EXAMPLES of BACK TITRATIONS

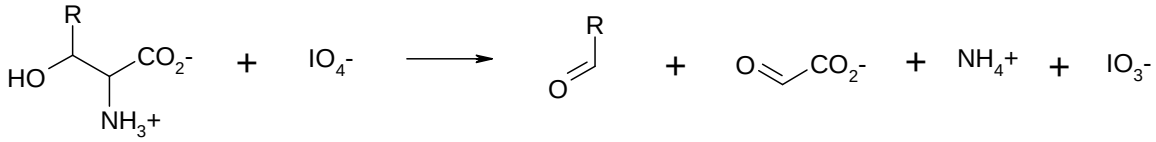
1. The quantity of organically bound nitrogen (org-**N**) released by acid digestion is referred to as *Kjeldahl* nitrogen. One method used to determine the *Kjeldahl* nitrogen content involves a back titration and is outlined below.

a) A 10.00 mL sample is diluted to 100 mL with distilled water.
b) A 25.00 mL aliquot of this diluted sample is pipetted into a digestion flask. Concentrated $\mathbf{H_2SO_4}$ and $\mathbf{H_2O_2}$ 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 $\mathbf{NH_4^+}$.
c) Concentrated **NaOH** is added to neutralize the excess $\mathbf{H_2SO_4}$ and to convert the $\mathbf{NH_4^+}$ to $\mathbf{NH_3}$, which is distilled into a flask containing 50.00 mL of 0.1011 N $\mathbf{H_2SO_4}$.
d) The excess $\mathbf{H_2SO_4}$ was determined by titration with 5.12 mL of 0.1266 N **NaOH**.

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

Note: Distillation of NH_3 prior to digestion gives the inorganic NH_3-N. This can be subtracted from the total Kjeldahl N to give the organic Kjeldahl N.

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.

$$\mathbf{IO_4^-} + 3\,\mathbf{I^-} + \mathbf{H_2O} \rightarrow \mathbf{IO_3^-} + \mathbf{I_3^-} + \mathbf{OH^-}$$

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

$$2\,\mathbf{S_2O_3^{2-}} + \mathbf{I_3^-} \rightarrow 3\,\mathbf{I^-} + \mathbf{S_4O_6^{2-}}$$

(Note: that in the presence of excess iodide ion, iodine is rapidly interconverted to triiodide ion; $\mathbf{I_2} + \mathbf{I^-} \equiv\equiv\equiv \mathbf{I_3^-}$)