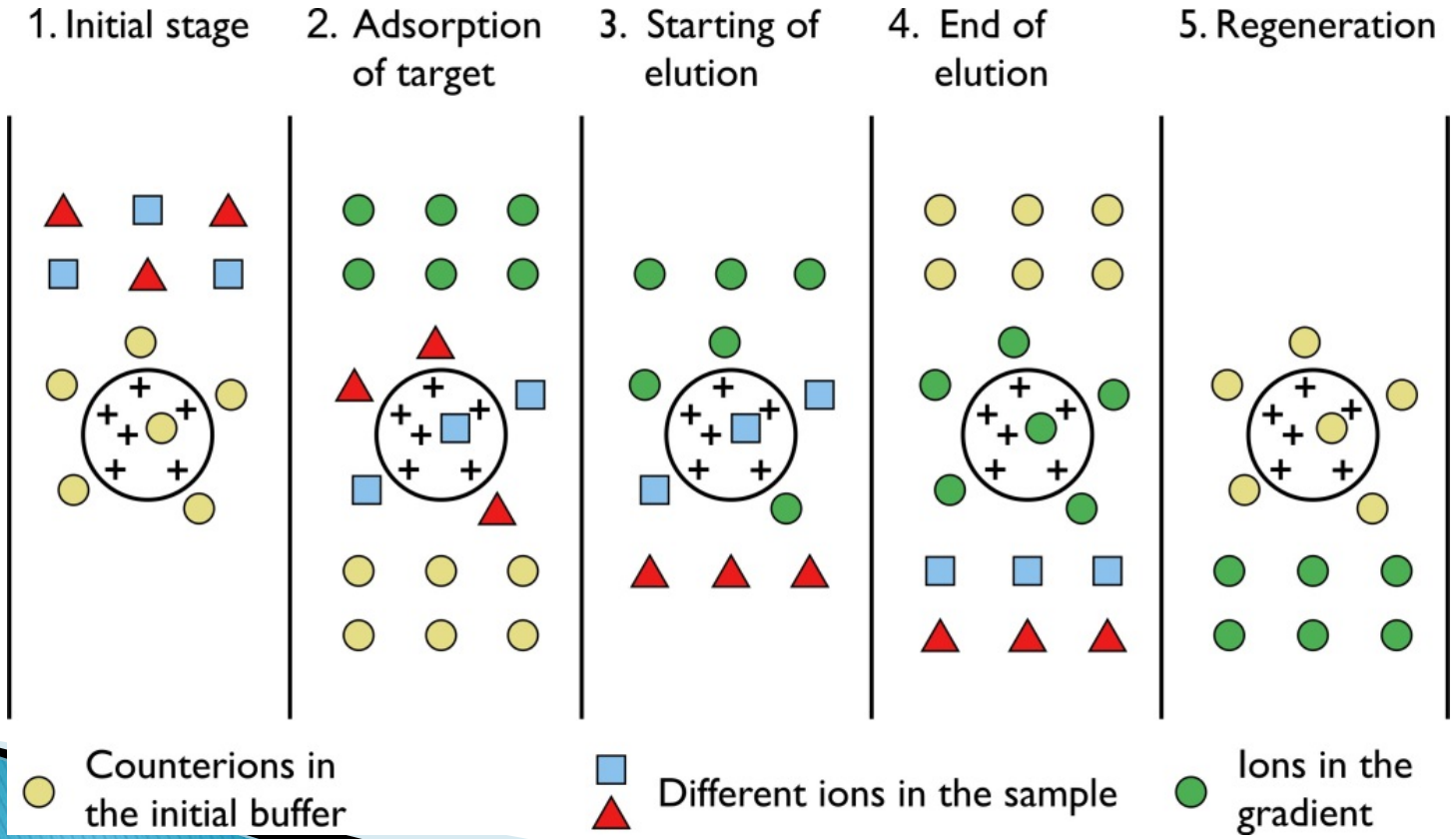
Method


Increasing time
Sample applied to top of column
Column flow
(a)
eluent
eluent
eluent
eluent
(b)
Amount of material
elution time

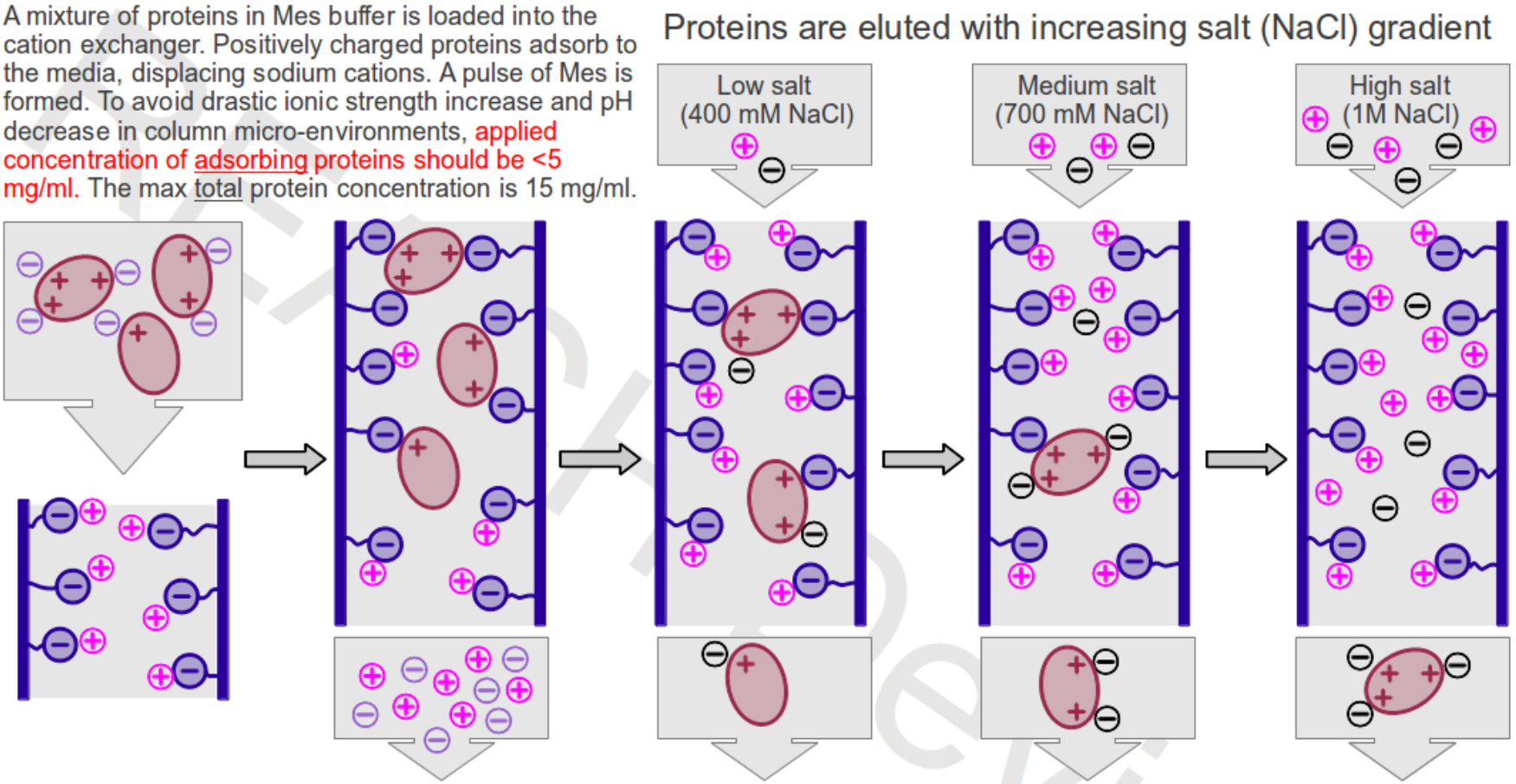
- A sample is introduced, either manually or with an **autosampler**, into a sample loop of known volume. A buffered aqueous solution known as the mobile phase carries the sample from the loop onto a column that contains some form of stationary phase material. This is typically a resin or gel matrix consisting of agarose or cellulose beads with covalently bonded charged functional groups. The target analytes(anions or cations) are retained on the stationary phase but can be eluted by increasing the concentration of a similarly charged species that will displace the analyte ions from the stationary phase.



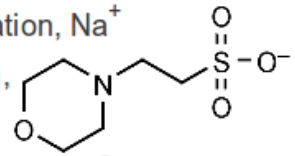
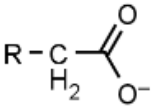
- For example, in cation exchange chromatography, the positively charged analyte could be displaced by the addition of positively charged sodium ions. The analytes of interest must then be detected by some means, typically by **conductivity** or **UV/Visible light absorbance.**

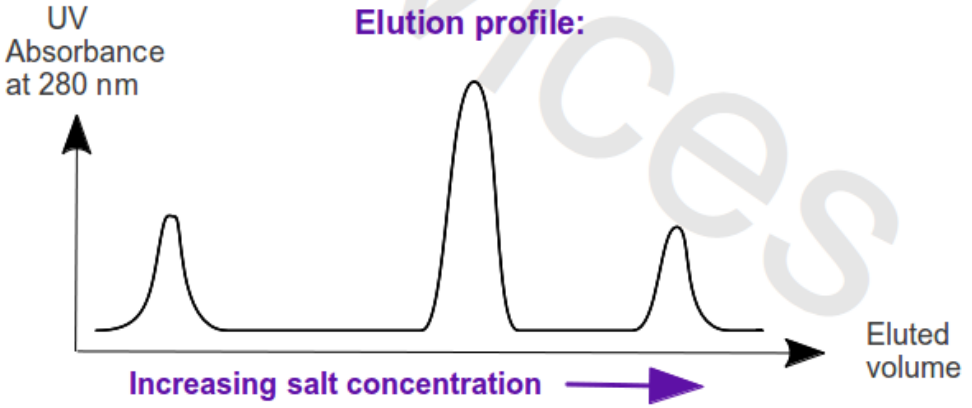


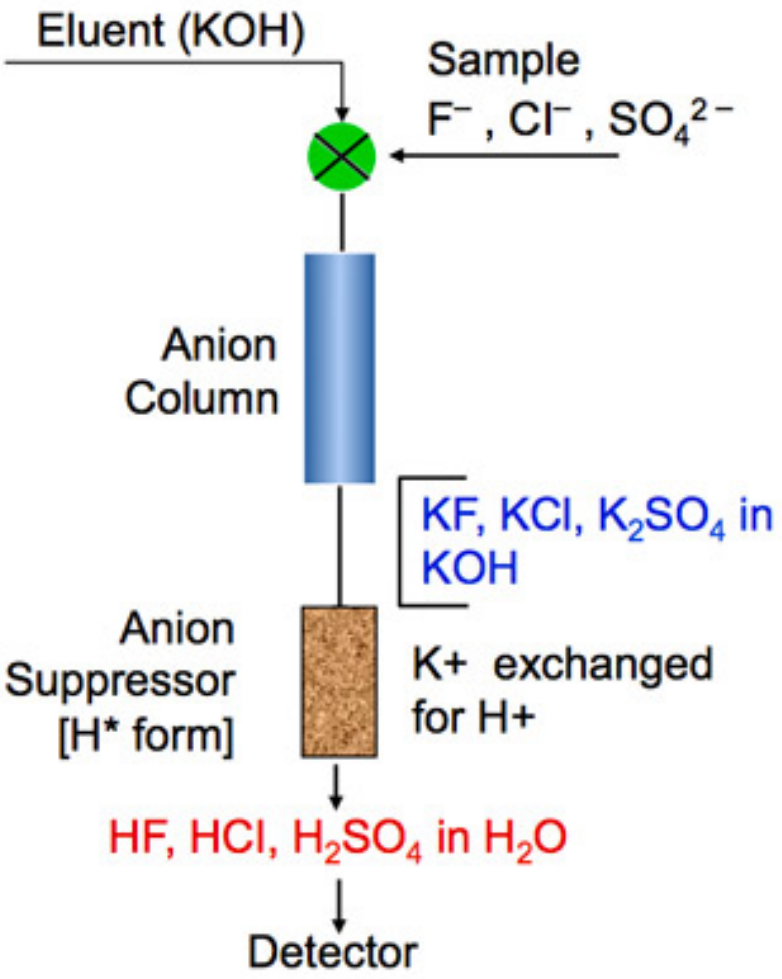
A mixture of proteins in Mes buffer is loaded into the cation exchanger. Positively charged proteins adsorb to the media, displacing sodium cations. A pulse of Mes is formed. To avoid drastic ionic strength increase and pH decrease in column micro-environments, applied concentration of adsorbing proteins should be <5 mg/ml. The max total protein concentration is 15 mg/ml.

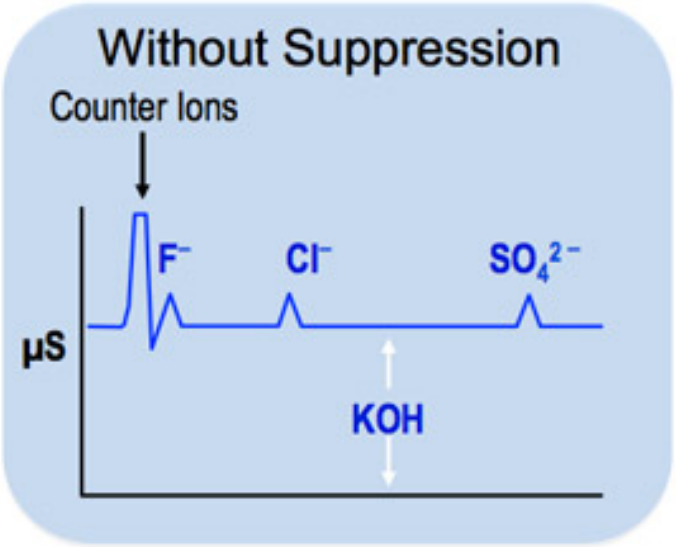


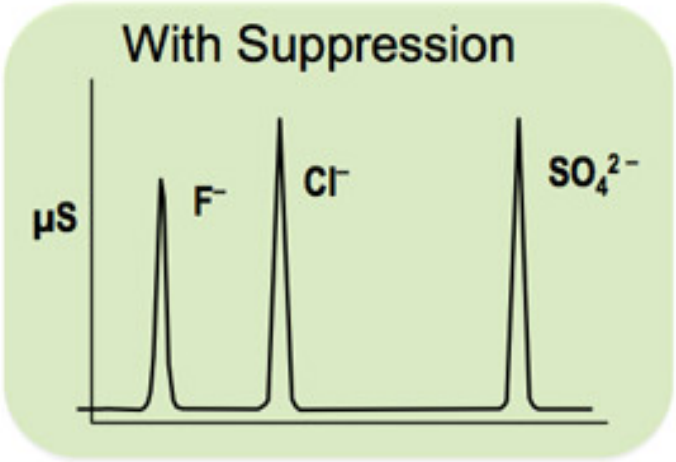
Legend:

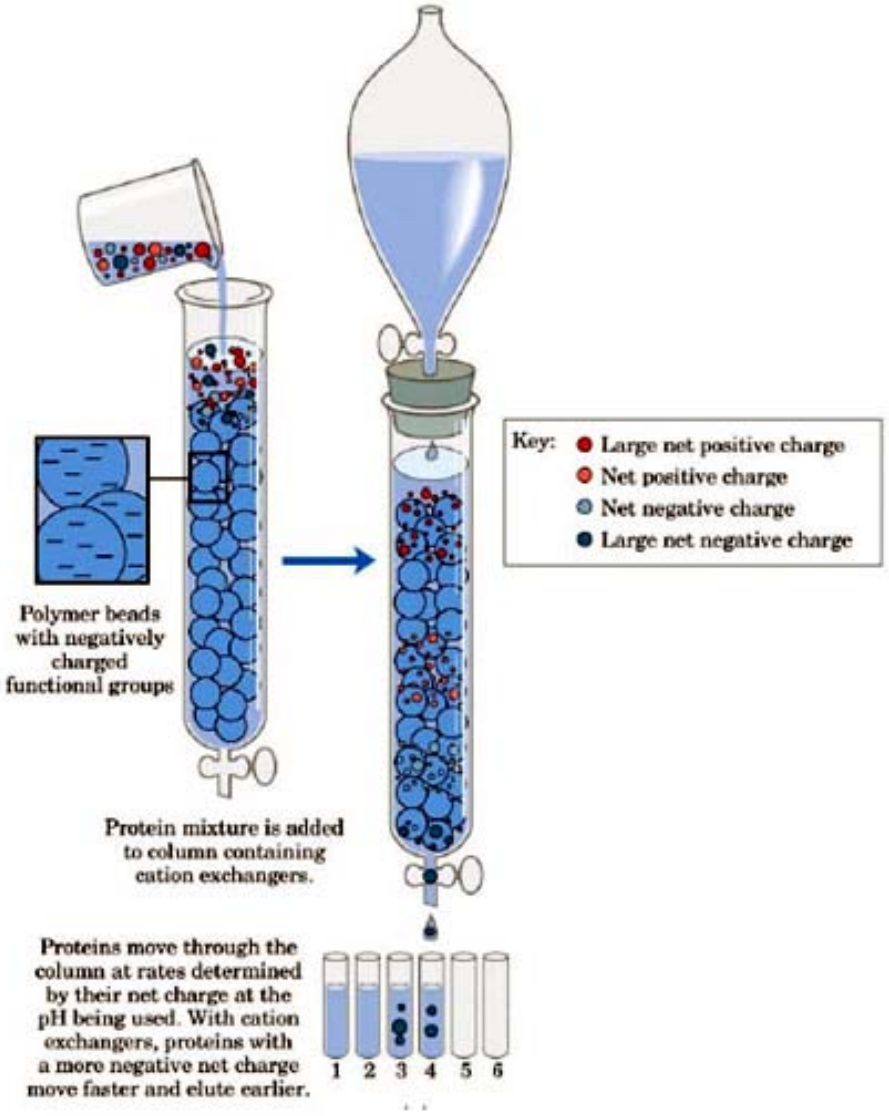
- ⊕ – Sodium cation, Na^+
- ⊖ – Mes anion, 
- ⊖ – Chloride anion, Cl^-
- ⊖ – Carboxymethyl anion (CM), 
- – Protein bearing a number of positive charges (as marked)

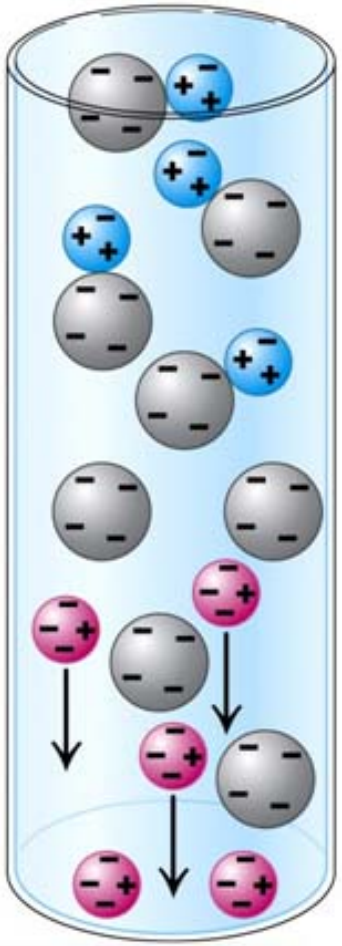



Eluent (KOH)
Sample
F^- , Cl^- , SO_4^{2-}
Anion
Column
KF, KCl, K_2SO_4 in
KOH
Anion
Suppressor
[H* form]
K+ exchanged
for H+
HF, HCl, H_2SO_4 in H_2O
Detector


Without Suppression
Counter Ions
F^-
Cl^-
SO_4^{2-}
µS
KOH


With Suppression
µS
F^-
Cl^-
SO_4^{2-}

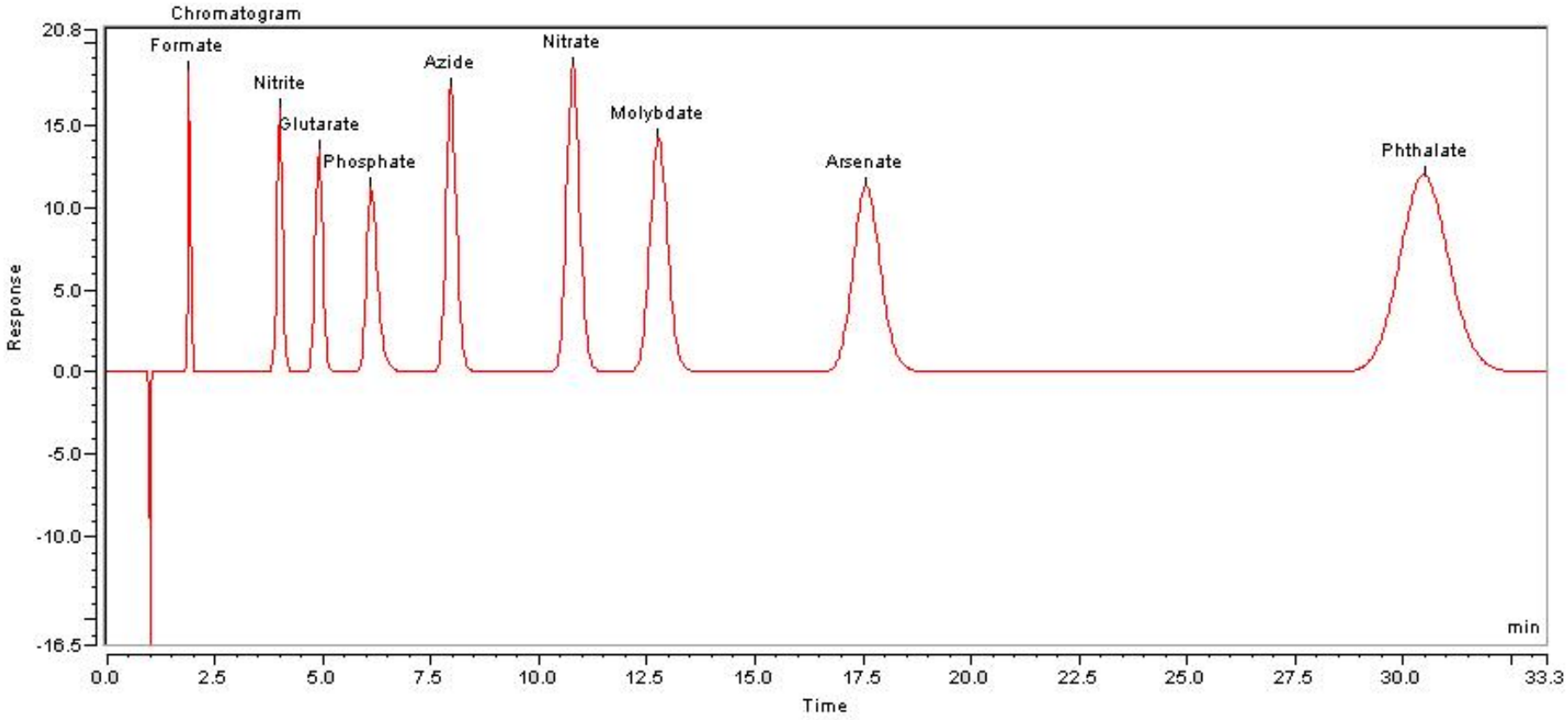




Positively charged protein binds to negatively charged bead

Negatively charged protein flows through

Results


Chromatogram
Formate
Nitrite
Glutarate
Phosphate
Azide
Nitrate
Molybdate
Arsenate
Phthalate
Response
Time
min
20.8
15.0
10.0
5.0
0.0
-5.0
-10.0
-16.5
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
30.0
33.3

Uses

- **Clinical utility**
  Used in measurement of porphyrin & water purification.
- **Industrial Applications** Allows for quantitative testing of electrolyte and proprietary additives of electroplating baths. It is an advancement of qualitative cell testing or less accurate UV testing. Ions, catalysts, brighteners and accelerators can be measured.

Operation Steps

- 1, Preparation of elutes.
- 2, Dilute the sample into a properly concentration.
- 3, Preparation of standard solutions. (0.2 mg/L to 10 mg/L)
- 4, Place sample and solutions on the autosampler.
- 5, Run the program

References

- 1, Eith, Claudia, Kolb Maximilian, and Seubert Andreas. "Introduction." Practical Ion Chromatography An Introduction. Ed. Viehweger Kai. Herisau: Metrohm, 2002. 160.
- 2, Sakash, J.B.; Kantrowitz, E.R. (2000). "The contribution of individual interchain interactions to the stabilization of the T and R states of Escherichia coli aspartate transcarbamoylase.". J Biol Chem 275 (37): 287017. doi:10.1074/jbc.M005079200. PMID 10875936.
- 3, Fairhead, M. (2013). "Plug-and-Play Pairing via Defined Divalent Streptavidins.". J Mol Biol 426 (1): 199-214. doi:10.1016/j.jmb.2013.09.016. PMID 24056174.
- 4, b Robert E. Smith (31 December 1987). Ion Chromatography Applications. CRC Press. ISBN 978-0-8493-4967-6.

Thank you!

To next lecture