⟨1857⟩ ULTRAVIOLET-VISIBLE SPECTROSCOPY—THEORY AND PRACTICE

Change to read:

THEORY

Ultraviolet-visible (UV-Vis) spectroscopy is an electronic transition spectroscopic technique in which the interaction between incident radiation and electrons results in the promotion of one or more of the outer or the bonding electrons from a ground state into a higher-energy state. This quantum effect results in a specific absorption of radiation, the frequency and wavelength of which are governed by the equation:

$$E = h\nu = \left(\frac{hc}{\lambda} \right) \times 10^9$$

where:

E = energy

h = Planck's constant ($6.63 \times 10^{-34} J \cdot s$)

ν = frequency (Hz), related to the energy change ΔE, induced when electromagnetic radiation is absorbed ($\Delta E = h\nu$ per photon)

c = velocity of light (2.998×10^8 m/s)

λ = wavelength (nm)

▲All absorption of ultraviolet (UV) and visible (Vis) radiation in organic molecules is restricted to certain functional groups (chromophores) that contain valence electrons of low excitation energy. The spectrum of a molecule containing these chromophores is complex. This is because the superposition of rotational and vibrational transitions on the electronic transitions gives a combination of overlapping lines. This appears as a continuous absorption band, in the form of a bell-shaped peak. As a general rule, small molecules absorb somewhere in the ultraviolet region, and the greater the extent to which the p electrons are delocalized over a ring or a larger complex multiple-ring or branched structure, the longer the wavelength of the first absorption band (i.e., the band of lowest energy and longest wavelength). This delocalization over multiple-ring structures can shift the wavelength into the visible region and is responsible for the observed color of the substance.▲ (USP 1-Dec-2022)

Change to read:

▲UV-VIS SPECTROMETERS▲ (USP 1-DEC-2022)

All modern UV-Vis measurements involve detecting and measuring the intensity ratio of the radiation at a certain wavelength in the presence or absence of the absorbing sample ▲(transmitted radiation and incident radiation). *Figure 1* shows schematics of four typically encountered optical designs: single-beam, single split-beam (where the reference detector is located within the internal optics of the system), double-beam, and reverse optic array spectrometers.▲ (USP 1-Dec-2022) Dispersion of light to achieve the desired resolution can occur before ▲(as in the first three configurations)▲ (USP 1-Dec-2022) or after introduction of the sample, but all commercial UV-Vis instruments share the following features to perform these functions:

- Continuum source
- Monochromator or polychromator
- Sampling area
- Detector

▲

Single Beam Spectrometer

Single /Split Beam Spectrometer

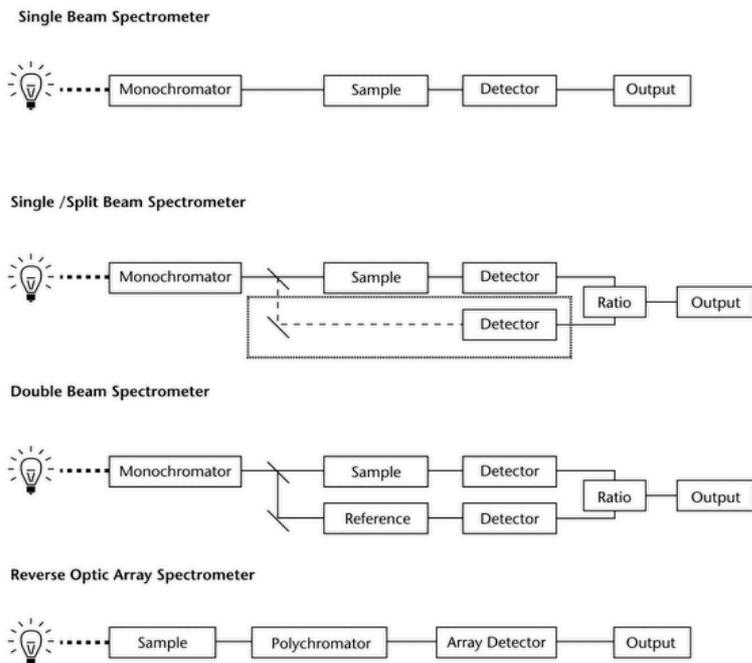**Double Beam Spectrometer**

Reverse Optic Array Spectrometer

Figure 1. Typical spectrometer designs.

▲ (USP 1-Dec-2022)

Care must be taken to ensure that the sample is not degraded by the incident light beam and that any heating effects are minimized. This is particularly important when ▲photodiode array▲ (USP 1-Dec-2022) instruments are used because the sample is irradiated at all wavelengths.

Continuum Source

Two major types of continuum source are currently in use: continuous and pulsed. Continuous sources include tungsten halogen for visible, deuterium arc for UV, and xenon arc for both. The source for pulsed radiation is the xenon flash lamp. Many UV-Vis instrument systems use a combination of deuterium and tungsten halogen to effectively cover the UV and visible regions, respectively. By necessity, source selection is achieved either by the use of a mirror ▲system to select the appropriate source, positioned manually or by using a hardware and software combination.▲ (USP 1-Dec-2022) This change usually is performed in the region of 320−350 nm, and thus qualification of the system must be performed using both source and mirror positions. Systems based on xenon lamps have the benefit of a single source and a higher energy output, but they are more expensive.

Monochromator

The wavelength scale can be encoded by either a scanning monochromator or a grating polychromator (see *Figure 2*), as is the case for ▲spectrometers▲ (USP 1-Dec-2022) equipped with linear or two-dimensional array detectors. A discussion of the specific benefits and drawbacks of each of the dispersive designs is beyond the scope of this general chapter. Any properly qualified instrument should be suitable for qualitative measurements. Care must be taken when selecting an instrument for quantitative measurements because dispersion, response linearity, and stray light may not be uniform across the full spectral range. ▲Appropriate qualification procedures can be found in *Ultraviolet-Visible Spectroscopy ⟨857⟩*.▲ (USP 1-Dec-2022)
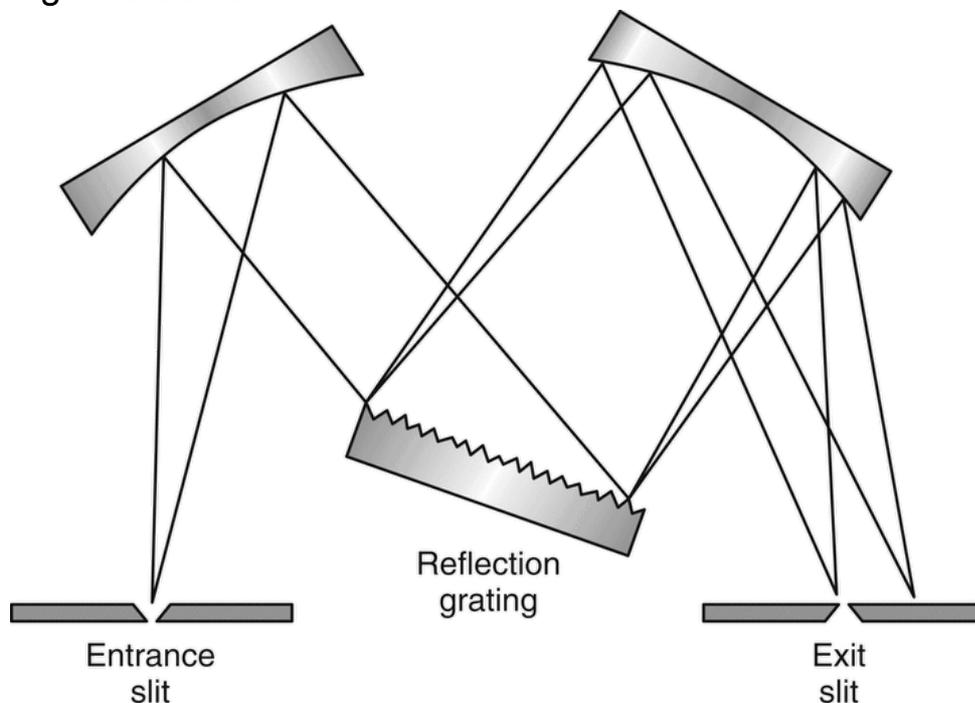
Figure 2. Grating monochromator.

Sampling Area

Numerous sampling arrangements are available in addition to the ▲cuvette▲ (USP 1-Dec-2022) holders that are designed to accommodate various path-length configurations based on conventional rectangular ▲cuvettes.▲ (USP 1-Dec-2022) These include flow ▲cuvettes,▲ (USP 1-Dec-2022) fiber-optic-based immersion probes, ▲multi-channel▲ (USP 1-Dec-2022) plate configurations, and automated sample changers, among others. Considerations such as sampling volume, speed of measurement, and reproducibility of sample presentation should be evaluated to optimize the sampling device for specific applications.

Detector

Photoelectric detectors, which are the most common form of UV-Vis detectors, generate an electric current that is directly proportional to the intensity of the radiant energy incident upon them. They may take the form of photosensitive semiconductor devices, either discrete detectors, linear or two-dimensional arrays, or photomultipliers. Photosensitive semiconductor devices include solid-state photodiodes, charge-coupled device arrays (CCDs), and phototransistors. The most common semiconductor material is silicon, which is sensitive to wavelengths of 400−800 nm, but some silicon devices have extended sensitivity from as short a wavelength as 190 nm to as long a wavelength as 1100 nm. The dynamic response of these detectors typically is four orders of magnitude. Most ▲photodiode▲ (USP 1-Dec-2022) array detectors are made of silicon and hence have a similar wavelength response. Other semiconductor materials can provide wavelength response to several micrometers.

In contrast, photomultipliers are vacuum detectors that have a photocathode in which photon energy releases electrons that are directed by the field applied to electron-sensitive plates. By a cascade effect, these dynodes amplify the electrons released initially by the absorption of the incident radiation. Photomultipliers have typical wavelength responses of 160−900 nm, although some photocathode materials can provide response to higher wavelengths. The dynamic response of these detectors typically is six orders of magnitude or higher.

Change to read:

ALTERNATIVE UV-VIS ▲SPECTROMETER▲ (USP 1-DEC-2022) CONFIGURATIONS

▲Photodiode Array Spectrometers

With photodiode array detection,▲ (USP 1-Dec-2022) the optical configuration is reversed from that in a conventional ▲spectrometer,▲ (USP 1-Dec-2022) and the light beam passes through the sample before being dispersed by the polychromator. This gives the benefit of fast, full spectral data with no moving parts that can wear out. The perception is, therefore, that ▲photodiode▲ (USP 1-Dec-2022) arrays are more reliable than other detectors. This is true, but the reverse-optics design requires that both the sample and optics before the dispersing element (usually a grating) are subjected to the full-spectrum radiation also encountered by these components in a conventional ▲spectrometer.▲ (USP 1-Dec-2022) In a conventional ▲spectrometer,▲ (USP 1-Dec-2022) the sample and optics are outside the monochromator, and optical beam deflection and/or scattering simply reduces the intensity of the light that enters the monochromator. In an array-based ▲spectrometer,▲ (USP 1-Dec-2022) deflection occurs within the ▲polychromator,▲ (USP 1-Dec-2022) causing scatter within the confined environment ▲of these devices,

resulting in▲ (USP 1-Dec-2022) an associated increase in stray light. ▲As detailed in *Stray Light*, that leads to a reduction in optimum photometric range as shown in *Figure 3*.▲ (USP 1-Dec-2022)

HPLC ▲UV-Vis▲ (USP 1-Dec-2022) Detectors

The design of a UV-Vis ▲spectrometer▲ (USP 1-Dec-2022) is always the result of a number of considered compromises. For example, optical performance (absolute wavelength resolution) is governed by the focal length of the monochromator, which in turn dictates the physical size of the instrument. In the case of HPLC ▲UV-Vis▲ (USP 1-Dec-2022) detectors ▲used for peak homogeneity assessment and identification procedures using the spectrum at the peak apex,▲ (USP 1-Dec-2022) the need to provide a high-stability, low S/N ratio output at high transmittance levels through a small-aperture flow ▲cuvette▲ (USP 1-Dec-2022) requires that by design these systems may not have the dynamic range or wavelength accuracy of their conventional spectroscopic counterparts.

Fiber-Optic-Based Modular Systems

In recent years there has been a rapid increase in the availability and use of systems built around the ability of fiber optics to channel and multiplex optical systems. Although these systems have the advantages of flexibility and ease of use and they allow measurements to be performed on ▲multi-channel plate readers, modular user-designed and -built systems, etc.,▲ (USP 1-Dec-2022) analysts must consider the following disadvantages:

- Some analysts assume that these array-based fiber-optic systems are immune to room light interference at the sample interface, which may or may not be true. This assumption is easily tested by using a black photographic film-changing bag or a simple black cloth. Use of a masking technique should not change the measured value if the assumption is true.
- Custom-built systems ▲may▲ (USP 1-Dec-2022) not have additional shuttering, stray light filtering, and other capabilities that are found in commercially designed ▲spectrometers.▲ (USP 1-Dec-2022) Light levels transmitted directly down fibers from high-intensity sources such as Xenon flash lamps may cause photodegradation ▲of the sample being measured by the system. In these▲ (USP 1-Dec-2022) systems, the source may be coupled by fiber directly to the sample interface, and the only control is the source on/off switch.
- ▲Therefore, by design, the performance characteristics, i.e., the optimum photometric range, may have to be significantly reduced, if possible, to allow the system to be qualified according to ⟨857⟩.▲ (USP 1-Dec-2022)

Add the following:

▲UV-VIS INSTRUMENT PERFORMANCE INDICATORS

Spectral Bandwidth—Effect on Resolution and Signal-to-Noise Ratio

Appropriate spectral bandwidth (SBW) is important to ensure adequate peak resolution. The selection of the SBW always requires balancing adequate resolution against maximization of the signal-to-noise (S/N) ratio. During method development, analysts should optimize the resolution by selecting the narrowest slit width that provides an adequate S/N ratio because optimum resolution is achieved when the S/N ratio is maximized. By design, the entrance and exit slits of the monochromator will be mechanically matched together to achieve the same physical separation and associated dispersion (usually triangular) to produce the required SBW. If access to a variable-bandwidth instrument is available, then the optimum setting can be defined as the narrowest slit width at which no significant reduction in peak intensity is observed. If accurate absorbance measurements must be made on benzenoid compounds or other compounds with sharp absorption bands (natural half-bandwidths of <15 nm), the SBW of the spectrometer used should not be greater than 1/8th of the natural half-bandwidth of the compound's absorption; i.e., this equates to a spectrometer with a SBW of 2 nm or less. In practical terms, for molecules of pharmaceutical interest in solution, this bandwidth of 2 nm is considered adequate in also providing the required balance of high resolution while maintaining good S/N ratio.

However, it should be noted that nicotinic acid, which is shown to be acceptable for use as a photometric accuracy reference, does have transmittance characteristics that are highly correlated to the SBW, and therefore it is always necessary to match the SBW of the certified absorbance values to those of the instrument system being qualified.

Stray Light

Stray radiation, commonly referred to as stray light, can be defined as radiant energy at wavelengths other than those indicated by the monochromator setting and all radiant energy that reaches the detector without having passed through the sample or reference solutions (see *Figure 3*). It may be caused by any scattered radiation from imperfections in the dispersing medium, which commonly is a grating. The use of a holographic grating substantially reduces the levels of this source of stray radiation. Higher-quality ruled gratings yield a low level of scattered radiation. In higher-performance instruments, stray radiation can be reduced by the use of double monochromators or double-pass monochromators. Stray radiation, or apparent stray radiation, also may be caused by light leaks in the system, incorrect wavelength calibration, incorrect optical alignment, reduced source output, or reduced detector response.
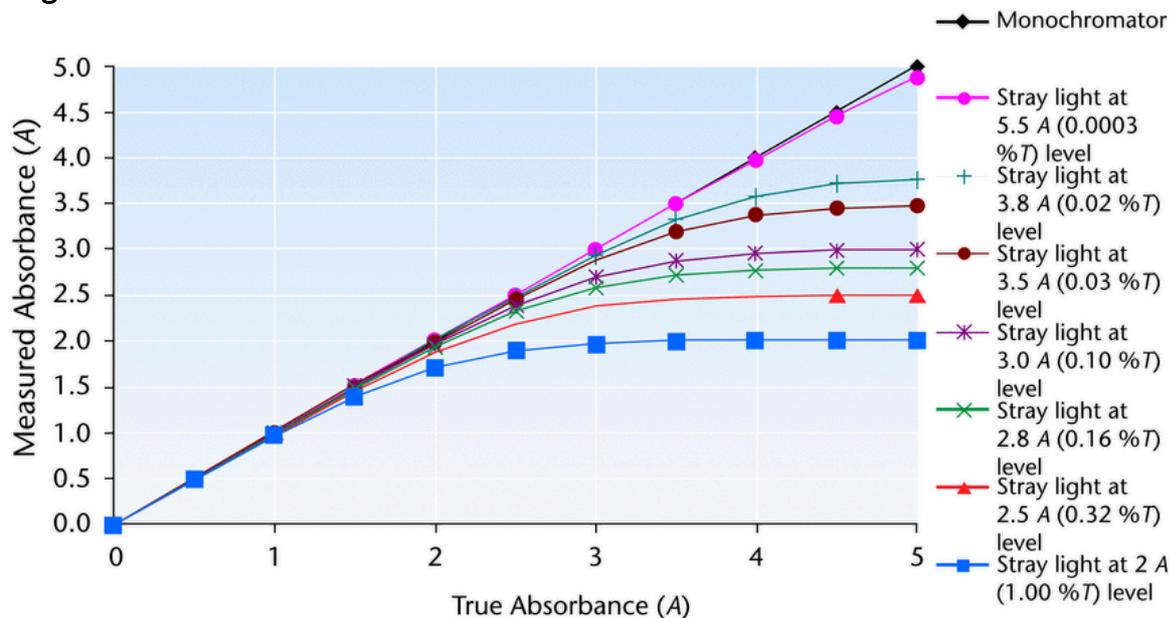
Figure 3. Effect of stray light on measured absorbance.

The usual way of assessing stray light is to measure, at the desired analytical wavelength, a sample that totally absorbs the radiation at that wavelength but transmits at all other wavelengths. Any light detected by the instrument is then stray light.

In 1982, Mielenz et al. at the National Institute of Standards and Technology (NIST) defined stray light as spurious radiant energy that has departed from its regular path in a spectrometer and then re-enters the path so that it is sensed by the detector and causes false readings of transmittance and absorbance. Mielenz also offered a refinement on the estimation method in current use at the time. This method has since been incorporated into ⟨857⟩, Qualification of UV-Vis Spectrometers, Estimation of the Limit of Stray Light (Stray Radiant Energy), Procedure A. In this method, the reference beam is attenuated by a 5-mm path length cuvette containing the same edge filter solution as used in a 10-mm path length cuvette placed in the sample beam. A maximum peak differential absorbance is observed (ΔA) while scanning through the edge absorbance, which is related empirically to the stray light level (s_λ) by:

$$s_\lambda = 0.25 \times 10^{-2\Delta A}$$

Table 1 provides a list of differential absorbance values, the "Mielenz measured absorbance", and their corresponding stray light levels.

Table 1. Value Comparison Table and Critical "Mielenz" Measured (Differential) Absorbance Values

"Mielenz" Measured Absorbance (ΔA)	Stray light level, s_λ, expressed as…		
	Transmittance (T)	% Transmittance (%)	Absorbance (AU)
0.3	0.063	6.3	1.2
0.5	0.025	2.5	1.6
0.7	0.01	1.0	2.0
1.0	0.0025	0.25	2.6
1.5	0.00025	0.025	3.6
2.0	0.000025	0.0025	4.6
25	0.0000025	0.00025	5.6

This method therefore avoids the potentially difficult procedure (see ⟨857⟩, Qualification of UV-Vis Spectrometers, Estimation of the Limit of Stray Light (Stray Radiant Energy), Procedure B) to find appropriate attenuation filters in the UV region required to "back-off" the system, especially if using a modern double-monochromator spectrometer, and also avoids the measurement performed at the limit of the instrumental range and analog-to-digital electronic resolution. Figure 4 shows spectral profiles acquired of a potassium chloride solution using both ⟨857⟩, Procedure A and ⟨857⟩, Procedure B.
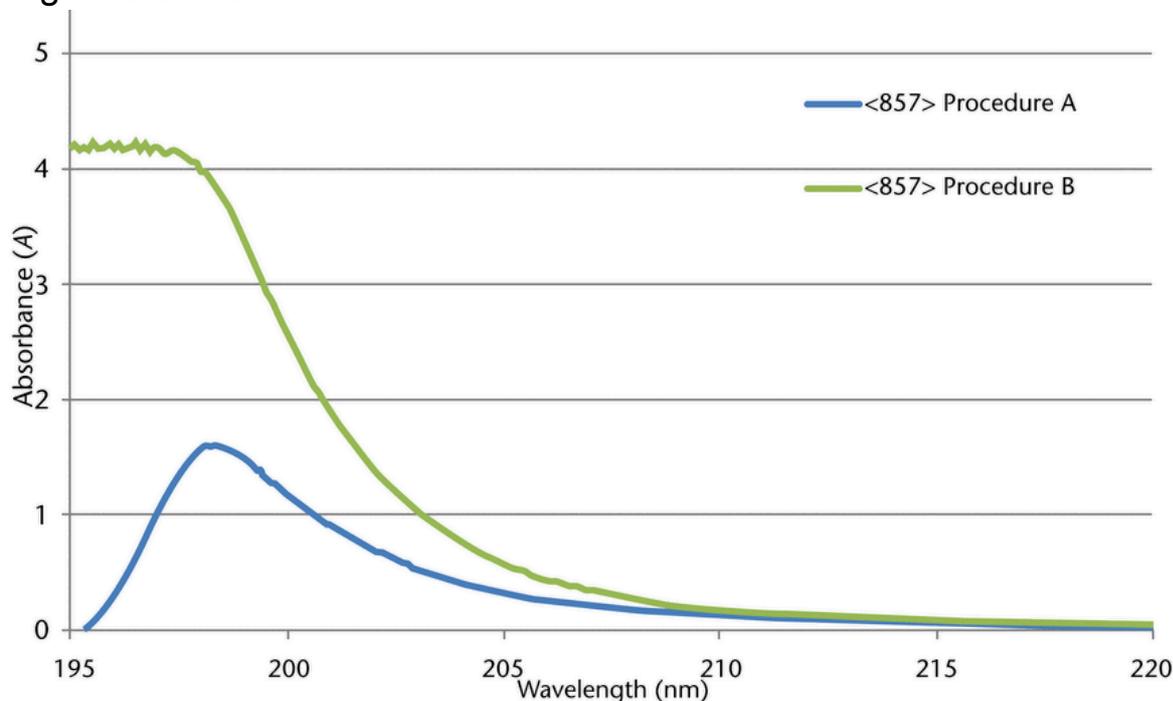
Figure 4. Typical spectrum of 1.2% aqueous potassium chloride vs. water as reference, 10-mm path length (*Procedure B*). Differential absorbance spectrum of 1.2% aqueous potassium chloride, 10-mm path length, vs. the same solution as reference, 5-mm path length (*Procedure A*).

Optimum Working Photometric Range

Determination of the optimum photometric range is fundamental to establishing the capability of a given instrument before and during method validation. The importance of this procedure can be shown by the fact that in 1945 Vandenbelt et al. attempted to establish the optimum absorbance range on the then-new Beckman DU spectrometer (*1*). They suggested a simple approach in which the molar absorptivities, $A_{1\,cm}^{1\%}$, of various compounds are measured at different concentrations. This approach is shown theoretically in *Figure 5*, where the center plateau is used to define the photometric range.
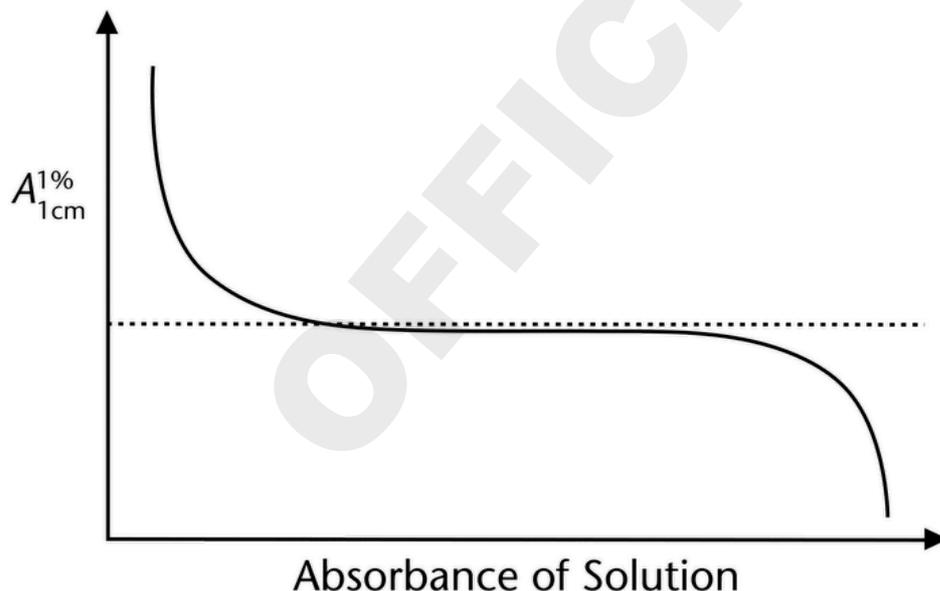

Figure 5. Theoretical photometric response curve.

In practice, data are more variable. *Figure 6* shows three typical curves.
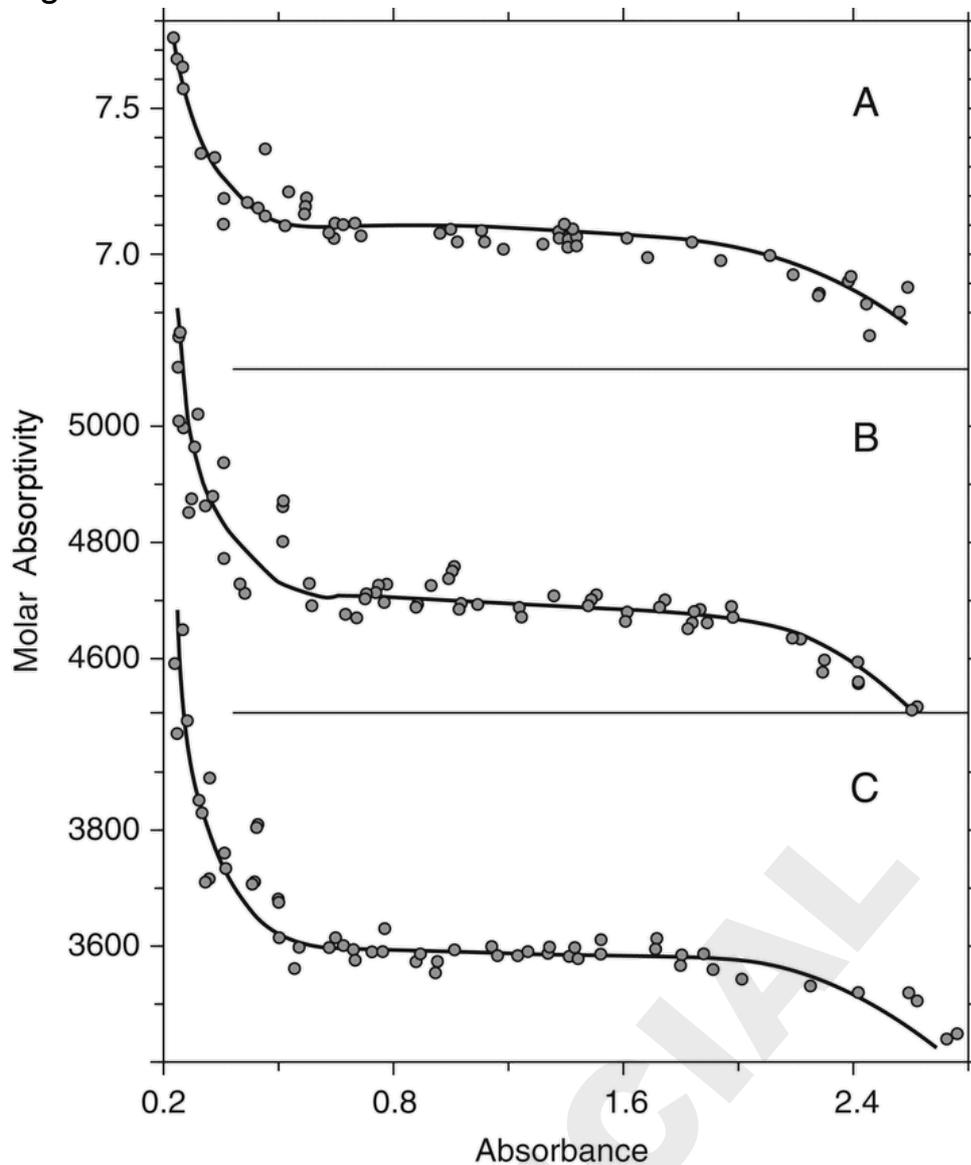
https://trungtamthuoc.com/

Figure 6. Molar absorptivity vs. absorbance for three solutions: A) aqueous potassium nitrate measured at 301 nm; B) potassium chromate in 50 mM potassium hydroxide at 373 nm; C) potassium chromate in 50 mM potassium hydroxide at 273 nm (2).

A more rigorous method for estimating an instrument's region of maximum precision is given by Youmans and Brown (3). The error in the measurement of the concentration of a solution of transmission T can be expressed as:

$$\Delta c = \frac{1}{\epsilon b}\log\frac{T_{av}}{T_{min}}$$

where Δc is the change in concentration, ε is the molar absorptivity of the compound, b is the path length, and T_{av} and T_{min} are the mean and minimum values, respectively, from a series of n measurements on the sample.

The statistical error is (see _Figure 7_):

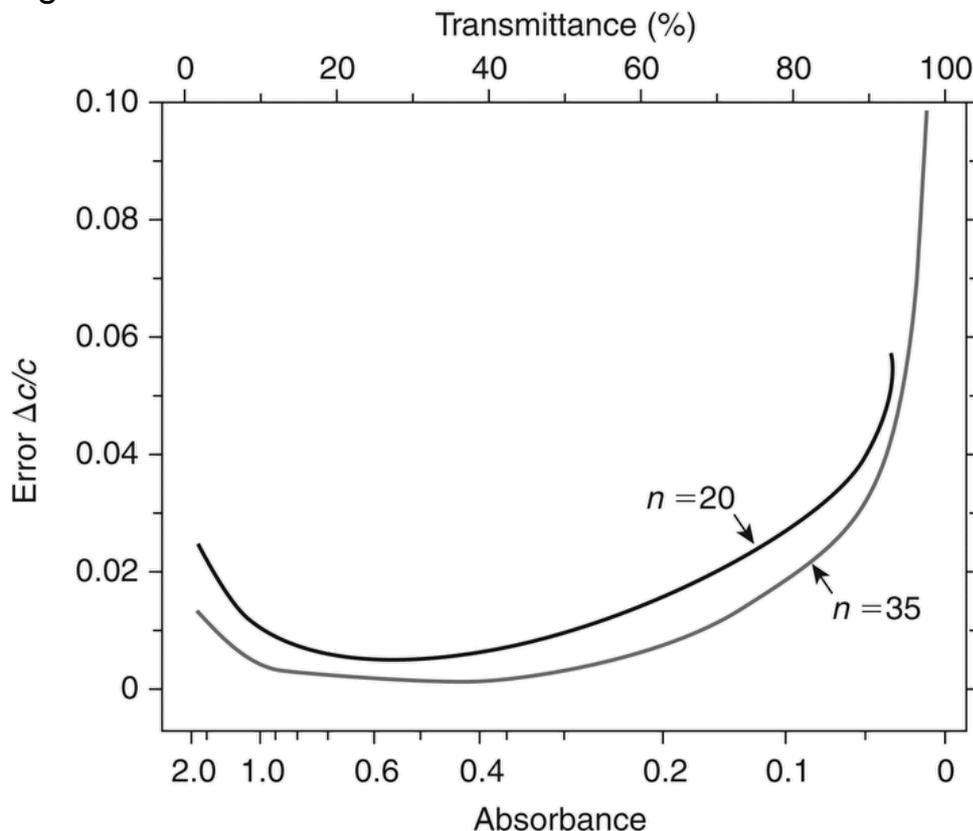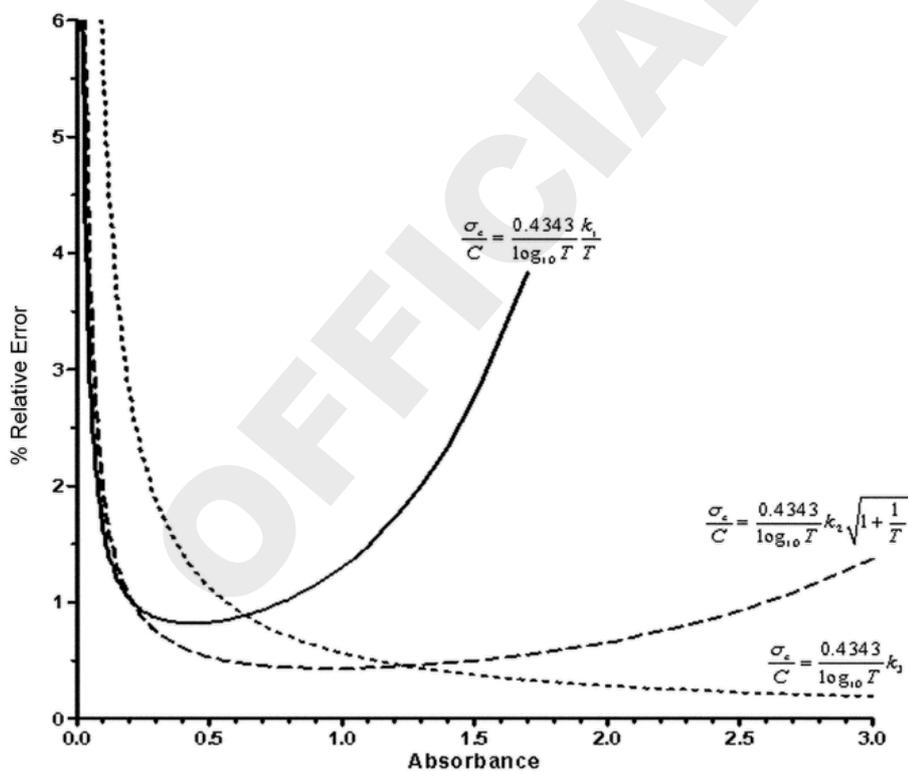$$\frac{\Delta c}{c} = \frac{1}{-\log(T_{av})}\log\frac{T_{av}}{T_{min}}$$

Figure 7. Youmans and Brown plot (2).

The noise levels, and hence the precision of measurement, depend primarily on detector noise type, as shown in *Figure 8*.

Standard deviation of a measurement σ_T	Source of variability	Relative error function
$\sigma_T = k_1$	Thermal detector, amplifier, and dark current noise. [Independent of T]	$\dfrac{\sigma_c}{C} = \dfrac{0.4343}{\log_{10} T} \dfrac{k_1}{T}$
$\sigma_T = k_2 \sqrt{T^2 + T}$	Shot noise from the detector	$\dfrac{\sigma_c}{C} = \dfrac{0.4343}{\log_{10} T} k_2 \sqrt{1 + \dfrac{1}{T}}$
$\sigma_T = k_3 T$	Cell positioning, non-parallelism errors and incident beam intensity fluctuations	$\dfrac{\sigma_c}{C} = \dfrac{0.4343}{\log_{10} T} k_3$

Figure 8. Measurement precision as a function of noise type (4).

Both approaches clearly show that in addition to the expected increasing error at higher absorbance levels caused by stray light and other factors, there is a similar, if not larger possibility for increasing error at low absorbance levels caused by instrumental variances in the form of noise from the detector, source, etc. This variance often is overlooked in the desire for economy of scale, when analysts use smaller samples and shorter path lengths and therefore lower measured absorbance values.

Good practice is to optimize the measured absorbance within 0.2–2.0 absorbance units (AU), where possible. In addition, the use of shorter path length cuvettes should be the preferred option to reduce a measured solution absorbance rather than the corresponding ratio dilution with solvent. Invariably, the path length tolerances on a modern manufactured cuvette are significantly smaller than those associated with volumetric glassware; and not diluting the sample avoids the risk of solution chemistry changes caused by dilution.▲ (USP 1-Dec-2022)

Change to read:

CALIBRATION

UV-Vis ▲spectrometer▲ (USP 1-Dec-2022) calibration involves two components: primary wavelength (*x*-axis) and ▲photometric scale▲ (USP 1-Dec-2022) (*y*-axis), and often is performed when the instrument is initialized. Most dispersive UV-Vis ▲spectrometers▲ (USP 1-Dec-2022) use atomic emission lines from either the source or a secondary lamp for primary wavelength (*x*-axis) calibration, in addition to the zero-order position in the monochromator. ▲How (and to what level of "user") these fundamental calibration procedures are made available will depend on the vendor's configuration of the instrument. Calibration of both the wavelength (*x*-axis) and the photometric scale (*y*-axis) is critical for successful quantitation and method transfer between instruments. In many cases, the effect of wavelength error in the *x*-axis can be minimized by the use of careful method selection, e.g., wavelength at a spectral peak.▲ (USP 1-Dec-2022) Although the fundamental measurement relies on a simple ratio measurement, modern instruments may use several gain settings or amplification factors to ensure a linear detector response, particularly at low intensity levels and high-power settings. These gain settings often are initially set by the manufacturer, but the settings may be recalibrated as the optics age or as other changes take place.

Detailed functional validation based on certified reference materials ▲as detailed in ⟨857⟩▲ (USP 1-Dec-2022) is recommended to demonstrate the suitability of laboratory instruments, even for instruments that possess an internal calibration capability. The use of external reference materials does not obviate the need for internal quality control procedures. Rather, it provides independent documentation of the fitness of the instrument to perform the specific analysis.

Change to read:

ANALYTICAL CONSIDERATIONS

▲Temperature and Coefficient of Expansion Effects

Temperature impacts absorption measurements and, thereby, the photometric accuracy in several significant ways. Changes in temperature can induce expansion or contraction of the solvent, leading to lower or higher concentrations and absorbances. While this is a particular issue with some organic solvents, careful temperature control, i.e., within ±2° may be required where high-accuracy absorbance values are required for aqueous solutions, for example, to verify compliance with photometric value assignments stated for a reference material and/or calculation of a molar extinction coefficient. In ⟨857⟩, this accurate control of temperature is particularly important when liquid photometric accuracy references are being used in the qualification process, i.e., nicotinic acid and acidic potassium dichromate solutions. In such cases, additional temperature monitoring and/or control of the sample measurement process may be required. Also, shifts in equilibria between the chemical forms of an analyte may cause the nature of the absorbing species to be changed.

Solvent Selection Effects

Solvents play an important role in UV spectra. In a spectrum, the compound peak can be obscured by the solvent peak. So, a most suitable solvent is one that does not itself absorb and is transparent in the region under investigation. As a general rule, it should be possible to measure a solvent at the wavelength of interest at a transmittance value of >75%.

A dilute solution of sample is always prepared for analysis. See *Table 2* for a list of most commonly used solvents. In this table, approximate wavelengths below which the solvent absorbance may be unacceptable are specified. For quantitative applications, the cut-off may be set at a wavelength (L_0) where the absorbance for the 10-mm path length of the solvent exceeds 0.05 AU (relative to water), i.e., $A_{1\,cm}$ > 0.05 AU. For qualitative work, it may still be feasible to operate at significantly lower wavelengths; most analysts accept a cut-off based on the wavelength (L_1) for $A_{1\,cm}$ > 1.0 AU.

Table 2. Solvent Cut-Off of Commonly Used Solvents

Solvent (Common Name)	L_0 (nm)	L_1 (nm)
Acetone	340	330
Acetonitrile	200	190
Isobutanol	250	200

Solvent (Common Name)	L_0 (nm)	L_1 (nm)
n-Butanol	245	215
n-Butyl acetate	275	255
Cyclopentane	220	195
Decalin	250	230
Dichloromethane	245	230
Diethyl ether	255	220
N,N-Dimethylformamide	300	270
Dimethylsulfoxide	330	285
Ethanol	240	205
Ethyl acetate	280	260
Heptane	230	200
Hexane	225	195
Methanol	240	205
Isopropanol	240	205
n-Propanol	250	210
Tetrachloroethylene	320	290
Tetrahydrofuran	280	220
Water	190	185

In addition, there are three other solvent related effects. First, dissolved gases may cause absorption at wavelengths above the pure solvent cut-off. For example, dissolved atmospheric gases may cause absorption to occur at 190–205 nm in an aqueous system. Second, organic solvents may contain other stabilizing chemicals that will absorb at higher wavelengths. Last, changes in physical solvent properties, e.g., polarity or degree of hydrogen bonding, may cause chromatographic shifts in the band spectrum.▲ (USP 1-Dec-2022)

Sample-Based Factors

The most important sample-based factors that negatively affect quantitative UV-Vis ▲spectrometry▲ (USP 1-Dec-2022) are fluorescence and light scattering. If the sample matrix includes fluorescent compounds, the measured signal usually will contain a contribution from fluorescence. The wavelength range and intensity of the fluorescence depend on the chemical composition of the fluorescent material. Suspended particles scatter light by the Tyndall effect, causing a decrease in the measured intensity that increases as the wavelength decreases. Unless there is no alternative, absorbance should not be determined on turbid samples. Procedures for removing turbidity include filtering, centrifuging, or flocculating the sample and are performed before any additional procedures that generate a chromophore, provided that they do not affect the concentration of the analyte or the chromophore in the test solution. Any measurements performed on a turbid solution are highly instrument specific and can be used only for comparative purposes in the same system. A further complication can arise at higher analyte concentrations with respect to the coordination chemistry and system matrix. Ionic association, complex formation, and similar factors can cause deviation from the expected linear response.

Sampling Factors

▲SOLUTION PREPARATION

As previously stated, all solutions used for measurement should be particle free and prepared as accurately as possible. The errors associated with any solution preparation can be minimized by ensuring that all gravimetric processes are used within the center median of the given working range of the balance(s) and that a gravimetric process, in preference to a volumetric process, is used for all subsequent required dilutions. Again, the use of reduced path length cuvettes should be chosen in preference to an additional dilution step.

CUVETTES▲ (USP 1-Dec-2022)

Quantitative absorbance measurements usually are made on solutions of the substance in liquid-holding ▲cuvettes.▲ (USP 1-Dec-2022) The most common ▲cuvette▲ (USP 1-Dec-2022) path length is 10 mm, although path lengths from 0.01–100 mm are commercially available (see

Figure 9). For samples with low absorbance, improved sensitivity generally can be obtained by increasing the ▲cuvette▲ (USP 1-Dec-2022) path length. For example, the theoretical absorbance of a solution in a 50-mm ▲cuvette▲ (USP 1-Dec-2022) is greater by a factor of 5 compared to the same solution in a 10-mm ▲cuvette.▲ (USP 1-Dec-2022) Errors in absorption readings arising from ▲cuvettes▲ (USP 1-Dec-2022) almost always are caused by dirty windows that can absorb a significant proportion of the incident light beam. Less frequent causes of error are an incorrect choice of ▲cuvette▲ (USP 1-Dec-2022) material for the wavelength required, e.g., use of glass ▲cuvettes▲ (USP 1-Dec-2022) less than 320 nm, nonrepeatability of ▲cuvette▲ (USP 1-Dec-2022) positioning, differences in ▲cuvette▲ (USP 1-Dec-2022) window thickness, nonparallel optical windows, or impurities in ▲cuvette▲ (USP 1-Dec-2022) window materials. A 10-mm ▲cuvette▲ (USP 1-Dec-2022) manufactured with a ±0.05 mm tolerance will add a 0.005 AU contribution to the overall uncertainty, when measuring absorbance values at the 1 AU level. There are 10-mm ▲cuvettes▲ (USP 1-Dec-2022) with a ±0.01 mm tolerance commercially available. As a general guide, the ▲cuvette▲ (USP 1-Dec-2022) chosen should have a transmittance value of >75% when filled with the appropriate solvent▲, as previously discussed,▲ (USP 1-Dec-2022) and measured at the wavelength of interest.
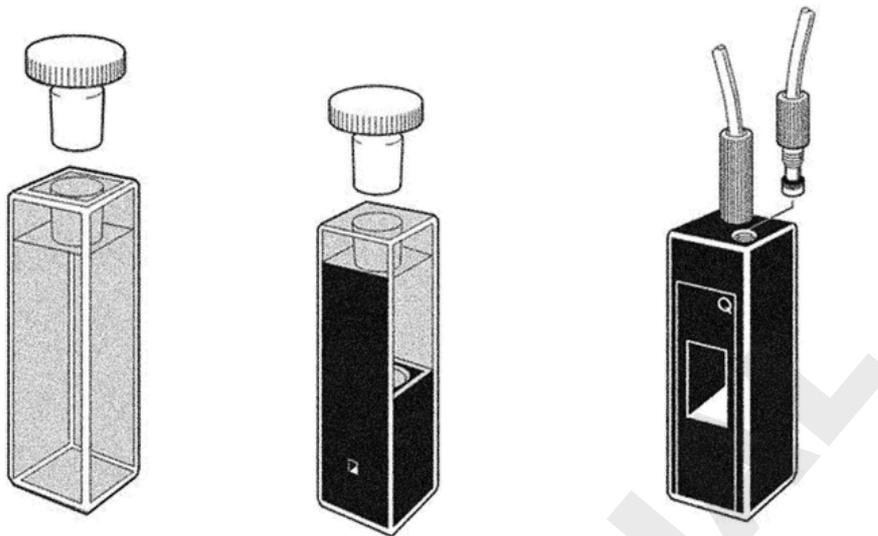

Figure 9. Commonly used ▲cuvette▲ (USP 1-Dec-2022) types: 10-mm regular, low-volume, and flow ▲cuvettes▲ (USP 1-Dec-2022) (from left to right).

Analysts can mitigate the variability introduced by ▲cuvettes▲ (USP 1-Dec-2022) by adhering to the following set of best practices. Carefully clean ▲cuvettes▲ (USP 1-Dec-2022) before use, and store them in a manner that avoids contamination when they are not in use. ▲Cuvettes▲ (USP 1-Dec-2022) in frequent and repeated use, e.g., flow ▲cuvettes,▲ (USP 1-Dec-2022) can be stored wet in high-purity water or 1% (v/v) nitric acid. Do not use cracked or scratched ▲cuvettes.▲ (USP 1-Dec-2022) Wipe ▲cuvettes▲ (USP 1-Dec-2022) carefully with a soft, clean, lint-free cloth before they are placed in the ▲spectrometer.▲ (USP 1-Dec-2022) Do not use lens-cleaning paper or any other form of paper cleaning tissue because these will either scratch or allow contamination of the optical face.

It is preferable to use a single ▲cuvette▲ (USP 1-Dec-2022) for all measurements of both the reference and samples▲, and where possible, never move the cuvette if you can move the solution. Liquid transfer should be made using plastic-tipped devices to avoid scratching the optical surfaces. This approach, which eliminates interreflection errors and nonparallelism effects, should always be used if work of the highest accuracy is needed. The use of a flow cuvette with peristaltic aspiration is recommended.▲ (USP 1-Dec-2022) When several sample ▲cuvettes▲ (USP 1-Dec-2022) must be tested against one reference ▲cuvette,▲ (USP 1-Dec-2022) they all should be closely matched in their characteristics. Whenever possible, measure standards and samples at the same time and under the same conditions to minimize the potential for bias. The reference ▲cuvette▲ (USP 1-Dec-2022) should contain the same solvent as the sample ▲cuvette▲ (USP 1-Dec-2022) and should be checked against the sample ▲cuvette▲ (USP 1-Dec-2022) at all wavelengths for which measurements are required. Analysts can apply corrections for differences between ▲cuvettes▲ (USP 1-Dec-2022) to absorbance readings obtained from the solutions under examination. The maximum allowable correction is ±0.01 AU. Best practices include using the same ▲cuvette▲ (USP 1-Dec-2022) in the reference beam with the same face incident to the light beam, and flushing the sample ▲cuvette▲ (USP 1-Dec-2022) three times with the sample solution before the final filling before measurement. ▲When solutions using volatile solvents are to be measured, it is advisable to use polytetrafluoroethylene (PTFE) stoppered-cuvettes, ideally one of the many screw-capped variants available.▲ (USP 1-Dec-2022) The use of flow ▲cuvettes▲ (USP 1-Dec-2022) effectively addresses many of these ▲handling and filling▲ (USP 1-Dec-2022) issues. The contents in both

the sample ▲cuvette▲ (USP 1-Dec-2022) and the reference ▲cuvette▲ (USP 1-Dec-2022) must be free from gas bubbles and particles. If flow ▲cuvettes▲ (USP 1-Dec-2022) are used, analysts may be required to degas the solution(s) before measurement in order to avoid the formation of air bubbles in the ▲cuvette.▲ (USP 1-Dec-2022)

A sequence of activities is used to monitor the quality of the measurement process and to ensure that the contents are representative of the sample under measurement. Drift of the ▲spectrometric▲ (USP 1-Dec-2022) baseline can be detected by measuring the blank at the start and end of the sample sequence▲and also at interim points if the time scale requires them.▲ (USP 1-Dec-2022) Adequate flushing of the ▲cuvette▲ (USP 1-Dec-2022) can be confirmed by alternating between measurement of the standard (high concentration) and the blank (zero concentration) at the start of the measurement sequence. The latter check is particularly important for automated systems that can generate reproducible but inaccurate results because of inadequate flushing.

CARE OF ▲CUVETTES▲ (USP 1-Dᴇᴄ-2022)

Contaminated ▲cuvettes▲ (USP 1-Dec-2022) are the greatest single source of error in ▲UV-Vis spectrometry. Cuvettes▲ (USP 1-Dec-2022) should never be handled by the optical polished faces, and analysts should rinse off residual or spilled solution. If ▲cuvettes▲ (USP 1-Dec-2022) are properly cared for, rigorous cleaning methods rarely will be needed, especially if the ▲cuvettes▲ (USP 1-Dec-2022) are cleaned immediately after use. Although acid solutions will simply contaminate surfaces, alkaline solutions can etch all types of polished glass surfaces, and the degree of attack depends on the pH and contact time. When cleaning ▲cuvettes,▲ (USP 1-Dec-2022) analysts should ensure that the optical faces are not scratched or chipped. Commercial cleansing solutions are available and can be used, provided that they are diluted before use in accordance with the manufacturers' recommendations. After they are cleaned with such agents, the ▲cuvettes▲ (USP 1-Dec-2022) should be carefully and thoroughly rinsed with high-purity water. If this fails to clean the ▲cuvettes,▲ (USP 1-Dec-2022) then soaking them in cold, concentrated nitric or hydrochloric acid is acceptable. Flow ▲cuvettes▲ (USP 1-Dec-2022) are best cleaned in situ, provided that the solvent does not interact with the connecting tubing. If the ▲cuvettes▲ (USP 1-Dec-2022) are stored for an extended period, then they should be dried quickly after cleaning by blowing dry with a compressed gas stream in a clean, dust-free environment. ▲Many laboratory "compressed air gas lines" may contain lubricating oil from the machinery and thereby deposit a film of oil on the optical face.▲ (USP 1-Dec-2022)

ALIGNMENT AND FILLING OF ▲CUVETTES▲ (USP 1-Dᴇᴄ-2022)

Analysts should align the ▲cuvettes▲ (USP 1-Dec-2022) so that the same optical face is always incident to the light beam. Most ▲cuvettes▲ (USP 1-Dec-2022) have type identification engraved on one face; this can be used to ensure consistent orientation. If there are no markings, a small mark can be made on one face outside the area of the light beam. The use of ▲cuvettes▲ (USP 1-Dec-2022) with no type markings is not recommended, but, if ▲cuvettes with type markings▲ (USP 1-Dec-2022) are used, analysts should confirm their suitability for the intended application. If ▲cuvettes▲ (USP 1-Dec-2022) are provided with the instrument, individual ▲cuvette▲ (USP 1-Dec-2022) holders should always be used in the same beam as identified, e.g., by use of appropriate markings.

▲CUVETTE▲ (USP 1-Dᴇᴄ-2022) CORRECTIONS

If multiple ▲cuvettes▲ (USP 1-Dec-2022) are used, differences in the optical transparency of individual ▲cuvettes▲ (USP 1-Dec-2022) can introduce a systematic bias unless the latter is accounted for by a ▲cuvette▲ (USP 1-Dec-2022) correction. ▲Depending on where the zero point is set, this correction can be either positive or negative; however,▲ (USP 1-Dec-2022) do not use corrections in excess of 0.01 ▲AU.▲ (USP 1-Dec-2022) To determine if ▲cuvette▲ (USP 1-Dec-2022) corrections are necessary, analysts can fill all ▲cuvettes▲ (USP 1-Dec-2022) with the appropriate solvent and measure the differences in absorbance at the required analytical wavelengths. These measurements are repeated after cleaning to ensure that differences in absorbance are related to the ▲cuvettes▲ (USP 1-Dec-2022) and are not the result of contamination. Analysts should fill and check the ▲cuvettes▲ (USP 1-Dec-2022) repeatedly until the results are consistent, and they should investigate any appreciable change in ▲cuvette▲ (USP 1-Dec-2022) correction because it indicates contamination, damage to the ▲cuvettes,▲ (USP 1-Dec-2022) or incorrect adjustment of the instrument.

APPENDIX

Other Sources of Information

1. Vandenbelt J M, Forsyth J, and Garrett A. *Anal. Chem. (Industrial and Engineering Chemistry, Analytical Edition)*. 1945;17(4):235.
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3. Youmans HL, Brown WD. *Anal. Chem.* 1976;48:1152.

4. Skoog DA, West DM, Holler FJ. *Fundamentals of Analytical Chemistry.* 7th ed.; 1996: Table 24-4.