

Recent Developments Expanding the Applications of UV-Visible Spectroscopy

Srividya Kailasam, PhD

Correlating absorbance or transmittance of liquid samples or reflectance of solid samples with the analyte concentration forms the basis of <u>ultraviolet-visible (UV-Vis) spectroscopy</u>. This simple, non-de-structive and cost-effective technique finds applications in fields as diverse as pharmaceutical, food and environmental analysis. In addition, easy portability of some UV-Vis spectrophotometers makes it suitable for onsite applications.

Although UV-Vis spectroscopy is a well-established method, it continues to evolve and is extensively used for the analysis of different types of molecules in varied matrices.

Recent developments in detectors, detection systems and detection strategies in UV-Vis spectrophotometry have enabled the measurement of very low concentrations of analytes in small sample volumes.¹

Combining the technique with near infrared (NIR) spectroscopies and chemometrics is also a powerful approach to identify, quantify and classify analytes in solid, liquid and gaseous phases. Non-targeted chemometric analysis is boosting UV-Vis analysis in fields from agriculture and environmental sciences to food and beverages, chemical and pharmaceutical analyses.²

This listicle showcases some recent developments in this technique as well as its applications.

Food analysis

Advances in instrumentation and chemometric tools for data analysis have made UV-Vis spectroscopy a technique of choice in the analysis of foodstuffs such as rice, tea, spices, meat, fish, oils and beverages. UV-Vis spectroscopy is used for quality control during production, to identify the variety of produce and determine their geographical origin.³

Eighteen starch-containing flours (e.g., type 00 flour, rye, barley, soybean, chestnut, potato, spelt, buckwheat, oat, millet, rice, durum wheat, amaranth, chickpea, sesame, corn, hemp and sunflower flours) were recently characterized with the help of NIR and UV-Vis spectroscopies coupled with chemometrics. The authors have suggested that UV-Vis spectroscopy can be used as a fingerprinting technique to identify the botanical source of starches.⁴ A simple, rapid and inexpensive method has been reported for the identification of adulteration of honey with sugar syrups and colorants using principal component analysis (PCA) along with random forest (RF), partial least squares-discriminant analysis (PLS-DA), data driven-soft independent modelling of class analogies (DD-SIMCA) and UV-Vis spectroscopy in the range of 220–550 nm.⁵

Phytochemicals

The phenolic compounds and flavonoids extracted from plants are known to be good antioxidants and are shown to have medicinal properties. UV-Vis spectrophotometry has been used to detect the presence of chromophores and aromatic rings in the phytochemicals extracted from *Dillenia pentagyna* (Roxb), using different organic solvents. The absorption bands helped to identify alkaloids, flavonoids, phenolic acids and tannins present in the extracts from the various parts (bark, leaves, sepals, fruits and seeds) of the plant.⁶

Water quality monitoring

Water quality parameters have been ascertained from UV-Vis spectral data by running high-precision mathematical models developed using innovative algorithms. The authors have cited numerous studies to determine the following parameters in water:

- Chemical oxygen demand
- Heavy metal (Zn²⁺, Co²⁺, Hg²⁺) concentration
- Nitrate concentration
- Dissolved organic carbon (DOC) levels

Further, the authors have suggested that early warning systems can be set up by combining UV-Vis spectrometry with 5G network-based communication technology for real-time water quality monitoring.²

The <u>American Public Health Association</u> (APHA) method for quantification of phosphate in water is based on the measurement of color intensity of molybdenum blue formed by the reduction of molybdophosphoric acid. The detection limit for phosphate (in freshwater) using this method is $30 \ \mu gL^{-1}$ with a 5 cm cuvette. Tripp et al. have developed a solventless visible spectroscopic method with a lower detection limit of 0.64 $\mu gL^{-1.8}$

Chemical engineering

In addition to fundamental sciences, UV-Vis spectroscopy has been extensively applied in chemical engineering to characterize polymer impregnation. For instance, zein fibers loaded with vanillin, a natural antimicrobial agent, are considered suitable for manufacture of biodegradable food packaging material. Vanillin content of impregnated zein fibers, dissolved in a water/ethanol solution, was measured with a UV-Vis spectrophotometer operated at 280 nm.²

Biomolecules and biopharmaceutical analysis

Oh and Kim have combined micro-volume UV-Vis spectrophotometry with a slope measurement technique to improve the accuracy and decrease the measurement time. This approach allows quantification without blank measurements, making it useful for the analysis of micro-volumes of nucleic acids or proteins samples.^{10,11}

UV-Vis spectroscopy has been used to study the conformational changes during complexation of caffeic acid with human serum albumin (HSA). The results obtained were further verified by fluorescence spectroscopy and molecular docking studies.¹²

A UV-Vis spectra-activated droplet sorter (UVADS) has been used to measure unlabeled bovine serum albumin (BSA) down to 10 μ M in concentration. This was achieved by collecting full droplet absorption spectra from 200 to 1050 nm at high speeds (up to 2,100 spectra per second) and subsequently processing and analyzing spectra within milliseconds.¹³

During purification of therapeutic proteins, their concentrations are measured by UV absorbance determination. The challenges of signal saturation and non-linearity observed at higher protein concentrations have been shown to be overcome by employing a multi-wavelength UV-based approach. This has enabled on-line protein estimation in the range of 0.8–100 g/L without the need for dilution. Partial least squares (PLS), a <u>chemometric</u> technique, has been adopted for rapid quantification of protein concentration from the spectra.¹⁴

Study of catalysts

UV-Vis spectroscopy has been used to study catalysts in solid states and liquid phases and also to understand the working principles of different types of catalysts – homogenous, heterogenous, photocatalysts and electrocatalysts.¹⁵

Characterization of nanoparticles

Nanoparticles are characterized by various analytical techniques such as UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM) and energy dispersive spectroscopy (EDS). Bindhu et al. have synthesized small-sized, monodispersed silver nanoparticles using extracts of *Moringa oleifera* flowers. They have reported antimicrobial activity as well as optical sensing of Cu²⁺ ions by these nanoparticles. UV-Vis spectroscopy was used to monitor the decrease in particle size of silver nanoparticles with increasing concentrations of *Moringa oleif-era* flower extract.¹⁶

Solar atmosphere

Using the spectral imaging of the coronal environment (SPICE) instrument on the European Space Agency/National Aeronautics and Space Administration (ESA/NASA) Solar Orbiter mission, extreme ultraviolet (EUV) spectra of the Sun have been collected to study the variability of the elemental composition in the solar atmosphere.¹⁷

Detector for liquid chromatography

A UV-Vis spectrophotometer is a commonly used detector for separation techniques like <u>high-perfor-mance liquid chromatography (HPLC)</u> or as an additional detector for <u>liquid chromatography (LC) coupled</u> to a mass spectrometer (LC-MS).

Pharmaceutical analysis

A simple and rapid reversed-phase (RP) HPLC method has been developed for quantification of paracetamol, caffeine and tramadol hydrochloride in tablets. The authors have reported low limit of detection (LOD) values of 0.2 μ g/mL, 0.1 μ g/mL and 0.3 μ g/mL for these compounds respectively using C₁₈ columns and a UV-Vis photodiode array (PDA) detector.¹⁸

Bioanalysis

Rufinamide, an antiepileptic drug, has been quantified in rat plasma and brain matrices using HPLC coupled with a UV detector. The limit of quantification (LOQ) values in plasma and brain were found to be 13.84 ng/mL and 105.24 ng/g respectively. The suitability of the method for pharmacokinetic studies was demonstrated by measuring the plasma concentrations and performing brain distribution studies following intravenous injections of the drug.¹⁹

Conclusion

UV-Vis spectroscopy remains a popular analytical technique used to test a variety of analytes. As discussed in this listicle, it continues to be used extensively for the analysis of organic and inorganic compounds, elements and even nanoparticles in matrices as diverse as food, pharmaceutical and environmental samples. Yet, there are several challenges outstanding, such as further improving the technique's sensitivity and specificity, the impact of temperature and stray light and limited linearity of calibration curves. Work to address these will enhance the potential of UV-Vis spectrophotometry further for multi-component analysis in complex matrices.

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About the author:

Srividya Kailasam completed her doctoral training in analytical chemistry at the Indian Institute of Technology, Madras. During her postdoctoral research in the United States, she worked on process analytical chemistry, analysis of toxic industrial chemicals and quantification of VOCs in biological matrices. After returning to India, Srividya joined Agilent Technologies in Bangalore, as an application scientist where she developed numerous LC, LC-MS and LC-MS/MS methods for the analysis of a variety of samples such as pharmaceuticals, bio-fluids, beverages and animal feed. She then moved to Thermo Fisher Scientific in Bangalore and worked as a subject matter expert for software development focused on the analysis of mass spectrometry data. Srividya started writing for Technology Networks in May of 2021.