PHARMACEUTICAL ANALYSIS

Pharmaceutical analysis may be defined as that branch of chemistry which deals with

- Resolution
- Separation
- Identification
- Determination and estimation of impurities that may be present in the sample.

The volumetric analysis is based on the fact that in all balanced chemical reactions utilized for the purpose, ie equivalent weight of one substance reacts with the equivalent weight of the other quantitavely.

Various types of volumetric assay depends upon the type of reactions involved. ie,

- Acid base titration (Neutralization titration)
- Non aqueous titration
- Redox titration
- Precipitation titration
- Complexometric titration

Fundementals in volumetric analysis

1. Principle: the volumetric analysis makes use ofa chemical reaction between the substance to be determined (Analyte) and the other substance (reagent0.
2. The reaction shoul be fast, quantitative and should take place according to a chemical reaction, such as

   aA + rR = Products

where a molecules of analyte "A" which reacts with "r" molecules of reagent "R".
ie, A given amount of analyte will react with a definite amount of reagent and this amount can be calculated with help of this Equation.

The volumetric titration of the analyte with the reagent involves various steps

(a) **Standard Solution** -The known amount of reagent dissolved in known volume of water, so that the concentration of reagent solution can be calculated. Such solution is called a standard solution. Always a volumetric standard flask is used to prepare the standard solution.

(b) **Sample Solution.-** With the help of pipette, a known volume of analyte soln is taken in a conical flask. The analyte soluton is also called sample soln, test soln or titrand.

(c) **Indicator -** A few drops of an indicator soln are added to the titrand soln, taken in the conical flask. The function of indicator is to show when the Reaction between the analyte and the reagent is just complete, by a change in colour of the solution.

(d) The standard solution of the reagent (titrant) is taken in burette and gradually added to the analyte in the conical flask until the reactionis just complete with a change in colour

(e) The volume of titrant needed is recorded and as the concentration of standard solution is known , the amount / concentration of analyte can be determined exactly

(f) **The Equivalence Point/ End Point** –The point during titration at which the reaction between titrand and titrant is just completed, is called equivalence point. The point at which indicator gives color change is called end point.

(g) **Titrant:** - The solution of accurately known concentration , usually added from burette.

(h) **Titrate /Analyte** :- The active substance in solution to be determined, usually taken in conical flask.

(i) **Titration Error-** In the ideal case of the titration, the visible end point and the theoretical end point(equivalence point) should coincide. But in practice this can never happen and usually a very small differences occurs b/w these 2 points and this difference is called titration error.

METHODS FOR THE DETERMINATION OF CONCENTRATION OF SOLUTIONS.

Ussualy the strength of the solutions refers to the weight of solute dissolved in a unit volume of solutions. It can be expressed in many ways.

1.**NORMALITY** :- When One gram equivalent of substance is dissolved in 1 dm^3 (1 litre), then

it is 1.0 N solution.

One gram equivalent of a Substance is that amount of it which, in a specified reaction, combines or releases that amount of hydrogen which is combined with 3 grams of Carbon 12 in methane ($^{12}CH_4$)

$$\text{Normality} = \frac{\text{No of gram equivalent of solute}}{\text{no of litre of solutions}}.$$

2. **MOLAL SOLUTIONS** :- It's a solution in which a known number of moles of solute is dissolved in 1 kg of solvent . ie 1.0 Molal solution contains 1 gm molecular weight of solute dissolved in kg of solvent

Molarity $= No: of\ moles\ of\ a\ component\ /_{kg\ of\ mass\ of\ solvent}$

(independent of temperature)

Molality= $No: of\ moles\ of\ a\ component\ /_{litre\ of\ solvent}$

(Varies with temperature)

3. **MOLE FRACTION**

$$\text{Mole fraction} = \frac{\text{no of moles of component}}{\text{total number of moles of all components}}$$

4**. FORMAL SOLUTION**

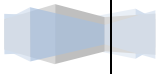
A formal solution is one which contains a formula weight of solute in one dm^3 of solution.its denoted by "F". Formula weight is the summation of atomic weights of all atoms in the chemical formula.

5. **WEIGHT PERCENT/PERCENTAGE COMPOSITION BY WEIGHT**

It indicates the number of grams of solute per grams of solutions.

$$P = \frac{W}{Wo} \times 100$$

Where, P= percentage by weight of solute



W = weight in grams of solute

W_0 = no of grams of solvent.

Ie, if 5 g of NaOH is dissolved in 45 g of water, then wt % ,

$$P = \frac{5}{5+45} \text{ x } 100$$

= 10% w/w.

6. **PERCENTAGE COMPOSITION BY VOLUME**.

Here the concentration is expressed in terms of volumes of solute and solvent . ie, if 20ml of ethanol is dissolved in 80ml of water., then

$$P = \frac{20}{20+80} \text{ x } 100$$

= 20 % w/v

7. **PARTS PER MILLION (PPM)**

Here the concentration is expressed in terms of grams of solute per million cm^3 of solution (mg of solute per 1 lt of solution). Thus a solution having 10mg/lt of a solute or 10 µg/ml of solution is 10ppm.

1mg/lt = 1µg/ml= 1ppm OR 10 mg/lt = 10 µg/ml= 10 ppm

8. **MOLE PERCENT**

Mole percent = mole fraction X 100 OR

$$\frac{= \text{ No of moles of a component .}}{\text{no of moles of solutes and solvent}} \text{ x } 100$$

PRIMARY STANDARD AND SECONDARY STANDARDS

Primary standard – the substances which are available in pure state and are not hygroscopic can be used for the preparation of primary standards by dissolving an accurately weighed amount

of these in water and making up the amount to the known volume by dilution with the solvent .such substances are known as primary standards.

A primary standard should satisfy the following conditions like,

- Must be cheap, easily available ,easily purifiable and driable at 110-120 ^{o} C and should remain in pure state for long time.
- Should remain unaltered in air during weighing, ie should not absorb moisture, should not oxidisable or reducable and should not affect by CO_2
- Should be capable of being tested for impurities by simple tests, amount of impurities Should not be greater or equal to 0.01 -0.02 %.
- Should have high equivalent weight so that weighing errors may be negligible
- Should be readily soluble and should be practically instant.

SECONDARY STANDARDS

The primary standard thus prepared should be then again standardized with a known concentration of primary standard. The primary standard whose concentration or strength thus formed out is termed as secondary standard.

It is the solution of known concentration derived from the primary standard

Examples –

Sl.no	Titration	Primary	Secondary
1.	Aid base titration	• Oxalic acid • Benzoic acid • Potassium hydrogen phthalate • Sulfamic acid	• Sodium carbonate • sodium hydroxide • hydrochloric acid • Sulfuric acid

2.	Redox titration	Oxidants • Potassium dichromate • Potassium bromate • Potassium iodate	Reductants • Oxalic acid • Sodium oxalate • Arsenic trioxide	
3.	Precipitation titration	• Silver nitrate • Silver chloride • Potassium chloride • Potassium bromide		
4.	Complexometric titration	• EDTA (Ethylene Diamine Tetra Acetic acid) • Pure metallic zinc and magnesium • ZnCl • $CaCl_2$		

SIGNIFICANT FIGURES

Significant figures are digits necessary to express the result of a measurement to the precision with which measurement is made, ie The number which expresses the result of a measurement such that only a last digit is in doubt. Eg, in the reading of a 50ml burette the small graduation is 0.1ml. Thus an analyst can be estimate the reading upto 0.01ml by mentally dividing the smallest division into 10 equal parts thus getting in figure 1, the reading around 1.42ml approximately . Thus in this value there are 3 significant figure, 2 certain and 1 uncertain (last digit).

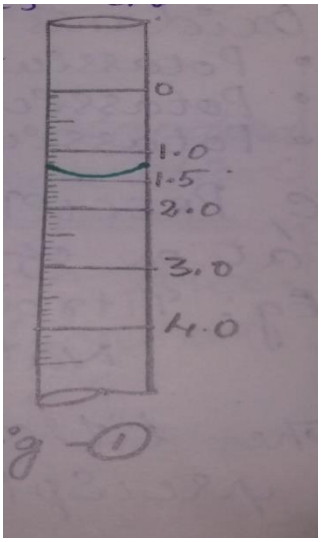


Fig. 1. Reading showing the burette reading to the approximate value.

4 RULES IN SIGNIFICANT FIGURES.

Rule 1.

All non zero digits are significant

- 2.345 in this there are 4 significant figures
- -9.66 In this there are 3 significant figures.

Rule 2.

Final zeros right to the decimals are significant

- 2.0 km :- 2 significant figures.
- 6.300 cm ;- 4 significant figures.

Rule 3.

Zeroes found between 2 significant figures

- 17.008m :- 5 significant figures.
- 28.0005 :- 6 significant figures.

Rule 4 .

Zeroes as place holders left to the decimal are not significant

- 0.00038 m: - 2 significant figures.
- 42 g :- 2 significant figures.

Rules for computation of significant figures.

1, Rounding off the numerical figures

In this all the digits to be rounded off are removed together and not one at a time. Eg-

A, 76.8437 → 76.8

All the numbers following the last figure to be retained is less than 5.

B, 59.873 →59.9

If the figure following the last digit to be retained is greater than 5, the last digit retained is increased by 1.

C, 69.752 →69.8

If the figure following the last digit to be retained is 5 and at least one of the other digit is non ero, the last digit retained is increased by 1.

D, 68.250 →68.2
68.350 → 68.4
In the figure, following the last digit to be retained if 5, all the other digits to be removed are zero, the last digit retained is not changed if it is even but it is increased by 1 if it is odd.

2, Addition And Subtraction

In addition and subtraction only as many decimal places are kept as occur in the number which has fewest decimal. Eg -15.23 + 9.145 – 4.6758 +121.4

The final answer should be expressed in only one decimal place. The number may be first rounded off to 2 decimal places, then find out the answer.

15.23 +		15.23 +
9.145 –	rounded off to 2 decimal point	9.14–
4.6758 +	⟶	4.68 +
121.4		121.4

		141.09

3, Multiplication And Division

In this the number of significant figures in the result is the same as the least reliable measurement. Eg. 0.165 m^3 X 10.487 kg/m^{3+} = 1.730355 kg/m^3

= 1.73 kg/m^3

4, Logarithms

Its made of 2 parts, a whole number and a decimal fraction. The whole number is not considered as the significant figure but the decimal fraction (mantissa) all digits are considered as significant. Eg. 2.4 x 10^5

From the log table .mantissa is 0.3802, the characteristic is 5 ,hence value is 5.38

ERRORS

Error is defined as the numerical difference between a measured value and a true value of an analytical determination.it can be classified as 1

1, Determinate Error (Systemic Error)

Depending upon the way of occurring

Depending upon the magnitude

2. Indeterminate Error (accidental error)

1. Operational error and personal error	1. Constant error	1. Physical error
2. Instrument and reagent error	2. Propotional error	2. Chemical error
3. Method Error		
3.Unknown Source		

1,. DETERMINATE ERROR- those errors which can be avoided or whose magnitude can be determined.

a. Operational And Personal Error

Due to the factor for which the experimenter is personally responsible and donot depend on method or procedure

- They arise from erractic personel judjement ,prejudice or biased, inability
    1. Eg- judgement of a colourof the solution at the end point
    2. Estimation of position of pointer in balance.
    3. Level of meniscus on a burette/pipette.
    4. Insufficient cooling of a crucible before weighing
    5. Allowing hygroscopic material to absorb moisture before /during weighing

Such errors can be varied from person to person and can be minimized by experienced and careful physical manipulation.

b. Instrumental And Reagent Error

These arise from the imperfections in measuring devices. Electronic devices are much prone to this because of voltage fluctuations

1. Eg. Unadjusted chemical balance
2. Uncalibrated weight's usage
3. Usage of uncalibrated glasswares
4. Reactions between chemicals and porcelien resulting in introduction of new undesirable material.
5. Usage of low grade chemicals.

c. Method Error

These errors arise from incorrect sampling and incomplete rezctions involved in the determination

1. Eg .In volumetric analysis, errors arise due to failure of reaction to proceed to completion, occurance of side reactions etc

2. In iodometric, determination of Cu,I gets adsorbed on starch which is difficult to remove completely

The determinate errors are further classified into constant errors and propotional errors

A, Constant error (Depends on Magnitude) – Independent of magnitude of measured quantity and becomes less significant as magnitude increases , also independent on concentration.

Error value remains same, its magnitude changes.

For example- In a number of titration if there is a constant error of 0.10 ml, then this indicates 1 % of 10 ml titrant and 0.2% or a 50 ml of titrant .

Ie, Constant Error decreases with increases in a measured quantity.

B, Proportional Error – Most common errors, because of presence of interfering impurities in sample. Here its magnitude will remain constant with the change in concentration.

Eg- In Iodometric titration of copper, if the sample is contaminated with Fe(III) impurity, it will also liberate I_2 from KI along with copper; hence resulting in a false result of high copper concentration. And if the concentration of sample is doubled, the error will also get doubled.

INDETERMINATE ERROR

Random errors arises from uncertainties which are associated with physical or chemical measurements. Such errors cannot be attributed to any known source or cause.

- They are random or accidental in nature .
- Lead to both high and low results with equal propability
- Cannot be eliminated or corrected
- And are the ultimate limitation on the measurement.

Minimization of errors –

They can be minimized by

- Calibration of apparatus and application of corrections.
- Analysis of standard samples.
- Running a blank determination
- Independent analysis- ie strength of HCl can be determined by titration with Na OH and again it can be determined by gravimetric precipitation with AgNO3 as AgCl. If the 2 results obtained are concordant, high propablity of correct values.

ACCURACY AND PRECISION

- There are certain basic concepts in analytical chemistry that are helpful to the analyst when treating analytical data. This section will address accuracy, precision, mean, and deviation as related to chemical measurements in the general field of analytical chemistry.

ACCURACY

- In analytical chemistry, the term 'accuracy' is used in relation to a chemical measurement. It can be defined as"closeness of the agreement between the result of a measurement and a true value." In theory, true value is that value that would be obtained by a perfect measurement.
- Accuracy is how close a measurement is to its desired or theoretical value.
- For example, if we need to dispense 25.0 mL of dilute HCl, then dispensing 24.9 mL is more accurate than dispensing 25.7 mL.
- Accuracy usually is reported as a percent error

$$\%\text{Error} = \frac{\text{actual value} - \text{expected value}}{\text{expected value}} \times 100$$

For analytical methods there are two methods for determining accuracy.

1. ABSOLUTE METHOD – In this Sample containing known amount is used. Various concentration of known amount is made and proceeded according to specified instructions
   The amount of constituent should be varied, because the determinate error is considered as a function

Eg 1, 100% known + 5% added ---------- 105%
2, 100% known + 6% added ---------- 106%
} Accurate

1, 100% known + 5% added ---------- 105%
2, 100% known + 6% added ---------- 105%
} Not Accurate

2. COMPARATIVE METHOD – sometimes when primary samples unavailable and are impossible to prepare it for analyst. In those cases a secondary standard is used in a same method. But this method is not usefull in analytical purpose.

PRECISSION

Precision is the reproducibility of a set of measurements. For example, Three identically prepared solutions with pH values of **6.76, 6.73,** and **6.78** are more precise than a duplicate set with pH values of **6.76, 6.54,** and **6.92**.

Precision usually is reported as a standard deviation,s,

which we define as $S = \frac{\sqrt{\sum(xi - \bar{x})^2}}{2n-1}$

Where '$\bar{x}$' is the average, or mean result, and 'xi' is one of the 'n' different results.

Another example - 2 analyst performing analysis for an accurate value 70%.

Analyst 1

70.07
70.18
70.14
70.08
70.02

NOT PRECISE

Analyst 2

70.20
70.21
70.23
70.21
70.20

MORE PRECISE

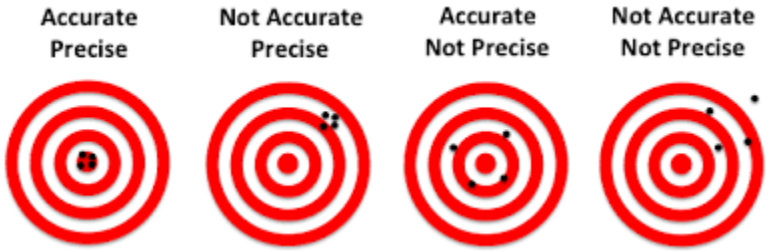


Figure 2: Clusters of five rifle shots illustrating the difference between accuracy and precision

PREPARATION AND STANDARDIZATION OF VARIOUS MOLAR SOLUTIONS.

1. **Oxalic acid (COOH)2**

Oxalic acid is available in pure state and its standard solutions can, therefore, be prepared by the direct method.

Eq. wt. of hydrated oxalic acid (C2H2O4.2H2O), being 63 its 0.1N solution would contain 6.3 gm/litre, and 0.05 N solution would contain 3.15 gm/litre.

These standard solutions are employed to find the strength of solutions of alkalies (NaOH and KOH) whose standard solutions cannot be prepared by the direct method.

PRINCIPLE – REDOX TITRATION

$KMnO_4$, potassium permanganate is a strong oxidizing agent. Oxalic acid is oxidized by potassium permanganate in acidic solution to produce CO_2 and H_2O

$$2KMnO_4 + 3\ H_2SO_4 + 5\ (COOH)_2 \longrightarrow 2MnSO_4 + K_2SO_4 + 10\ CO_2 + 8H_2O$$

HCl cannot be used in place of sulphuric acid as it readily get oxidized to chlorine in presence of KMnO4

Preparation Of 0.1N Oxalic Acid Solution

Weigh accurately 6.3 gm of oxalic acid & dissolve in distilled water & finally make up the volume to one liter in a volumetric flask.

Standardization Of 0.1 N Oxalic Acid

- Clean and dry al the glasswares as per standard laboratory procedure.
- Take 20 ml of prepared oxalic acid in a conical flask
- Add 5 ml of sulphuric acid , and warm at 70 ^{0}C
- Rinse the burrete with distilled water and pre rinse with the portion of potassium permanganate soln.
- Start the titration with 0.1N $KMnO_4$ until the end point.

- End point is the appearance of pink colour that persists for more than 30 seconds.
- Record the reading repeat the titration 3 times to get th precise values.

Calculation

$$\text{Normality of Oxalic Acid} = \frac{\text{Weight Taken} \times \text{Expected Normality}}{\text{Equivalent Weight Factor} \times \text{Titre Value}}$$

Sl no	Content in conical flask	Burette reading		Indicator/color change	Volume used.
		Initial	Final		
1.	20ml oxalic acid + 5ml Sulphuric acid			KMnO4/persistence of pink colour.	
2.					

2. SODIUM HYDROXIDE

Sodium hydroxide is hygroscopic and absorbs water from the air when you place it on the balance for massing. This water will prevent you from being able to find the exact mass of sodium hydroxide.

In order to determine the exact concentration of a sodium hydroxide solution you must standardize it by titrating with a solid acid that is not hygroscopic.

Potassium hydrogen phthalate, KHC8H4O4 (abbreviated KHP/PHP), is a non-hygroscopic, crystalline, solid that behaves as a monoprotic acid.

It is water soluble and available in high purity. Because of its high purity, you can determine the number of moles of KHP directly from its mass and it is referred to as a primary standard. You will use this primary standard to determine the concentration of a sodium hydroxide solution

-

PRINCIPLE – ACID BASE TITRATION

An acid-base titration is a procedure used to compare the amount (moles) of acid in one sample with the amount (moles) of base in another. In this laboratory exercise you will carry out such a titration to standardize (determine the exact concentration of) a NaOH

solution by measuring accurately how many milliliters of it are required to exactly neutralize a known amount of acid. A buret filled with the titrant (NaOH solution) is used to measure the volume of NaOH solution added to the known amount of acid in a flask. An indicator is added to signal the endpoint has been reached

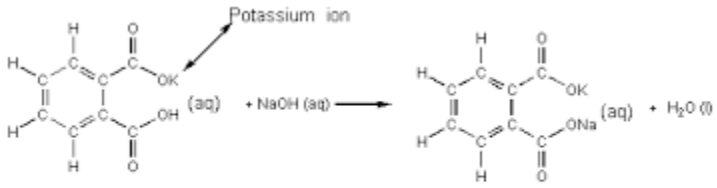


Figure 1. Neutralization reaction of potassium hydrogen phthalate with sodium hydroxide forming sodium potassium phthalate and water.

Procedure

1. Preparation of 0.1 M NaOH

- Weigh about 402 g of sodium hydroxide in a 1000ml volumetric flask. Add water mix the solution well and make up to 1000ml with distilled water

2. Standardization of 0.1M Sodium hydroxide

- Weigh accurately about 0.500g of Pottasium hydrogen phthalate, previously powdered and dried at 120 ^{0}C for 2 hrs and dissolve in 75ml of CO2 free water in a conical flask.
- Add 0.1ml phenolphthalein solution
- Fill the burette with prepared 0.1M NaOH
- Titrate the PHP with 0.1M NaOH until thr colour change of a permanent pink colour.
- Repeat the titration for 3 times to get the precise reading
- Equivalent weight factor of PHP for 0.1 M NaOH = 0.020422 g

Calculation

$$\text{Normality of Sdoium hydroxide} = \frac{\text{Weight Taken} \times \text{Expected Normality}}{\text{Equivalent Weight Factor} \times \text{Titre Value}}$$

3. HYDROCHLORIC ACID

Principle- A known concentration or strength of sodium carbonate is titrated directly with HCl. End point can be detected by using methyl orange or methyl red indicator. Reaction involves,

$Na_2CO_3 + 2\ HCl \longrightarrow 2\ NaCl + H_2O + CO_2$
(Sodium carbonate)

Preparation of 0.1 M HCl

Pipette out 8.5 ml of HCl and dilute upto 1000 ml with distilled water.

Standardization of 0.1 M HCl.

- Clean and dry al the glasswares as per standard laboratory procedure.
- Rinse the burette with distilled water and again rinse the same with a portion of 0.1M Hcl. This is necessary to ensure that all of the solution in the burette is of desired solution not of diluted or contaminated solution.
- Weigh accurately 0.150 g of sodium carbonate heated previously for 1 hr, t 270 ^{0}C and transfer it into a conical flask.
- Add 20 ml of distilled water and shake well or sonicate for 5 min.
- Add 2 drops of methyl orange indicator
- Titrate with 0.1 m HCl by adding small quantities of HCl soln from burrette.
- End point is the appearance of pink colour that persists for 30 seconds.
- Repeat the titration for 3 times to get the precise reading
- Equivalent weight factor of sodium carbonate for 0.1 M HCl= 0.00529 g

Calculation

$$\text{Normality of 0.1 N HCl} = \frac{\text{Weight Taken x Expected Normality}}{\text{Equivalent Weight Factor x Titre Value}}$$

4, **SODIUM THIOSULPHATE**

Sodium thiosulfate is an inorganic sodium salt composed of sodium and thiosulfate ions in a 2:1 ratio. It has a role as an antidote to cyanide poisoning, a nephroprotective agent and an antifungal drug.

- Synonyms: SODIUM THIOSULFATE
- Molecular Formula: $Na_2O_3S_2$ or $Na_2S_2O_3$

It is typically found in its pentahydrate form which is either white in colour, or colourless altogether. This pentahydrate of sodium thiosulfate is described by the following chemical formula: Na2S2O3.5H2O.

In its solid form, it is a crystalline solid which has a tendency to readily lose water. Sodium thiosulfate is readily soluble in water and is also referred to as sodium hyposulfite**.**

PRINCIPLE

-Redox Titration (IODOMETRY)

The principle of standardization of sodium thiosulphate is based on redox iodometric titration with potassium iodate (primary standard).

Potassium iodate is a strong oxidizing agent, it is treated with excess potassium iodide in acidic media which liberates iodine which is back titrated with sodium thioslphate.

Uniformity of reactions between iodine and sodium thiosulphate forms basis for utilizing the standard solution of iodine in the analysis of sodium thiosulphate.

. $KIO_3 + 5KI + 3H_2SO_4 \rightarrow 3K_2SO_4 + 3I_2 + 3H_2O$

I_2 $3I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$

PROCEDURE:

Preparation of 0.1N Sodium thiosulphate

- Dissolve 24.8g of sodium thiosulphate pentahydrate($Na_2S_2O_3.5H_2O$) in 800 ml of freshly boiled and cooled water and mix thoroughly by shaking for approximately 15 minutes.
- Make up the volume to 1000 ml.

Preparation of 0.1N Potassium Iodate

- Weigh accurately about 356 mg of KIO_3 and dissolve in 100 ml distilled water.

Preparation of Starch indicator

- Take 1 gm of soluble starch and triturate with 5 ml of water and add it to 100 ml of Boiling water containing 10 mg of Mercuric iodide with continous stirring.

Standardisation of 0.1N sodium thiosulphate

- Take 10 ml of Potassium Iodate solution .
- Add 2 gm of Potassium Iodide and 5 ml of dilute H_2SO_4,keep it in dark for 10 minutes.
- add 2 to 3 drops of starch indicator and titrate with sodium thiosulphte using starch solution as indicator until the blue colour is disappeared.

- Record the reading , Repeat the titration for 3 times to get the precise reading
- Equivalent weight factor of sodium carbonate for 0.1 M HCl= 0.00529 g

Calculation

$$\text{Normality of 0.1 N sodium thio sulphate} = \frac{\text{Weight Taken x Expected Normality}}{\text{Equivalent Weight Factor x Titre Value}}$$

-