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DETERMINATION OF CRUDE PROTEIN BY KJELDAHL's METHOD

INTRODUCTION: ***Allyl Isothiocyanate present in mustard oil is also known as Pungency with chemical formula as CH2=CH=CH2–N=C=S and molecular weight 99.18g/mole. To test AITC is very sensitive as it's conc. is very low and is volatile at room temperature (flash pt 46). It is insoluble in water but good soluble in ethanol. This AITC or pungency test is important to distinguish between expeller oil or cold pressed oil.***

METHODOLOGY: ***The Allyl Isothiocyanate in oil is transferred by steam distillation into a measuring flask containing excess of known silver nitrate solution where the reactions between takes place. The remaining unused $AgNO_3$ is calculated by titrating with standard solution of ammonium thiocyanate and similarly a blank titration is performed to find the actual total $AgNO_3$ taken.After finding the value of used silver nitrate and its relation with wt. of AITC, we will derive a formula to calculate the Allyl Isothiocyanate% related to titration values .***

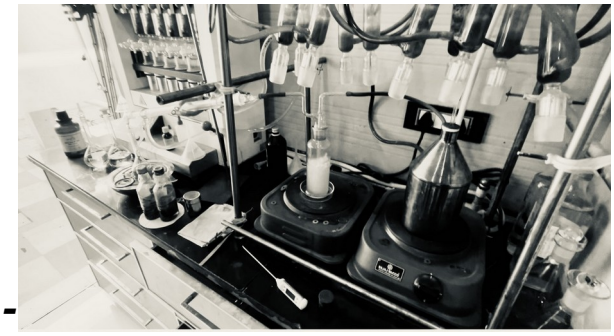
APPARATUS & EQUIPMENTS :

1.Distillation flask 500ML.
2.Weighing balance.
3.100ml beaker with dropper
4. ***Reflux air condenser.***
5.Steam generator.
6. Long reflux water condenser.
7. Measuring flask 250 ML
8. Water bath.
9. 50ml Burette with stand
10. 25ml Pipette with filler
11. 500ml titration flasks-2
12. Funnel with filter paper

CHEMICALS & REAGENTS

1. ***Ethyl alcohol***
2. ***N_1=N/20 $AgNO_3$ solution***
3. ***N_2=N/20 NH_4SCN solution***
4. ***10% NH_4OH solution***
5. ***Conc. Nitric acid***
6. ***10% Ferric ammonium sulphate***

PROCEDURE STEPS : To better understand we have divided the complete procedure of Allyl Isothiocyanate determination into following 6steps:-



1.CHEMICAL & REAGENTS PREPARATION

2.SAMPLE & BLANK PREPARATION

3.STEAM DISTILLATION

4.FILTRATION

5.FORMULATION

6.TITRATION (Sample & blank)

STEP1 CHEMICAL & REAGENTS PREPARATION

Ethyl alcohol ➡ As the ethyl alcohol becomes acidic timely therefore neutralise it with N/10 NaOH solution using phenolphthalein as indicator

N_1=N/20 $AgNO_3$ ➡ dissolve 4.247 g of AR grade silver nitrate in 500 ML of distilled water and standardise with NaCL solution using potassium chromate as indicator to find the actual normality

N_2=N/20 NH_4SCN ➡ Dissolve 1.962 g of AR grade ammonium thiocyanate in 500 ML of distilled water and standardise with N/20 $AgNO_3$ solution

10% Ferric alum as indicator ➡ dissolve 10 g of ammonium ferric sulphate in 100ml of distilled water

STEP2 SAMPLE & BLANK PREPARATION

<u>Sample preparation</u> ➡ we will weight accurately near about 5g of oil in distillation flask by mixing the sample thoroughly. If direct weighing of oil in distillation flask on the balance is not possible due the over weight of distillation flask then use the indirect method. For that take approximately 50ml of the oil in the 100ml beaker and weight it along with the dropper and note the reading W1. Now carefully transfer about 5g of oil from the beaker to the distillation flask using this dropper and again take the weight of oil beaker along with used dropper and note this as final reading W2. By substracting final

reading from the initial reading we will have the accurate weight of oil taken in the distillation flask. Now add to it 25ml of ethyl alcohol to dissolve all the AITC present in the oil and then add 150ml of distilled water. Close the distillation flask with glass stopper as the compound AITC is volatile at room temperature.

<u>Blank preparation</u> ➡ Take 500ml empty conical titration flask and add to it exactly 25ml of N/20 AgNO3 solution (as same as that we will take in measuring flask for sample) and 150ml of distilled water.

STEP 3 STEAM DISTILLATION

In this process we will connect the distillation flask on one side with steam generator and on other side with long reflux water condenser using the glass joint assembly as shown in the above picture. Now we will take a 250ml of measuring flask and after adding to it exactly 25ml N/20 AgNO3 solution and 10ml of 10% ammonium hydroxide solution, connect to the lower end of condenser. The steam generated passes through the side hole to the distillation flask and the mixture starts boiling. The vapours of AITC formed get condensed through the condenser and are collected as droplets into the measuring flask containing AgNO3 solution and NH4OH solution where the reaction between takes place. After collecting about 150ml of the distillate stop the distillation process and immediately remove the measuring closing with stopper.

Now either keep this measuring flask in a dark cool place overnight to have complete reaction between the chemicals or heat on a water bath by attaching the reflux air condenser for 1hour to accelerate the reaction. Cool down the mixture distillate to room temperature by keeping the measuring flask under the running water tap.

STEP4 FILTRATION PROCESS:

As shown below in the reactions, the AITC does not react directly with AgNO3, initially the AITC reacts with the ammonia liberated by ammonium hydroxide to produce unstable compound THIOSINAMINE and this compound further reacts with silver nitrate to form Allyl cyanamide and silver sulfide. Now our distillate contains both the unused AgNO3 to be measured and unwanted Ag2S , so we will remove Silver Sulfide by filtering the complete distillate into a 500ml conical titration flask using funnel and filter paper followed by rinsing the emptied measuring flask twice with distilled water.

<u>CHEMICAL REACTION PROCESS</u>

CH2=CH-CH2-N=C=S + NH3 ➡ CH2=CH-CH2-NH-CS-NH2

Allyl Isothiocyanate Ammonia Thiosinamine

CH2=CH-CH2-NH-CS-NH2 + 2AgNO3 ➡ CH2=CH-CH2-NH-CN + Ag2S

Thiosinamine Silver nitrate Allyl cyanamide Silver sulfide

STEP5 FORMULATION : To find the value of AITC firstly we will find the relation between the wt of AITC equivalent to volume of used or reacted AgNO3 and secondly we will find the volume of used AgNO3 by having the value of unused AgNO3 and actual total AgNO3 through titration processes.

In the both reactions, 2 moles of AgNO3 reacts with 1 moles of AITC

Therefore 1L of 1N AgNO3 Mo***l.wt. of AITC/2***

↪Or ***1000ml of 1N AgNO3*** 99***.18/2*** ***49***.5***9g of AITC***

↪***Or*** 1***ml of 1N AgNO3*** ***0.***04***959g of AITC***

↪***Or*** 1m***l of N1 normality of AgNO3*** ***N1×***0.***0***4959***g AITC***

Or wt of AITC for used volumeV of N1 AgNO3 ***N1×V×0***.04***9***59***g***

EQUATION FOR AITC in (w/v) 0.04959***×V×N1***

Now i***n t****he next step we will find the value of V and put in this equation*

STEP 6 TITRATI***ON(SAMPLE & BLANK) :***

For titration process we will add standard solution of ammonium thiocyanate of normality N2=N/20 in the burette.

SAMPLE T**ITRATION: Take t****he sample titration flask containing filtered distillate and add 5ml of conc. nitric acid and few drops of indicator ferric alum. Note the initial reading on the burette and start the titration by adding titrant NH4SCN dropwise. When the colour change from grey white to red orange stop the the titration process and note the final reading on the burette. By subtracting initial reading from the final reading we will have the volume of NH4SCN used as Vs*

Now by applying Normality equality equation NxVx=NyVy

Putting the values in the equation ➡N1V1=N2V***s➡v1=vs×***N***2/N1***

Vo***l of unused AgNO3 ➡V1=Vs×***N***2/N1***

BL***ANK TI*****TRATION:Similarly** titrate the blank titration flask containing total volume of AgNO3 and note the volume of NH4SCN used as Vb*

Now by applying Normality equality equation NxVx=NyVy

And putting the value in the equation ➡N1V2=N2V***b***

Vol of total AgNO3 ➡V2=Vb×N***2/N1***

V= ***vol of used AgNO3 =Vol of total AgNO3 —Vol of unused AgNO3***

V =V2—V1=(Vb—Vs)N2/N1

Now put this value of V in the AITC equation and finally we get

Equation for Wt. of AITC = 0.04959(Vb—Vs)N2

Now AITC% = wt of AITC ×100

wt of oil

Therefore **FORMULA for**

Allyl Isothiocyanate %(w/v)

$$\text{AITC\%} = 0.04959\frac{(Vb—Vs)\ N\times 100}{W}$$

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Where

Vb=volume of NH4SCN used in blank titration

Vs=volume of NH4SCN used in sample titration

N=normality of NH4SCN

W= weight of oil used for test

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SAMPLE TITRATION PROCESS

$C_3H_5NCS + AgNO_3 + AgNO_3 \rightarrow C_3H_5\text{–}NH\text{–}CN + Ag_2S$

AITC USED UNUSED

$AgNO_3 + NH_4SCN \rightarrow\rightarrow AgSCN + NH_4NO_3$

unused Ammonium Thiocyanate Silver Thiocyanate Ammonium Nitrate

BLANK TITRATION PROCESS

$AgNO_3 + NH_4SCN \rightarrow\rightarrow AgSCN + NH_4NO_3$

Total Ammonium Thiocyanate Silver Thiocyanate Ammonium Nitrate

$NH_4SCN + NH_4Fe(SO_4)_2 \rightarrow (FeSCN)^{+2}$ ions

(Indicator) **(Red colour free ions)**

During TITRATION after complete absorption of unused AgNO3 -then very next drop of NH4SCN reacts with indicator ferric alum to produce orange red colour free ions: