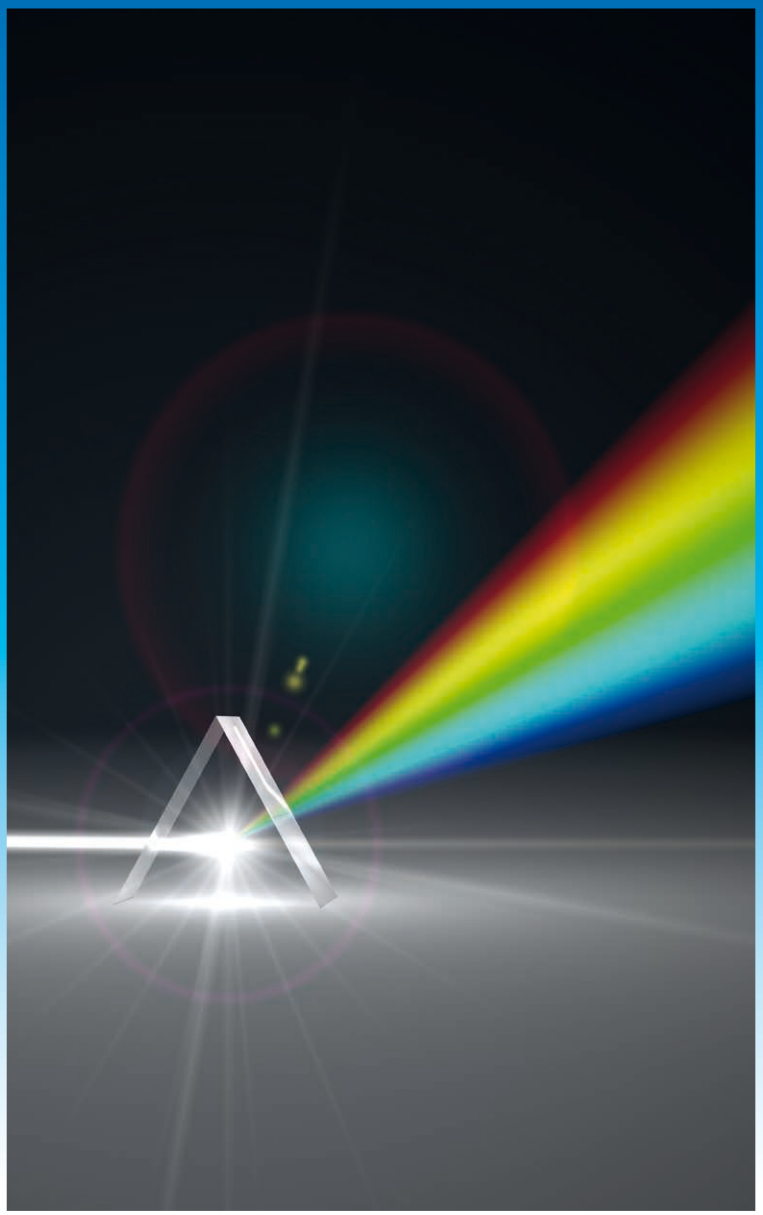
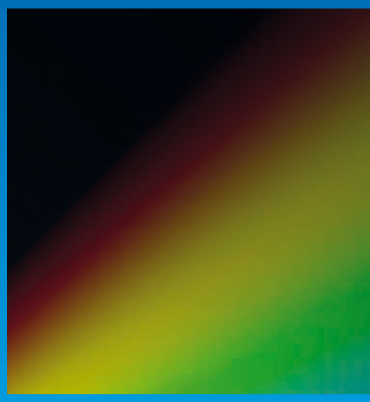
UV/Vis Spectrophotometry





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Fundamentals and Applications

METTLER TOLEDO

Introduction

Besides chemical analysis, the characterization of pure as well as mixtures of substances is achieved with physical methods. Among other techniques, such as the determination of melting point, refractive index, and density, optical spectroscopy in the ultraviolet (UV) and visible (Vis) light range is widely applied in almost all market segments and workplaces in research, production, and quality control for the classification and study of substances. UV/Vis spectroscopy is based on the absorption of light by a sample. Depending on the amount of light and its wavelength absorbed by the sample, valuable information can be obtained, such as the purity of the sample. Moreover, the amount of absorbed light is related to the amount of sample; thus, quantitative analysis is possible by optical spectroscopy.

Content

1. UV/Vis Spectroscopy

1.1. What is UV/Vis spectroscopy?

Optical spectroscopy is based on the interaction of light with matter. The following figures illustrate what happens when light is shining onto an object:

Figures 1 and 2: The light which is not absorbed by the object is reflected and can be seen by the eye.

Both objects are illuminated by visible or white light, represented by a rainbow: the different colors represent the different components of visible light. When light rays are shining onto an object, they might be absorbed by the object – in particular, one or more light components (i.e., its colors) are specifically absorbed.

The colors which are not absorbed by the objects are reflected. In our example, red light is reflected by the red shell of the tomato (Figure 1), whereas the green light is reflected by the green surface of the zucchini (Figure 2). The two objects absorb all other colors. The eyes then see the reflected light: the tomato is seen in red while the zucchini are green.

Each color has a specific wavelength, e.g., red light has a wavelength of 660 nm, while green light has a wavelength of 520 nm. Thus, the different components of light are characterized by a specific wavelength. The sum of all components, i.e., of all wavelengths, is called a **spectrum**. More specifically, a spectrum represents a distribution of radiant energy. For instance, the electromagnetic spectrum of visible light ranges from approximately 390 nm up to about 780 nm.

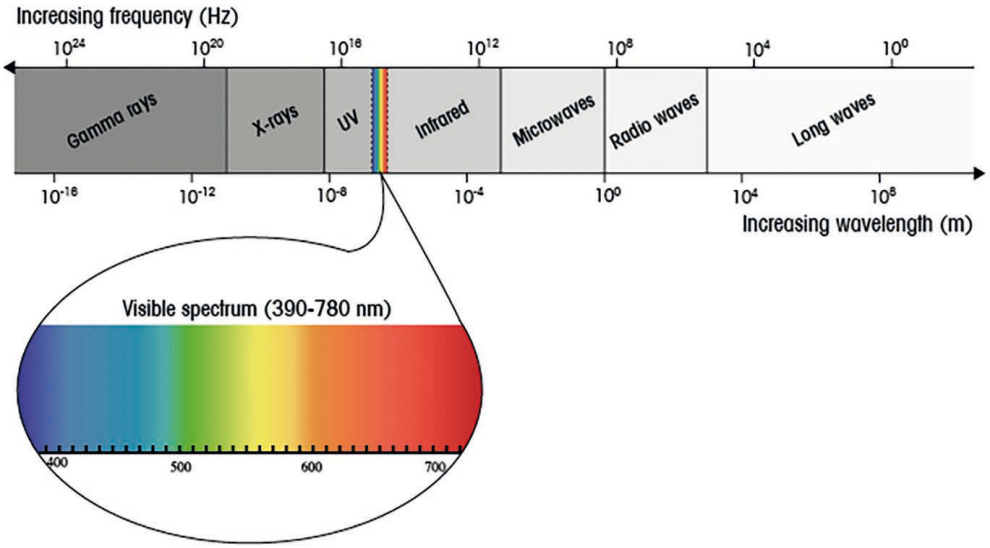

Figure 3: The visible spectrum (390 – 780 nm) represents only a tiny portion of the whole electromagnetic spectrum.

Absorption of light as analytical tool

Light absorption can be used in analytical chemistry for characterization and quantitative determination of substances. UV/Vis spectroscopy is a technique based on the absorption of light by an unknown substance or by an unknown sample. Here, the sample is illuminated with electromagnetic rays of various wavelengths in the visible (Vis, i.e., the different colors) and adjacent ranges, i.e., ultraviolet (UV) and part of the lower infrared region (near IR) of the spectrum. Depending on the substance, light is partially absorbed. The remaining light, i.e., the transmitted light, is recorded as a function of wavelength by a suitable detector, providing the sample's UV/Vis spectrum.

As a result, a unique and specific relationship exists between the substance and its UV/Vis spectrum because each substance absorbs light differently. The spectrum can then be used to identify or quantify a substance.

Even though the explanation above sounds complicated, we can easily relate it to our daily experiences. Without even realizing it, we perform optical spectroscopy constantly in our everyday life. Let us consider something as simple as a glass of syrup. If we have three glasses of syrup in front of us; one with raspberry syrup, one with lemon syrup, and one with mint syrup; we will have no difficulties telling which one is which without even needing to taste them. That is because our brain and our eyes are performing optical spectroscopy! Because of the specific way the molecules of each syrup type interact with visible light, every glass will appear in a different color. The glass with raspberry syrup is red, the one with lemon syrup is yellow, and the last one with mint syrup is green.

We are using visible light and how it interacts with our samples (the glasses with syrup) as an analytical tool to gather information about the samples (e.g., which glass contains the raspberry syrup). All we need to perform optical spectroscopy are three key components: a light source (e.g., the sun or a light bulb), a sample (i.e., the object you want to gather information about), and a detector system (i.e., our eyes) and a recorder (i.e., our brain). Figure 4 illustrates this principle.

Figure 4: Light passing through a sample solution is partially absorbed by the components.

Obviously, this rudimentary way of performing spectroscopy is unreliable as an analytical tool for precise measurements. Every person perceives and describes colors slightly differently (everyone has argued, "is it red or is it pink" or "is it green or is it turquoise" at least once). Moreover, the appearance of the sample is affected by lighting conditions (an object's color can change if exposed to natural sunlight or artificial lighting) and even the shape of the sample's container (i.e., the shape of the glass). On top of that, not all substances interact with visible light. For example, it isn't easy to distinguish between a glass of water and one of colorless liquor just by looking at them.

We need an instrumental technique to ensure our spectroscopic measurements are objective and consistent. Additionally, we need a way to extend the measurement range outside the visible range and into the ultraviolet range. This is achieved by UV/Vis spectroscopy, a measurement technique in which the recording of the absorption spectra of different samples using ultraviolet (UV) and visible (Vis) light is performed by a spectrophotometer, i.e., an instrument able to measure the spectrum of a sample in the UV/Vis range.

UV/Vis spectroscopy is usually applied to organic molecules, inorganic ions, or complexes in solutions, although solid materials such as films or glass can also be analyzed. The obtained UV/Vis spectra are very useful for quantitative measurements of a specific compound. The concentration of an analyte in a solution can be determined by measuring the absorbance at a specific wavelength. From the absorbance value of the sample, its concentration can be calculated; see the description in chapter 1.4.

1.2. Measurement principle

UV/Vis spectrophotometers measure the intensity of light passing through a sample solution in a cuvette and compares it to the intensity of the light before it passes through the sample. The main components of a UV/Vis spectrophotometer are a consistent and stable light source, a sample container, and a suitable detector and recorder, as illustrated in Figure 6.

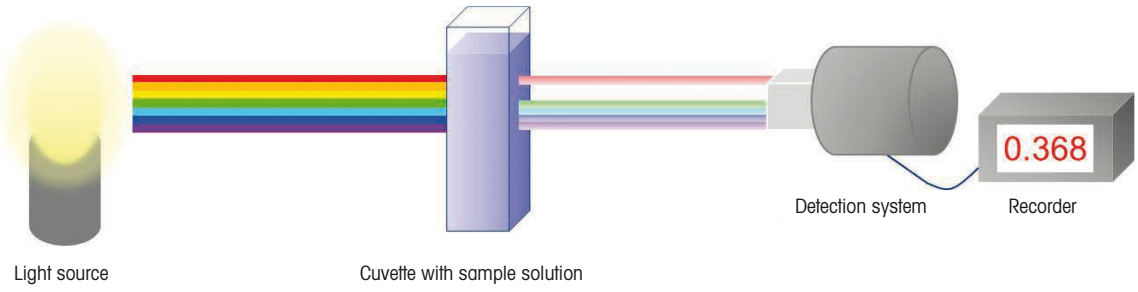

Figure 5: Measurement principle in UV/Vis spectroscopy.

If we compare this instrumental set up with the concept described in Figure 5, we see that it has the same main constituents. Yet, we have replaced non-standardized elements, like the light source and sample containers, and the subjective detection system with stable, standardized components and an objective electronic detection system. This enables accurate, consistent, and comparable measurements across many samples.

The working principle of a spectrophotometer is based on the following steps:

Blank (measurement of the intensity of light transmitted through the solvent):

1. The solvent (e.g., water or alcohol) is added into a suitable, transparent, and not absorbing container – a cuvette.
2. A light beam emitted by the light source passes through the cuvette with the solvent.
3. The intensity of the transmitted light at different wavelengths is then measured by a detector, positioned after the cuvette with the solvent, and recorded.

This is known as the **blank**, which is needed for the sample measurement.

Sample determination:

1. A sample is dissolved in the same solvent used for the blank measurement and added into the cuvette.
2. A light beam emitted by the light source passes through the cuvette with the sample.
3. When passing through the cuvette, the light is partially absorbed by the sample molecules in the solution.
4. The transmitted light is then measured by the detector.
5. The light intensity change at different wavelengths is calculated by dividing the transmitted intensity of the sample solution by the corresponding values of the blank. This ratio is finally stored by a recorder.

1.3. Transmittance and absorbance

A UV/Vis spectrophotometer's detector measures the light intensity after passing through the sample solution. This fraction of light collected by the detector is called the transmitted intensity, I. The sample solution attenuates the intensity of the transmitted light due to, for instance, absorption of light at specific wavelengths. Therefore, its value is lower than the original intensity I_0 at the light source.

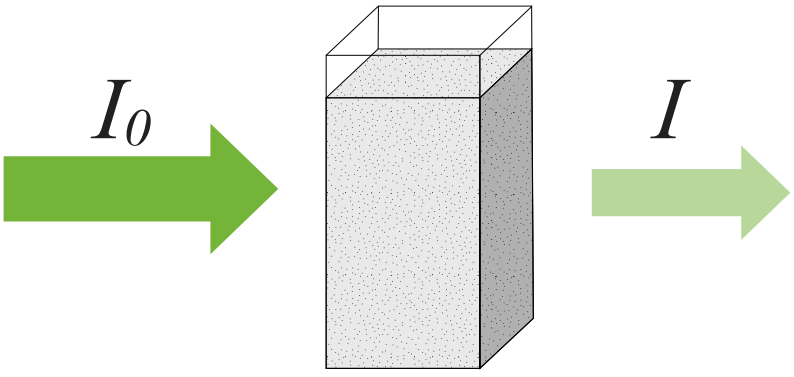

Figure 6: Light attenuation by absorption of sample molecules in solution.

The ratio between the two intensities I / I_0 is defined as Transmittance T, and its unit is %.

$$T = \frac{I}{I_0}$$

Figure 7: Transmittance is the ratio of the transmitted intensity I to the original intensity I_0.

The transmittance is the primary value determined by UV/Vis spectroscopy, but it is not the only one. The absorbance A represents an additional result widely used when recording UV/Vis spectra. It is defined as the negative logarithm of the transmittance, and it has a significant advantage, which we will see in the next chapter.

$$A = -log(T)$$

Figure 8: Absorbance is the negative logarithm of the transmittance value.

Note that the absorbance A does not have any unit of measurement. In other words, it is a dimensionless value. However, it is often represented using the letter "A" or as AU for absorbance units. For example, 0.3 A or 0.3 absorbance units, respectively.

The result of a measurement using a UV/Vis instrument is shown in the following figures. We can see how the transmission spectrum (on the left) is mirrored when compared to the absorbance spectrum (on the right). This makes sense since, in a wavelength range, where the sample doesn't absorb (0 Absorbance), all the light will be transmitted through the sample (100% Transmittance), whereas in ranges where the sample absorbs large amounts of light, less light will be transmitted through the sample.

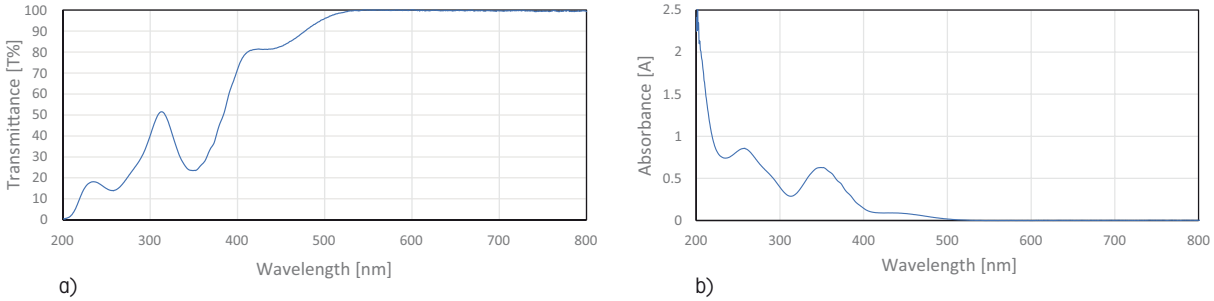

Figure 9: a) Transmittance spectrum of a potassium dichromate solution as a function of wavelength, b) Absorbance spectrum of the same solution.

The transmittance spectrum of a sample is recorded as a function of the wavelength. The sample absorbs light mainly at 257 nm, 350 nm, and below 220 nm. The light absorption is marked by a sharp decrease in the transmittance at these wavelengths.The light absorption is marked by a sharp decrease of the transmittance at these wavelengths.

Generally, a UV/Vis spectrum is graphically represented as absorbance as a function of wavelength. The advantage of this representation is obvious; the height of the absorption peaks is directly proportional to the concentration of the species.

1.4. Lambert-Beer law

When passing through a transparent cuvette filled with the sample solution, the light intensity is attenuated proportionally to the sample concentration. In other words, a higher concentrated sample solution will absorb more light. In addition, the attenuation is also proportional to the length of the cuvette; a longer cuvette will lead to higher absorption of light. The more light a liquid absorbs in the visible range, the darker it will appear (a completely black liquid, for example, absorbs all visible light).

Going back to the syrup example, we instinctively know that the more syrup we add to the water, the darker the liquid in the glass will appear. Adding more syrup to the water means increasing the concertation of the syrup molecules in the glass, which increases the amount of light they absorb, causing the liquid to become darker. This is clearly illustrated in Figure 10, where three samples of increasing concentration are shown, along with the corresponding absorbance spectra.

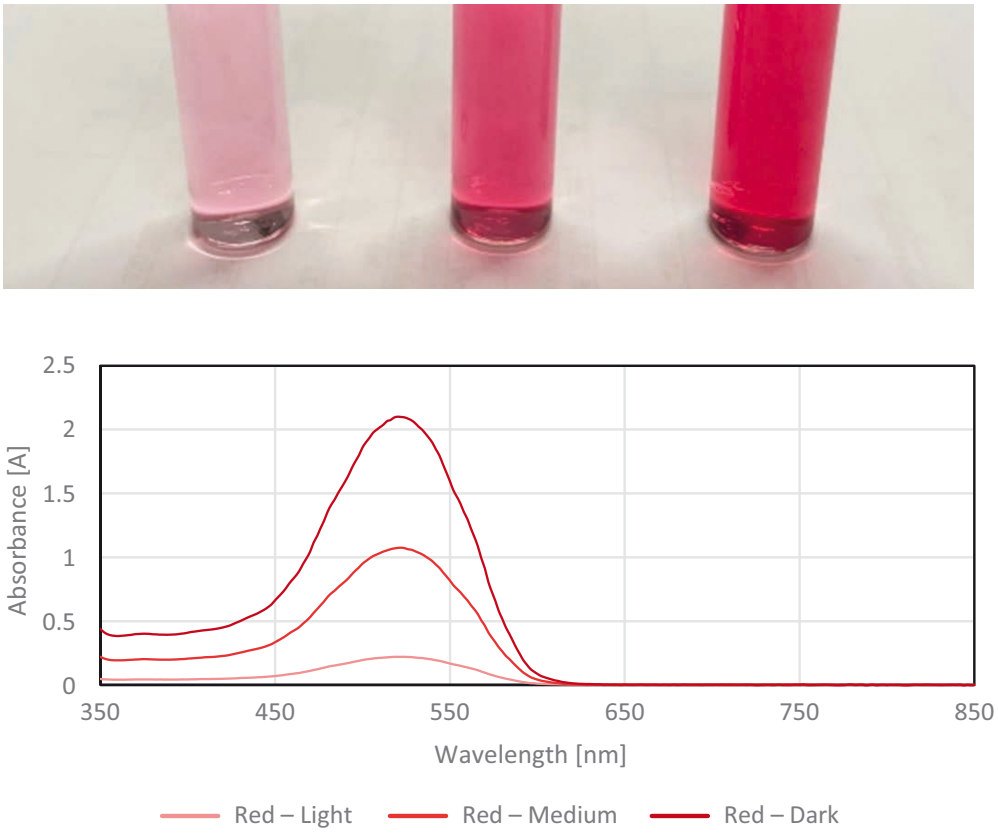

Figure 10: Three samples of increasing concentration and the corresponding spectra. The most concentrated sample (right) is darker because it absorbs more light and clearly shows the highest absorption value in the spectrum.

At the same time, the same concentration of syrup will appear lighter in a small, narrow glass than in a larger, wider glass. That is because, in a narrow glass, the light will traverse less liquid and interact with fewer syrup molecules than in a wide glass, where the optical path length is longer.

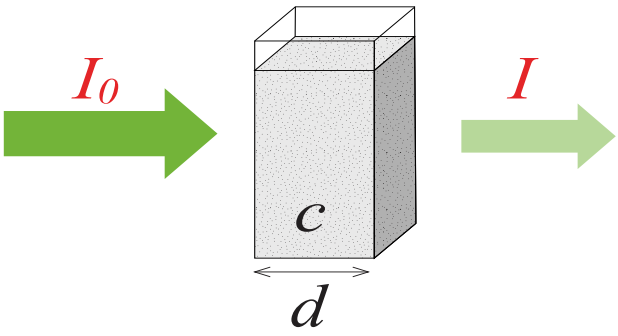

Figure 11: The attenuation of the light intensity is proportional to the concentration of the sample solution as well as the length of the cuvette.

Both factors can be summarized by expressing the absorbance A as a function of the concentration and the cuvette length. In particular, the absorbance A is equal to the product of the extinction coefficient ε, the concentration c, and the path length d:

$$A = \varepsilon \cdot c \cdot d$$

This relationship is called the **Lambert-Beer** law, where:

1. The **sample concentration** c is given in mol / L or g / mL, respectively
2. The **path length** d of the cuvette is given in cm,
3. The **extinction coefficient** ε (epsilon) is a sample specific constant describing how much the sample is absorbing at a given wavelength (in L / (cm*mol) or mL / (cm*g), respectively).

The equation clearly shows a linear correlation between the concentration of the sample and the measured absorbance if the path length is kept constant (i.e., using the same cuvette). In other words, if we double the sample concentration, the measured absorbance will double as well; increase the concentration 10-fold, and the absorbance will increase 10-fold, and so on. The same applies to the path length: while keeping the concentration constant (i.e., measuring the same sample), a 2 cm cuvette will produce an absorbance two times higher than a 1 cm cuvette.

The Lambert-Beer law allows to determine the sample concentration from the measured absorbance value. If the extinction coefficient ε and the path length d are known, then concentration c can be calculated from absorbance A as given below:

$$c = \frac{A}{\varepsilon \cdot d}$$

2. UV/Vis Spectroscopy in Analytical Chemistry

2.1. Why do we measure UV/Vis spectra?

There are three main reasons to measure UV/Vis spectra:

- UV/Vis spectra allow components present in the sample solution to be identified. More precisely, the position and, to some extent, the profile of the absorption peaks allow specific compounds to be identified. For example, organic compounds can be identified by their spectra, or solvent purity can be easily checked by UV/Vis spectroscopy.
- Absorption peaks can be used to quantify the investigated sample. For example, the sample concentration can be calculated from the absorbance value of the peak based on the extinction coefficient of the substance of interest. Additionally, based on the linear relationship between absorbance and sample concentration, the concentration of the sample can be determined by using a calibration curve.
- Determination of the rate of a reaction is possible by monitoring the absorption spectra as a function of time (also known as kinetic measurements).

UV/Vis spectroscopy applications mainly focus on qualitative and quantitative analysis, which will be addressed in more detail in the next chapter.

2.2. Qualitative analysis: Identification

Qualitative analysis is based on the specificity of UV/Vis spectroscopy. Samples absorb light of one or more distinct wavelengths with specific maximum absorbance values. For this reason, each sample has a characteristic and unique UV/Vis spectrum that can be used for its identification. Generally, this is achieved by comparing the spectrum of the sample with spectra of known, pure compounds.

Figure 12 shows colored photographs of four known samples and their corresponding absorbance spectra. We can see that each sample has a unique UV/Vis spectrum, which we can use for identification.

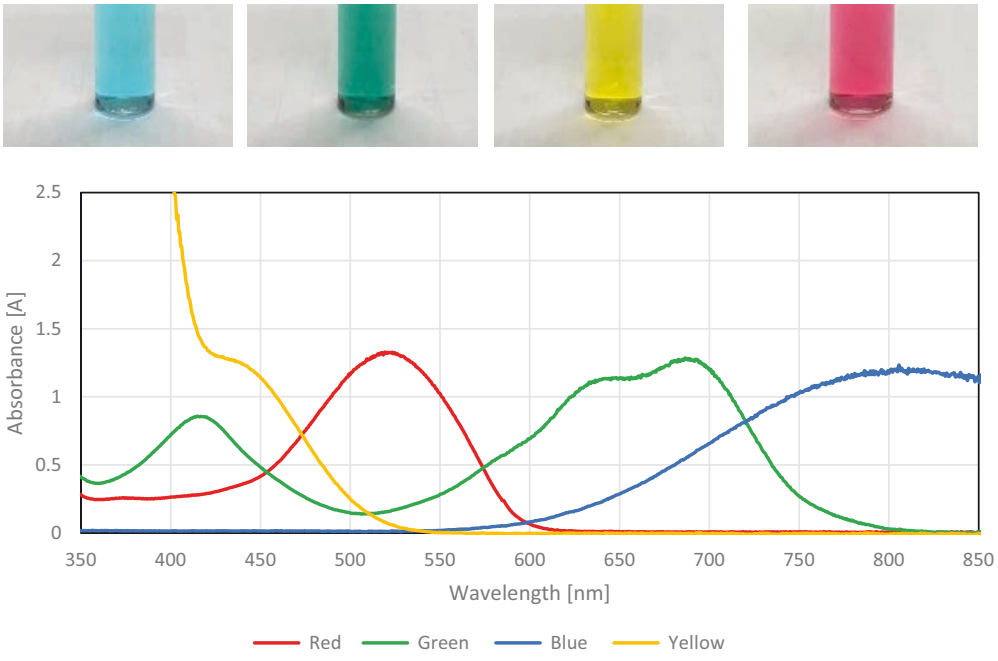

Figure 12: Four colored samples and the corresponding measured UV/Vis spectra

If we now take an unknown sample (exemplified by a black and white picture shown in Figure 13), we cannot confirm its identity based on its color.

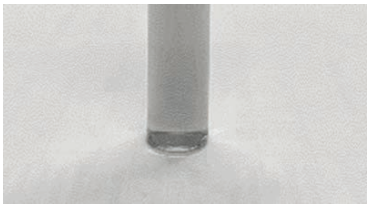

Figure 13: Black and white photograph representing an unknown sample.

However, given the UV/Vis spectrum of the unknown sample, as shown in Figure 14, it becomes easy to identify the unknown sample as a red sample by comparing it to the reference spectra of the four known samples.

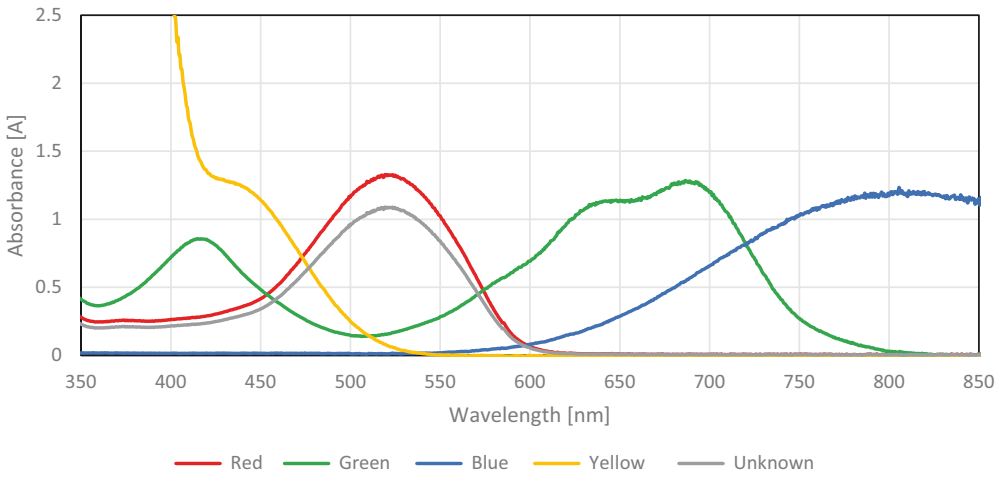

Figure 14: The absorbance spectrum of the unknown sample and the reference spectra of the four known samples. The spectrum of the unknown sample closely matches the spectrum of the red reference, allowing us to identify the unknown sample as red.

2.3. Quantitative analysis: Concentration determination

Based on the Lambert-Beer Law, the concentration of a compound in a solution can be determined quantitatively by UV/Vis spectroscopy. A calibration curve is first determined by measuring the absorption of several standard solutions of known concentration. In this way, the concentration of samples can be determined. The linear relationship between absorbance and concentration of a sample opens the door for a variety of quantitative analyses.

2.3.1 Calibration curve

A calibration curve must first be created to determine an unknown concentration of a sample solution by UV/Vis spectroscopy. This is done by measuring the light absorption of several standard solutions of different known concentrations at a predefined, fixed wavelength. In the following example, five standard solutions of increasing concentrations were measured at a predefined wavelength. A calibration curve was obtained by plotting the measured absorbance values as a function of the concentration. Finally, a linear regression of the measured values gives the calibration curve:

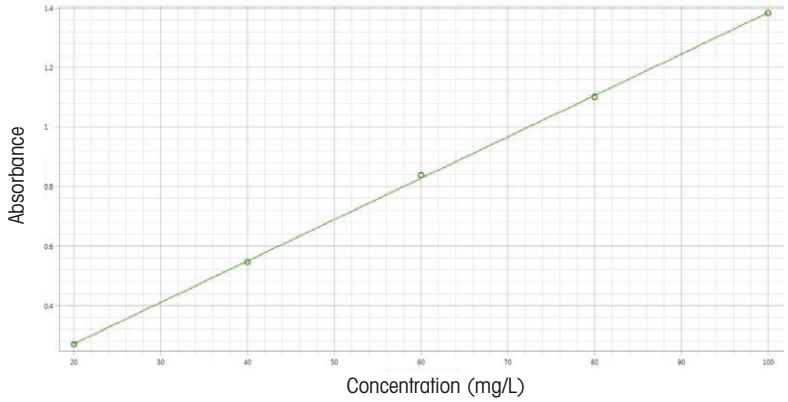

Figure 15: The absorbance values of standard solutions are determined. A linear regression of the data points gives the calibration curve.

2.3.2 Sample determination

Using the calibration curve, an unknown sample can now be determined from its absorbance:

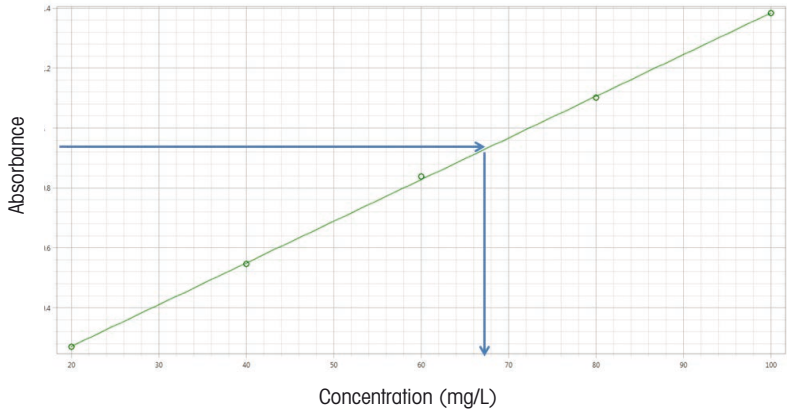

Figure 16: Concentration determination – The absorbance of an unknown sample solution is measured and compared with the calibration curve to determine its concentration.

3. Spectrophotometer Design

A digital spectrophotometer generally consists of four components:

1. A suitable **light source** that covers the UV/Vis spectrum of interest. In general, a lamp containing a gas such as xenon or a combination of two different lamps such as tungsten and deuterium is used.
2. An appropriate **sample holder** is needed to hold the sample.
   - **Liquid samples** are best placed in optical or quartz glass cuvettes. For less demanding measurements, disposable plastic cuvettes can be used as an alternative. However, glass and acrylic plastic do not transmit UV light and should only be used for measurements in the visible light range.
   - **Solid samples** can be mounted into a suitable holder to be positioned in the optical path of the spectrophotometer for measurement of the transmitted light.
3. A **dispersion element** is needed to distribute the light into separate wavelengths. It can be either a **quartz prism** or a **diffraction grating**, i.e., an optical component with a periodic structure able to diffract light.
4. Finally, the transmitted light intensity is recorded by a suitable **detector** such as a photomultiplier, a multi-channel array (e.g., a photodiode array, or PDA), or a charge-coupled device (CCD), similarly to a digital camera. Both PDA and CCD detectors use a photosensitive semiconductor material to convert the light into an electronic signal which is then recorded by the instrument.

3.1. Design comparison

UV/Vis spectrophotometers can be classified according to the geometry of the components building up the optical system for recording spectra. The following two configurations are generally used in UV/Vis spectroscopy:

- Scanning spectrophotometer
- Array spectrophotometer

3.1.1 Scanning spectrophotometer

The working principle of a conventional scanning spectrophotometer is based on measuring the absorbance value at every single wavelength sequentially. The light is first dispersed into individual wavelengths using a reflection grating. The grating is rotated to individually select each wavelength that is then sent through a cuvette. The transmittance at this specific wavelength is recorded. The whole spectrum is obtained by continuously changing the wavelength of the light (i.e., scanning) incoming onto the sample solution by rotating the grating:

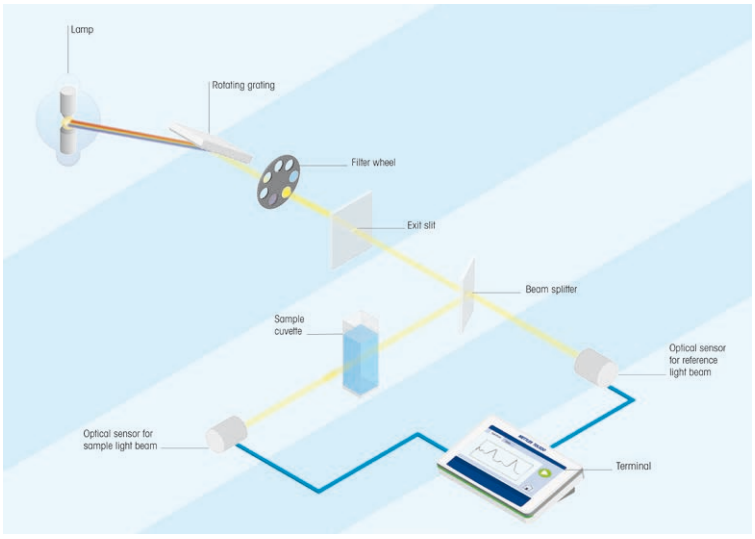

Figure 17: Scanning spectrophotometer: Light beams of specific wavelengths are directed onto the cuvette.

Note that scanning spectrophotometers take some time for a full spectrum scan because the grating must be mechanically rotated by a motor.

3.1.2 Array spectrophotometer

In this configuration, the sample is illuminated by a light beam consisting of all spectral components of the UV/Vis range – a continuum. In other words, the sample in the cuvette simultaneously absorbs different wavelengths of light. The transmitted light is then diffracted by a reflection grating located after the cuvette, as shown in the diagram below.

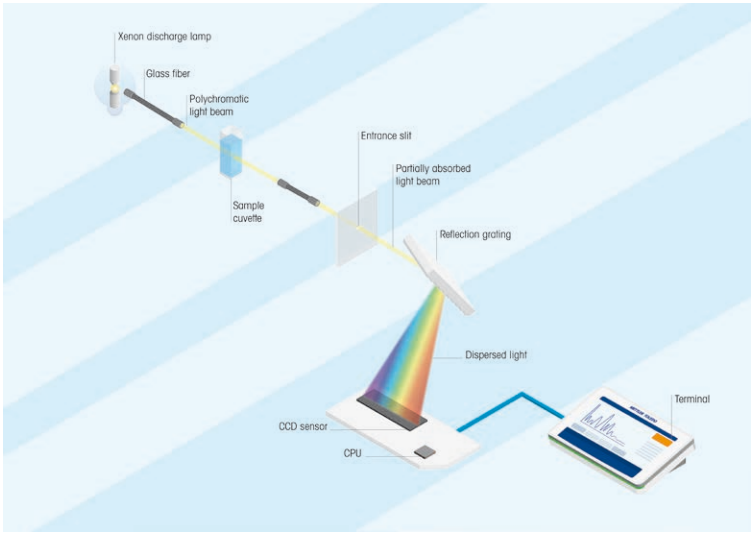

Figure 18: Array spectrophotometer: Light of the whole spectral range is directed onto the sample cuvette.

This design is also known as "reverse optics"; only after passing through the sample, the light is diffracted by the grating. Subsequently, the diffracted light of various wavelengths is directed onto the detector. With its long array of photosensitive semiconductor material, the detector allows for simultaneous measurement of all wavelengths of the transmitted light beam. With this setup, measuring the whole UV/Vis spectrum is generally faster than using a conventional scanning spectrophotometer since the spectrum is recorded simultaneously at all wavelengths. The robust design without any moving optical parts ensures excellent optical performance.

3.1.3 Optical pathways

UV/Vis spectrophotometers can have either a single beam or double beam optical pathway, i.e., the way that a light beam from the lamp passes through the sample cuvette to reach the detector.

- **Single beam configuration**
  The single-beam configuration is the simplest and easiest UV/Vis spectroscopy setup. The light beam is directly guided through the sample onto the detector. A cuvette containing only the solvent must be measured first to determine the blank value (see previous chap.). After measuring the blank value, the solvent cuvette is replaced by a cuvette containing the sample. The latest is measured to get the absorption spectrum of the sample.

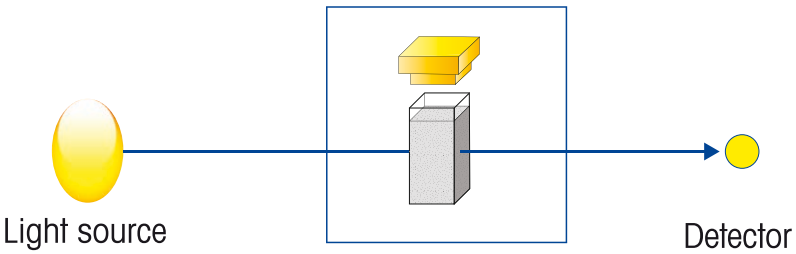

Figure 19: Single-beam optical pathway.

- **Dual-beam configuration**
  The dual beam configuration is similar to the single beam configuration but with the addition of a non-accessible reference channel. The reference channel compensates for eventual fluctuations in the system in real-time. Like in the single-beam design, blank and sample are measured sequentially.

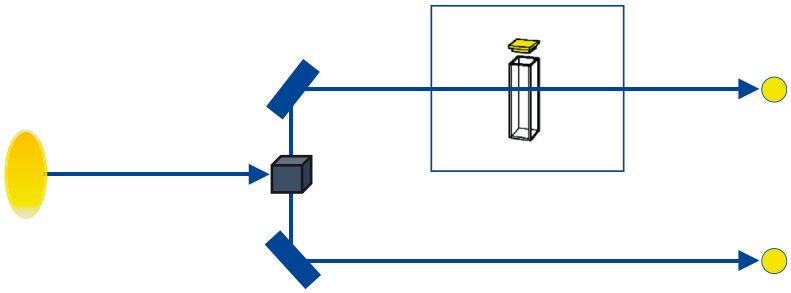

Figure 20: Dual-beam optical pathway.

- **Double-beam configuration**
  In a double-beam configuration, the light beam is split into an accessible reference and a sample beam. Blank and sample are measured contemporaneously.

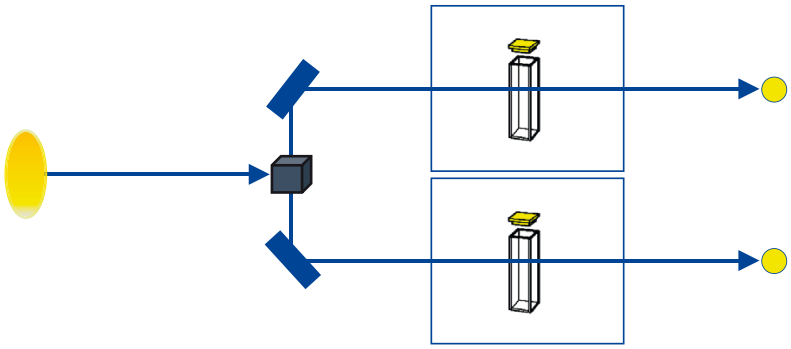

Figure 21: Double-beam optical pathway.

3.2. Micro-volume UV/Vis spectroscopy

In addition to cuvette-based spectrophotometers, METTLER TOLEDO provides a spectrophotometer capable of performing micro-volume UV/Vis measurement. This instrument is capable of measuring very small volumes and highly concentrated samples. The method is straightforward. The sample is pipetted directly onto the measuring platform without further dilution. Therefore, manipulation errors are avoided. Moreover, selecting a specific path length allows for the measurement over a large concentration range with as little as 1 µL of sample. Micro-volume analysis is an ideal choice for biomolecular analysis. The most common applications are the concentration determination of nucleic acids – DNA and RNA – the purity of nucleic acids, and the concentration determination of proteins.

To learn more about micro-volume spectroscopy, consult the **UV/Vis Micro-Volume Measurement Guide** and the **UV/Vis Life Science Applications Guide**. Both are available for download at mt.com.

4. Applications

4.1. Photometric

The Photometric measurement, sometimes also called Fixed Wavelength measurement, is the most straightforward application of a spectrophotometer. It is a single or multiple wavelength measurement, and the result can be reported in absorbance or transmittance. The wavelength is usually chosen at a peak maximum, i.e., at the peak of the absorption band. Further calculations can be done to obtain the final result, for example, a concentration of a substance based on its extinction coefficient.

Photometric measurements are widely used in quality control (QC). Generally, the expected absorbance (or transmittance) values and the corresponding tolerances are known during the quality control of a product. Therefore, measurement at a fixed wavelength can suffice to assess the quality of the product.

A typical example of photometric application is the verification of the purity and degree of oxidation of olive oil. It enables the product to be classified as "Extra Virgin", "Virgin", or simply "Olive Oil". The International Olive Committee's (IOC) olive oil standards specify exactly the measurement methodology and the threshold which must be met for oils to be graded as extra virgin, virgin, and so on. The olive oil samples are prepared in accordance to the procedure described the norm and absorbance is measured at four wavelengths (232, 266, 270 and 274 nm). Based on the measured absorbance, three quality criteria a calculated: K232, K270 and ΔK. Low values correlate with high-quality oil, as UV absorbance detects early and later states of oxidation, while high values indicate oil of lower quality.

Other examples of photometric applications are the determination of the purity of alcohol in the chemical industry and the determination of Vitamin a in fortified foods.

4.2. Scanning

In contrast to the fixed wavelength measurement, the spectral scanning measurements determine the absorbance or transmittance of a sample over a specified wavelength range or the full spectrum range, typically from 190 to 1100 nm. Following the scan measurement, the most often applied analysis is the detection of peaks and valleys in the spectrum. A peak is where the absorbance reaches a maximum, and a valley is where the absorbance is smallest between two peaks. The height and location of peaks and valleys are of interest as they give an indication of the sample composition and purity. Furthermore, the identity of a compound can be confirmed by comparing the spectrum to a known compound spectrum from a database.

Examples of scanning applications are the analysis of sunscreen in cosmetics,the identification of cyanocobalamin (vitamin B12) and the determination of nicotinamide in food and beverages.

4.3. Concentration determination by quantification

The quantification or concentration determination of a substance by UV/Vis spectroscopy is based on the Lambert-Beer Law, described in chapter 1.4, which states that the absorbance of a solution is directly proportional to the concentration of the absorbing substance in the solution and the path length of the cuvette. Thus, for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the absorbing substance in a solution. Quantitative measurements are usually performed at a single wavelength (ideally at a peak maximum). However, it is necessary to know how much the absorbance changes with concentration at that specific wavelength. This can be easily determined from a calibration curve, which represents the correlation between the concertation of a sample and the measured absorbance at the wavelength of interest.

To create a calibration curve, standards of known concentration are measured at the chosen wavelength. The absorbance of the standards is then plotted against the concentration, as shown in Figure 15 of chapter 2.3.1. The calibration curve for a spectrophotometric analysis should approximate the sample as closely as possible and encompass a suitable range of concentrations. Ideally, at least three different concentrations of the substance are measured, although a single one can be applied. In practice, five different concentrations will produce a more accurate calibration curve. The absorbance presents a linear relationship to the concentration, and a first-order regression curve can be fitted to the data points.

After the calibration curve establishes the correlation between concentration and measured absorbance, the concentration of an unknown sample can be determined by measuring its absorbance at the chosen wavelength. The corresponding concentration is then easily calculated based on the calibration curve.

Examples of quantitative applications are the determination of caffeine in soft drinks, the determination of iron in beer and the determination of Sulphur dioxide in packaged food.

4.4. Kinetics

UV/Vis spectrophotometry is often used to monitor the change of the concentration of either the reactant or the products by absorbance at a specific wavelength over time. This is a reaction as a function of time and, therefore, often called rate measurements. Kinetics measurements are often used to investigate enzyme activity or reaction rates and the affinity of the enzyme-substrate interaction. This analysis type is especially prevalent in the field of biotechnology, medicine, and food, as well as in chemistry.

Kinetic methods are beneficial for samples where some interfering components are present in varying concentrations from sample to sample. For example, UV/Vis absorption spectroscopy is applied in colored samples such as whole blood, bottled/canned soft drinks, and juices. A specific analysis can be performed by measuring the rate of change in absorbance of a sample without having to do complicated and time-consuming chemistry to eliminate the interfering, colored background or to apply some separation method.

Examples of kinetic applications are the determination of enzymatic activity (e.g., alkaline phosphatase) and the determination of the reaction rate of the oxidation of Iodide by hydrogen peroxide.

Please note that kinetic applications can only be performed with selected METTLER TOLEDO UV/VIS Excellence spectrophotometers (UV5Bio, UV5Nano and UV7).

4.5. Water analysis

The term "water analysis" describes various procedures to analyze water quality and safety. To meet the criteria under which water is judged fit for consumption or industrial processes - outlined under regulatory requirements set out by local authorities - a large amount of water testing work is performed daily in laboratories worldwide. Various technologies and instruments can be employed to implement a water testing workflow. Among them, UV/Vis spectroscopy has been proven reliable, fast, and easy to perform, requiring minimal bench space and the ability to measure down to low detection limits with sophisticated, yet affordable instruments.

By this method, a sample of water containing the analyte of interested is mixed with specific reagents. The analyte undergoes a color reaction, forming a product than can be measured photometrically with a UV/Vis spectrophotometer.

The EasyPlus UV/VIS spectrophotometer installs more than ready-to-use 150 methods, for the assessment of over 48 parameters. The methods have been validated with Spectroquant® test kits (under the brand of Supelco). The Spectroquant® test kits are ready-to-use, eliminating the time-consuming and costly preparation for chemistries and chemical reagents, while METTLER TOLEDO's UV/Vis Spectrophotometers deliver accurate and reliable measurement results with the predefined and validated methods. This solution dramatically facilitates workflows for waste, drinking and process water testing in the corresponding industrial segments.

A water testing workflow usually starts with the selection of a test kit that provides a suitable analyte concentration range. The water samples are then prepared by mixing with the reagents from the kits, with additional procedures such as heating when necessary. Once the sample preparation is completed, the methods can be simply started by scanning the 2D-Barcodes on the cells or the AutoSelector.

A typical example of water testing applications is the determination of phosphate in wastewater. Phosphate will readily react with ammonium molybdate in the presence of suitable reducing agents to form phosphormolybdenum blue, a blue colored complex. The intensity of the blue color is directly proportional to the concentration of phosphate in the solution. The corresponding test kits provide all reagents required to perform the reaction. After the sample preparation is carried out following the step-by-step instructions provided with the test kit, the absorbance at 690 nm is measured and the concentration of phosphate is automatically calculated by the EasyPlus UV/VIS spectrophotometer.

Other examples of water analysis applications are the determination of Chemical oxygen demand (COD) and the quantification of inorganic anions and cations in water (e.g., nitrate, chloride, ammonium, etc.)

For further information about water analysis with UV/Vis spectroscopy, download the **Easy Water Testing Guide**.

Spectroquant® is a trademark of Merck KGaA, Darmstadt, Germany.

4.6. Color determination

Color is an essential parameter in many industries, including food, cosmetics, and the medical industry. This importance shows that there is a real need for quantitative analysis of color, ensuring the results meet the industry's quality standards and company-specific standards.

As discussed in this guide in chapter 1.1, however, color perception is very individual and can be affected by the lighting conditions. Color determination based on spectral data measured with a UV/Vis spectrophotometer offers the accuracy and consistency required for quality control. It allows standardizing the measurement and delivers an objective assessment of the chromatic characteristics of the sample.

For example, in the beer industry, color measurements are an important parameter in the quality control and process control. In Europe, the current standard is EBC color scale (European Brewery Convention). Based on the EBC color scale, beer types can be distinguished according to their color. the e.g. a Pale Ale should have an EBC value of about 18.

The EBC color value is determined by measuring the absorbance of the decarbonated beer sample at 430 nm and 700 nm. The measured absorbance is automatically converted into the corresponding EBC value by the EasyPlus UV/VIS spectrophotometers. The absorbance at 700 nm is used as a turbidity check. If the value exceeds a specific threshold, the beer sample is too turbid and should be filtered through a membrane before being measured again. Multiple filtration cycles may be required until the absorbance at 700 nm is within the allowed range.

Other examples of color determination applications are the determination of the chromatic characteristics of wine and the determination of water color based on the APHA/Pt-Co/Hazen color scale.

For further information about color determination with UV/Vis spectroscopy, download the **Basics of Color Measurement Guide**.

5. Tips and Hints for Successful UV/Vis Measurements

The next chapter will provide tips and hints to assure successful UV/Vis measurements.

In addition, we recommend that you follow the Good UV/Vis practice program. GUVP™ makes sure that appropriately trained operators use the right equipment in a suitable environment. It forms the basis for minimizing risks, eliminating error sources, and thus achieving reliable results throughout the complete lifecycle of the instrument. You will find **more information** on mt.com.

5.1. Proper Cuvette Handling

The cuvette used to measure a sample is a part of the spectrophotometer's optical system. Therefore, the cuvette's position, geometry, and cleanliness significantly affect results and repeatability. We recommend observing the following steps when selecting the cuvette:

1. Cuvettes must have windows fabricated from a transparent material in the spectral region of interest. For measurements in the UV range, UV-transparent glass needs to be used, such as quartz glass or SUPRASIL® glass. For the visible range (>400 nm), optical glass, disposable PMMA (Polymethylmethacrylate), or PS (Polystyrene) cuvettes are frequently used. For more information about available cuvettes visit mt.com.
2. The windows of the cuvette must be cleaned before every use. For thorough inner and outer cleaning, use a 60% isopropanol/deionized water solution and wipe with an optical cleaning cloth or lint-free tissue (to avoid scratches from lint on the surface and avoid residues on the windows of the cuvette).
3. Hold cuvettes only on the frosted sides and be careful not to leave fingerprints on the cuvette. Wearing laboratory gloves can also help prevent fingerprints on the cuvette.
4. For best results, the reference and sample measurements must be done using the same type of cuvette. A matched pair of Standard cuvettes or Excellence Cuvettes with a low manufacturing tolerance is best. Mix the sample well before filling it into the cuvette. Avoid using glass pipettes to fill the cuvette, as they could scratch the optical surface. Pipettes with disposable plastic tips are ideal. Let the solution run down the glass wall into the cuvette to avoid bubble formation. Do not exceed filling over 80% of the cuvette's capacity.
5. For best measurement practice, the cuvette should remain in the cuvette holder between the measurements. If removed, care should be taken to always position the cuvette in the same direction in the cuvette holder, i.e., with the label towards the light source. This ensures that the optical effects are identical for reference and sample measurements.
6. The sample should be removed immediately after the measurement to avoid damage to the cuvette by evaporating solvents.
7. For short-term storage of the cuvettes, special cuvette holders are available. The delivered box should be used for long-term storage to avoid scratches and dust on the glass surface.

5.2. Solvent selection

A solvent for UV/Vis spectroscopy must be transparent throughout the applied region and should dissolve a sufficient quantity of the sample to give well-defined peaks. Every solvent has a UV/Vis absorbance cut-off wavelength. The solvent cut-off is the wavelength below which the solvent itself absorbs all the light. So, when choosing a solvent, it is important to be aware of its absorbance cut-off and where the compound under investigation is expected to absorb. If they are close, a different solvent should be chosen.

Care must be taken when working below 300 nm, where solvent absorption may be so high that the incremental absorption due to the sample is small compared to total absorption. The following table lists common solvents and the lower limit of their useful wavelength range.

Solvent	**UV absorbance cut-off (nm)**
Acetone	329
Benzene	278
Dimethylformamide	267
Ethanol	205
Toluene	285
Water	180

6. Performance Verification

The instrumental performance is the main factor directly affecting the accuracy and reproducibility of the measurements. The instrument must perform according to specification for critical UV/Vis measurements, especially in clinical, pharmaceutical, or industrial quality control. In the laboratories working according to the pharmacopeia, the instrument performance must be monitored regularly, and documentary evidence must be present. Furthermore, this is mandatory for laboratories that offer an accredited measurement service (e.g., in accordance with ISO 17025). The validation is also an essential requirement for laboratories working according to the Good Laboratory Practice (GLP).

Regular performance verification tests assess the quality of the measurements. For UV/Vis measurements specifically, this is the spectrum. The tests must guarantee:

- that the wavelength positions (x-axis) are correct (Wavelength accuracy) and stable (Wavelength repeatability)
- that the intensities, absorbances, or transmittances (y-axis) are correctly measured (Photometric accuracy) and stable (Photometric repeatability)
- that the measured absorbance (y-axis) increases linearly with increasing concertation (Photometric linearity)
- that the measured shape of the spectrum is correct and not distorted (Resolution Toluene, Stray light)

To perform the calibration of UV/Vis spectrophotometers, the use of certified reference materials (CRMs) is highly recommended. These materials are certified either for the absorbance value or the wavelength position at several wavelengths in the UV and visible spectral regions. Comparing the measured values with the certified values allows objectively assessing the spectrophotometer's performance.

Please note that only the UV7 Excellence spectrophotometer is compliant with the current pharmacopeia regulations. To learn more about performance verification and its regulatory implication, download the whitepaper "United States and European Pharmacopeia – What Every UV/Vis Lab Should Know" at www.mt.com/uvvis-calibration or visit www.mt.com/UV-VIS-Pharmacopeia.

GUVP™ Lifecycle Services
5 Steps to Minimize Risks

GUVP – Good UV/VIS Practice improves measurement quality by minimizing risks through a 5-step lifecycle program including specific services. It provides professional evaluation and selection tools, comprehensive installation and qualification services and tailored training and maintenance programs to ensure correct operation. As each step is thoroughly documented, traceability and up-to-date regulatory compliance are guaranteed.

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