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INAF INSTITUTE OF NUTRITION AND FUNCTIONAL FOODS

UNIVERSITÉ LAVAL
Faculty of Agriculture and Food Sciences

# Methods of Analysis for Organic Chemical Contaminants in Food

## Module 4 - Liquid Chromatography

# Learning Objectives

Basic Analytical Techniques

1. Understand the principles of the analytical technique of liquid chromatography

1. Understand the detection techniques used with liquid chromatography

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# LESSON 1
# General Chromatography

# What is Chromatography?

Basic Analytical Techniques

- Separation of a mixture
- Solution or suspension or as a vapor
- Medium in which the components move at different rates

# What is Chromatography?

Basic Analytical Techniques

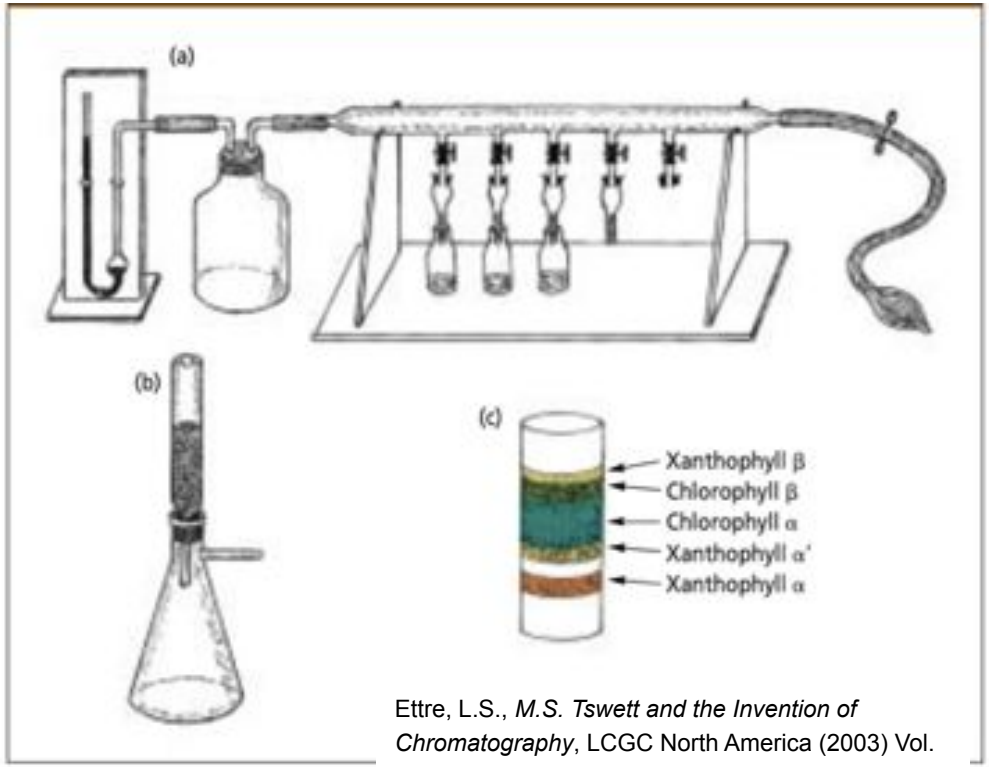
The separation of a mixture by passing it in solution or suspension or as a vapor (as in gas chromatography) through a medium in which the components move at different rates

Oxford Dictionnary

# Mikhail S. Tswett (1903)



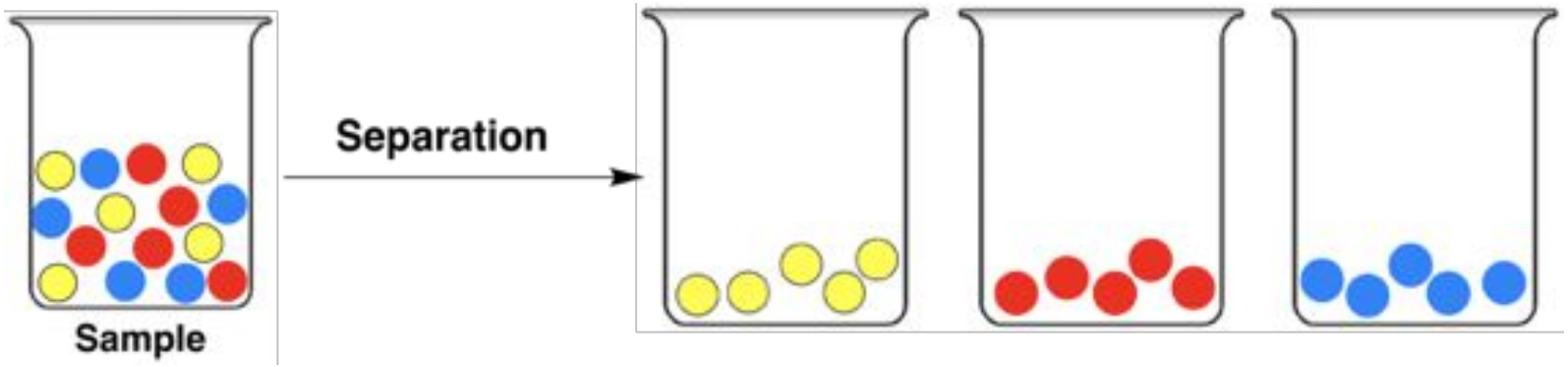
Objective was to separate plant pigments




Ettre, L.S., *M.S. Tswett and the Invention of Chromatography*, LCGC North America (2003) Vol. 21, No 5., pp458-467 Available at: https://cdn.sanity.io/files/0vv8moc6/chroma/1d8bd9c34045ef61d93b9002b40e9335d19de2ed.pdf

# Chromatography - with colors

Basic Analytical Techniques



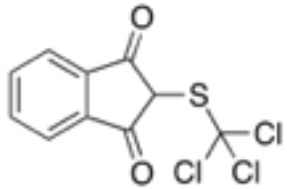

waters.com

# Objective of Using Chromatography

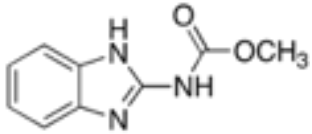
Basic Analytical Techniques

- Separate contaminants of interest from:
  - Matrix (water, proteins, fat, carbohydrates, etc)
  - Other contaminants

Separation



Folpet



Carbendazim

# State of the Sample



- Solution:
  - Liquid chromatography
  - Ion chromatography
  - Thin layer chromatography (also paper)
- Suspension:
  - Size exclusion chromatography
- Vapor:
  - Gas chromatography

# Principles of Separation

Basic Analytical Techniques

- Binding interactions
  - Affinity chromatography
- Charge
  - Ion exchange chromatography
- Hydrophobic interactions
  - Hydrophobic interaction chromatography
- Size
  - Size exclusion chromatography

# Affinity Chromatography

Basic Analytical Techniques

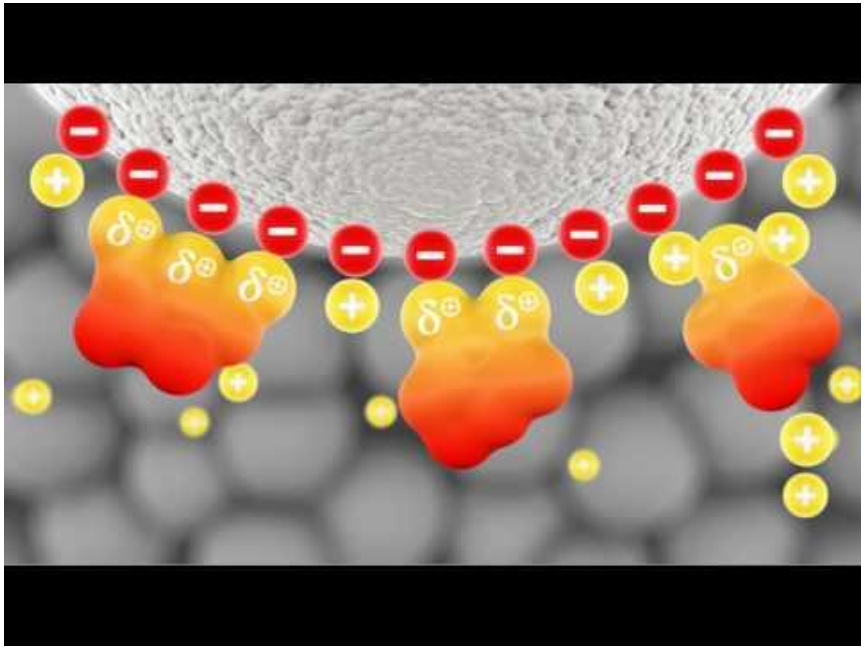
- Specific interactions between molecules
  - Protein – antibody
  - Enzyme – substrate
  - Polarity

- Advantage: Uses the biological structure or function of the molecule

# Ion Chromatography

Basic Analytical Techniques

- Electrostatic interactions between opposite charges
  - Proteins based on isoelectric point
  - Uses pH
- Advantage: Separate similar molecules by manipulating their charge



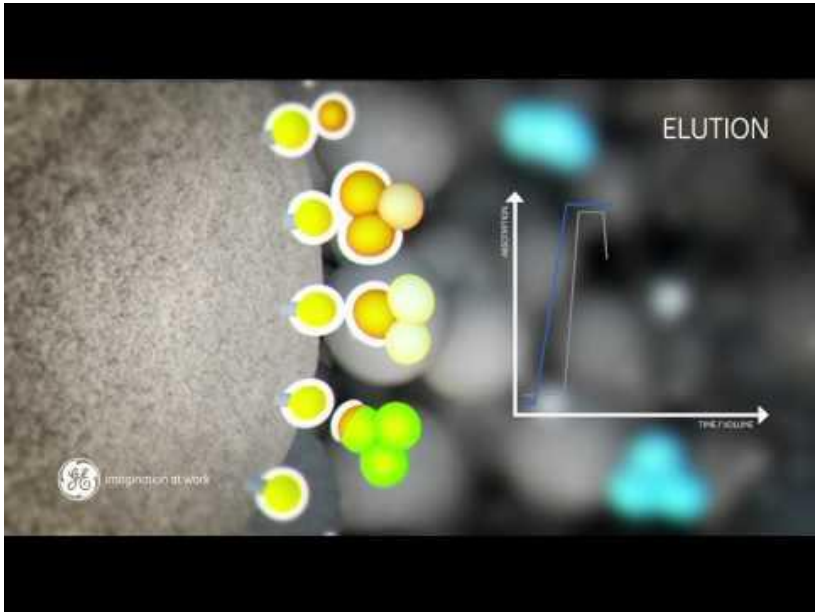
https://youtu.be/lp40a7mtc4E

# Hydrophobic Interactions Chromatography



- Hydrophobicity
  - Interaction can be modulated by differing salt concentration, pH, temperature, and organic solvent concentration

- Advantage: Very good for purifying proteins while maintaining biological activity

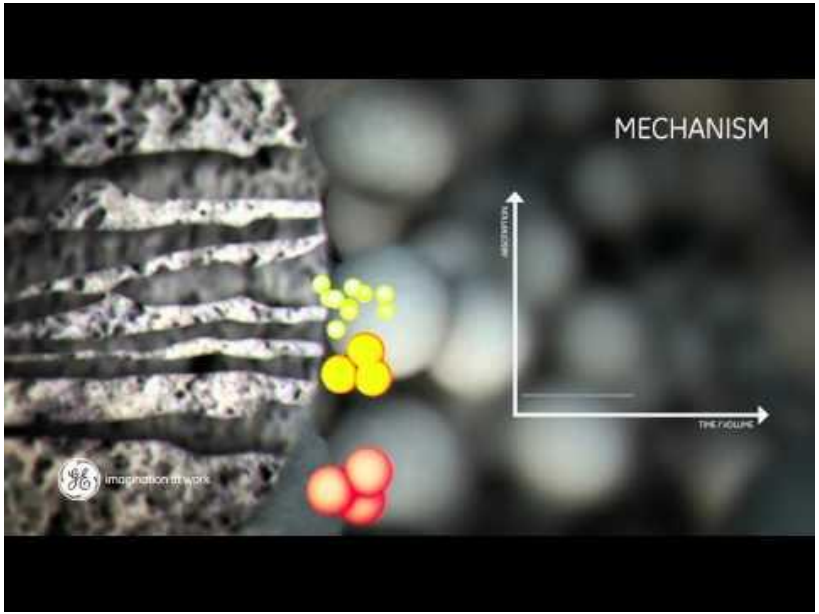



https://youtu.be/v6SPK6ZovgA

# Size Exclusion Chromatography

Basic Analytical Techniques

- Size
  - Gel composed of beads with known pore size is used
  - Very useful for fractionation and desalting
  - Aka Gel permeation chromatography
- Advantage: No chemical interaction, so complementary to other chromatographic separation principles.




https://youtu.be/oV5VB5kO3tQ

# Terminology

Basic Analytical Techniques

- Chromatography means a set of laboratory ***techniques*** for the separation of mixtures
- Chromatograph is a piece of ***equipment*** used to produce a chromatogram
- Chromatogram is a ***chart*** generated by a chromatograph
- You run a technique called liquid chromatography; the instrument used is called a liquid chromatograph; the data generated is called a chromatogram.

# Chromatography in Food Safety

Basic Analytical Techniques

- ➔ Chromatography, especially liquid chromatography, is essential for contaminants separation, quantitation and confirmation
- ➔ Modern sample preparation methods AND flexibility of modern columns allow for relatively simple methods with broad scope
- ➔ Organic food contaminants are mostly measured by liquid chromatography

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# LESSON 1
## End