

Application of UV-vis spectroscopy in the detection and analysis of substances

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Abstract. Ultraviolet-visible (UV-vis) spectroscopy is a spectral measurement technique that relies on Lambert-Beer's law. Due to its user-friendly nature, rapid analytical capabilities, broad applicability, and non-destructive properties, it has gained significant prominence across various scientific disciplines. In the realm of environmental science, UV-vis spectroscopy can be employed for effectively detecting water quality by determining the concentration of heavy metals and related chemicals in water sources. Within the field of clinical medicine, UV-vis spectroscopy plays an indispensable role in disease diagnosis and patient health monitoring. Agriculture and plantation industries greatly benefit from UV-vis spectroscopy due to its capability in facilitating precise analysis of soil and plant nutrients. The field of materials science heavily relies on ultraviolet-visible spectroscopy for the examination of optical properties pertaining to various materials. Moreover, UV-vis spectroscopy finds extensive application in organic chemistry for compound identification and purity evaluation. This research aims to provide a comprehensive overview of multiple applications of UV-vis spectroscopy while exploring its potential synergies with specific technologies across different fields.

Keywords: UV-vis spectroscopy; Detection; Mechanism.

1. Introduction

Spectroscopy is a technique used to analyze the absorption of specific wavelengths of light energy by molecules and atoms in incident light, leading to electron transitions and the formation of characteristic spectra [1]. Ultraviolet-visible (UV-vis) spectroscopy involves the absorption of UV-vis energy by molecules or functional groups, resulting in distinctive band spectra. The acquired UV-vis spectra exhibit distinct characteristics that can be effectively utilized for both qualitative and quantitative analysis of organic and inorganic substances, encompassing elemental and compound analysis, examination of molecular structure, as well as identification of material composition [2].

The UV-vis spectroscopy approach is widely utilized and plays a pivotal role in various fields today. In clinical medicine, it is employed to determine target extracts (zinc oxide nanoparticles) and aid in their extraction from aloe vera as an antibiotic. In experimental chemistry, UV-vis spectroscopy provides a precise quantitative reference for accurately measuring the amount of O₂ produced through absorbance bleach observed during experiments assisted by specific probes. In geology and environmental science, UV-vis spectroscopy accurately assesses water quality based on the concentration of multiple pollutants such as oxygen demand, heavy metal ions, nitrate nitrogen, and dissolved organic carbon. Additionally, in agriculture, UV-vis spectroscopy determines phosphorus content in animal feed and facilitates conformity control measures. These operations are built upon the foundation of UV-vis spectroscopy integration with other disciplines to achieve desired objectives.

UV-vis spectroscopy is based on the Beer-Lambert Law, which assumes a linear relationship between optical absorbance and substance concentration. Therefore, its applicability is limited to cases where this relationship remains linear, as required by the Beer-Lambert Law [3]. Consequently, UV-vis spectroscopy is suitable only for trace analysis; for high concentrations of substances (generally >0.01mol/L), the deviation from linearity renders UV-vis spectroscopy unsuitable. Moreover, due to various factors that affect the Beer-Lambert Law such as non-monochromatic light, stray light, noise and chemical interferences among others, strict requirements are imposed on both



the light source and experimental environment in UV-vis spectroscopy. In addition, UV-vis spectroscopy necessitates a substantial number of representative samples for modelling chemical analysis and establishing corresponding chemical systems. Apart from this standard requirement, UV-vis spectroscopy offers the convenience and efficiency in obtaining results. Firstly, UV-vis spectroscopy operation is straightforward and convenient, devoid of complex procedures, allowing direct measurement in the cupola. Additionally, UV-vis spectroscopy analysis exhibits rapid speed with most general samples completed within 1-2 minutes. Thirdly, the sample remains undamaged during the UV-vis spectroscopy detection process, enabling non-destructive testing and facilitating repeated experiments. Lastly but not least importantly, compared to other spectral measurement methods, UV-vis spectroscopy boasts a wide detection range that reflects material molecules' absorption characteristics to electromagnetic waves within the 200-760 nm range [4].

2. Water quality detection

Due to its numerous advantages, such as high precision, efficiency, non-destructive sampling, environmental friendliness, and affordability, UV-vis spectroscopy has increasingly gained recognition as the optimal method for identifying pollutants in aquatic environments. UV-vis spectroscopy enables the evaluation of water pollution levels through customized scales and algorithms that adapt to different indicators of water quality by developing relevant models for absorbance, organic matter concentration, and inorganic matter concentration. For instance, Ma et al. utilized an enhanced Water Quality Index (WQI) combined with principal component analysis to identify the key variables influencing water quality in aquaculture areas [5]. Similarly, Giudicianni et al. employed principal component analysis (PCA) and wavelet transform techniques to calculate the water quality index [6]. Conclusions are derived by integrating methods of UV-vis spectroscopy deformations and simplifications with the detection results. However, the direct utilization of UV-vis spectroscopy and the original spectrum for modeling would result in a complex model that necessitates extensive computational time, thereby impeding practical usage and hindering its widespread acceptance. To identify the essential components for measurement effectively, PCA efficiently reduces computational complexity by employing principles of linear algebra to determine feature wavelengths and decrease data dimensionality. PCA is employed in the processing of UV-vis spectroscopy water quality parameter data to effectively reduce the dimensionality of spectral data, thereby simplifying model complexity and extracting valuable information from the spectra. This is accomplished by encapsulating the unique characteristics of the primary pollutant species within the spectral matrix and subsequently employing Chi-square analysis to assess local anomalies based on their distribution within the principal molecular space. Furthermore, a comparative analysis of the modelling outcomes with and without implementing PCA dimensionality reduction reveals a clear enhancement in prediction accuracy for input data when utilizing PCA. The evaluation of water quality in the PAC test encompasses the assessment of crucial parameters including chemical oxygen demand (COD), heavy metal content, nitrate nitrogen ($\text{NO}_3\text{-N}$), dissolved organic carbon (DOC), and turbidity. The distinctive absorption properties of pollutants give rise to their respective spectral curves. Nitrate and nitrite, commonly utilized for water quality monitoring, exhibit a spectral absorption range spanning from 200 to 250 nm. Unsaturated aldehydes, conjugated dienes, and unsaturated ketones all absorb light within the wavelength range of 220-250 nm. Additionally, both turbidity and organic matter effectively absorb light in the range of 380-750 nm.

3. Detection of singlet oxygen

The singlet oxygen ($^1\text{O}_2$), the lowest electron-excited state of molecular oxygen, possesses distinct spin properties in comparison to its triplet ground state [7]. Owing to its exceptional reactivity, it holds significant importance in diverse chemical and industrial applications, as well as plays a pivotal role in various biological processes such as intracellular signaling and cellular defense against bacterial infections. Notably, this latter function serves as the fundamental basis for photodynamic treatment (PDT), wherein an appropriate photosensitizer is administered to a specific tissue region

prior to light exposure, resulting in the generation of $^1\text{O}_2$. This methodology enables the concurrent targeting of specific regions via drug delivery while co-localizing with light sources. The employed photosensitizers consist of organic dye molecules that demonstrate absorbance bands within the visible range of the electromagnetic spectrum. Upon absorption of light, these photosensitizers undergo intersystem crossing and effectively transfer energy to molecular oxygen, resulting in the generation of $^1\text{O}_2$. To identify reactions involving $^1\text{O}_2$, a molecular singlet oxygen probe is typically employed for monitoring changes in various characteristics. UV-vis spectroscopy is commonly utilized as a detection tool for $^1\text{O}_2$ due to its slightly reduced sensitivity compared to fluorescence but enhanced resistance against artifacts caused by absorbance quenching or trace impurities that may dominate the absorbance spectrum of compounds with high extinction coefficients. UV-vis spectroscopy can be effectively integrated with a range of carefully selected chemicals, namely 1,3-diphenylisobenzofuran (DPBF), 9,10-anthracenediyl-bis(methylene) dimalonate (ABDA), or Rose Bengal (RB). These substances possess distinctive characteristics and can be suitably employed under diverse experimental conditions and objectives. In addition, there are significant variations in the spectral ranges of these molecules. RB absorbs light at approximately 550 nm, while ABDA exhibits absorption within the range of 350 to 400 nm. On the other hand, DPBF shows absorption between 400 and 450 nm. By employing these chemically diverse compounds in conjunction with UV-vis spectroscopy techniques, precise detection of molecular oxygen becomes feasible across a wide range of research contexts. Notably, DPBF demonstrates exceptional sensitivity towards $^1\text{O}_2$ through a diffusion-limited mechanism. DPBF exhibits remarkable efficiency in capturing up to 50% of singlet oxygen generated in alcohol/water or micellar solutions, owing to its distinctive property. Furthermore, the utilization of deuterium oxide (D_2O) as a solvent medium instead of regular water (H_2O) can lead to an enhanced capacity for capturing singlet oxygen. In situations where there is minimal production of singlet oxygen, it is highly recommended to employ 1,3-diphenylisobenzofuran (DPBF) due to its exceptional performance.

4. Particle size evaluation

The determination of the average size of gold nanoparticles has predominantly relied on UV-vis spectroscopy [8]. To accurately calibrate the dumping frequency of surface plasmon resonance, the Gans ellipsoid model is employed to account for non-spherical gold nanoparticles (AuNPs) while fitting UV-vis spectroscopy data to the Mie model. This approach has been successfully applied to free and functionalized gold nanoparticles ranging in size from 4 to 25 nm across various solvents, exhibiting an impressive accuracy of approximately 6% compared to transmission electron microscopy (TEM). Furthermore, the fitted model offers additional insights that TEM cannot provide, such as estimating the concentration of AuNPs and quantifying the percentage of non-spherical particles in a given sample. These findings hold significant value in enhancing our understanding of nanoparticle aggregation mechanisms.

AuNPs have emerged as a crucial component in the field of nanotechnology due to their exceptional surface plasmon resonance (SPR), ease of surface functionalization or biocoupling, remarkable chemical stability, and intrinsic biocompatibility. To accurately evaluate the optical, electrical, chemical, and biological characteristics of AuNPs, precise control over their size, concentration, and aggregation level is indispensable. While TEM remains the most reliable method for determining the average size and size distribution of gold nanoparticles, it lacks real-time monitoring capabilities and insights into AuNPs aggregation and concentration. Therefore, UV-vis spectroscopy holds immense significance in this specific context owing to its ability to determine the size, concentration, and degree of aggregation of gold nanoparticles. Additionally, the wide-ranging applicability of UV-vis spectroscopy, its non-invasive sampling technique, and rapid data acquisition all significantly contribute to its attractiveness. The utilization of Mie theory and Gans model of spheres enables precise determination of nanoparticle size and metal permittivity within their physical and chemical environments through direct measurement of individual nanoparticles as well as nanoparticle ensembles. UV-vis spectroscopy can be conveniently employed for examination by recording AuNP

extinction spectra. Furthermore, the MY-GANS fitting model investigates the morphological properties of AuNP while studying associated extinction spectra to ascertain the influence of the physical and chemical environment on SPR. Non-aggregated, spherical gold nanoparticles often exhibit a prominent peak in the UV-visible spectrum at approximately 520 nm, which is attributed to surface plasmon resonance. Furthermore, these nanoparticles demonstrate a distinct absorption edge resulting from D-band electron interband transitions. The resolution Mi model, based on Maxwell's equations in spherical coordinates, can be employed for further analysis of experimental data. The Mie model specifically designed for compact spheres enables precise computation of the extinction cross section of spherical AuNPs. Based on our research findings, the average particle size of diverse AuNP solutions, ranging from 4 to 25 nanometers in diameter, is approximately 6%. It has successfully developed a robust model that facilitates precise evaluation of the distribution of AuNP particle sizes. This model has yielded promising outcomes by expanding the fitting range of the UV-visible spectrum up to 200 nm.

5. Detection of chlorophyll and carotenoids

UV-vis spectroscopy is a crucial and reliable technique in biochemistry for accurately quantifying chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids in total pigment extracts from green plant tissues. However, it should be noted that spectral overlap may occur due to the absorption of light by different plant pigments in similar regions. To ensure precise determination of Chl a, Chl b, and total carotenoids within the same pigment extract obtained from leaves or fruits, specific equations are employed. Nevertheless, possessing prior knowledge about the spectral characteristics and absorption coefficients of Chl a, Chl b, and carotenoids is essential for achieving accurate quantitative analysis.

The provided absorption spectra of Chl a and Chl b serve as an illustrative example, having been separated using ether. Both Chl a and b exhibit their maximum absorption in the blue region (around 428 and 453 nm) as well as in the red region (around 661 and 642 nm). The carotenoids in isolation exhibit a wide absorption spectrum, characterized by three distinct peaks spanning from 400 to 500 nm or an additional spectral range resembling a shoulder. It is worth noting that the solvent type used significantly influences the maximum absorbance of extracted pigments, while the choice of spectrophotometer also plays a role to some extent. For example, when the polarity of the solvent is increased, there is a shift towards longer wavelengths in the maximum absorption of red light for Chl a (from 660 nm to 665 nm), and for Chl b (from 642 nm to 652 nm). Similarly, there is also observed a rightward shift for the maximum blue absorption wavelength from 428 nm to 432 nm for Chl a and from 452 nm to 469 nm for Chl b. These variations in the wavelengths of maximum absorption are closely associated with changes in the absorption coefficients used for accurately quantifying Chl a, b, and carotenoids. It is imperative to accurately evaluate the absorption of pigment extracts at precise wavelengths that correspond to the peak values of pure Chl a and pure Chl b in the solvent being utilized. When determining pigment content, it is essential to employ appropriate equations that consider solvent-specific extinction coefficients. Depending on the type of spectrophotometer employed, there may be slight discrepancies in locating the exact wavelength maximum. Consequently, differences in wavelength positions can vary by approximately 1.0 or 1.5 nm.

To ensure accurate measurement of the spectral characteristics of green plant tissue extracts, it is imperative to determine the peak positions on the red spectrum for pure solutions of Chl a and Chl b using their respective spectrophotometers, as shown in Figure 1. These values should then be compared with those on the calibration scale to establish precise comparisons. When there are wavelength deviations exceeding 1nm, these self-determined maximum values should be employed for measuring pigment extracts instead of relying solely on literature values. For a specific solvent, as long as the difference in wavelength position does not exceed 2 nm, the same equation can be applied. However, if there is a deviation of 2 nm, adjustments need to be made either by calibrating the spectrophotometer or ensuring that an incorrect or impure solvent is not utilized. In determining carotenoids within the same extract, maintaining a consistent wavelength position of 470 nm suffices

since even a slight shift of 1 nm has negligible impact on total carotenoid levels compared to individual levels of Chl a and b.

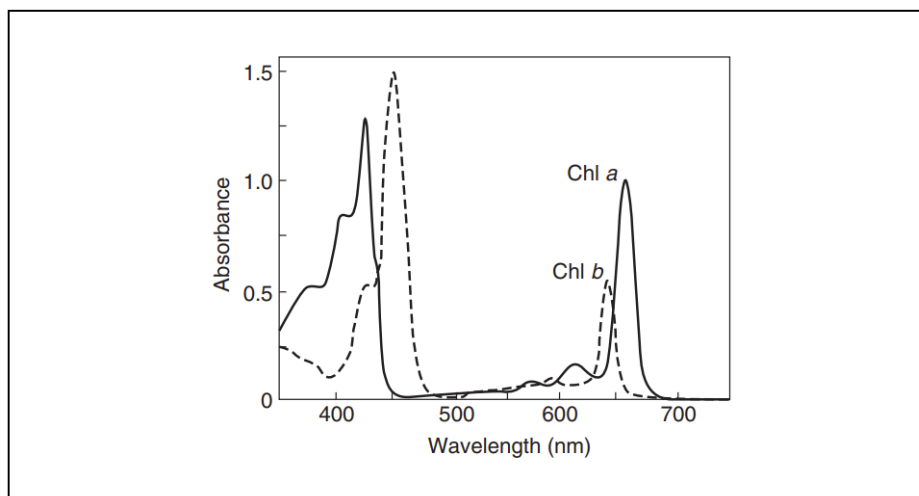


Figure 1. UV-vis spectra of Chl a and Chl b [9].

6. Identification of adulteration in various herbs and spices

Due to its inherent simplicity, cost-effectiveness, and non-invasive nature, UV-vis spectroscopy emerges as an exceptional analytical technique for the precise detection of contaminants in herbs and spices [10]. The remarkable potential of UV-vis spectroscopy lies in its capability to significantly contribute to taxonomic research, herbal development, quality assurance, process monitoring, identification of counterfeit products, and assessment of geographical origin within the pharmaceutical and food industries.

The UV-vis absorption bands observed in this case indicate the presence of structurally related combinations or functional groups rather than a specific chemical entity. UV-vis spectroscopy is commonly employed to identify UV-absorbing chemical groups that contain carbon-carbon bonds or conjugated polycarbonyl heteroatoms, which include substances such as olefins, aromatics, and various heterocyclic compounds. Furthermore, for categorization, comparative analysis, and identification of pharmaceutical materials, chemometrics is often combined with UV-vis spectroscopy. In this study, we propose a novel method utilizing the UV spectrum obtained from ethanol extracts of thymus samples collected at 20 different locations to effectively distinguish thymus from other closely related species. To achieve this objective, the obtained spectra underwent rigorous analytical techniques including PCA, analog-like soft independent modeling (SIMCA), and hierarchical cluster analysis (HCA). The aim was to establish a clear differentiation between commonly encountered thyme species and closely related ones. A comprehensive analysis was conducted on twelve commercially available thyme species sourced from plants other than thyme, encompassing *Origanum Satureja*, *Eriocephalus*, *Plectranthus*, and various additional thyme species. Relevant techniques such as UV-vis and IR spectroscopy were utilized to conduct a comprehensive assessment of the quality of gentian. Subsequently, a robust dataset was generated by capturing these spectra, followed by the application of advanced statistical methodologies including partial least squares discriminant analysis (PLSDA) and support vector machine discriminant analysis (SVM). Furthermore, the Folin-Ciocalteu reaction can be employed to establish a determination model for evaluating the polyphenols and tannins content. The authors investigated on these compounds in diverse plant components, encompassing leaves, stems, and flowers of *Plantago L.*, utilizing multivariate analysis (MA) in conjunction with PCA to elucidate the interrelationships among distinct groups.

7. Conclusion

This exemplifies the extensive applicability of UV-vis spectroscopy across various disciplines, as it enables precise quantification of heavy metal levels, nitrate nitrogen concentrations, and dissolved organic carbon content in aqueous environments. It facilitates the development of simplified models to tackle complexity. In the identification of $^1\text{O}_2$, a specifically designed and synthesized detection probe is employed to achieve selective reaction with $^1\text{O}_2$, enabling accurate quantification and presence assessment. This methodology holds immense potential for applications in photodynamic therapy research, free radical generation studies, and other associated fields. When evaluating the average size of gold nanoparticles, UV-vis spectroscopy is utilized to infer their mean dimensions by monitoring alterations in absorption intensity at specific wavelengths. In the determination of total pigment extracts from green plant tissues, UV-vis spectroscopy scans are employed to analyze Chl a, Chl b, and carotenoids. This approach enables the calculation of corresponding peak absorption intensities for different types of chlorophyll and carotenoids present in plant tissues, thereby providing valuable insights into their proportion or content within the sample. For detecting adulterants in herbs and spices, UV-visible spectroscopy swiftly identifies authenticity while also ascertaining the presence of any adulteration. In addition, UV-vis technology is extensively employed in various domains, playing a pivotal role in medical diagnostics, food safety monitoring, environmental protection, and other areas. However, UV-vis spectroscopy encounters certain challenges. For example, the superposition model proves excessively intricate to demonstrate the presence of specific compounds. Nevertheless, it is anticipated that through technological advancements and enhanced integration of models with other devices, UV-vis spectroscopy can be more effectively applied across diverse fields.

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