

Agilent Cary 3500 Flexible UV-Vis Spectrometer: Benefits of a Wide Dynamic Range

Enabling fast, easy, routine quantitative measurements of low to high concentration samples

Abstract

Measuring sample concentrations of analytes by ultraviolet visible (UV-Vis) spectroscopy is a well-established approach used in many laboratories. With its wide linear dynamic range, the Agilent Cary 3500 Flexible UV-Vis spectrophotometer offers best-in-class research performance in a routine bench-top UV-Vis spectrophotometer. Instrument performance was investigated by analyzing potassium permanganate solutions ranging from 0.03 to 490 mg/L.



Figure 1. The Agilent Cary 3500 Flexible UV-Vis spectrophotometer offers superior photometric performance in a routine system for direct measurements of liquid and solid samples. It is ideal for analyzing low to high-concentration samples.

Introduction

The Agilent Cary 3500 Flexible UV-Vis is a double-beam spectrophotometer equipped with a powerful xenon flash lamp eliminating daily warmup. The lamp comes with a 10-year warranty (for Cary 3500 instruments purchased from Agilent or participating partners) that drastically reduces the frequency and cost of lamp replacement for ultimate peace of mind. The Cary 3500 Flexible UV-Vis spectrophotometer offers advanced photometric performance in a routine system for direct measurements of liquid and solid samples. For added flexibility, the instrument includes a large sample compartment that can accommodate a range of accessories for the analysis of a variety of sample sizes and types. The linear dynamic range (LDR) of the Cary 3500 Flexible UV-Vis goes beyond 6 Abs, providing best in class research performance in a routine benchtop UV-Vis spectrophotometer. The wide LDR allows for direct measurements of highly absorbing samples, minimizing sample preparation time, and increasing the efficiency and accuracy of the analysis.

In addition to an extended LDR, the Cary 3500 Flexible UV-Vis is configured for routine and flexible use. The system has a large sample compartment to facilitate sample handling and user access, while maintaining a small footprint. The Cary 3500 Flexible UV-Vis is also equipped with a custom cell holder base that can accommodate both 1 cm and longer pathlength cells. The cell holder base is designed so that cells can be loaded quickly in a fixed position, ensuring accurate placement of samples irrespective of user-experience or expertise.

This technical overview demonstrates how high (up to 490 mg/L), and low (0.03 mg/L) concentration solutions of potassium permanganate (KMnO_4) were measured using the Cary 3500 Flexible UV-Vis. The required LDR for the measurements was achieved by switching between a standard 1 cm pathlength cell and a 10 cm pathlength cell. Changing the variable pathlength cell holder to the desired pathlength is quick and easy, with no tools required. Once in position, no further alignment of the cell holder is necessary, allowing many different sample types to be analyzed with speed, accuracy, and confidence.

Experimental

Standard KMnO_4 solutions

- A series of aqueous KMnO_4 solutions of known concentration from 0.03 to 500 mg/L were prepared in 20 mL volumetric flasks through appropriate dilution using distilled water.
- A standard 1 cm pathlength cell was used to measure KMnO_4 solutions at 5, 50, 100, 200, 300, 350, 400, 420, 460, 490, and 500 mg/L.
- A rectangular 10 cm pathlength cell was used to measure the absorbance of the KMnO_4 solutions at 0.03, 0.04, 0.05, 0.1, 0.3, 0.5, 0.8, 1, 2, 3, 5, 8, and 10 mg/L.

Note: It is important to rinse the sample cell with each solution between measurements. Make sure that the optical faces are dry and free from fingerprints before each measurement using lint-free tissues.

Instrumentation

The Cary 3500 Flexible UV-Vis spectrophotometer was fitted with the standard 1 cm cell holder (part number G9886-68005) and the variable pathlength rectangular cell holder (part number G9886-68003). Depending on the concentration of the KMnO_4 standard solutions, the standards were added to either 1 or 10 cm pathlength quartz cells. Absorbance measurements for KMnO_4 solutions were acquired using the [Agilent Cary UV Workstation software](#), version 1.4. Instrument operating parameters are shown in Table 1.

Table 1. Experimental parameters for the Agilent Cary 3500 Flexible UV-Vis spectrophotometer.

Parameter	Setting
Wavelength Range (nm)	400 to 750
Data Interval (nm)	0.5
Signal Averaging Time (s)	1.0
Baseline	Distilled water

Results and discussion

Analysis of high concentration samples using a standard 1 cm pathlength cell

The LDR of the Cary 3500 Flexible UV-Vis spectrophotometer was determined by measuring 5, 50, 100, 200, 300, 350, 400, and 420 mg/L of KMnO_4 solutions in a 1 cm standard cell. The UV-Vis spectra of the eight standard solutions are shown in Figure 2A. Figure 2B shows the calibration plot of absorbance taken at 525 nm versus concentration (mg/L). An excellent linear calibration graph up to 6.6 Abs with a correlation

coefficient of $R^2 = 0.9995$ was obtained. These results confirm that quantitative analysis of high concentration KMnO_4 solutions up to 420 mg/L (6.60 Abs) is achievable with the Cary 3500 Flexible UV-Vis using a standard 1 cm cell.

However, by increasing the signal averaging time to 10 seconds, higher absorbance measurements were achievable for KMnO_4 solutions up to 490 mg/L (Figure 3A). Corresponding calibration curve is shown in Figure 3B and has an excellent linear trend with a correlation coefficient of $R^2 = 0.9997$.

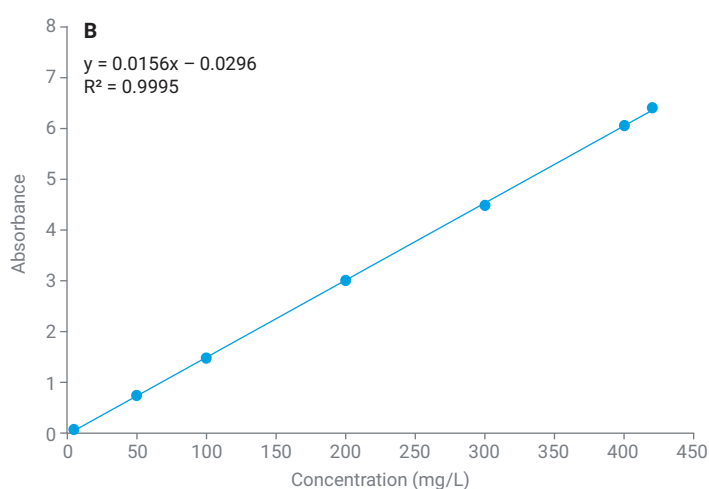
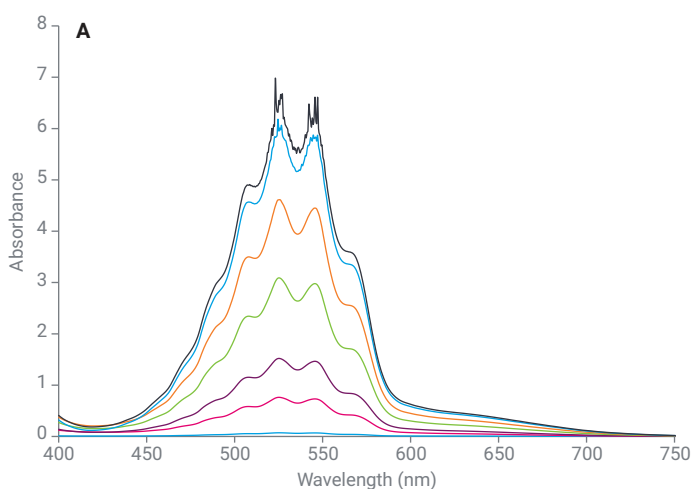


Figure 2. (A) Spectra of aqueous KMnO_4 solutions measured using a 1 cm standard pathlength cell and signal averaging time of 1 second. (B) Calibration plot of absorbance (at 525 nm) versus concentration (mg/L) with excellent linearity, as shown by the correlation coefficient of $R^2 = 0.9995$.

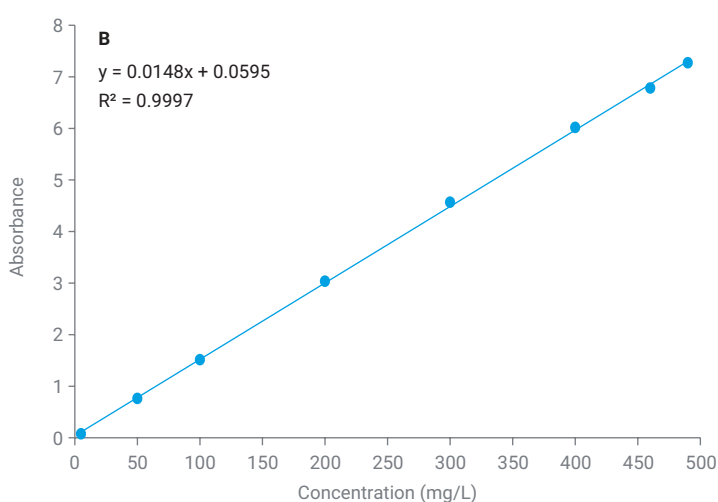
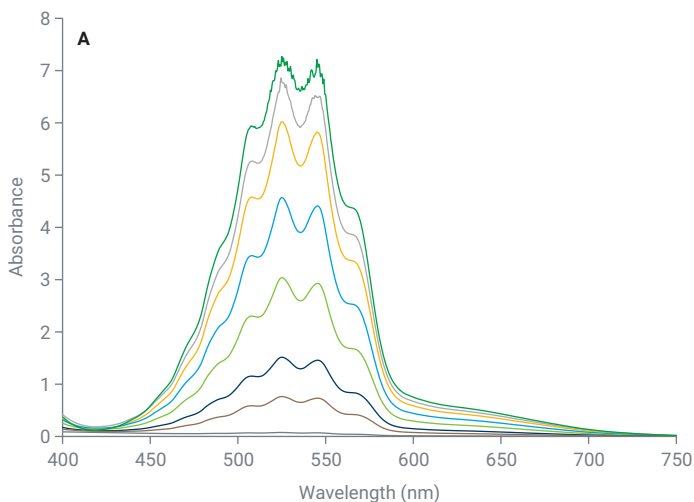


Figure 3. Analysis of high concentrations of aqueous KMnO_4 solutions up to 7 Abs using a standard 1 cm pathlength cell and signal averaging time of 10 seconds. (A) The UV-Vis spectra of the eight standard solutions (B) Calibration plot of absorbance (at 525 nm) versus concentration (mg/L) with excellent linearity ($R^2 = 0.9997$).

Analysis of low concentration samples using a 10 cm pathlength cell

The Cary 3500 Flexible UV-Vis spectrophotometer is equipped with a specially designed cell holder base that is designed to accommodate cell holders with different pathlengths. The cell holder includes a variable pathlength holder for rectangular cells (2, 4, 5, and 10 cm) and a variable pathlength holder for cylindrical cells (2, 4, 5, and 10 cm). The variable pathlength cell holders come with predefined positions to ensure the optimized setting is used for the desired cell pathlength.

Figure 4A shows the variable pathlength rectangular cell holder adjusted to support the 10 cm pathlength cell. The cell holder is adjusted by sliding the carriage ends over the guided

rail of the cell holder base at the correct pathlength and tightening the thumbscrews. No further alignment of the cell holder is necessary. Low concentration KMnO_4 solutions, less than 10 mg/L, were analyzed using the 10 cm long pathlength cell, as shown in Figure 4B.

Qualitative and quantitative analysis of low concentration KMnO_4 solutions in the range of 0.03 to 10 mg/L are shown in Figure 5. The inset of Figure 5A shows that the spectral profiles of the spectra obtained for the lowest concentration samples (0.10, 0.05, and 0.03 mg/L) were identical to the profiles of the more concentrated samples. The corresponding calibration plot shown in Figure 5B has excellent linearity ($R^2 = 0.9996$). The lowest

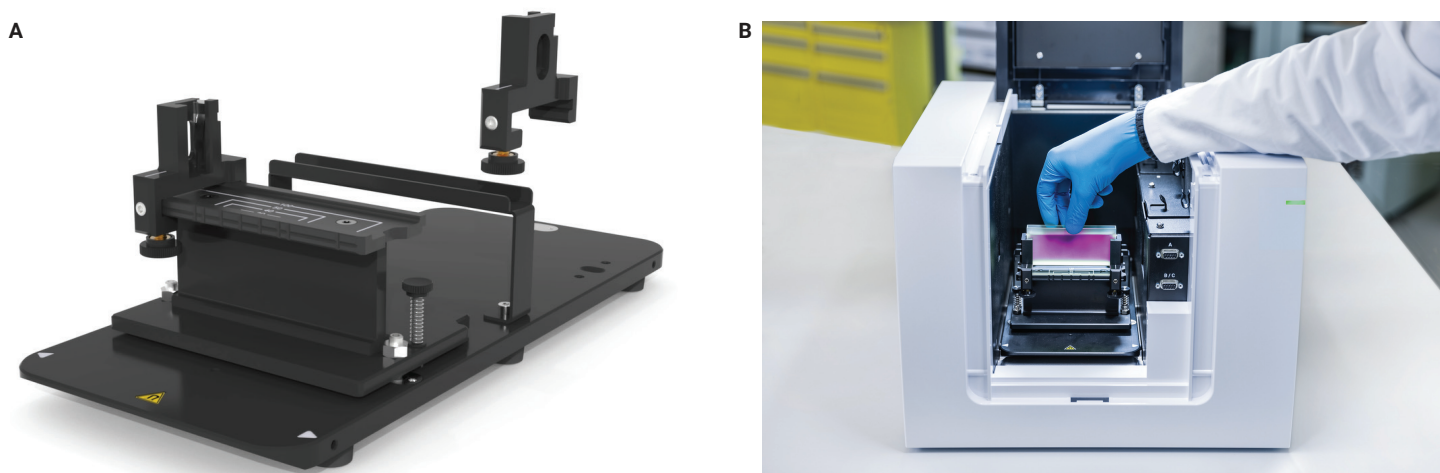


Figure 4. (A) The variable pathlength rectangular cell holder for the Agilent Cary 3500 Flexible UV-Vis spectrophotometer. Changing the variable pathlength cell holder to the desired pathlength is quick and easy, requiring no tools or further alignment of the cell holder. (B) The Cary 3500 Flexible UV-Vis spectrophotometer with a 10 cm pathlength cell filled with a KMnO_4 solution.

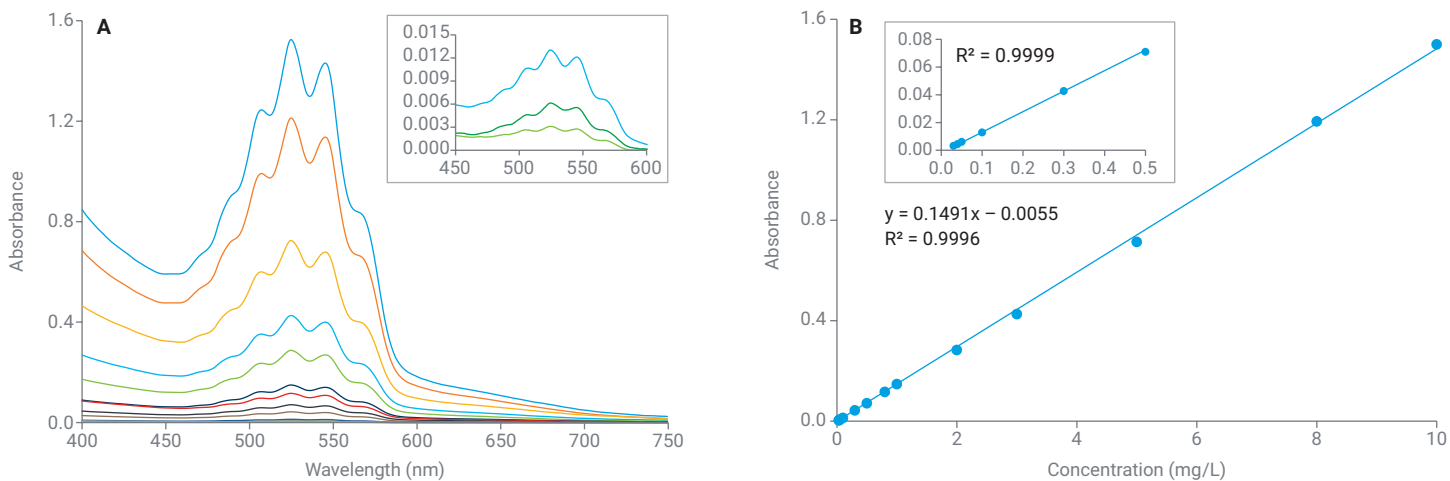


Figure 5. (A) Spectra of standard KMnO_4 solutions measured using a rectangular 10 cm pathlength cell. The inset shows the spectra obtained for the low concentration standards 0.10, 0.05, and 0.03 mg/L. (B) Linear calibration plot of absorbance (at 525 nm) versus concentration (mg/L) with a correlation coefficient of $R^2 = 0.9996$. The inset shows the calibration plot for the lowest concentration range of 0.03 to 0.50 mg/L.

concentration range of the calibration curve (inset of Figure 5B) demonstrates the excellent photometric linearity (R^2 of 0.9999) of the Cary 3500 Flexible UV-Vis from 0.03 to 0.5 mg/L.

Limit of quantification

The limit of quantification (LOQ) is defined as the lowest possible concentration of an analyte in a test sample that can be quantified by an analytical method. The standard deviation method as described by Equation 1 was used to determine the quantification limit of KMnO_4 .

Equation 1.

$$\text{LOQ} = 10 \times \sigma/S$$

Where σ is the standard deviation of an appropriate number of blank sample measurements and S is the slope of the calibration curve.¹

Note: According to the guidelines given by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), the estimation of σ may be carried out in different ways. The standard deviation of the blank is one such method that involves calculating the standard deviation by analyzing an appropriate number of blank samples.

The LOQ of KMnO_4 was determined using the Cary 3500 Flexible UV-Vis spectrophotometer fitted with a standard 1 cm pathlength cell and a rectangular 10 cm pathlength cell. Corresponding calibration curves are shown in Figures 2B and 5B. To calculate the standard deviation (σ) of the blank, sample measurements for each pathlength, absorbance spectra were acquired during 10 replicate determinations of the blank, and absorbance values were taken at 525 nm. As can be seen in Table 2, the LOQs for KMnO_4 for the 1 and 10 cm pathlength cells were calculated to be 0.029 and 0.003 mg/L, respectively. The LOQ demonstrates that extremely low concentration samples, such as residual and impurity absorptions, can be measured using the Cary 3500 Flexible UV-Vis spectrophotometer with a 10 cm pathlength cell.

Table 2. Determination of limit of quantification using 1 and 10 cm pathlength cells.

Scan	Absorbance of Blank at 525 nm	
	1 cm Pathlength	10 cm Pathlength
1	0.000063	0.000205
2	0.000079	0.000205
3	0.000171	0.000199
4	0.000175	0.000200
5	0.000176	0.000188
6	0.000188	0.000190
7	0.000192	0.000187
8	0.000188	0.000182
9	0.000190	0.000177
10	0.000188	0.000022
Standard Deviation (σ)	0.000046	0.000052
Slope of the Calibration Curve (S)	0.0156	0.1491
LOQ (mg/L)	0.029	0.003

Conclusion

The Agilent Cary 3500 Flexible UV-Vis is an innovative double beam UV-Vis spectrophotometer that offers advanced photometric performance over a wide LDR—ideal for routine UV-Vis measurements. It is a double beam instrument equipped with a powerful xenon flash lamp. The xenon lamp has a 10-year warranty ensuring uninterrupted operation while avoiding expensive and time-consuming lamp replacements. The benchtop Cary 3500 Flexible UV-Vis includes a large sample compartment to facilitate easy sample handling and user access. It is also equipped with a specially designed cell holder base that can accommodate variable pathlength cell holders. Switching and positioning cell holders to achieve the desired pathlength can be done quickly and accurately without the need for tools, minimizing time-consuming accessory handling.

The quantitative analysis of aqueous KMnO_4 solutions demonstrated the excellent linear dynamic range, beyond 6 Abs, for the Cary 3500 Flexible UV-Vis spectrophotometer. High and low concentrations of aqueous KMnO_4 samples were quantified accurately using a standard 1 cm cell and a 10 cm long pathlength cell, respectively. The LOQ for KMnO_4 was estimated to be 0.003 mg/L with 10 cm pathlength, demonstrating the sensitivity of the Cary 3500 Flexible UV-Vis spectrophotometer for analyzing extremely low concentration samples.

The Cary 3500 Flexible UV-Vis measures highly absorbing liquid or solid samples directly over a wide LDR, minimizing sample preparation time and increasing the sensitivity and accuracy of the analysis.

References

1. ICH Q2, Validation of Analytical Procedures: Text and Methodology, November **2005**.

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](#)

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Printed in the USA, March 7, 2023
5994-5840EN

