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Original Article

UV-visible, Infrared, Fourier transform infrared and Raman spectroscopy, spectrophotometry: Principles and Applications

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Abstract:- One way to find out how much of a known substance is in an unknown medium is to use spectroscopy, which includes spectrophotometers and other forms of the technique. The most practical and accurate way to analyse unknown samples quantitatively and qualitatively is with spectroscopy. Spectroscopic and spectrophotometric methods of many kinds are invaluable in scientific study for analysing samples down to the sub-ppm level. These methods work on the basic premise that the quantity of a certain kind of radiation—a photon—that a sample absorbs or reflects is proportional to the strength of the incident light at that wavelength. Using these methods, one may quantitatively determine the concentration of a material or solution, as well as its purity, percentage content in a combination, the type of reactions or chemical interactions that have taken place, and the absorption or reflectance of colour. For the most part, scientists have relied on spectroscopic and spectrophotometric methods, such as infrared, Raman, X-ray fluorescence, ultraviolet, and visible light spectrophotometry, among others, to help with substance identification and characterization. Quantitative measurements of various substances and elements are also made possible through atomic absorption and emission spectroscopy.
Keywords: UV-visible spectroscopy, infrared spectroscopy, Raman spectroscopy

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Introduction:

Light, or electromagnetic radiation, can be absorbed, transmitted, or reflected by any chemical found in nature. Because of this quality of the chemicals, spectrophotometric methods allow for more precise quantitative measurements. The measurement of the light's interaction with materials is the focus of spectrophotometry. When light strikes a substance having the properties of being either reflected, transmitted, scattered, or absorbed, and simultaneously the absorbed light can release light of a different frequency, this phenomenon is called light refraction. Picture 1. This happens because of the light's warmth (incandescence) or the energy it gains (e.g., electroluminescence) [1]. It is common practice in research, industry, and chemical labs to employ spectrophotometry and other forms of spectroscopy to determine the identity and concentration of chemicals. One example is the use of the Beer- Lambert-Bouguer Law in UV-visible spectrophotometry, a fundamental method for sample analysis in the chemical and pharmaceutical sciences. The concentration of biomolecules in a solution can be determined by using spectrophotometric analysis, which is widely used in biochemistry and molecular biology [2]. Spectrophotometry, both manual and automated, is widely used in clinical laboratories to determine the composition of various bodily fluids, including blood, urine, and more [3].

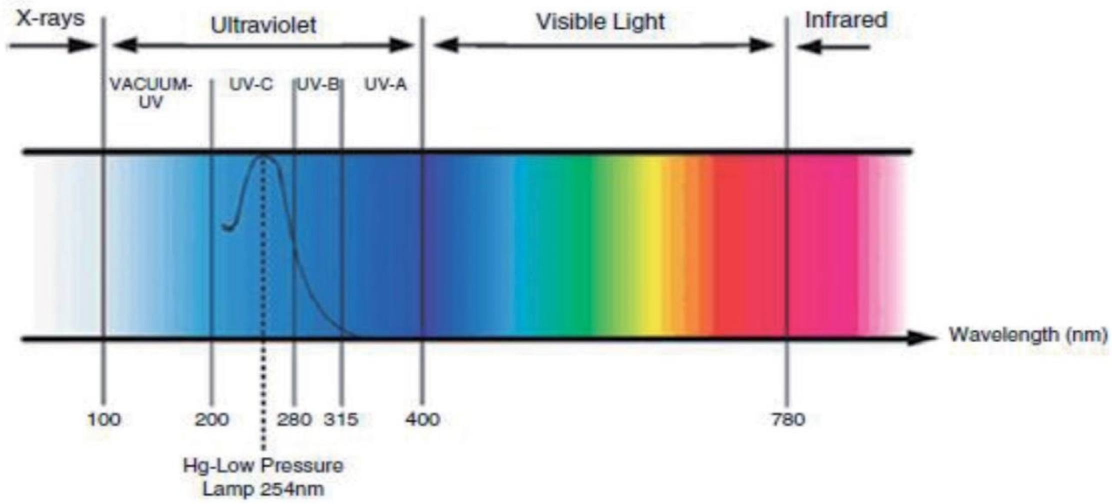

Figure 1. Electromagnetic Spectrum

The materials are analysed using a variety of spectroscopic and spectrophotometric methods. Among these, two main methods are widely used: absorption spectrophotometry, which measures the amount of light that a material absorbs at specific wavelengths to create an absorption spectrum, and UV-visible spectrophotometry, which measures the amount of light that a material reflects across the visible and ultraviolet portions of the electromagnetic spectrum [4].

The theory, technology, and practical uses of spectrophotometric methods

Light or radiation source, collimator, monochromator, and detector are the four main parts of a spectroscopic or spectrophotometric equipment. A moving exit slit, a dispersing element (such as a prism or diffraction grating), and a stationary input slit make up the monochromator (Figure 2) [6].

UV-visible spectrophotometry

Visual and ultraviolet spectrophotometry are based on the law of absorption. Beer-Lambert law, sometimes known as Beer's law, concerns the relationship between the concentration of the sample solution and the thickness of the absorbing medium. As stated in this law, the absorbing substance's concentration and the absorbing material's thickness are directly proportional to the amount of light that is absorbed [7].

Applications of UV-visible spectrophotometry

The pharmaceutical industry has made extensive use of ultraviolet-visible spectroscopy as a method for determining medication concentrations. The quantification of etravirine in both its bulk and pharmacological forms is one application of this method. The vapour phase, which is placed above the sample in its condensed state, is studied using a UV-visible spectrophotometer in the vaporisation of low-volatile chemicals [10]. Pure analytes, those that have not been decomposed, can also be identified using UV-visible spectroscopy; this is especially true for nucleic acid identification. The newly discovered genetic materials in different species of bacteria and other organisms can be identified with the help of this investigation. Using the UV-visible spectroscopy method, we were able to quantify and identify chemical molecules. The pharmaceutical industry finds this method to be particularly useful for evaluating newly produced medications [12]. One common method in biochemistry for finding compounds in bodily fluids at micromolar quantities is ultraviolet-visible spectrophotometry. Its other uses include researching biological processes and identifying species [13]. In order to learn how gold nanoparticles (AuNPs) interact with human serum albumin (HAS), a case study utilising combined UV-visible spectroscopy and chemometrics has been underway. Picture 2. Thermodynamic, kinetic, and structural characteristics were used to determine the evolution of the protein nano-conjugate based on data obtained from UV-visible spectroscopy and chemometrics, which concerned the interaction of HAS with AuNPs.

Infrared spectrophotometry

The potential for vibrational transitions in molecules arises when they absorb light with a greater wavelength than ultraviolet and visible light. The infrared spectrum is created by the vibrational transitions of the molecules.

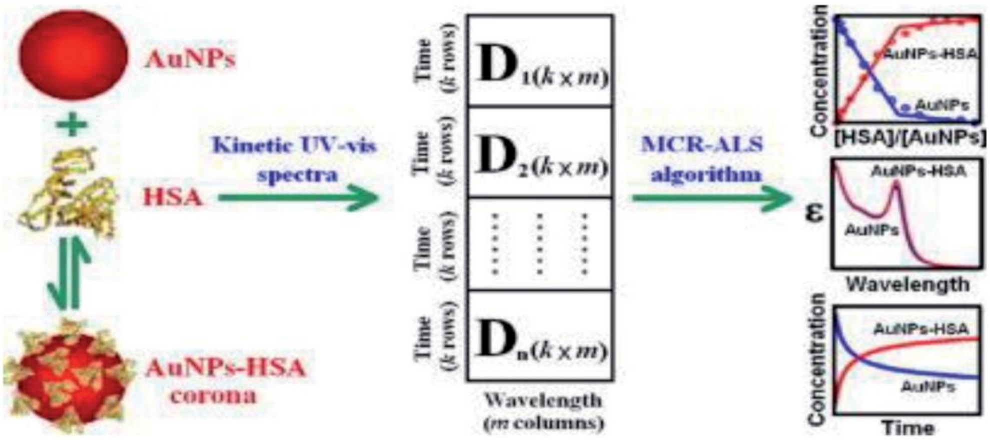

Figure 2. The relationship between citrate-capped gold nanoparticles and human serum albumin (HAS).

Applications of IR spectroscopy

Identification of compounds: Infrared spectroscopy is useful for identifying a wide range of organic molecules and their constituent parts, including aliphatic and aromatic hydrocarbons, amino acids, ether and hydroxyl groups, halogens, silicon, sulfur-oxy compounds, nitrogen, phosphorus, and silicon. The stretching and bending vibrations of C—H and C—C can be used to study aromatic and aliphatic hydrocarbons. The majority of these vibrations are exclusive to each molecule and are typically referred to as skeletal vibrations. Bending and stretching vibrations are diagnostic of the C-C-C bond in aromatic ring structures [18]. One molecule of hexene, for instance, exhibits the signature absorptions of a double bond in its spectrum. The bond stretching at 3080 cm^{-1} is associated with the alkene bonds. When the C—C double bond is stretched, it causes absorption at 1642 cm−1. The infrared spectra of 1-hexene are shown in the figure.

When it comes to studying the physicochemical properties of drug nanocarriers and identifying the functional groups on the surface of the developed nanoparticles that are involved in the drug targeting system, infrared spectroscopy has proven to be an invaluable tool [19]. To better understand how to optimise sensitivity between the interacting molecules, IR spectroscopy plays a significant role in surface biology research by studying the surface interaction of medicines, antibodies, and cell surface proteins and other biological molecules [20].

Reaction rate: for many functional groups, infrared spectra provide fantastic clue. Therefore, infrared spectroscopy can be used to evaluate enzymatic reactions that incorporate these functional groups, whether they are eaten or produced during the activity. As an example, phosphoenol pyruvate has been used to study the enzymatic activity of the pyruvate kinase. This has provided a characteristic spectrum that helps us understand how the substrate is used and the product is generated. The following [21] displays the details. How molecules interact with one another: Inter-chain hydrogen bonding are formed by polypeptide chains. The two branches of DNA do the same. The use of infrared spectroscopy has proved highly fruitful in the study of hydrogen bonding. One useful method for determining mineral structures is infrared spectroscopy. An example of this would be in a case study with the modifications.

Fourier transform infrared spectroscopy (FTIR)

FTIR is based on the same premise as infrared spectroscopy. However, compared to infrared spectroscopy, the equipment is distinct. Similar to an infrared spectrophotometer, a Fourier transform infrared spectrometer (FTIR) has a light source, a sample holder, a monochromator, and a detector; however, the interferometer is what really sets this instrument apart as being state-of-the-art. Compensator plates, beam splitters, fixed mirrors, and scanning mirrors are the special components of an interferometer that are coupled to a detector. Spectrum quality, data collecting speed, data repeatability, and ease of use and maintenance are some of the benefits of Fourier transform infrared compared to the current dispersive infrared instrument. When applied to real-world materials, Fourier transform infrared (FTIR) spectroscopy may reveal structures in a variety of forms and identify chemical components with remarkable precision. A number of biodiesel and antioxidant samples have had their fuel stability evaluated using Fourier transform infrared spectroscopy (FTIR). To do this, we can use the Fourier transform infrared spectroscopy (FTIR) to determine whether organic and inorganic molecules are present in the sample [24]. Functional groups in polymers and co-polymers have been identified using FTIR. An example of a co-polymer that uses FTIR to identify functional groups is poly-3-hydroxybutyrate (PHB). PHB demonstrates peaks at 1724 cm^{-1} for C═O stretching and 1279 cm^{-1} for the adsorption band in the ester group, respectively [25].

The biochemical information on postmortem interval estimation based on rabbit pericardial fluids was obtained through forensic analysis using Fourier transform infrared (FTIR) spectroscopy with attenuated total reflectance (ATR) accessories. Evaluation of active chemical immobilisation in biomedical material matrices has also made use of Fourier transform infrared (FTIR) spectroscopy with attenuated total reflectance (ATR). The FTIR method was used to study the biomaterial's surface for the presence of Sparfloxacin. The Sparfloxacin exhibited a wide array of absorption bands in the FTIR spectrum, which provides evidence of antibiotic-bacterium binding success [26].

Raman spectroscopy

The Raman effect, a groundbreaking finding by C.V. Raman in 1928, laid the groundwork for Raman spectroscopy, which relies on light scattering. When monochromatic radiation of a specific frequency interacts with vibrating molecules in a sample, the Raman effect states that the resulting dispersed light has a different frequency than the original light. The underlying principle is that of incident radiation's inelastic scattering [27]. After interacting with the molecules in the sample, monochromatic light in Raman spectroscopy scatters in all directions. Rayleigh scattering accounts for a large portion of this scattered radiation. Raman scattering only applies to a tiny subset of the dispersed radiation that is inherently elastically scattered. Typically, Stokes lines will show up in a Raman spectrum because the incident radiation frequency is greater than the dispersed radiation frequency. In contrast, anti-Stokes lines come into view in the Raman spectrum when the incident radiation frequency is less than the scattered radiation frequency.

The detectors employed in early types of dispersive Raman spectrophotometers were photodiode array detectors and thermoelectrically cooled photomultiplier tubes. More sensitive charge transfer devices (CTDs), like charge-coupled devices (CCDs) and charge-injection devices (CIDs), are now in use as a result of improvements in instrumentation technology. These array-shaped devices serve as detectors.

Methods of cell treatment, including Raman spectroscopy, are being developed. In order to treat numerous degenerative and fatal diseases, cell treatment sometimes involves implanting a live cell into the patient. When it comes to generating therapeutic cells, understanding their biochemical and functional characteristics, and successfully implementing them for therapy, Raman spectroscopy is a crucial tool [31].

Cervical cancer molecular testing: One of the most useful applications of Raman spectroscopy in biochemistry is the determination of molecular, cellular, and tissue structures. Researchers have found this method to be particularly useful in their quest to identify cervical cancer-causing malignant neoplasms [32].

Early identification of plant diseases is a common application of Raman spectroscopy in the agriculture, food, and biosystems industries. Like when it comes to spotting Citrus Huanglongbing and rose rosette disease (RRD) in their early stages. The impact of Tomato spotted wilt virus (TSWV) and Tomato yellow leaf curl Sardinia virus (TYLCSV) on tomatoes can be understood through the use of Raman spectroscopy in conjunction with chemometric studies. One of the useful methods for detecting food fraud is Raman spectroscopy. As an example, the detection of butter adultera-tion makes use of Raman spectroscopy in conjunction with chemometric techniques. SERS is employed to ascertain the mycotoxin deoxyniva-lenol's hazardous impact in maize, kidney beans, and oats [33].

Raman spectroscopy, similar to infrared spectroscopy, is utilised in the field of chemistry for the purpose of identifying functional groups and chemical structures through the provision of a fingerprint region in the spectrum. The biopharmaceutical business makes use of Raman spectroscopy, a potent technology. The pharmaceutical sector relies on it for a number of reasons, including the measurement and analysis of particle size during drug preparation and the identification of impurities emerging from various sources such as pipes, valves, bags, filters, etc. Raman spectroscopy plays a significant role in the biopharmaceutical industry by identifying protein structures, glycosylation, stability, and aggregation, as well as in protein formulations and identity tests [34].

Spectro-Fluorometry

A molecule exhibits fluorescence when it absorbs radiation and then releases radiation with a larger wavelength. To return to the ground state after absorbing radiation, a molecule can do it in a single step by emitting radiation of the same wavelength or in a stepwise way by producing quanta of radiation with a larger wavelength matching to each energy step. Fluorescence spectra are created as a result of this process. Fluorescence can give information on events that happen in less than 10−7 seconds because it is a very transient phenomena. Similarly, the basis of operation of fluorometry is based on the Beer-Lambert law.

One of the most useful analytical tools for determining the concentration of compounds that fluoresce at extremely low concentrations is fluorometry. Aside from a few subtle differences, a spectrophotometer and a spectrofluorometer are essentially different instruments. Figure 3.

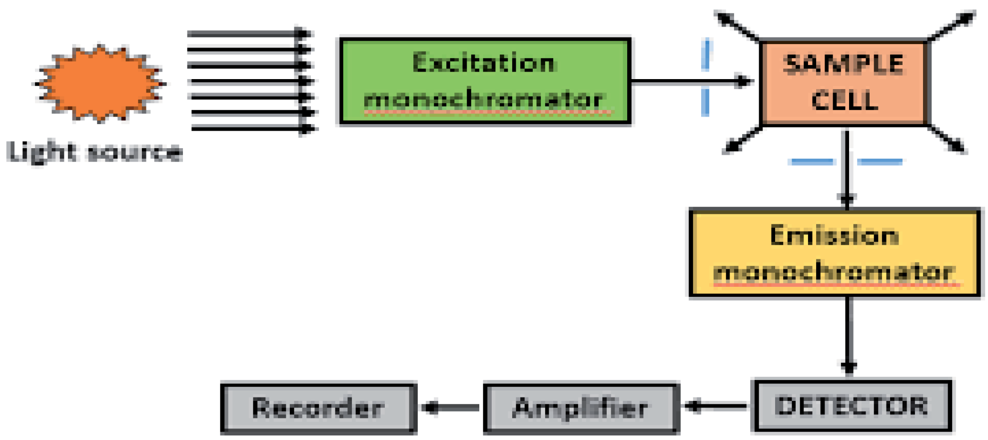

Figure 3. Instrumentation of spectrofluorometer.

In contrast to a spectrophotometer, which uses a single monochromator, this device uses two: one is positioned before the sample holder, while the other is placed after it. The sample holder incorporates a mechanism to keep the temperature between 25 to 30°C, as that is the optimal range for fluorescence. 3D protein structure determination: 20 amino acids come together to form a protein. It is crucial to understand the relationship between a structure's three-dimensional structure and its functions in computational drug discovery. Both X-ray crystallography and electron microscopy may fail to produce the desired results when asked to supply this data. In order to get around this issue, scientists have turned to fluorescence spectrophotometry, which preserves the native structure of proteins while providing accurate information about their three-dimensional structure [35].

Several dietary components, adulterants, additives, and pollutants can be identified with the help of fluorescence spectroscopy [36].

As a sensitive and quick analytical tool, fluorescence spectroscopy is ideal for use in food processing quality control. For instance, a number of writers have lately used fluorescence spectra to investigate how heat treatment affects vitamin A in milk samples. Since the milk samples were heated at 75°C for 10 minutes, the fluorescence spectra reveal that the heat treatment reduced the fluorescence intensity at 320 and 290 nm. In comparison to milk samples that were cooked at 55°C for the same duration.

Spectrophotometers are commonly used for qualitative and quantitative analyses, with the former including the monitoring of substances like riboflavin and thiamine as well as hormones like oestrogens, cortisol, serotonin, and dopamine; the latter for drugs like barbiturates and lysergic acid; and the former for pesticides made of organophosphorus. Porphyrins, cholesterol, and even some metal ions; and research into protein structure (proteins containing FAD).

Nuclear magnetic resonance spectroscopy (NMR)

When it comes to determining the structure of molecules, nuclear magnetic resonance (NMR) is a powerful and effective approach. Natural magnetic resonance spectroscopy (NMR) works on the premise that a radiofrequency transmitter and a powerful magnetic field can excite the atomic nuclei of sample molecules, resulting in the formation of spectral lines in the resulting spectra [40].

The following parts make up an NMR spectrometer: A radiofrequency transmitter (RF) is the source of radiation since it produces the radiofrequency current. The signal is utilised to excite protons in the magnetic field, and it is created by the current that is delivered to the transmitting coil. A magnet that generates a magnetic field within its own volume—a superconducting magnet. A magnetic field of 1–10 T can be generated. The sophisticated nuclear magnetic resonance (NMR) spectrometers are made comprised of solenoids that produce a magnetic field stronger than 3.5 T. The magnet is designed with a hole to accommodate the sample probe and an RTS coil assembly to minimise field inhomogeneity throughout the active sample volume. Free induction decay (FID) is the digital version of the absorbed signal that a receiver receives.

Neutrinometric resonance (NMR) is an effective tool for metabolic studies in biochemistry. It quickly became the go-to technique for learning about metabolic network dynamics and compartmentalization [41]. This aside, NMR is also helpful for studying whole biological samples containing 31P isotope, including kidneys, hearts, and skeletal muscles.

Using nuclear magnetic resonance spectroscopy, pharmaceutical researchers may see molecules and individual atoms in a variety of liquids and solids [42]. Nondestructive molecular imaging (NDI) offers insight into the chemical make-up and dynamic properties of organic molecules in living systems, as well as the ability to quantify and elucidate the structures of a wide range of organic compounds [35]. Antibiotics like ciprofloxacin, azithromycin, and valinomycin, as well as amino acids, proteins, and carbs, have their structures studied to learn more about their roles.

For the purpose of confirming the identity of newly synthesised compounds, scientific publications typically demand NMR spectroscopy in the field of chemistry because of its unambiguous utility in identifying novel molecules. Various in vivo and synthetic membrane transport systems have been investigated using nuclear magnetic resonance (NMR). The transfer of Na+ ions in red blood cells (RBCs) in humans is one area that has made use of this method. Metabolite concentrations have been quantitatively determined using NMR. One such application is the use of nuclear magnetic resonance (NMR) to measure phosphocreatine concentrations in human muscle [7]. In the petroleum business, nuclear magnetic resonance (NMR) is a highly valuable technology for identifying and quantifying hydrocarbons. For instance, in FACE Petrol F, the quantitative assessment of liquid hydrocarbons is aided by the 1H and 13C nuclear magnetic resonance. Its high spectral resolution makes it a valuable tool for measuring hydrocarbon concentrations in FACE Petrol F liquids [43].

Conclusion

In many different scientific disciplines, including chemistry, biology, physics, engineering, agriculture, and medicine, spectroscopy is a crucial analytical tool for analysing different molecules. Methods like ultraviolet-visible spectrophotometry, infrared spectroscopy, Raman spectroscopy, nuclear magnetic resonance spectroscopy, and electrochemical scatter spectroscopy are employed in this process. The data that these methods are yielding is incredibly helpful for academics, students, and instructors. Nondestructive, consistent, and dependable, these analytical procedures necessitate no or no sample preparation. We are able to utilise these techniques on samples that are solid, liquid, or powdered. Detection limits at concentrations as low as sub-ppm can be readily and concurrently provided by these, making them useful for a variety of elemental analyses. Because they give accurate information on the elements to be studied, spectro-photometric techniques are, hence, extremely valuable in elementary analysis in nearly every subject.

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