Spectroscopy

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- Application of UV absorption Spectroscopy to Organic Compounds
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Objectives.

After going through the unit you would be in position

- to Understand the basic techniques of spectroscopy
- Know about the importance of spectroscopy
- To learn about elements of practical spectroscopy
- Getting the basic concepts of spectroscopic instruments like absorption and emission spectroscopic techniques
- know about physical basis and steps in AES
- To know the important applications of various techniques.

Introduction to Spectroscopy:

The Spectroscopic techniques form the wide range and most important techniques used in Chemistry and Physics.

These techniques provide a large range of qualitative and quantitative data. Most of the spectroscopic techniques are more or less depending on the emission or absorption of electromagnetic radiation of certain energy changes within an atomic or molecular system.

The changes in energy are associated with a complex series of discrete or quantized energy levels in which atoms or molecules are assumed to exist.

The use of spectroscopic techniques in analytical chemistry is in the determination atomic and molecular structure, including functional groups' photochemical process and stereochemical arrangements.

Various spectroscopic techniques are used currently because they require a very small amount of material and short interval of time for analysis.

Modern spectroscopy and its advantages form a significant part of modern chemistry, physics and technology. The word 'spectroscopy' means 'the watching of images', but the modern subject covers the interaction of electromagnetic radiations with matter.

The very important effect of such interaction is that energy is absorbed or emitted by the matter in discrete value called quanta.

The absorption or emission processes of the electromagnetic spectrum range from the gamma region (nuclear resonance absorption or the Mossbauer Effect) to the radio region (nuclear magnetic resonance).

When the measurement radiation frequency is done experimentally, its gives a value for the change of energy involved and one can draw the conclusion of studied about the set of possible discrete energy levels of the matter.

The experimental measurements of radiation frequency (emitted or absorbed) and the energy levels derived from these comprise the practice of spectroscopic techniques.

In many spectroscopy branches the system interacts with the electric field, but, in case of magnetic resonance spectroscopy it interacts with the magnetic field.

Spectroscopy is powerful technique available for the study of atomic and molecular structure and is used in the study of a wide range of samples to get the data. The study of spectroscopy technique can be carried out as follows:

(a) **Atomic Spectroscopy:** The interaction of electromagnetic radiation with atoms which are most commonly in their lowest energy state called the ground state.

Monoatomic substance normally exists in the gaseous state and is able to absorb electromagnetic radiation, leads to transitions of electrons from one electron energy level to another.

The electronic absorption of electromagnetic energy can occur only if the photon has an energy which is equal to the energy difference between two quantized energy levels, i.e., $\Delta E = h\nu$, where 'ΔE' is the energy different between two levels and 'ν' is the frequency of photon which can results in the electronic excitation. Applications of atomic spectroscopy in the field of physics and chemistry are few. However its demand has increased by the development of lasers.

(b) **Molecular Spectroscopy:** The interaction of electromagnetic radiation with molecules, these results in transitions between rotational and vibrational energy levels in addition to electronic transitions. Which gives, the spectra of molecules are much more completed than those of atoms.

Molecular information may be obtained about molecular vibrations and rotations that reveal a great deal about molecular structure.

The detailed information regarding molecular structure (molecular symmetry, bond distances and bond angles) and physicochemicals properties like electronic distribution, bond strength and intra-and inter-molecular processes are obtained from the atomic and molecular spectroscopy.

The spectroscopic investigation find were first carried out by Sir Issac Newton in the seventeenth century; the quantitative analysis was possible only after the intervesion of quantum mechanics. Initially, it was atomic spectra that provided the empirical evidence for the various quantum mechanical ideas.

Range of the spectrum

We have observed the regions into which electromagnetic radiations have been divided in and boundaries are not precise. The molecular process associated with each region is quite different and are noted along with the corresponding regions the brief explanation of the following few lines:

(i) Radio frequency range $3x10^6$ to $3x10^{10}$ c/s; 10 cm to 1 cm wavelength, Nuclear magnetic resonance and electron spin resonance spectroscopy, reversal of spin of a nucleus or electron is the cause of energy change.

(ii) Microwave region: $3x10^{10}$ to $3x10^{12}$ c/s; 1 cm to 100 μm wavelength (Rotational spectroscopy).

(iii) Infrared region: $3x10^{12}$ to $3x10^{14}$ c/s; 100 μ to 1 μ wavelength (Vibrational spectroscopy).

(iv) Visible and ultra-violet region: $3x10^{14}$ to $3x10^{16}$ c/s; 1 μ to 100 Å wavelengths (Electron spectroscopy).

(v) X-ray region: $3x10^{16}$ c/s; and above; 100 Å wavelength.

(c) **Near Infra-red Spectra.** In the absence of any of the electronic transition (no change in electronic state) vibrational transition can occur, i.e. there is a possibility of a transition from one vibrational energy level to another with the same electronic (ground) state of the molecules involving in energy changes of about 0.1 eV ($1.6X10^{-20}$erg/mole) ; frequency of radiation will be

$$\nu = \frac{1.6X10^{-20}}{3X10^{8}\,6.6X10^{-34}} = 8X10^{4}\ \mathrm{m}^{-1\nu} \qquad \text{or} \qquad \text{-} = 125000\text{Å} = 12.5\mu$$

This observation shows these radiations accompanying vibrational changes within the electronic ground state, should appear in near infra-red portion of the spectrum.

The vibrational transition within particular electronic state would be associated with different possible rotational changes which results to the presence of a number of closely spaced spectral lines (vibration rotation bands).

These are observed in absorption in the wavelength region of about 1 to 20μ.

For a diatomic molecule, the mode of vibration is only one hence only one vibration-rotation band for each vibrational transition.

(d) Far infra-red Spectra. In the far infra-red region, transitions occur that are purely rotational in character, unaccompanied by changes in electronic and vibrational energies. Such a transition involves an energy change of 0.005 eV and thus frequency of radiation is

$$\lambda = \frac{19.8}{800}\ \text{cm.} = 247\text{x}10^{-2}\ \text{cm} = 2470000\ \text{Å} = 247\ \mu.$$

It is observed that these radiations will lie in far infra-red region. The bands in the far infra-red regions are therefore called rotational spectra.

Importance of Spectroscopy

The electromagnetic spectrum is one of the most widely used techniques of analysis. It is used in all branches of chemistry and environmental laboratories because many substances can be selectively converted to a colored derivative. The technique is readily available and generally fairly easy to study.

(i) it will explain the absorption of radiation by molecules and its relation to molecular structure;

(ii) make accurate calculations, relating the amount of energy absorbed to the concentration of an absorbing analyze; and

(iii) explain the instrumentation required for making data. Data can be made in the infrared, visible, and ultraviolet regions of the spectrum.

The radiation energy region of choice will depend upon the availability of instruments, whether the analyte is coloured or can be converted to a coloured derivative, whether it contains functional group that absorbs the ultraviolet or infrared region and whether other absorbing species are present in the solution.

IR technique is generally less suited for quantitative measurements but better suited for qualitative or fingerprinting information than are ultraviolet and visible technique.

Visible optical technique is generally less expensive and more available than UV spectroscopy.

Table 1: Spectroscopic techniques, their principle and applications.

Name of Technique	Principle	Applications
arc /spark spectroscopy or spectrography plasma emission spectrometry flame photometry	Atomic emission	Qualitative and quantitative determination of metals, largely as minor or trace constituents quantitative determination of metals as minor or trace constituents
X-ray fluorescence Spectroscopy	atomic fluorescence emission	Qualitative and quantitative Determination of elements heavier than nitrogen as trace to major constituents.
atomic fluorescence spectroscopy	atomic fluorescence emission	Quantitative determination of metals as minor or trace constituents
atomic absorption spectroscopy γ-spectroscopy	atomic absorption nuclear emission	qualitative and quantitative determination of elements at trace levels
ultraviolet spectroscopy Visible spectroscopy Infrared spectroscopy	molecular absorption	Quantitative determination of elements and compounds, mainly at trace levels quantitative determination of elements and compounds mainly as trace minor constitutes.
Nuclear magnetic resonance spectroscopy mass spectroscopy	nuclear absorption structural fragmentation	Identification and structural analysis of organic compounds identification and structural analysis of organic compounds.

Elements of Empirical Spectroscopy:

The spectroscopic methods used in various regions of the spectrum naturally differ widely from each other in construction. These experimentally observed differences will be discussed, but here it will probably be helpful to indicate the basic ideas which are common to all types of spectroscopic techniques separately.

1. Absorption techniques: The block diagram shows the components of an absorption spectrometer which might be used in the IR, visible, and ultra-violet regions.

The radiation energy from a white source is incident on the sample, and then it is connected to analyzer. This leads to the required frequency reaching the detector at the time interval.

The signal later pass to a recorder which is connected to analyzer so as to get a trace of the absorbance as the frequency (energy) varies.

The sample and the analyzer is placed in between a modulator; this electronic device interrupts the radiation beam a certain number of times per second, usually fixed somewhere between 10 and 1000 times, and this detector sends an alternating current signal to the recorder with a fixed frequency of 10-1000 Hz.

The two main advantages of this techniques are (a) the amplifier in the recorder can be of a.c type i.e, simpler to construct and more reliable in operation than a d.c amplifier and

(b) the amplifier can be tuned to select only that frequency which the modulator imposes on the signal, neglecting all other signals. By this method other extraneous signals are removed from the spectral trace and a better, accurate spectrum results.

In the microwave and radiofrequency ranges the monochromatic sources whose emission frequency can be varied over a range are to be selected. Fig 1(b) shows, no analyzer is necessary, the source being, in a sense, its own analyzer.

Now it is necessary for the recorder to be synchronized with the source –scanning device in order that a spectral trace be obtained.

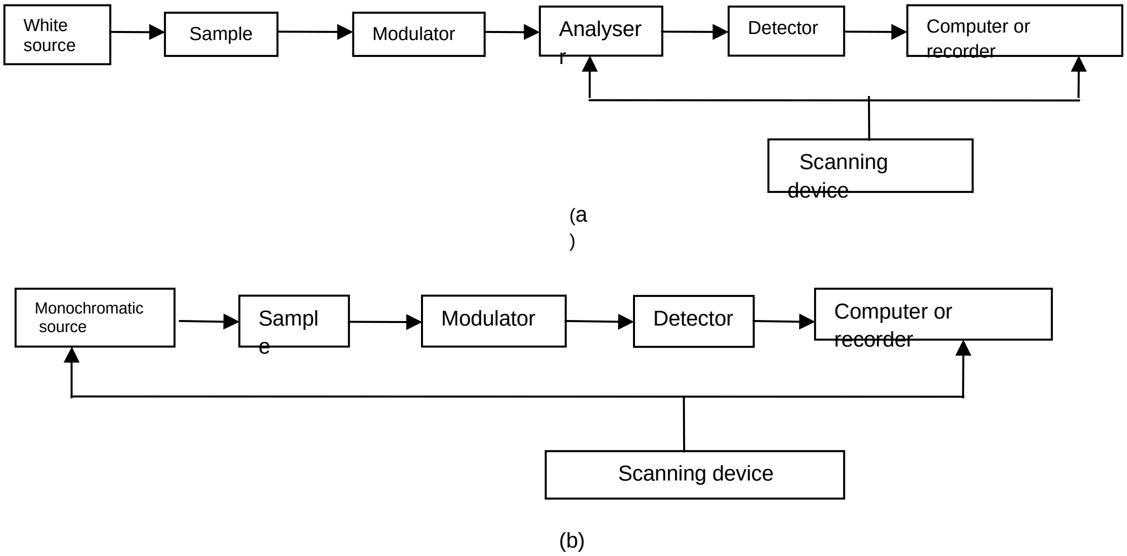

Fig:1 Typical diagram of scanning absorption spectrophotometer.

(a) Infrared, visible and ultraviolet regions where polychromatic source is used.

(b) Microwave and radiofrequency regions whose source can be varied over the range of frequencies.

2. Emission instruments: Figure 1.2 shows schematically, a typical spectrometer. The excitation can be thermal or electrical, but usually takes form of electromagnetic radiation.

In the latter stage it is essential that the detector does not collect radiation directly from the exciting beam and the two are placed at right angles as shown (Figure 1.2).

A modulator fixed between the source and the sample, together with a tuned detector-amplifier, ensures that the only emission recorded from the sample arises directly from excitation by neglecting other emission.

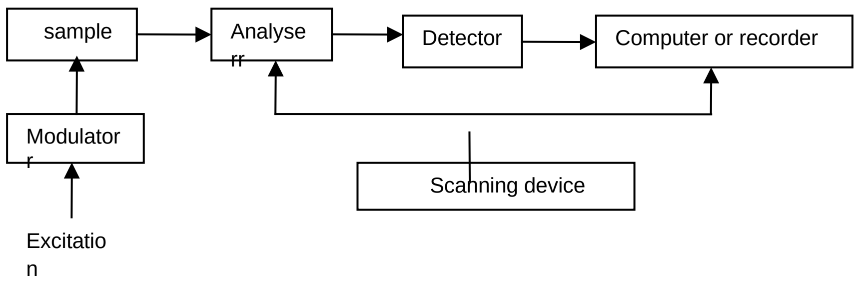

Fig:2 Block diagram of a typical emission spectrophotometer.

Electromagnetic Spectrum and Absorption of Radiations

The types of electromagnetic radiations in order of their increasing wavelengths or decreasing frequencies are known as complete electromagnetic spectrum.

The visible spectrum represents only a small part of the electromagnetic spectrum.

If all types of electromagnetic radiations in order of their increasing wavelengths, then the portion above the visible region is called Infrared while that lower it is the ultra-violet region.

Infra-red have higher wavelength (less energetic). Cosmic rays carry high energy while radio waves (least energetic).

Microwaves have larger wavelengths and are used in telephone transmission (higher range energy).

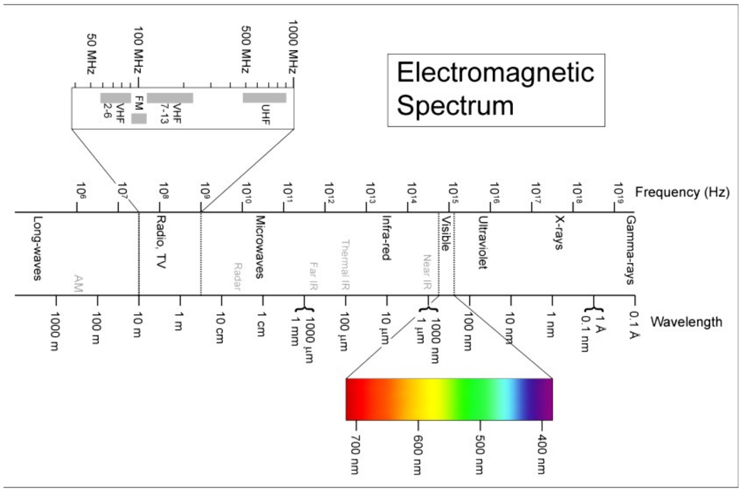

Fig 3: Typical Electromagnetic spectrum.

All types of radiations travel as waves with the same velocity but differ in their properties, example X-rays can pass through glass and muscle tissues.

Radio waves can pass through air. Visible, Ultraviolet and Infra-red radiations can be bent by reflection or diffraction in a prism.

When light radiation is penetrated through an organic sample, some of the wavelengths are absorbed while others belonging to that radiation remain unaffected.

A molecule can absorb radiation of certain frequencies, if there exists within a molecule an energy transition of magnitude $\Delta E = h\nu$.

It is observed that when light radiations are passed through an organic sample, then electrons of the sample's atoms are excited to higher level.

Also, the vibrational and the rotational energies of the molecules are completely quantized. Therefore the wavelength of light absorb by molecule will be due to the changes in the electronic, vibrational or rotational energy levels allowed for the atoms.

The wavelengths' absorption can be measured by using spectrometer.

If we plot the changes in absorption v/s wavelength, then we get some absorption band which is highly characteristic of a sample and the experiment provides a tool to measure the molecular structure of an unknown compound.

The electromagnetic spectrum can explain the followings:

(i) The visible and ultra-violet region radiations' wavelength (200-800 mμ): the absorption of radiation in this region results the excitation of π electron in a conjugated or an unconjugated system. In the conjugated system, the separation between the ground state and the excited energy level will be less and hence absorption occurs at a longer wavelength. In the case of carbonyl group, an aldehyde or a ketone absorbs some characteristic wavelength. Hence, an UV or visible spectrum is useful for the determination of conjugation, carbonyl group and will not give any other information about the remaining part of the molecule.

(ii) Infra-red radiations covers the wavelength (from 0.8 to 2.5 μ) is called near IR region (15 to 25 μ) is called far IR region and 2.5 to 15 μ is useful region for IR spectroscopy.

Hence these radiations are of higher wavelengths and less energetic.

The absorption of radiation by an organic sample in 2.5 to 15 μ range causes the changes in the vibrational levels are resulted by the changes in rotational levels.

Thus, certain bands appear which characteristically absorb for the stretching vibrations and are very useful in structure determination.

The absorptions at higher wavelength in the IR region are the characteristic of a compound and also help in distinguishing one compound from the other (Finger print region).

Although, more helpful than ultra-violet method, it does not give complete information about the environmental effects in a molecule.

(iii) NMR spectroscopy gives a complete insight into the environment and the arrangement of atoms within a molecule.

For this method, radiations of longest wavelength range- Radio waves are useful.

A simple under finding is placed in strong magnetic field and irradiated with radio waves.

By using the strength of the magnetic field applied, radiations of definite wavelength (or frequency) will be absorbed which gives the nuclear magnets specific orientations with respect to the applied magnetic field.

Application of UV absorption Spectroscopy to Organic Compounds

1. **Detection of conjugation:** It gives the relationships between different groups, particularly with respect to conjugation; the conjugation may be (a) relation between two or more carbon-carbon double (or triple) bonds, (b) relation between carbon-carbon and carbon-oxygen double bonds or (c) relation between double bonds and an aromatic ring. It can observe the presence of an aromatic ring itself and the number and locations of substitutes attached to the carbons of the conjugated system.
2. **Detection of geometrical isomers:** In the geometrically isomeric compounds, the Trans isomers exhibit λ_{max} at slightly longer wavelengths and have larger extinction coefficients than cis isomers.
3. **Detection of functional groups:** It is possible to determine the presence of some functional groups using UV spectrum. Even the absence of any absorption above 200 nm is of some utility since it shows the absence of conjugation, carbonyl group and benzene rings in the compound.
4. **Qualitative Analysis:** UV absorption spectroscopy can give the information about those types of compounds which absorb UV radiation. These compounds are with unbonded electrons (n electron) or those with the conjugated double-beam system (π electron) and aromatic compounds. Determination is done by using the absorption spectrum with the spectra of known compounds.

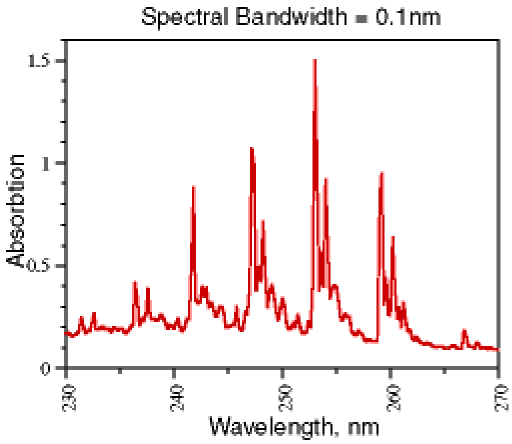

Fig: 4 Typical UV absorption spectrum of benzene.

To record UV absorption spectrum, the amount of radiation absorbed at various wavelengths by moving the slit across the dispersed beam of light from the monochromatic source. A typical UV absorption spectrum of benzene vapour is shown in fig. 1.4. In UV absorption spectroscopy, the absorption curve is influenced by the whole molecule as well as by the particular group that contains the absorbing electrons. UV absorption method is useful for characterizing aromatic compounds and conjugated olefins.

5. **Determination of Impurities:** UV absorption spectroscopy method is used for detecting impurities in organic compounds. The main aims of the superiority of this method are, (i) the bands due to impurities are very intense. i.e, an impurity having an amount of 0.05% has a ε value of 2000. Therefore, such an impurity can be observed in a transparent major component. (ii) The organic compounds can be classified into saturated compounds having little absorption and unsaturated compounds having strong absorption bands. The common impurity in cyclohexane is benzene. Its presence can be easily detected by its absorption at 255nm.

6. **Quantitative Analysis. Q**uantitative determination of compound that absorbs UV. This determination is based on Beer's law

Molecular Weight Determination. Molecular weights of compounds can be measured using UV-Visible spectrometer provided suitable derivate of these compounds could be prepared. This technique is based upon the formation of a derivative such as a picrate and this is determining from the molecular weight of any amine.

First it is converted into amines picrate. Then, a known concentration of the amine picrate is dissolved in a liter of solution and its optical density is measured at λ_{max}=380nm.

Spectrofluorimeter.

There are two types of instruments mainly (i) those consisting of fluorescence attachment for a spectrophotometer.

(ii) Self-contained instruments usually with two monchromators.

A symmetric septrofluorimeter consists of an exciting source, usually mercury or xenon lamp from illumination of the sample at a 90^0 angle.

The source is dispersed by a prism or grating blazed for high efficiency at shorter wavelengths and another prism or grating blazed at somewhat greater wavelengths to disperse the emitted fluorescence.

The fluorescent intensity can be obtained with help of photo tube attached to a micro photometer and a recorder or an oscilloscope.

Two types of information may be made available with the help of spectrometer. (i) The wavelength of best excitation. (ii) Wavelength of the strongest emission. There are two curves, (i) excitation curve and (ii) emission curve, which are generally plotted or the recorded for each fluorescent material.

The excitation spectrum is obtained by plotting wavelength of the exciting source versus the intensity of emission.

The exciting wavelength producing the higher intensity of emission would observe to best exciting wavelength for a light source and grating.

To get the spectrum, the curve may be corrected for certain intensity of light source for each wavelength.

The data for obtaining such a correction may be obtained by the intensity of the excitation source, at the sample compartment with a thermopile or by fluorescent solution for the study.

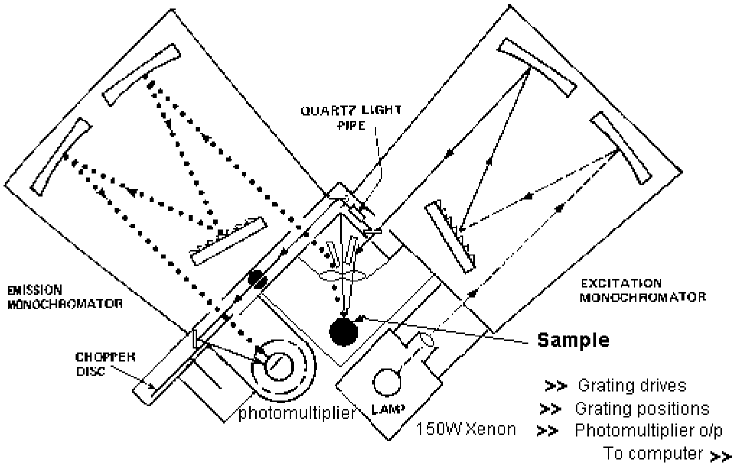

Fig: 5 Fluorescence spectrophotometer (Spectrofluorimeter.)

Thus a fluorescence spectrometer can be operated by the following steps.

i. A suitable wavelength in the emission spectrum is chosen with the help of preliminary studies.
ii. Second monochromator is set at this point.
iii. An excitation spectrum is plotted by scanning the first monochrmator.
iv. An emission spectrum is resulted by scanning the second monochromator with the first set a suitable observed value.

Advantages

i. The spectrophotometer technique is sensitive, about one part in 10^8 or 1.0 μg/ml in many determinations.

ii. Flurometric techniques possess greater specificity than spectrophotometric technique, because there is selection wavelength not only for the radiation emitted, but also for the light which excites the sample.

iii. The fluorometer results affected not only the sensitivity, but also the precision (accuracy).

General Applications

i. Finding the Chemical Structures and Processes.

ii. Analysis i.e, determining of Impurity. Estimation of single component-Vitamin, investigation of Fluorescent Intensity of the sample compound.

iii. Finding of 3-4 Benzpyrene.

iv. Finding of Rare earth Terbium.

v. Investigation of Bismuth.

Applications of Fluorimetry

Fluorimetry is a well established analytical method. We will describe some of these as follows.

(a) The determination of uranium in salts by fluorimetry: This is used extensively in the field of nuclear physics. The uranium sample is evaporated with nitric acid to bring about oxidation. After, the sample is fused with sodium fluoride to a melt having fluorides of sodium and uranium. On cooling, this solidifies to a glass which is examined in a specially designed fluorimeter.

(b) Some of inorganic ions do not exhibit fluorescence. However, some of these inorganic ions from fluorescent chalets with non-fluorescent organic molecules. This result has provided the basis for very sensitive analysis of many elements.

(c) Fluorescent Indicators**-** The intensity and colour of the fluorescence of many samples depend upon the pH of the solution. These are shown as fluorescent indicators. These are mainly used in acid base titrations. These can be useful in the titration of coloured solutions in which the changes in colour of indicators get masked. Some examples of fluorescent indicators are shown in table 2

Table 2 Various Fluorescent Indicators

Name of compound	Approx. pH Range	Change in colour
Eosin	3.0 – 4.0	Colourless to green
Fluroescein	4.0 – 6.0	Colourless to green
Quinine sulphate	3.0 – 5.0	Blue to violet
Acridine	5.2 – 6.6	Green to violet-blue

(d) Organic analysis Fluorimetry has been used to carry out qualitative as well quantitative analysis in many aromatic compounds observed in cigarette smoke, air-pollutant, concentrates and automobile exhausts gases. Specific example is the determination of benzopyrene in the nanogram range.

(f) Pharmaceutical chemistry: Aneurine hydrochloride in pharmaceutical preparation such as medicines and elixirs and in foodstuffs such as flour is relatively easily obtained by oxidation to the highly fluorescent thiochrome. The obtained product is soluble in isobutyl alcohol and result easily extracted from the reaction mixture from measurement.

Conclusion

The important technique of spectroscopy unit explains the techniques of spectroscopy with modern spectrometer. The study of these instruments used in spectroscopy to explain in detail for various uses of UV and fluorescence spectrometers presented properly for studying detail usage of UV and fluorescence with examples explained which can be important role for many applications. Detailed advantages of UV and fluorescence with examples are given in this unit.