Calculation :

25 ml of NH_4^+ solution ≡ X ml S(N) NaOH solution

≡ X.S ml 1(N) NaOH solution

From equation (1),

1 ml 1(N) NaOH soln. ≡ 0.017 g of NH_3 ≡ 0.018 g of NH_4^+

∴ X.S ml 1(N) NaOH soln. ≡ 0.018 × X × S g of NH_4^+

∴ 25 ml NH_4^+-salt solution contains 0.018 × X × S g of NH_4^+

∴ 1000 ml NH_4^+-salt solution contains 0.018 × X × S × 40 g of NH_4^+

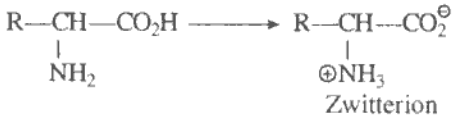
∴ The strength of supplied NH_4^+-salt solution

= 0.018 × X × S × 40 g/litre.

11. Estimation of amino acids by sorensen formol titration :

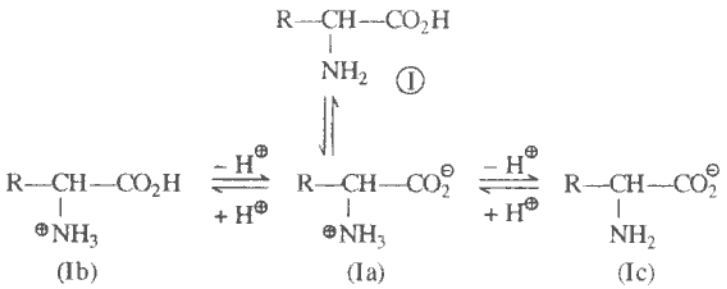
Principle :

The ammonium salt of carboxylic acid is formed when an amine is added to a carboxylic acid. This takes place by the transfer of a proton from carboxylic acid group to the amino group. However, when both the groups are present in the same molecule as in the case of amino acids, the proton transfer occurs internally to give internal salt or 'dipolar ion' or 'zwitterion'.



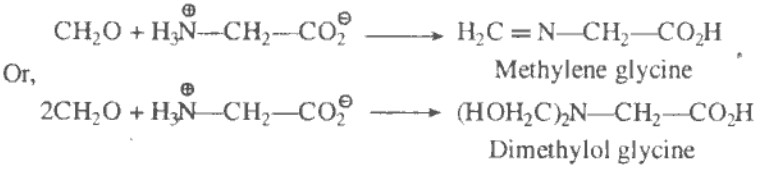
Zwitterion

Because of the dipolar nature, they are amphoteric and in aqueous solution following species are in equilibrium (water plays the role of both an acid and a base by donating H^+ to a stronger base and accepting H^+ from a stronger acid respectively) :



(Ib) (Ia) (Ic)

Absence of acidic and basic properties of an amino acid in spite of the presence of $—NH_2$ and $—CO_2H$ group can be accounted by zwitterion structure. That is why, amino acid cannot be titrated directly with alkali. When formalin solution is added to glycine, the following mixture of products are formed :



Methylene glycine

Or,

Dimethylol glycine

The process is a nucleophilic addition of an amino group to the carbonyl group of formaldehyde. This treatment marks the amino group and titration of carboxylic acid group by alkali becomes feasible.

Preparation of required reagents and chemicals :

(i) 250 ml standard $\left(\frac{N}{10}\right)$ oxalic acid solution : About 1.5758 g but accurately weighed oxalic acid (A.R.) is dissolved in 250 ml volumetric flask, diluted up to the mark with distilled water and mixed thoroughly.

(ii) 1000 ml $\left(\frac{N}{10}\right)$ NaOH solution : 4.0 g/litre.

(iii) $\left(\frac{N}{10}\right)$ Glycine solution (unknown) : ~7.5 g/litre.

Procedure :

(a) Standardisation of NaOH solution : Standardisation of NaOH solution is made as usual with standard oxalic acid solution.

(b) Estimation of glycine : 25 ml of distilled water and a drop of phenolphthalein are added to 10 ml of formalin, taken in a 250 ml conical flask by a measuring cylinder. The resulting solution is neutralised by standard $\left(\frac{N}{10}\right)$ NaOH solution by adding it dropwise from a burette to just appearance of pink colour. The titre value is ignored.

25 ml of unknown (supplied) glycine solution is pipetted into a 250 ml conical flask and a drop of phenolphthalein is added to it. The resulting solution is neutralised by $\left(\frac{N}{10}\right)$ NaOH solution adding dropwise from a burette to just appearance of pink colour. The titre value is ignored and adjusted to zero mark of the burette by filling same NaOH solution.

Then neutralised formalin solution is added to the neutralised glycine solution and titrated with standard $\left(\frac{N}{10}\right)$ NaOH solution until just appearance of pale pink colour.

Experimental Results :

Table 1 : Preparation of $\left(\frac{N}{10}\right)$ standard oxalic acid solution

Initial weight w_1 g	Final weight w_2 g	Weight taken : $w_1 - w_2 = w$ g

Table 2 : Standardisation of NaOH solution

No. of titrations	Volume of oxalic acid solution in ml	Required volume of NaOH solution in ml	Mean volume of NaOH solution in ml
1	25	x	$V_1 = (x + y + z)/3$
2	25	y	
3	25	z	

Table 3 : Estimation of glycine

No. of titrations	Volume of glycine solution in ml	Required volume of NaOH solution in ml	Mean volume of NaOH solution in ml
1	25	p	$V_2 = (p + q + r)/3$
2	25	q	
3	25	r	

Calculation :

$$\text{Strength of NaOH solution} = \frac{w \times 25}{1.5758 \times V_1}\left(\frac{N}{10}\right) = S(N)$$

$$\text{where, } S = \frac{w \times 25}{1.5758 \times V_1 \times 10}$$

25 ml glycine solution ≡ $V_2 \times S(N)$ NaOH solution.

Again, 1000 ml 1(N) NaOH solution ≡ 75 g of glycine.

∴ 1 ml 1(N) NaOH solution ≡ 0.075 g of glycine.

∴ V_2 ml S(N) NaOH solution ≡ $(0.075 \times V_2 \times S)$ g of glycine.

∴ Strength of glycine in supplied solution

$$= 0.075 \times V_2 \times S \times \frac{1000}{25} \text{ g/litre}$$

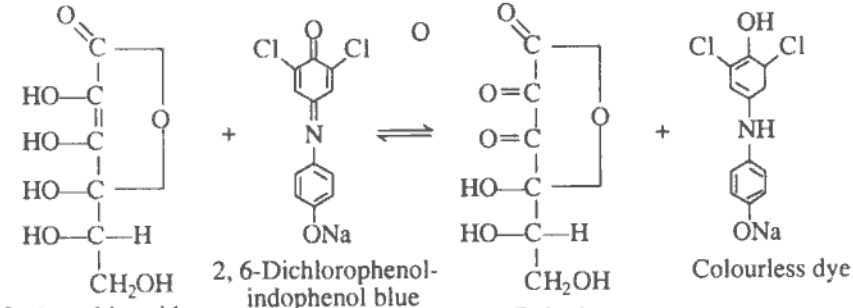
$$= 0.075 \times V_2 \times S \times 40 \text{ g/litre.}$$

12. Estimation of reduced vitamin C (ascorbic acid) by 2, 6-dichlorophenol-indophenol blue :

Principle :

Naturally occurring vitamin C (ascorbic acid) is reduced form of L-ascorbate and can be oxidised to oxidised form L-dehydroascorbate by 2, 6-dichloroindophenol, the latter being reduced accordingly.

The quantity of ascorbic acid present in an unknown (supplied) solution can be estimated by titrating with 2, 6-dichlorophenol-indophenol blue which is a dye and itself acts as an indicator. The dye is to be standardised by standard ascorbic acid solution. The method is based on redox conversion of ascorbic acid and 2, 6-dichlorindophenol blue. The blue dye becomes colourless on reduction because of the loss of extended conjugation.



The colour is discharged by other compounds present as impurities in ascorbic acid but interfering substances react slowly in presence of metaphosphoric acid to give acceptable result. The dye is blue in alkali but red in acid.

Titration must be carried out quickly as far as practicable to avoid aerial oxidation of the dye.

Preparation of required reagents and chemicals :

(i) Standard ascorbic acid solution :

Approximately, accurately weighed 100 mg of L-ascorbic acid (A.R. quality) is dissolved in 3% metaphosphoric acid solution in a 100 ml volumetric flask and volume is made up to the mark and the solution is shaken thoroughly. Again, 10 ml of above solution is pipetted out into 100 ml volumetric flask and volume is made up to the mark and shaken thoroughly to get homogeneous solution.