Application Note

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Concentration Analysis of Ultra-microvolume Samples by UV-Vis Spectroscopy

Simple, precise, and nondestructive concentration analysis with Agilent Cary 60 UV-Vis and TrayCell 2.0

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Abstract

The Agilent Cary 60 UV-Vis spectrophotometer fitted with a TrayCell 2.0 Ultra-Microvolume Cell was used for the analysis of protein and nucleic acid samples. The non-destructive measurement of microliter-sized sample volumes is beneficial in terms of sample preservation, sample handling, and eliminating the need to perform tedious and error-prone dilutions. Using the TrayCell 2.0 is quick and easy, resulting in workflow improvements for users.

Introduction

Modern labs are increasingly looking for accurate, precise, and easy-to-use methods to analyze samples such as DNA, RNA, and proteins without dilution. The Agilent Cary 60 UV-Vis spectrophotometer with a TrayCell 2.0 Ultra-Microvolume Cell fitted with a suitable pathlength cap provides a convenient and easy-to-use platform for the direct measurement of ultra-microvolume quantities of sample. High and low concentration samples can be analyzed using a short and long pathlength cap, respectively, ensuring a wide dynamic range. The method is nondestructive, allowing recovery of precious samples, and the TrayCell 2.0 is easy to clean, increasing the usability of the technique.

The Cary 60 UV-Vis spectrophotometer (Figure 1) is designed for repetitive, routine applications as well as more advanced applications. It is a double-beam instrument equipped with a powerful, highly focused xenon flash lamp. The lamp maximizes the level of light that passes through the sample, ensuring high-quality photometric results. The Cary 60 UV-Vis is therefore ideal for measuring small sample volumes accurately and reproducibly. The xenon flash lamp only illuminates the sample when data is acquired, protecting sensitive samples from photodegradation and reducing power consumption. Also, the Cary 60 UV-Vis spectrophotometer is immune to the distorting effects of room light. Room light immunity of the Cary 60 UV-Vis allows the operation with an open sample compartment, enabling easy access, and reducing the risk of compromised data due to handling errors. Compared to existing methods, which can produce inaccurate or unrepeatable results due to instrument limitations, the high-performance Cary 60 UV-Vis provides better quality data.

Figure 1. The Agilent Cary 60 UV-Vis spectrophotometer.

Towards a more sustainable lab

The Cary 60 UV-Vis has been independently audited for its environmental impact and has received the ACT (Accountability, Consistency, Transparency) Label, verified by My Green Lab. The label provides information about the environmental impact of the Cary 60 UV-Vis throughout its entire life cycle (Figure 2).

The Cary 60 UV-Vis improves the environmental impact of laboratories without impeding productivity or scientific progress.

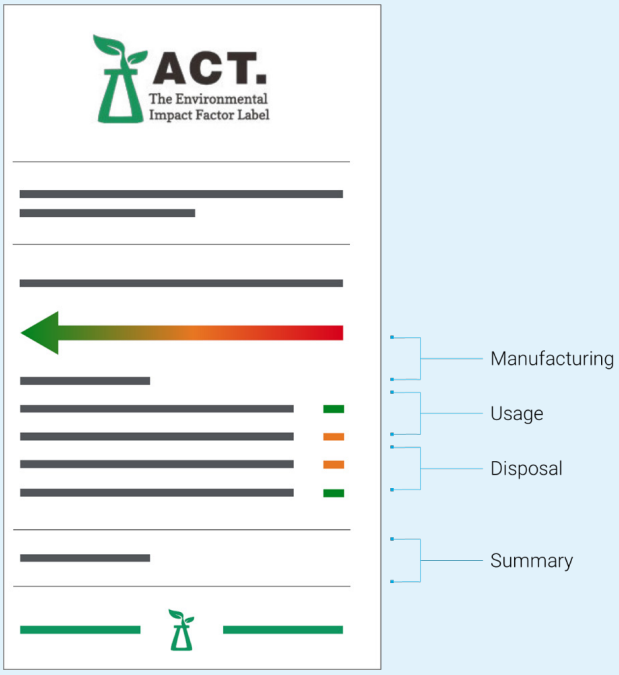

Figure 2. The ACT label provides information about the environmental impact of manufacturing, using and disposing of a product, and its packaging.

In a typical absorbance measurement, the TrayCell 2.0 Ultra-Microvolume Cell (part number G6871C) is placed in the standard cell holder of the Cary 60 UV-Vis spectrophotometer. The TrayCell 2.0 can be fitted with exchangeable caps with four different pathlengths, 2.0 (part number G6871-68005), 1.0 (part number G6871-68004), 0.2 (part number G6871-68003), and 0.1 mm (part number G6871-68002), providing a wide dynamic range. An ultra-microvolume aliquot of the sample (0.7 to 10 µL, depending on the pathlength) is pipetted onto the measuring window of the TrayCell 2.0 and the corresponding cap with the selected pathlength is placed on top. The highly focused light beam from the Cary 60 UV-Vis is directed through the sample via fiber optics in the TrayCell 2.0 (Figure 3). The light beam is then reflected by the mirror in the cap back to the spectrophotometer detector, repassing through the sample, as outlined in Figure 4.

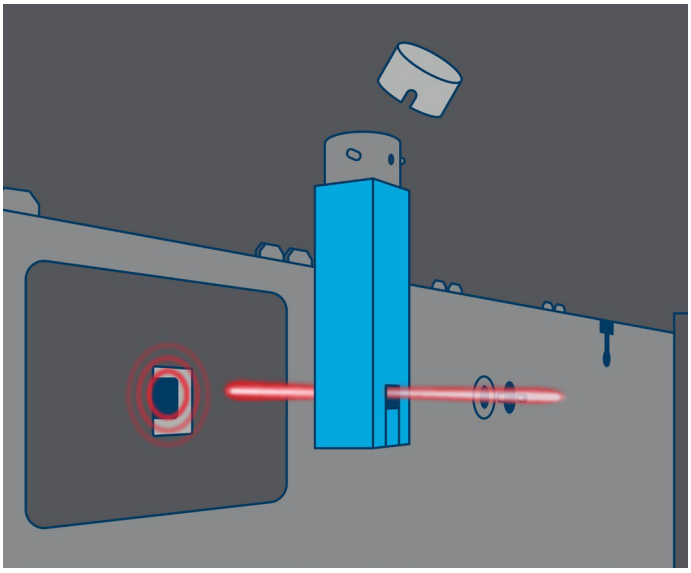

Figure 3. Schematic of the Agilent Cary 60 UV Vis spectrophotometer fitted with a TrayCell 2.0 Ultra-Microvolume Cell for the direct measurement of ultra-microvolume quantities of sample. Representation of the highly focused beam of the Cary 60 UV-Vis passing through the TrayCell 2.0.

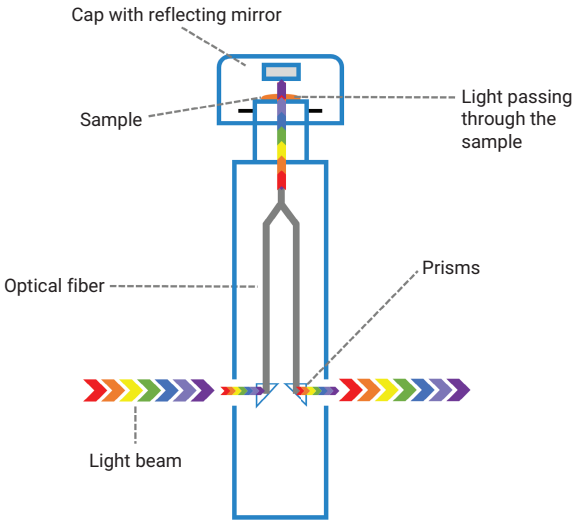

Figure 4. Optical design of the TrayCell 2.0 Ultra-Microvolume Cell.

Before the next sample is introduced onto the measuring window of the TrayCell 2.0, the window and the mirror inside the cap need to be cleaned. There is no need to remove the TrayCell 2.0 from the cell holder during cleaning of the window. The precisely defined spacing between the window and the mirror inside the cap ensures that the optical pathlength is accurate and remains constant for every measurement.

In this study, the Cary 60 UV-Vis spectrophotometer fitted with a TrayCell 2.0 Ultra-Microvolume Cell was used to measure ultra-microvolume samples of protein and DNA. Different pathlength cap options for the TrayCell 2.0 were used to reduce dilution and extend the measurable concentration range of the sample. The photometric performance of the Cary 60 UV-Vis spectrophotometer with the TrayCell 2.0 was demonstrated using Bovine Serum Albumin (BSA) and herring sperm DNA samples.

Instrumentation and materials

- Agilent Cary 60 UV-Vis spectrophotometer
- TrayCell 2.0 Ultra-Microvolume Cell
- BSA protein: to prepare a 400 mg/mL stock solution, a known amount of BSA protein (Sigma-Aldrich, CAS 9048-46-8) was dissolved in PBS buffer (phosphate buffered saline). The stock solution was serially diluted to prepare a set of samples at different concentrations.
- Herring sperm DNA: to prepare 5 mg/mL stock solution, a known amount of herring sperm DNA (Sigma-Aldrich, CAS 438545-06-3) was dissolved in PBS buffer. The stock solution was serially diluted to prepare a set of samples at different concentrations.

An ultra-microvolume (3 µL) of sample was placed on the measuring window using a pipette, and data acquisition was carried out using the Agilent Cary WinUV software, version 5.1.3.1042.

Results and discussion

Photometric reproducibility at low concentrations

To assess the usable absorbance range of the Cary 60 UV-Vis spectrophotometer with the TrayCell 2.0, both high- and low-concentration samples were measured. For low concentration measurements of limited volume samples, the TrayCell 2.0 is ideal, as it requires only ultra-microvolume aliquots of sample. Also, the Cary 60 UV-Vis is sensitive enough to measure low concentrations, whereas many spectrophotometers do not have the sensitivity to measure concentrations less than 25 ng/µL. The performance capabilities of the spectrophotometer are vital at these low concentrations, so wavelength scans need to be performed to ensure that a typical sample curve is observed. Ten repeated wavelength scans of a 20 ng/µL herring sperm DNA sample were collected using the Cary 60 UV-Vis with the TrayCell 2.0 fitted with a 1.0 mm cap. Absorbance for this sample is 0.04, which is equivalent to 0.4 Abs in a standard 10 mm cuvette. As illustrated in Figure 5, the Cary 60 UV-Vis produced highly reproducible wavelength scans, which is important for ultra-microvolume analysis.

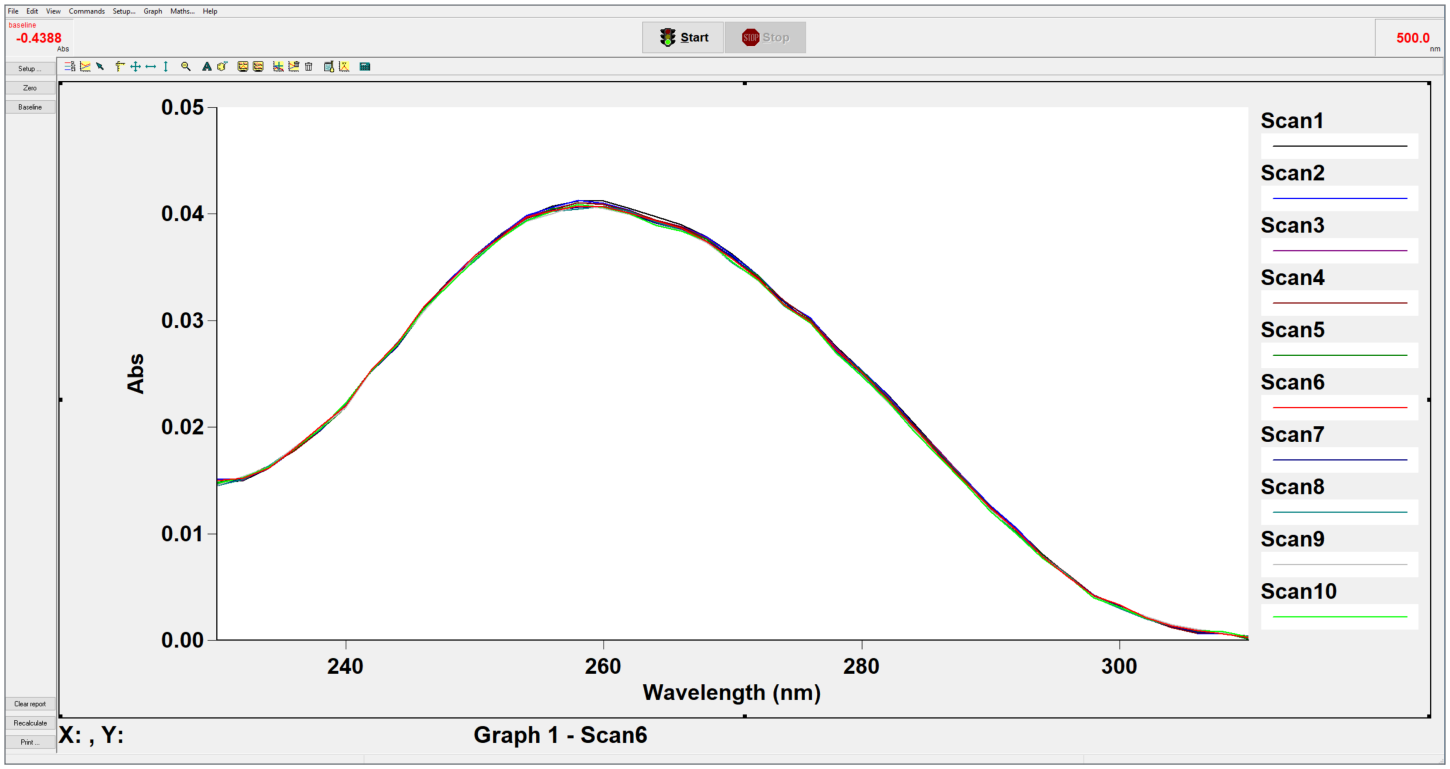

Figure 5. Ten repeat wavelength scans of herring sperm DNA (20 ng/µL, 0.04 Abs at 260 nm) acquired using the Agilent Cary 60 UV-Vis spectrophotometer with the TrayCell 2.0 Ultra-Microvolume Cell fitted with a 1.0 mm pathlength cap.

Photometric linearity

The quantitative and qualitative measurements of herring sperm DNA and BSA protein were acquired using the Cary 60 UV-Vis fitted with a TrayCell 2.0. The 1.0 mm pathlength cap is designed for the measurement of sample volumes in the range of 3 to 5 µL, which is ideal for routine working concentrations of DNA, RNA, and proteins.

Photometric linearity measurements for herring sperm DNA

Single wavelength absorption measurements at 260 nm were taken for the herring sperm DNA samples in the concentration range of 5 to 1,500 ng/µL. As shown in Figure 6, excellent photometric linearity up to 2.4 Abs was obtained using the TrayCell 2.0 with 1.0 mm pathlength cap. At 2.4 Abs, the corresponding concentration of the herring sperm DNA was 1,500 ng/µL. Excellent photometric linearity was observed for extremely low concentrations of the DNA sample, as low as 5 ng/µL (0.008 Abs, equivalent Abs 0.08), with 1.0 mm pathlength cap (Figure 6, inset).

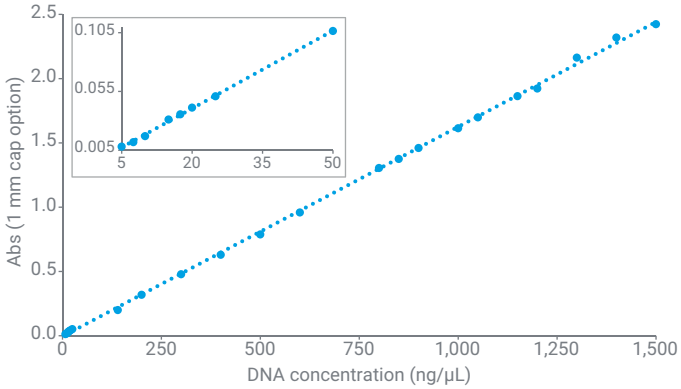

Figure 6. Photometric linearity for herring sperm DNA samples up to 1,500 ng/µL. The inset shows the results for low concentration samples in between 5 to 50 ng/µL.

Photometric linearity measurements for BSA protein

Single wavelength absorption measurements for BSA protein were collected at 280 nm (1 to 400 mg/mL concentration range) using the Cary 60 UV-Vis spectrophotometer. Like for the herring sperm DNA sample, excellent photometric linearity up to 2.4 Abs was obtained using the 1.0 mm pathlength cap (Figure 7A). At 2.4 Abs, the corresponding concentration of the BSA protein was 40 mg/mL. However, highly concentrated BSA protein samples were accurately analyzed by simply swapping the TrayCell 2.0 cap with shorter pathlength options. BSA protein concentrations up to 200 mg/mL were analyzed using the 0.2 mm pathlength cap (Figure 7B). The corresponding absorbance for the 200 mg/mL BSA protein was 2.3, which is equivalent to 115 Abs for a standard 10 mm cuvette. The 0.1 mm pathlength cap, which is the shortest pathlength cap available within the TrayCell 2.0, was used to measure concentrations up to 400 mg/mL (Figure 7C).

These results demonstrate that direct measurement of BSA protein samples up to 400 mg/mL can be easily performed using the Cary 60 UV-Vis with TrayCell 2.0, without time-consuming and error-prone dilutions (Table 1).

Table 1. Comparison of equivalent absorbance for BSA protein using 1.0, 0.2, and 0.1 mm pathlengths.

	TrayCell 2.0 Ultra-Microvolume Cell		
Pathlength (mm)	1.0	0.2	0.1
Highest Concentration Measured (mg/mL)	40	200	400
Measured Absorbance	2.4	2.3	2.0
Equivalent Abs to 10 mm Standard Cuvette	24	115	200

Note: Equivalent Abs is the absorbance value calculated for a 10 mm pathlength. The benefit of being able to measure up to 400 mg/mL is that highly concentrated protein solutions can be measured directly, eliminating dilutions.

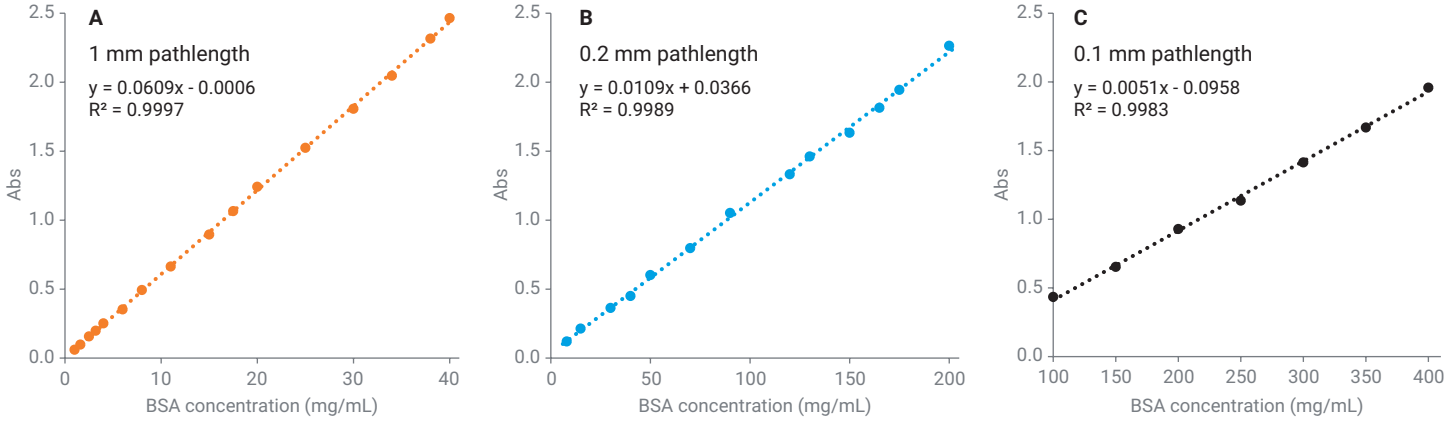

Figure 7. Photometric linearity measurements for BSA concentration up to 40 mg/mL using the 1.0 mm pathlength cap (A), up to 200 mg/mL using the 0.2 mm pathlength cap (B) and up to 400 mg/mL using the 0.1 mm pathlength cap (C).

The Cary WinUV Concentration module for quick and easy data acquisition and analysis

The Cary WinUV software includes powerful features and streamlined methods for data collection, analysis, storage, and display, while reducing complexity. It also includes different modules that are designed to cover a range of applications including qualitative wavelength scan or reads, concentration analysis, enzyme kinetics, and many more.

In this study, full wavelength scans were performed using the **Scan** module, while photometric linearity measurements were performed as single wavelength absorbance measurements using the **Concentration** module. To demonstrate the instrument setup and related results-report generated by the **Concentration** module, the photometric linearity measurements were carried out for BSA protein samples using a 0.1 mm pathlength cap. (The same results are shown in Figure 7C). Setting up the Cary WinUV software is quick and easy, requiring a few simple steps as follows and illustrated in Figure 8:

1) Open the **Concentration** module and click the **Setup** tab to open the instrument setup window.
2) In the **Cary** tab, enter the wavelength for single wavelength measurements in **Wavelength** option (for BSA proteins, enter 280 nm).
3) Enter the number of replicates that are needed for each standard using **Replicates** or **Sample/Std Averaging**. In this example, data were collected for each BSA standard solution as the average of three consecutive measurements.
4) In the **Standards** section, enter concentrations of standard samples in ascending order and select the **Fit type**. In this example **Fit type** was selected as **Linear** with minimum R^2 (**Min R^2**) of 0.9500.
5) Both the software and the instrument are ready for analysis. Click the **Start** button to start the analysis and simply follow the standard/sample loading instructions to continue the analysis.

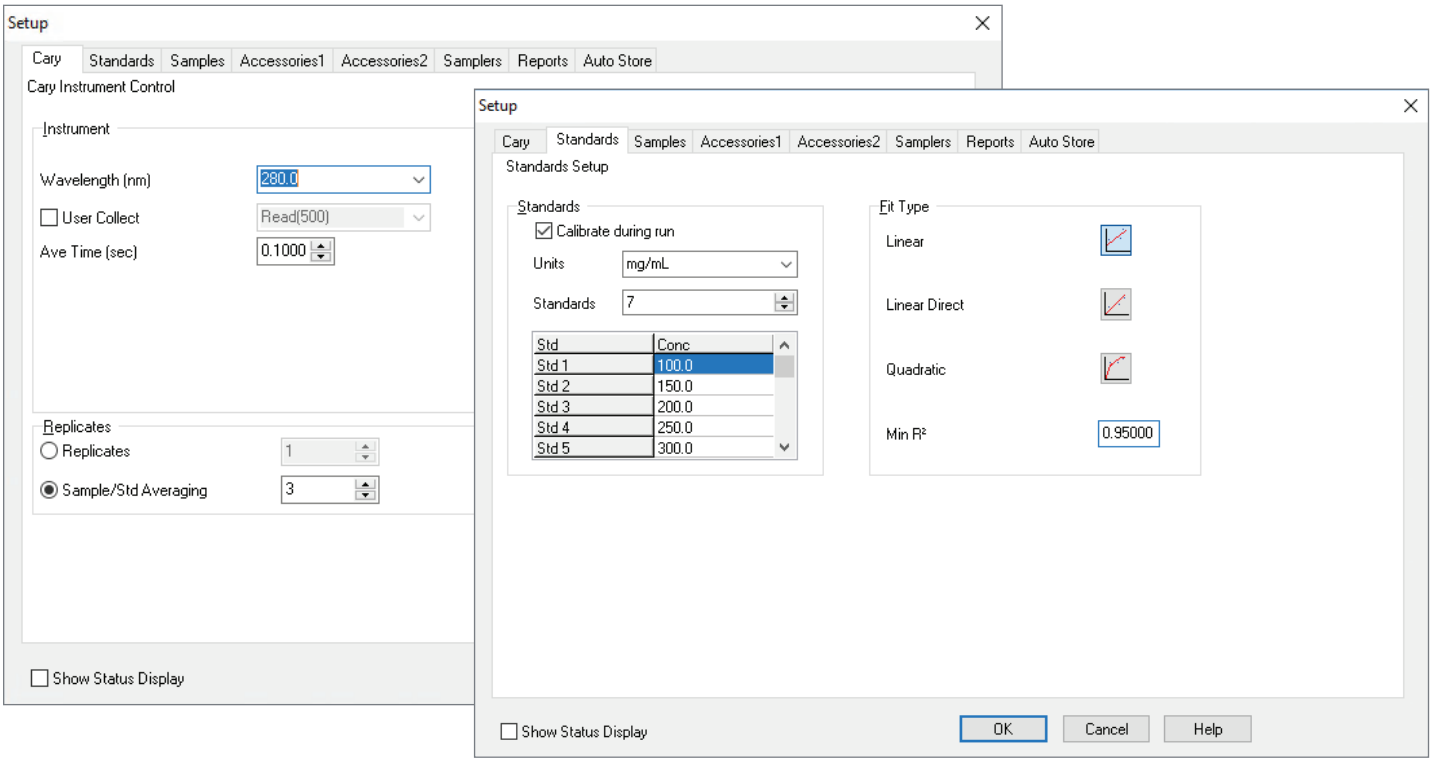

Figure 8. Instrument setup for data acquisition and analysis using the **Concentration** module in the Agilent Cary WinUV software.

Following the data acquisition, the corresponding concentration versus absorbance graph (calibration curve) is automatically generated by the software (Figure 9), reducing time-consuming data workup procedures. The calibration curve is saved in the **Concentration** module. When analyzing an unknown sample, the software automatically uses the calibration curve to calculate and report the sample concentration.

Cleaning of TrayCell 2.0 Ultra-Microvolume Cell

Figure 10 outlines the simple steps needed to clean and load a sample in the TrayCell 2.0. The sample window and cap are wiped clean with a lint-free swab or lint-fee lab cloth and the next sample is loaded with a pipette. These procedures eliminate time-consuming cleaning steps commonly associated with conventional cuvettes and reduce the risk of sample carryover.

Fully flexible solution

A standard 10 mm pathlength cuvette is recommended for extremely dilute samples, which cannot be measured using different cap options available with the TrayCell 2.0. Standard 10 mm pathlength cuvettes are available in a range of volumes, from 40 µL to 3.0 mL.

To perform a wider range of measurements, the Cary 60 UV-Vis spectrophotometer can be fitted with long pathlength cells, automated multicell changers, as well as temperature-controlled cuvette holders. The range of measurements include quantitation, sample automation, or analyzing kinetic processes. Components are easily interchangeable, making the Cary 60 UV-Vis spectrophotometer a widely used instrument for routine biological UV-Vis measurements.

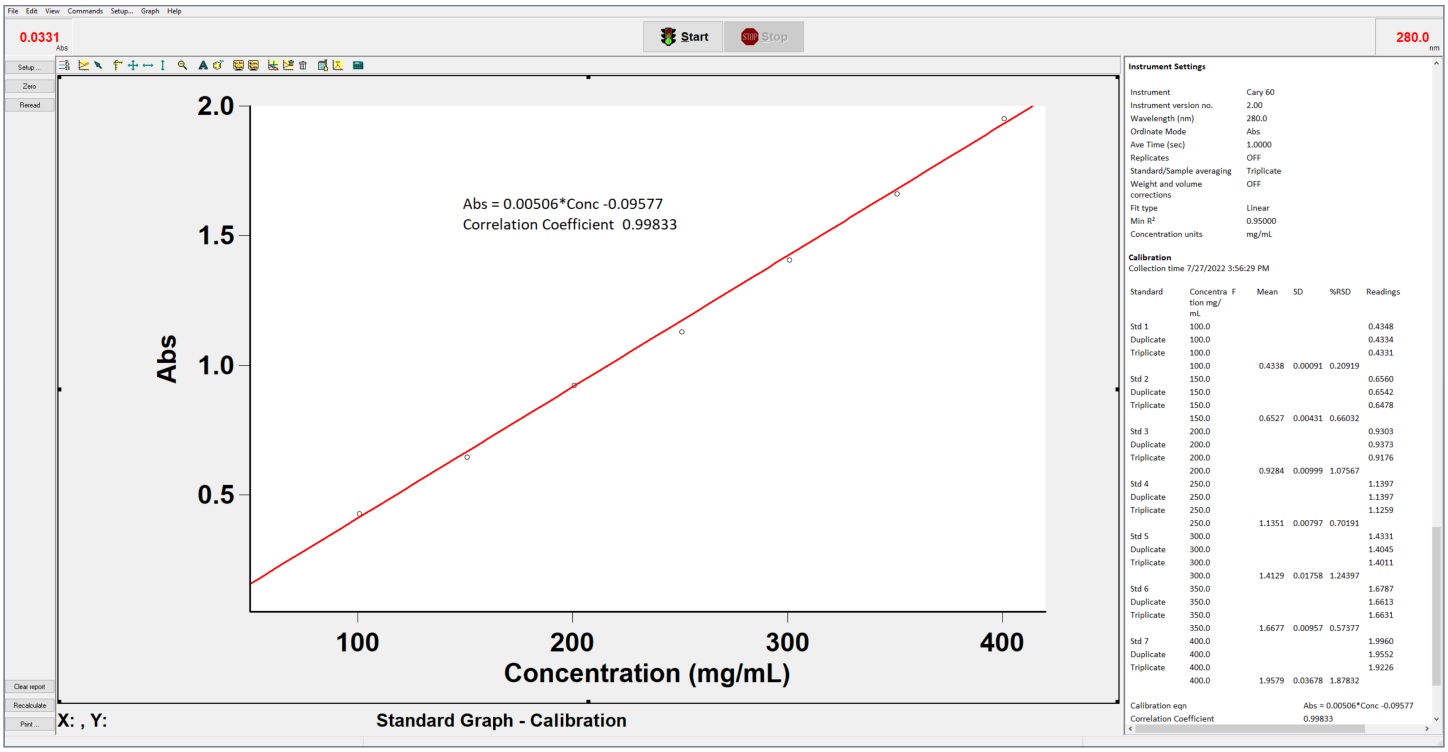

Figure 9. Calibration curve and the concentration analysis report generated automatically by the Agilent Cary WinUV software for the BSA protein using the TrayCell 2.0 with 0.1 mm pathlength cap. The same graph is shown in Figure 7C.

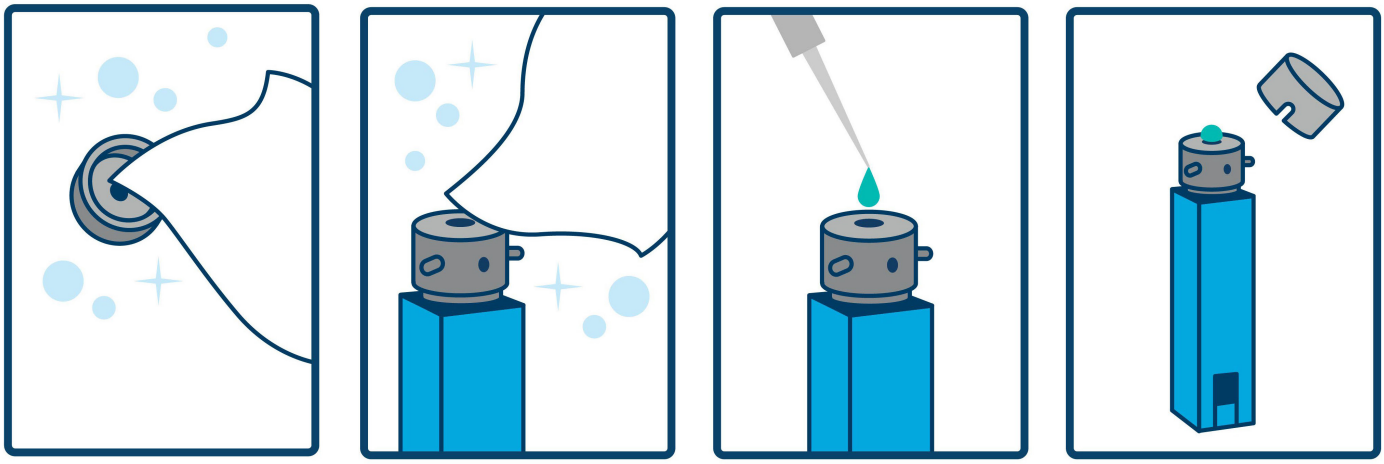

Figure 10. Schematic of cleaning and loading a sample in the TrayCell 2.0.

Conclusion

The Agilent Cary 60 UV-Vis spectrophotometer fitted with the TrayCell 2.0 Ultra-Microvolume Cell provides a convenient and easy-to-use platform for measuring ultra-microvolume samples such as nucleic acids and proteins. Repeated qualitative wavelength scans of a 20 ng/µL herring sperm DNA sample demonstrated the sensitivity and reproducibility of the Cary 60 UV-Vis for the analysis of ultra-microvolume samples. Using four different caps for the TrayCell 2.0 (and therefore pathlengths), extended the analytical range of the method, alleviating the need to perform tedious and error-prone dilutions. A linear photometric range was observed for herring sperm DNA and BSA protein samples in the range from 5 to 1,500 ng/µL and 1 to 400 mg/mL, respectively. The streamlined workflow, versatility, and photometric performance of the Cary 60 UV-Vis spectrophotometer with the TrayCell 2.0 make it suitable for accurate measurements of undiluted nucleic acids and proteins.

Further information

- Agilent Cary 60 UV-Vis spectrophotometer
- Agilent Cary 3500 UV-Vis spectrophotometer
- UV-Vis Spectroscopy Learning Tools
- UV-Vis & UV-Vis-NIR Instrument Selection Guide
- UV-Vis Spectrophotometer Applications Overview
- UV-Vis Spectroscopy FAQs

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