

Experiment-6: Titration Methods for Estimation of Amino acids

Aim: Estimation of Amino acids by Titration methods

Principle:

Amino acids react with formalin to form methylene amino acids. But it was found that the reaction was more complex and the main product is monomethylol and di methylol amino acids. Amino acids exist in zwitter ionic form and cannot be titrated directly with alkali. Thus amino groups of amino acids are blocked by reaction with formaldehyde. But formaldehyde does not react with the charged amino groups ($-\text{NH}_3^+$), thus first the amino acid reacts with sodium hydroxide solution to give glycine which condenses with formaldehyde to give a stable anion.

Further formaldehyde solution contains formic acid and also amino acids are not exactly neutral species, thus it is necessary that both the formaldehyde and amino acid solution should have the same pH before mixing and for this purpose each solution is first made just alkaline to phenolphthalein by means of dilute sodium hydroxide solution.

Reagents:

a. Neutral formalin solution

It is prepared by neutralization of formalin solution with alkali to phenolphthalein end point.

b. Standard Glycine solution

An accurately weighed 0.8 grams of Glycine is dissolved in distilled water in a measuring flask 100 mL.

c. Unknown Glycine solution

d. Sodium hydroxide solution 0.1 N

e. Phenolphthalein indicator

Procedure:

1. A standard glycine solution (20 mL) is pipetted out into a conical flask and phenolphthalein (2-3 drops) added.
2. It is titrated with sodium hydroxide solution until a faint pink color appears.
3. Then the neutralized formaldehyde solution (10 mL) is added.
4. The pink color of the solution in the titration flask disappears immediately and the solution becomes acidic.
5. Then the contents of the flasks are titrated with sodium hydroxide solution till the pink color is obtained.
6. Same experiment repeated with unknown glycine solution.

Observations:

Weight of Glycine dissolved in 100 mL = w g

Volume of sodium hydroxide solution used with standard glycine solution = v_1 mL

Volume of sodium hydroxide solution used with unknown glycine solution = v_2 mL

Volume of glycine solution taken = 20 mL

Calculations

V1 mL of NaOH in 20mL standard glycine solution= (w/5) g of glycine

V2mL of Na OH in 20mL of unknown glycine solution= (w/5) x (v2/v1) g of glycine

Thus strength= (w/5) (v2/v1) (1000/20) g/L

$$= (wxv2x10)/v1 \text{ g/L}$$

Result:

Reference:

- 1) Sadasivam S and Balasubramanian T (1985). Practical Manual (Undergraduate), Tamil Nadu Agriculture University, Coimbatore, p.2.
- 2) Sadasivam S, Manickam A (2008). Biochemical Methods, New Age International Publishers, 3rd Edition, ISBN: 978-81-224-2140-8.