TECHNICAL NOTE

Selecting the Best Cuvette for UV-Vis and Fluorescence Spectroscopy

TN_FL_01; Julia Gardiner

Introduction

Cuvettes are small laboratory vessels used to hold samples (typically liquid) for spectroscopic measurements, such as UV-Vis spectrophotometry and fluorescence spectroscopy (Figure 1). They allow for precise and reproducible spectroscopic measurements by providing a consistent optical path. Different varieties of cuvettes are available, some of which can be optimal depending on the methodology and requirements of the application. This Technical Note will discuss the factors to consider when selecting the most suitable cuvette for spectroscopic measurements

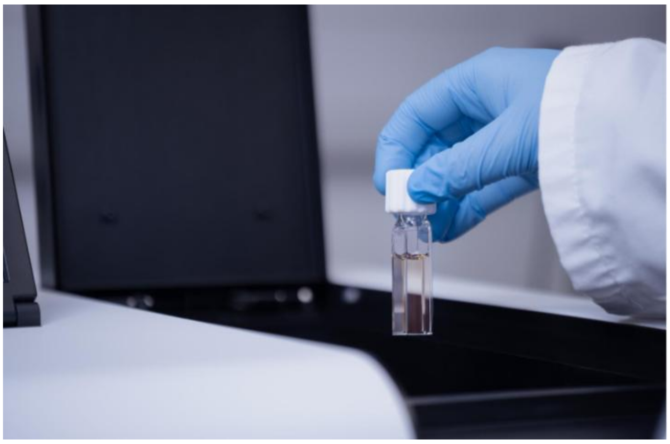

Figure 1. A cuvette used for spectral measurements.

UV-Vis *vs.* Fluorescence Cuvettes

UV-Vis and fluorescence spectroscopy differ in how light is measured. In UV-Vis spectrophotometry, the light passes through the sample in a straight line (180°) and the fraction of light transmitted through the solution detected (Figure 2a). In contrast, fluorescence spectrometers typically use a 90° measurement geometry, in which the emitted fluorescence is collected perpendicular to the excitation light (Figure 2b). This setup avoids interference from the transmitted excitation light, reducing the background signal and improving sensitivity.

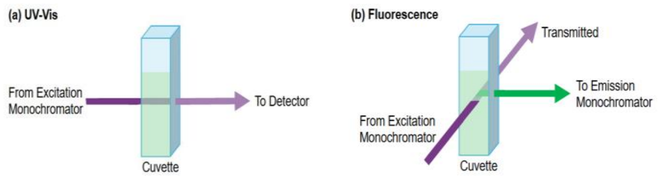

Figure 2. Light path in **(a)** UV-Vis spectroscopy, where light travels through the sample at 180°, and **(b)** fluorescence spectroscopy, where light is collected at a 90° angle.

Due to the difference in measurement geometry, different cuvettes are used for each technique. UV-Vis cuvettes have two clear optical windows that allow light to pass through the sample and two frosted sides for ease of handling. Fluorescence cuvettes have four clear optical windows due to the 90° angle light collection. Two-sided UV-Vis cuvettes cannot be used for fluorescence measurements as they scatter the excitation light and reduce sensitivity.

Material

Cuvettes are made from a variety of materials, with the most common being quartz (molded or bonded type), optical glass, and plastic (PS, PMMA or UV-grade). Specialised materials such as sapphire can also be used, however they are beyond the scope of this note. The choice of material depends on the instrumental and experimental requirements, including factors such as spectral range, durability, and budget. A brief overview can be found below (Table 1), followed by more detailed descriptions of each factor.

Table 1. Summary of cuvette material comparison.

Material	Molded Quartz	Bonded Quartz	Optical Glass	UV Plastic	PS/PMMA
Spectral Range	190–2500 nm (UV-Vis-NIR)	190–2500 nm (UV-Vis-NIR)	340–2500 nm (Vis-NIR)	230–900 nm (UV-Vis)	380–780 nm (Vis)
Chemical Resistance	High	Moderate	Moderate	Moderate	Low
Thermal Resistance	≤ 1200 °C	~ 80–120 °C	≤ 90 °C	≤ 60 °C	≤ 60 °C
Cost	High	Moderate	Moderate	Low	Very Low

Spectral Range

The material of a cuvette determines its transparency at different wavelengths – it is important that the cuvette transmits light at the wavelength(s) used in the experiment, particularly for measurements in the UV region (Figure 3).

- **Quartz** cuvettes have a wide spectral range from ~190–2500 nm, covering the UV, visible, and near-infrared (NIR) regions.
- **Optical glass** is transparent in the range ~340–2500 nm, so cuvettes made from this material can be used for visible and NIR measurements.
- **UV plastic** materials are transparent from ~230–900 nm, allowing for measurements in the UV and visible regions.
- **PS/PMMA** cuvettes transmit light in the range of ~380–780 nm, making them suitable for measurements in the visible region.

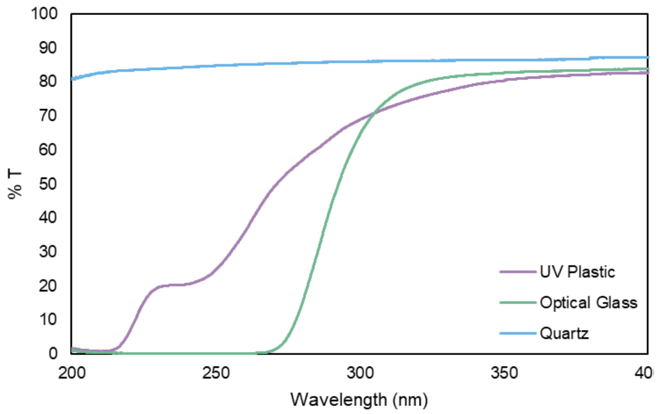

Figure 3. Transmission spectra of different cuvette materials in the UV region. Acquired on an Edinburgh Analytical DB30 UV-Vis Spectrophotometer.

Since neither optical glass nor PS/PMMA cuvettes are transparent in the UV region, they are not suitable for UV measurements, e.g. DNA quantitation at 260 nm (A_{260}). Quartz is the gold standard for UV applications, with a high transmission rate of >80% from the UV to the NIR. UV plastic provides a more convenient and cost-effective alternative to quartz, which is well suited for routine analyses, however it is a less transmissive and robust material.[1]

Chemical Resistance

Another important consideration for cuvette selection is the chemical resistance of the material, i.e. how resistant the cuvette is to aggressive solvents.

- **Molded quartz** cuvettes are formed from monolithic fused quartz. This makes them highly chemically inert and resistant to most organic solvents, acids and bases, and aqua regia. Quartz cuvettes are suitable for handling a majority strong acids (e.g. HCl, H_2SO_4) at different temperatures.

  Exceptions include hot concentrated phosphoric acid (H_3PO_4) which can damage the quartz at temperatures above 150 °C; and hydrofluoric acid (HF), which can dissolve the material even at low temperatures and concentrations.[2] Quartz cuvettes also have a high alkali resistance and can handle strong bases (e.g. NaOH, KOH); however, prolonged exposure and heating can reduce the cuvette's transparency and precision.

- **Bonded quartz** has less chemical resistance than molded quartz. The glue used to bond the faces of the cuvette together can be compromised by certain solvents. So, while the quartz itself may be inert to these solvents, the glue may degrade or dissolve, causing cuvette failure.

  These cuvettes are compatible with common acids and strong alkalis, though this is usually limited to short-term use or exposure as the adhesive will degrade over time. They are not compatible with organic solvents such as toluene or chloroform, nor with aqua regia.[2]

- **Optical glass** cuvettes have a moderate chemical resistance – they are suitable for short-term storage of strong acids and bases. Similar to quartz, optical glass gets corroded by HF. Long-term exposure to strong bases also causes damage, especially at high temperatures.

- **UV plastic** offers a similar level of chemical compatibility to glass. It can be used with most polar organic solvents, and most acids and bases. However, the material is not resistant to chloroform or hexane.

- **PS/PMMA** cuvettes are the least resistant out of these materials. They are compatible with aqueous solutions, but they can be damaged and/or dissolved by organic solvents such as acetone, chloroform, and toluene.

Thermal Resistance

Reaction temperature can also affect the choice of cuvette material. It is important to consider for applications involving high temperatures or risk of thermal shock.

- **Molded quartz** cuvettes are the most thermally resistant, remaining stable up to 1200 °C.[2] These cuvettes are well suited for high-temperature applications, i.e. catalytic research or metal oxide studies. However, it is recommended to keep the temperature under 800 °C to maintain the longevity of the cuvette.[3]

- **Bonded quartz** has a much lower tolerance for high temperatures compared to molded quartz. While the actual quartz material is still stable up to 1200 °C, the adhesive holding the cuvette together fails at much lower temperatures. This results in bonded quartz cuvettes only being able to handle temperatures of up to ~80–120 °C.[2]

- **Optical glass** offers a moderate resistance to heat, up to approximately 90 °C when it begins to deform.[2]
- **Plastic** (UV-grade and PS/PMMA) is best suited for lower temperature use, as it begins to melt around 60 °C.[2]

Cost

Another factor to consider is the cost of each cuvette material. Depending on the intended applications and methods,

- **Quartz** offers a broad spectral range and high durability, making it a lot more expensive compared to glass and plastic. Bonded quartz cuvettes are less expensive than molded ones, though they have disadvantages in terms of chemical and thermal resistance, so it is important to consider the intended applications and reaction conditions. Regardless of the price, investing in quartz cuvettes is typically recommended for high-precision analysis and research applications.
- **Optical glass** is more affordable than quartz and is also suitable for long-term use. However, it is not an appropriate material for UV measurements, or experiments requiring extreme thermal conditions and long-term exposure to harsh solvents.
- **Plastic** cuvettes come at a low cost, and they are disposable and shatterproof, though they have much more limited transparency and durability. UV plastic is more expensive than PS/PMMA, though it has the added advantage of UV transparency and improved solvent compatibility. This makes UV plastic cuvettes a very cost-effective alternative to quartz, if used for routine analysis under appropriate reaction conditions.

Path Length

The path length of a cuvette is the distance that the incident light travels through the sample, and is equivalent to the distance between the interior walls of the cuvette. The path length (l) has a direct, linear relationship with the absorbance (A) of the sample solution in accordance with the Beer-Lambert Law ($A = \varepsilon lc$).

Path lengths typically range between 1 mm and 100 mm. Most commonly, cuvettes are designed with a standard path length of 10 mm, which is compatible with most spectrometers; however, short and long path lengths are also available (Figure 4a–c).

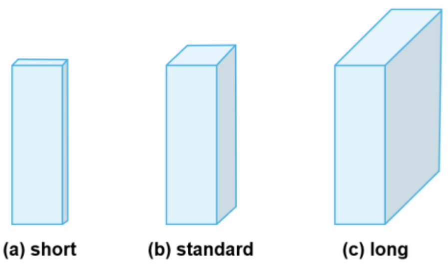

Figure 4. Cuvettes with **(a)** short, **(b)** standard, and **(c)** long pathlengths.

- **Short path length** cuvettes (Figure 4a) are most suitable for applications involving high-concentration samples, e.g. dense bacterial cultures. Using a shorter path length allows transmission through high absorbance solutions in UV-Vis measurements, and helps reduce the Inner Filter Effect during fluorescence applications. This approach also allows for easier and more accurate measurements by avoiding additional serial dilutions.
- **Long path length** cuvettes (Figure 4c) are typically used for very dilute and low-concentration samples. The longer path length allows light to pass through more of the sample, which increases the absorbance and improves sensitivity. These cuvettes are particularly useful for trace analysis and environmental testing, e.g. impurities and pollutants in water.

Volume

Cuvette volume is the maximum amount of liquid sample that a cuvette can safely hold. Different cuvette volumes may be more appropriate for certain applications depending on sample availability and concentration. It is important to note that cuvettes can still have the standard exterior dimensions (12.5 × 12.5 × 45 mm) while having a different internal capacity (e.g. a narrower inner width or a shorter inner height). This allows the cuvette to hold a smaller volume of sample and still fit into the standard cell holder of most UV-Vis and fluorescence spectrometers.

Maximum cuvette volume can be determined using Equation 1. The volume is calculated at 80% capacity: it is not recommended to fill a cuvette any further, to prevent overflow and unwanted spillages.

$$\text{Inner Length} \times \text{Inner Width} \times \text{Inner Height} \times 80\% = \text{Cuvette Volume} \qquad \text{Eq. 1}$$

Different categories of cuvettes are available depending on the desired volume (Table 2).[3]

- **Standard** 10 mm path length cuvettes hold a volume of 3.5 mL and are suitable for most analytical applications. Typically, UV-Vis and fluorescence spectrometers will come with a standard sample chamber which can readily accommodate a 10 mm cuvette.
- **Macro** cuvettes are designed for volumes greater than 3.5 mL. They are useful for low-concentration samples (due to the longer path length) and temperature-sensitive applications, as they provide better surface contact with temperature-controlled holders.[4]
- **Semi-micro** and **sub-micro** cuvettes are used when a sample volume smaller than 3.5 mL is required or available – they are ideal for applications involving biochemical assays, DNA and protein analysis, or clinical samples.

It is important to note that many small volume cuvettes have a shorter inner height, or a restricted sample window: therefore, they have a specific Z-dimension which must match that of the spectrometer. More details on Z-dimensions can be found in the next section.

Table 2. Categories of cuvette sizes and the volumes they can hold.

Category	Volume (mL)
Macro	>3.5
Standard	3.5
Semi-micro	0.35-3.5
Sub-micro	<0.35

Z-Dimension

The Z-dimension (ZD) of a cuvette is the vertical distance between the bottom of the cuvette to the centre of the sample window/aperture, through which the incident light passes. The ZD is an important factor, particularly for small volume and short height cuvettes, as it must be matched to the light beam path of the spectrometer. The most common ZD are 8.5 mm, 15 mm, or 20 mm (Figure 5a-c).[1]

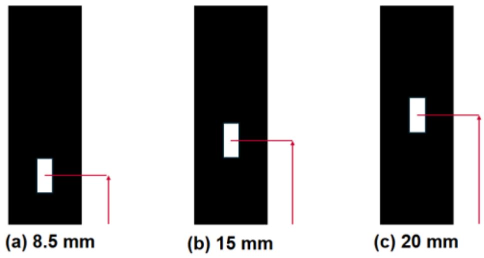

Figure 5. Sub-micro cuvettes in common Z-dimensions: **(a)** 8.5 mm, **(b)** 15 mm, and **(c)** 20 mm.

It is important to ensure the compatibility of the cuvette and spectrometer ZD, otherwise the light will not be able to pass through the sample. The UV-Vis and fluorescence spectrometry products offered by Edinburgh Analytical (DB30 UV-Vis Spectrophotometer and FE30 Fluorescence Spectrometer) and Edinburgh Instruments (FS5 Spectrofluorometer and FLS1000 Photoluminescence Spectrometer) all have a ZD of 15 mm (Figure 4b).

Conclusion

This Technical Note has discussed the considerations when selecting a cuvette for UV-Vis spectrophotometry and fluorescence spectroscopy. Different factors – measurement geometry, material, path length, volume, and Z-dimension – are of significance depending on the experiment. The most commonly used cuvette has a standard 10 mm pathlength and a volume of 3.5 m. For material, molded quartz is the gold standard with the highest transparency, durability, chemical and thermal resistance. Glass and plastic are lower cost alternatives and are suitable for many applications.

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