INSTRUMENTATION

Computer session 2

Linear regression and calibration curves
The case of concentration analysis of a solute using spectroscopy.

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Useful definitions for analyte concentration analysis

Consider monochromatic light transmitted through a solution; with an incident intensity of I_0 and a transmitted intensity of I.

The **transmittance**, *T*, of the solution is defined as the ratio of the transmitted intensity, *I*, over the incident intensity, I_0 and takes values between 0 and 1. However, it is more commonly expressed as a **percentage transmittance** (x100%).

$$T = \frac{I}{I_0}$$

The **absorbance**, *A*, of the solution is related to the transmittance and incident and transmitted intensities through the following relations:

$$A = \log_{10} \frac{I_0}{I}$$
$$A = -\log_{10} T$$

Beer’s law : relationship between concentration and absorbance

The relationship between **absorbance** A and **concentration** C is defined by Beer-Lambert Law.

Beer’s Law states that the **absorbance** of light absorbing matter in water **is directly proportional to its concentration**, expressed by the following equation:

$$A = \varepsilon \times b \times C$$

Where:

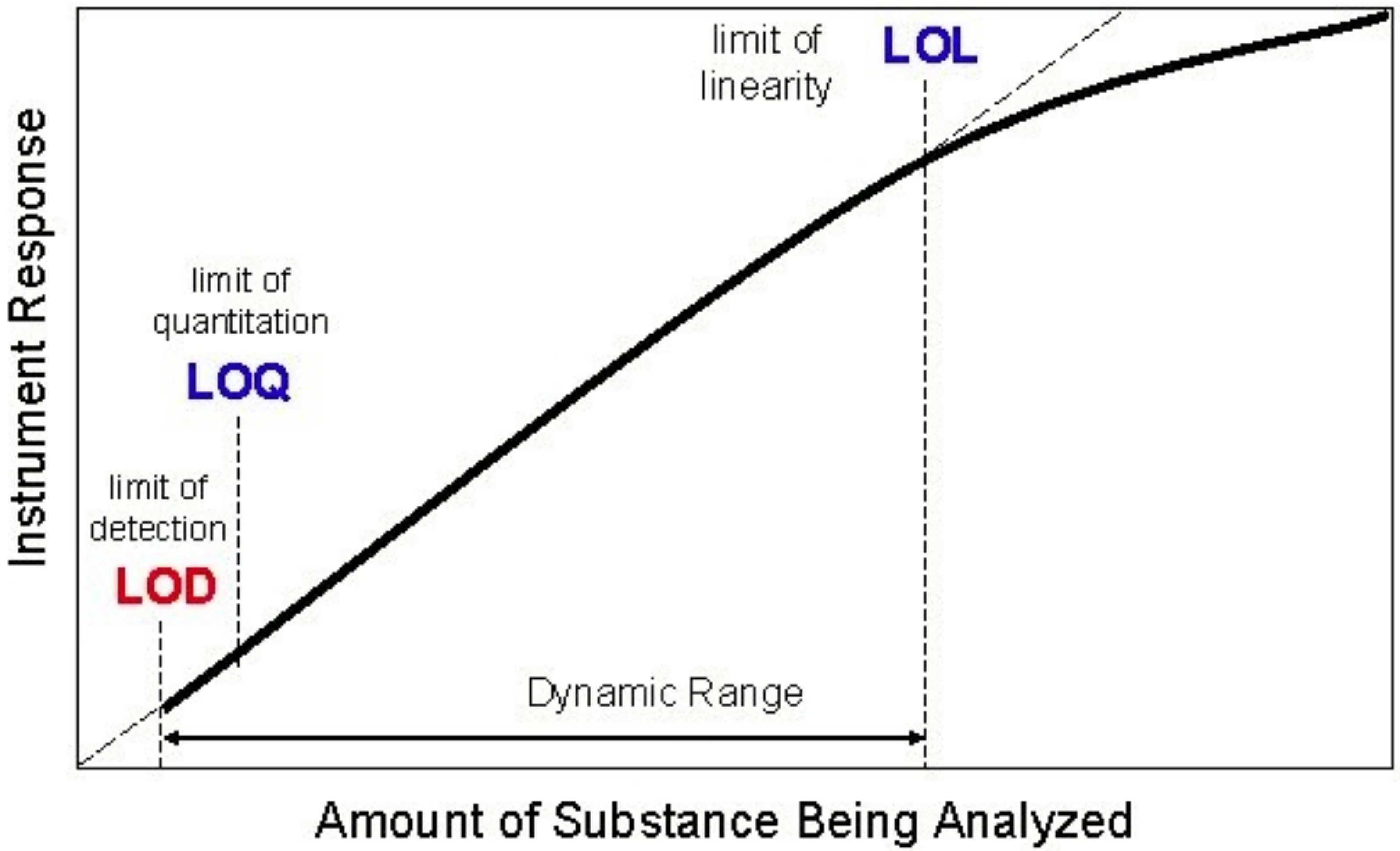
ε is the molar absorbtivity of the particular type of matter in the water sample,

b is the path length of the water sample,

c is the concentration of matter in the water sample.

Calibration curves

Signal
S_A



Concentration
C_A

What is being calibrated?

- For each analyte (A), we calibrate the proportion between concentration (C_A) and signal (S_A)
- The proportionality constant is k_A

$$S_A = k_A \cdot C_A$$

Sensitivity

- Single-point standardization is less desirable than multiple-point standardization

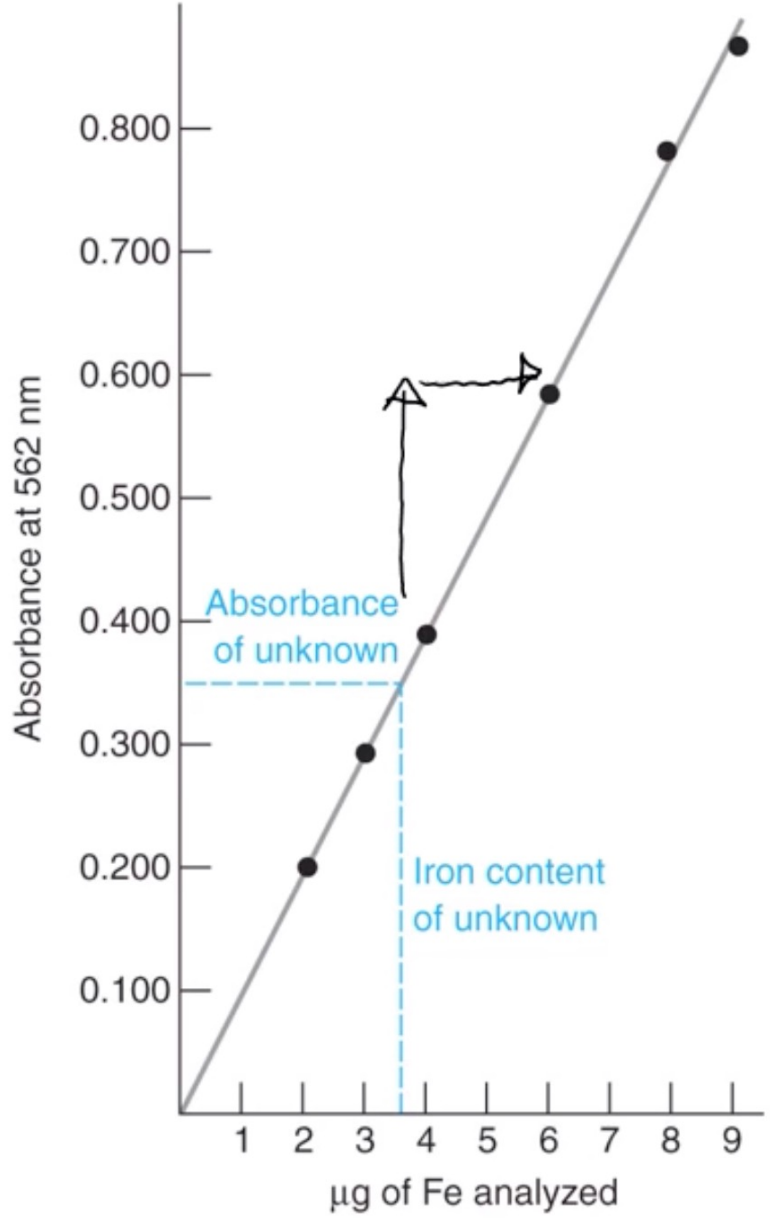
Selectivity and Sensitivity

- **Sensitivity** is the capability of responding reliably and measurably to changes in analyte concentration

$$\textit{Sensitivity} = \text{slope of calibration curve}$$

$$K_A = \frac{\text{change in signal}}{\text{change in analyte concentration}}$$

- **Selectivity** (or **specificity**) is the ability to distinguish the analyte from other species in the sample
  - Selectivity is avoiding interference



Linearity

- How well does a calibration curve follow a straight line?
- If you know the target analyte concentration, **prepare standards ranging from 0.5 to 1.5 times the expected analyte concentration**

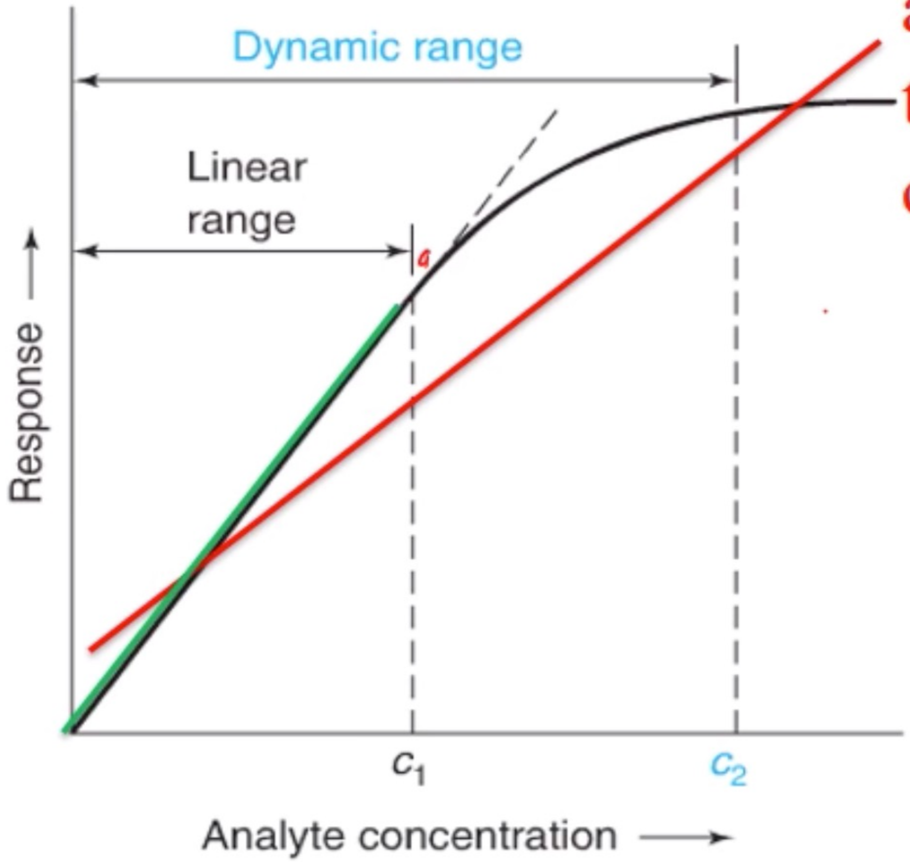
- <u>Measures of linearity are:</u>
- Square of the correlation coefficient: $\mathbf{R^2}$
  - R^2 close to 1 is a very good fit, 0.995 or 0.999 are typical cutoffs
- May also consider the **y-intercept** of the calibration curve. It should be small (≤10%) compared to the response for the high end of the calibration curve
  - This tests how good the blank subtraction is

$y = mx + b$ **y-intercept**

Keep your eyes open when making calibration curves

You can use the rest of the dynamic range if need be, but it needs a non-linear fit!

Don't just fit a line blindly to the whole curve



Only fit a line to the linear dynamic range

Range

- **Range** is the concentration interval over which specifications are met for linearity, accuracy and precision

- *Don't confuse* this with
- Linear Range = concentration range over which a calibration curve is linear
- Dynamic Range = concentration range over which there is a measurable response

Webography - references

https://realtechwater.com/blog-post/what-is-the-relationship-between-absorbance-and-concentration/

https://www.youtube.com/watch?v=XGlUFE8UMB4

https://www.edinst.com/fr/blog/the-beer-lambert-law/