UNIT 2 UV-VISIBLE SPECTROMETRY

Structure

2.1 INTRODUCTION

In the previous unit you have learnt about the electromagnetic radiation in terms of its nature, characteristics and properties. You also learnt that the interaction of radiation with matter brings about transitions among some of the quantised energy levels of the matter and study of such an interaction is called spectroscopy. Further, the interaction of radiation with matter may be manifested in terms of its selective absorption or absorption followed by emission or scattering.

In this unit you would learn about the ultraviolet-visible (UV-VIS) spectrometry. This concerns the measurement of the consequences of interaction of EM radiations in the UV and/or visible region with the absorbing species like, atoms, molecules or ions. In such determinations the extent to which radiation energy is absorbed by a chemical system as a function of wavelength, as well as, the absorption at a fixed predetermined wavelength of the radiation is measured. Since such measurements need an instrument called spectrometer the technique is known as UV-VIS spectrometry.

The UV-VIS spectrometry is one of the oldest instrumental techniques of analysis and is the basis for a number of ideal methods for the determination of micro and semi-micro quantities of analytes in a sample. We will begin by understanding the theory behind UV-VIS spectrometry in terms of the origin and the features of the UV-VIS spectrum. Then we will discuss the fundamental principles that form the basis of the analytical applications of UV-VIS spectrometry. It will be followed by an account of the essential components of a UV-VIS spectrometer and their functions. Thereafter,

we will discuss the qualitative and quantitative applications of UV-VIS spectrometry in diverse areas. In the next unit you will learn about the study of infra- red spectrometry which concerns the interaction of electromagnetic radiation in the infrared region with matter.

Objectives

After studying this unit, you should be able to:

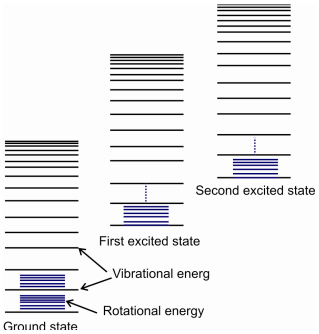
- explain the origin and the characteristics of UV-VIS spectrum,
- outline different types of species absorbing in the UV-VIS region,
- explain the Lambert's and Beer's laws,
- explain the factors leading to deviation from Beer-Lambert's law,
- describe the components of UV-VIS instruments,
- state the principles of different types of UV-VIS instruments,
- elaborate the methodology of quantitative applications of UV-VIS spectrometry, and
- state different applications of UV-VIS spectrometry.

2.2 ORIGIN AND CHARACTERISTICS OF UV-VIS SPECTRUM

UV-VIS spectrum results from the interaction of electromagnetic radiation in the UV-VIS region with molecules, ions or complexes. It forms the basis of analysis of different substances such as, inorganic, organic and biochemicals. These determinations find applications in research, industry, clinical laboratories and in the chemical analysis of environmental samples. It is therefore important to learn about the origin of the UV-VIS spectrum and its characteristics. Let us try to understand the origin of the UV-VIS spectrum.

2.2.1 Origin of UV-VIS Spectrum

The absorption of radiation in the UV-VIS region of the spectrum is dependent on the electronic structure of the absorbing species like, atoms, molecules, ions or complexes. You have learnt in Unit 1 that the absorption spectrum of atomic species consists of a number of narrow lines that arise as a consequence of the transition amongst the atomic energy levels. You would also recall that in molecules, the electronic, vibrational as well as the rotational energies are quantised. A given electronic energy level has a number of **vibrational energy levels** in it and each of the vibrational energy level has a number of **rotational energy levels** in it. When a photon of a given wavelength interacts with the molecule it may cause a transition amongst the electronic energy levels if its energy matches with the difference in the energies of these levels.



The absorption of radiation in the UV-VIS region of the spectrum causes the transitions amongst the electronic energy levels.

In the course of such transitions, for the sample in gaseous or vapour phase, the spectrum consists of a number of closely spaced lines (Fig 2.1(a)), constituting what is called a **band spectrum.** However, in the solution phase, the absorbing species are surrounded by solvent molecules and undergo constant collisions with them. These collisions and the interactions among the absorbing species and the solvent molecules cause the energies of the quantum states to spread out. As a consequence, the sample absorbs photons spread over a range of wavelength. Thereby, the spectrum acquires the shape of a smooth and continuous absorption peak in the solution phase. A typical UV-VIS spectrum in the solution phase is depicted in Fig. 2.1 (b).

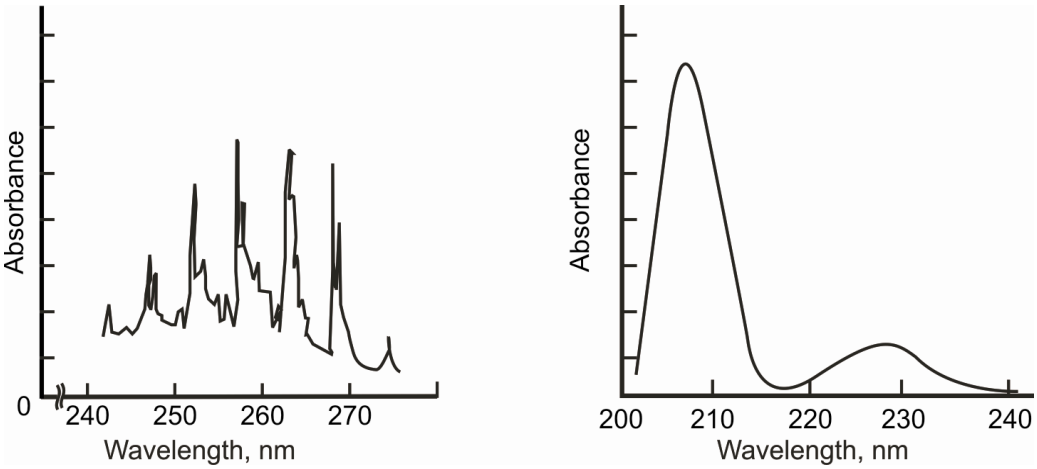


Fig. 2.1: Representative UV spectrum: a) in vapour phase and b) in solution phase

2.2.2 Characteristics of UV-VIS Spectrum

In order to obtain a UV-VIS spectrum the sample is ideally irradiated with the electromagnetic radiation varied over a range of wavelength. A monochromatic radiation i.e., a radiation of a single wavelength is employed at a time. This process is called **scanning**. The amount of the radiation absorbed at each wavelength is measured and plotted against the wavelength to obtain the spectrum. Thus, a typical UV spectrum is a plot of wavelength or frequency versus the intensity of absorption.

Have a look again at Fig. 2.1 (b). The abscissa (x-axis) indicates the wavelengths absorbed and therefore, is marked in wavelength though sometimes frequency may also be used. The ordinate (y-axis) on the other hand represents the intensity of absorption and is generally represented in terms of absorbance (explained in subsec. 2.2.3). The UV spectra of substances are characterised by two major parameters, namely, the **position** of the maximum of the absorption band called λ_{max}, and the **intensity** of the bands. The λ_{max} refers to the wavelength of the most absorbed radiation and is a measure of the difference in the electronic energy levels involved in the transition. The intensity on the other hand is indicative of the **probability of the transition** i.e., whether the transition is allowed or not. It is also is a measure of the concentration of the absorbing species. The relationship between the intensity of absorption and the concentration is explained in Section 2.3.

Analyte: The chemical species which is to be determined in the sample under investigation.

The characteristics of UV-VIS spectrum depend on the structure and concentration of the absorbing species in solution. Therefore, these spectra are extensively used in the characterisation and in the quantitative estimations of the analyte.

2.2.3 Absorbing Species

As discussed above, the UV-VIS spectra arise due to electronic transitions in the absorbing species. The electronic transitions do not occur in all the species when a radiation in UV region is passed through the sample. Generally three types of species show the mentioned transitions, these are organic, inorganic and complexes formed by change transfer. Let us learn about these species and the nature of the transitions involved in them.

Organic Molecules

The molecular absorptions in the UV-VIS region depend on the electronic structure of the molecule. The wavelength of the radiation absorbed by an organic molecule is determined by the difference in energy between the ground state and the various excited electronic states of the molecule. You would recall from your earlier

knowledge of bonding in organic molecules that the constituent atoms are bonded through σ and π bonds. In addition, these have nonbonding electrons on the atoms like, N,O,S and halogens etc. There are a number of transitions possible involving the bonding and the nonbonding electrons. The generalised molecular orbital energy level diagram and possible transitions for organic compounds is given in Fig. 2.2.

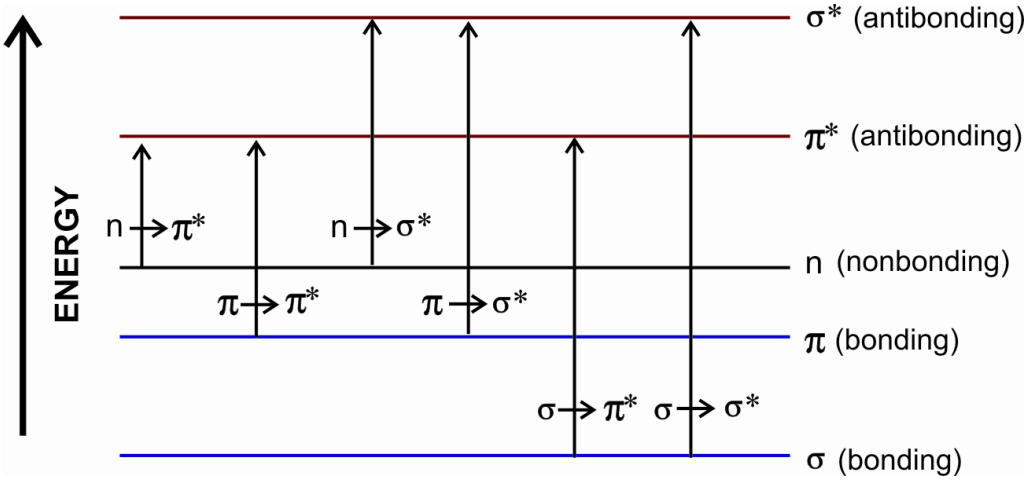


Fig. 2.2: Generalised molecular orbital energy level diagram and possible transitions for organic compounds

As a rule, the transitions occur from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) in a molecule. In most of the organic compounds, the bonding and sometimes the nonbonding orbitals also are filled and the antibonding orbitals are empty. Of the six possible transitions indicated in the figure, only the two of the lowest energy ones ($n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$) can be achieved by the energies available in the 200 to 800 nm region. Let us learn about these two in the following paragraph.

$\pi \rightarrow \pi^*$ **transitions:** These transitions are observed in molecules containing π= electrons and occur at wavelengths approaching near ultraviolet regions The hydrocarbons containing double and triple bonds are typical examples. For example, ethylene absorbs at 170 nm. The conjugation of unsaturation further increases λ_{max}; but-1, 3-diene absorbs at 217 nm. The effect of conjugation is quite important in aromatic molecules. For example single ring aromatics absorb in the vicinity of 250 nm, naphthalene in the vicinity of 300 nm and anthracene in the vicinity of 360 nm.

$n \rightarrow \pi^*$ **transitions:** These transitions are observed in molecules containing lone pairs or nonbonding electrons. In such transitions one of the nonbonding electrons may be excited into an empty π^* orbital. The energies required for these transitions are lower than that for $\pi \rightarrow \pi^*$ transitions and result in the absorption in the ultraviolet and visible region. The presence of atoms or groups containing *n* = electrons, can cause remarkable changes in the spectrum. Thus, nitrogen, sulphur and halogens tend to move absorption to higher wavelengths.

The $\pi \rightarrow \pi^*$ transitions are generally intense while the $n \rightarrow \pi^*$ transitions are weak. For example, acetone, exhibits a high intensity $\pi \rightarrow \pi^*$ transition at 195 nm and a low intensity $n \rightarrow \pi^*$ transition at 274 nm in its absorption spectrum (Fig 2.3).

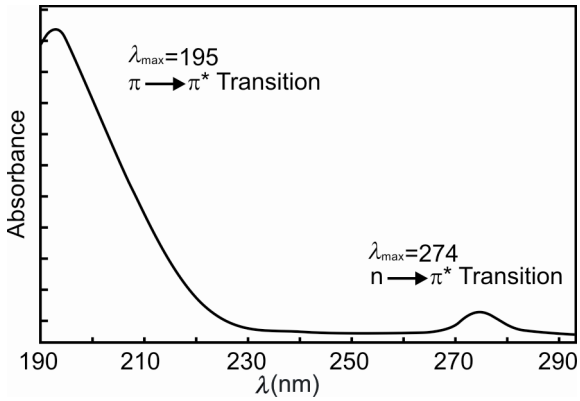


Fig. 2.3: UV spectrum of acetone showing the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions

Thus, only those molecules are likely to absorb light in the 200 to 800 nm region which contain π-electrons and may also have atoms with non-bonding electrons. Such groups that absorb light in the UV-VIS region are referred to as **chromophores**. Chromophore in Greek means, "colour bearing". However, the term chromophore is now not limited to colours only but is used in a general way for groups which are responsible for causing an absorption of electromagnetic radiation between about 175 to 800 nm. A list of some simple chromophoric groups and their light absorption characteristics is given in Table 2.1.

Table 2.1: Absorption characteristics of some common chromophoric groups

Chromophore	Example	λ_{max}	Type of Transition
>C = C<	C_6H_{13} CH = CH_2	177	$\pi \rightarrow \pi^*$
–C ≡ C–	C_5H_{11} C ≡ C – CH_3	178	$\pi \rightarrow \pi^*$
>C = O	CH_3CO CH_3	186 280	$n \rightarrow \sigma^*$ $n \rightarrow \pi^*$
	CH_3CHO	180 293	$n \rightarrow \sigma^*$ $n \rightarrow \pi^*$
–COOH	CH_3COOH	204	$n \rightarrow \pi^*$
$–CONH_2$	CH_3CONH_2	214	$n \rightarrow \pi^*$
–N = N–	$CH_3N = NCH_3$	339	$n \rightarrow \pi^*$
$–NO_2$	CH_3NO_2	280	$n \rightarrow \pi^*$
–N = O	C_4H_9 NO	300 665	- $n \rightarrow \pi^*$

Inorganic Species

A large number of inorganic salts containing atoms with electrons in *d*-orbitals give weak absorption bands in the visible range. The ions and the complexes of the elements of the first two transition series belong to this group and are coloured. The colour of these species is due to transitions amongst *d*-orbitals. The complex formation of these ions with solvent molecules or with other ligands lifts the degeneracy of the five *d* orbitals. As a consequence, these split into groups having different energies. The electronic transitions from the lower energy *d* orbitals to higher energy *d* orbitals are responsible for the observed colour. These transitions are called ***d-d* transitions**.

The blue colour of the aqueous solutions of copper sulphate and the violet colour of potassium permanganate are some of the examples.

The ions of the lanthanides and actinides are also coloured, however, these involve ***f-f* transitions**. The nature of spectrum in case of these ions is different because the *f*-electrons are relatively less affected by external influences due to shielding by the occupied orbitals of higher principal quantum number. These ions absorb the radiation of ultraviolet and visible region in narrow bands. The representative visible spectra of the ions of transition and inner transition elements are given in Fig. 2.4.

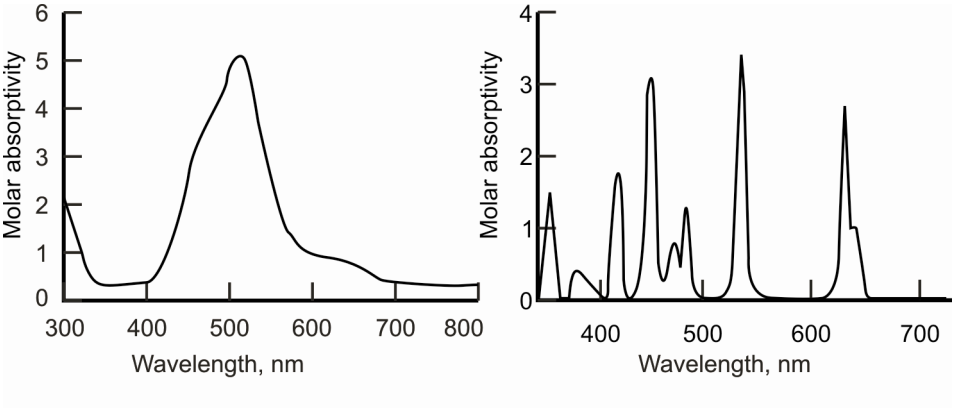


Fig. 2.4: Representative absorption spectra for the a) transition and b) inner transition element ions

Charge Transfer Complexes

Many a times a given compound that is transparent in the UV region starts absorbing after interacting with another species. This happens if one of the species has an electron **donor** group and the interacting species has an electron **acceptor** group. When the two species bind to each other, the resulting species is intensely coloured. This is due to the formation of a complex between the two species. Such a complex is called **charge transfer complex**. For example, the blood red color of the complex ion, thiocyanatoiron (III) ion, Fe (SCN) $^{2+}$ is due to the formation of a charge transfer complex.

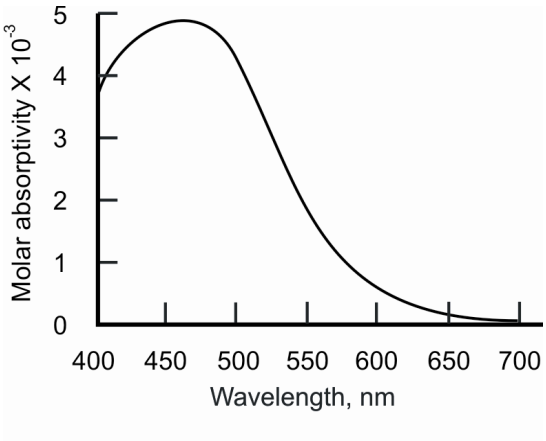


Fig. 2.5: The charge transfer spectrum of the complex ion, Fe (SCN)$^{2+}$

The radiation absorbed by this product or complex causes the transfer of an electron from an orbital on the donor to an orbital of the acceptor. This has been schematically illustrated in Fig. 2.6. The transitions in the donor and the acceptor species individually are of high energy and absorb outside the UV-VIS region. However, in the complex, the energies of the orbitals are such that the HOMO to LUMO transition is of much lower energy and falls in the visible region.

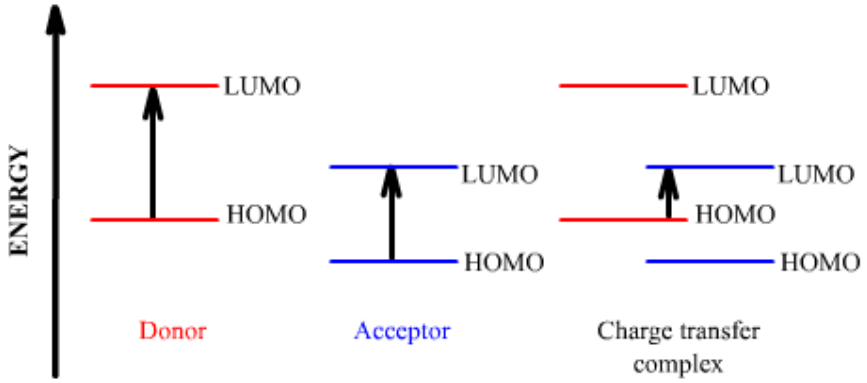


Fig. 2.6: Schematic diagram showing the disposition of the molecular orbitals of the donor, acceptor and the charge transfer complex species

A large number of chelating agents are used for the spot tests. The intense colour of such metal chelates is often due to charge transfer transitions. They simply involve the movement of electrons from the metal ion to the ligand, or vice-versa. In the complex between thiocyanate (SCN^-) and the Fe (III) ions, the absorption of photon transfers an electron from the thiocyanate ion to Fe (III) ion. Besides metal ions, non-metals and their ions also form coloured substances which produce characteristic spectra. For example, the alkaloid brucine reacts with nitrate ion in concentrated sulphuric acid to form a red compound which quickly turns yellow.

It was mentioned earlier that UV-VIS spectrometry can be used for qualitative and quantitative determinations of the analytes. The quantitative determinations are based on the relationship between the absorption of radiation and concentration of the analyte. Let us learn about the principle of spectrometric determinations. Before that why don't you assess your understanding by answering the following self assessment question?

SAQ 1

List different types of species that may absorb in the UV-VIS region of the spectrum and indicate the type of transitions involved in them.

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2.3 PRINCIPLE OF UV-VIS SPECTROMETRY

In a typical absorption spectral measurement a monochromatic radiation is made to fall on a sample taken in suitable container called **cuvette**. In such a situation a part of the radiation is reflected, a part is absorbed, and a part is transmitted. The intensity of original radiation, P_o is equal to the sum of the intensities of reflected (P_r), absorbed (P_a) and transmitted (P_t) radiation.

The transmitted radiation has information about the nature and the amount of the absorbing species.

$$P_o = P_r + P_a + P_t$$

The effect of reflection can be compensated by passing equal intensities of beams through the solution and through the solvent contained in the same or similar container and comparing the transmitted radiations. We can then write the above equation as follows:

$$P_o = P_a + P_t$$

The intensity of the transmitted light is measured and is found to depend on the thickness of the absorbing medium and the concentration, besides the intensity of the incident radiation. This dependence forms the basis of spectrometric determinations

and is given in terms of two fundamental laws. One is **Bouguer's law** or **Lambert's law**, which expresses the relationship between the light absorption capacity of the sample and the thickness of the absorbing medium; and the other is **Beer's law**, which expresses the relationship between the light absorptive capacity of the sample and its concentration. The two laws are combined together to give **Beer-Lambert's law**. Let us discuss these laws one by one.

2.3.1 Lambert's Law

Lambert (1760) and Bouguer independently studied the decrease in the intensity of radiation when it passes through a substance and made the following observations:

i) The amount of monochromatic light absorbed by a substance is proportional to the intensity of the incident light i.e. the ratio of the intensity of the transmitted and incident light is constant.

ii) The intensity of the transmitted light decreases exponentially when the thickness of the substance, through which the light is passing, increases linearly.

These observations, called Lambert's law can be translated into a mathematical expression as described below.

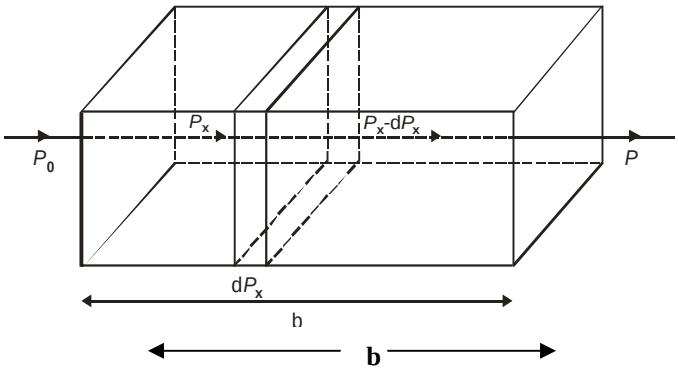

Fig. 2.7: Illustration for establishing the Lambert's Law

In Fig. 2.7, if P_o represents the radiant power of incident light and P represents the radiant power of transmitted light after passing through a slab of thickness b, consider a small slab of thickness dx, then the change in power (dP_x), is proportional to the power of incident light (P_x) multiplied by the change in thickness (dx) of the slab through which the light is passed. That is,

$$dP_x \propto P_x dx$$

or
$$dP_x = -k P_x dx \quad \ldots (2.1)$$

where k is the proportionality constant and the negative sign indicates that radiant power decreases with absorption. Eq. 2.1 can be rearranged to Eq. 2.2.

$$\frac{dP_x}{P_x} = -k dx \quad \ldots (2.2)$$

Integrating Eq. 2.2 within the limits of P_o to P for intensity and 0 to b for the thickness we get,

$$\int_{p_0}^{p} \frac{dP_x}{P_x} = -k \int_{0}^{b} dx$$

$$\ln \frac{P}{P_o} = -kb \qquad \ldots (2.3)$$

Eq. 2.3 is the mathematical expression for Bouguer-Lambert law or Lambert's law. Changing this equation to base 10 logarithms and rearranging we get,

$$\log \frac{P_o}{P} = \frac{k}{2.303} b = k'b \qquad \ldots (2.4)$$

Note that the ratio P/P_o has been inverted to remove the negative sign. Lambert's law applies to any homogeneous non-scattering medium, regardless of whether it is gas, liquid, solid, or solution.

2.3.2 Beer's Law

In 1852, Beer and Bernard independently studied the dependence of intensity of transmitted light on the concentration of the solution. It was found that the relation between intensity of the transmitted light and concentration was exactly the same as found by Lambert for the intensity of the transmitted light and the thickness of the absorbing medium. Mathematically, the Beer's observations can be expressed as follows.

Consider a monochromatic radiation beam of intensity P_x traversing any thickness of solution of a single absorbing substance of concentration c. If c is changed by a small amount dc to c + dc, the change in transmitted power dP_x is proportional to the incident intensity P_x and dc. We can express it as follows.

$$\begin{aligned} dP_x &\propto P_x \, dc \\ dP_x &= -k'' P_x \, dc \end{aligned} \qquad \ldots (2.5)$$

where k'' is the proportionality constant and the negative sign indicates that radiant power decreases with absorption. This equation can be rearranged to:

$$\frac{dP_x}{P_x} = -k'' \, dc \qquad \ldots (2.6)$$

On integration within the limits of P_o to P for intensity and 0 to c for concentration we get

$$\begin{aligned} \int_{P_o}^{P} \frac{d\, P_x}{P_x} &= -k'' \int_{o}^{c} dc \\ \text{or} \qquad \ln \frac{P}{P_o} &= -k''c \\ \text{or} \qquad \log_{10} \frac{P_o}{P} &= \frac{k''}{2.303} c \end{aligned} \qquad \ldots (2.7)$$

Eq. 2.7 is the mathematical expression for Beer's law. The Lambert's and Beer's laws are combined and are expressed as per Eq. 2.8.

$$\log \frac{P_o}{P} = abc \qquad \ldots (2.8)$$

In this expression, 'a' is a constant (combining two constants k', k'' and the numerical factor) and is called absorptivity (earlier called extinction coefficient) whose value depends on unit of concentration used and is a function of wavelength of the

monochromatic light used. The concentration is generally expressed in terms of grams per dm^3 and *b* in cm. Therefore, it has units of $cm^{-1}g^{-1}\,dm^3$.

However, if the concentration is expressed as mol dm^{-3} and b in cm then it is called **molar absorptivity** (earlier called molar extinction coefficient) and expressed as ε. Its units are $cm^{-1}\,mol^{-1}dm^3$. The modified expression for the Beer-Lambert's law becomes,

$$\log\frac{P_0}{P} = \varepsilon bc \qquad \ldots (2.9)$$

Absorbance is defined as the logarithm of the ratio of the intensities of the incident and transmitted radiations.

The term log P_o/P is called **absorbance** and is represented as '*A*'.

$$A = \log\frac{P_0}{P}$$

The expressions for Beer-Lambert's law then becomes,

$$\log\frac{P_0}{P} = A = abc \;\; or \;\; \varepsilon bc \qquad ...(2.10)$$

Eq. 2.10: use 'a' if concentration is expressed in g/L and path length in cm and 'ε' if the concentration in mol dm^{-3} and b in cm.

The absorbance, *A* is related to another important term called **transmittance** which is defined as the fraction of the incident radiation transmitted by the absorbing medium. Mathematically,

$$\text{Transmittance, } T = \frac{P}{P_0} \qquad \ldots (2.11)$$

It is generally expressed as a percentage and is expressed as,

$$\textbf{Percentage transmittance, } \%T = \frac{P}{P_0}\times 100\% \qquad \ldots (2.12)$$

You may note here that though transmittance and absorbance are equivalent expressions which can be interconverted, it is the absorbance which is directly proportional to the absorbed fraction of the radiation.

$$\text{It is related to absorbance as, } \; A = -\log T \qquad \ldots (2.13)$$

In typical measurements the radiant power transmitted by a solution is measured and compared with that observed with solvent (also called a blank). The ratio of transmitted powers through solution and that through blank or solvent is called as transmittance.

It is desirable that you get acquainted with the expressions of the Beer-Lambert's law and learn to use them. Let us take some examples illustrating the application of these expressions.

Example 1

The molar absorptivity of a substance is $2.0 \times 10^4\,cm^{-1}\,mol^{-1}\,dm^3$. Calculate the transmittance through a cuvette of path length 5.0 cm containing 2.0×10^{-6} mol dm^{-3} solution of the substance.

Solution

As per the Lambert Beer's law,

Absorbance, A = $\varepsilon\, c\, b$

Given:

$\varepsilon = 2.0 \times 10^4\,cm^{-1}\,mol^{-1}\,dm^3$, $c = 2.0 \times 10^{-6}$ mol dm^{-3} and $b = 5.0$ cm

Substituting the values we get,

$A = 2.0 \times 10^4 \times 2.0\ 10^{-6} \times 5.0 = 0.2$

=> log $l/T = 0.2$ $\therefore A = \log 1/T$

Taking antilog on both sides, we get

$l/T = 1.585$

$\therefore T = 0.63$

Example 2

A solution containing 36.5 mg of potassium dichromate per 500 cm^3 was taken in a cuvette having a path length of 2 cm and its transmittance was measured at 455 nm. If the percentage transmittance is found to be 12, calculate the molar absorptivity of potassium dichromate.

Solution

The molarity of given potassium dichromate solution can be calculated as

$$= \frac{1000 \times 0.0365}{500 \times 294} = 2.48 \times 10^{-4} \text{ mol dm}^{-3}$$

(M_m of potassium dichromate = 294 g mol^{-1})

12% transmittance means $T = 0.12$

$\therefore A = 1/0.12 = 0.921$

$\therefore \varepsilon = A/cb = 0.921 / (2.48 \times 10^{-4} \times 2) = 0.921/4.96 \times 10^{-4}$

$\therefore \varepsilon = 1.86 \times 10^3 \text{ cm}^{-1} \text{ mol}^{-1} \text{ dm}^3$

From the Lambert-Beer's law expression given above, absorbance ($A = \log P_0 / P$) is a direct measure of the concentration of the analyte if the thickness of the absorbing medium is kept constant. This proportionality is exploited in the determination of analyte concentration. However, some factors challenge the validity of the Beer's law and need to be controlled to take up quantitative determinations. Let us learn about the possible reasons for the deviation from Beer's law. Why don't you answer the following SAQs before proceeding further?

SAQ 2

In a spectrophotometer set at the λ_{max} of a sample the value of P_0 (with the solvent) was found to be 85.4 using cuvettes of 2.00 cm path length. The value of P for a solution of the sample having a concentration of 1×10^4 M was measured in the same cuvette and was found to be 20.3. Calculate the molar absorptivity of the sample.

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SAQ 3

A substance has a molar absorptivity of 14,000 cm^{-1} mol^{-1} dm^3 at the wavelength of its maximum absorption. Calculate the concentration of the substance whose solution in a cuvette of path length 1 cm has an absorbance of 0.85.

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2.3.3 Deviations from Beer-Lambert's Law

As per the Beer's law discussed above, there is a direct proportionality between the absorbance and concentration. A plot of absorbance versus concentration is expected to be a straight line passing through origin. However, this is not always true; there are certain limitations. The law does not hold for all species under every condition. Many a times instead of a straight line, a curvature in the plot may be observed as shown in Fig. 2.8. The upward curvature, curve (a), is known as **positive deviation** and the downward curvature, curve(c), as **negative deviation**.

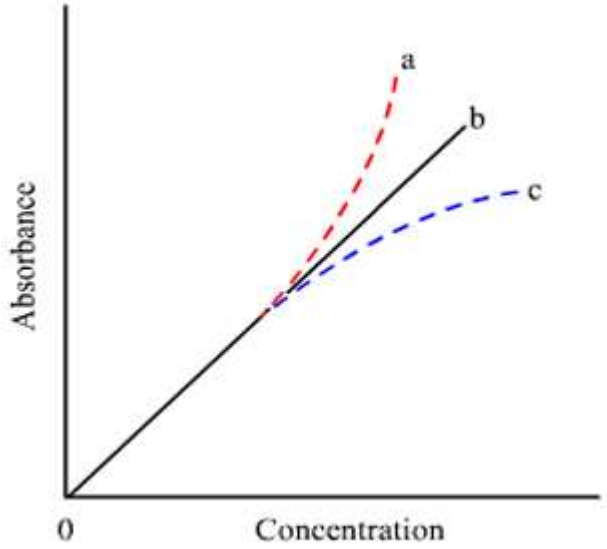

Fig. 2.8: Beer-Lambert law plots; the curvatures show deviations from the law

Some of the factors responsible for the deviation from Beer's law are as follows.

Presence of Electrolytes

The presence of small amounts of colourless electrolytes which do not react chemically with the coloured components does not affect the light absorption as a rule. However, large amounts of electrolytes may affect the absorption spectrum qualitatively as well as quantitatively. This is due to the physical interaction between the ions of the electrolyte and the coloured ions or molecules. This interaction results in a deformation of the later, thereby causing a change in its light absorption property.

Hydrogen Ion Concentration

There are a number of substances whose ionic state in solution is greatly influenced by the presence of hydrogen ions. For example, the aqueous solution of potassium dichromate involves the chromate ion-dichromate ion equilibrium as shown below:

$$\underset{\text{Chromate ion}\ (\lambda_{max}, 375\ \text{nm})}{2CrO_7^{2-}} + 2H^+ \rightleftharpoons \underset{\text{Dichromate ion}\ (\lambda_{max}\ 350,450\ \text{nm})}{Cr_2O_7^{2-}} + H_2O$$

The chromate ion has a single λ_{max} at 375 nm whereas dichromate ion has two peaks in the spectra; λ_{max} at 350 and 450 nm. The position of equilibrium depends on the pH of the solution and yellow colour of solution changes to orange on increasing the concentration of hydrogen ions. Therefore, the results of the determination of chromate ion (CrO_7^{2-}) concentration will depend on the pH. Thus, it is imperative that substances, whose colour is influenced by change in hydrogen ion concentration, must be studied under the condition of same pH.

In some cases, two absorbing species are in equilibrium and have a common value of absorptivity at a certain wavelength. For example, in case of bromothymol blue the absorption spectra at different pH values are different. However, at wavelength of 501 nm, we see that all species have same molar absorptivity (see Fig. 2.9). Therefore, no matter to what extent does one species change into the other, there is no change in the total absorption. Such a wavelength is known as **isosbestic point**. At this wavelength the Beer's law holds, though the measurements have low sensitivity. However, such wavelengths should be avoided for the quantitative work.

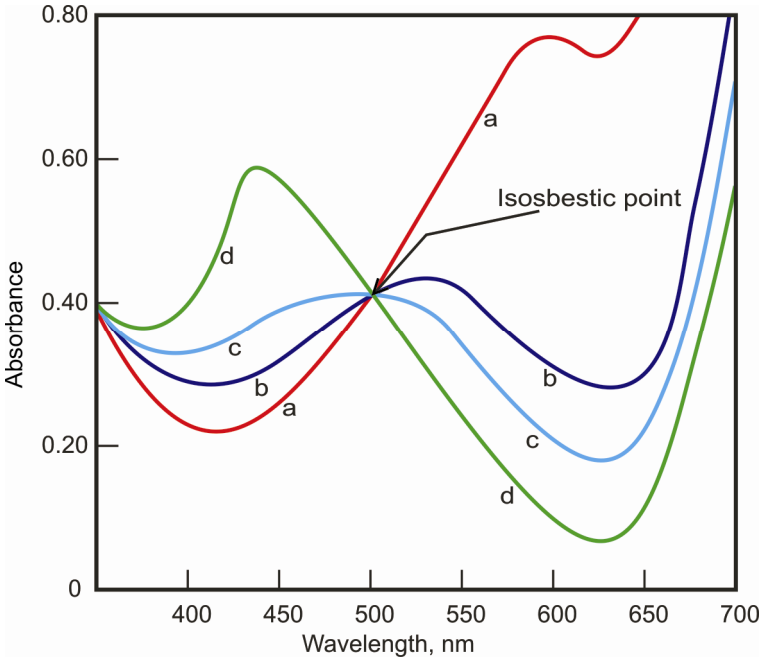

Fig. 2.9: The absorption spectra for bromothymol blue at different pH values showing the isosbestic point at 501 nm

Complexation, Association or Dissociation

Some salts have a tendency to form complexes whose colours are different from those of the simple compounds. For example, the colour of cobalt chloride changes from pink to blue due to the following complex formation.

$$2\ CoCl_2 \rightleftharpoons Co[CoCl_4]$$
pink blue

The degree of complex formation increases with increase in concentration, therefore, Beer's law does not hold at high concentrations. Similar discrepancies are found when the absorbing solute dissociates or associates in solution because the nature of the species in solution depends on the concentration.

Non-monochromatic Nature of the Radiation

In order for the Beer's law to hold, it is necessary that monochromatic light is used. However, most spectrophotometers and all filter photometers, discussed later employ a finite group of frequencies. The wider the bandwidth of radiation passed by the filter or other dispersing devices, the greater will be the apparent deviation of a system from adherence to Beer's law.

Concentration of the Analyte

As per Beer and Lambert's law, the plot of absorbance versus the concentration of absorbing substance should be a straight line when ε and b are constant. The path

length can always be held constant but there are some factors which affect ε and it is found that at high concentration, ε is not constant. Therefore, at higher concentrations ($>10^{-3}$ mol dm^{-3}) there may be deviation from the law.

Temperature

The temperature is not considered as an important factor since ordinarily the measurements are made at a constant temperature. However, changes in temperature sometimes may shift ionic equilibrium and the absorptivity. For example, the colour of acidic ferric chloride solution changes from yellow to reddish brown on heating due to change in λ_{max} and absorptivity.

SAQ 4

Why is it important to know about the possible reasons for deviation from Beer-Lambert's law?

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2.4 INSTRUMENTATION FOR UV-VIS SPECTROMETRY

Today a wide range of instruments are available for making molecular absorption measurements in the UV-visible range. These vary from simple and inexpensive machines for routine work to highly sophisticated devices that can be used for specialised work and of course the routine jobs also. However, the basic components of these instruments remain the same. The five essential components of UV-VIS instruments are as follows

- A stable radiation source
- Wavelength selector
- Sample holder
- Radiation detector or transducer , and
- Signal processing and output device

The general layout of the essential components in a simple absorption instrument is given in Fig. 2.10.

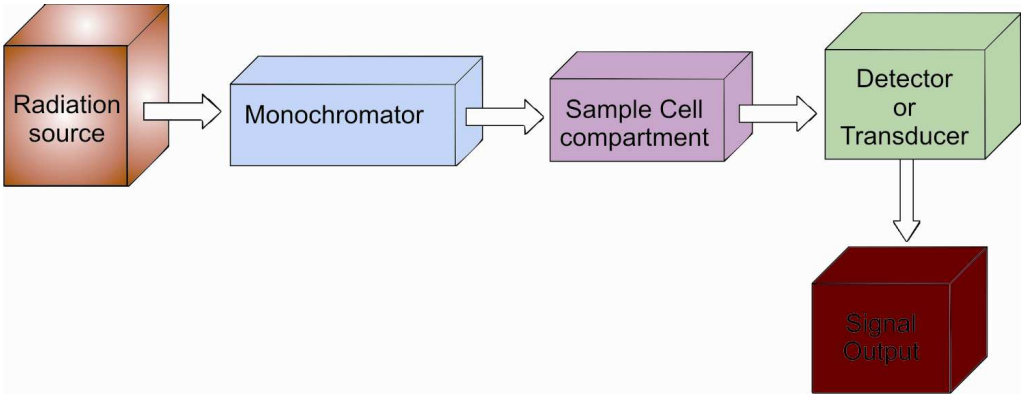


Fig. 2.10: General layout of the essential components in a simple absorption instrument

Let us learn about these components one by one.

2.4.1 Radiation Sources

A spectrophotometric radiation source must provide a stable high energy output over a broad range of wavelengths. There is no inexpensive source available that may provide stable output over the entire UV-visible range (190 nm to 780 nm). The radiation sources commonly used for the UV and visible range are described below.

Sources for UV Region

For measurements in the UV region, electric discharge sources like hydrogen or a deuterium lamp are used. In these, the excitation of the gaseous molecules is brought about by the passage of electrons through the gas at low pressures. A hydrogen lamp is commonly used in the spectrophotometers and gives light in the wavelength region of 160-375 nm. The radiant power of the hydrogen lamp is low and these are replaced by deuterium lamps but it increases the cost of the instrument.

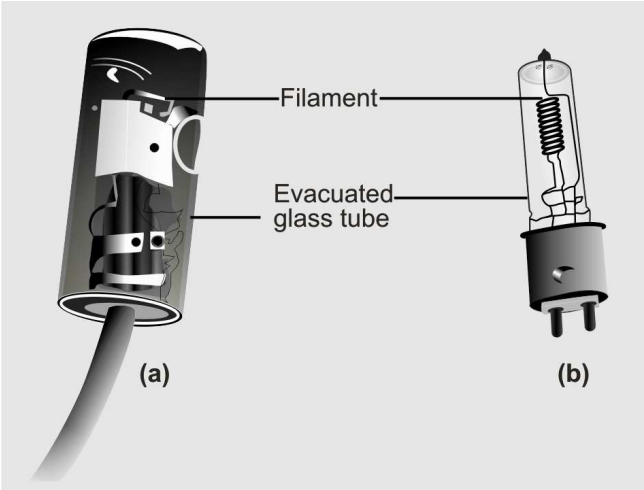


Fig. 2.11: Radiation sources: a) deuterium lamp for UV range b) tungsten lamp for visible range

Sources for Visible Region

In the beginning of development of instruments for colorimetric work, sunlight was used as source for measurements in the visible range. The modern instruments use a tungsten filament lamp as the radiation source. This consists of a thin, coiled tungsten wire that is sealed in an evacuated glass bulb. This gives radiations in the range of 350-2200 nm. As the output depends on the voltage, the tungsten lamp is energised by a 6 or 12 volt storage battery or by the output of a constant voltage transformer.

Now a days some instruments use tungsten-halogen lamps that contain a small amount of iodine in the quartz bulb housing the tungsten filament. The presence of iodine extends the output wavelength range of the lamp from 240 -2500 nm.

2.4.2 Wavelength Selectors

You would recall that a radiation consisting of a single wavelength is called a monochromatic radiation.

In spectrophotometric measurements we need to use a narrow band of wavelengths of light. This enhances the selectivity and sensitivity of the instrument. Less expensive instruments use a filter to isolate the radiant energy and provide a broad band of the wavelengths. In many applications we need to continuously vary the wavelength over a defined range. This can be achieved by using monochromators. Most modern instruments use monochromators that employ a prism or diffraction grating as the

dispersing medium. The selection and use of fillers and monochromators is described below.

Absorption Filters

In low cost instruments catering to measurements in the visible range, coloured glass filters are used to cut off undesirable wavelengths. A typical filter is a coloured piece of glass, which absorbs light of certain wavelength and allows that of the other to pass through. You know that white light is made up of seven different colours; the acronym is **VIBGYOR** for violet, indigo, blue, green, yellow, orange and red. These seven colours add up to give white light back. When white light falls on an object, a part of it is absorbed and rest is transmitted. These transmitted components add up to give the observed colour of the object. The absorbed component and the observed colour can again add up to give white light. These are therefore called as **complementary** to each other. An object of a particular colour looks of that colour because this colour is transmitted and its complementary colour is absorbed.

In order to select a suitable filter for a measurement of absorbance of a solution, we need to have an understanding of complementary colours. The colour of the filter should be the complementary to colour of the solution to be measured. If a solution appears orange, this implies that orange light is not being absorbed but that the complement to orange i.e. blue is being absorbed. Therefore, for measuring absorbance of a orange solution you would employ a blue filter. The use of such a filter is illustrated in Fig. 2.12.

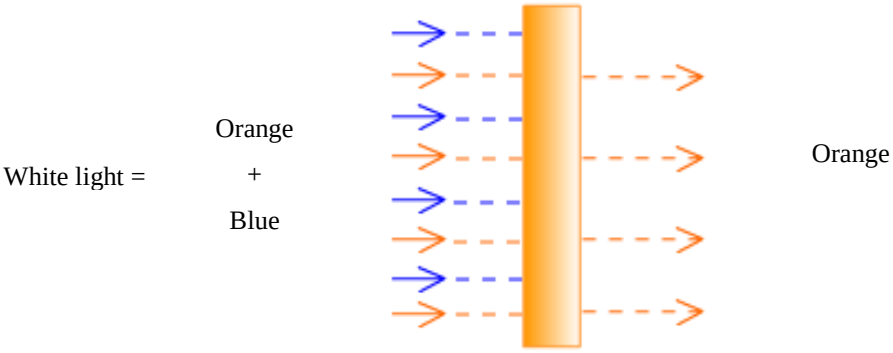


Fig. 2.12: Use of an absorption filter for the measurement of absorbance

Since these filters work by absorbing part of the radiation incident on them therefore they do not provide monochromatic light. In order to get a narrow band of wavelengths we can use certain filters which actually contain two glass filters; one filter absorbs strongly above a certain wavelength, while the second absorb strongly below a certain wavelength.

In order to find the colour of the filter to be used we can take the help of **colour wheel** shown in Fig. 2.13, or consult a table of complementary colours given in Table 2.2.

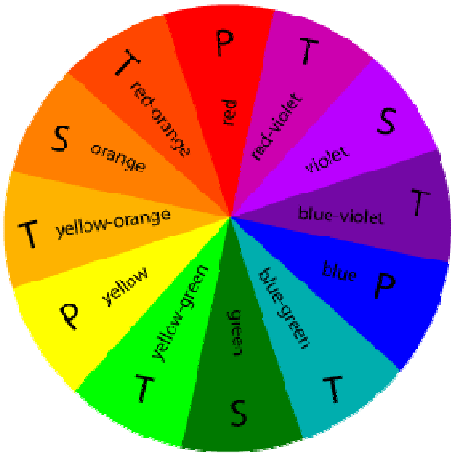


Fig. 2.13: The colour wheel

In a colour wheel, the colours, which face one another, are said to be complementary to each other. In order to measure a red coloured solution, its complementary, that is, a green coloured filter should be used. In the colour wheel P, S and T refer to the primary, secondary and tertiary colours, respectively.

Table 2.2: Complementary Colours Chart

Wavelength region eliminated by absorption (nm)	Colour absorbed	Complementary colour of the residual light (as seen by the eye)
400-450	violet	yellow-green
450-480	blue	yellow
480-490	green-blue	orange
490-500	blue-green	red
500-560	green	purple
560-580	yellow-green	violet
580-600	yellow	blue
600-650	orange	green –blue
650-750	red	blue –green

Absorption filters are simple and are totally adequate for many applications in visible range. However, for extended ranges we need **interference filters**. The interference filters cover a wider range than the absorption filters. Interference filters are essentially composed of two transparent parallel films of silver, which are so close as to produce interference effects. Such interference filters are available for ultraviolet, visible and near- infrared region. The performance characteristics of interference filters are significantly superior to those of absorption (coloured) filters. The effective bandwidths of these filters are narrower than absorption filters.

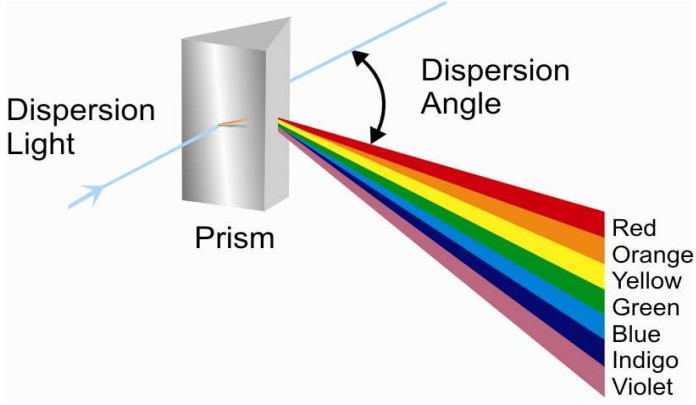
2.4.3 Monochromators

As mentioned above, monochromators are devices that can selectively provide radiation of a desired wavelength out of the range of wavelengths emitted by the source. These are of two types; the prism and grating monochromators. These are described in the following paragraphs.

Glass prisms and lenses can be used in the visible region. However, since glass absorbs ultraviolet light, a quartz or fused silica is a better choice for the material of the prism because it can be used in both the regions.

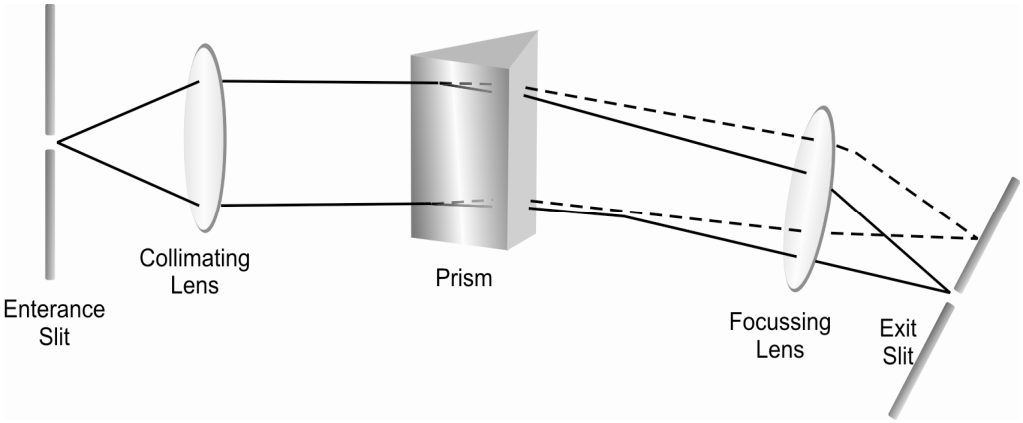
Prism Monochromators

You know that a prism disperses sunlight into seven different colours. This occurs due to the refraction of the light when it passes through the prism. The radiations of different colours having different wavelengths are refracted to different extent due to the difference in the refractive index of glass for different wavelengths. Shorter wavelengths are refracted more than longer wavelengths as depicted in Fig. 2.14 (a).



(a)

Fig. 2.14: a) Dispersion of radiation by prism



(b)

Fig. 2.14: b) Schematic diagram of the prism monochromator

If a prism is rotated, different wavelengths of the radiation, coming out after refracting through it, can be made to pass through the exit slit. In a **prism monochromator**, shown in Fig. 2.14 (b), a fine beam of the light from the source is obtained by passing through an entrance slit. This is then collimated on the prism with the help of a lens. The refracted beams are then focused on an exit slit. The prism is then rotated in a predetermined way to provide the desired wavelength from the exit slit.

Grating Monochromators

A grating is made by cutting or etching a series of closely spaced parallel grooves on the smooth reflective surface of a solid material as shown in Fig. 2.14 (a). The surface is made reflective by making a thin film of aluminium on it and the etching is done with the help of a suitably shaped diamond tool. The intensity of radiation reflected by a grating varies with the wavelength, the wavelength of maximum intensity being dependent on the angle from which the radiation is reflected from the surface of the line of the grating as shown in Fig. 2.15.

The gratings used for the ultraviolet and visible region generally contain about 1200-1400 grooves/mm.

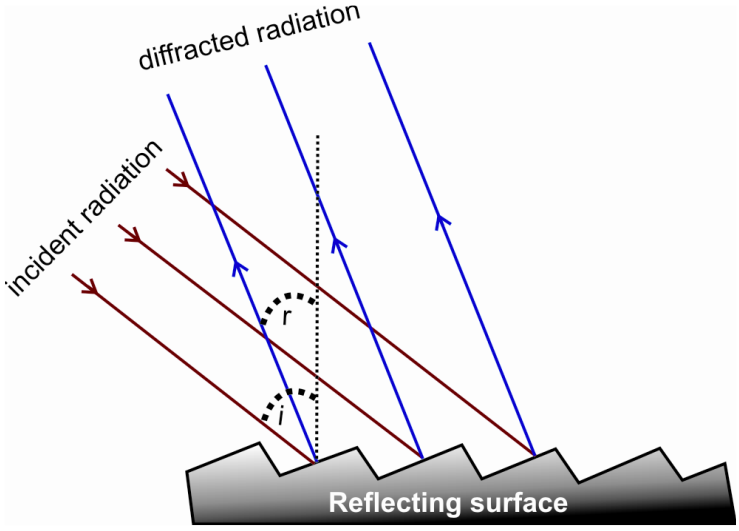


Fig. 2.15: Diffraction of Radiation by a grating

In grating monochromator (Fig. 2.16), a fine beam of the light from the source falls on a concave mirror through an entrance slit. This is then reflected on the grating which disperses it. The dispersed radiation is then directed to an exit slit. The range of wavelengths isolated by the monochromator is determined by the extent of dispersion by the grating and the width of the exit slit. Rotation of the grating in a predetermined way can be used to obtain the desired wavelength from the exit slit.

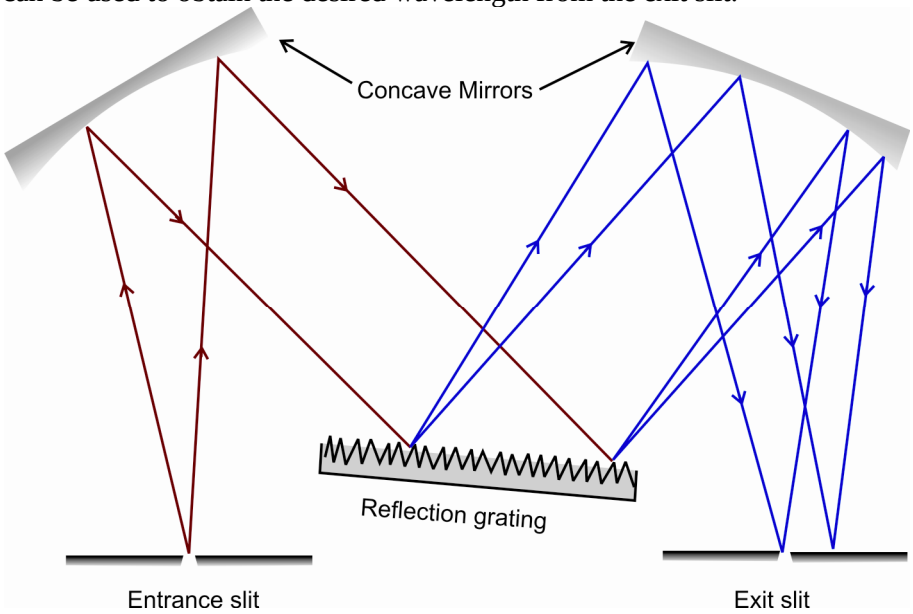


Fig. 2.16: Schematic diagram of a grating monochromator

2.4.4 Sample Handling

The UV-VIS absorption spectra are usually determined either in vapour phase or in solution. In order to take the UV spectrum of the analyte it is taken in a cell called a **cuvette** which is transparent to the wavelength of light passing through it. A variety of quartz cuvettes are available for the spectral determination in the vapour phase. These are of varying path lengths and are equipped with inlet and outlets. For measurements on solutions in the visible region the cuvettes made of glass can also be used. However, since glass absorbs the ultraviolet radiations, these cannot be used in the UV region. Therefore, most of the spectrophotometers employ quartz cuvettes (Fig 2.17), as these can be used for both visible and UV region. Usually square cuvettes having internal path length 1.0 cm are used, though cuvettes of much smaller path lengths say of 0.1 mm or 0.05 mm are also available.

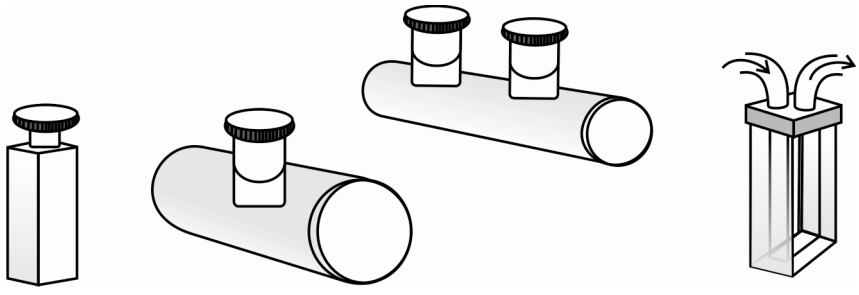


Fig. 2.17: Quartz cuvettes for measurements in solution and in vapour phase

The sample whose spectrum is to be measured is dissolved in a solvent that is transparent in the UV region. It means that it does not absorb in this region. Hexane, 95% ethanol, methanol and 1,4 dioxane are commonly employed as solvents. Now a days 'spectral grade' solvents are available which have high purity and have negligible absorption in the region of absorption by the chromophore.

The eye of a common person is quite sensitive to notice differences in radiant power transmitted through two coloured solutions. We can say that eye is a natural photosensitive detector in the visible range. Therefore, early instruments used eye or photographic plate as the detector.

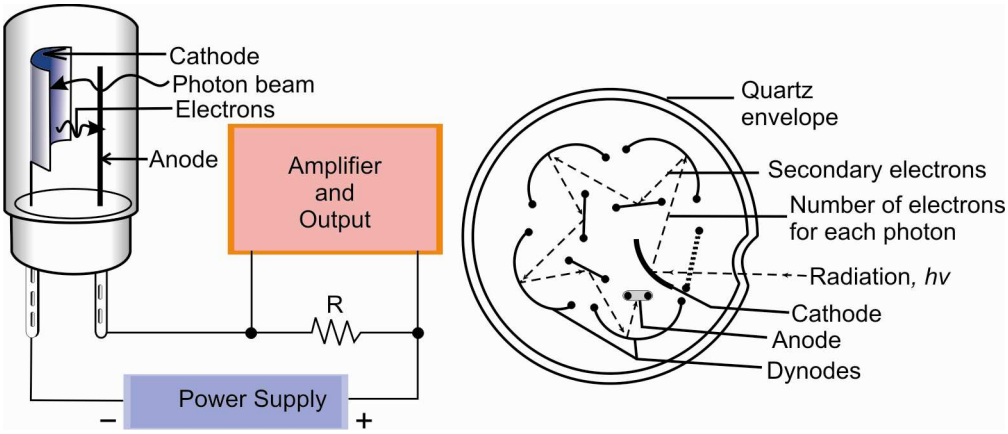
In a typical measurement of a UV spectrum, the solution of the sample is taken in a suitable cuvette and the spectrum is run in the desired range of the wavelengths. The absorption by the solvent, if any, is compensated by running the spectrum for the solvent alone in the same or identical cuvette and subtracting it from the spectrum of the solution. This gives the spectrum only due to the absorption species under investigation. In double beam spectrometers, the sample and the solvent are scanned simultaneously (subsection 2.5.2).

2.4.5 Detectors

The detectors are used to convert a light signal to an electrical signal which can be suitably measured and transformed into an output. The detectors used in most of the instruments generate a signal, which is linear in transmittance i.e. they respond linearly to radiant power falling on them. The transmittance values can be changed logarithmically into absorbance units by an electrical or mechanical arrangement in the signal read out device. There are three types of detectors which are used in modern spectrophotometers. These are described in the following paragraphs.

1. Phototube

A phototube consists of a photoemissive cathode and an anode in an evacuated tube with a quartz window as shown in Fig. 2.18 (a). These two electrodes are subjected to high voltage (about 100 V) difference. When a photon enters the tube and strikes the cathode, an electron is ejected and is attracted to the anode resulting in a flow of current which can be amplified and measured. The response of the photoemissive material is wavelength dependent and different phototubes are available for different regions of the spectrum.



(a) (b)

Fig. 2.18: Detectors of UV-VIS radiation; a) Phototube and b) Photomultiplier tube

2. Photomultiplier (PM) Tube

A photomultiplier tube (Fig. 2.18 (b)), consists of a series of electrodes, called **dynodes**. The voltage of successive electrodes is maintained 50 to 90 volt more positive than the previous one. When a radiation falls on the cathode an electron is emitted from it. This is accelerated towards the next photoemissive electrode by the potential difference between the two. Here, it releases many more secondary electrons. These, in turn are accelerated to the next electrode where each secondary electron releases more electrons. The process continuous upto about 10 stages of amplification. The final output of the photomultiplier tube gives a much larger signal than the photocell.

3. Diode Array Detector

These detectors employ a large number of silicon diodes arranged side by side on a single chip. When a UV-VIS radiation falls on the diode, its conductivity increases significantly. This increase in conductivity is proportional to the intensity of the radiation and can be readily measured. Since a large number of diodes can be arranged together, the intensity at a number of wavelengths can be measured simultaneously. Though the photodiode array is not as sensitive as the photomultiplier tube, the possibility of being able to measure a large number of wavelengths makes it a detector of choice in the modern fast instruments.

2.4.6 Signal Processing and Output Devices

The electrical signal from the transducer is suitably amplified or processed before it is sent to the recorder to give an output. The subtraction of the solvent spectrum from that of the solution is done electronically. The output plot between the wavelength and the intensity of absorption is the resultant of the subtraction process and is characteristic of the absorbing species.

Having learnt about different components of UV-VIS instruments and their importance; you are now equipped to learn about the types of instruments used. But before proceeding further, try to answer the following SAQ.

SAQ 5

In what way a diode array detector is better than a photomultiplier tube?

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2.5 TYPES OF UV-VISIBLE SPECTROMETERS

Having learnt the basics of the different components of UV-Visible spectrometers let us now see how these individual components are arranged in a spectrometer. Broadly speaking there are three types of spectrometers. Let us learn about their set ups.

2.5.1 Single Beam Spectrometers

The instruments using filters and photoelectric detectors are called photometers.

As the name suggests, these instruments contain a single beam of light. The same beam is used for reading the absorption of the sample as well as the reference. The schematic diagram of a typical single beam UV- Visible spectrometer is given in Fig. 2.19. The radiation from the source is passed through a filter or a suitable monochromator to get a band or a monochromatic radiation. It is then passed through the sample (or the reference) and the transmitted radiation is detected by the photodetector. The signal so obtained is sent as a read out or is recorded.

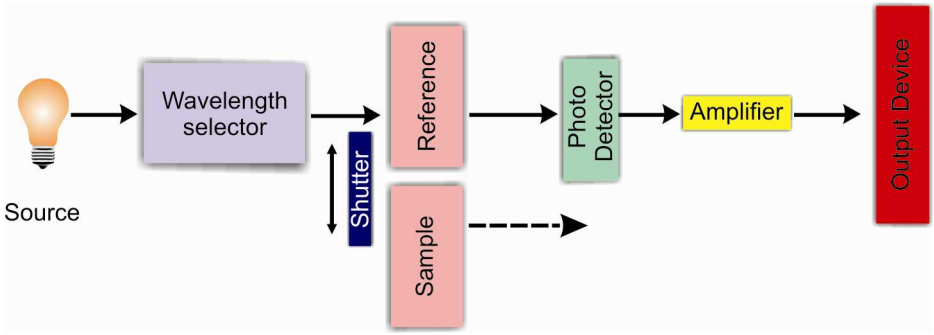

Fig. 2.19: Schematic diagram for a single beam UV-VIS spectrometer

Typically, two operations have to be performed – first, the cuvette is filled with the reference solution and the absorbance reading at a given wavelength or the spectrum over the desired range is recorded. Second, the cuvette is taken out and rinsed and filled with sample solution and the process is repeated. The spectrum of the sample is obtained by subtracting the spectrum of the reference from that of the sample solution.

2.5.2 Double Beam Spectrometers

In a double beam spectrometer, the radiation coming from the monochromator is split into two beams with the help of a beam splitter. These are passed simultaneously through the reference and the sample cell. The transmitted radiations are detected by the detectors and the difference in the signal at all the wavelengths is suitably amplified and sent for the output.

The general arrangement of a double beam spectrometer is shown in Fig. 2.20. There could be variations depending on the manufacturer, the wavelength regions for which the instrument is designed, the resolutions required etc.

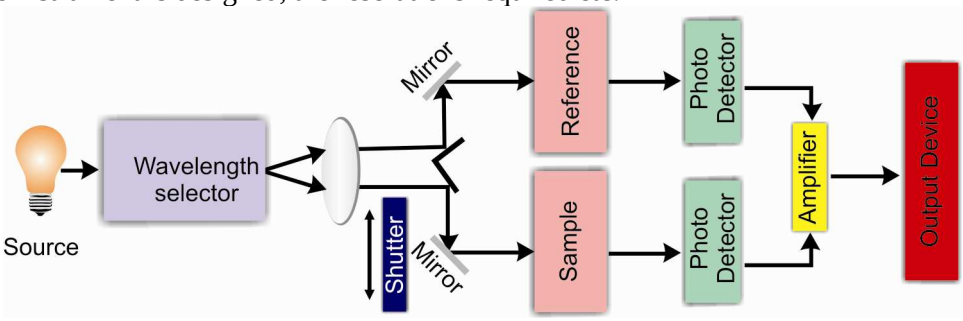

Fig. 2.20: Schematic diagram for a double beam spectrometer

2.5.3 Photodiode Array Spectrometer

In a photodiode array instrument, also called a multi-channel instrument, the radiation output from the source is focused directly on the sample. This allows the radiations of all the wavelengths to simultaneously fall on the sample. The radiation coming out of the sample after absorption (if any) is then made to fall on a reflection grating. The schematic arrangement of a diode array spectrometer is given in Fig. 2.21.

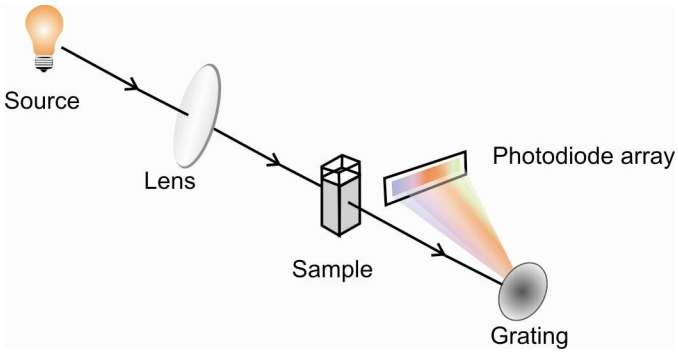

Fig. 2.21: Schematic diagram for a photodiode array spectrometer

The grating disperses all the wavelengths simultaneously. These then fall on the array of the photodiodes arranged side by side. In this way the intensities of all the radiations in the range of the spectrum are measured in one go. The advantage of such instruments is that a scan of the whole range can be accomplished in a short time.

SAQ 6

What colour absorption filter should be used to measure each of the following absorptions? (You may use Table 2.2 to answer this question.)

a) Iron salicylate, λ_{max} = 530 nm

b) Ti (H_2O_2), λ_{max} = 410 nm

c) Glucose (*o*-toluidine), λ_{max} = 625 nm.

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It was mentioned earlier that UV-VIS spectrometry can be used for qualitative and quantitative determinations of the analyte. The quantitative determinations are based on the relationship between the absorption of radiation and concentration of the analyte. Let us learn about this relationship and other applications.

2.6 ANALYTICAL APPLICATIONS OF UV-VISIBLE SPECTROMETRY

UV-VIS spectrometry provides a technique that may be used to detect one or more components in a solution and measure the concentration of these species. The primary advantage of this technique is that even traces of substances can be determined in a simple way which is not possible with classical analytical methods like gravimetric and volumetric procedures. In addition, UV spectrometry is also used for obtaining structural information of substances; particularly the organic compounds and it may as well help in establishing the identity of a molecule. We will discuss some of qualitative and quantitative applications of UV-VIS spectrometry.

2.6.1 Qualitative Applications

In terms of qualitative analysis of the analyte, the UV-VIS spectrometry is of a secondary importance for the identification and the determination of structural details. The information obtained from it needs to be supplemented by that from IR, NMR and mass spectrometries. This is because of simple and broad nature of the spectra. Nonetheless, it can still provide information about the presence or absence and the nature of the chromophore in the molecule. For example, the presence of one or more signals in the 200-400 nm region of the spectrum is a clear indication of the presence of unsaturation in the molecule.

Though lack of fine structure in the UV-visible spectrum does not allow unambiguous identification of the molecule, yet sometimes the spectrum can be quite useful in distinguishing two molecules having similar colour. For example, Fig. 2.22 gives the visible spectra of two purple coloured species of distinctly different structures. One of the spectra happens to be of an azo dye and the other is of permanganate solution. The shapes of the absorption curves help in distinguishing; the smooth curve with a single maximum is for the dye.

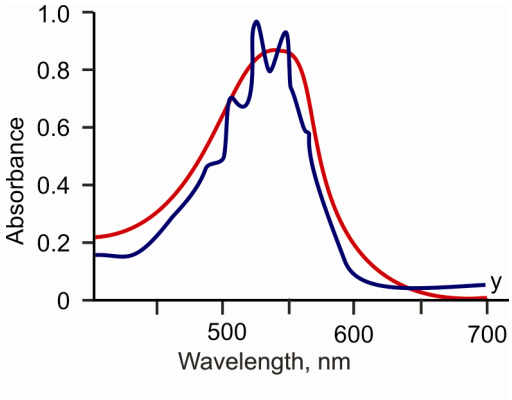

Fig. 2.22: Absorption spectra of two distinctly different compounds but with similar colour

Historically speaking, the UV spectroscopy has played an important role in the study of a wide array of natural products of plant and animal origin. UV spectrometry was used by Woodward to assign chemical structures of closely related compounds; differing in only the location of a carbonyl group or a carbon-carbon double bond. In fact extensive studies of the UV spectra of a large number of molecules led to the establishment of empirical rules for calculating the λ_{max} for organic molecules. The rules proposed by Fieser, Woodward and Scott could be used to predict to a reasonable degree of accuracy the λ_{max} of unsaturated organic compounds. The details of these rules and their applications can be obtained from any book on organic spectroscopy.

The changes in spectra due to the changes in the pH of the solution or the solvent can provide useful information about the nature of the analyte. The change in the polarity of the solvent alters the energies of the orbitals. This leads to change in the absorption maxima. On increasing the polarity of the solvent, the $n \rightarrow \pi^*$ transitions are shifted to lower wavelengths while the $\pi \rightarrow \pi^*$ transitions are shifted to longer wavelengths. This helps in the identification and distinction of two closely associated molecules.

2.6.2 Quantitative Applications

As discussed above, UV-VIS spectrometry has limited applications in qualitative analysis of the analytes; however, it is probably the most useful tool available for quantitative determinations in diverse areas. It is due its versatility, accuracy and sensitivity. It can be used for direct determination of a large number of organic, inorganic and biochemical species accurately at fairly low concentrations; viz., 10^{-4} to 10^{-5} or even lower. In addition to these, the convenience of conducting the determination and its reasonable selectivity make it a method of choice for quantitative determinations.

Another significant feature of this technique is that it may be used for the quantitative determination of analyte which do not absorb in the UV-VIS region. It is achieved by making them react with a reagent that gives a product which absorbs in the region. The same technique can be used to undertake quantitative determination of all such species which have an absorption in the region but are present in system which contain other constituents that also absorb in the same region. Thus, absorbance measurements in visible or in UV region, are used in diverse areas. Some of the common ones are related to the following quantitative aspects of solution chemistry.

1. Analytical determination of metals and non-metals
2. Analytical determination of organic compounds
3. Determination of dissociation constants of organic acids and dyes
4. Determination of metal-ligand formation constants
5. Determination of kinetic stability of complexes

The basis for the first two aspects of quantitative analysis lies in the Beer and Lambert's law discussed earlier. It involves comparing the extent of absorption of radiant energy at a particular wavelength by a solution of the test material with a series of standard solutions. In the earlier times, visual comparators were used for such determinations which were carried out with natural light (white light) and employed human eye to judge the colour intensity. In such an approach, a series of standard solutions of the analyte to be determined were prepared in tubes of constant depth and diameter. The sample solution was transformed to a duplicate tube and diluted to the extent that its colour matched with one of the standard solutions. The concentration of the test solution was determined from the concentration of the standard solution by accounting for the dilution factor.

The rest three aspects of solution chemistry require measurements of the UV-visible spectrum of the analyte as a function of pH or ligand concentration etc. Today, a given analytical determination can be made in a number of possible ways. These methods depend on the nature of the species being determined and follow a kind of methodology. Let us learn about the methodology of quantitative determinations.

2.6.3 Quantitative Determination Methodology

The methodology followed for the quantitative determinations have certain essential steps. These are,

- Forming an absorbing species
- Selection of the measurement wavelength
- Controlling factors influencing absorbance
- Validation of Beer and Lambert's law

Let us briefly discuss these one by one.

Forming an Absorbing Species

There are only a few analytes that have a strong absorption in the UV or Visible region and can be subjected to direct determinations. However, for most of the analytes we have to form a absorbing species by reacting them with a suitable reagent. In any case, the absorbance of the solution should be stable and should not change with minor variations in the pH, temperature and the ionic strength of the solution. Further, the reagent should be selective towards the analyte and its reaction with the analyte should be quantitative i.e. 100%.

Selection of the Measurement Wavelength

The wavelength chosen for quantitative work is called the analytical wavelength.

In the absence of interfering substances, the measurements are made at the wavelength of the maxima (λ_{max}) of the largest peak in the spectrum. At this wavelength the absorbance is most sensitive to the concentration. Further, as in many cases there is broadening of peak so within certain interval of wavelength near the λ_{max}, there is no appreciable change in the value of absorbance i.e. the measurement is not very sensitive to the wavelength. This is quite advantageous because even if the spectrophotometer fails to resolve λ_{max}, the determination does not suffer.

Ordinarily the absorbance should be measured at a wavelength where there is no interference from other absorbing species in the solution.

However, in systems where the reagent, metal and products all absorb light, it may so happen that at λ_{max} there is not much of difference in the absorbance value for pure reagent and the metal complex. In such cases we need to identify such a wavelength at which there is large difference in the absorbance values of pure reagent and the complex.

Controlling Factors Influencing Absorbance

As discussed earlier, a number of factors can affect the absorption spectrum of the analyte. These include solvent polarity, pH, temperature, ionic strength and interferences from other absorbing species. It is therefore, essential for a reliable quantitative determination that the conditions of the determination are so chosen that there is minimal effect of these factors.

Validation of Beer and Lambert's law

Let us rewrite the expression for the Beer and Lambert's law as

$$\log P_o / P = A = abc \quad \text{or} \quad \varepsilon bc$$

According to this expression, if we know the values of a (or ε) and b then we can determine the concentration directly from the absorbance value. However, generally the value of ε is not known accurately for the species being determined under the conditions of the determination. Quite often even the validity of the Beer and Lambert's law expression is questionable. Therefore in most of the methods, a calibration curve is obtained by measuring the absorbance values for a series of standard solutions of the analyte being determined at a fixed wavelength. These solutions should be under similar solution conditions and in the range of the concentration of the analyte. For the law to be valid, the plot of absorbance, A versus the concentration, c for the standard solutions should be a straight line passing through origin.

Such a curve is known as **calibration curve** and is handy in determination of concentration of unknown solutions from a measurement of absorbance value. For example, the unknown concentration of a solution of iron can be determined by using a calibration curve obtained by plotting the absorbance values of a series of standard solutions of iron measured at 562 nm depicted in Fig. 2.23 (a).

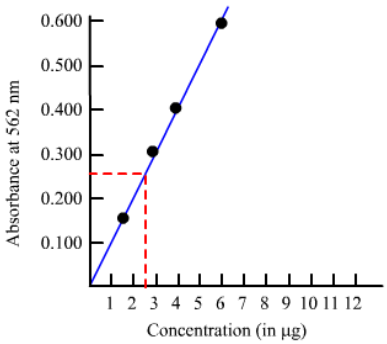

Fig. 2.23: Calibration curve: a) Standard solution method

Sometimes, it may so happen that all the constituents in the sample may not be known; so a standard solution with the same chemical composition cannot be prepared. In such a case, a method called **standard addition method** is used which eliminates any error arising from molar absorptivities (ε) being different in the standard solution from that in the sample solution. In this method known amounts of the standard is added to identical aliquots of the sample and the absorbance is measured. The first reading is the absorbance of sample alone and the second reading is absorbance of sample containing analyte plus, a known amount of analyte and so on. The readings so obtained are then plotted to obtain the calibration curve. If the Beer's law is obeyed, i.e. a straight line is obtained; the unknown concentration of the solution can then be obtained by the extrapolation of the calibration curve as shown in Fig. 2.23 (b).

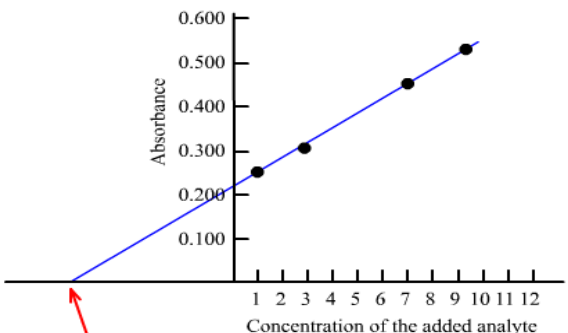

Fig. 2.23: Calibration curve: b) Standard addition method

2.6.4 Simultaneous Determination

Sometimes we come across a situation wherein the analyte contains two species which have overlapping spectra. In order to determine these species we need to find two wavelengths where molar absorptivity of two species is different. In such a case, measurements are made on the solution of the analytes at two different wavelengths. This gives a set of simultaneous equations which could be solved for the concentrations of the individual constituents. For best results it is desirable to select two such wavelengths where the ratio of molar absorptivities is largest. Consider the spectra given in Fig. 2.24.

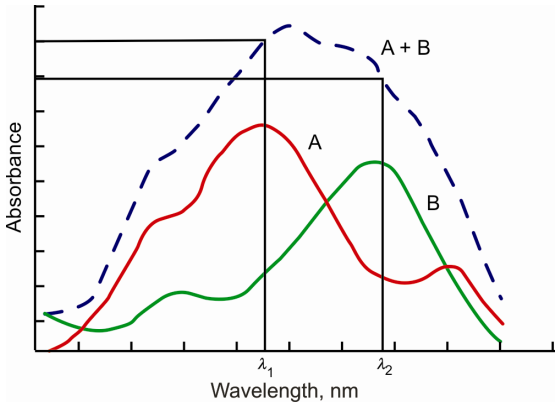

Fig. 2.24: Schematic UV spectra of analyte having two species with overlapping spectra

Let us select the wavelengths λ_1 and λ_2 on these two spectra. The molar absorptivity of spectra 1 and 2 can be found out by taking known amounts of the substance and recording their absorbance value. Let these values be $(\varepsilon_1)_{\lambda_1}$, $(\varepsilon_2)_{\lambda_1}$, $(\varepsilon_1)_{\lambda_2}$, and $(\varepsilon_2)_{\lambda_2}$. If the absorbance of the solution at the wavelength λ_1 and λ_2 are A_{λ_1} and A_{λ_2} respectively then we can write, for 1 cm path,

$$A_{\lambda_1} = C_1(\varepsilon_1)_{\lambda_1} + C_2(\varepsilon_2)_{\lambda_1}$$

$$A_{\lambda_2} = C_1(\varepsilon_1)_{\lambda_2} + C_2(\varepsilon_2)_{\lambda_2}$$

The above two simultaneous equations could be solved for C_1 and C_2. The expressions are found to be.

$$C_1 = \frac{A_{\lambda_1}(\varepsilon_2)_{\lambda_2} - A_{\lambda_2}(\varepsilon_2)_{\lambda_1}}{(\varepsilon_1)_{\lambda_1}(\varepsilon_2)_{\lambda_2} - (\varepsilon_2)_{\lambda_1}(\varepsilon_1)_{\lambda_2}}$$

$$C_2 = \frac{A_{\lambda_2} - (\varepsilon_1)_{\lambda_2} C_1}{(\varepsilon)_{\lambda_2}}$$

Let us take an example to understand it.

Example

The absorbance values of a mixture of $K_2Cr_2O_7$ and $KMnO_4$ at 440 nm and 545 nm using a cell of 1 cm path length were found to be 0.405 and 0.712 respectively. The absorbance values of pure solutions of $K_2Cr_2O_7$ (0.001 M) and $KMnO_4$ (0.0002 M) in similar conditions were as follows:

A (Cr,440 nm) = 0.374, A (Cr, 545 nm) = 0.009
A (Mn, 440nm) = 0.019, A (Mn, 545 nm) = 0.475

Using the given data determine the concentration of $Cr_2O_7^{2-}$ ions and MnO_4^- ions in the mixture.

Solution: Using the given absorbance values for the pure solutions, we can calculate the molar absorptivities as for Cr and Mn at 440 and 545 nm as follows:

$0.374 = \varepsilon_{Cr,\,440} \times 1.0 \times 1.0 \times 10^{-3}$ => $\varepsilon_{Cr,\,440} = 374$
$0.009 = \varepsilon_{Cr,\,545} \times 1.0 \times 1.0 \times 10^{-3}$ => $\varepsilon_{Cr,\,545} = 9$
$0.019 = \varepsilon_{Mn,440} \times 1.0 \times 2.0 \times 10^{-4}$ => $\varepsilon_{Mn,440} = 95$
$0.475 = \varepsilon_{Mn,545} \times 1.0 \times 2.0 \times 10^{-4}$ => $\varepsilon_{Mn,545} = 2.38 \times 10^3$

For the mixture we can write the simultaneous equations as

$$A_{440} = \varepsilon_{Cr,\,440}[Cr_2O_7^{2-}] + \varepsilon_{Mn,440}[MnO_4^-]$$
$$A_{545} = \varepsilon_{Cr,\,545}[Cr_2O_7^{2-}] + \varepsilon_{Mn,545}[MnO_4^-]$$

Substituting the values, we get

$$0.405 = 374\,[Cr_2O_7^{2-}] + 95\,[MnO_4^-]$$
$$0.712 = 9[Cr_2O_7^{2-}] + 2.38 \times 10^3[MnO_4^-]$$

Solving simultaneous equations, we get

$[Cr_2O_7^{2-}] = 1.0 \times 10^{-3}$ mol dm^{-3}
$[MnO_4^-] = 2.95 \times 10^{-4}$ mol dm^{-3}

Let us conclude our section on applications by leaving the following SAQ for you to solve.

SAQ 7

What is the advantage of the standard addition technique as compared to a calibration curve method?

……...
……...
……...
……...
……...

2.7 SUMMARY

UV-VIS spectrometry is an instrumental technique that provides ideal methods for the determination of micro and semi-micro quantities of analytes in a sample. The UV-VIS spectrum arises due to the transitions amongst the electronic energy levels of the absorbing species. For the sample in gaseous phase, the spectrum consists of a number of closely spaced lines whereas in the solution phase, the spectrum has the shape of a smooth and continuous absorption peak. This is due to the collisions and the

interactions among the absorbing species and the solvent molecules. A typical UV spectrum is a plot of wavelength versus the intensity of absorption and is characterized by two major parameters-the position of the maximum of the absorption band (λ_{max}) and the intensity.

There are three types of species that absorb in the UV-VIS region. These are organic molecules, inorganic salts containing the ions of the transition and inner transition elements and the species that involve formation of charge transfer complexes. The relationship of the absorption with the concentration and the path length of the absorbing sample is expressed in terms of Beer-Lambert's law. This law forms the basis of quantitative determinations of the analyte. A number of factors like pH, concentration of electrolytes, wavelength, the possibility of association and dissociation etc may cause deviations from the law. These need to be considered in such determinations.

There are five essential components of UV-visible instruments. These are: radiation source, wavelength selector, sample holder, detector and a signal processing and output device. For UV region a deuterium lamp is used as the source whereas for visible range a tungsten filament lamp is employed. The wavelength selection is achieved by absorption filters in low cost instruments for visible region. In the modern instruments this is done by using suitable monochromators. The sample is taken in quartz cuvettes, though for visible region even glass cells can be used. The detection of the transmitted radiation is done generally by a phototube or a photomultiplier tube. Nowadays, the modern machines employ diode arrays for detection purposes. These components are assembled into single beam, double beam and diode array spectrometers.

UV-VIS spectrometry can be used to detect one or more components in a solution and measure the concentration of these species. In addition, it may also used for obtaining structural information of substances; particularly the organic compounds and it may as well help in establishing the identity of a molecule. The most important qualitative application of UV-VIS spectrometry is to detect the presence of unsaturation in the molecule. In addition, the changes in spectra due to the changes in the pH of the solution or the solvent can provide useful information about the nature of the analyte. On the other hand, due to its versatility, accuracy and sensitivity UV-VIS spectrometry can be used for direct determination of a large number of organic, inorganic and biochemical species accurately at fairly low concentrations of the order of 10^{-4} to 10^{-5} M or even lower. The quantitative determinations follow a general methodology involving four steps as, identifying or forming an absorbing species, selection of the measurement wavelength, controlling factors that influence absorbance and validation of Beer and Lambert's law.

2.8 TERMINAL QUESTIONS

1. The UV-VIS spectra in the solution phase have band structure whereas in the vapour phase we get sharp lines. Explain.

2. The *d-d* transitions are forbidden transitions so most of the transition metal ions produce very weak bands in aqueous solution with various ligands. How do you account for the intense blood red colour produced in a solution of Fe (III) ion when the ligand like thiocyanate is added?

3. Outline the factors that may cause deviations from Beer and Lambert's law.

4. The absorbance of a solution of ferrous 1, 10-phenanthroline complex containing 1.00 microgram of iron (II) in 10 cm^3 of solution was measured at 515 nm in 1 cm cuvette. It was found to be 0.0200. Calculate the molar absorptivity of the complex.

5. What are monochromators? Describe the functioning of a grating monochromator.

6. Why do we need to take UV spectra in quartz cuvettes?

7. Enlist the essential steps of quantitative determination methodology.

8. What is the principle of the photomultiplier tube?

9. A mixture of Ni^{2+} and Co^{2+} is analyzed by forming the salicyaldoxime complexes in chloroform solvent. The molar absorptivities for Ni and Co ions at 500 nm are, 10 and 1000 respectively. However, at 400 nm both molar absorptivities are $5.0 \times 10^3\ cm^{-1}mol^{-1}dm^3$. The absorbances were recorded using 1 cm cuvette and were found to be 0.091 at 500 nm and 0.615at 400 nm. Calculate the concentrations of Ni^{2+} and Co^{2+} in the given mixture.

2.9 ANSWERS

Self Assessment Questions

1. Different types of species absorbing in the UV-VIS region and type of transition occurring in them are as follows

Species	Type of Transition
Organic molecules	$n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$
Inorganic molecules	*d-d* or *f-f* transitions
Charge Transfer complexes	Donor HOMO to acceptor LUMO

2. $P_o = 85.4$ $b = 2.00cm$

   $P = 20.3$ $c = 1 \times 10^{-4}$ M ϵ =?

   A = log P_o/P = A = log 85.4/20.3 = 0.624

   Since $A = \epsilon\, b\, c$; $\epsilon = A/b.c$

   Substantially the value $\varepsilon = \dfrac{0.624}{2.0\,\text{cm} \times 1 \times 10^{-4}\ \text{M}}$

   => 3120 $cm^{-1}mol^{-1}dm^3$

3. ϵ =14000 $cm^{-1}mol^{-1}dm^3$

   c = ? b = 1cm A= 0.85

   A = $\epsilon\, cb$ c = A/ $\epsilon.b$

   c = 0.85/14000 × b

   = 6.07×10^{-5} M

4. According to Beer's law there is a direct proportionality between the absorption and concentration of the absorbing species taken in a cuvette of unit path length. It implies that a plot of concentration versus absorption is a straight line. In order to use this property for quantitative determination of the concentration of the absorbing species there should not be any deviation from the linearity. It therefore becomes important to know about the factors that may cause deviation so that these could be suitably controlled.

5. A diode array detector is better than a photomultiplier tube as it allows simultaneous determination of the intensity of a number of electromagnetic radiations of different wavelengths.

6. i) purple
   ii) yellow-green
   iii) green-blue

7. The standard addition method allows the determination of the concentration of the species in such solution also in which the other constituents are not fully known.

Terminal questions

1. The UV-VIS spectra arise as a consequence of the interaction of the radiation with absorbing species causing a transition amongst their electronic energy levels. In case of gaseous samples, the absorbing species the spectrum consists of a number of closely spaced discreet lines that resemble a band and the resulting spectrum is called a band spectrum.
   In the solution phase the energies of the quantum states of the molecules are somewhat spread out due to constant collisions with the surrounding solvent molecules. Therefore the sample absorbs photons spread over a range of wavelength and the spectrum acquires the shape of a smooth and continuous absorption peak.

2. The intense red colour produced on addition of thiocyanate ion to Fe(III) ions is due to the charge transfer complex formed between them.

3. Some of the factors responsible for the deviation from Beer and Lambert's law are as follows.
   - Presence of Electrolytes
   - Hydrogen Ion Concentration
   - Complexation, Association or Dissociation
   - Non monochromatic nature of the radiation
   - Concentration of the Analyte
   - Temperature

4. The atomic weight of iron is 56, therefore the molar concentration of the solution containing 1.00 microgram of iron (II) present in 10 mL of solution will be

$$\frac{1\times 10^{-6}\,\text{g}}{56}\times\frac{1000\text{mL}}{10\text{mL}} = 1.786\times10^{-6}\ \text{mol per dm}^3.$$

$$\varepsilon = A/b.c \qquad \Rightarrow \qquad \frac{0.02}{1.786\ \text{mol dm}^{-3}\times 1\ \text{cm}}$$

$$= 1.12\times10^{4}\ \text{dm}^{-3}\ \text{mol}^{-1}\ \text{cm}^{-1}$$

5. A monochromator is a device that can selectively provide radiation of a desired wavelength out of the range of wavelengths emitted by the source. In grating monochromator, a fine beam of the light from the radiation source is made to fall on a concave mirror through an entrance slit. After reflection, it falls on the grating which causes it to disperse. The dispersed radiation is then emerges out of an exit slit. The range of wavelengths isolated by the monochromator is determined by the extent of dispersion by the grating and the width of the exit

slit. Rotation of the grating in a predetermined way used to obtain the desired wavelength from the exit slit.

6. In order to take the UV absorption spectra the analyte is taken in a quartz cuvette because it is transparent to the electromagnetic radiations of the UV range. As glass absorbs the ultraviolet radiations, these cannot be used for the purpose.

7. The essential steps of the quantitative determinations methodology are,
   - Forming an absorbing species
   - Selection of the measurement wavelength
   - Controlling factors influencing absorbance
   - Validation of Beer and Lambert's law

8. A photomultiplier tube is based on the principle of successive increase in the number of electrons. The output signal by photoemission from a series of electrodes called dynodes is much larger than the original signal.

9. We know that for simultaneous determination of two absorbing species the absorbance is measured at two different wavelengths. For measurements in a cuvette of 1 cm path length, the absorbances, concentrations and the extinction coefficients are related as:

$$A_{\lambda_1} = C_1(\varepsilon_1)_{\lambda_1} + C_2(\varepsilon_2)_{\lambda_1}$$

$$A_{\lambda_2} = C_1(\varepsilon_1)_{\lambda_2} + C_2(\varepsilon_2)_{\lambda_2}$$

With given data we can write as:

$A_{500\ nm}$ = $10 \times [Ni^{2+}] + 1000 \times [Co^{2+}] = 0.091$ ... (A)

$A_{400\ nm}$ = $5.0 \times 10^3 \times [Ni^{2+}] + 5.0 \times 10^3 \times [Co^{2+}] = 0.615$ ... (B)

Multiplying (A) by 5 and subtracting from (B) we get,

$4950\ [Ni^{2+}] = 0.615 - 0.455 = 0.160$

$[Ni^{2+}] = 0.160 / 4950 = 3.2 \times 10^{-5}$ mol dm^{-3}

Substituting it in (A) or (B) and solving we get

$[Co^{2+}] = 9.1 \times 10^{-5}$ mol dm^{-3}