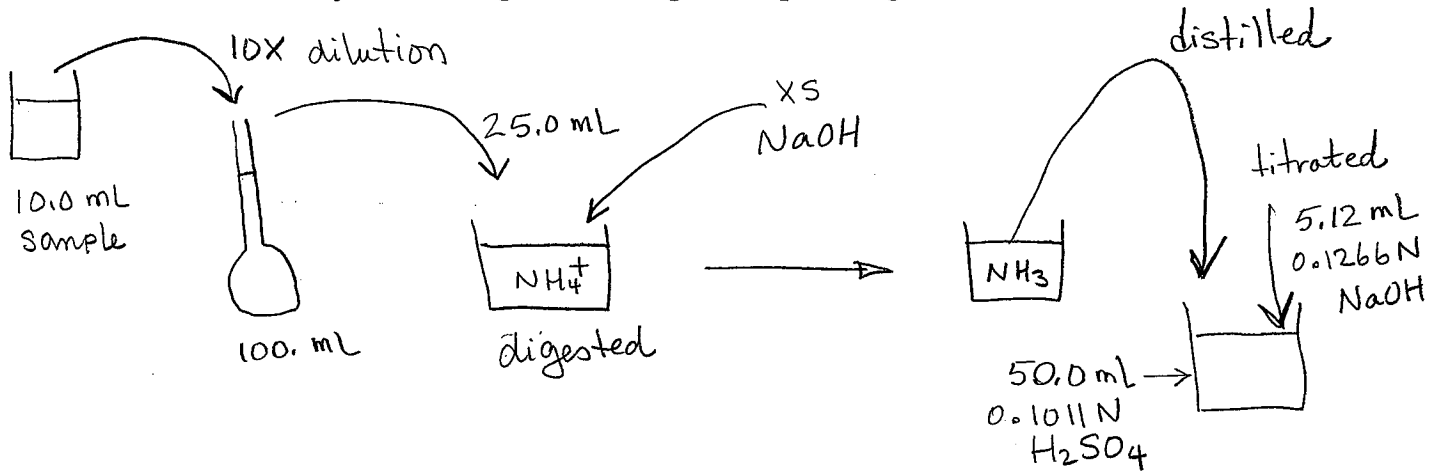


EXAMPLES of BACK TITRATIONS

1. The quantity of organically bound nitrogen (org-N) released by acid digestion is referred to as *Kjeldahl* nitrogen. One method used to determine the *Kjeldahl* nitrogen content involves a back titration and is outlined below.

- A 10.00 mL sample is diluted to 100 mL with distilled water.
- A 25.00 mL aliquot of this diluted sample is pipetted into a digestion flask. Concentrated H_2SO_4 and H_2O_2 are added and the solution is heated for 45 mins (digestion). Under these conditions, the organic molecules are broken down and all the organic nitrogen is converted to NH_4^+ .
- Concentrated NaOH is added to neutralize the excess H_2SO_4 and to convert the NH_4^+ to NH_3 , which is distilled into a flask containing 50.00 mL of 0.1011 N H_2SO_4 .
- The excess H_2SO_4 was determined by titration with 5.12 mL of 0.1266 N NaOH .

What is the mass of *Kjeldahl* nitrogen in the original sample in mg/L?



(see attached)

Note: Distillation of NH_3 prior to digestion gives the inorganic $\text{NH}_3\text{-N}$. This can be subtracted from the total Kjeldahl N to give the organic Kjeldahl N.

$$4. \quad \frac{\text{mass N (mg)}}{L} = \frac{\text{mass N}}{V_N \text{ sample}}$$

$$V_N \text{ sample} = 10.00 \text{ mL} \times \frac{1 \text{ L}}{10^3 \text{ mL}} \times \frac{25.00 \text{ mL}}{100.00 \text{ mL}}$$

$$\text{mass N} = \underbrace{N_N \times \text{M.W. N}}_{\text{method ①}} \text{ or } \underbrace{\# \text{equiv. N} \times \text{E.W.}}_{\text{method ②}}$$

$$\text{method ①} \quad N_N = N_{\text{NH}_3} \times \frac{1 \text{ mol N}}{1 \text{ mol NH}_3}$$

$$N_{\text{NH}_3} = N_{\text{H}_2\text{SO}_4}^{\text{reacted}} \times \frac{2 \text{ mol NH}_3}{1 \text{ mol H}_2\text{SO}_4}$$

$$N_{\text{H}_2\text{SO}_4}^{\text{reacted}} = N_{\text{H}_2\text{SO}_4}^{\text{initial}} - N_{\text{H}_2\text{SO}_4}^{\text{final}}$$

$$N_{\text{H}_2\text{SO}_4}^{\text{initial}} = 0.1011 \frac{\text{equiv}}{\text{L}} \times 50.00 \text{ mL} \times \frac{1 \text{ L}}{10^3 \text{ mL}} \times \frac{1 \text{ mol}}{2 \text{ equiv}}$$

$$= 2.527 \times 10^{-3} \text{ mol H}_2\text{SO}_4$$

$$N_{\text{H}_2\text{SO}_4}^{\text{final}} = N_{\text{NaOH}} \times \frac{1 \text{ mol H}_2\text{SO}_4}{2 \text{ mol NaOH}}$$

$$N_{\text{NaOH}} = M_{\text{NaOH}} \times V_{\text{NaOH}} = 0.1266 \frac{\text{mol}}{\text{L}} \times 5.12 \text{ mL} \times \frac{1 \text{ L}}{10^3 \text{ mL}}$$

$$= 6.481 \times 10^{-4} \text{ mol}$$

$$\therefore N_{\text{H}_2\text{SO}_4}^{\text{final}} = 3.240 \times 10^{-4} \text{ mol}$$

$$\text{and } N_{\text{H}_2\text{SO}_4}^{\text{reacted}} = 2.527 \times 10^{-3} \text{ mol} - 3.240 \times 10^{-4} \text{ mol} = 2.204 \times 10^{-3} \text{ mol}$$

$$\text{and } N_{\text{NH}_3} = 4.407 \times 10^{-3} \text{ mol} \quad \text{so } N_N = 4.407 \times 10^{-3} \text{ mol}$$

$$\text{and mass N} = 4.407 \times 10^{-3} \text{ mol} \times 14.00 \frac{\text{g}}{\text{mol}} = 0.06170 \text{ g}$$

$$\text{so, } \frac{\text{mass N (mg)}}{L} = \frac{0.06170 \text{ g} \times \frac{10^3 \text{ mg}}{1 \text{ g}}}{10.00 \text{ mL} \cdot \frac{1 \text{ L}}{10^3 \text{ mL}} \cdot \frac{25.00 \text{ mL}}{100.0 \text{ mL}}} = 2.47 \times 10^4 \frac{\text{mg}}{\text{L}}$$

method ②

$$\text{mass N} = \# \text{equiv N} \times \text{E.W.}$$

$$\# \text{equiv N} = \# \text{equiv. H}_2\text{SO}_4 \text{ (reacted)}$$

$$\# \text{equiv H}_2\text{SO}_4 \text{ (reacted)} = \# \text{equiv H}_2\text{SO}_4 \text{ (initial)} - \# \text{equiv. H}_2\text{SO}_4 \text{ (final)}$$

$$\# \text{equiv. H}_2\text{SO}_4 \text{ (initial)} = N_{\text{H}_2\text{SO}_4} \times V_{\text{H}_2\text{SO}_4}$$

$$\begin{aligned} &= 0.1011 \frac{\text{equiv}}{\text{L}} \times 50.00 \text{ mL} \times \frac{1 \text{ L}}{10^3 \text{ mL}} \\ &= 5.055 \times 10^{-3} \text{ equiv} \end{aligned}$$

$$\# \text{equiv H}_2\text{SO}_4 \text{ (final)} = \# \text{equiv NaOH}$$

$$\begin{aligned} &= N_{\text{NaOH}} \times V_{\text{NaOH}} = 0.1266 \frac{\text{equiv}}{\text{L}} \times \frac{5.12 \text{ L}}{10^3} \\ &= 6.482 \times 10^{-4} \text{ equiv} \end{aligned}$$

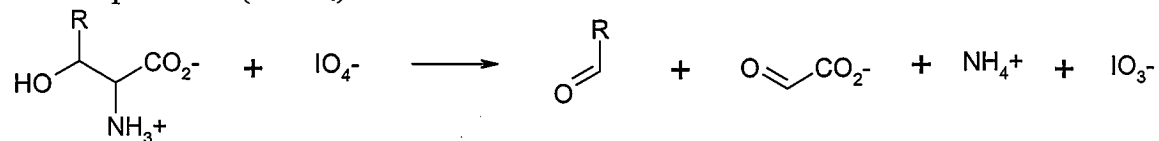
$$\begin{aligned} \text{therefore } \# \text{equiv. H}_2\text{SO}_4 \text{ (reacted)} &= 5.055 \times 10^{-3} \text{ equiv} - 6.482 \times 10^{-4} \text{ equiv} \\ &= 4.407 \times 10^{-3} \text{ equiv} \end{aligned}$$

$$\text{and } \# \text{equiv. N} = 4.407 \times 10^{-3} \text{ equiv.}$$

$$\begin{aligned} \text{mass N} &= 4.407 \times 10^{-3} \text{ equiv} \times 14.00 \frac{\text{g}}{\text{equiv}} \\ &= 0.06170 \text{ g} \quad \text{since } K=1(\text{NH}_3), \text{ E.W.} = \text{M.W.} \end{aligned}$$

$$\text{therefore } \frac{\text{mass N (mg)}}{\text{L}} = \frac{0.06170 \text{ g} \times \frac{10^3 \text{ mg}}{1 \text{ g}}}{10.00 \text{ mL} \times \frac{1 \text{ L}}{10^3 \text{ mL}} \times \frac{25.00}{100.00}} = 2.47 \times 10^4 \frac{\text{mg}}{\text{L}}$$

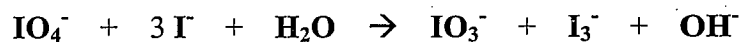
2. A 64.3 mg sample of a protein (MW = 58,600) was treated with 2.00 mL of 0.0487 M sodium periodate (NaIO_4) to react all of the serine and threonine residues.



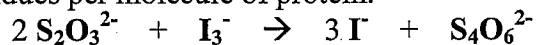
R = H, serine

R = CH_3 , threonine

The solution was then treated with excess iodide ion to convert the unreacted periodate into iodine.



Titration of the iodine required 823 μL of 0.0988 M thiosulfate. Calculate the number of serine plus threonine residues per molecule of protein.



(Note: that in the presence of excess iodide ion, iodine is rapidly interconverted to triiodide ion; $\text{I}_2 + \text{I}^- \rightleftharpoons \text{I}_3^-$)

(see class notes)