1000 ml of (N) glycine ≡ 75 g of glycine

So V_2 ml of S_1(N) NaOH ≡ $(75 \times V_2 \times S_1)/1000$ g of glycine

25 ml of glycine solution contains $(75 \times V_2 \times S_1)/1000$ g of glycine

So 1000 ml of glycine solution contains

$(40 \times 75 \times V_2 \times S_1)/1000$ g of glycine

$= (40 \times 75 \times V_2 \times 25w/V_1 \times 0.7879 \times 20)/1000$ g of glycine

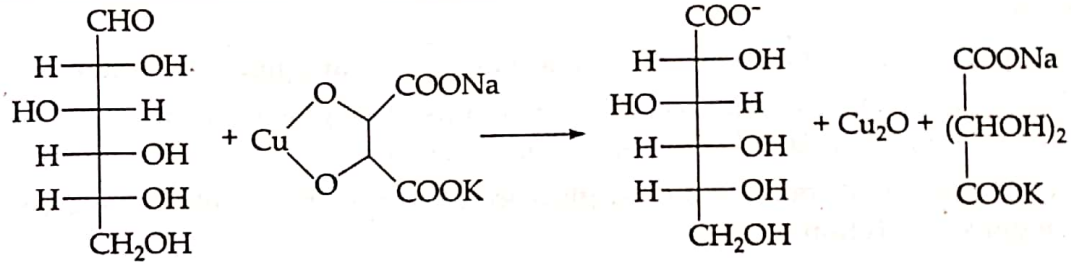
$= 4.7595 \times (wV_2/V_1)$ g of glycine.

6.1.2 Estimation of Glucose (using Fehling's Solution)

Principle :

Glucose, a reducing sugar, is quantitatively oxidized by Fehling's solution under boiling conditions to gluconate along with precipitation of red cuprous oxide.

Fehling's solution is a (1:1 by volume) mixture of an aqueous solution of copper sulfate (Fehling A) and a strongly alkaline solution of sodium potassium tartarate also known as Rochelle salt (Fehling B)



D-Glucose Fehling's solution

■ Chemical required :

① **Standard glucose solution :** Weigh out accurately ~ 0.5 g of (A.R) glucose in a 100 ml volumetric flask. Dissolve it in distilled water and make up to the mark with distilled water and mix uniformly.

② **Fehling's solution A :** Dissolve 17.32 g of copper sulfate pentahydrate in distilled water and dilute to 250 ml and mix uniformly.

③ **Fehling's solution B :** Dissolve 86.5 g of sodium potassium tartarate and 25 g of sodium hydroxide in distilled water. Dilute to 250 ml with distilled water and mix uniformly.

④ **Unknown glucose solution :** Weigh out accurately ~ 0.4-0.5 g of (A.R) glucose in a 100 ml volumetric flask. Dissolve it in distilled water and make up to the mark with distilled water and mix uniformly.

■ Procedure :

① **Standardisation of Fehling's solution :** Pipette out 10 ml of Fehling's solution A and 10 ml of Fehling's solution B in a 250 ml conical flask. Boil the mixture gently on an asbestos centred wire gauge. Add standard glucose solution from a burette to the boiling Fehling's solution till the supernatant liquid becomes colourless with the precipitation of bright brick red cuprous oxide.

② **Estimation of unknown glucose solution :** Use unknown glucose solution instead of the known glucose solution and repeat the procedure as described under the heading of "Standardisation of Fehling's solution".

■ Results :

① **Standardisation of Fehling's solution :**

Volume of Fehling's solution (ml)	Volume of Known glucose solution (ml)	Mean volume of Known glucose solution (ml) (V_1)
10+10	a	
10+10	b	$(a+b+c)/3$
10+10	c	

② **Estimation of unknown glucose solution :**

Volume of Fehling's solution (ml)	Volume of unknown glucose solution (ml)	Mean volume of unknown glucose solution (ml) (V_2)
10+10	x	
10+10	y	$(x+y+z)/3$
10+10	z	

Calculation :

Let the weight of glucose taken for the preparation of standard glucose solution is w g.

If V_1 ml of standard glucose solution and V_2 ml of unknown glucose solution are needed for the titration of (10+10) ml i.e. 20 ml of Fehling's solution then

Weight of glucose in V_1 ml of standard glucose solution = the weight of glucose in V_2 ml of unknown glucose solution.

Now 100 ml of standard glucose solution contains w g of glucose

So V_1 ml of standard glucose solution contains $[(w \times V_1)/100]$ g of glucose

Hence V_2 ml of unknown glucose solution contains $[(w \times V_1)/100]$ g of glucose

Thus 100 ml of unknown glucose solution contains $[(w \times V_1)/V_2]$g of glucose

So the strength of unknown glucose solution is $[(w \times V_1)/V_2]\%$.

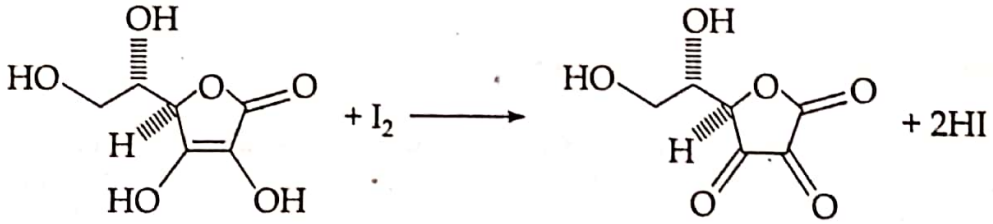
NOTE :

1. During titration always keep the Fehling's solution in gently boiling conditions. So adjust the flow of glucose solution from the burette such that boiling of Fehling's solution does not stop during titration.
2. Methylene blue indicator may be used in this titration. 2-3 Drops of Methylene blue indicator may be added near the end point. However, its use is not essential.

6.1.4 Estimation of Vitamin C (Ascorbic acid)

■ Principle :

Vitamin C (ascorbic acid) is quantitatively oxidized by iodine to dehydroascorbic acid.



Vitamin C (ascorbic acid) Dehydroascorbic acid

A known volume of an aqueous solution of vitamin C is treated with a measured excess of standard iodine solution and the excess iodine is back titrated against a standard solution of sodium thiosulfate. Iodine consumed by vitamin C is thus estimated.

$$I_2 + 2\,Na_2S_2O_3 = Na_2S_4O_6 + 2NaI$$

So 1000 ml of (N) thiosulfate ≡ 1000 ml of (N) iodine ≡ 1000 ml of (N) vitamin C ≡ 88.06 g of vitamin C [equivalent weight of vitamin C = 88.06]

■ Chemical required :

① **Standard (N/20) $K_2Cr_2O_7$ solution :** Weigh out accurately ~ 0.6129 g (w) of (A.R) $K_2Cr_2O_7$ in a 250 ml volumetric flask, dissolve in distilled water and make up to the mark with distilled water. Mix the solution uniformly.

② **(N/20) I_2 solution :** Mix ~ 1.6 g of solid iodine and 2 g of potassium iodide. Add 10-15 ml of water and stir to dissolve. Dilute the solution to 250 ml with distilled water.

③ **(N/20) sodium thiosulfate solution :** Dissolve 3-4 g of sodium thiosulfate pentahydrate in distilled water, dilute to 250 ml and mix uniformly.

④ **10% potassium iodide solution**

⑤ **1% Starch indicator**

⑥ **Sample vitamin C solution :** Dissolve ~ 1-1.2 g (weighed accurately) of vitamin C in distilled water, dilute to 250 ml and mix uniformly.

■ Procedure :

① **Standardisation of sodium thiosulfate solution against standard potassium dichromate solution :** Pipette out 25 ml of standard (N/20) $K_2Cr_2O_7$ solution in a 500 ml conical flask. Add 25 ml of 4(N) sulfuric acid and 10 ml of 10% KI (or 1 g of solid KI) solution to it .Cover the flask with a small watch glass and allow to stand in dark for 4-5 minutes. Dilute with 150 ml of distilled water to adjust the acidity to ~ 0.5(N). Titrate with sodium thiosulfate solution till the appearance of a straw yellow colour. Add 2 ml of 1% starch solution when the solution becomes intense blue. Continue addition of sodium thiosulfate till the blue colour disappears with the appearance of a light green colour.

② **Standardisation of iodine solution against standard sodium thiosulfate solution :** Pipette out 25 ml of iodine solution in a 500 ml conical flask. Add 75 ml of distilled water to it. Titrate with standard sodium thiosulfate solution till the appearance of a straw yellow colour. Add 2 ml of 1% starch solution when the solution becomes intense blue. Continue addition of sodium thiosulfate till the disappearance of the blue colour. **(end point : colourless)**

③ **Estimation of vitamin C solution :** Pipette out 25 ml of the unknown vitamin C solution in a 500 ml conical flask. Add 25 ml of distilled water to it. Then add 1 ml of 4(N) sulfuric acid to adjust the acidity to ~ 0.1(N). Add a measured excess (25 × x ml) of standard iodine solution so that the colour of iodine persists in solution. Allow to stand for ~ 1 minute. Add 2 ml of 1% starch solution when the solution becomes intense blue. Titrate against standard sodium thiosulfate solution till the disappearance of the blue colour. **(end point : colourless).**

■ Results :

① **Standardisation of sodium thiosulfate solution against standard potassium dichromate solution :**

Volume of $K_2Cr_2O_7$ (ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_1)
25	a	
25	b	$(a+b+c)/3$
25	c	

Strength of $K_2Cr_2O_7$ solution = $(w/0.6129)(N/20)$

So the strength of $Na_2S_2O_3$ solution = $S_1 = (25 \times w)/(V_1 \times 0.6129)(N/20)$

② **Standardisation of iodine solution against standard sodium thiosulfate solution :**

Volume of I_2 (ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_2)
25	x	
25	y	$(x+y+z)/3$
25	z	

③ Estimation of vitamin C solution :

Volume of Vitamin C solution (ml)	Volume of I_2 Solution(ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_3)
25	$25 \times x$	d	
25	$25 \times x$	e	$(d+e+f)/3$
25	$25 \times x$	f	

■ **Calculation :**

25 ml of iodine solution ≡ V_2 ml of S_1(N) sodium thiosulfate solution

So $(25 \times x)$ ml of iodine solution ≡ $(V_2 \times x)$ ml of S_1(N) sodium thiosulfate solution

Thus iodine consumed by vitamin C≡ $(xV_2 - V_3)$ ml of S_1(N) sodium thiosulfate solution

Now 1000 ml of (N) thiosulfate ≡ 1000 ml of (N) iodine ≡ 1000 ml of (N) vitamin C ≡ 88.06 g of vitamin C [equivalent weight of vitamin C = 88.06]

So (xV_2-V_3) ml of S_1(N) sodium thiosulfate ≡ $[88.06 \times (xV_2-V_3)S_1]/1000$ g of vitamin C

$= [88.06 \times (xV_2-V_3)25 \times w]/(1000 \times V_1 \times 0.6129 \times 20)$g of vitamin C

Thus 25 ml of vitamin C solution contains

$= [88.06 \times (xV_2-V_3)25 \times w]/(1000 \times V_1 \times 0.6129 \times 20)$g of vitamin C

Hence 1000 ml of vitamin C solution contains

$= [88.06 \times (xV_2-V_3) \times 1000 \times w]/(1000 \times V_1 \times 0.6129 \times 20)$g of vitamin C

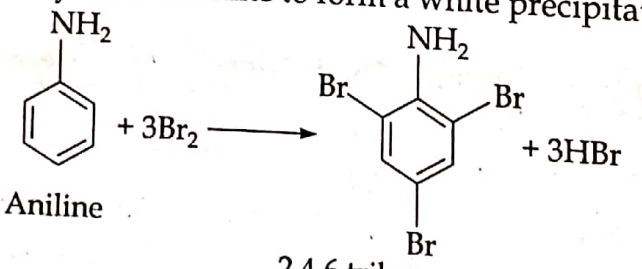
$= [88.06 \times (xV_2-V_3) \times w]/(V_1 \times 0.6129 \times 20)$g of vitamin C

$= 7.1839 \times (xV_2-V_3) \times w]/(V_1)$ g of vitamin C.

6.1.5 Estimation of Aniline (Bromate-Bromide Method)

■ **Principle :**

Aniline reacts quantitatively with bromine to form a white precipitate of 2,4,6 tribromo aniline.



Aniline

2,4,6 tribromoaniline

Thus 6000 ml (N) bromine ≡ 93 g of aniline

So 1000 ml of (N) bromine ≡ (93/6)gi.e. 15.5 g of aniline

Hence the equivalent weight of aniline is 15.5

The aniline solution is treated with a measured excess of (KBr + $KBrO_3$) solution which is equivalent to bromine in acidic medium. The excess bromine is allowed to react with sufficient potassium iodide. Bromine oxidizes iodide to iodine. This iodine is estimated by titration against a standard solution of sodium thiosulfate. Thus the amount of bromine left after consumption by aniline is determined. Difference between added and remaining bromine corresponds to the amount of aniline in the volume of sample (aniline) solution taken for titration.

$$KBrO_3 + 5KBr + 6HCl = 3Br_2 + 6KCl + 3H_2O$$

$$Br_2 + 2KI = 2KBr + I_2$$

$$I_2 + 2Na_2S_2O_3 = Na_2S_4O_6 + 2NaI$$

Thus 1000 ml of (N) $Na_2S_2O_3 \equiv$ 1000 ml of (N) $I_2 \equiv$ 1000 ml of (N) $Br_2 \equiv$ 1000 ml of (N) aniline $\equiv$ 15.5 g of aniline.

In acid medium bromate reacts according to the following equation.

$$BrO_3^- + 6H^+ + 6e = Br^- + 3H_2O$$

So the equivalent weight of $KBrO_3$ = (molecular weight of $KBrO_3$/6) = (167/6) = 27.8334.

Chemicals required :

① **Standard (N/20) $KBrO_3$-KBr solution :** Weigh out accurately ~ 0.35 g (w) (exactly 0.3479 g) of (A.R) $KBrO_3$ and ~ 5 g of KBr in a 250 ml volumetric flask. Dissolve in distilled water and make up to the mark with distilled water.

② **(N/20) Sodium thiosulfate solution :** Dissolve 3-4 g of sodium thiosulfate pentahydrate in distilled water and dilute to 250 ml with distilled water.

③ **10% Potassium iodide solution**

④ **1% Starch solution**

⑤ **Sample aniline solution :** Dissolve 3-4 ml of freshly distilled aniline in 20-25 ml of conc. HCl and dilute to one litre with distilled water. Dilute 20-25 ml of this stock solution to 100 ml with distilled water in a 100 ml volumetric flask. 25 ml of this diluted solution may be used for titration.

Procedure :

① **Standardisation of sodium thiosulfate solution against standard $KBrO_3$-KBr solution :** Pipette out 25 ml of standard $KBrO_3$-KBr solution in a 500 ml conical flask. Add 10 ml of 10% potassium iodide solution or 1 g of solid potassium iodide to this solution. Then add 5 ml of conc. HCl to adjust the acidity to ~ 1.5-2(N). Cover the flask with a small watch glass and keep in dark for 3-4 minutes. Dilute with 100 ml of water to adjust the acidity of the resulting solution to ~ 0.5(N). Titrate the liberated iodine with sodium thiosulfate solution till the appearance of a straw yellow colour. Add 2 ml of 1% starch solution when the solution becomes intense blue. Continue addition of sodium thiosulfate till the disappearance of the blue colour. **(end point : colourless)**

② **Estimation of aniline :** Transfer the sample aniline solution into a volumetric flask of definite size as directed and make up to the mark with distilled water.

Pipette out 25 ml of diluted aniline solution in a 500 ml conical flask. Add 10-12 ml of conc. HCl to maintain the acidity at ~ 3(N). Add a measured excess (25 × x) ml of standard $KBrO_3$-KBr solution so that a permanent yellow colour due to an excess of free bromine persists. Cover the flask with a small watch glass, shake well and allow to stand at room temperature in the dark for 5 minutes with occasional shaking. Add 10 ml of 10% potassium iodide solution or 1 gm of solid potassium iodide. Dilute with 150 ml of distilled water to adjust the acidity to ~ 0.5(N). Titrate the liberated iodine with sodium thiosulfate solution till the appearance of a straw yellow colour. Add 2 ml of 1% starch solution when the solution becomes intense blue. Continue addition of sodium thiosulfate till the disappearance of the blue colour leaving white precipitate of 2,4,6 tribromoaniline in the solution.

■ **Results :**

① **Standardisation of sodium thiosulfate solution against standard $KBrO_3$-KBr solution :**

Volume of $KBrO_3$-KBr (ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_1)
25	a	
25	b	$(a+b+c)/3$
25	c	

Strength of $KBrO_3$-KBr solution = $(w/0.3479)(N/20)$

So the strength of $Na_2S_2O_3$ solution = $S_1 = (25 \times w)/(V_1 \times 0.3479)(N/20)$

② **Estimation of aniline :**

Volume of aniline solution (ml)	Volume of $KBrO_3$-KBr Solution(ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_2)
25	$25 \times x$	x	
25	$25 \times x$	y	$(x+y+z)/3$
25	$25 \times x$	z	

25 ml of $KBrO_3$-KBr solution ≡ V_1 ml S_1(N) $Na_2S_2O_3$ solution

So $(25 \times x)$ ml of $KBrO_3$-KBr solution ≡ $(V_1 \times x)$ ml S_1(N) $Na_2S_2O_3$ solution

Bromine left after consumption by aniline ≡ V_2 ml S_1(N) $Na_2S_2O_3$ solution

Hence bromine consumed by aniline ≡ (xV_1-V_2) ml S_1(N) $Na_2S_2O_3$ solution

Now 1000 ml of (N) $Na_2S_2O_3$ ≡ 15.5 g of aniline

So (xV_1-V_2) ml S_1(N) $Na_2S_2O_3$ ≡ $[15.5 \times (xV_1-V_2) \times S_1]/1000$ g of aniline

25 ml of aniline solution contains $[15.5 \times (xV_1-V_2) \times S_1]/1000$ g of aniline

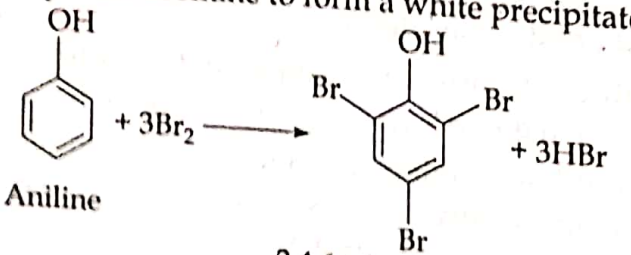
So 1000 ml of aniline solution contains $[15.5 \times (xV_1-V_2) \times S_1]/(25)$g of aniline

= $[15.5 \times (xV_1-V_2) \times w/(V_1 \times 0.3479 \times 20)]$ g of aniline = $2.2276 \times (xV_1-V_2) \times w/V_1$ g of aniline.

6.1.6 Estimation of Phenol (Bromate-Bromide Method)

■ **Principle :**

Phenol reacts quantitatively with bromine to form a white precipitate of 2,4,6 tribromo phenol.



2,4,6 tribromophenol

Thus 6000 ml (N) bromine ≡ 94 g of phenol

So 1000 ml of (N) bromine ≡ (94/6)g i.e. 15.67 g of phenol

Hence the equivalent weight of phenol is 15.67

The phenol solution is treated with a measured excess of (KBr + $KBrO_3$) solution which is equivalent to bromine in acidic medium. The excess bromine is allowed to react with sufficient potassium iodide. Bromine oxidizes iodide to iodine. This iodine is estimated by titration against

a standard solution of sodium thiosulfate. Thus, the amount of bromine left after consumption by phenol is determined. Difference between added and remaining bromine corresponds to the amount of phenol in the volume of sample (phenol) solution taken for titration.

$$KBrO_3 + 5KBr + 6HCl = 3Br_2 + 6KCl + 3H_2O$$

$$Br_2 + 2KI = 2KBr + I_2$$

$$I_2 + 2Na_2S_2O_3 = Na_2S_4O_6 + 2NaI$$

Thus 1000 ml of (N) $Na_2S_2O_3 \equiv$ 1000 ml of (N) $I_2 \equiv$1000 ml of (N) $Br_2 \equiv$ 1000 m l of (N) phenol $\equiv$ 15.67 g of phenol.

In acid medium bromate reacts according to the following equation

$$BrO_3^- + 6H^+ + 6e = Br^- + 3H_2O$$

So the equivalent weight of $KBrO_3$ = (molecular weight of $KBrO_3/6$) = (167/6) = 27.8334.

■ Chemicals required :

① **Standard (N/20) $KBrO_3$-KBr solution :** Weigh out accurately ~ 0.35 g (w) (exactly 0.3479 g) of (A.R) $KBrO_3$ and ~ 5 g of KBr in a 250 ml volumetric flask. Dissolve in distilled water and make up to the mark with distilled water.

② **(N/20) Sodium thiosulfate solution :** Dissolve 3-4 g of sodium thiosulfate pentahydrate in distilled water and dilute to 250 ml with distilled water.

③ **10% Potassium iodide solution**

④ **1% Starch solution**

⑤ **Sample phenol solution :** Dissolve 3-4 g of pure phenol in distilled water and dilute to one litre with distilled water. Dilute 20-25 ml of this stock solution to 100 ml with distilled water in a 100 ml volumetric flask. 25 ml of this diluted solution may be used for titration.

■ Procedure :

① **Standardisation of sodium thiosulfate solution against standard $KBrO_3$-KBr solution :** Pipette out 25 ml of standard $KBrO_3$-KBr solution in a 500 ml conical flask. Add 10 ml of 10% potassium iodide solution or 1 g of solid potassium iodide to this solution. Then add 5 ml of conc. HCl to adjust the acidity to ~ 1.5(N). Cover the flask with a small watch glass and keep in dark for 3-4 minutes. Dilute with 100 ml of water to adjust the acidity of the resulting solution to ~ 0.5(N). Titrate the liberated iodine with sodium thiosulfate solution till the appearance of a straw yellow colour. Add 2 ml of 1% starch solution when the solution becomes intense blue. Continue addition of sodium thiosulfate till the disappearance of the blue colour. **(end point : colourless)**

② **Estimation of phenol :** Transfer the sample phenol solution into a volumetric flask of definite size as directed and make up to the mark with distilled water.

Pipette out 25 ml of diluted phenol solution in a 500 ml conical flask. Add 10-12 ml of conc. HCl to maintain the acidity at ~ 3(N). Add a measured excess (25 × x) ml of standard $KBrO_3$-KBr solution so that a permanent yellow colour due to an excess of free bromine persists. Cover the flask with a small watch glass, shake well and allow to stand at room temperature in the dark for 5 minutes with occasional shaking. Add 10 ml of 10% potassium iodide solution or 1 gm of solid potassium iodide. Dilute with 150 ml of distilled water to adjust the acidity to ~ 0.5(N). Titrate the liberated iodine with sodium thiosulfate solution till the appearance of a straw yellow colour. Add 2 ml of 1% starch solution when the solution becomes intense blue. Continue addition of sodium thiosulfate till the disappearance of the blue colour leaving white precipitate of 2,4,6 tribromophenol in the solution.

■ **Results :**

① **Standardisation of sodium thiosulfate solution against standard $KBrO_3$-KBr solution :**

Volume of $KBrO_3$-KBr (ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_1)
25	a	
25	b	$(a+b+c)/3$
25	c	

Strength of $KBrO_3$-KBr solution = $(w/0.3479)(N/20)$

So the strength of $Na_2S_2O_3$ solution = $S_1 = (25 \times w)/(V_1 \times 0.3479)(N/20)$

② **Estimation of phenol :**

Volume of phenol solution (ml)	Volume of $KBrO_3$-KBr Solution(ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_2)
25	$25 \times x$	x	
25	$25 \times x$	y	$(x+y+z)/3$
25	$25 \times x$	z	

25 ml of $KBrO_3$-KBr solution ≡ V_1 ml S_1(N) $Na_2S_2O_3$ solution

So $(25 \times x)$ ml of $KBrO_3$-KBr solution ≡ $(V_1 \times x)$ ml S_1(N) $Na_2S_2O_3$ solution

Bromine left after consumption by phenol ≡ V_2 ml S_1(N) $Na_2S_2O_3$ solution

Hence bromine consumed by phenol ≡ $(xV_1 - V_2)$ ml S_1(N) $Na_2S_2O_3$ solution

Now 1000 ml of (N) $Na_2S_2O_3$ ≡ 15.67 g of phenol

So (xV_1-V_2) ml S_1(N) $Na_2S_2O_3$ ≡ $[15.67 \times (xV_1-V_2) \times S_1]/1000$ g of phenol

25 ml of phenol solution contains $[15.67 \times (xV_1-V_2) \times S_1]/1000$ g of phenol

So 1000 ml of phenol solution contains $[15.67 \times (xV_1-V_2) \times S_1]/25$ g of phenol

$= [15.67 \times (xV_1-V_2) \times w/(V_1 \times 0.3479 \times 20)]$ g of phenol $= 2.2521 \times (xV_1-V_2) \times w/V_1$ g of phenol.

6.1.7 Estimation of Formaldehyde (Formalin)

■ **Principle :** Formaldehyde (formalin) is quantitatively oxidized by iodine in weakly alkaline medium to formate.

$$HCHO + I_2 + 3NaOH = 2NaI + HCOONa + 2H_2O$$

Thus 2000 ml (N) iodine ≡ 30 g of formaldehyde

So 1000 ml (N) iodine ≡ 15 g of formaldehyde

A known volume of formalin solution is allowed to react with a measured excess of iodine solution. The iodine left after consumption by formalin is estimated by titration against a standard solution of sodium thiosulfate. Thus the amount of iodine left after consumption by formalin is determined. Difference between added and remaining iodine corresponds to the amount of formalin in the volume of sample (formalin) solution taken for titration.

$$I_2 + 2Na_2S_2O_3 = Na_2S_4O_6 + 2NaI$$

Thus 1000 ml of (N) $Na_2S_2O_3$ ≡ 1000 ml of (N) I_2 ≡ 1000 ml of (N) formalin ≡ 15 g of formalin.

■ **Chemicals required :**

① **Standard (N/20) $K_2Cr_2O_7$ solution :** Weigh out accurately ~ 0.6129 g (w) of (A.R) $K_2Cr_2O_7$ in

a 250 ml volumetric flask, dissolve in distilled water and make up to the mark with distilled water. Mix the solution uniformly.

② **(N/20) I_2 solution :** Mix ~ 1.6 g of solid iodine and 2 g of potassium iodide. Add 10-15 ml of water and stir to dissolve. Dilute the solution to 250 ml with distilled water.

③ **(N/20) sodium thiosulfate solution :**

Dissolve 3-4 g of sodium thiosulfate pentahydrate in distilled water, dilute to 250 ml and mix uniformly.

④ **10% potassium iodide solution**

⑤ **1% Starch indicator**

⑥ **Sample formalin solution :** Dissolve 4 - 5 ml of formalin in distilled water and dilute to one litre with distilled water.

■ Procedure :

① **Standardisation of sodium thiosulfate solution against standard potassium dichromate solution :** Pipette out 25 ml of standard (N/20) $K_2Cr_2O_7$ solution in a 500 ml conical flask. Add 25 ml of 4(N) sulfuric acid and 10 ml of 10% KI (or 1 g of solid KI) solution to it. Cover the flask with a small watch glass and allow to stand in dark for 4-5 minutes. Dilute with 150 ml of distilled water to adjust the acidity to ~ 0.5(N). Titrate with sodium thiosulfate solution till the appearance of a straw yellow colour. Add 2 ml of 1% starch solution when the solution becomes intense blue. Continue addition of sodium thiosulfate till the blue colour disappears with the appearance of a light green colour.

② **Standardisation of iodine solution against standard sodium thiosulfate solution :** Pipette out 25 ml of iodine solution in a 500 ml conical flask. Add 75 ml of distilled water to it. Titrate with standard sodium thiosulfate solution till the appearance of a straw yellow colour. Add 2 ml of 1% starch solution when the solution becomes intense blue. Continue addition of sodium thiosulfate till the disappearance of the blue colour. **(end point : colourless)**

③ **Estimation of formalin :** Transfer quantitatively the sample formalin solution into a volumetric flask of definite size as directed and make up to the mark with distilled water. Shake well to ensure uniform mixing.

Pipette out 25 ml of the unknown formalin solution in a 500 ml conical flask. Add a measured excess (25 × x ml)of standard iodine solution so that the colour of iodine persists in solution. Then add 5% NaOH solution drop wise till the solution assumes light yellow colour and this yellow colour persists after keeping the mixture for 15 minutes. After 15 minutes add 15 ml of 5% HCl solution. Then add 2 ml of 1% starch solution when the solution becomes intense blue. Titrate against standard sodium thiosulfate solution till the disappearance of the blue colour. (end point : colourless)

■ Results :

① **Standardisation of sodium thiosulfate solution against standard potassium dichromate solution :**

Volume of $K_2Cr_2O_7$ (ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_1)
25	a	
25	b	$(a+b+c)/3$
25	c	

Strength of $K_2Cr_2O_7$ solution = $(w/0.6129)(N/20)$

So the strength of $Na_2S_2O_3$ solution = $S_1 = (25 \times w)/(V_1 \times 0.6129)(N/20)$

② **Standardisation of iodine solution against standard sodium thiosulfate solution :**

Volume of I_2 (ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_2)
25	x	
25	y	$(x+y+z)/3$
25	z	

③ **Estimation of formalin solution :**

Volume of formalin solution (ml)	Volume of I_2 Solution(ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_3)
25	$25 \times x$	d	
25	$25 \times x$	e	$(d+e+f)/3$
25	$25 \times x$	f	

■ **Calculation :** 25 ml of iodine solution ≡ V_2 ml of S_1(N) sodium thiosulfate solution

So $(25 \times x)$ ml of iodine solution ≡ $(V_2 \times x)$ ml of S_1(N) sodium thiosulfate solution

Thus iodine consumed by formalin ≡ $(x\ V_2 - V_3)$ ml of S_1(N) sodium thiosulfate solution

Now 1000 ml of (N) thiosulfate ≡ 1000 ml of (N) iodine ≡ 1000 ml of (N) formalin ≡ 15 g of formalin [equivalent weight of formalin = 15]

So $(xV_2 - V_3)$ ml of S_1(N) sodium thiosulfate ≡ $[15 \times (xV_2 - V_3) \times S_1]/1000$ g of formalin

$= [15 \times (xV_2 - V_3) \times 25 \times w]/(1000 \times V_1 \times 0.6129 \times 20)$g of formalin

Thus 25 ml of formalin solution contains = $[15 \times (xV_2 - V_3)25 \times w]/(1000 \times V_1 \times 0.6129 \times 20)$g of formalin

Hence 1000 ml of formalin solution contains = $[15 \times (xV_2 - V_3) \times w]/(V_1 \times 0.6129 \times 20)$g of formalin = $1.224 \times [(xV_2 - V_3) \times w]/(V_1)$g of formalin.

6.1.8 Estimation of Acetic Acid in Commercial Vinegar

■ **Principle :**

Commercial vinegar contains 4-8% (V/V) acetic acid. Acetic acid can be estimated by titration against a strong alkali like sodium hydroxide.

$$CH_3COOH + NaOH = CH_3COONa + H_2O$$

■ **Chemicals required :**

① **Standard (N/20) oxalic acid solution :** Weigh out accurately ~ 0.8 g (w) (exactly 0.7879 g) of A.R oxalic acid, dissolve it in distilled water in a 250 ml volumetric flask and make up the volume with distilled water upto the mark. Mix well to ensure homogeneity of the solution.

② **(N/20) NaOH solution :** Dissolve ~1 g of solid NaOH in 500 ml of distilled water.

③ **Phenolphthalein indicator :** 0.5% in (1:1) ethanol.

④ **Sample commercial vinegar solution :** Mix 75-80 ml of commercial vinegar in distilled water and dilute to one litre with distilled water.

■ **Procedure :**

① **Standardisation of NaOH against standard oxalic acid solution :** Pipette out 25 ml of standard oxalic acid solution in a 250 ml conical flask. Add 2-3 drops of phenolphthalein indicator. Add NaOH solution from a burette until the appearance of a faint pink colour.

② **Estimation of acetic acid :** Dilute the supplied vinegar solution as directed. Pipette out 25 ml of the diluted unknown vinegar solution in a 250 ml conical flask. Add 2-3 drops of phenolphthalein indicator. Add NaOH solution from a burette until the appearance of a faint pink colour.

■ **Results :**

① **Standardisation of sodium hydroxide solution against standard oxalic acid solution :**

Volume of oxalic acid (ml)	Volume of NaOH (ml)	Mean volume of NaOH(ml) (V_1)
25	a	
25	b	$(a+b+c)/3$
25	c	

Strength of oxalic acid solution = $(w/0.7879) \times (N/20)$

So the strength of NaOH solution = $S_1 = (25 \times w)/(V_1 \times 0.7879) \times (N/20)$

② **Estimation of acetic acid in vinegar :**

Volume of vinegar (ml)	Volume of NaOH (ml)	Mean volume of NaOH (ml) (V_2)
25	x	
25	y	$(x+y+z)/3$
25	z	

■ **Calculation :**

1000 ml of (N) NaOH ≡ 1000 ml of (N) acetic acid ≡ 60 g of acetic acid

So V_2 ml of S_1(N) NaOH ≡ $(60 \times V_2 \times S_1)/1000$ g of acetic acid

25 ml of vinegar solution contains $(60 \times V_2 \times S_1)/1000$ g of acetic acid

So 1000 ml of vinegar solution contains $(40 \times 60 \times V_2 \times S_1)/1000$ g of acetic acid

$= (40 \times 60 \times V_2 \times 25w/V_1 \times 0.7879 \times 20)/1000$ g of acetic acid

$= 3.8076 \times (wV_2/V_1)$ g of acetic acid.

6.1.9 Estimation of Urea (Hypobromite Method)

■ **Principle :** Urea is quantitatively oxidized by hypobromite in presence of alkali.

$$CO(NH_2)_2 + 3\,^-OBr + 2\,^-OH = N_2 + 3\,Br^- + CO_3^{2-}$$

An aqueous solution of urea is treated with a measured excess of standardized alkaline solution of hypobromite. Unreacted hypobromite is then allowed to react with an excess of potassium iodide solution in the presence of dilute sulfuric acid. The liberated iodine is then back titrated against a standard solution of sodium thiosulfate.

$$^-OBr + 2I^- + 2H^+ = I_2 + Br^- + H_2O$$

$$I_2 + 2Na_2S_2O_3 = Na_2S_4O_6 + 2NaI$$

So $^-OBr \equiv I_2 \equiv 2S_2O_3^{2-}$ and $CO(NH_2)_2 \equiv 3\,^-OBr \equiv 3I_2 \equiv 6\,S_2O_3^{2-}$

Thus 1000 ml of (N) $Na_2S_2O_3$ ≡ (Molecular weight of urea/6) g of urea ≡ (60/6) g ≡ 10 g of urea

Hypochlorite is unstable when prepared directly from bromine and alkali. Hence it is prepared in the reaction medium using an excess of bromide and a solution of hypochlorite.

$$^-OCl + Br^- = {}^-OBr + Cl^-$$

Therefore $^-OBr \equiv {}^-OCl$

■ Chemicals required :

① **Standard (N/20) $K_2Cr_2O_7$ solution :** Weigh out accurately ~ 0.6129 g (w) of (A.R) $K_2Cr_2O_7$ in a 250 ml volumetric flask, dissolve in distilled water and make up to the mark with distilled water. Mix the solution uniformly.

② **(N/20) sodium thiosulfate solution :** Dissolve 3-4 g of sodium thiosulfate pentahydrate in distilled water, dilute to 250 ml and mix uniformly.

③ **10% potassium iodide solution.**

④ **(N/20) calcium hypochlorite solution.**

⑤ **1% Starch indicator.**

⑥ **Sample urea solution :** Dissolve ~ 0.4-0.5 g of urea in one litre of distilled water.

■ Procedure :

① **Standardisation of sodium thiosulfate solution against standard potassium dichromate solution :** Pipette out 25 ml of standard (N/20) $K_2Cr_2O_7$ solution in a 500 ml conical flask. Add 25 ml of 4(N) sulfuric acid and 10 ml of 10% KI (or 1 g of solid KI) solution to it. Cover the flask with a small watch glass and allow to stand in dark for 4-5 minutes. Dilute with 150 ml of distilled water to adjust the acidity to ~ 0.5(N). Titrate with sodium thiosulfate solution till the appearance of a straw yellow colour. Add 2 ml of 1% starch solution when the solution becomes intense blue. Continue addition of sodium thiosulfate till the disappearance of the blue colour with the appearance of a light green colour.

② **Preparation of (N/20) calcium hypochlorite solution :** Shake thoroughly 1-2 g of bleaching powder with 100 ml of distilled water in a 250 ml conical flask. Filter the slurry through a Whatman No. 1 filter paper to remove iron oxide, excess of calcium hydroxide and any other insoluble material. Dilute the filtrate with distilled water to 250 ml.

③ **Standardisation of hypochlorite solution :** Take an aliquot of 25 ml of hypochlorite solution in a 500 ml conical flask. Add 25 ml of 4(N) sulfuric acid and ~ 2 g of solid potassium iodide. Cover the flask with a small watch glass and keep the solution in dark for 3-4 minutes. Dilute with 150 ml of distilled water. Titrate with sodium thiosulfate solution till the appearance of a straw yellow colour. Add 2 ml of 1% starch solution when the solution becomes intense blue. Continue addition of sodium thiosulfate till the disappearance of the blue colour.

④ **Estimation of Urea :** Pipette out 25 ml of the unknown urea solution in a 500 ml conical flask. Add 2 g of potassium bromide and 0.5 g of sodium bicarbonate. Shake thoroughly to ensure complete dissolution of the added salts (KBr and $NaHCO_3$). Add a measured excess ($25 \times x$ ml) of standard hypochlorite solution till a permanent yellow colour due to free Br mine persists in the solution. Cover the flask with a small watch glass and allow to stand for 5 minutes. Then slowly add 10 ml of 6(N) sulfuric acid to the solution followed by addition of 1 g of solid potassium iodide. Cover the flask with a small watch glass and keep the solution in dark for 3-4 minutes. Dilute to 200 ml with distilled water. Titrate the liberated iodine with standard sodium thiosulfate solution till the appearance of a straw yellow colour. Add 2 ml of 1% starch solution when the solution becomes intense blue. Continue addition of sodium thiosulfate till the disappearance of the blue colour.

■ **Results :**

① **Standardisation of sodium thiosulfate solution against standard potassium dichromate solution :**

Volume of $K_2Cr_2O_7$ (ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_1)
25	a	
25	b	(a+b+c)/3
25	c	

Strength of $K_2Cr_2O_7$ solution = $(w/0.6129)(N/20)$

So the strength of $Na_2S_2O_3$ solution = $S_1 = (25 \times w)/(V_1 \times 0.6129)(N/20)$

② **Standardisation of hypochlorite solution :**

Volume of hypochlorite (ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_2)
25	x	
25	y	$(x+y+z)/3$
25	z	

③ **Estimation of urea solution :**

Volume of urea solution (ml)	Volume of hypochlorite Solution(ml)	Volume of $Na_2S_2O_3$ (ml)	Mean volume of $Na_2S_2O_3$ (ml) (V_3)
25	$25 \times x$	d	
25	$25 \times x$	e	$(d+e+f)/3$
25	$25 \times x$	f	

■ **Calculation :**

25 ml of hypochlorite solution ≡ V_2 ml of S_1(N) sodium thiosulfate solution

So (25 × x) ml of hypochlorite solution ≡ ($V_2 \times x$) ml of S_1(N) sodium thiosulfate solution

Thus hypochlorite (i.e hypobromite) consumed by urea ≡ $(xV_2 - V_3)$ ml of S_1(N) sodium thiosulfate solution

Now 1000 ml of (N) thiosulfate ≡ 10 g of urea

So $(xV_2 - V_3)$ ml of S_1(N) sodium thiosulfate ≡ $[10 \times (xV_2 - V_3)S_1]/1000$ g of urea

$= [10 \times (xV_2 - V_3)25 \times w]/(1000 \times V_1 \times 0.6129 \times 20)$g of urea

Thus 25 ml of urea solution contains = $[10 \times (xV_2 - V_3)25 \times w]/(1000 \times V_1 \times 0.6129 \times 20)$g of urea

Hence,

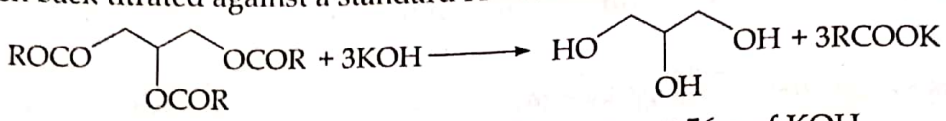
1000 ml of urea solution contains = $[10 \times (xV_2 - V_3) \times 1000 \times w]/(1000 \times V_1 \times 0.6129 \times 20)$g of urea

$= [0.8158 \times (xV_2 - V_3) \times w]/(V_1)$g of urea

6.1.10 Estimation of Saponification Value of Oil

■ **Principle :** Saponification value of an oil/fat/ester is the number of milligrams of KOH needed to completely saponify 1 g of oil/fat/ester. A weighed quantity of oil/fat/ester is completely

saponified by boiling with a measured excess of standard alcoholic KOH solution. The excess KOH is then back titrated against a standard HCl solution.



1000 ml of (N) HCl solution ≡ 1000 ml of (N) KOH solution ≡ 56 g of KOH

So 1 ml of (N) HCl solution ≡ 1 ml of (N) KOH solution ≡ 56 mg of KOH

Chemicals required :

① **Standard (N/20) oxalic acid solution :** Weigh out accurately ~ 0.8 g (w) (exactly 0.7879 g) of A.R oxalic acid, dissolve it in distilled water in a 250 ml volumetric flask and make up the volume with distilled water upto the mark. Mix well to ensure homogeneity of the solution.

② **Coconut oil or mustard oil**

③ **(N/2) alcoholic KOH solution :** Dissolve ~ 7.0 g of KOH in minimum volume of distilled water. Dilute to 250 ml with ethanol. Then dilute 10 ml of this solution to 100 ml in a 100 ml volumetric flask to prepare ~ (N/20) KOH solution.

④ **(N/2) HCl solution :** Dilute 10-12 ml of Conc. HCl to 250 ml with distilled water. Then dilute 10 ml of this solution to 100 ml in a 100 ml volumetric flask to prepare ~ (N/20) HCl solution.

Procedure :

① **Standardisation of KOH against standard oxalic acid solution :** Pipette out 25 ml of standard oxalic acid solution in a 250 ml conical flask. Add 2-3 drops of phenolphthalein indicator. Add KOH solution from a burette until the appearance of a faint pink colour.

② **Standardisation of HCl against standard KOH solution :** Pipette out 25 ml of ~ (N/20) HCl solution in a 250 ml conical flask. Add 2-3 drops of phenolphthalein indicator. Add KOH solution from a burette until the appearance of a faint pink colour.

③ **Estimation of saponification value :** Weigh out accurately ~ 2.0 g (w_1) of coconut or mustard oil in a 250 ml conical flask fitted with an air condenser. Add 50 ml of standard ~ (N/2) alcoholic KOH solution to it. Reflux the resulting solution on a hot water bath till the complete disappearance of the oily layer. Allow the mixture to cool to room temperature and add 2-3 drops of phenolphthalein indicator to it. The solution turns pink. Titrate the unreacted KOH by the standard (N/2) HCl solution till the disappearance of the pink colour forming a colourless solution.

Perform a blank experiment under the same conditions using the same quantity of (50 ml) of KOH without using the oil. Titrate the solution against standard (N/2) HCl solution using phenolphthalein indicator up to a colourless end point.

Results :

① **Standardisation of potassium hydroxide solution against standard oxalic acid solution :**

Volume of oxalic acid (ml)	Volume of KOH (ml)	Mean volume of KOH(ml) (V_1)
25	a	
25	b	$(a+b+c)/3$
25	c	

Strength of oxalic acid solution = $(w/0.7879)(N/20)$

So the strength of KOH solution = $(25 \times w)/(V_1 \times 0.7879)(N/20)$

Hence the exact strength of (~ N/2) KOH solution = $S_1 = (25 \times w)/(V_1 \times 0.7879)(N/2)$

② **Standardisation of HCl solution against standard KOH solution :**

Volume of HCl (ml)	Volume of KOH (ml)	Mean volume of KOH (ml) (V_2)
25	x	
25	y	$(x+y+z)/3$
25	z	

So the strength of HCl solution = $[(25 \times w)/(V_1 \times 0.7879)(N/20)] \times V_2/25$

$= [(V_2 \times w)/(0.7879 \times V_1)](N/20)$

Hence the exact strength of (~ N/2) HCl solution = $S_2 = [(V_2 \times w)/(0.7879 \times V_1)](N/2)$

③ **Estimation of the saponification value :**

Weight of Mustard/coconut oil (g)	Volume of (N/2) KOH Solution (ml)	Volume of (N/2) HCl (ml) (V_3)
w_1	50	d

④ **Blank experiment :**

Volume of (N/2) KOH Solution (ml)	Volume of (N/2) HCl (ml) (V_4)
50	e

■ Calculation :

V_4 ml S_2 (N) HCl is needed for 50 ml of S_1 (N) KOH.

V_3 ml S_2 (N) HCl is needed for back titration of S_1 (N) KOH after consumption by w_1 g of oil/fat/ester.

Hence KOH consumed by w_1 g of oil/fat/ester $\equiv (V_4-V_3)$ ml of $(V_2 \times w/0.7879 \times V_1)(N/2)$ HCl.

$\equiv (V_4-V_3)$ ml of $[(V_2 \times w)/(0.7879 \times V_1)](N/2)$ KOH.

$\equiv [(V_4-V_3) \times V_2 \times w]/(2 \times 0.7879 \times V_1)$ ml (N) KOH

$\equiv [56 \times (V_4-V_3) \times V_2 \times w]/(2 \times 0.7879 \times V_1)$ mg of KOH

[As 1 ml of (N) KOH ≡ 56 mg of KOH].

Thus saponification value of the oil/fat/ester

= number of milligrams of KOH consumed by 1.0 g of oil/fat/ester

$= 56 \times (V_4-V_3) \times V_2 \times w/2 \times w_1 \times 0.7879 \times V_1$

■ Alternative procedure :

① **Standard (N/2) oxalic acid solution :** Prepare a solution of (N/2) oxalic acid by dissolving ~ 8 g (w) (exactly 7.879 g) of oxalic acid in distilled water and making up to the mark in a 250 ml volumetric flask with distilled water.

② **Estimation of saponification value :** Weigh out accurately ~ 2.0 g (w_1) of coconut or mustard oil in a 250 ml conical flask fitted with an air condenser. Add 50 ml of (~ N/2) alcoholic

KOH solution to it. Reflux the resulting solution on a hot water bath till the complete disappearance of the oily layer. Allow the mixture to cool to room temperature and add 2-3 drops of phenolphthalein indicator to it. The solution turns pink. Titrate the unreacted KOH by the standard oxalic acid solution till the disappearance of the pink colour forming a colourless solution.

Perform a blank experiment under the same conditions using the same quantity (50 ml) of KOH without using the oil. Titrate the solution against standard oxalic solution using phenolphthalein indicator up to a colourless end point.

Results :

① Estimation of the saponification value :

Weight of Mustard/coconut oil (g)	Volume of (N/2) KOH Solution (ml)	Volume of oxalic acid (ml) (V_3)
w_1	50	d

② Blank experiment :

Volume of (N/2) KOH Solution (ml)	Volume of oxalic acid(ml) (V_4)
50	e

Calculation :

KOH consumed by w_1 g of oil/fat/ester $\equiv (V_4-V_3)$ ml of $(w/7.879)$(N/2) oxalic acid

$\equiv (V_4-V_3)$ ml of $(w/7.879)$(N/2) KOH.

$\equiv [(V_4-V_3) \times w/(2 \times 7.879)]$ (N) KOH

$\equiv [56 \times (V_4-V_3) \times w]/(2 \times 7.879)$ mg of KOH

[As 1 ml of (N) KOH ≡ 56 mg of KOH].

Thus saponification value of the oil/fat/ester = number of milligrams of KOH consumed by 1.0 g of oil/fat/ester = $[56 \times (V_4-V_3) \times w]/(2 \times 7.879)/w_1$

Saponification values of some oils/fats/esters :

Oils/fats/esters	Saponification value
Coconut Oil	250-265
Mustard Oil	172
Neem Oil	197
Olive Oil	184-196
Palm Oil	190-205
Rapeseed Oil	175
Rice Bran Oil	185-195
Soybean Oil	180-200

Oils/fats/esters	Saponification value
Sunflower Oil	188-194
Peanut Oil	190
Mango butter	183-198
Lanolin Oil	90-110
Kokum butter	187-193
Goat tallow	195
Beef tallow	196