Biochemistry of Proteins BCH 303 [Practical]

Lab (2) Quantitative amino acids estimation by Ninhydrin method

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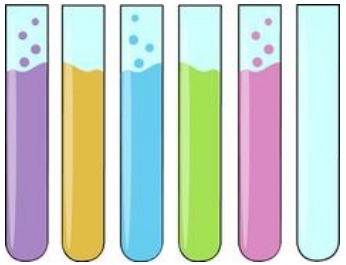
Types of assay

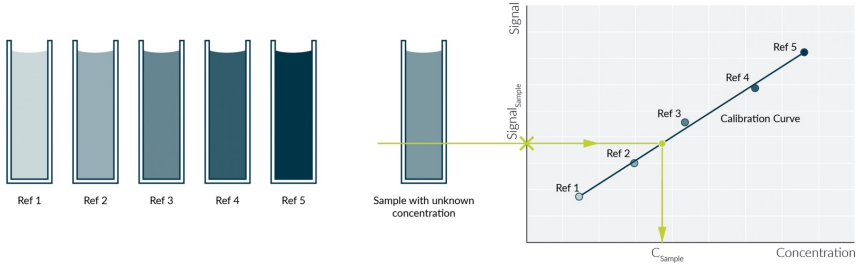
Qualitative assays

Determine if specific substance is present or not, by color or some other quality.
i.e. is the amino acid present in the sample or not?



Quantitative assays

Determine the concentration of a substance (numerical value).
i.e. what is the concentration of the amino acid in the sample?



Amino acid analysis

- **Amino acid analysis** is the most accurate way to determine the composition and quantity of protein in a sample.
- **Are fundamental biochemical techniques used for:**
  1. Quantitation of free amino acids, as well as amino acids released from macromolecules such as *peptides, proteins or glycoproteins.*
  2. Determining the amino acid composition or content of *proteins*, *peptides*, and other *pharmaceutical* preparations.

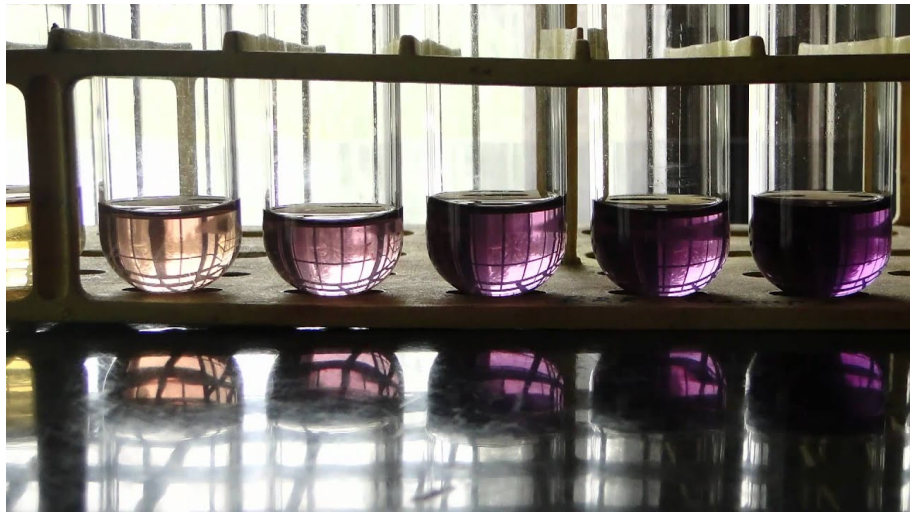
- Rapid and accurate.
- Important to understand the underlying biochemistry of multiple physiological and disease state, food science, drug samples and others.

Ninhydrin

- One of the most important method of detecting amino acids
- Used to detect their microgram amounts.
- When amino acids with a **free alpha amino group** are treated with an excess of ninhydrin, they yield a **purple-colored** product.
- Although this is a fast and sensitive test for the presence of alpha-amino acids, because of the non-selectivity, it cannot be used to analyze the relative individual contents of a mixture of different amino acids.
- Other reagents which can react with the alpha amino group to form colored or fluorescent derivatives. These include **fluorescamine**, **dansyl chloride**, **dabsyl chloride** → used in the detection of trace amounts of amino acids at the nanogram level.

Quantitative estimation of amino acid using Ninhydrin reagent

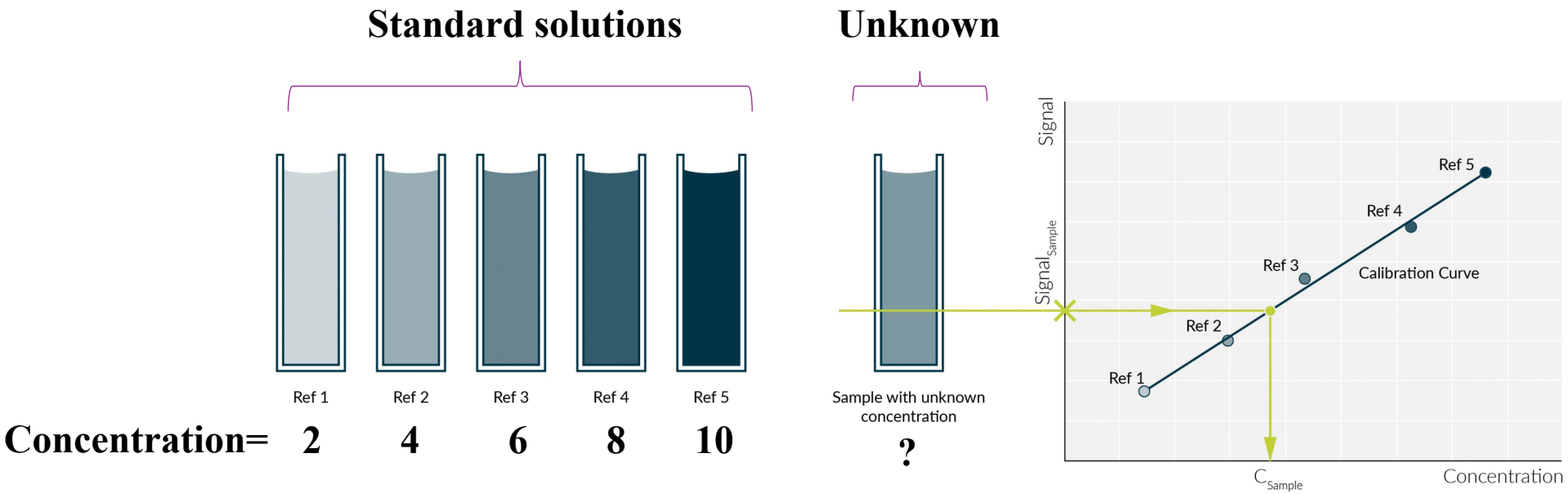
- Ruhemann's purple (RP) was discovered by *Siegfried Ruhemann* in 1910.
- In the quantitative estimation of amino acid using Ninhydrin reagent, the absorbance of the Ruhemann's purple formed by the reaction at 570 nm is measured, whereas for imino acids, the absorption happens at 440 nm.
- Under appropriate conditions, the color intensity produced is proportional to the amino acid concentration.



↑ *Color* ↑ *Concentration* ↑ *Absorbance*

Standard curve

- Direct relationship between color and concentration ➔ direct relationship between concentration and absorbance.
- Since there is a proportional relationship between the concentration and absorbance, a standard curve could be constructed to determine an unknown concentration of an amino acid sample.



Standard curve

- The standard curve (also called calibration curve): is a type of graph used as a quantitative research technique that shows the relationship between different known concentrations of a substance and the absorbance at a specific wave length.

- Is most commonly used to determine the concentration of a substance (unknown), using serial dilution of solutions (standard solutions) of known concentrations.

PAUSE AND THINK ➔ How an unknown concentration could be determined by knowing its absorbance at given wavelength?

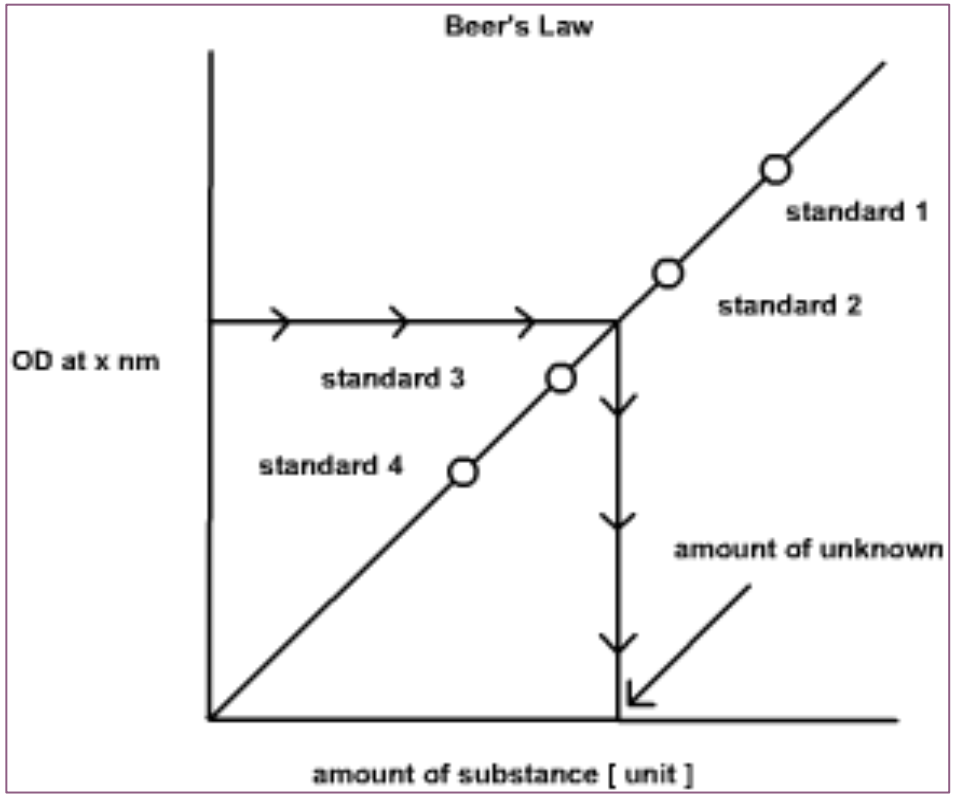


Figure 1. A standard curve showing the relation between the absorbance of different concentrations of a substance.

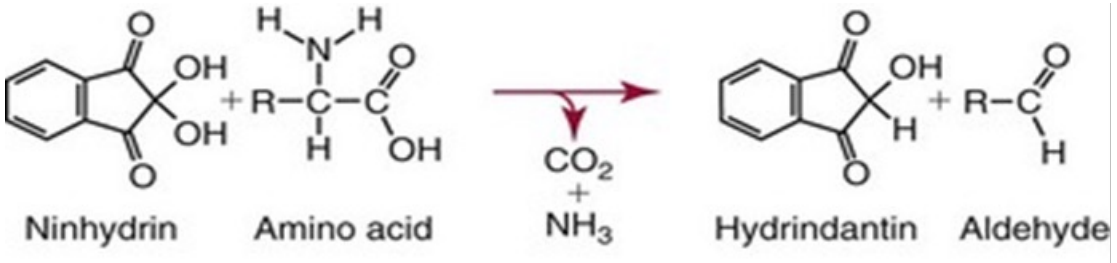
Practical part 🧪

Aims

- Determination of amino acids quantity using **ninhydrin reaction**.
- Understanding and constructing a **standard curve.**

Principle

- At neutral pH, ninhydrin destroys each primary α-amino acid
- **Ninhydrin** reacts with the released **NH_3** → a deep purple chromogen referred to as Ruhemann's purple
- Ruhemann's purple maximum absorption at ~570 nm
- **Proline** and other imino acids yields a yellow-orange product at neutral pH *(Why?)*
- The intensity of the color resulted is linearly proportional to the concentration of the amino acids present in the solution.



(1)

(2)

Purple Complex (Ruhemann's purple)

Results

Test tube	Amino acid concentration [µg/ml]	Absorbance at 570 nm
Blank		
A		
B		
C		
D		
E		
Unknown sample	_____	

Table 1. Concentration od standard amino acid solution and their absorbance at 570 nm.

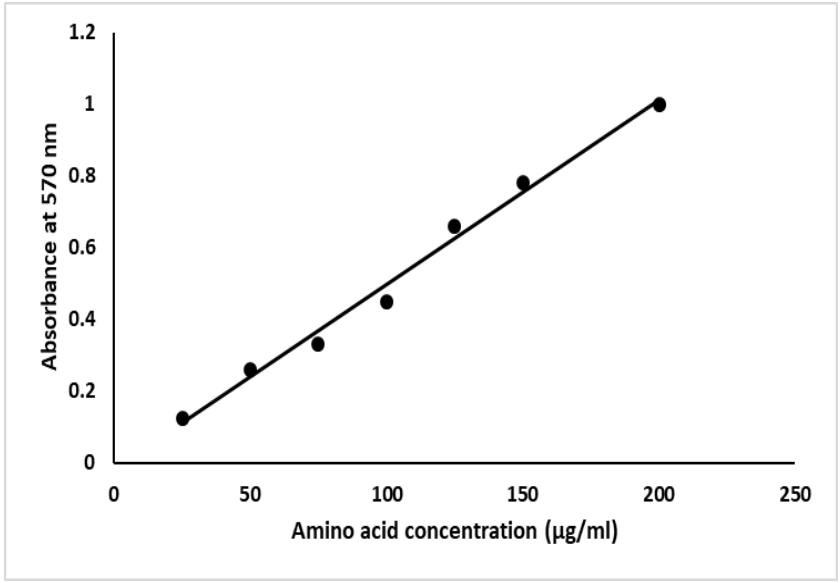


Figure 1. standard curve of amino acid using ninhydrin method.

Homework

- Name 3 techniques used in amino acid analysis.