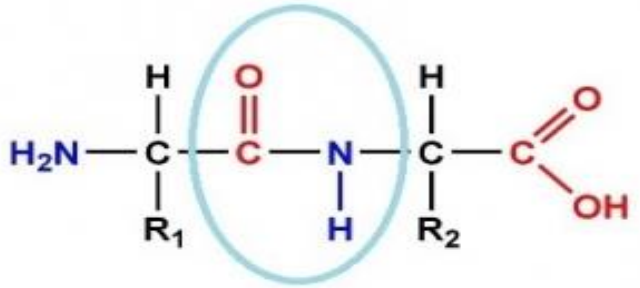
Quantitative amino acids estimation by ninhydrin method

Amino acid analysis:

- Allows for amino acid **quantitation** of free amino acids, as well as amino acids released from macromolecules such as peptides, proteins or glycoproteins.

- Critical to understanding the underlying biochemistry of multiple physiological and disease state, food science.

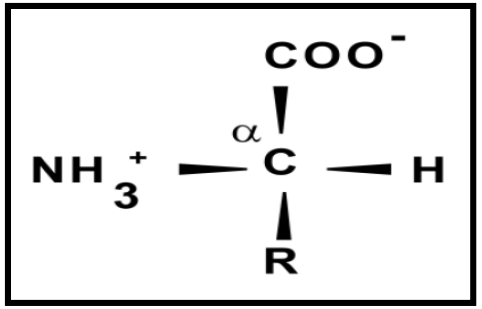


Ninhydrin reaction:

- 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** called Ruhemann's purple (RP) .

- fast and sensitive test for the presence of alpha-amino acids
- (**Non-selectivity**), it cannot be used to analyze the relative individual contents of a mixture of different amino acids



- The color intensity produced is **proportional** to the amino acid concentration.

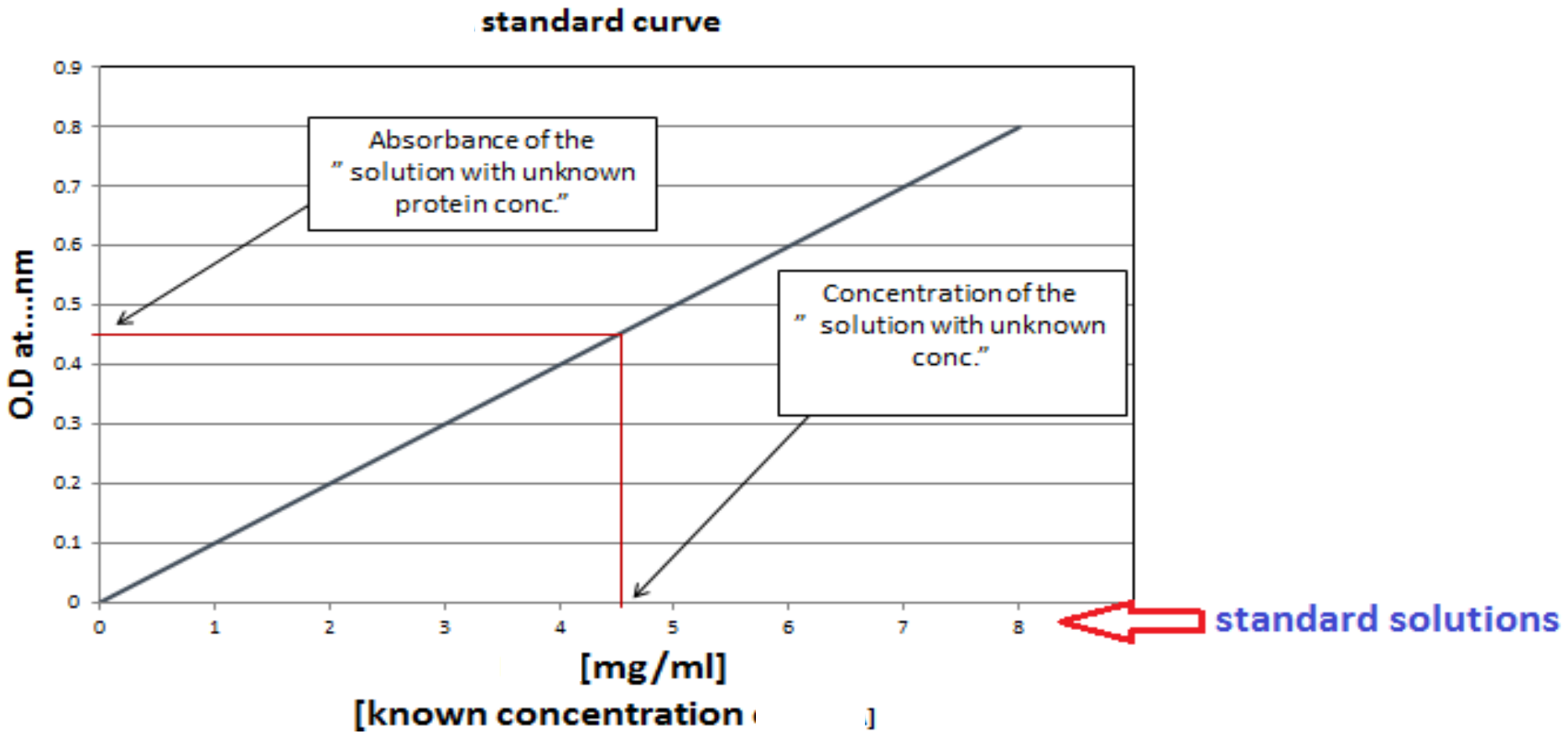
- Since there is a **proportional relationship** between the concentration and absorbance, a **standard curve** could be constructed to determine an unknown concentration of an amino acidsample.

Increased concentration => Increased color intensity => Increased absorbance value

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 wavelength.

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


standard curve
O.D at....nm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
8
Absorbance of the " solution with unknown protein conc."
Concentration of the " solution with unknown conc."
[mg/ml]
[known concentration
standard solutions

- Practical part

-Objective:

Determination of amino acids quantity using ninhydrin reaction.

-Principle:

At nuetral pH, ninhydrin destroys each primary α-amino acid and also reacts with the released NH3 to form a deep purple chromogen referred to as Ruhemann's purple, which has a maximum absorption at about **570 nm**. The reaction with proline and other imino acids yields a yellow- orange product at neutral pH, as the cyclised N-group is not released. The intensity of the color resulted is linearly proportional to the concentration of the amino acids present in the solution.

Method:

Tube	Standard amino acid solution (500 µg/ml) (ml)	Distilled water (ml)	Unknown sample	Ninhydrin reagent (ml)
Blank	-	4		1
A	0.2	3.8		
B	0.4	3.6		
C	0.6	3.4		
D	0.8	3.2		
E	1	3		
Unknown sample	-	-	4	

2.Mix the contents of the tubes by vertexing/shaking the tubes.

3. Cover the mouth of the tubes with aluminium foil.

4. Place all the test tubes in boiling water bath for 15 minutes.

5. Cool the test tubes in cold water, the add 1 ml of ethanol to each test tube and mix well.

6. Record the absorbance of all tubes against the blank at 570 nm using a colorimeter (spectrophotometer).

7. Calculate the amino acid concentration for each standard amino acid solution using

C1 x V1 = C2 x V2 formula.

8. Plot standard curve for absorbance against amino acids concentration (µg/ml) using results for solutions (A-E).

9. From the standard curve, estimate the concentration of the amino acids present in your unknown sample.

Result:

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