Lab (2)

Quantitative amino acids estimation by ninhydrin method

✏ Materials:

Chemical
Standard amino acid stock solution (100 µg/ml), 8% w/v of ninhydrin reagent, 50% v/v ethanol, distilled water.

Equipment and Glassware
Test tubes, rack, pipette, pipette pump, aluminum foil, plastic cuvette, spectrophotometer, water bath.

Protocol:

1. **Set up 7 test tubes as following:**

Tube	Standard amino acid solution (100 µg/ml) (ml)	Distilled water (ml)	Unknown sample	Ninhydrin reagent (ml)
Blank	-	4		
A	1.2	2.8		
B	1.6	2.4		
C	2	2		1
D	2.4	1.6		
E	2.8	1.2		
Unknown sample	-	-	4	

1. Mix the contents of the tubes by vertexing/shaking the tubes.
2. Cover the tubes with aluminium foil.
3. Place all the test tubes in 80°C for 5 minutes.
4. Cool the test tubes in cold water, the add 1 ml of ethanol to each test tube and mix well.
5. Record the absorbance of all tubes against the blank at 570 nm using a colorimeter (spectrophotometer).
    🞂 *PAUSE AND THINK* ➔ *What the blank should contain? Why?*
6. Calculate the amino acid concentration for each standard amino acid solution using C1 x V1 = C2 x V2 formula.
7. Plot standard curve for absorbance against amino acids concentration (µg/ml) using results for solutions (A-E).
8. From the standard curve, estimate the concentration of the amino acids present in your unknown sample.

🖊 **Results:**

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

✍ **Supporting materials:**

- A video explains the mechanism of reaction between ninhydrin and amino acid:
  https://www.youtube.com/watch?v=P-iK4QUU9t0

- A video shows the practical steps of the experiment step by step:
  -Part 1: https://www.youtube.com/watch?v=wvVYF8T7uiE
  -Part 2: https://www.youtube.com/watch?v=B4EgYeFqV5Q