Novelty Journals

ISSN 2394-9651

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 12, Issue 1, pp: (53-60), Month: January - April 2025, Available at: www.noveltyjournals.com

Ultraviolet-Visible Spectroscopy, Importance, Principle, Structure and Most Important Applications: A Study Review

Azhar S. Alaboodi[1], Shaymaa Awad Kadhim[2], Ahmed Shaker Hussein[3]

[1,2]University of Kufa/ Faculty of Science/ Physics Department/ Najaf/ Iraq

Department of Basic Science /College of Dentistry / University of Babylon / Iraq.

DOI: https://doi.org/10.5281/zenodo.14965556

Published Date: 04-March-2025

Abstract: **Most research studies aim to reach new methods in diagnosis, whether for diseases or materials used in various fields of science such as medicine, physics, agriculture, etc., which help in early detection of most difficulties, whether a disease or a material used to solve a specific problem. One of the techniques used for such purposes is ultraviolet spectroscopy. UV spectroscopy, one of the earliest automated analytical methods, has shown itself to be a flexible and essential instrument in many scientific domains. It is a useful tool in analytical chemistry because of its capacity to differentiate between various kinds of materials. This technique is frequently employed to ascertain the identification, potency, calibre, and purity of various materials. UV spectroscopy's fundamental idea is that samples absorb particular light wavelengths, and it offers important information about how materials react to this absorption.**

In UV spectroscopy, the use of Beer's Law, a general theory, describes how samples absorb radiant radiation. Hundreds of studies have demonstrated the value of this device as a preliminary determination in the fields of agriculture, nanomaterials, and medicine for a variety of diseases. It is noteworthy that this method is characterized by its accuracy, simplicity, and broad range of applications. Colourless and coloured chemicals in the visible and ultraviolet spectrums can be analyzed thanks to the device's operation in the 200–800 nm wavelength range. To put it briefly, UV spectroscopy is a vital analytical method with many uses that forms the basis of scientific investigation and analysis. These methods should become more clinically accepted as a result of future immunological research.

Keywords: **UV Spectroscopy, monohromator, detector, Adenine, guanine, cytosine.**

1. INTRODUCTION

Spectroscopy is the science that studies the interaction of electromagnetic radiation with matter. This science began when Isaac Newton divided light with a prism, which was previously called optics, and thus the study of visible light, which was originally called “colour” later came to include the entire electromagnetic spectrum, within the framework of the studies of James Clerk Maxwell[1]. The interaction between light or electromagnetic radiation and matter shows a result of great importance, as “matter absorbs or emits energy in the form of discrete quantities called quanta”[2]. Ultraviolet spectroscopy is known as the absorption or reflection spectroscopy of ultraviolet rays, while the visible parts close to the electromagnetic spectrum are known as ultraviolet spectroscopy, or ultraviolet-visible spectroscopy may be referred to as (UV-Vis or UV/ Vis)[3]. In order for a substance to absorb ultraviolet light, it must form a chromophore[4]. This methodology is widely used in applied and basic applications due to its low cost and ease of implementation[5]. Using light with wavelengths between 200 and 800 nanometers, which fall into the ultraviolet or visible zone, UV spectroscopy is shown to be an effective

Novelty Journals

ISSN 2394-9651

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 12, Issue 1, pp: (53-60), Month: January - April 2025, Available at: www.noveltyjournals.com

analytical instrument. This method can evaluate both coloured compounds in the visible range (400-800 nm) and colourless substances in the UV region (200-400 nm). The discrete wavelengths of UV or visible light absorbed or transmitted by a sample compared to a reference or blank are primarily determined by UV spectroscopy. This feature, which is linked with some complexity to the composition of the sample, gives the ability to detect the components of the sample and their concentrations[2].

Researchers have conducted a simple spectroscopic study of some natural oils used in medical applications, such as castor oils, eucalyptus, and glycerin. The study shed light on the form of ultraviolet-visible (UV-vis) spectroscopy as a non-destructive and rapid technique in determining the absorption spectrum of some natural oils used for medical purposes. The maximum quantity of the absorption spectrum was at 320 nm, or 1.6574%, while the absorption value of castor oil at 340 nm was around 3.2324%. At a wavelength of 380 nm, the absorption spectrum's greatest value, or 0.0401%, occurs[6].

Other researchers have also studied natural blood samples from healthy people using spectroscopic techniques not only to understand the biological nature of the disease, but also to diagnose the disease[7].

Researchers have used quantitative UV and visible spectroscopy for clinical and pre-clinical applications in cancer treatment, and this study included new and emerging studies linking optically measured parameters to independent measures such as immunohistochemistry, which should help increase the clinical acceptance of these techniques[8].

Current uses of UV/VIS spectroscopy are to estimate biomarkers, analyze blood samples, and detect certain compounds in body fluids. This technology diagnoses and monitors various diseases, including liver disorders, kidney diseases, and certain types of cancer, because it provides healthcare professionals with valuable insights into patients' health as well as helping them develop effective treatment strategies.

Ultraviolet rays

When white light is shined on a prism or slit, it will be resolved into a spectrum. Red light (lowest frequency and lowest energy) is at the beginning of the spectrum, while purple light (highest frequency and highest energy) is at the end of the spectrum, as shown in the figure 1[5]:

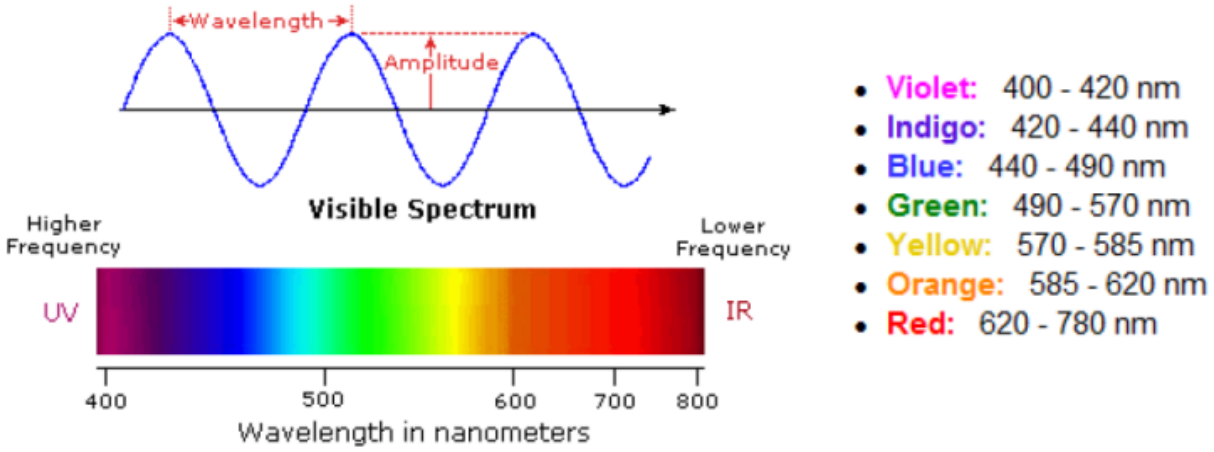

Fig.1. Electromagnetic radiation is in the form of a sine wave[2].

Outside of this side is the invisible region known as electromagnetic radiation. Beyond the high-energy ultraviolet (UV) rays, there are various types of invisible lights. Long-term exposure to sunlight damages human skin due to UV radiation. According to the International Organization for Standardization's (ISO) classification in ISO 21348:2007, ultraviolet radiation is classified into four categories (sectors) based on its wavelength (energy): EUV, MUV, FUV, and NUV. Near ultraviolet radiation, located near X-rays, the most active of the four types, at the end of the ultraviolet spectrum, is known as extreme ultraviolet (EUV). Between (EUV) and (MUV) are medium ultraviolet (MUV) and far ultraviolet (FUV). Figure 2 defines sectors and their wavelengths in accordance with ISO 21348:2007 [5]:

Novelty Journals

ISSN 2394-9651

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 12, Issue 1, pp: (53-60), Month: January - April 2025, Available at: www.noveltyjournals.com

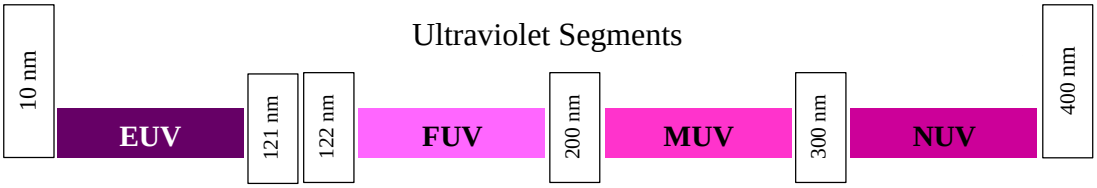

Fig.2. Wavelengths of Segments of ultraviolet rays.

Principle

Spectroscopy is unique spectra that are based on the principle of ultraviolet-visible radiation. These spectra are produced when ultraviolet or visible light is absorbed by chemicals. Spectroscopy is based on the interaction of light and matter. The material will undergo phases of excitation and re-excitation when light is absorbed by it, creating a spectrum. When an electromagnetic wave hits matter, it can undergo a number of processes, including transmission, absorption, reflection, and dispersion[9].

When the structure of a molecule or ion is electronically transformed by radiation, the object will exhibit absorption in the visible or ultraviolet range. When light is absorbed by the sample in the ultraviolet or visible range, the molecules of the sample will be electronically altered, Therefore; the electrons will be promoted from their ground state orbit to higher energy orbits excited by the energy of absorbed light or antibonding orbit. There are three possible types of ground state orbits involved: σ (bonding) molecular, π (bonding) molecular orbital and n (bonding) atomic orbital, in addition to two types of antibonding orbitals that may participate in the transition process: σ* (sigma star) orbital and π* (pi star) orbital. Note that there is no antibonding orbital n* because the n electrons don't form bonds, and as a result electronic transitions can occur by absorbing ultraviolet and visible light[10].

Absorption of ultraviolet and visible light leads to types of electronic transformations, the most important of which are:

1. σ → σ* Transitions: The electron in the bonding orbital σ is stimulated to the anti-orbit that it corresponds to, and this stimulation requires a lot of energy[11].

2. n → σ* Transitions: These transformations occur in saturated compounds that contain atoms with lone pairs, or unpaired electrons, and they usually require less energy than the energy needed to convert σ to σ*[12].

3. n → π* and π → π* Transitions: Electron transitions from n or π to the excited state π*. It is the basis for most absorption spectroscopy of organic compounds. This is because of the spectral range (between 200 and 700 nanometers) suitable for experimentation[13].

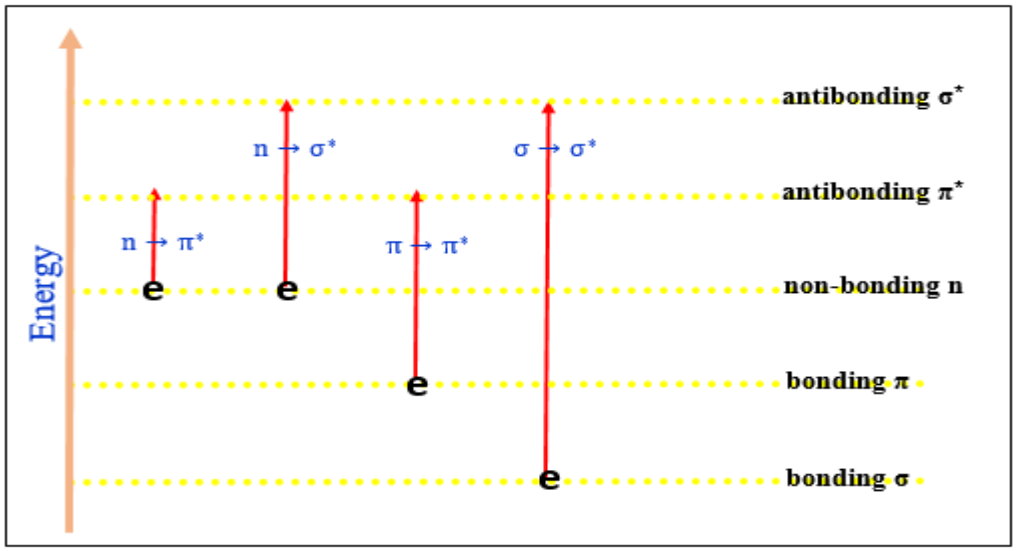

Fig.3. Electronic transitions [2].

Novelty Journals

ISSN 2394-9651

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 12, Issue 1, pp: (53-60), Month: January - April 2025, Available at: www.noveltyjournals.com

Beer-Lambert Law

When a beam of electromagnetic radiation passes through an absorbing material, the intensity of the incident radiation (I_o) will be higher than the transmitting radiation (I). To know how materials absorb radiant energy, the general rule known as Beer's law can be used to describe[4].

According to this law, the amount of radiation absorbed (absorption, A) or transmitted by a solution or medium is inversely related to the amount of absorbed substance present, c (mole per liter), and the length of the absorbed substance. Passage of radiation through the sample, b. (poison), Therefore; the plot of absorption versus concentration should be a straight line with slope equal to εb, passing through the origin[14].

$$A = -\log\frac{I}{I_0} = \varepsilon bc$$

Where A represents the absorbance, ε represents the molar extinction coefficient, which is equal to $\varepsilon = \frac{K}{2.303}$, b represents the path length of the light inside the sample and c represents the concentration of the absorbing sample.

To get a constant route length that may be used to calculate the absorbent concentration in the sample, it is essential to understand how quickly absorbance changes with concentration.

Components of ultraviolet-visible spectroscopy

The UV-VIS spectrophotometer consists of the following essential components: light source, monochromator, sample & reference cells, detector and recorder[15].

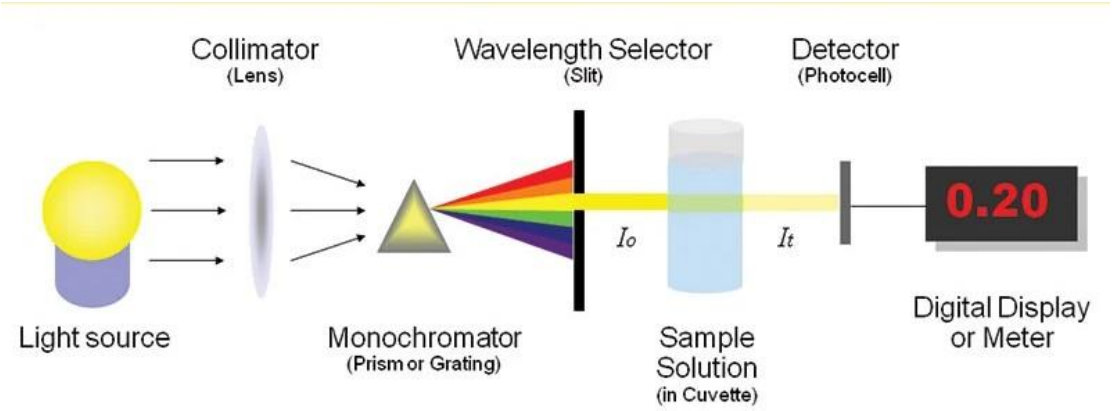

Fig.4. Instrumentation of UV Spectrophotometer(Ultraviolet-spectroscopy single beam)[16].

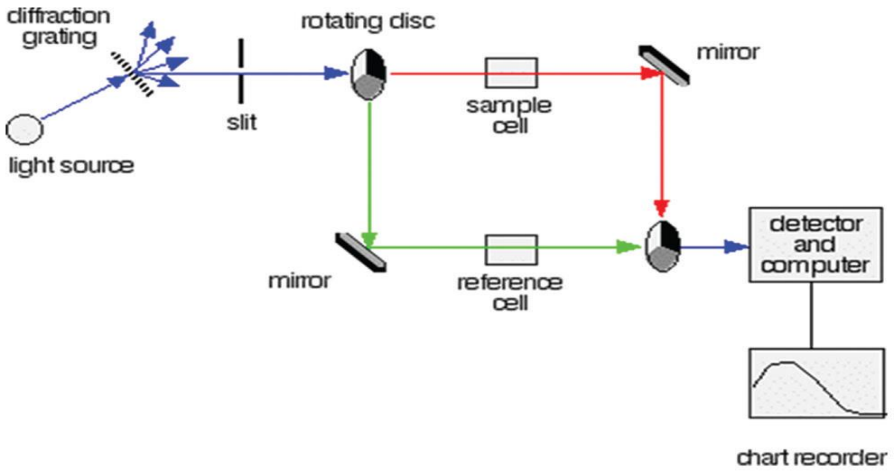

Fig.5. Instrumentation of UV Spectrophotometer (Ultraviolet-spectroscopy double beam)[17].

1. Sources of ultraviolet radiation and the light source: The strength of the radiation source varies steadily and unsurprisingly over its wavelength. Also, the light provided by the light source must be constant and stable. The light source used must provide constant and stable light. When deuterium or hydrogen is excited under the influence of low pressure, it produces ultraviolet radiation in the form of a continuous spectrum, where an excited molecular species is formed, and thus this molecular species is broken, producing an ultraviolet photon and two atomic species[18]. Various sources of ultraviolet radiation include:

1.a. Hydrogen lamp: They are highly reliable and stable lamps. The radiation emitted from it is continuous and its range ranges between (160-380) nm[2].

1.b. Deuterium lamp: It is the source that emits ultraviolet radiation and is symbolized by the symbol D2. The wavelength of the radiation emitted from it ranges from about (160-450) nm, and it is more expensive than a hydrogen lamp.

1.c. Tungsten lamp: It is the most common source used in spectrophotometers, as it operates in the wavelength range between 330 and 900 micrometers[3].

1.d. Xenon Lamp: It is a high-energy light source. The wavelength of the light emitted from it ranges from about (250-600) nm in the ultraviolet and visible spectra. The xenon lamp flashes at a frequency of 80 Hz, making it longer lasting than other lamps. In terms of manufacturing, it is the highest cost[5].

2. Monochromators: Monochrome devices are used to convert multicolored or heterochromatic light into monochromatic light, and are considered better and more efficient than filters. There are two types of Monochromators: prism monochromator and grating monochromator[3].

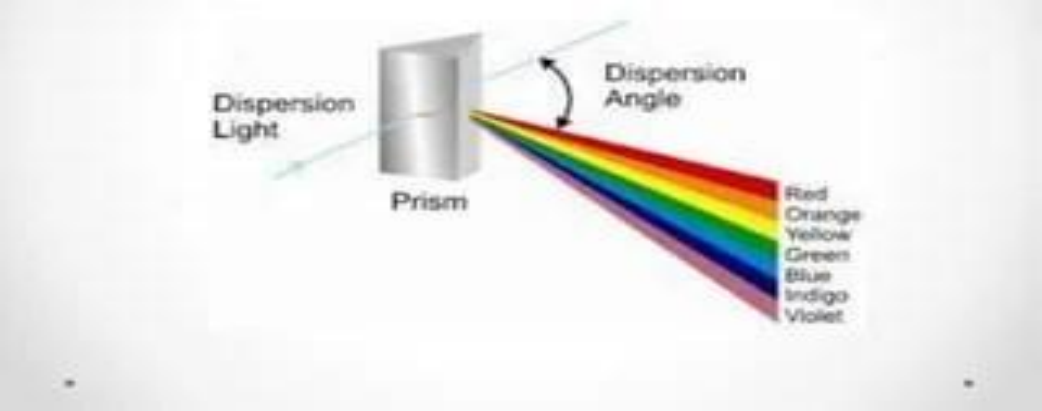

Fig.6. prism monochromator

3. sample & reference cells: they need transparent containers for radiation to pass through. Quartz or fused silica are required for spectroscopic analysis in the UV region. These cells are also transparent in the visible region. Silicate glass can be used to manufacture cuvettes for use in the wavelength between 350 and 2000 nanometers in the visible region, while it cannot be used for the same purpose in the ultraviolet region, because this material absorbs ultraviolet rays[2].

4. Detectors: It is a device that converts light energy absorbed by the sample into electrical signals and displays them on reading devices. The incidence of transmitted radiation on the detector is determined by the intensity of the radiation absorbed by the sample. There are several advantages that must be available in the detector: that it gives quantitative responses, has high sensitivity and low noise level, short response time, and responds to a large amount of incoming radiation. In absorption spectrophotometers the following types of detectors are used[2]:

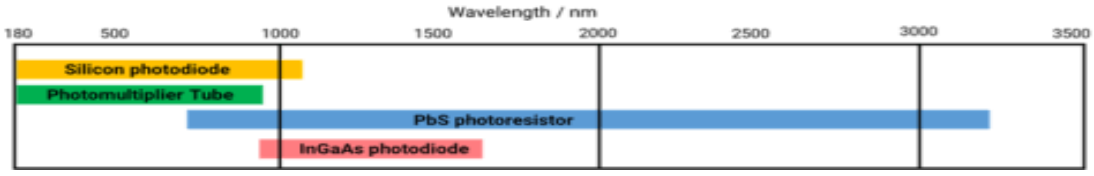

Fig.7. Detector option wavelength ranges

The most important applications of ultraviolet and visible spectroscopy

1. **DNA AND RNA ANALYSIS:** Determining the purity and concentration of RNA and DNA is a common application, which can be summarized in two tables 2 and 3[19].

Table 2. Absorption ratios of 260/280 and 260/230 provide beneficial absorption of UV rays[20].

Wavelength used in absorbance analysis in (nm)	Absorption of ultraviolet radiation at this wavelength indicates the presence of:	Causes of absorption of ultraviolet rays at this wavelength?
230	Protein	Protein
260	DNA and RNA	Adenine, guanine, cytosine, thymine, uracil
280	Protein	Mostly tryptophan and tyrosine

Table 3. Expected absorption ratios of UV rays 260/280 and 260/230 for DNA and RNA analysis[20].

Absorbance ratio	**Typical values**
260/280	The usual absorbance ratio for pure DNA is 1.8. The usual absorbance ratio for pure RNA is 2.0.
260/230	For RNA and DNA, the absorbance ratio typically ranges from 2.15 to 2.50.

2. **PHARMACEUTICAL ANALYSIS:** Screening and characterization of potential therapeutic compounds is one application, where UV spectroscopy plays a vital role in drug discovery[21].
3. **BACTERIAL CULTURE:** To estimate cell concentration and Tracking growth in the process of culturing bacteria. The wavelength of 600 nm is often used in UV spectroscopy, due to the optical properties of the bacteria[22].
4. **BEVERAGE ANALYSIS:** This method is employed in the examination of bacterial cultures in order to pinpoint particular substances found in drinks. For instance, by matching what are known as the peak absorption wavelengths, they can be readily identified in wine for quality control purposes utilizing UV absorption[23].

The advantages of UV-Vis spectroscopy

UV-visible spectroscopy has many advantages, the most important of which are[24]:

1. Accuracy in measurement.
2. Ease of use.
3. Provide robust operation.
4. Ease of operation.
5. Low cost.
6. Covers all wavelengths in the ultraviolet and visible spectrum.
7. Possibility of use in qualitative and quantitative analysis.
8. Possibility of obtaining the graph derived using UV-VIS a spectrophotometer.
9. It is used in drug analysis and analyzes containing chromophores.

The Disadvantages of UV-Vis Spectroscopy

Although the UV-Vis spectrometer has important advantages, it has some weaknesses, the most important of which are[25]:

1. Only molecules containing chromophores and liquid samples can be analysed.
2. pH, temperature, contaminants and impurities do not affect the absorption results.
3. It takes time to prepare to use it.
4. Handling of the cuvette can affect the reading.

2. CONCLUSION

In conclusion, by analyzing the interaction of a sample with electromagnetic radiation, UV-Vis spectroscopy is an essential and indispensable technique in characterization, as it provides deep insights into the properties of that sample. It looks at how nanofillers improve the properties of nanocomposites. UV-Vis spectroscopy is the ideal method for estimating the concentration of absorbed species if used with the correct standard curve and applied to various samples of blood, tissue, saliva, urine and pathogens, especially since it is considered low cost. As a result, it enables analysts to extract information from the spectra corresponding to different samples and in all fields of science, medicine and nano agriculture. This facilitates the ability of analysts to extract meaningful information from spectra of different concentrations that correspond to different wavelengths because the derivatives of the UV spectrum provide useful information for understanding many substances used in pharmaceutical formulations.

REFERENCES

[1] Pate, S., et al., *Prof. Mitali Dalwadi, Dr. Umesh Upadhyay, A Review on "Uv Visible Spectroscopy".* International Journal of Pharmaceutical Research and Applications, 2022. **7**(5): p. 1144-1151.

[2] Mandru, A., J. Mane, and R. Mandapati, *A Review on UV-visible spectroscopy.* Journal of Pharma Insights and Research, 2023. **1**(2): p. 091-096.

[3] Akash, M.S.H., et al., *Ultraviolet-visible (UV-VIS) spectroscopy.* Essentials of pharmaceutical analysis, 2020: p. 29-56.

[4] Shukla, R., et al., *Chromophore-an utility in uv spectrophotometer.* Inventi Rapid: Pharm Ana & Qual Assur, 2012. **2012**(3): p. 334.

[5] Perera, K.S., *UV-visible Spectroscopy*. 2022.

[6] Numan, N.H., et al., *Characterization of Some Natural Oils Used for Medical Purposes by Ultraviolet–Visible spectroscopy.* journal of the college of basic education, 2019. **25**(105): p. 36-44.

[7] Kanagathara, N., et al., *FTIR and UV-Visible spectral study on normal blood samples.* IJPBS, 2011. **1**(2): p. 74-81.

[8] Dixon, A.R., et al., *Recent developments in multiplexing techniques for immunohistochemistry.* Expert review of molecular diagnostics, 2015. **15**(9): p. 1171-1186.

[9] Rocha, F.S., et al., *Experimental methods in chemical engineering: Ultraviolet visible spectroscopy—UV-Vis.* The Canadian Journal of Chemical Engineering, 2018. **96**(12): p. 2512-2517.

[10] Verma, G. and M. Mishra, *Development and optimization of UV-Vis spectroscopy-a review.* World J. Pharm. Res, 2018. **7**(11): p. 1170-1180.

[11] Kumar, S.G. and L.G. Devi, *Review on modified TiO2 photocatalysis under UV/visible light: selected results and related mechanisms on interfacial charge carrier transfer dynamics.* The Journal of physical chemistry A, 2011. **115**(46): p. 13211-13241.

[12] Hou, D.-B., et al., *Water quality analysis by UV-Vis spectroscopy: A review of methodology and application.* Spectroscopy and Spectral Analysis, 2013. **33**(7): p. 1839-1844.

[13] Schoonheydt, R.A., *UV-VIS-NIR spectroscopy and microscopy of heterogeneous catalysts.* Chemical Society Reviews, 2010. **39**(12): p. 5051-5066.

[14] Jentoft, F.C., *Ultraviolet–visible–near infrared spectroscopy in catalysis: theory, experiment, analysis, and application under reaction conditions.* Advances in catalysis, 2009. **52**: p. 129-211.

[15] Mangam, V.T., et al., *Novel colorimetric approach for amikacin estimation in pure powder and its pharmaceutical formulations.* World Journal of Biology Pharmacy and Health Sciences, 2023. **14**(1): p. 270-279.

[16] Yang, Z. and M. Zhu, *Case Studies: Ultraviolet-Visible (UV-Vis) Spectroscopy*, in *Springer Handbook of Advanced Catalyst Characterization*. 2023, Springer. p. 265-283.

[17] Lange, R. and C. Balny, *UV-visible derivative spectroscopy under high pressure.* Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology, 2002. **1595**(1-2): p. 80-93.

[18] Sirajuddin, M., S. Ali, and A. Badshah, *Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltametry.* Journal of Photochemistry and Photobiology B: Biology, 2013. **124**: p. 1-19.

[19] Lai, Q., et al., *Ultraviolet-visible spectroscopy of graphene oxides.* AIP advances, 2012. **2**(3).

[20] Korshin, G.V., C.-W. Li, and M.M. Benjamin, *Monitoring the properties of natural organic matter through UV spectroscopy: a consistent theory.* Water Research, 1997. **31**(7): p. 1787-1795.

[21] Wondimkun, Z.T., et al., *The determination of caffeine level of wolaita zone, Ethiopia coffee using UV-visible spectrophotometer.* American Journal of Applied Chemistry, 2016. **4**(2): p. 59-63.

[22] Gendrin, C., Y. Roggo, and C. Collet, *Pharmaceutical applications of vibrational chemical imaging and chemometrics: a review.* Journal of pharmaceutical and biomedical analysis, 2008. **48**(3): p. 533-553.

[23] Yu, J., et al., *Review of recent UV–Vis and infrared spectroscopy researches on wine detection and discrimination.* Applied Spectroscopy Reviews, 2018. **53**(1): p. 65-86.

[24] Pinheiro, H.M., E. Touraud, and O. Thomas, *Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters.* Dyes and pigments, 2004. **61**(2): p. 121-139.

[25] Malarat, N., et al., *Electrochemical platform based on activated carbon/graphene oxide-gold nanoparticle composites for the electrochemical sensing of methylparaben in cosmetic samples.* Microchemical Journal, 2023. **188**: p. 108473.