Introduction

The study of science began with the exploration of the behavior of objects of ordinary size, but today is exclusively concerned with matters on an extremely small scale which cannot be dealt with normal perception. Such tiny materials are studied usually by observing the bulk behavior with the aid of various methods, notably the spectroscopic techniques. The laws governing these tiny objects differ from those for material in bulk. It is, however, possible to coordinate the experimental results by using appropriate models founded on the properties of large-scale matter.

Spectroscopic techniques are used to study, in microscopic level, the interaction of electromagnetic waves and matter for studying the structures of atoms and molecules. Such studies make it possible to investigate their structures in detail, including the electron configurations of ground and various excited states. Spectroscopy also provides a precise analytical method for finding the constituents in material having unknown chemical composition. In a typical spectroscopic analysis, a concentration of a few parts per million of a trace element in a material can be detected. In astronomy, the study of the spectral emission lines of distant galaxies led to the discovery that the universe is expanding rapidly and isotropically. The finding was based on the observation of a Doppler shift of spectral lines. That discovery was followed by another discovery of a low level of isotropic microwave radiation which is identified as a product of the big bang that marks the birth of the universe and the beginning of its rapid expansion. So, spectroscopy has a broad spectrum of applications which is expanding in a rapid rate.

1.1. History of spectroscopy

It is worthwhile to recall some of the landmark achievements of classical spectroscopy. Although the spectral nature of light is present in the rainbow, before Sir Isaac Newton no one could recognize its significance. In the year 1666 Newton showed that the white light from the sun could be dispersed into a continuous series of colors, called "spectrum." He designed the first spectroscope to analyze light using a small aperture to define a beam of light, a lens to collimate it, a glass prism to disperse it, and a screen to display the resulting spectrum. The basic concept in devising a spectrometer is still the same. Gradually, it became clear that the sun's radiation has
components outside the visible portion of the spectrum through the contributions from W Herschel (1800) and J.W. Ritter (1801) \(^{15}\). Joseph Fraunhofer was the first to provide the quantitative basis for spectroscopy (1814) \(^{15}\). He also studied spectra of the stars and planets, using a telescope objective to collect the light and these laid the foundation for the science of astrophysics \(^{14}\). He also developed the diffraction grating, an array of slits, which disperses light in much the same way, as does a glass prism, but with important advantages \(^{16}\). Despite his enormous achievements, Fraunhofer could not recognize the origin of the observed spectral lines. After a long gap, Gustav Kirchhoff and Robert Bunsen (1859) discovered that each element and compound has its own unique spectrum, and that by studying the spectrum of an unknown source, one can determine its chemical composition \(^{17}\). Thereafter, spectroscopy became a reliable scientific tool for probing atomic and molecular structure. These techniques are used today to analyze both terrestrial and stellar objects, and it is still the only way to study the chemical elements present in stars \(^{11},^{12}\).

It was known from early time that light induces physical and chemical changes in matter \(^{18}\). The medicinal effects of the sun were also recognized early in human history. In ancient India, leprosy was treated with photosensitizing plant pigments \(^{19}\). The first scientific observations of the effects of light on pure substances were discovered by Scheele, who noticed that silver halide salts blackened in sunlight \(^{20}\). K. Grotthaus (1820) stated on the basis of theoretical considerations that chemical or physical changes in a substance can be brought about by the absorbed part of the incident light only. The proposition was supported experimentally by J. Herschel in 1842 and J. Draper in 1843 and thereafter it became known as the Grotthus-Draper law \(^{4},^{21}\). It is more commonly known as the first law of photochemistry since it marks the beginning of the science of photochemistry. However, the theory of photochemistry must start with Planck’s law which establishes the quantum nature of radiation: the energy of a monochromatic resonator is not continuous but divided into smaller and discontinuous but unique energy packets \(^{22}\). The next major contribution came from Johannes Stark and Albert Einstein, who independently formulated the second law of photochemistry between 1908 and 1913, also known as the Stark–Einstein law \(^{4}\). It states that, the fraction of incident radiation absorbed by a transparent medium is independent of the intensity of incident radiation but
depends on the concentration of the absorbing species and that each successive layer of the medium absorbs an equal fraction of incident radiation.

1.2. Classifications of molecular spectroscopy

An electromagnetic radiation may be considered as a simple harmonic train of transverse sine waves propagated from a source and travelling in straight lines. The properties which undulate during the passage of the wave are interconnected electric and magnetic fields. It is these undulatory fields which interact with matter and gives rise to a spectrum. It is essential to mention that electromagnetic radiation can bring about different types of various types of atomic or molecular processes. Hence, electromagnetic radiation has been classified into various regions. These regions are rather arbitrary and have no precise boundaries but these help to understand the different nuclear, molecular or electronic transitions initiated by the electric or magnetic fields associated with such regions of radiation.

- **Radiofrequency region** corresponds to the energy change arising from the reversal of spin of a nucleus or electron. The wavelengths are in the range of 10 m to 1 cm. Nuclear magnetic resonance (NMR) and electron spin resonance (ESR) are the techniques used to study such processes.

- **Microwave region** ranges from 1 cm to 100 μm wavelength and corresponds to the separations between the rotational levels of molecules. Rotational spectroscopic techniques are used to study molecular rotations.

- **Infra-red region** has wavelengths in the range of 100 μm to 1 μm and corresponds to the separation between the vibrational levels of molecules. Different vibrational modes of molecules are studied using infrared spectroscopy.

- **Visible and ultra-violet regions** range from 1 μm to 10 nm wavelength which matches the separations between the energy levels of valence electrons. Electronic spectroscopic tools are used to investigate the electronic transitions.

- **X-ray region** has wavelengths ranging from 10 nm to 100 pm and hence is similar to the energy changes of the inner-shell electrons of an atom or molecule. X-ray spectroscopic techniques reveal elemental composition of a compound, its three-dimensional structures etc.
γ-ray region ranges from 100 pm to 1 pm in wavelength and corresponds to the rearrangement of nuclear particles. Gamma-ray spectroscopy is the quantitative study of the energy spectra of gamma-ray sources, in the nuclear industry, geochemical investigation, and astrophysics.

1.3. Applications of electronic spectroscopy

Combination of the different spectroscopic tools yields complete understanding on molecular structure, properties, interactions etc. Electronic spectroscopic tools, however, are the most popular for its widespread applicability and easier instrumentation 23. Electronic absorption spectroscopy is the most basic of all electronic spectroscopic tools but it has various essential applications 24, 25. It is often used for the structure elucidation and characterization of aromatic or unsaturated aliphatic compounds. It can also be used for the quantitative determination of such compounds or detection of impurities in it. It is also used to study molecular interactions, e.g. determination of the association constants for complex formation reactions, estimation of the dissociation constants of acids and bases, studies on reaction kinetics, determination molecular weight, evaluation the chemical and physical affinity of the molecule for its surroundings without photoexcitation. Transient absorption spectroscopy is a special type of electronic spectroscopy that is used to study short-lived reaction intermediates which helps to investigate the corresponding reaction mechanisms 26-29.

Absorption of visible and ultra-violet radiation triggers excitation of valence electrons to higher electronic states. Following excitation various relaxation processes typically occur among which fluorescence and phosphorescence are the radiative processes 4, 7, 30. Analyses of these radiations further broaden the scope of electronic spectroscopy and reveal finer details. Fluorescence is the more common phenomenon and hence fluorescence spectroscopy is widely used to study fluorophores, the molecules which emit fluorescence. Fluorescence spectroscopy provides further insights into molecular properties and molecular interactions and is distinguished by ultimate sensitivity, high temporal and spatial resolution and versatility. It reports the nature of electronic excited states of fluorophores and also the studies on the excited state interaction with molecules or solvents 7, 31-35.
Fluorophores are often used as reporter molecules to study various industrial, analytical and biological processes. The first famous use of fluorescence reporter was in 1877 in southern Germany to prove that the River Danube and Rhine are connected by underwater streams. 10 Kg of fluorescein was placed in Danube and about 60 hours later it appeared in an affluent of the Rhine. Today fluorescent reporters are finding their increased use in optical molecular imaging in small animals. Its optimal use and implementation is very important for increasing the sensitivity, precision, multiplexing power and also the spectral, temporal and spatial resolution in different methods of research and practical analyses.

Fluorescence sensing is also a flourishing area of research which targets nearly the whole world of natural and synthetic compounds being detected in different media including living bodies. The principle of fluorescence sensors uses the emission behavior of specifically sensitive dyes which depends on the concentration of spectroscopically silent analytes such as Cl\(^-\), Na\(^+\) or Ca\(^{2+}\), onwards which they react selectively. Fluorescence sensors have various applications ranging from control of industrial processes to environmental monitoring and clinical diagnostics.

New fluorescence based techniques are coming out every year which help in accelerating the pace of research and development in basic and applied life sciences, including genomics, proteomics, bioengineering, medical diagnosis and industrial microbiology. These are widely used to address fundamental and applied questions in the biological and biomedical sciences. Examples of current use of fluorescence-based technology include assays for biomolecules, metabolic enzymes, DNA sequencing, research into biomolecule dynamics cell signaling and adaptation, and fluorescence in situ hybridization (FISH) to identify specific DNA and/or RNA sequences in tissues. Recently, molecular methods have been applied to fuse the gene for Green Fluorescent Protein (GFP) to other genes leading to its expression in living cells. This allows sophisticated analysis of gene expression and cellular location of important structural proteins and enzymes. Extreme selectivity of fluorescent labels that can target specific organisms open new avenues to resolve industrially and medically relevant problems in areas such as public health, safety of foods and environmental monitoring. Innovative fluorophores, new techniques including spectrally and temporally resolved fluorescence and purpose-engineered instrumentation create niche commercial opportunities and lead towards tangible industrial outcomes.
Fluorescence techniques also find extensive uses in various areas of forensic science. Much of this work has been spurred on by the development of laser methods of visualization. One of the major beneficiaries of this attention to lasers and fluorescence has been in fingerprints. A great deal of research has been done on the development of new fluorescent dyes for visualizing fingerprints with a laser. Fluorescence has also been applied extensively to questioned-document analyses, principally in characterization of inks. Other types of evidence that use fluorimetric analyses include drugs, glass, petroleum products, and biological samples.