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UV-Vis Spectroscopy or Ultraviolet-visible spectroscopy or Ultraviolet-visible spectrophotometer (UV-Vis) is also called absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. Electron transition takes place, so it is also called electron spectroscopy. It is a cost-effective, simple, versatile, and non-destructive technique that allows the sample to be used again for further analysis. It is a qualitative, quantitative, and analytical technique that compares a sample with a blank or reference sample to measure the amount of discrete ultraviolet and visible light absorbed or transmitted through a particular sample using Beer-Lambert law. It studies under vacuum conditions. The wavelength of UV-vis spectroscopy ranges from 190 nm to 800 nm. The UV region ranges from 190 to 400 nm, and the visible region from 400 to 800 nm. Near UV region is 190 nm to 400 nm, and far UV region is below 200 nm. The shorter the wavelength, the higher will be the frequency and energy. It occurs in UV region. Similarly, the higher the wavelength, the lower the frequency and energy in the visible region. Light Spectrum Its properties depend on sample composition and concentration. It helps to identify, assess purity, and quantify the components of the sample by analyzing the pattern of absorption and transmission of light. It may apply in several sample types, such as monolithic solids, liquids, glass, powders, and thin films. Absorbance (A): It, also known as optical density (OD), is the amount of light absorbed by the object and can be expressed as follows: Absorbance (A)= -log(T) Transmittance (T): It is measured by dividing the intensity spectrum of light transmitted through a sample (I) by the intensity spectrum of light transmitted through the blank (I0).T= I/Io When a specific wavelength of light hits a molecule, that molecule gets excited. Once the electron excites, it excites from the ground (lower) energy state to the higher energy state. When an electron jumps off, it absorbs light energy because electrons in the orbital at a lower energy state utilize energy to move to a higher energy level. Energy is neither created nor destroyed but can transform energy from one form to another. On passing EMR (UV- Vis range 200- 800 nm), only light possessing the precise amount of energy that can cause transitions from one level to another will absorb because matter's energy levels are quantized. If the energy is utilized, the intensity of light received is lost. At this time, the energy absorbed by the electrons will equal the energy difference between the two energy levels. During this stage, electron transition occurs. So, after the interaction of electromagnet radiation, the spectra received are called absorption spectra. Hence, it is called electron spectroscopy. Similarly, when electrons in the orbital at a higher energy level move to the ground energy level, the spectra received are called emissions. Beer-Lambert Law equation is the principle behind absorbance spectroscopy. The concentration of the sample can be determined directly from the absorption of spectra produced by these samples at specific wavelengths using the Beer-Lambert law. When a beam of light allows it to pass through a transparent medium, the rate at which an intensity decreases with medium thickness is directly proportional to the light beam's intensity. According to the Beer-Lambert Law, the absorbance is directly proportional to the concentration of the substance in the solution. Therefore, a sample's concentration can also be determined using UV-visible spectroscopy. The Beer-Lambert Law can be expressed in the form of the following equation: A = –log T = –log (I/Io) = log(Io/I)= ecl A = ecl Where A = absorbance l = optical path length of the cell or cuvette or sample holder(cm) c = concentration of the solution (mol dm-3) e = molar absorptivity of the compound or molecule in solution, which is constant for a particular substance at a particular wavelength (dm3 mol-1 cm-1) Following the Beer-Lambert Law, the plot of absorbance versus concentration should be linear if the absorbance of a series of sample solutions with known concentrations is measured and plotted against equivalent concentrations. This graph is known as a calibration graph. The main components of UV- Vis spectrophotometer are: Light Source Wavelength selector Sample container Detectors It is essential for emitting light in a wide range of wavelengths to work in a UV-Vis spectrometer. Commonly, a high-intensity light source used for both UV and Visible ranges is a xenon lamp. In contrast to tungsten and halogen lamps, it is less stable and more costly. So, the two lamps in this instrument are a deuterium lamp for UV light and a halogen or tungsten lamp for visible light as a source of light. The two lamps provide good intensity. While measuring the intensity of the light, the spectrometer ought to switch. A smoother transition is possible when the switchover occurs between 300 and 350 nm because the light emission for both visible and UV light sources is the same amount at that wavelength. In order to allow sample examination using the wavelengths that the light source emits, wavelength selection helps to ascertain which wavelength is appropriate for the type of analyte and sample. The commonly used wavelength selector in the UV-Vis spectrometer is the monochromator. It separates light into a narrow band of wavelength. From the entrance slit, radiation of different wavelengths will enter the monochromator. At a particular angle, the beam will collide and strike the dispersing element. A monochromator contains a prism that separates all different wavelengths of light in a single beam. It bends the monochromatic light and produces non-linear dispersion. Only single radiation or color of a specific wavelength will allow it to leave the monochromator and pass through its ultimate chain or exit slit. In a single-beam spectrophotometer, all the radiation coming from the light source passes through the sample as one beam. Single-beam spectrophotometers can determine color by comparing the light sources' intensities before and after a sample is inserted. The wavelength range measure is 190–750 nm; however, it may go up to 1100 nm. In a double-beam spectrophotometer, all the radiation coming from the light source splits into two beams: one passes through the sample, and the other only passes through the reference. Similarly, Double-beam spectrophotometers offer a wavelength range of 190 to 1100 nm. Moreover, the double-beam spectrophotometer measures absorbance versus wavelength or sample and reference beam ratio. The reference detector is used to adjust lamp brightness fluctuations for each measurement. After collecting the sample, the sample detector is measured in the sample position and deducted from the sample spectrum. It contains both a reference chamber and a sample chamber. The sample is kept in a flat, transparent container called a cuvette or sample chamber. The solvent in which the sample dissolves is kept in the reference chamber, also known as the blank. The sample cell's choice depends on the path length, shape, size, and transmission characteristics at the desired wavelength and the relative expense. For each wavelength, the light intensity passes through the beam separator to the reference chamber (Io) and sample chamber (I). The intensity of light symbolizes as Io measures the number of photons per second. When the light passes through the blank solution, it does not absorb light, referred to as (I). If sample I is less than Io, the sample has absorbed some light. The absorbance (A) of the sample is related to I and Io according to the following equation: Absorbance (A)= -log(T)= -log (I/Io) This equation shows the relationships between absorbance and transmittance. Also, the fraction I divided by Io is called transmittance (T), which expresses how much light has passed through a sample. T= I/Io T= I/Io = e–kbc Where: – I o is the incident intensity – I is the transmitted intensity – e is the base of natural logarithms – k is a constant – b is the path length (usually in cms). The lighter the refracted, the more transmittance occurs. The lower the absorbance, the higher the transmittance. In UV and visible regions, fused silica or quartz cuvettes are commonly used. Detectors rely on photoelectric coatings or semiconductors. It converts the incoming light from the sample into an electric signal or current. The higher the current, the greater the intensity. It has properties of low noise and high sensitivity, so it gives a linear response. It can detect a variety of wavelength ranges and different sensitivity. Finally, The data recorder usually plots the absorbance against wavelength (nm) in the UV and visible section of the electromagnetic spectrum. It focuses on verifying the concentration and purity of DNA and RNA, which plays a crucial role in downstream applications like sequencing. It ensures whether the DNA or RNA samples prepared for sequencing are contaminant or pure. Since pure DNA has an absorbance ratio of 1.8 and pure RNA has a ratio of 2, the 260 nm/280 nm absorbance ratio is crucial for displaying protein contamination in nucleic acids. 260nm/230nm absorbance ratio varies for RNA and DNA (2.15 to 2.50). It is essential in drug discovery and development, quantifying impurities in drug ingredients, dissolution testing of solid oral dosage forms like tablets, and chemical identification and quantification. It allows overlapping absorbance peaks in the original spectra using mathematical derivatives to identify pharmaceutical compounds. Likewise, the Identification of pharmaceutical compounds, Chlortetracycline (antibiotic) and benzocaine (anesthetic) in veterinary powder formulation, by overlapping the absorbance peaks in UV spectra using mathematical derivatives. It applies to assessing the sensory attributes, nutritional components of food and its products such as beer, wine, juices, energy and soft drinks, waters, other thin and thick liquids (honey, oils), fruits, vegetables, caffeine content, etc., and the chemical composition of ingredients and detect contaminants or adulterant to ensure the product is safe and healthier. It can be used in quality control in wine by identifying anthocyanin in blueberries, raspberries, and cherries. It can evaluate food and food product color, flavor, and aroma. It is essential in the biomass growth curve. It is used in culturing bacteria by estimating cell concentrations and growth tracking in measuring optical density at 600 nm. 600 nm is best to preserve the optical properties of culture media where bacteria grow and to avoid cell damage when there is a need for continuous experimentation. In the cosmetic industry, it is used to evaluate photostability agents and color index, quantify dyes and antioxidants, and detect adulteration. It is used in material science, like the characterization of small nanoparticles and to determine battery composition. It is used to examine structural protein changes by tracking changes in peak wavelength absorbance. In wastewater treatment, it is employed in kinetics and monitoring studies of dyes and dye byproducts to ensure adequate dye removal by comparing their spectra over time. It is used in cancer research to estimate hemoglobin concentration. It is used to measure color index to monitor transformer oil as a preventive measure to ensure electric power is delivered safely. It is used in petrochemistry for characterizing crude oil, quality of crude oil gravity, formulation of indices for aromatic content, and sulfur content. In the biochemistry and genetic fields, it is used to quantify DNA, protein/enzyme, and thermal denaturation of protein. It is non-destructive and reusable. It is easy to operate and the fastest method to interpret data because it gives accurate readings. It is an inexpensive technique. It is more convenient. It may take time to prepare using the machine. Spectrometer reading might be affected if it keeps with any electronic noise, outside light, and other contaminants. That could affect the accuracy of the machine's measurement could be impacted by stray light from defective equipment design because the linearity range and substance absorbency measuring are likely to be reduced by stray light. About Author UV Vis spectroscopy is a scientific technique used to measure the amount of light that is absorbed or transmitted by a sample at different wavelengths of ultraviolet (UV) and visible (Vis) light.The process involves shining a beam of UV Vis light through the sample and measuring the amount of light that passes through it. By analyzing the pattern of absorption and transmission of light, scientists can identify and quantify the components of the sample. A unique relationship exists between the substance and its UV Vis spectrum when a substance absorbs the maximum light at a specific wavelength. This relationship can be used for:Qualitative analysis, i.e., determining the presence of certain substancesQuantitative analysis, i.e., determining the amounts of certain substances.UV Vis spectrophotometry is commonly used in many fields of science, including chemistry, biology, and physics, to study the properties of materials and their interactions with light. It is also widely used in industry for quality control and analysis of materials such as drugs, food, and cosmetics.This page will give you essential knowledge about UV Vis Spectroscopy and its applications.UV Vis spectroscopy is a type of absorption spectroscopy in which a sample is illuminated with electromagnetic rays of various wavelengths in the ultraviolet (UV) and visible (Vis) ranges. Depending on the substance, the UV or visible light rays are partially absorbed by the sample. The remaining light, i.e. the transmitted light, is recorded as a function of wavelength by a suitable detector. The detector then produces the sample's unique UV Vis spectrum (also known as the absorption spectrum).To learn more about basics of UV Vis spectroscopy, download the METTLER TOLEDO guide "Spectrophotometry Applications and Fundamentals"When light hits an object, it can be absorbed by the object, typically because the wavelength of the absorbed light corresponds to an electronic excitation in the object. The remaining light is transmitted, i.e. it passes through the object.In a UV Vis spectrophotometer the transmittance is measured by dividing the intensity of light through a sample solution in a cuvette and compares it to the intensity of the light before it passes through the sample. The intensity of light passing through the blank (I0).Absorbance (A), also known as optical density (OD) is the amount of light absorbed by the object and can be expressed as follows Transmittance (T) To learn more about fundamental knowledge on UV Vis spectroscopy techniques, download the guide, "Spectrophotometry Applications and Fundamentals".The Beer-Lambert Law states that the amount of energy absorbed by a solution is proportional to the path length and concentration. Put simply, a more concentrated solution absorbs more light than a dilute solution does.The mathematical statement of Beer's law is: A = ε.d.cWhere ε = molar absorptivity, d = path length and c = concentration. Molar absorptivity is a unique physical constant of the sample that relates to the sample's ability to absorb light at a given wavelength. ε has the unit is L·mol-1·cm-1.To learn more about basics of UV Vis spectroscopy, download the METTLER TOLEDO guide, "Spectrophotometry Applications and Fundamentals"A UV Vis spectrophotometer is an instrument designed to measure the absorbance in the UV region by a sample using the Beer-Lambert law. It measures 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 light source, a sample holder, a dispersive device to separate the different wavelengths of the light, and a suitable detector. Scanning Spectrophotometer Conventional scanning spectrophotometers work on the principle of taking consecutive transmittance measurements at each defined wavelength. The light is split into different wavelengths by a diffraction grating. A sample cuvette is placed between the diffraction grating and the detector. Array Spectrophotometer In an array spectrophotometer, the sample is illuminated by a continuum, i.e. all spectral components of light at once, thus it absorbs light of different wavelengths simultaneously. The transmitted light is then diffracted by a reflection grating. This instrumentation helps to acquire the UV Vis spectrum faster than it can be obtained using a traditional scanning spectrophotometer.A spectrophotometry instrument must perform according to its specification for critical UV Vis measurements, especially in clinical, pharmaceutical or industrial quality control. Therefore, performance verification must be carried out regularly. Calibration results must also be recorded and stored.Download "How Should UV Vis Labs Do Spectrophotometer Calibration" for insight into the importance of calibration relative to UV Vis chapter revisions found in the Ph. Eur. 10 and USP42 NF37.The major parameters to be calibrated for a UV Vis spectrophotometer are shown in the following table.Performance testCertified reference material (CRM)Instrument Test ParameterAcceptance criteriaUSP 42 NF 37Ph. Eur. 10Wavelength accuracy &repeatabilityHo(ClO4)3: 4 % Ho2O3 in 10 % v/v HClO4Blank: Air14 wavelengths(240 nm – 650 nm)Xe: 2 wavelengths (260.6, 528.6 nm)UV (200 – 400 nm): ± 1 nmVis (400 – 780 nm): ± 2 nm(S.D.) < 0.5 nmUV (< 400 nm):± 1 nmVis (> 400 nm):± 3 nmPhotometricaccuracy &repeatability**K2Cr2O7 in 0.001 M HClO4Blank: 0.001 M HClO460 mg/L0 A – 2 A,235, 257, 313, 350 nmFor absorbance ≤ 1AAccuracy : ± 0.010AARepeatability:S.D. ≤ 0.005 AFor absorbance > 1AAccuracy: ± 1%Repeatability:S.D. ≤ 0.5%Accuracy: ± 0.010 A or ± 1 %, whichever is greaterNicotinic acid in0.1 M HClBlank: 0.1 M HCl12 mg/L0.26 A – 1.6 A213, 261 nmPhotometric linearityK2Cr2O7 in 0.001 M HClO4Blank: 0.001 M HClO46 – 200 mg/L, up to 3.0 A,235, 257, 313, 350 nmAll measured filters fulfill photometric accuracy acceptance criteriaR2> 0.999Nicotinic acid in0.1 M HClBlank: 0.1 M HCl6 – 60 mg/L, up to 2.5 A213, 261 nmStray light according to procedure A(SFRM)1.2 % w/v KCl/H2O;10 mm path lengthBlank: 1.2 % w/v KCl/H2O, 5 mm path lengthAmax at 198 nm≥ 0.7 A(NA)Stray light according to procedure B (SWM)1.2 % w/v KCl/H2O;10 mm path lengthBlank: H2O, 10 mm path lengthAmax at 198 nm≥ 2.0 A≥ 2.0 AResolution0.02 % v/v toluene in n-hexaneBlank: n-hexane/n-heptane (Ph. Eur. 10)Amax,269/Amin,267>1.3Levels are stated in the respective monograph** No specification of Photometric Repeatability (Precision) in Ph. Eur.S.D. - Standard deviationColors make our world more interesting. When we see an object, the light reflected from the object enters our eyes and is collected by several types of photoreceptors in the retina. Depending upon photoreceptor sensitivity, different people may perceive the same color differently.To accept the accuracy of a specific color universally, numerical values must be assigned. In short, measurement equipment such as spectrophotometers and colorimeters deliver color results as values to ensure color-determination accuracy and repeatability.Spectrophotometers quantify color data by collecting and filtering wavelengths transmitted through a sample. A mathematical equation is applied to the spectral data to map the color onto a color scale.A CIE (Commission internationale de l'éclairage) color scale is defined using three parameters: hue, chroma and lightness.Hue is the dominant color of an object. Primary and secondary colors combined make hue.Chroma, also known as saturation, describes how vivid or dull a color is.Lightness is the luminous intensity of the color (whether it is dark or light).Each CIE color system uses three coordinates to locate a color on a scale. The three primary color scales are Tristimulus CIE XYZ, CIE L*a*b*, and CIE L*u*v*.For example, when a color is expressed in CIE L*a*b*:L* defines lightnessa* denotes the red/green valueb* denotes the yellow/blue valueColor is a key factor for instant recognition.Providing an overall successful visual experience for consumers can influence the decision to buy. Therefore, color is important in the definition of brand identity and product consistency. Different color scales are established to uniquely define a product according to industrial standards. These scales include:ScaleStandardApplications SayboltASTM D156, ASTM D6045To determine if fuel (kerosene, gasoline, diesel, naphtha, etc.) is contaminated or has degraded in storageAPHA/Pt-Co/HazenASTM D1209Yellowness index used as a metric for purity checks in the water, chemical, oil, and plastics industriesGardnerASTM D1544/D6166, DIN EN ISO 4630-2For testing products such as resins, fatty acids, varnishes and drying oils that have attained color through heatingCIELABDIN EN 11664-4, DIN 5033-3, 4630, ASTM Z 58.7.1 DIN 6174Quality control for the flavor & fragrance and food & beverage industriesCIELab Color Measurement - UV Vis SpectroscopyEBCMEBAK Method 2.13.2, EBC Method 8.5, EBC Method 9.6To measure color intensity and turbidity (haze) in EBC units of beer, malts, caramel, etc.USP/EUPUSP-24 Monograph 631, EP method 2.2.2Quality control of drugsHess-IvesDGK test method F 050.2Used to test chemicals and surfactant liquids (mainly in the cosmetics industry)4. Microvolume Analysis Using a UV Vis SpectrophotometerHow to Perform Microvolume Analysis in UV Vis Spectroscopy? A micro-volume spectrophotometer measures sample volumes as low as 1 µl. The concentration of nucleic acids in a sample is usually of the order of nano or microgram per milliliter.Diluting such micro-volumes and getting accurate results is challenging. Therefore, microanalysis without dilution becomes important for downstream analysis of nucleic acids.During analysis of nucleic acids the micro-volume sample is pipetted into the fine compartment on the pedestal surface. The light beam from the lamp source is guided by the fiber optics to the micro-volume platform. As the path length is reduced to the order of a millimeter, higher concentration of analyte can be analyzed precisely without multiple dilutions.A micro-volume system uses fiber optic technology along with the inherent properties of the sample (such as surface tension) to retain the sample on the pedestal platform and determine the real-time absorbance of the samples at low volumes.With these advantages, micro-volume analysis becomes an ideal choice for biomolecular analysis.Attend the on-demand webinar "Maximize Workflow Accuracy through Good UV/VIS Practice in Nucleic Acid Analyses" for tips and tricks on microvolume analysis.Quality Control of Nucleic Acids Nucleic acid quantification is an essential pre-analytical method for obtaining accurate and reliable results in many molecular biology assays such as Next-Generation Sequencing (NGS), Polymerase Chain Reaction (PCR), Real-Time PCR (quantitative PCR; qPCR), cloning and transfection.Qualitative and quantitative control of nucleic acids can be performed by determining the purity and the concentration of nucleic acids.DNA Analysis Methods A260 gives the correlation of the concentration of nucleotides and A280 gives that of the residual proteins. The amino acids tyrosine and tryptophan absorb at 280 nm and phenylalanine absorbs well at 260 nm. This allows calculation of the ratio A260/A280 for DNA purity using direct absorbance measurements. Good-quality DNA will have an A260/A280 ratio of 1.7–2.0 Protein Quantification Assays Different methods of total protein quantitation include A280, Bicinchoninic acid (BCA), Bradford, Lowry, Pierce and other novel assays. Proteins in solutions have maxima at 280 nm due to amino acids with aromatic rings and minima at around 220 nm due to the presence of peptide bonds.The concentration is calculated by using the Warburg formula:Protein concentration (mg/ml) = 1.55 X (A280 reading) – 0.76 X (A260 reading)To learn more about DNA analysis with UV Vis spectroscopy, download the application editorial "260/280 Ratio: Indicator of Protein Contamination".Good accuracy and precision in UV Vis measurements can be attained by taking precautions to avoid errors. Typical error risks that should be accounted for when taking UV Vis measurements includes:Spectral characteristics. Spectral characterization is performed during the calibration process. Major factors that may lead to erroneous results are wavelength accuracy, spectral bandwidth, stray light, and linearity. Photometric characteristics. Photometric characteristics include the spectral sensitivity of the light source, the temperature-dependent sensitivity of the light source and detector, etc.Optical interactions. The radiations of the lamp source may interact with the cuvette material, altering the intensity of sample absorbance. Such optical interactions can be avoided by selecting the right cuvette material.Miscellaneous factors. Other factors such as temperature, line voltage fluctuations, vibrations, contamination, or heating of the sample by the photometer also affect measurements adversely.Although not all errors can be avoided, errors can be minimized for better results. 'Download the Good UV/VIS Practice brochure "Trustworthy Results for UV/Vis Spectroscopy". How to choose the right cuvette for analysis?Choosing the right cuvette involves selecting the right material and the correct size based on your sample and instrumentation.The material of cuvette should have a sufficient transmission at a given wavelength. Light attenuation on the cuvette walls should not affect the outcome of an analysis. Glass cuvettes are not used in the UV region for analysis below 370 nm as they absorb the radiation. It is recommended to use them only in the visible region.The chart that follows gives the usable transmission ranges of cuvettes:MaterialTheoretical transmission range (nm)Far UV quartz170-2700Optical glass320-2500Near IR quartz220-3800UV silica220-2500UV plastic220-900Disposable PS cell340-750Disposable PMMA cell285-750The size of the cuvettes also affect measurement capabilities. The nominal radiation path length of the cuvette is 10 mm. Depending upon the samples, the length can be varied from 1 mm to 100 mm.Standard cuvettes can be used for most of samples under study. Common absorption and fluorescence cuvettes have an external base of 12.5 mm x 12.5 mm, a height of 45 mm, and internal dimensions 10 mm x 10 mm.Long path cuvettes (cuvettes having a pathlength more than 10 mm) are used when the sample is too dilute or the sample vaporizes or undergoes a chemical change during the measurement process.Short path cuvettes (cuvettes having a pathlength less than 10 mm) are used when absorbance is high and dilution is difficult.How to handle cuvettes correctly?When handling cuvettes, always carry the cuvette using the frosted sides. Avoid touching the transparent optical surfaces with your fingers, as fingerprints can cause significant absorbance and thus impact accuracy.Avoid using glass pasteur pipettes to fill the cuvette, as they could scratch the optical surface causing further interference. Pipettes with disposable plastic tips are recommended.The cleanliness of cuvettes has a major effect on results, so we must consider this as a very important factor.The following steps are recommended for deep cleaning of quartz cuvettes:Soak the cuvettes in the cleaning solution.Remove the cleaning solution and rinse the cuvettes with deionized water.Wash the cuvettes with ethanol or acetone, except in cases of proteins (see table below).Dry the cuvettes by wiping with a lint-free tissue followed by air drying or oven drying.The chart that follows gives the usable transmission ranges of cuvettes.Aqueous solutionsOrganic moleculesDifficult to remove particlesProteinsHeavy metalsFatty acidsCleaning solutionsEqual parts by volume of 3 M HCl and ethanolWash with 50% nitric acidConcentrated HNO3 or 2 M HClEqual parts by volume of ethanol and 3 M HClIncubate at room temperature with trypsin(Ethanol and acetone are not recommended for cleaning.)Equal parts by volume of sulfuric acid 2 M and 50% deionized waterAqua regiaEqual parts by volume of IPA and Deionized waterSoaking time*10 minutes10 minutes30 secondsOvernight20 minutesWipe*The soaking time stated in the table is rough estimation; however, it is only recommended that you soak cuvettes until stains/contaminants are removed.Glass cuvettes can be cleaned by rinsing the cuvettes with acetone or ethanol, followed by rinsing with water. Air-drying is recommended.Plastic cuvettes can be washed with deionized water several times. Washing plastic cuvettes with chemicals is not recommended. Prepare the sample Instrument sensitivity may be low for diluted concentrations of biological samples. To increase the sensitivity of such samples, consider taking higher concentration of the sample. Micro volume measurements typically need 1-2 µl of sample volume. Use calibrated pipettes for taking the sample. Care must be taken that a homogeneous sample is prepared and taken for analysis.Clean the micro volume platform Ensure that the micro volume pedestal surface and the the mirror are cleaned properly. Simply wipe off the surfaces with a lint-free tissue using deionized water. If using a buffer solution, detergents or a sticky sample, clean the surface multiple times before proceeding for the next sample.Place the sample on the platform Slowly and steadily, place the sample onto the surface to avoid bubble formation.Work within detection limits Know the instrument's lowest and highest detection limits and work within that range.Be careful while preparing the sample and pipetting it into a cuvette or onto a microvolume platform. The sample should be homogeneous.If any suspended solid particles are present in the sample, the light may scatter. In such cases, filter the sample using a syringe filter.Fill the sample in a cuvette considering the z dimension of the sample holder. This will ensure that the light is passing through the sample. z-dimension is the distance from the bottom of a cuvette to the height at which the light beam passes through the sample.Before every measurement, clean the cuvette with a lint-free tissue. Use new tissue every time.Use the same solvent/solution buffer that was used in the sample preparation as a blank.UV Vis spectroscopy is a versatile analytical technique with a wide range of applications in various industries. Some of the significant applications of UV Vis spectroscopy in different industries are:Food & BeverageUV Vis spectroscopy determines the quality and composition of food and beverage products. It can be used to analyze the color (e.g., wine), flavor, and aroma of food products, as well as to detect the presence of contaminants or adulterants.PharmaceuticalUV Vis spectroscopy analyzes the purity, concentration, and identity of drugs and other pharmaceutical products. It is also used to monitor the stability of pharmaceuticals over time.CosmeticsEvaluation of photostability of agents for formulations, particle characterization of UV blocking agent, assessing the color index, detecting adulteration (perfume industry), study of optical properties, quantification of dyes, antioxidants, etc.PetrochemicalCharacterization of crude oil, calculation of asphaltene fractions, formulation of indices for aromatic content, quality of crude oil gravity, sulfur content, calculating Hildebrand solubility factor.(Extended to bitumen, heavy & shale oils and oils from fluid catalytic cracking, coking, or coal liquefaction)ChemicalDetermining chemical properties, final quality assessment of finished product, study of polymer composition, qualification of waste water, determination of purity & dyeing efficiency, photocatalytic degradation of polymers/dyes, pesticides residues in soil or waterBiotechnologyConcentration and purity of nucleic acid, proteins (A280, BCA, Biuret, Bradford Lowry, OD 600), microbial cell culture measurements, denaturation of protein, kinetic studies (enzymatic activity), biological samples such as blood, plasma, serum, etc.The different spectroscopic techniques are mainly differentiated by the radiation they use, the interaction between the energy and the material, and the type of material and applications they are used for. The spectroscopic techniques commonly used for chemical analysis are atomic spectroscopy, ultraviolet and visible spectroscopy (UV Vis spectroscopy), infrared spectroscopy, Raman spectroscopy and nuclear magnetic resonance.Type of Spectroscopy Type of RadiationInteractionsWavelength ϒ-ray spectroscopyϒ-raysAtomic nuclei< 0.1 nm X-ray fluorescence spectroscopyX – raysInner shell electrons0.01 – 2.0 nmVacuum UV spectroscopyUltraviolet (UV)Ionization2.0 – 200 nmUV Vis spectroscopyUV VisValance electrons200 – 800 nmInfrared & Raman spectroscopyInfraredMolecular vibrations0.8 – 300 mmMicrowave spectroscopy MicrowavesMolecular rotations1 mm to 30 cmElectron spin resonance spectroscopyElectron spinNuclear magnetic resonance spectroscopyRadio wavesNuclear spin0.6 – 10 mWhat Are the Different Molecular Interactions in the UV Region?The absorption of UV light results in electronic transitions from lower energy levels to higher energy levels. Absorption of ultraviolet radiation in organic molecules is restricted to certain functional groups (chromophores) that contain valence electrons of low excitation energy. The molecular transitions/interactions that take place due to UV absorption are:π- π* (pi to pi star transition) – bonding to anti-bonding orbitaln - π* (n to pi star transition) – non-bonding to anti-bonding orbitalThese transitions need an unsaturated group in the molecule to provide the π electrons.σ (bonding) to σ* (anti-bonding) transitions require higher energy and therefore cannot be detected using UV Vis spectroscopy.Consider a functional group containing atoms with one or more lone pairs of electrons that do not absorb ultraviolet/visible radiation. However, when this functional group is attached to a chromophore, it alters the intensity and wavelength of absorption. This phenomena is called an auxochrome or a color-enhancing group.The presence of an auxochrome causes the position shift of a peak or signal to a longer wavelength, which is called a bathochromic or red shift. The functional groups contributing to bathochromic groups are substituents such as methyl, hydroxyl, alkoxy, halogen and amino groups.The auxochrome that causes position shift of a peak or signal to shorter wavelength is called a hypsochromic or blue shift. Actually, the combination of chromophore and auxochrome behaves like a new chromophore having a different absorption maxima (λmax). For example, benzene shows λmax at 256 nm, whereas aniline shows λmax at 280 nm. Hence, the NH2 group acts as an auxochrome and causes the shift of λmax to a larger value.The spectral bandwidth (SBW) of a spectrophotometer is related to the physical slit-width and optical dispersion of the monochromator system. Resolution is the ability of an instrument to separate light into finite, distinct wavelength regions and to distinguish each finite region. Spectral bandwidth is typically used for scanning instruments, whereas resolution is typically used for array instruments.For most pharmacopeia quantitative purposes, a spectral bandwidth of less than 2 nm is sufficient and the acceptance criteria for the ratio is 1.3. Spectral resolution can be used for comparison with spectral bandwidth.The table shows the resolution of METTLER TOLEDO's UV/VIS Excellence spectrophotometers, which is measured using toluene in hexane, and the equivalent SBW.InstrumentSpectral resolution Equivalent SBW (nm)UV5> 1.5< 2.0UV5Bio> 1.5< 2.0UV5Nano> 1.7< 1.5UV7> 1.9≤ 1.0The best light source would be one that provides good intensity with low noise across all ultraviolet and visible wavelengths and offers stability over a long period. There is a range of light sources which are commonly employed as mentioned below.Light SourceWavelength Range(nm)RegionLifetimeTungsten filament lamp350 – 2500VIS + IR3,000 hrDeuterium arc lamp190 – 400UV1,000 hrHydrogen lamp190 – 400UV1,000 hrXenon flash lamp190 – 1100UV + VIS + NIR5,500 hr** Corresponds to 50 Hz flashes at constant operationPrisms and diffraction grating are typical dispersive elements. A prism achieves dispersion due to the difference in the material refractive index according to the wavelength. However, a diffraction grating uses the difference in diffraction direction for each wavelength due to interference. Both prisms and diffraction gratings can spread light spectra into many colors for analysis. However, a diffraction grating is less sensitive to the color of the light and can be made to spread colors over a larger angle than a prism. The glass in a prism is clear to visible light, but it absorbs and blocks light in the infrared and ultraviolet part of the spectrum. A diffraction grating with a few hundred lines per inch can deflect light in the middle of the visible spectrum by at least 20 degrees. The deflection angle of a glass prism is generally much smaller than this.Molecules can be analyzed using UV Vis spectroscopy if they possess any functional group or conjugation, or if they produce a color complex. As inorganic compounds do not contain any functional group or conjugation, the common method for analyzing them is by reaction with a suitable compound. This produces a color complex whose absorbance can be photometrically measured in the visible region and correlated with its actual concentration. For example, iron is commonly analyzed by a reaction with 1, 10-phenthroline to produce a red color complex. The absorbance of the complex is measured at 570 nm to estimate iron concentration.The main difference between a single beam and double beam spectrophotometer follows.Single beam spectrophotometer: A single beam from the light source passes through the sampleDouble beam spectrophotometer: The light beam from the light source is split into two parts: one part goes through the sample, and the other part passes through the referenceBeam splitting in a double beam spectrophotometer is achieved in two ways:statically, with partially transmitting mirrors or a similar deviceattenuating the beams using moving optical and mechanical devicesHow to Analyze Solid Polymer Film Using UV Vis?The analysis of a solid sample is performed mainly by estimating its absorbance, transmittance and reflectance. Common parameters determined for solid polymers include % transmittance, cutoff wavelength, and yellowness index. The sample is mounted on a holder specifically designed for solid samples and readings are taken in the same manner as they are for liquid samples. A solid sample holder enables measuring of solid samples such as films or glass.Temperature affects absorbance values. Different solvents undergo different interactions at different temperatures. Solution parameters that change due to temperature changes are:Rate of reaction. The rate changes when temperature is elevated. This can cause a change in the activity of the sample. Enzymatic/biomolecular reactions are very sensitive to temperature.Solubility of a solute. Solubility is affected with variations in temperature. Poor solubility may result in imprecise absorption.Expansion or contraction of the solvent. This may lead to a change in the concentration of the solution and affect the absorbance, as absorbance is linearly related to concentration.Schlieren effect. This effect may occur with temperature changes, leading to a series of convective currents which may change the true absorbance.Optical performance parameters such as photometric noise, wavelength accuracy/repeatability, photometric repeatability and stray light are not influenced by temperature within a range of 10 – 40 °C.Whereas, optical parameters like photometric resolution (toluene/hexane ratio) and photometric accuracy wavelengths (K2Cr2O7 in HClO4) show a temperature dependency ranging from 0.014 to -0.034/unit within 10 – 40 °C.Temperature control for UV Vis spectrophotometry can be achieved using high-performance thermostating systems like CuveT and CuvetteChanger. Learn more here.What Is Stray Light?Stray light is defined as light that reaches the detector which is not from the instrument's light source and does not follow the optical path, causing a deviation at the correponding wavelength. Therefore, the light intensity measured by the detector is higher than it actually should be. Conversely, this also means that the measured absorbance is lower than the true absorbance because it is reduced by the contribution of stray light. This effect is more prominent at higher absorbance values (high sample concentrations).Download the whitepaper to learn more about the origin and accurate measurement of stray light:The sample compartment in UV Vis array spectrophotometers is open due to the fact that array instruments use reverse optics and the simultaneous detection of all wavelengths of the spectrum.Reverse optics: The light is diffracted after it has gone through the sample. Due to this, only a small fraction of the external ambient light contributes to the signal in a given wavelength region.Simultaneous detection: Using an array detector which provides 2048 light intensity signals at the same time, full spectrum is recorded within one second. Because the measurement is very fast, the effect of ambient light is significantly reduced. The Agilent Uv-Vis Spectroscopy technique is a technological advancement widely used in various areas of Science. It's used for bacterial culturing, nucleic acid purity checks, drug identification, quantitation, and quality control in chemical research and the beverage industries. This innovative technology makes the lives of researchers and scientists easier. Let's learn about Uv-Vis spectroscopy, how it works, and its advantages and limitations. What is Uv-Vis Spectroscopy? Uv-Vis Spectroscopy is a quantitative and analytical technique that measures the amount of visible or UV light a chemical substance absorbs through a Uv-Vis spectrometer. The technique is done by measuring light's intensity in wavelengths that passes through a particular sample and then comparing it with a blank or a reference sample. Generally, Uv-Vis Spectroscopy is widely used in several sample types: liquids, glass, and thin films. It is an advanced technique that sees beyond the visible light spectrum our eyes can see, including ultraviolet and infrared light. How does Uv-Vis Spectroscopy Work? To give you a better understanding of how Uv-Vis spectroscopy works, let's talk about its main components and the processes of how light is absorbed and measured by the spectrometer. 1. Components and How They Function Even though Uv-Vis spectrometers come in various forms, all these machines can only function optimally when these components are complete and properly working: 2. Lightsource For a Uv-Vis spectrometer to work, a light source is essential. One of the most common high-intensity light sources used for visible and UV ranges is a xenon lamp. However, it's more expensive and less stable compared to halogen and tungsten lamps. If a spectrometer needs two lamps, a halogen or a tungsten lamp is typically used as visible light and a deuterium lamp as the UV light. Because these are two different light sources, the spectrometer should switch when measuring the light's intensity. Generally, the switchover occurs at 300 to 350 nanometers, when the light emission is the same for both the visible and UV light sources, allowing smoother transition. 3. Wavelength Selector Wavelength selection is done to determine which wavelength is suited to the type of analyte and sample to allow sample examination from the wavelengths the light source emits. The most widely used selector in Uv-Vis spectrometer is the monochromator, and this contains the following parts: Entrance slit Collimating lens Dispersing device, commonly a grating or a prism Focusing lens Exit slit Polychromatic radiation or radiation of multiple wavelengths will enter the monochromator from the entrance slit. The beam will collimate and strike the dispersing element through a specific angle and split into several component wavelengths using the prism or the grating. Single radiation of a specific wavelength will leave the monochromator via the exit slit. Some Uv-Vis spectrometers use monochromators and filters to narrow light wavelengths, allowing more precise measurements and improving the signal-to-noise ratio. 4. Sample Container The sample container and reference solutions should be transparent compared to the radiation passing through. Fused silica or quartz cuvettes are commonly used for spectroscopy in both UV and visible regions. 5. Detectors The most common detector used in Uv-Vis spectroscopy is the photomultiplier tube, which contains a photoemissive cathode, dynodes, and an anode. A photoemissive cathode emits electrons when it's struck by radiation photons, while the dynodes emit multiple electrons each time an electron strikes them. When a radiation photon enters the tube, it strikes the cathode, which will emit several electrons. The emitted electrons will accelerate and strike the first dynode, which will emit more electrons as each incident electron strikes it. Again, the electrons emitted will accelerate to the second dynode, repeating the process until the electrons reach the anode. Once the electrons reach the anode, the photon from the beginning of the process has already produced millions of electrons, and the resulting current will be measured and amplified. The Purpose and Applications of Uv-Vis Spectroscopy Uv-Vis Spectroscopy has been widely used in various sample testing today. This technique has the following famous innovative applications: 1. RNA and DNA Analysis One of the most widespread applications of the spectrometer is verifying the RNA or DNA purity and concentration. The Uv-Vis spectroscopy ensures that the DNA or RNA samples prepared for sequencing or other applications are not contaminated with any chemicals that could negatively affect the results. 2. Pharmaceutical Analysis The mathematical derivatives of a Uv-Vis spectrometer have been an effective machine to detect individual pharmaceutical compounds in overlapping absorbance peaks or different powder formulations. 3. Bacterial Culture Uv-Vis spectroscopy is also widely used in culturing bacteria, estimating their cell concentrations, and tracking their growth. The wavelength measurement commonly used is 600 nm to preserve the bacterial culture media properties and the cells when they are needed for continuous and consecutive experimentation. 4. Beverage Analysis Uv-Vis spectroscopy has also effectively identified the quantitative content of certain compounds in various beverages, such as caffeine content and colored substances like anthocyanin in wine. 5. Other Applications Uv-Vis spectroscopy has proven its success and efficiency in various other applications, including the following: Food and agriculture Cosmetic industry Petrochemistry Material Science Quality Control Astrology Advantages of Uv-Vis Spectroscopy The best advantage of utilizing Uv-Vis spectrometers is their optimal accuracy. These machines are guaranteed to give you accurate readings, which are essential when you need to prepare chemical solutions or record the movement of the celestial bodies. Uv-Vis spectroscopy is also easy to understand with its simple analysis ability. The spectrometers are convenient and easy to operate, and there is only a rare chance that you will get the readings wrong. Disadvantages of Uv-Vis Spectroscopy The main disadvantage of Uv-Vis spectrometers is their challenging assembly, and it may take time to prepare using them. Ensure that the area where you'll place the device is clear of any electronic noise, outside light, and other contaminants that could affect the measurements and readings of the spectrometer. A Uv-Vis spectrometer is sensitive to external factors, so you must ensure your working area is clean and dust-free. Aside from that, the device's stray light caused by a faulty equipment design could also influence the accuracy of the machine's measurement. This is because stray light will likely reduce the linearity range and substance absorbency it's measuring. Uv-Vis Spectroscopy Limitations Even an advanced technique like Uv-Vis spectroscopy has its limitations, too. You can grasp what these are below: 1. Sample While this technique works well with liquids and other solutions, the readings may not be as accurate when the sample is a suspension of different solid particles. The sample will likely scatter the light rather than absorb it with solids, affecting the data. Generally, UV-vis spectrometers are more efficient in analyzing liquids and solutions. 2. Choice of The Solvent or Container When choosing what solvent to use, ensure that its absorbance cutoff is not close to the sample or compound to be tested. Aside from the solvent, the material of the container or cuvette is also critical because it also has a UV-vis absorbance cutoff. Generally, a quartz container or cuvette is more effective and practical because its absorbance cutoff is only around 160 nm. 3. Reference and Calibration Calibration references used to compare the sample readings should be accurately prepared to determine the sample's concentration to be tested. UV-Vis Spectroscopy is the Future UV-vis spectroscopy provides researchers and scientists with more efficient methods to measure light wavelengths, providing accurate readings that are helpful in various biological and chemical analyses. The UV-vis spectrometer device is precise and easy to operate, provided that you maintain a clean working area free from any external noise and dust that can affect the machine's readings. Uv-Vis SpectroscopyWhat Is Uv-Vis Spectroscopy

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