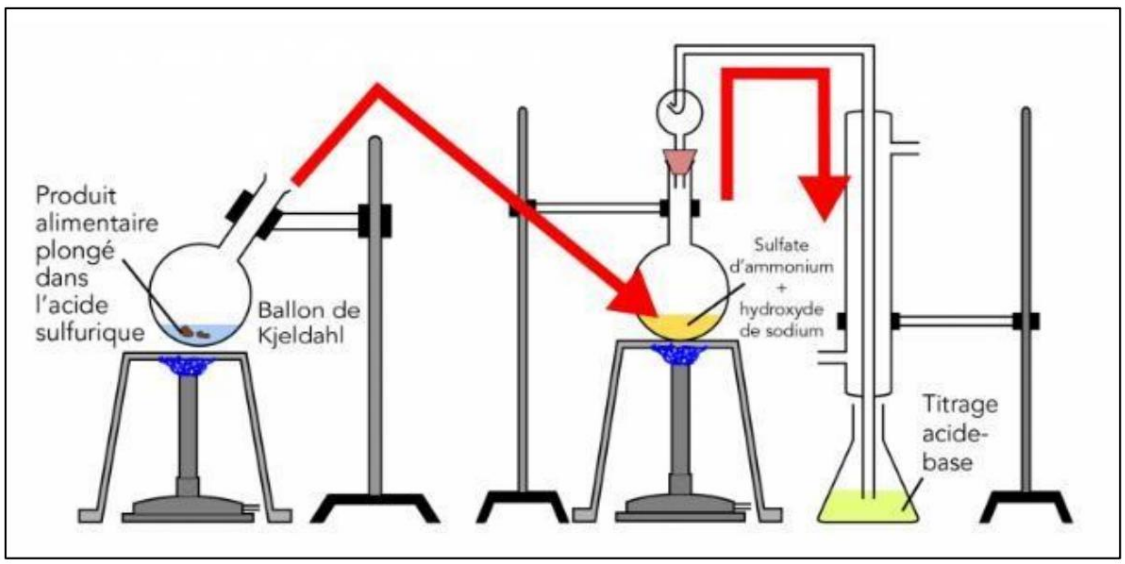
TP4: Determination of total nitrogenous matter (TNM) by Kjeldahl method

1.. Determination of total nitrogenous matter by Kjeldahl method (AOAC, 1999; 1995; 1999)

The Kjeldahl method involves three main successive stages:

- The organic nitrogen contained in the sample is transformed into mineral nitrogen by presence of a concentrated acid: ***this is mineralization***
- The mineral nitrogen formed is displaced in the presence of soda and by steam entrainment then collected quantitatively in a standard receiving solution: ***this is distillation***
- The nitrogen thus collected is titrated with an acid of known normality: ***this is titration.***



Reagents

- Concentrated H2SO4 (95%)
- 40% NaOH
- 0.1N HCl
- Catalyst (80g potassium sulfate + 20g copper sulfate + 2g selenium)
- Receiving solution (per liter of solution): dissolve 40 g of boric acid in a little water distilled then add 10ml of RB dye solution (200mg of methylene blue and 100mg of methyl red in 100ml of 95° alcohol), the whole is brought to 1000ml.

Figure: Nitrogen mineralizer and distiller

Operating mode

a- Mineralization

- Accurately weigh about 500mg of sample (after homogenization) on

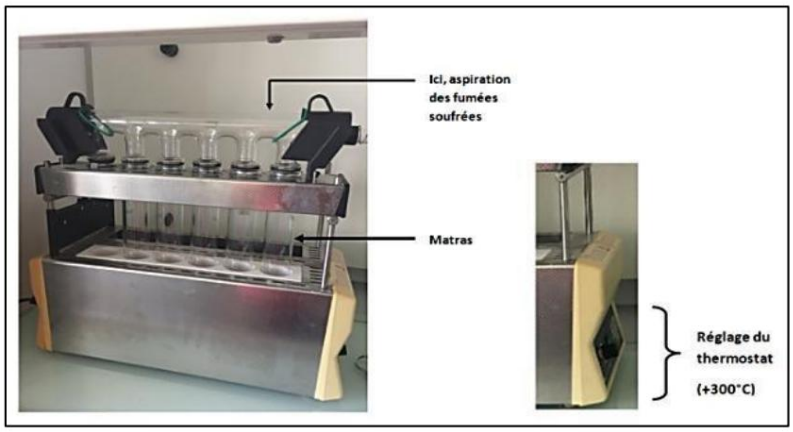
Joseph paper (paper made without nitrogen) folded into a papillote and introduced into the mattresses and add the catalyst.

- Add very slowly and while stirring 20ml of concentrated H2SO4 . This operation must be

absolutely carried out with caution: work under a hood, do not orient the tube or the vial when adding the acid towards the direction of the face and wear gloves, goggles and a protective apron.

- Place on a mineralization ramp heated to 420 - 450°C. Start the system

vapor suction. Generally, the start of mineralization is accompanied by a formation of foam which can sometimes overflow the flask. To avoid losses, it is It is advisable to remove the support from the tubes to let them cool for 5 to 10 minutes and then put back again.

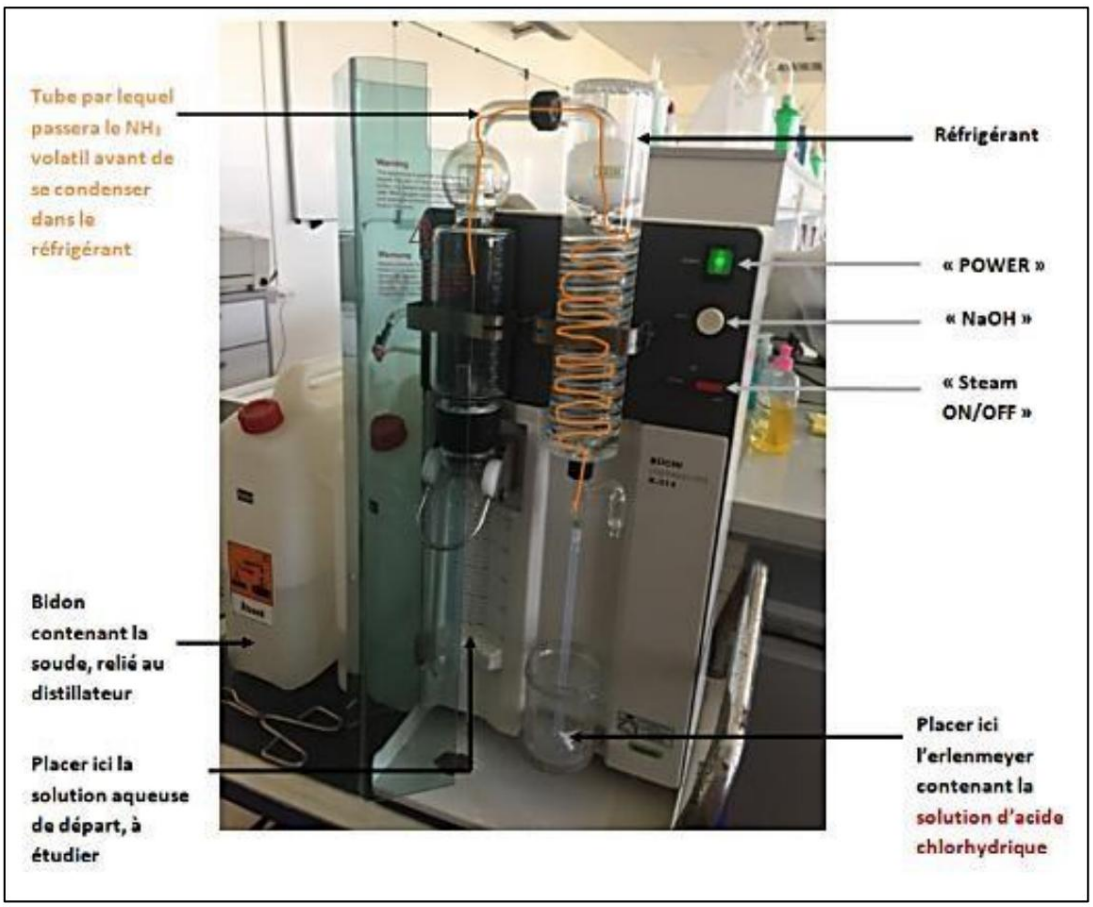


- Stop the mineralization ramp when the solution becomes clear (pale green) and leave cool for about 15 to 20 minutes (do not stop the fume extractor immediately)

Slowly add, while stirring, 150 ml of distilled water (if a precipitate forms, it is necessary to shake the matrass vigorously to dissolve it) **(Le Coq, 1965).**

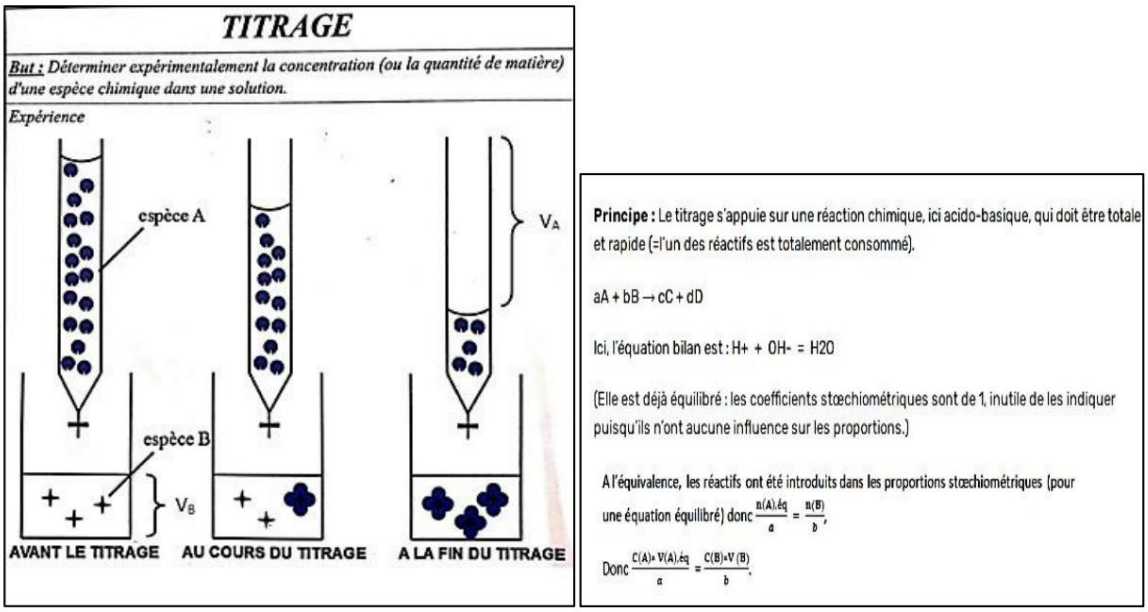
b- Distillation and titration

- Place 50 ml of receiving solution in an Erlenmeyer flask. Place the Erlenmeyer flask below the unit distillation. Place the flask on the distillation unit while properly securing the stopper then transfer 70 ml of soda (it is sometimes advisable to add a few pieces of stone think to avoid bursting of the flasks when boiling). Turn on the distillation until obtaining 200 ml of greenish solution in the Erlenmeyer flask (do not forget to turn on the refrigeration system).

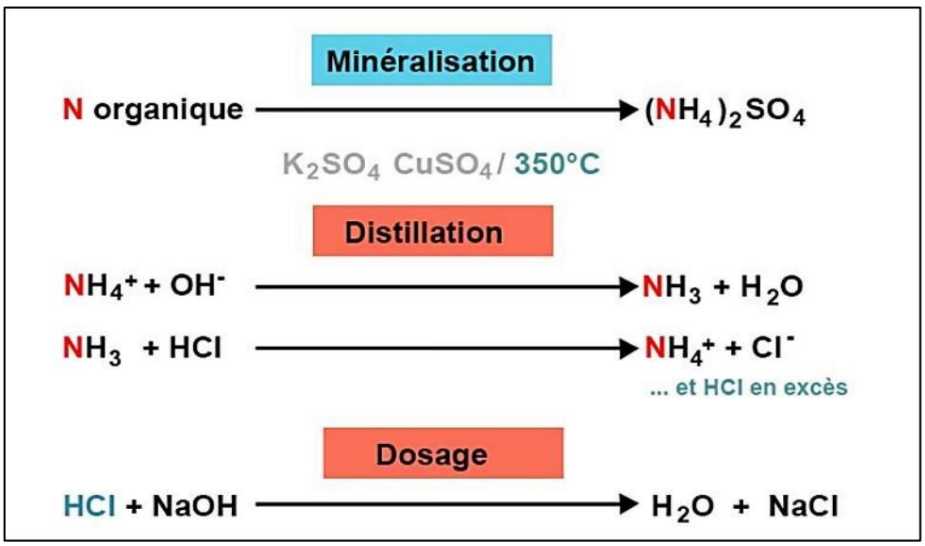


- Titrate the contents of the Erlenmeyer flask with 0.1N HCl solution until the color changes.

from green to dirty gray.



On the other hand, it is absolutely necessary to carry out blanks during each series of analyses in order to avoid the possible effect of any kind of impurities which may exist in particular in the reactive on subsequent results. These white analyses consist of following the different steps of the method, but in the absence of the sample to be analyzed.



The nitrogen or MAT content of the sample is thus calculated as follows:

$$\%N = (((V - V_o) \times N \times 14{,}01) / PE \times MS_a) \times 100$$

$$\%MAT = \%N \times 6{,}25$$

V : number of ml of HCl used for sample titration

Vo : average number of ml of HCl used for the titration of blanks

N : normality of the HCl used

14.01 : equivalence factor, 1 ml of 1N HCl contains 14.01 mg of nitrogen

PE : test sample (in mg)

MSa: % MSa / 100.