Chapter

Spectroscopy and Spectrophotometry: Principles and Applications for Colorimetric and Related Other Analysis

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Abstract

Spectrophotometry and different types of spectroscopy are the technique that involved in identifying and quantifying the amount of a known substance in an unknown medium. Spectroscopy is the most convenient method for analysis of unknown samples both qualitatively and quantitatively with a good percentage of accuracy. Different types of spectroscopic and spectrophotometric techniques are very helpful in analyzing the samples even at sub-ppm level particularly in the field of scientific research. These techniques based on the simple principle that the amount of specific radiation i.e. ray or light (photon) absorbed or reflected by the sample relative to the intensity of the incident ray/light at a particular wavelength. These techniques are using, for analyzing purity, % content in mixture, type of reactions/chemical interactions occur/absorption or reflectance of color for a colored substances/solutions are detectable and quantitatively determinable quantitative determination. Most of the scientists have been using different spectroscopic and spectrophotometric techniques like Infrared spectroscopy, Raman spectroscopy, X-ray fluorescence and UV VIS spectrophotometry etc., which are playing an important role in the identification and characterization of substances, apart from this the atomic absorption spectroscopy and atomic emission spectroscopy are also being used for quantitative measurement of different substances or elements.

Keywords: spectroscopy, spectrophotometry, UV-visible spectroscopy, infrared spectroscopy, Raman spectroscopy, X-ray fluorescence

1. Introduction

Every compound, that is present in the nature, has a property to absorb, transmit, or reflect light (electromagnetic radiation) at a certain wavelength. This property of the compounds, helps to measure quantitatively by using spectrophotometric techniques. Spectrophotometry is a technique which deals with the measurement of the interaction of light with materials. When light falls on a material that can be reflected, transmitted, scattered, or absorbed, and at the same time the material on which light has fallen can emit absorbed light with different frequency. This is due to the gained energy from the light (e.g., electroluminescence) or due to its temperature (incandescence) [1]. Different types of spectroscopy and spectrophotometry is well known and widely used technique to identify and quantify
2. Principle, instrumentation and applications of various spectrophotometric techniques

A spectroscopic/spectrophotometric instrument basically consists of four important components: a light/radiation source, a collimator, a monochromator, and a detector. The monochromator comprehends a fixed entrance slit, a dispersing element such as a prism or a diffraction grating, and a moving exit slit (Figure 2) [6].

2.1 UV-visible spectrophotometry

2.1.1 Principle

Law of absorption is the basic principle of UV-visible spectrophotometry. This law discusses the relation between thickness of the absorbing material and the concentration of the sample solution, which is popularly known as Beer-Lambert law or simply Beer’s law. This law states that the amount of light absorbed is proportional to the concentration of the absorbing substance and to the thickness of the absorbing material [7].
\[ \log_{10} = \frac{I_0}{I} = abC \quad (1) \]

where \( I_0 \) = the intensity of the incident light, \( I \) = the intensity of the transmitted light, \( a \) = absorption, \( b \) = the absorbing thickness, \( C \) = the concentration of the absorbing material.

2.1.2 Instrumentation

The UV-visible spectrophotometer consists of a light source, sample holders, a monochromator, and a detector [8].

**Light source:** Hydrogen lamp and the deuterium lamps are used as UV light source, whereas for visible source tungsten filament lamp is the most used.

**Sample holders:** In UV and Visible ranges, cuvettes are used as sample holders, which are made from quartz or ordinary glass. Generally, in the UV region quartz or silica cell are used, whereas in the visible region glass cell are used and these cuvettes have a standard path length is usually 1 cm.

**Monochromators:** A monochromator converts polychromatic radiation into monochromatic radiations by which the wavelengths of these radiations translate into very narrow bands.

**Detectors:** Photovoltaic cells, phototubes and photomultiplier are commonly used detectors in the UV and visible range [7]. The following block diagram (Figure 3) shows main parts of UV-Visible spectrophotometer [7].

2.1.3 Applications of UV-visible spectrophotometry

a. Pharmaceutical analysis: UV-visible Spectrophotometry has been widely used technique in the determination of drug concentration in pharmaceutical analysis.

For example, this technique is used in the determination of etravirine in bulk and pharmaceutical formulations. The spectrum of etravirine is shown below. This is
acting as an anti-viral drug, and it showed the maximum absorption at 414 nm (visible range) by reacting with NaOH and 1,2-napthaquinone 4-sulphonate. The details are shown in the following (Figure 4) [9].

b. Vaporization studies of low-volatile compounds: UV-visible spectrophotometer is involved in the determination of vaporization of low-volatile compounds in the vapor phase located above the sample in the condensed state [10].

c. UV-visible spectroscopy is also used in the identification of pure analytes which are not subjected to decomposition, particularly this is used for identification of nucleic acids. This study has an advantage to identify the newly found genetic materials in various microbes and other species. The following (Figure 5) shows absorption of DNA in UV light [11].

d. Quantification and identification of organic compounds has been achieved by using UV-visible spectroscopy technique. This technique is very helpful for the analyzing of newly developed drugs in the pharmaceutical industries [12].

e. UV-visible spectrophotometry is widely used technique in biochemistry for the determination of micromolar concentrations of substances in blood, urine, and other body fluids. It is also used both for the determination of species and for studying biochemical processes [13].

f. UV-vis spectroscopy also involved in the assessing the Color Index of Transformer Insulating Oil (Figure 6) [14].

g. A case study has been conducting on combined UV-visible spectroscopy and chemometrics to determine the interaction of human serum albumin (HAS) and gold nanoparticles (AuNPs). The data which has been recovered from the UV-visible spectroscopy and chemometrics about protein (HAS) interaction with nanoparticles (AuNPs) were apply to the thermodynamic, kinetic and structural parameters to establish the evolution of protein nano-conjugate (Figure 7) [15].
2.2 Infrared spectrophotometry

2.2.1 Principle

When a molecule absorbs the light of higher wavelength rather than UV and visible, then there is a possibility of vibrational transitions in the molecules. These vibrational transitions of the molecules lead to the formation of an IR spectrum.
These vibrational transitions due to occurrence of electronic transitions when a given substance absorbs light energy.

2.2.2 Instrumentation

Like UV-visible spectrophotometer, IR spectrometer also consists of a light source, a sample holder, a monochromator, and a detector [7].

*Light source:* Xenon and tungsten lamps are typically used as light sources in the near IR-region [16].

*Sample holders:* In IR region quartz cuvettes usually used as sample holders.

*Monochromator:* Gratings are used as monochromators in the IR region.

*Detectors:* Commonly used detectors in the IR spectrophotometry are indium gallium arsenide (InGaAs) semiconductor materials.

2.2.3 Methodology

The radiation energy is the main element in the IR spectrophotometer. It acts as a function of wavelength and provides energy to reach a maximum at a wavelength (\(\mu\text{m}\)) equal to \(2897/T\), where \(T\) is the absolute temperature (K). This radiation energy is usually providing a short-wavelength limit of the spectrum (\(\sim 2\ \mu\text{m}\)) and it decreases as the wavelength gets longer [17]. The radiation energy coming from the source falls on to the samples, causing the excitations followed by molecular vibrations in the sample by which it can give IR spectrum that can be detected by detectors. The detectors can change the radiation energy into an electrical signal that can be amplified and processed to yield a spectrum. The spectrum gives information about various functional present in the sample.

2.2.4 Applications of IR spectroscopy

a. Identification of compounds: IR spectroscopy assists in finding out various chemical compounds and functional groups in organic molecules, such as aliphatic, aromatic, saturated and unsaturated hydrocarbons, amino acids, ether and hydroxyl groups, halogens, nitrogen, phosphorous, silicon, sulfur-oxy compounds etc.
The aliphatic and aromatic hydrocarbons can be analyzed by C–H and C–C stretching and bending vibrations, most of these vibrations are unique for each molecule, and are generally described as skeletal vibrations. The C=C–C bond in the ring structure of aromatic compounds is diagnosed by characteristic stretching and bending vibrations [18]. For example, the spectrum of 1-hexene shows characteristic absorptions of a double bond. The C–H stretch at 3080 cm⁻¹ corresponds to the alkene –C–H bonds. The absorption at 1642 cm⁻¹ results from stretching of the C=C double bond. The diagram represents the IR Spectra of 1-hexene (Figure 8) [18].

b. IR spectroscopy successfully involved in the characterization of nano particles particularly in the study of physicochemical characteristics of drug nanocarriers and in the identification of

c. Functional groups on the surface of the developed nano particles which are involved in drug targeting system [19].

d. IR spectroscopy takes an important role in the surface biology research to study the surface interaction of drugs, antibodies with cell surface proteins and other biological molecules, which helps in understanding how to optimize sensitivity in between the interacted molecules [20].

e. Rate of reactions: infrared spectra give wonderful indication for many functional groups. Thus, enzymatic reactions involving these functional groups—either these groups are consumed or generated in the enzymatic reaction—can be assayed with the help of infrared spectroscopy. For example, the enzymatic activity of the pyruvate kinase has been studied with its substrate phosphoenol pyruvate, which gave a characteristic spectrum for understanding how the substrate consumed and the product formed. The details are shown in the following (Figure 9) [21].

f. Interaction between molecules: polypeptide chains form inter chain hydrogen bonds. So do the two stands of DNA. Hydrogen bonds have been studied very profitably using infrared spectroscopy.

g. Infrared spectroscopy is an important tool in structural determination of minerals. For example, in one of the case studies which has been done on the alternations

Figure 8.
IR spectra of 1-hexene.
of chondrule in NWA 2086 CV3 meteorite by using IR spectroscopy along with optical microscopy and electron microprobe. This study revealed that the alternations have brought changes in intensity and wavelength positions of olivine peaks with the advancement of alteration and related Fe/Mg substitution inward of the chondrule and also it was identified that there is a good correlations between Fo% composition and positions of 830 and 860 cm\(^{-1}\) IR peaks [22].

2.3 Fourier transform infrared spectroscopy (FTIR)

2.3.1 Principle

FTIR works on the principle of IR spectroscopy. Nevertheless, the instrumentation is different from IR spectroscopy.

2.3.2 Instrumentation

FTIR spectrometer consists of a light source, a sample holder, a monochromator, and a detector which are like that of IR spectrophotometer, but the major difference is an interferometer, which makes this instrument highly advanced than normal IR spectrophotometer. The interferometer specially consists of a compensator plate, a beam splitter, a fixed mirror, and a scanning mirror, which are connected to a detector. The advantages of FTIR over the existing dispersive infrared instrument are spectral quality, data collection speed, reproducibility of data, and ease of maintenance and use. Instrumentation of the FTIR is shown in the following (Figure 10) [23].

2.3.3 Applications

Fourier transform infrared (FTIR) spectroscopy is a powerful analytical tool in identifying chemical constituents and elucidating structures in various forms in real-world samples.
a. FTIR has been used for the characterizing the unpredictability in fuel stability of various biodiesel and antioxidant samples. This can be achieved by identifying the presence of various organic and inorganic compounds in the sample through the FTIR spectrum [24].

b. FTIR has been used for the identification of functional groups in polymers and co-polymers. For examples, FTIR used in the identification of functional groups in one of the co-polymers i.e., poly-3-hydroxybutyrate (PHB), which
shows peaks at 1724 cm\(^{-1}\) and 1279 cm\(^{-1}\) corresponding to C=O stretching and the adsorption band respectively in the ester group. The following (Figure 11) shows the C=O stretching and the adsorption band of poly-3-hydroxybutyrate (PHB) [25].

c. Fourier transform infrared (FTIR) spectroscopy with attenuated total reflectance (ATR) accessory was used in forensic analysis to get biochemical information on postmortem interval estimation based on pericardial fluids of rabbit.

d. Fourier transform infrared (FTIR) spectroscopy with attenuated total reflectance (ATR) has also been used for the assessment of the immobilization of active substances in the matrix of biomedical materials. The presence of Sparfloxacin on the surface of the biomaterial was studied by using FTIR method. In this study the Sparfloxacin has shown many absorption bands in FTIR spectrum, which reveals the information that the successful binding of an antibiotic with bacterium (Figure 12) [26].

2.4 Raman spectroscopy

2.4.1 Principle

Raman spectroscopy based on the scattering of light, which was described by C.V. Raman in 1928 through his outstanding study i.e., Raman effect. According to Raman effect when a certain frequency of monochromatic radiations incident on a sample, the incident light is scattered through interaction with vibrating sample molecules, the frequency of scattered light is different from that of the incident light. It is based on the inelastic scattering of incident radiation [27]. In Raman spectroscopy, when a monochromatic radiation strikes the sample, it scatters in all directions after its interaction with sample molecules. Much of this scattered radiation elastically that constitutes Rayleigh scattering. Only a small fraction of scattered radiation in-elastically scattered that constitutes Raman
scattering. Usually, in Raman spectrum there is an appearance of Stokes lines, this is due to the frequency of incident radiation is higher than frequency of scattered radiation. On the other hand, there is an appearance of anti-Stokes lines in Raman spectrum, when the frequency of incident radiation is lower than frequency of scattered radiation occurs.

2.4.2 Instrumentation

Instrumentation for modern Raman spectroscopy consists of following components:

a. Light source

b. Prism or grating

c. Detectors

a. Light source: during 1960s Mercury lamps were used as light source. From late 1960s various kinds of lasers were started to use as light sources as they provide stable and intensive beam of light in Raman spectrophotometers that makes the Raman spectroscopy more versatile than ever. Long wavelength sources such as diode or Nd:YAG lasers are preferred as they have advantage and can be operated at much higher power without causing photodecomposition of sample and eliminates or reduces fluorescence in most cases [28].

b. Prism or grating: prism or grating uses in dispersive Raman spectrophotometer while Michelson interferometer uses in non-dispersive Raman spectrophotometer. The grating monochromators are used to separate relatively weak Raman lines from intense Rayleigh scattered radiations.

c. Detectors: in earlier models of dispersive Raman spectrophotometers, thermoelectrically cooled photomultiplier tubes and photodiode array detectors were used. Now a days, due to the advancement in instrumentation technology, more sensitive charge transfer devices (CTDs) such as charge-coupled devices (CCDs) and charge-injection devices (CIDs) are in usage. These devices act as a detector and use in the form of arrays.

The (Table 1) one shows some common laser sources which are generally use in Raman Spectroscopy [29] and (Figure 13) shows the instrumentation of Raman Spectrophotometer [30].

<table>
<thead>
<tr>
<th>Laser type</th>
<th>Wavelength, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argon ion</td>
<td>488.0 or 514.5</td>
</tr>
<tr>
<td>Krypton ion</td>
<td>520.9 or 647.1</td>
</tr>
<tr>
<td>Helium-neon</td>
<td>632.8</td>
</tr>
<tr>
<td>Diode</td>
<td>785 or 830</td>
</tr>
<tr>
<td>Nd:YAG</td>
<td>1064</td>
</tr>
</tbody>
</table>

Table 1. Some common laser sources for Raman spectroscopy.
2.4.3 Raman spectrum

Typically Raman spectrum is plotted signal intensity vs. Raman shift (Raman shift, in cm$^{-1}$ = energy of photon in − energy of photon out). Figure 14 shows the Raman spectrum.

2.4.4 Applications

a. Cell therapy: Raman spectroscopy involved in the development of cell therapies. Cell therapy is typically done by introducing a living cell into the patients to cure many degenerative and deadly diseases. Raman spectroscopy plays an important role in basic understanding of biochemical and functional characteristics of therapeutic cells, in manufacturing them and to effectively implement them for therapy [31].

b. Molecular diagnosis of cervical cancer: Raman spectroscopy is powerful tool in biochemical investigation, particularly in identifying and characterizing the
structure of biomolecules, cells, and tissues. This approach is very helpful for the scientists to diagnose the malignant neoplasm which leads to the cervical cancer [32].

c. In agriculture, food and biosystems: Raman spectroscopy is often used for early detection of plant diseases. For example, for the early detection of Citrus Huanglongbing and rose rosette disease (RRD). Raman spectroscopy combined with chemometric analysis provides the information about the effect of Tomato yellow leaf curl Sardinia virus (TYLCSV) and Tomato spotted wilt virus (TSWV) on tomato. Raman spectroscopy is one of the effective tools in identifying the food adulteration. For examples Raman spectroscopy combined with chemometric methods are used in the determination of butter adulteration. SERS is used in the determination of toxic effect of mycotoxin deoxynivalenol in corn, kidney beans, and oats [33].

d. Raman spectroscopy like IR spectroscopy used in chemistry to identify the chemical structures by providing the fingerprint region in the spectrum and it is involved in the functional group identification.

e. Raman spectroscopy is one of the powerful techniques in biopharmaceutical industry. It helps in measurement and analysis of particle size while preparation of the drug and it involved in the identification of contaminants which are coming out from the pipes, valves, bags, filters, etc. during formulation the drug in pharmaceutical industry.

f. Raman spectroscopy involved in the identification protein structure, protein glycosylation, protein stability and aggregation, and also in protein formulations and identity testing which are main important aspects in the biopharmaceutical manufacturing [34].

2.5 Spectro-fluorometry

2.5.1 Principle

The phenomenon, where a molecule after absorbing radiations emits radiation of a longer wavelength, is known as fluorescence. When a compound absorbs radiation, it goes to the exited level and thereafter comes to the ground level either in one step by emitting radiation of the same wavelength that it absorbed or in a stepwise manner by emitting quanta of radiation corresponding to each energy step with longer wavelength. This phenomenon leads to the formation of fluorescence spectra. Fluorescence is an extremely short-lived phenomenon (10^{-7} s or less) and therefore can provide information about events which takes less than 10^{-7} s. Fluorometry also follow the Beer-Lambert law in its working principle.

2.5.2 Instrumentation

Fluorometry is an important analytical tool for the determination of extremely small concentrations of substances which exhibit fluorescence.

2.5.3 Instrumentation

The instrumentation of a spectrofluorometer differs from that of the spectrophotometer in two important respects besides other minor variations.
a. There are two monochromators instead of one as in a spectrophotometer; one monochromator is placed before the sample holder and one after it, and

b. As fluorescence is maximum between 25 and 30°C, the sample holder has a device to maintain the temperature.

The main components of a spectrofluorometer indicated in Figure 15 are:

2.5.4 Applications

a. Identification of 3-D structure of protein: proteins are made by the combined cluster of 20 amino acids. For computational drug discovery it is very important to know that the correlation between three-dimensional structure and its functions. This data may not be successfully provided by the X-ray crystallography and electron microscopy. Hence, to resolve this problem the fluorescence spectrophotometry provides the reliable information about three-dimensional structure of protein by preserving the native structure of protein [35].

b. In food: fluorescence spectroscopy plays an important role in the determination of numerous food components, adulterants, additives, and contaminants [36].

c. Quality control in food processing: fluorescence spectroscopy is a rapid and sensitive analytical method for characterizing the food products. For example, recently, some authors have studied on impact of heat treatment on vitamin A in milk sample by using fluorescence spectra. The fluorescence spectra shows that the heat treatment induced a decrease in the fluorescence intensity at both 320 and 290 nm since milk samples heated at 75°C for 10 min. Compared with milk samples heated at 55°C during the same time. The details are shown in the (Figure 16) [37].

d. The more common applications of spectrofluorometer include qualitative analysis, quantitative analysis (applications include assay of riboflavin, thiamine, hormones such as cortisol, estrogens, serotonin and dopamine, organophosphorus pesticides, tobacco smoke carcinogens, drugs such as lysergic acid and barbiturates. Porphyrins, cholesterol, and even some metal ions); and studies on protein structure (FAD containing proteins).
2.6 Atomic absorption spectrophotometry (AAS)

2.6.1 Principle

When the sample molecules are subjected to volatilization, the produced atoms absorb certain wavelength of light from a source which produce a characteristic atomic spectrum of the molecule.

2.6.2 Instrumentation for AAS

The basic components of an atomic absorption spectrophotometer are [38]

a. Atomizers: most used atomizers in AAS are electrothermal atomizers (ETAs). These are involved to convert sample molecules into an individual gaseous atom that can absorb light from the source.

b. Light source: generally, hallow cathode lamp used as light source in AAS to produce a certain wavelength of light that are ideally absorbed by the gaseous atoms. Now a days, instruments with single and double beam optics are available.

c. Isolation and quantification of wavelengths of interest can be done by detectors and to control instrument operation and collect and process data the computer system helps.

2.6.3 Applications

a. AAS is a sensitive and highly selective spectrometric technique for the determination of many elements at trace and ultra-trace levels viz., Cd, Cr, Zn, Cu, Ag, Mn, Mg, Hg, As, Sc etc., at the picogram levels in soil, sediments and in plant samples. For example, this technique is very helpful for the scientists who are working on finding out the impacts of mining and industrial activities.
b. Atomic absorption spectrometry (AAS) can be used in the estimation of metals in body fluids like in serum, whole blood, apart from urine and in tissues for toxicological investigation in clinical studies [39].

c. AAS found to be a very good tool especially for the determination of food quality. In this concern, it is involved in the determination of alkaline and alkaline-earth metals because these elements act as a micro component in food samples.

d. To check the quality of water the AAS is one of the very useful techniques.

2.7 Nuclear magnetic resonance spectroscopy (NMR)

2.7.1 Principle

The nuclear magnetic resonance (NMR) is one of the most useful and powerful techniques in determination of molecular structure. The principle behind NMR is that when a strong magnetic field and a radiofrequency transmitter are applied on sample molecules, the atomic nuclei of those molecules get excited and forms spectral lines in the spectrum [40].

2.7.2 Instrumentation

The components of NMR spectrometer are

a. Radiation source: a radiofrequency transmitter (RF), which generates the radiofrequency current. This current deliver to the transmitting coil which creates a signal used to excite protons in the magnetic field.

b. A superconducting magnet, which produces magnetic field in the central volume of the magnet. The produced magnetic field varies from 1 to 10 T. The advanced NMR spectrometers consist superconducting solenoids that generates magnetic field above 3.5 T. The magnet has a bore to hold the sample probe and a room-temperature shim (RTS) coil assembly to reduce inhomogeneity of the magnetic field across the active sample volume.

c. A receiver receives absorbed signal in digitalized form generally called as free induction decay (FID).

d. A computer, an amplifier, and an analog-to-digital converter (ADC).

2.7.3 Applications

a. Biochemistry: NMR is a powerful technique in metabolic research. It became a method of choice for discovering the dynamics and compartmentation of metabolic pathways and networks [41]. Apart from this NMR is also useful in the study of intact biological specimens such as heart, kidney, and skeletal muscle with $^{31}$P isotope.

b. Pharmacy: NMR spectroscopy permits the visualizing single atoms and molecules in various liquids as well as in solid state [42]. It is nondestructive and gives an idea on molecular structure that allows the structure elucidation and quantification of various organic molecules and provides information about the
chemical structure and the dynamics of organic molecules in biological systems [35]. These structural studies provide an information related to functions of organic molecules such as amino acids, proteins, carbohydrates, and antibiotics such as ciprofloxacin, azithromycin and valinomycin.

c. Chemistry: NMR spectroscopy is used unambiguously to identify novel compounds, and as such, is usually required by scientific journals for identity confirmation of synthesized new compounds.

d. NMR has been used to study in vivo or synthetic membrane transport system. For example, it has been used for study of transport of Na⁺ ions in human RBCs.

e. NMR has been used for the quantitative determination of the concentration of metabolites. Using NMR in the determination of concentration of phosphocreatine in human muscle is an example of the same [7].

f. NMR is a very useful techniques in identifying and quantifying hydrocarbons in petroleum industry. For example, the ¹H and ¹³C nuclear magnetic resonance is involved in the quantitative measurement of liquid hydrocarbons in FACE Gasoline F. It gives a good resolution in the spectrum which is useful for quantifying liquid hydrocarbons in FACE Gasoline F [43].

2.8 Electron spin resonance spectroscopy (ESR)

Electron spin resonance (ESR) or electron paramagnetic resonance (EPR) spectroscopy is an analytical technique for detecting and characterizing the paramagnetic species.

2.8.1 Instrumentation

Figure 17 illustrates the basic components of an ESR spectrometer. Fields of 50–500 mT, required for accurate work are generated by electromagnets. Auxiliary sweep generators with a capacity of 10–100 mT are also provided. Monochromatic microwave radiation might be readily obtained by using a klystron oscillator.
Samples for ESR must be solids. Biological samples which contain a large amount of water are therefore frozen in liquid nitrogen before ESR experiment. Figure 18 shows the Basic components of an ESR [7].

2.8.2 Principle

The principle of ESR spectroscopy is based on the fundamental properties of electrons. An unpaired electron in atoms or molecules possesses paramagnetic character and shows both magnetic moment and angular momentum. In an external magnetic field, the spin magnetic moment aligns parallel or antiparallel to the field, and the spinning electrons are split or divided into high and low energy states. These split electrons form spectral lines, and they can be determined [44].

2.8.3 Applications

a. Electron spin resonance spectrometry is used in polymer chemistry to identify paramagnetic species in polymers. During polymer synthesis or degradation, the paramagnetic species are produced due to free radical mechanism. These paramagnetic species may damage the quality of polymer. Hence, ESR provides an information about the number of paramagnetic species [45].

b. ESR has been used in studying reaction mechanisms which proceed through free radical intermediates in metabolic reactions.

c. ESR is one of the important methods to study transition metals present in biological systems such as iron in hemoglobin, and cytochromes, copper in cytochrome oxidase, molybdenum in xanthine oxidase etc.

3. Conclusion

Spectroscopy is one of the most important analytical tools for the analysis of various compounds in various fields including chemistry, physics, biology, agriculture, engineering, and medicine. This is working with different principles which are projected through various instrumentation techniques like UV-visible spectrophotometry, IR spectroscopy, Raman spectroscopy, NMR spectroscopy and ESR spectroscopy. The applications of these techniques are providing very useful
information to the teachers, students, and researchers. These analytical methods are nondestructive, consistent, and reliable and require no or very little sample preparation. We can apply these methods to solid, liquid, and powdered samples. They can be used for a wide range of elemental analysis and provide detection limits at the sub-ppm level concentrations easily and simultaneously. Therefore, the spectrophotometric techniques are very useful in elementary analysis in all most all fields by providing reliable information about elements to be analyzed.

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