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 :

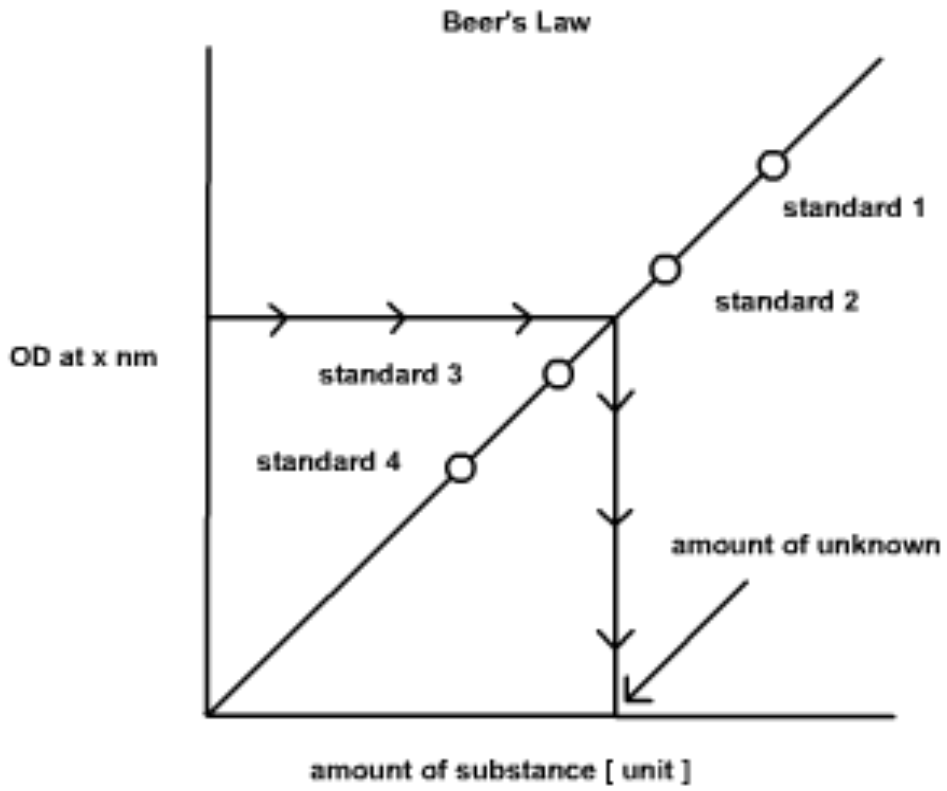

Figure 1. A standard curve showing the relation between the absorbance of different concentrations of a substance.

Practical part

Aim:

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

Principle:

- At nuetral pH, ninhydrin destroys each primary α-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 od standard amino acid solution and their absorbance at 570 nm.

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

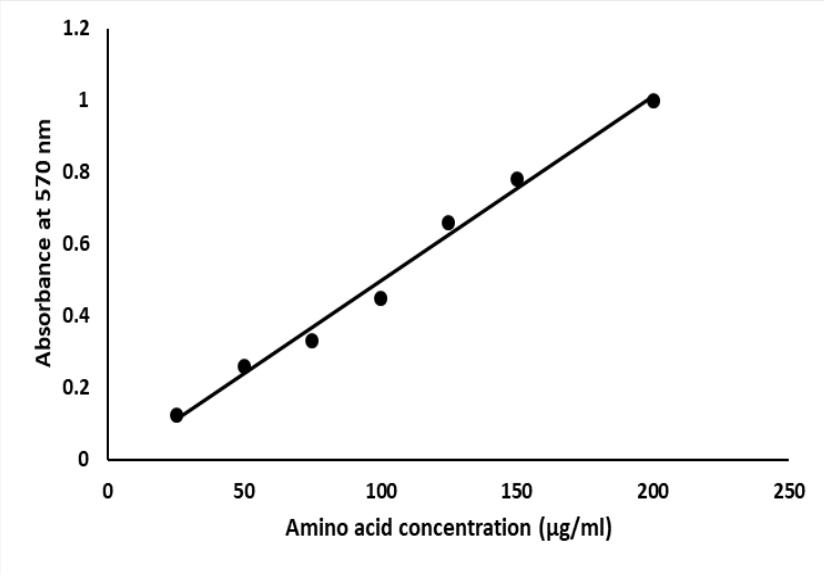

Figure 1. standard curve of amino acid using ninhydrin method.