

# Quantitative amino acids estimation by Ninhydrin method

BCH303 [Practical]

# Amino acid quantitation :

- Free amino acids, as well as amino acids released from macromolecules such as peptides, proteins or glycoproteins.
- Rapid and accurate.
- **Importance ?**

# Amino acid analysis :

- Refers to the methodology used to determine the amino acid composition or content of proteins, peptides, and other pharmaceutical preparations.
- **Methods ?**

# Ninhydrin:

- Detecting amino acids.
- Microgram amounts.
- Non-selectivity ?
- Other convenient reagents are available which can react with the alpha amino group to form colored or fluorescent derivatives. These include fluorescamine, dansyl chloride, dabsyl chloride, etc., **used in the detection of trace amounts of amino acids at the nanogram level.**

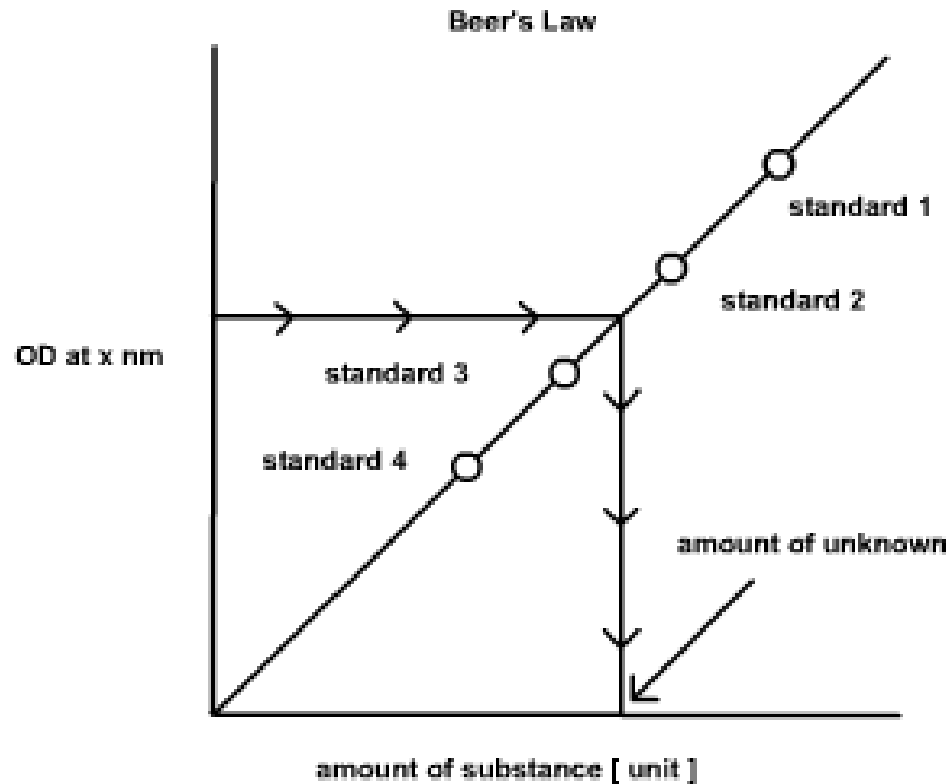
# Quantitative estimation of amino acid using Ninhydrin reagent:

- Ruhemann's purple (RP) was discovered by Siegfried Ruhemann in 1910.
- Ruhemann's purple formed by the reaction at 570 nm is measured, whereas for **imino acids**, the absorption happens at 440 nm.
- **The color intensity produced is proportional to the amino acid concentration.**
- **So ?**

# Standard curve :

- **Direct relationship between color and concentration → direct relationship between concentration and absorbance.**
- **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.
- **How ?**

# Standard curve :



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

# Practical part



## Aim:

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

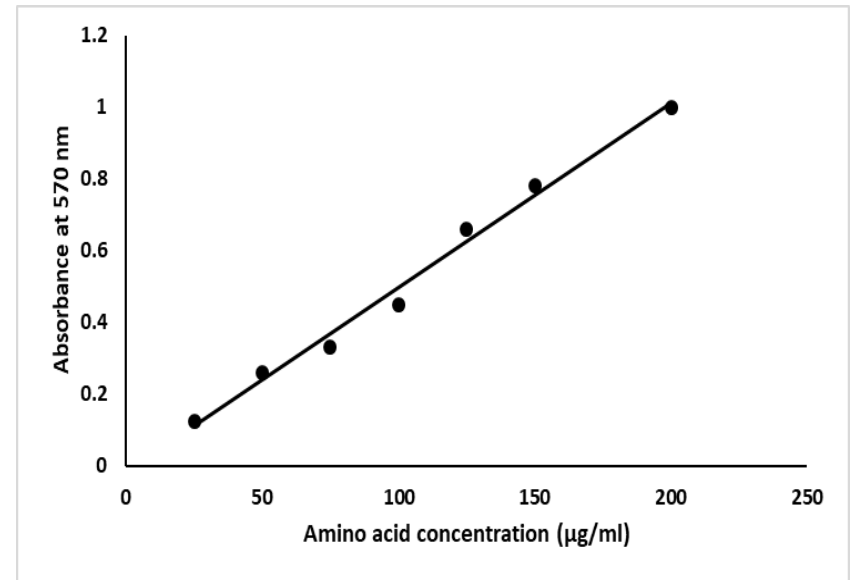
# Principle:

- At neutral pH, ninhydrin destroys each primary  $\alpha$ -amino acid to form Ruhemann's purple.
- Maximum **absorption at about 570 nm.**
- Imino acids yields a yellow- orange product at neutral pH.
- The intensity of the color resulted is linearly proportional to the concentration of the amino acids present in the solution

# Results:

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

Test tube	Amino acid concentration [ $\mu\text{g/ml}$ ]	Absorbance at 570 nm
Blank		
A		
B		
C		
D		
E		
Unknown sample	_____	



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

## Home Work:

1. Search for a method used in amino acid analysis aimed to identify the identity or composition of individual amino acids.