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.
A quick look at the IC
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:

\[ R-X^+C^+ + M^+B^- \rightleftharpoons R-X^-M^+ + C^+ + B^- \]

Anion exchange chromatography retains anions using positively charged functional group:

\[ R-X^+A^- + M^+B^- \rightleftharpoons R-X^+B^- + M^+ + A^- \]

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
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**.

|------------------|-------------------------|------------------------|------------------|----------------|

- **Counterions in the initial buffer**
- **Different ions in the sample**
- **Ions in the gradient**
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 a drastic ionic strength increase and pH decrease in column micro-environments, the applied concentration of adsorbing proteins should be <5 mg/ml. The maximum total protein concentration is 15 mg/ml.

Proteins are eluted with increasing salt (NaCl) gradient:
- Low salt (400 mM NaCl)
- Medium salt (700 mM NaCl)
- High salt (1 M NaCl)

Legend:
- Sodium cation, Na⁺
- Mes anion, $\text{O} - \text{N} - \text{O} - \text{S} - \text{O} -$
- Chloride anion, Cl⁻
- Carboxymethyl anion (CM), $\text{R} - \text{C} - \text{O} - \text{H}_2$
- Protein bearing a number of positive charges (as marked)

UV Absorbance at 280 nm

Elution profile:
- Increasing salt concentration
- Eluted volume
Polymer beads with negatively charged functional groups

Protein mixture is added to column containing ion exchangers.

Proteins move through the column at rates determined by their net charge at the pH being used. With ion exchangers, proteins with a more negative net charge move faster and elute earlier.

Key:
- Large net positive charge
- Net positive charge
- Net negative charge
- Large net negative charge

Positively charged protein binds to negatively charged bead

Negatively charged protein flows through
Results
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.
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


Thank you!

To next lecture