Questions on ion exchange chromatography

1. Two amino acids, serine (pI = 5.68) and tryptophan (pI = 5.89), are to be separated by cation exchange chromatography on beads coated with sulfonic acid groups (pKa = -2).

a) If the mobile phase is at pH 4, which amino acid forms the stronger interaction with the beads (and will elute second)? Justify.
b) If the pH is increased slowly during the separation, would this help better separating the two amino acids?

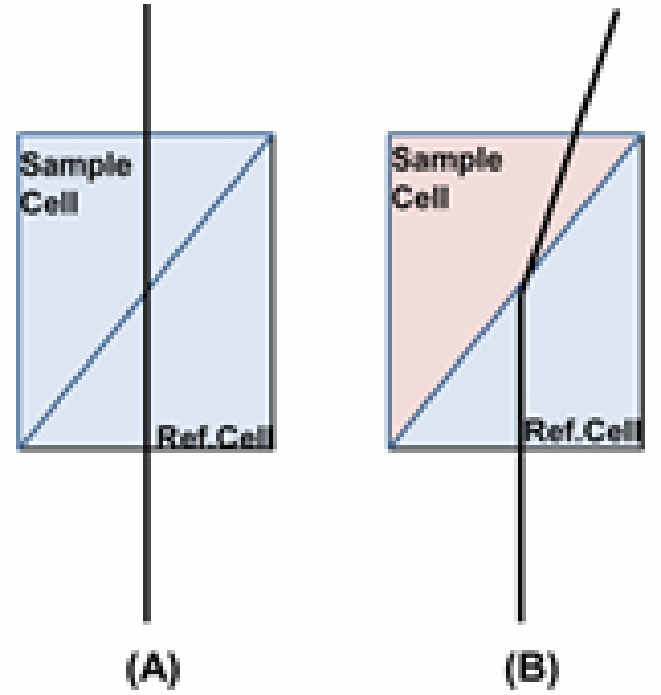
2. What kind of stationary phase would be appropriate for anion exchange chromatography?

3. Ion exchange chromatography experiments may be conducted with gravity columns or HPLC columns using the same type of stationary phase. Compare both methods with respect to particle size, resolution, efficiency of separation, and time of analysis

HPLC detection methods other than UV and fluorescence

Refractive-Index Detector

- Glass cell divided into two chambers, the sample cell and the reference cell.
- Effluent from LC column flows through sample cell while reference cell is filled with only mobile phase.
- With no analyte in LC effluent, solvents are the same (A).
- With components other than the mobile phase, the refraction index is modified. Monitoring this change (B) is used to detect compounds and their concentrations.



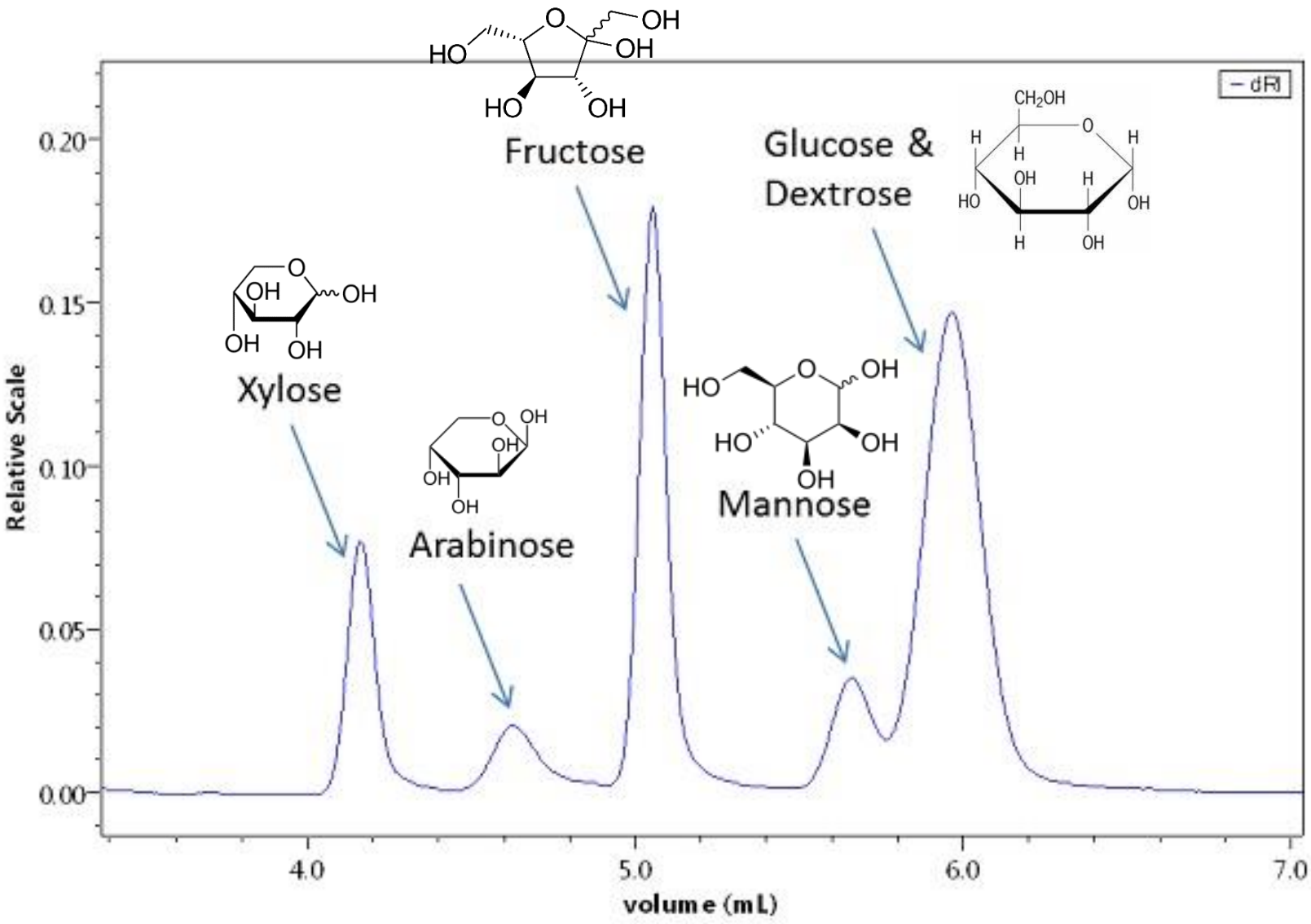
https://shodexhplc.com/lessons/lesson-6-detectors-for-hplc/

Refractive-Index Detector

Lower sensitivity compared to UV detector for UV-abs compounds, and cannot be used with a solvent composition gradient.

A few advantages:

- Suitable for detecting all components. Sugars, alkanes, alcohols, inorganic ions cannot be detected by UV detector. Changes in refraction index occur for all analytes.
- Can be used with solvents that absorb in UV.
- Provides direct relationship between intensity and analyte concentration.

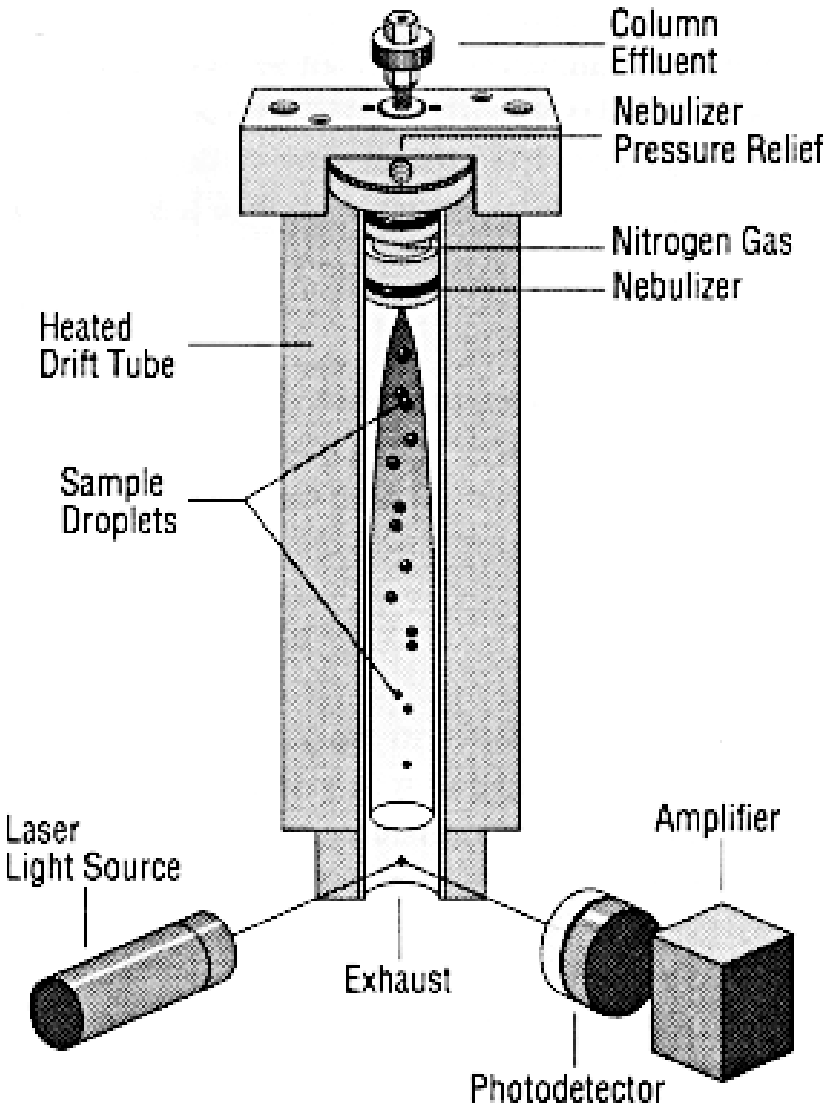


https://www.azom.com/article.aspx?ArticleID=10390

Evaporative Light Scattering Detector (ELSD)

- Good sensitivity for non-volatile analytes at the ng level.
- Column effluent nebulized, then solvent evaporated to form fine particles.
- Analyte is then irradiated with a laser beam and the scattered radiation is detected.
- Target samples include lipids, sugars, and high MW compounds.
- Gradient method is possible for mobile phase composition.

Evaporative Light Scattering Detector (ELSD)

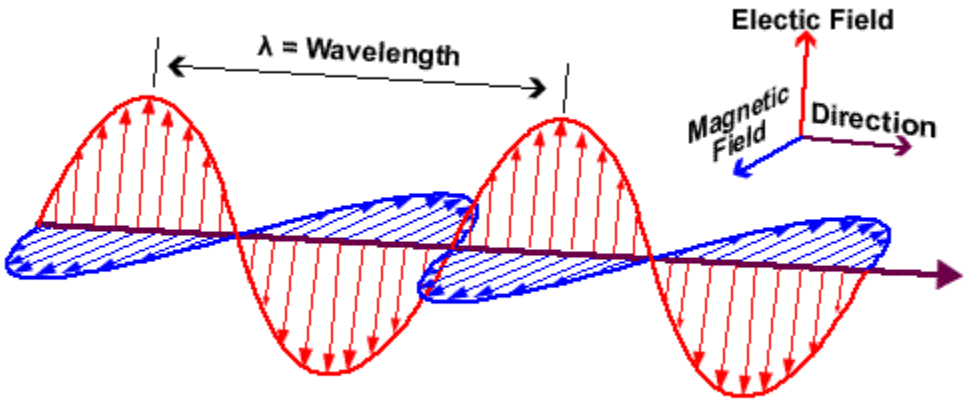


http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_ltsc.html

- In a typical light scattering experiment, a single frequency, polarized light beam from a laser illuminates a macromolecules or nanoparticles, either dry or in solution.

- The electric field of the polarized light beam is produced perpendicular to the plane in which the intensity and angular dependence of the subsequently scattered light is to be measured.

- By convention, the polarization direction is denoted ‘vertical’ and the measurement plane ‘horizontal’.



- The overall intensity carries information about the molar mass.

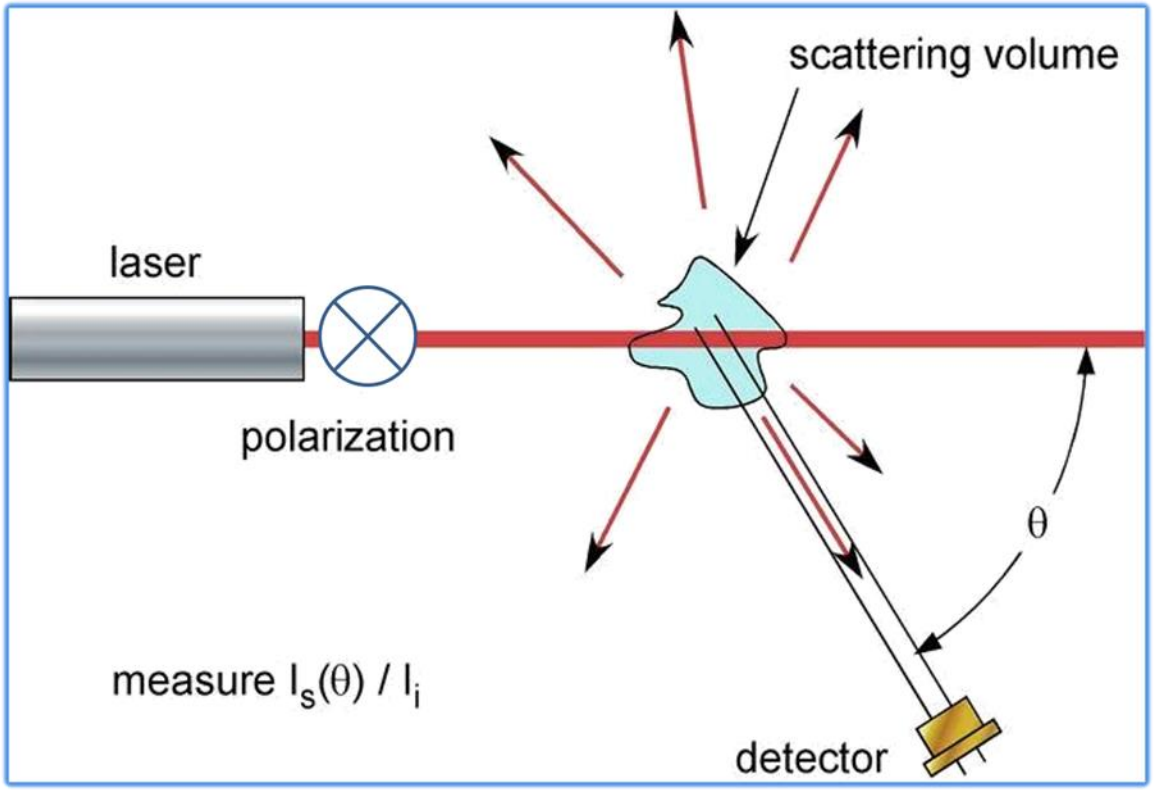
- The oscillating electric field of the light induces an oscillating dipole within the macromolecules analytes link

- This oscillating dipole will re-radiate (scatter) light, much like the antenna for a radio station sends out radio waves.

- The intensity of the radiated light depends on the magnitude of the dipole induced in the macromolecule.

- The more polarizable the macromolecule, the larger the induced dipole, and hence, the greater the intensity of the scattered light.

- In general larger molecules produce more intense scattered light as they are more polarizable.



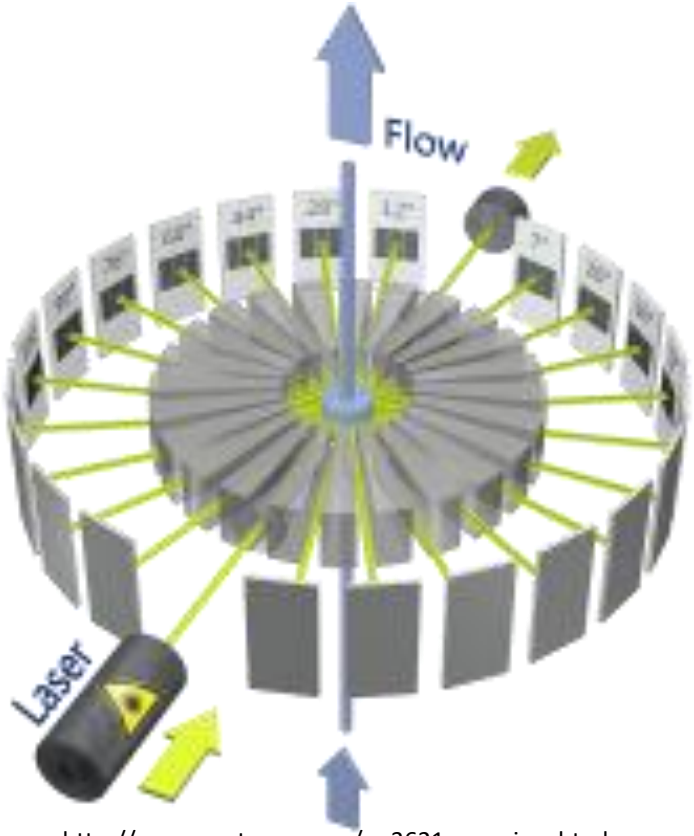
https://www.wyatt.com/solutions/techniques/sec-mals-molar-mass-size-multi-angle-light-scattering.html

Multi-Angle Light Scattering Detector (MALS)

- For GPC/SEC analysis, MW of analyte is usually estimated from a calibration curve built using known MW standards.

- With MALS, MW can be determined, + shape of the molecule.

- The overall intensity carries information about the molar mass, while the angular dependence within the horizontal plane carries information about the size of the macromolecule.



http://www.postnova.com/pn3621-overview.html

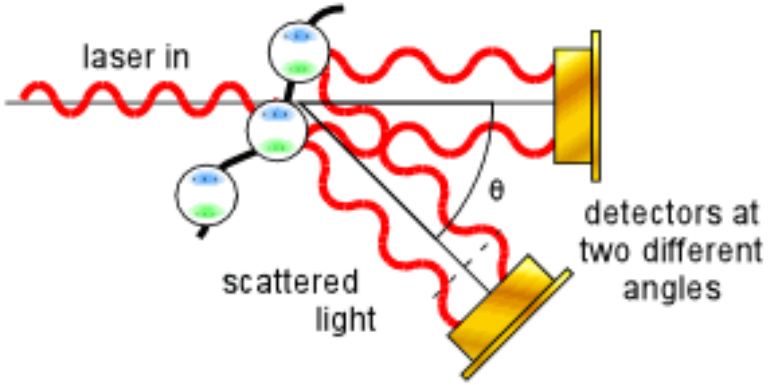
- Molecules much smaller than the wavelength of the incident light can be treated as if they were point scatterers.

- For such very small molecules, the light scattered into the perpendicular plane is independent of scattering angle. It is the same at every scattering angle – **small molecules scatter light isotropically**.

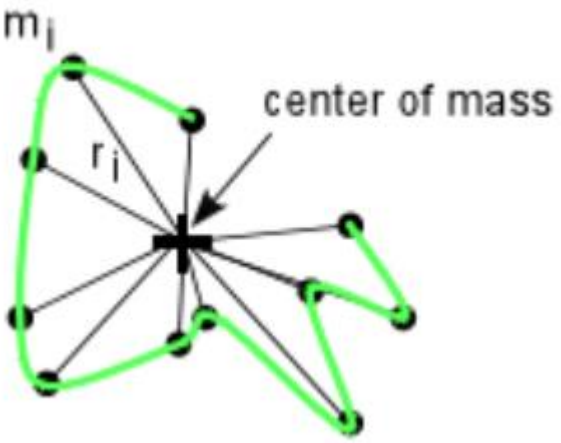
- For larger macromolecules, light scattered from different parts of the macromolecule reach the detector with different phases.

- This can lead to destructive or constructive interference of the overall light wave at the detector.

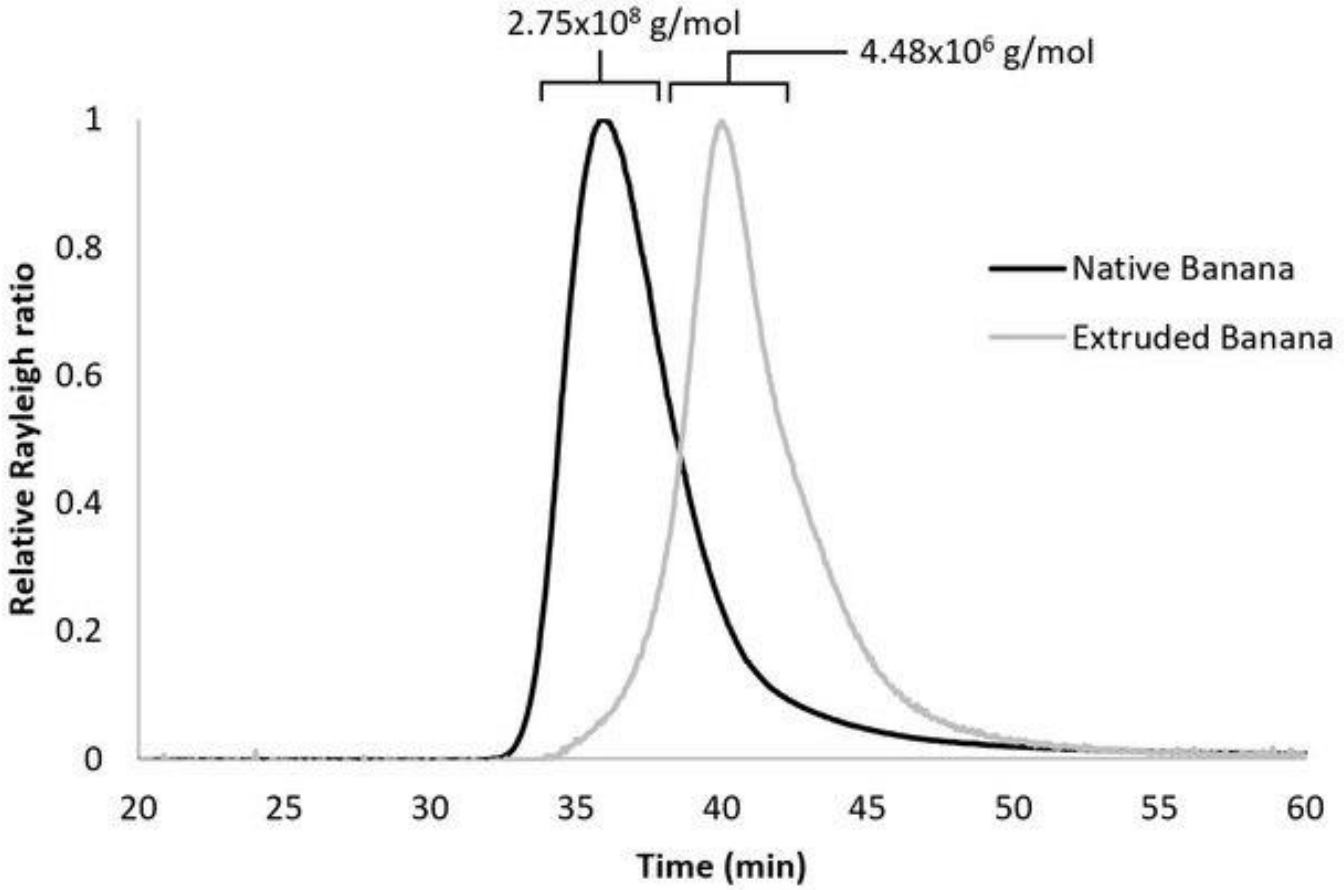
- The net result is that the intensity of light scattered away from the direction of propagation is lower, and varies with the scattering angle.



- If the angular dependence of the scattered light is measured in the horizontal plane, it is possible to determine the size of the molecule.
- This size measurement is known as the root mean square (rms) radius, or sometimes the "radius of gyration," r_g.
- The rms radius is a measure of the molecule's size weighted by the mass distribution about its center of mass.
- If the molecule's conformation is determined to belong to a specific class (e.g., random coil, sphere, or rod), the rms radius can be related to its geometrical dime



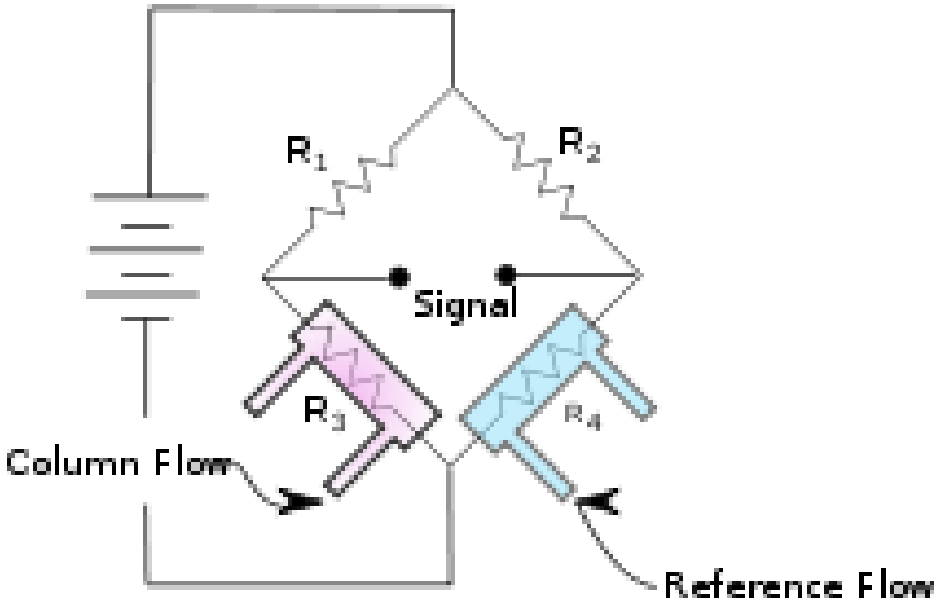
https://www.wyatt.com/library/theory/understanding-multi-angle-static-light-scattering.html



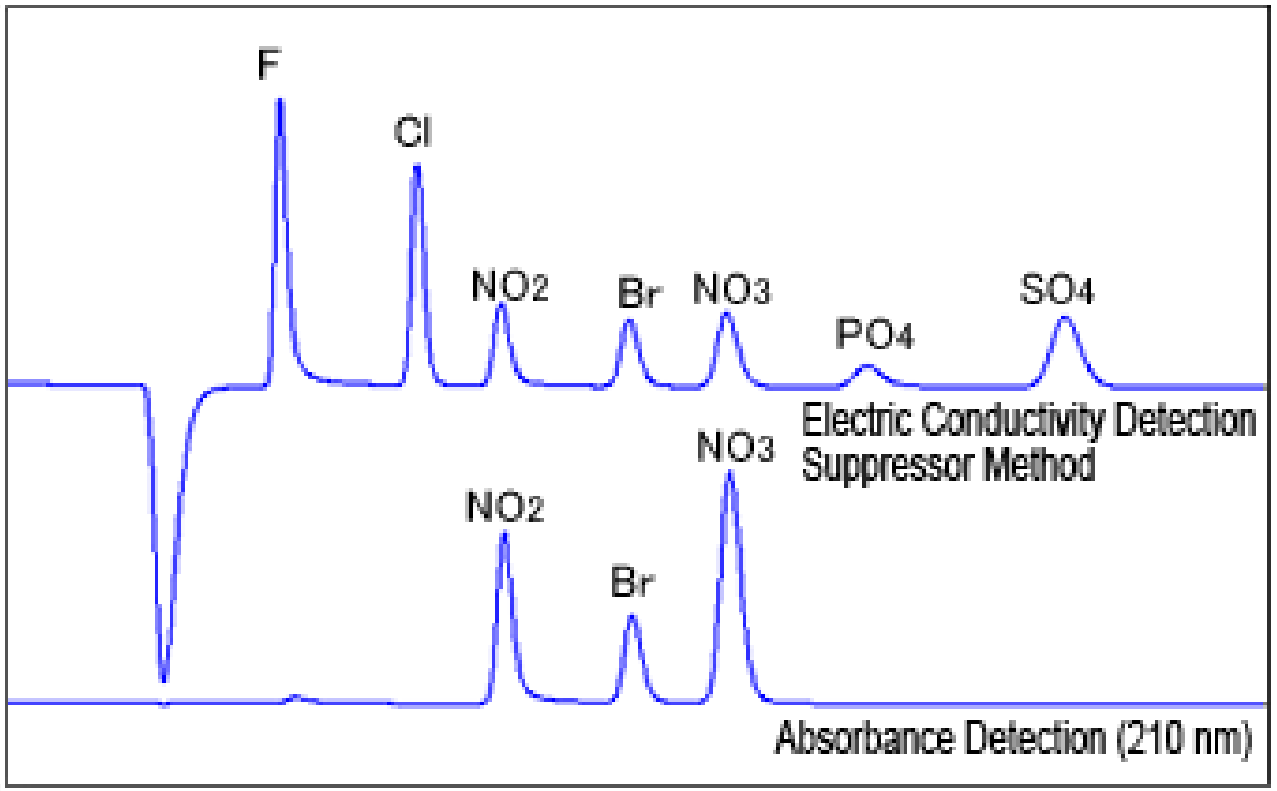
https://www.researchgate.net/publication/327427136_Banana_starch_and_molecular_shear_fragmentation_dramatically_increase_structurally_driven_slowly_digestible_starch_in_fully_gelatinized_bread_crumb/figures?lo=1

Conductivity Detector

- Solutions containing ionic components will conduct electricity.
- The detector measures the ability of the mobile phase to conduct a current in a flow cell between two electrodes.
- The detector measures the electric resistance, which is inversely proportional to the concentration of ions in solution.
- Generally used for ion exchange chromatography.

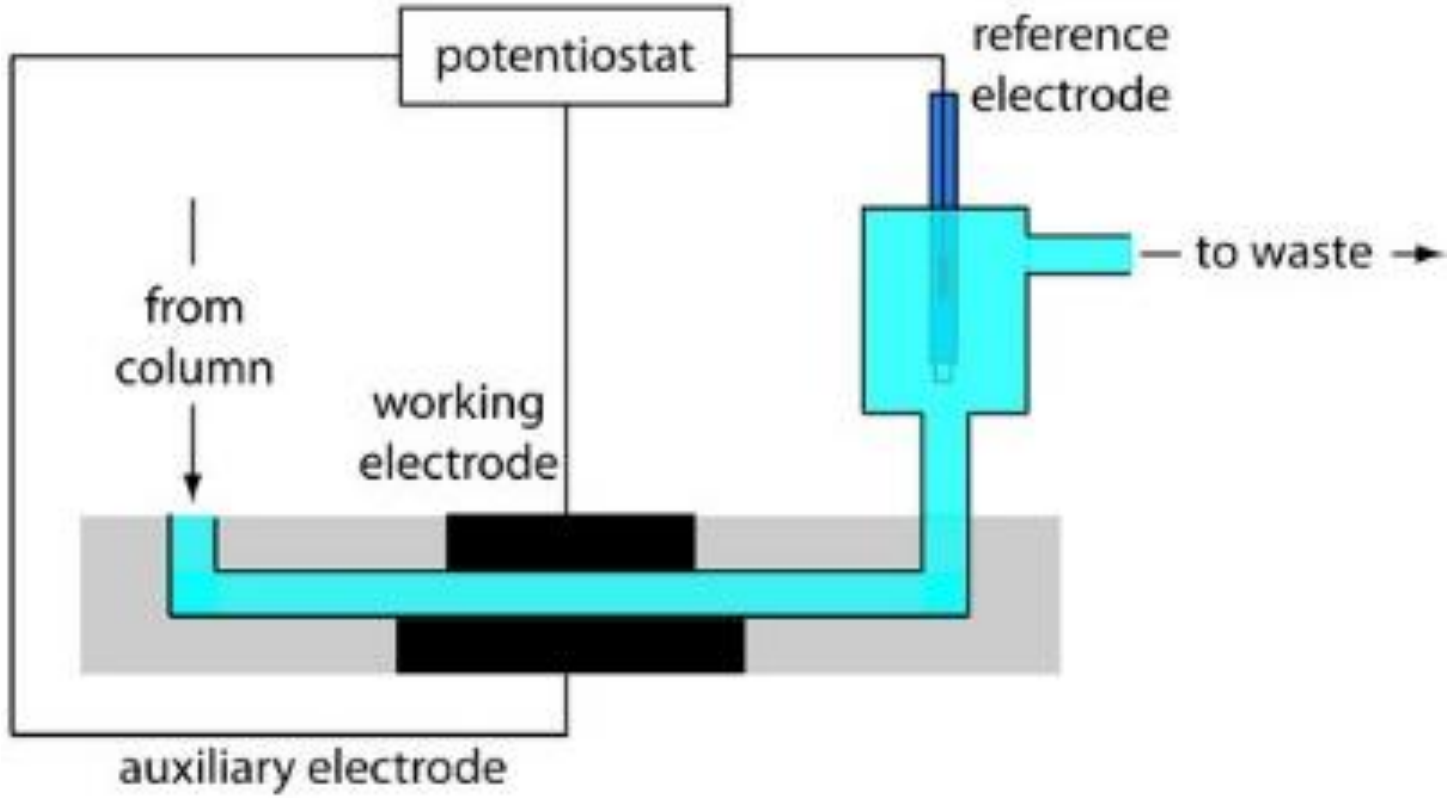


Conductivity Detector



https://www.shimadzu.com/an/hplc/support/lib/lctalk/64intro.html

Amperometric Electrochemical Detector



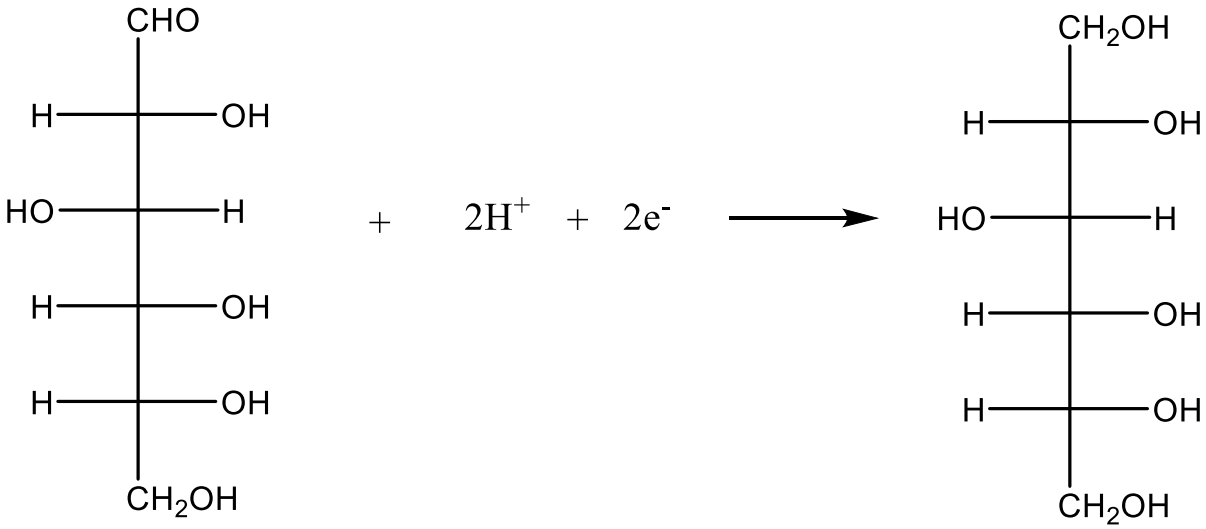
https://chem.libretexts.org/Textbook_Maps/Analytical_Chemistry/Book%3A_Analytical_Chemistry_2.0_(Harvey)/12_Chromatographic_and_Electrophoretic_Methods/12.5%3A_High-Performance_Liquid_Chromatography

Amperometric Electrochemical Detector

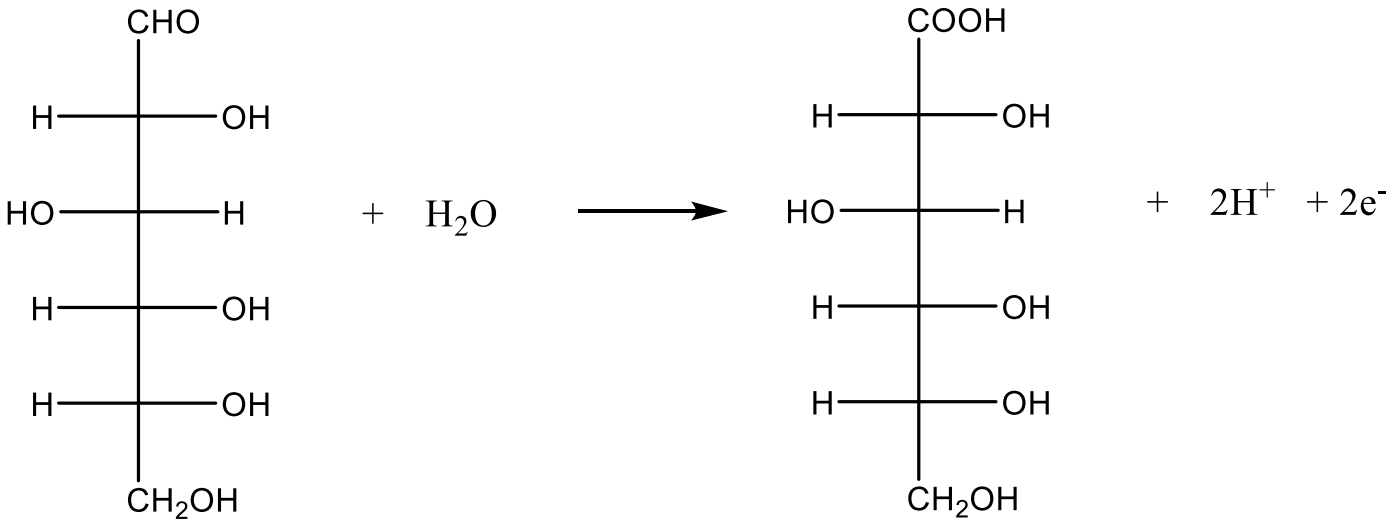
Amperometry relies on the detection of ions in solution based on electric current or changes in electric current.

Some compound are easily reduced or oxidized. When these reactions are initiated by a potential applied to a set of electrodes, electrons are exchanged and a current is formed.

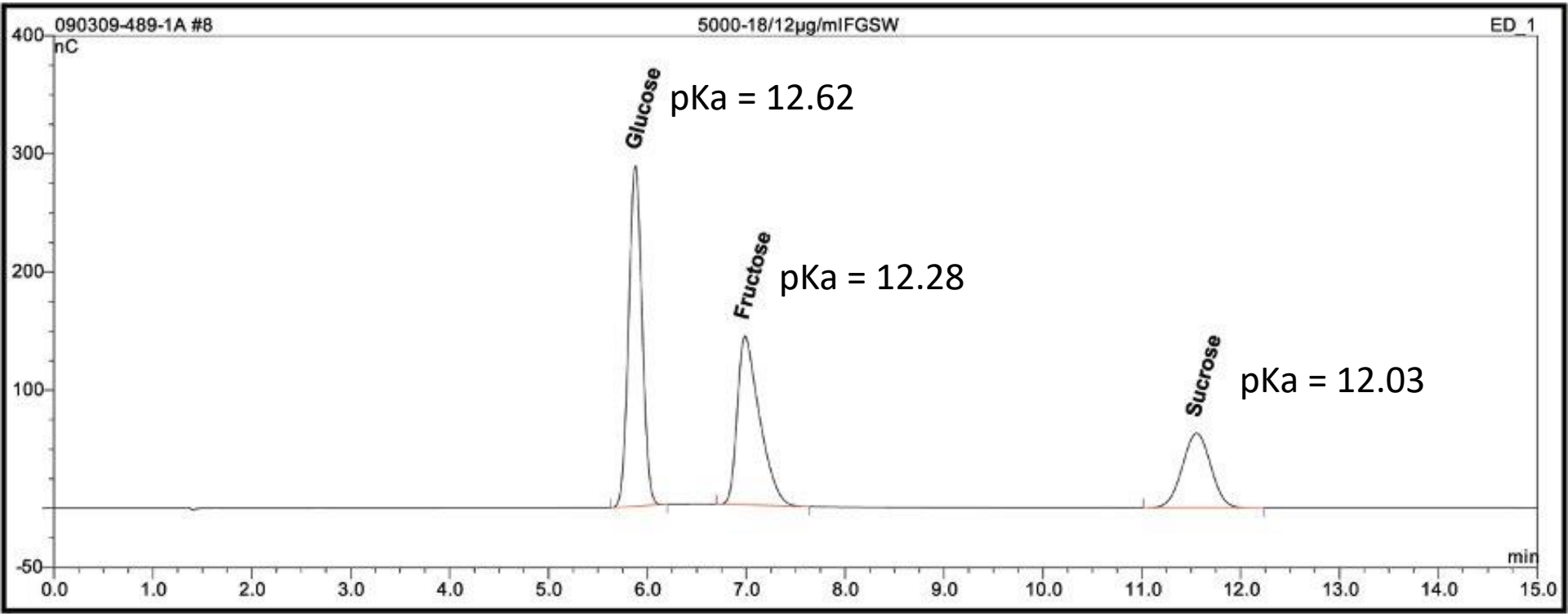
Example: sugars can be reduced…



…. or oxidized.



HPLC separation coupled with amperometric detection



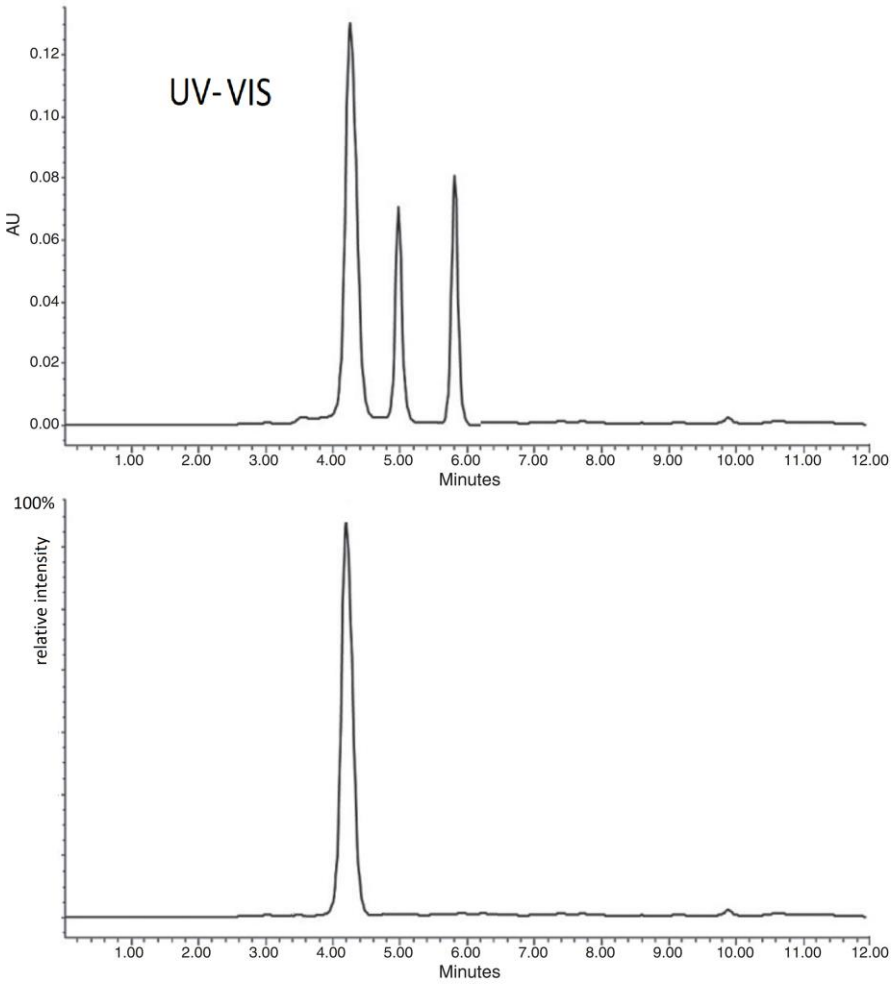
https://americanlaboratory.com/914-Application-Notes/35296-The-Power-of-Pulsed-Amperometric-Detection-Coupled-With-Chromatography-for-Analyzing-Carbohydrates/

Mass Spectrometry (MS)

- Two main ionization techniques allow LC/MS interfacing :
- Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI)
- Column effluent sprayed directly into the ionization source of the mass spectrometer.
- Compounds detected by MW and fragmentation patterns.

Questions

1. Both chromatograms shown here were obtained under the same HPLC conditions but different detectors. What type of detector was used for the bottom chromatogram? More than one answer possible. Discuss 3 cases.



2. How is a conductivity detector different from an amperometric detector?

3. If a mass spectrometer is available to measure MW, why would MALS be necessary?