Unit 2:Analytical methods

Instrumental methods of analysis:

The instrumental methods of analysis are based on the measurement of various physical and or chemical properties of the analytes. These methods require the sensing probes or electronic gadgets to measure and quantify the physical/chemical property. The following table-2 shows some of the methods and the corresponding measuring characteristic properties.

Table-2: List of modern methods of analysis

Sl. No.	*Instrumental method*	*Characteristic property*
1	Kinetic methods	Rate of reaction
2	Conductometry	Electrical resistance
3	Potentiometry	Electrical potential
4	Polarimetry	Rotation of radiation
5	Refractometry	Refraction of radiation
6	Spectrophotometry and photometry (X-ray, UV, Visible, IR)	Absorption of radiation
7	Emission spectroscopy	Emission of radiation
8	Raman spectroscopy	Scattering of radiation

There is a group of instrumental procedures that are used for separation and resolution of closely related compounds. Most of these procedures are based on chromatography, solvent extraction or electrophoresis. One of the characteristics listed in the above table is usually used to complete the analysis following the chromatographic separations.

Some instrumental techniques are more sensitive than classical techniques. With certain combinations of elements or compounds, an instrumental method may be more selective, but with others, a gravimetric or volumetric approach may have less interference. The instrumental procedures employ is more sophisticated or costlier apparatus.

Potentiometric titration

Potentiometric titration is an important electroanalytical technique based on the measurement of the potential of electrochemical cells without drawing appreciable current. In this technique absolute potentials w.r.t a standard half-cell is not required and the measurements are made when the titration is in progress. The potential of an electrode depends upon the concentration of the ion to which it is reversible in accordance with Nernst equation.

In other words, potentiometric titration is a technique similar to direct titration of a redox reaction. No indicator is used; instead the voltage across the analyte, typically an electrolyte solution is measured. The potential of an electrode depends upon the concentration

of the ion to which it is reversible in accordance with Nernst equation. Potentiometric titrations are those titrations which involve the measurement of electrode potentials with the addition of the titrant. To do this, two electrodes are used, a neutral electrode and a standard reference electrode. The voltage is recorded at intervals as the titrant is added. A graph of voltage against volume added can be drawn and the end point of the reaction is half way between the jump in voltage. EMF of the cell depends on the concentration of the electrolytes with which the electrodes are in contact. Therefore, the electrode reaction is

$M^{n+} + ne^{-}$----->M

As the concentration of M^{n+} changes, the EMF of the cell also changes correspondingly. Thus the potentiometric titration involves measurement of EMF between indicator electrode and reference electrode, with the addition of titrant.

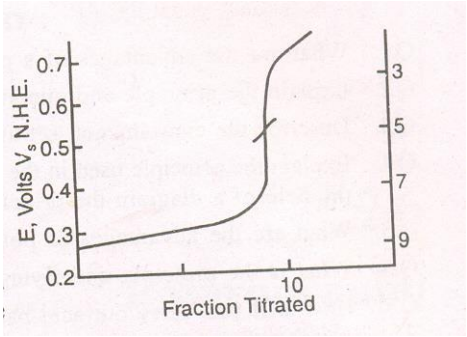
Nowadays the number of potentiometric measurements made on a daily basis is staggering. Manufacturers measure the pH of many consumer products; clinical laboratories determine blood gases as important indicators of disease states; industrial and municipal effluents are monitored continuously to determine pH and concentrations of pollutants; and oceanographers determine carbon dioxide and other related variables in sea water. Potentiometric measurements are also used in fundamental studies to determine thermodynamic equilibrium constants such as K_a , K_b ,and K_{sp}. In more recent methods, ion concentrations are measured directly from the potential of ion-selective membrane electrodes. These electrodes are relatively free from interferences and provide a rapid, convenient, and nondestructive means of quantitative determining numerous important anions and cations.

Procedure

The emf of the cell containing the initial solution is determined and relatively large amounts of the titrant solution are added until the equivalence point is approached. The emf is determined after each addition. The approach of the end point is indicated by a somewhat more rapid exchange of emf. Thus the pilot run is made to locate the end point. Then again fresh solution is taken and in the vicinity of the end point, equal increments (0.1or 0.05ml) of titrant is added to locate where exactly the equivalence point lies.

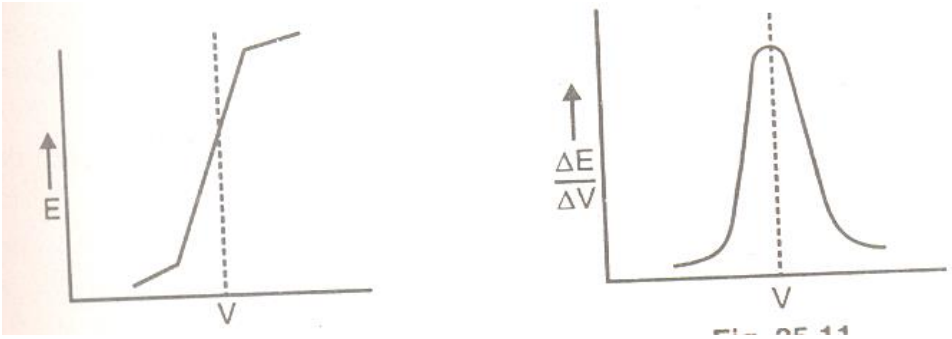
Location of end point

The important factor in titration is the recognition of the point at which the quantities of the reacting species are present in equivalent amounts- the equivalence point of the titration. In potentiometric titration, the end point can be fixed by plotting emf readings obtained against volume of titrant added.



The cell emf varies gradually, but near the end point, the cell emf changes abruptly as log of concentration changes rapidly. The end point can be located on the steeply rising portion of the curve, at the point of inflection, the point which corresponds to the maximum rate of change of cell emf for unit volume of titrant added. When the curve shows a very clear marked steep portion, one can give an approximate value of the end point as being midway along the steep portion of the curve.

The end point can be located more precisely by employing derivative methods in which the first derivative $\Delta E/\Delta V$ against V or the second derivative $\Delta^2E/\Delta V^2$ against V is plotted.



The first derivative curve gives a maximum at the point of inflection of the titration curve ie at the endpoint. In the second derivative curve, $\Delta^2E/\Delta V^2$ is zero at the end point.

Advantages

1. The apparatus required is generally inexpensive, reliable and readily available

2. It is easy to interpret titration curves

3. The method can be used for colored solutions

4. The method is applicable for analysis of dilute solutions

5. Several components can be titrated in the same solution without the possibility of indicators interfering with each other. Eg bromide and iodide may be titrated together.

Application

1. Calculation of p^H of buffer mixture of weak acid and its salt (Henderson- Hasselbalch equation)

   Consider buffer solution of weak acid HA and its highly dissociatd salt NaA. The hydrogen ion concentration of such a solution is given by

   $[H^+] = K_a$ [Acid]/ [Salt]

   $-\log[H^+] = -\log K_a + \log$ [Acid]/ [salt]

   $pH = pK_a + \log$ [Acid]/ [Salt] ---- Henderson- Hasselbalch equation helps to calculate the p^H of buffer mixture of weak acid and its salt. Also it enables calculation of the ratio in which acid and salt must be mixed in order to get a buffer solution of a definite pH.

<u>Conductometric titration</u>

The end point of titration can be determined with the help of conductivity measurements. In a conductometric titration, the titrant is added from the burette and the conductivity is measured during the course of titration. The principle of conductometric titration process can be stated as follows – *During a titration process, one ion is replaced with another and the difference in the ionic conductivities of these ions directly impacts the overall electrolytic conductivity of the solution.* The values of conductivities are then plotted against the volume of titrant. Since the measured conductivity is a linear function of the concentration of ions present, two lines will be obtained which will intersect each other at a point known as the equivalence point. In order to get straight line graphs, it is essential that the total volume of the solution remains constant during the titration. In Conductometric titration conductometer is used for measuring conductance. This is the reason it does not require any indicator as conductance or increase/decrease in ions is measured by a conductometer. That's why it is most suitable for titration of colored solutions. In Conductometric titration, concentration of the titrant must be 10 times as the solution being titrated. Because a sharp observable change in the value will be observed by the addition of a single drop of titrant when the end point is near.

Terms used in Conductometric Titration

Titrant – A solution used in titration whose concentration is known and is added to another solution of unknown concentration to determine its concentration.

Analyte – The solution used in titration whose concentration is unknown.

Equivalence Point – The point in conductometric titration at which conductivity undergoes a sudden change.

Conductometric titration is based on the measurement of conductance of the solution. The conductance of the solution (analyte + titrant) depends on following three factors –

- The number of free ions
- The charge on free ions
- The mobility of the free ions

Advantages of Conductometric Titration

Conductometric titration techniques are used in various fields due to their various advantages over other titration techniques. A few advantages of conductometric titration are listed below

- It does not require indicators as titration is based on the conductance of the solution and endpoint or neutralization point is determined graphically.
- It is suitable for colored solutions as well.
- As the endpoint is determined graphically, results are more accurate with minimum error.
- It is used for the analysis of turbid suspensions, weak acids, weak bases, a mix of weak and strong acids, etc.

Limitations of Conductometric Titration

conductometric titration has few limitations as well which are listed below

- By conductometric titration technique, only a few specific redox titrations can be carried out.
- It shows less accurate results when the total electrolytic concentration is high in solution. It makes it less satisfactory.

Different type of acid-base conductometric titration

1.Titration of Strong acid with strong base

Eg: HCl Vs NaOH

During the titration of a strong acid with a strong base, the following reaction is going to takes place:

$$HCl + NaOH \rightarrow NaCl + H_2O$$

Before NaOH is added, the conductance is high due to the presence of highly mobile hydrogen ions. When the base is added, the conductance falls rapidly due to the replacement of fast-moving hydrogen ions by slow moving Na^+ ions and also H+ ions react with OH^- ions to form undissociated water. This decrease in the conductance value continues till the equivalence point

reaches in the titration. At the equivalence point, the solution contains only NaCl. After the equivalence point, the increase in conductance value is observed due to the large conductivity of OH^- ions. The variation in conductance value is as shown in the Figure 1.

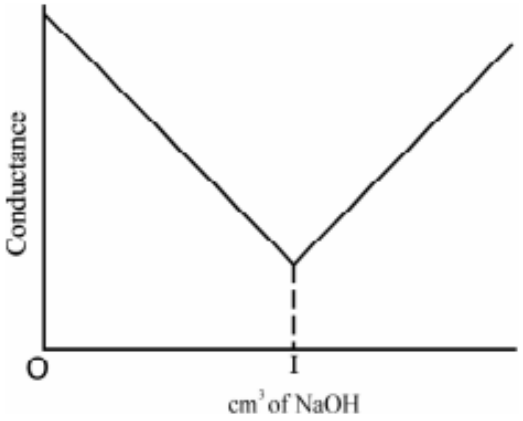


Figure 1: Conductometric titration of a strong acid (HCl) vs. a strong base (NaOH)

2. Titration of Strong acid with weak base

Eg: HCl Vs NH_4OH

During the titration of a strong acid with a weak base, the following reaction is going to takes place:

$$HCl + NH_4OH \rightarrow NH_4Cl + H_2O$$

Before NH_4OH added, Strong acid HCl dissociates completely into H^+ and Cl^- and hence number of ions are maximum in the solution. Furthermore, as H^+ ion has very large ionic mobility and hence ionic conductance, the solution has high conductance.

When the base is added dropwise, the conductance falls rapidly due to the replacement of fast-moving H^+ ion by slow moving NH_4^+ ion and also OH^- ion consumes H^+ to form water. After reaching equivalent point, when all the H^+ ion is consumed, further addition of NH_4OH have almost no effect as NH_4OH will not be dissociated further as it is weak base and due to common ion effect. So, the conductance value is unchanged. The variation in the conductance value is as shown in the Figure 2.

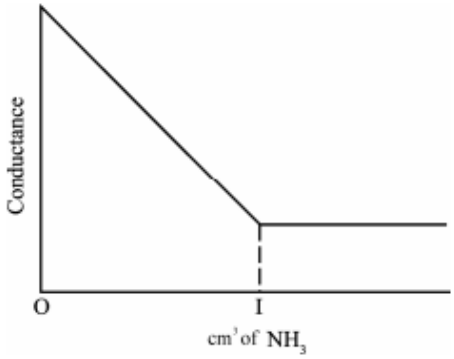


Figure 2- Conductometric titration of a strong acid (HCl) Vs. a weak base (NH_4OH)

3.Titration of weak acid with strong base

Eg: CH_3COOH Vs NaOH

During the titration of a weak acid with a strong base, the following reaction is going to takes place:

$$CH_3COOH + NaOH \rightarrow CH_3COONa + H_2O$$

The shape of the curve will depend on the concentration and dissociation constant of the acid. When a small amount of NaOH is added to CH_3COOH, the conductivity decreases initially and then increases.

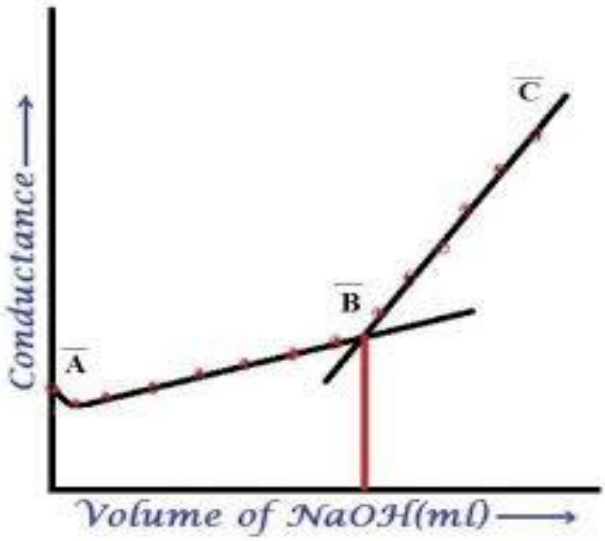


Figure 3: Conductometric titration of a weak acid (CH_3COOH) Vs. a strong base (NaOH)

It decreases initially as the anion formed suppresses the ionization of the weak acid and again increase in conductance value was observed up to the equivalence point. This is because the conversion of the non-conducting weak acid into its conducting salt. The two opposing effects act here- decrease in conductance is due to the common ion effect and increase is due to formation of conducting salt. Beyond the equivalence point, further addition of NaOH introduces OH^- ions, as a result there is a sharp increase in conductance value is observed. The variation in the conductance value is as shown in the Figure 3.

4.Titration of a weak acid with weak base

Eg:CH_3COOH Vs NH_4OH

During the titration of a weak acid with a weak base, the following reaction is going to takes place:

$$CH_3COOH + NH_4OH \rightarrow CH_3COONH_4 + H_2O$$

Acetic acid is a weak acid, it dissociates partially into H^+ and CH_3COO^- ions and hence number of ions are less in the solution as a result initially low conductance value is observed. When weak base is added dropwise initially slight decrease in conductance value is observed due to the common ion effect. With further addition of NH_4OH dropwise, gradual increase in conductance value is observed. This is due to the formation of conducting CH_3COONH_4 salt.

After the equivalence point an excess of NH_4OH in solution has little effect on the conductance value as its dissociation is suppressed by ammonium salt in the solution. The variation in the conductance value is as shown in the Figure 4.

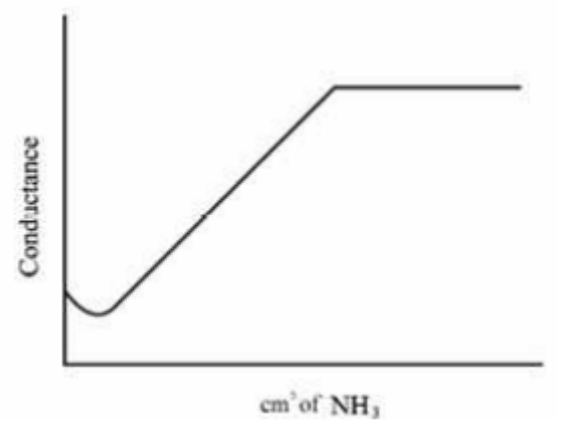


Figure 4: Conductometric titration of a weak acid (CH_3COOH) Vs. a weak base (NH_4OH)

Note- The nature of curve before the equivalence point is similar to the curve obtained by titrating weak acid against strong base. After the equivalence point, conductance virtually remains same as the weak base which is being added is feebly ionized and, therefore, is not much conducting.

Spectrophotometry:

Beer and Lambert's law and its derivation:

Lambert's Law:

When a monochromatic light passes through a transparent medium, the rate of decrease in intensity with the thickness of the medium is proportional to the intensity of the light. This is equivalent to stating that the intensity of the emitted light decreases exponentially as the thickness of the absorbing medium increases arithmetically.

We can write it as

$$-\frac{dI}{dt} = kI \qquad (1)$$

Where I = intensity of the incident light of wave length, λ

t = thickness of the medium

k = proportionality constant

Integrating eq. 1, and putting $I = I_0$ when t = 0

$$-\int \frac{dI}{dt} = \int kI$$
$$-\int \frac{dI}{I} = k \int dt$$
$$\ln \frac{I_0}{I_t} = kt$$
$$I_t = I_0\, e^{-kt} \qquad (2)$$

Where I_0 = intensity of the incident light falling upon the absorbing medium of thickness, t

It = intensity of the transmitted light

k = constant for the wavelength and the absorbing medium

we can write the eq. 2 as

$$\frac{I_t}{I_0} = e^{-kt}$$
$$= 10^{\frac{-k}{2.3036}t}$$
$$= 10^{-0.4343kt}$$
$$I_t = I_0\, 10^{-Kt} \qquad (3)$$

Where 'K' is called as absorption coefficient.

The absorption coefficient (K) is generally defined as the reciprocal of the thickness (t, cm) required to reduce the light to $1/10^{th}$ of its intensity.

i.e., in eq. 3, $\frac{I_t}{I_0} = 0.1$

$= 10^{-Kt}$

or Kt = 1 and K = 1/t

The ratio, $\frac{I_t}{I_0}$ is the fraction of the incident light transmitted by a thickness 't' of the medium and is termed as the transmittance (T). The reciprocal of transmittance i.e., $\frac{I_o}{I_t}$ is opacity. The absorbance (A) of the medium is given by

$$A = \log \left(\frac{I_0}{I_t}\right) \qquad (4)$$

Beer's Law:

"The intensity of a beam of monochromatic light decreases exponentially as the concentration of the absorbing substance increases arithmetically"

This can be written as

$$I_t = I_0\, e^{-k'C}$$

$$= I_0\, 10^{-0.4343k`C}$$

$$= I_0\, 10^{-KC} \qquad (5)$$

Where 'C' is the concentration, k` and K` are constants.

Combining eq. (3) and eq. (5),

$$\frac{I_t}{I_0} = 10^{-aCt}$$

$$\log \left(\frac{I_t}{I_0}\right) = aCt \qquad (6)$$

This **(eq. 6) is the mathematical expression for Beer-Lambert's law**.

The value 'a' depend upon the method of expression of the concentration of the solution. If 'C' is expressed in mol/l and 't' in cm; then 'a' is given the symbol, ε and is called the molar absorption coefficient or molar absorptivity.

It is clear that, there is a relationship between the absorbance (A), the transmittance (T) and the molar absorption coefficient.

$$A = \log \left(\frac{I_0}{I_t}\right) = \log(1/T) = -\log T \qquad (7)$$

∴ A = -log(T)

A = εCt

The scales of spectrophotometers are often calibrated to read directly in absorbance and frequently in % transmittance also.

A = εCt

ε = A/Ct

If C = 1 mol/dm^3, t = 1 cm; then ε = A

i.e., the molar absorption coefficient is the specific absorption coefficient. Specific absorption coefficient is defined as the absorption per unit thickness and unit concentration.

Limitations of Beer-Lambert law

The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. The probable causes of nonlinearity are:

1. Deviations in absorptivity coefficients at *high concentrations (>0.01M)* due to electrostatic interactions between molecules in close proximity
2. Scattering of light due to particulates in the sample
3. Fluoresecence or phosphorescence of the sample
4. Changes in refractive index at high analyte concentration
5. Shifts in chemical equilibria as a function of concentration
6. Non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band
7. Stray light

Applications of Beer Lambert's law in qualitative and quantitative analysis:

Estimation of Iron:

Iron is one of the many minerals required by the human body. It is biologically relevant as oxygen-carrying proteins, haemoglobin and myoglobin. A deficiency of iron in the body can leave a person feeling tired and can lead to a disorder called anemia. Many of the foods we eat contain small quantities of iron. The trace quantity of iron also may be present in water sources. Other application of iron is in industrial metallurgy. So, a simple method to estimate iron at very low concentrations is desired using colorimetric analysis.

Method:

In this analysis the iron present in the samples (like an iron tablet or a sample of food is extracted to form a solution containing Fe^{3+} (ferric) ions. To make the presence of these ions in solution visible, thiocyanate ions (SCN^-) are added. These react with the Fe^{3+} ions to form a blood-red colored complex as given below.

$$Fe^{3+}(aq) + SCN^-(aq) \rightarrow [FeSCN]^{2+}(aq)$$

By comparing the intensity of the colour of this solution with the colours of a series of standard solutions, with known Fe^{3+} concentrations, the concentration of iron in the tablet, food or any other sample may be determined. A plot of absorbance vs solution of known concentration and the by comparison of the absorbance of the unknown solution, one can quantitatively estimate the amount of iron present.

Numerical:

1. The molar extinction coefficient of phenanthroline complex of iron (II) is 1200 dm^3 mol^{-1} cm^{-1} and the minimum detectable absorbance is 0.01. Calculate the minimum concentration of the complex that can be detected in the experiment if the path length is 1.00 cm.

$A = \varepsilon bc$

$c = A/\varepsilon b = 0.01 / 1.00 * 1200$

$= 8.33 \times 10^{-6}$ M

2. A chemist has a sample of Adenine with an absorbance of 0.67 at a wavelength of 260 nm. The Molar absorption coefficient (ε_{260}) is 7100 $M^{-1}cm^{-1}$. The path length of light is 1.00 cm. What is the concentration of the sample?

Ans: In the example problem we are given the absorbance of the sample, the Molar absorption coefficient, and the path length of light. Concentration of the sample needs to be calculated.

Step 2: Using the Beer-Lambert Law equation, we can rearrange to solve for concentration (c):

$A=\varepsilon cl$

or

$c=A/\varepsilon l=0.67 \ / \ (7100M^{-1}cm^{-1})*(1.00cm) = 9.4\cdot10^{-5}M$

3. There is a substance in a solution (4 g/liter). The length of cuvette is 2 cm and only 50% of the certain light beam is transmitted. What is the extinction coefficient?

Using Beer-Lambert Law, we can compute the absorption coefficient. Thus,

$$A= -\log(It/Io)$$

$$A=-\log(0.5/1.0)$$

$$\text{and } A=2*4*\epsilon$$

Then we obtain that $\epsilon = 0.0376$

4. In Example above, how much is the beam of light is transmitted when 8 g/liter?

Ans: We can calculate the transmission using Beer-Lambert Law.

Thus, $\log (1) - \log(I_t) = 0 - \log (I_t) = 0.0376 \times 8 \times 2 = 0.6016$

Therefore $\log (I_t) = -0.6016$

Hence, $I_t = 0.2503 = 25\%$

5. The absorption coefficient of a glycogen-iodine complex is 0.20 at light of 450 nm. What is the concentration when the transmission is 40 % in a cuvette of 2 cm?

Ans: It can also be solved using Beer-Lambert Law. Therefore,

$-\log(It/Io) = -\log_{10}(0.4) = 0.20 \times c \times 2$

Then c = 0.9948

Flame photometric determination of Na^+ and K^+ ions

Background

Sodium ion is the major cation of the extra cellular fluid whereas potassium is the major ion found inside the cells. The body maintains a delicate balance of these ions across the cellular membrane and any alteration in their normal values has significant physiological consequences. For example, an abnormal increase of potassium (hyperkalemia) or decrease of potassium (hypokalemia) can significantly affect the nervous system and heart, and if the levels become extreme, it can be fatal. In other words, an accurate determination of these ions in the body fluids can serve as important diagnostic tool.

Principle

When the solution containing a metallic compound of Na, K, Li, Ca and Ba is aspirated into a flame, a vapour containing the atoms of the metal may be formed. Some of these gaseous metal atoms may be raised to an energy level which is sufficiently high to permit the emission of radiation, which is characteristic of that metal (Eg, the characteristic yellow colour imparted to flames by compounds of sodium and Lilac colour imparted to flames by compounds of Potassium). This radiation can be measured by the detectors.

Instrumentation:

Air at a given pressure is passed in to the atomizer and the suction thus produced draws a solution of the sample in to the atomizer, where it joins the air stream as a fine mist and passes to the burner. Here in a small mixing chamber called nebulizer, the air meets the fuel supplied to the burner at a given pressure and the mixture is burnt. Radiations from the resulting flame passes through a lens and finally through an optical filter which permits only the radiation characteristic to the element under investigation to pass through the photocell. The output from the photocell is measured on a suitable digital readout system. The layout of a simple flame photometer is shown below.