

Effortlessly Change Path Length to Enhance Photometric Performance

Using an Agilent Cary 3500 Flexible UV-Vis spectrophotometer and variable-path-length cell holder



Introduction

In UV-Vis spectroscopy applications, the path length is defined as the distance that light travels through a sample. Selecting the right cuvette path length is essential for the accurate quantification of sample concentration.

Quantification of concentration using spectroscopy

One of the most common applications for the UV-Vis spectrophotometer is the simple quantification of concentration. Sample solutions exhibit higher UV or visible (or both) light absorptions with increased concentration. This can be used to measure several standards of known concentrations and create a calibration curve. Once the calibration curve is established, it can be used to efficiently determine the concentration of an unknown sample (Figure 1).

In UV-Vis measurements, the photometric response of the spectrophotometer follows the Beer–Lambert law (Equation 1). Following this law, a linear relationship exists between absorbance and sample concentration. The simple, linear relationship and the relative ease of measurement of UV-Vis light have together made UV-Vis spectroscopy a fundamental tool for many routine quantitative analytical methods.

Equation 1.

$$A = \epsilon bc$$

Where:

A = absorbance

ϵ = molar absorptivity ($M^{-1}cm^{-1}$)

b = path length (cm)

c = concentration (M)

Choosing an appropriate path length

The path length is the distance that the incident light travels through a sample. To accurately quantify sample concentration, the right cuvette path length should be used based on the lowest/highest absorbance. In the following example (Figure 2), phenylalanine was measured using a 1 cm cuvette, and the maximum absorbance was observed at 257 nm. Also, a second peak was observed at 438 nm and was considered to be a contaminant. Accurate quantification of the contaminant in this case could be difficult due to low peak intensity. Based on the Beer–Lambert law, increasing the cuvette path length from 1 to 10 cm would increase the absorbance of this contaminant, which could help in more accurate quantification. Alternatively, if sample concentration is high and exceeds the linear dynamic detection range of the system, a cuvette with a shorter path length could be used, or the sample could be diluted further. Selecting the path length of the cuvette within the linear dynamic range of the system can provide more accurate quantification of sample components.

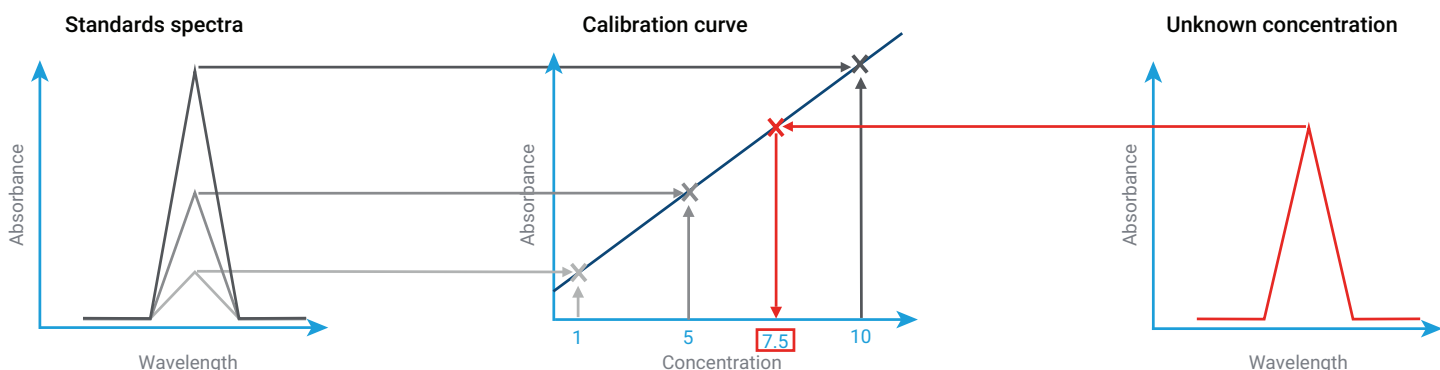


Figure 1. Illustration of simple quantification of concentration using UV-Vis spectroscopy.

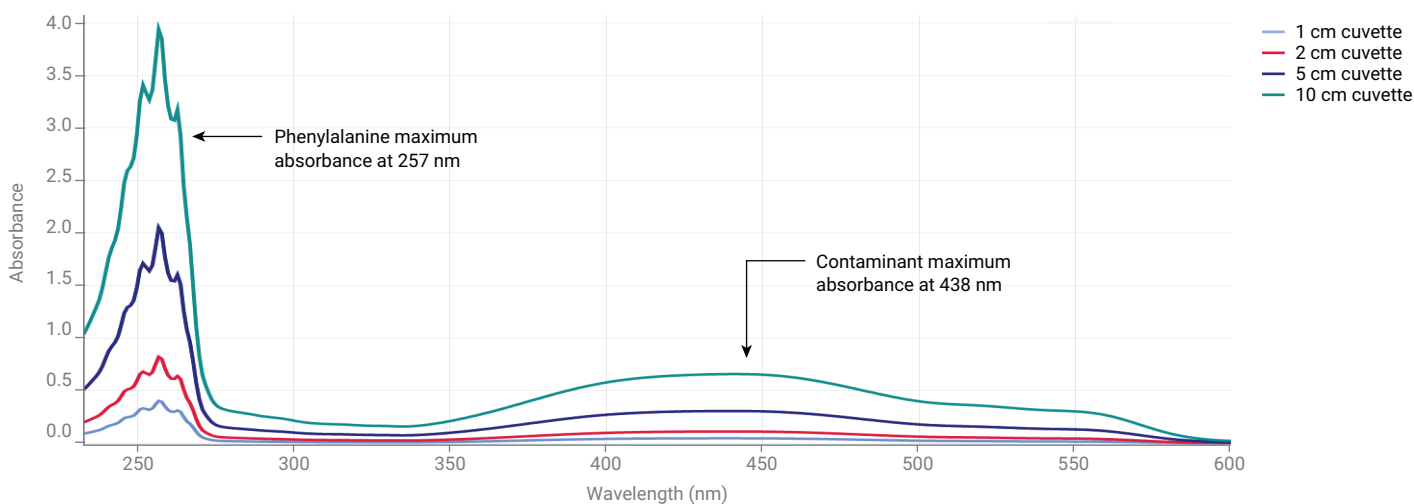
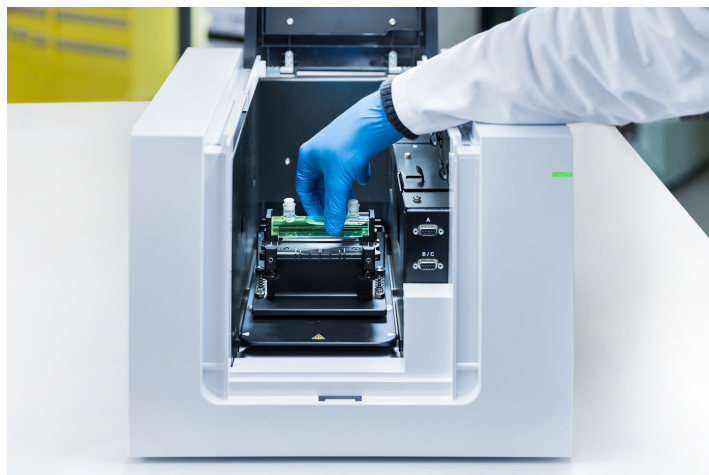
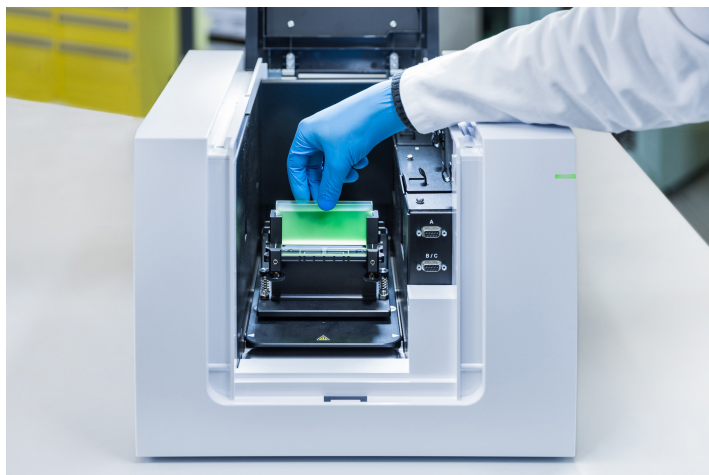


Figure 2. UV-Vis spectrum of phenylalanine (0.4 mg/mL) and a contaminant, analyzed on an Agilent Cary 3500 Flexible UV-Vis spectrophotometer equipped with a standard 1 cm cuvette and Agilent variable-path-length cell holders (2, 5, and 10 cm cuvettes).

Effortlessly change path length with the Cary 3500 Flexible UV-Vis spectrophotometer variable-path-length cell holder

The Cary 3500 Flexible UV-Vis spectrophotometer is complemented by a range of accessories to enable the handling of various sample sizes and types. The Cary 3500 Flexible UV-Vis spectrophotometer features unique, variable-path-length rectangular or cylindrical cell holders, which enable you to quickly and reproducibly change the path length (2, 4, 5, and 10 cm). This design guides the customer to the required path length in an easy, toolless process, and eliminates the time-consuming alignment procedure.

The path length can be changed easily and quickly following the steps described in Figure 3. A wide range of cuvettes with different path lengths are provided by Agilent, as described in Table 1.



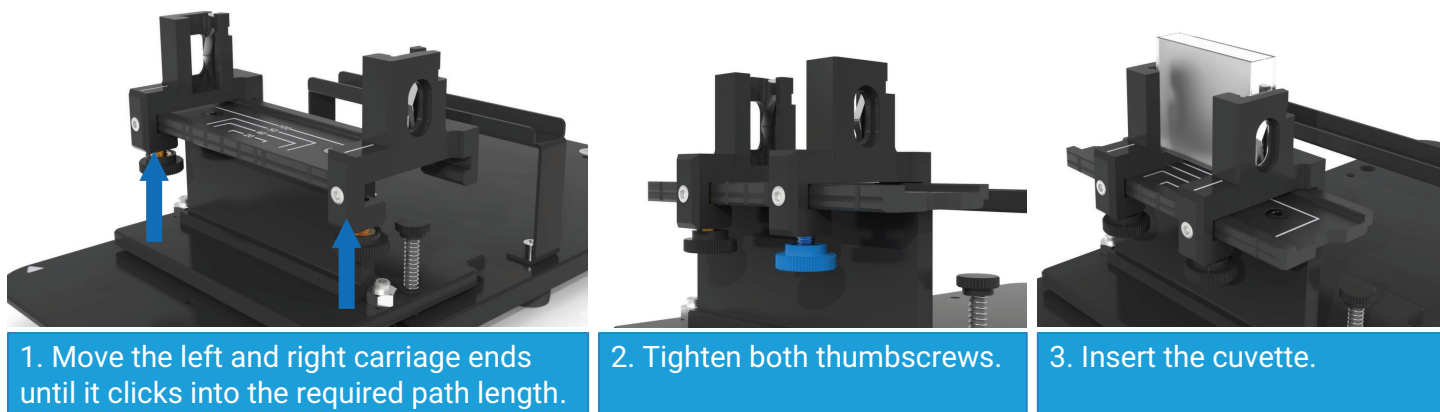



Figure 3. The Agilent Cary 3500 Flexible UV-Vis spectrophotometer with variable-path-length cell holder. The path length can be changed easily and quickly following the steps described.

Table 1. Agilent UV-Vis cuvettes with different path lengths.

Part Number	Illustration	Type	Volume (mL)	Path Length (cm)
5061-3387		Rectangular with PTFE lid	3.5	1
6610016200		Rectangular with PTFE lid	7	2
6610016100		Rectangular with PTFE lid	17.5	5

Part Number	Illustration	Type	Volume (mL)	Path Length (cm)
6610016000		Rectangular with PTFE lid	35	10
6618000600		Cylindrical with a single PTFE stopper	2.8	1
6610002200		Cylindrical with PTFE stoppers	14.1	5
6610002300		Cylindrical with PTFE stoppers	28.2	10

Conclusion

The advanced photometric performance of the Agilent Cary 3500 Flexible UV-Vis spectrophotometer combined with the unique design of Agilent variable-path-length cell holders provide an easy and quick solution for low- or high-concentration samples to be measured accurately. The cell holders can accommodate various path lengths (2, 4, 5,

and 10 cm) with both rectangular and cylindrical cuvettes. With this combination, analysts can achieve more accurate quantification over a broad concentration range.

Further information

- Cary 3500 Flexible UV-Vis Spectrophotometer
- Cary UV Workstation software
- Data Integrity Options for GMP Facilities for the Agilent Cary 3500 UV-Vis Spectrophotometer Series
- UV-Vis Spectroscopy & Spectrophotometer FAQs

www.agilent.com/chem/cary3500flexible

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