Application Note

Biological QC testing

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Benefits of the Cary 3500 Multicell UV-Vis for Protein Analysis

Productivity and reproducibility improvements for qualitative and quantitative measurements of very low volumes

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Introduction

Measurements made by a UV-Vis spectrophotometer provide a fast and reliable quality control check of a sample. They can also be used to calculate specific activity or estimate yield after purification, and to identify fractions containing protein or amino acids.

Proteins containing amino acids with aromatic side chains absorb light at approximately 280 nm. This fact enables quantitative analysis by a UV-Vis spectrophotometer. Following Beer-Lambert's law (*1*), the concentration of proteins containing these amino acids can be determined when the absorption coefficient is known. Often a protein sample is limited, and being able to do these measurements at a minimal volume is important for sample preservation. Performing a wavelength scan can provide information about potential contaminants.

The Cary 3500 Multicell UV-Vis has an integrated, permanently aligned multicell holder, idea for reproducible and reliable qualitative, and quantitative measurements with ultra-microvolume cuvettes (Figure 1).

Figure 1. The Cary 3500 UV-Vis has a highly collimated beam, which is less than 1.5 mm wide. The small beam size makes it ideal for use with small aperture cuvettes, such as the one shown here.

This study demonstrates the benefits of the Cary 3500 Multicell UV-Vis spectrophotometer for qualitative and quantitative low volume protein analysis. Bovine serum albumin (BSA) is a commonly used protein standard with a known absorption coefficient and was used for this analysis.

Experimental

Samples

A 0.01 M phosphate buffer solution (PBS) was prepared at pH 7.0. A 10 mg/mL stock BSA solution was prepared and then diluted to 0.75, 1.50, 2.25, 3.00, and 3.75 mg/mL.

Six 70 µL ultra-microvolume cuvettes with an aperture measuring 2 x 2.5 mm, and a 10 mm optical pathlength were used for measurements (Figure 1). One was used as a reference, and five were used as samples. A standard, 3.5 mL 10 mm pathlength quartz cuvette was used for reproducibility measurements. PBS buffer was used as the reference solution.

Instrumentation and method

A Cary 3500 Multicell UV-Vis spectrophotometer was used for all measurements. A wavelength scan was performed from 250 to 350 nm with 1 second signal averaging time and 1 nm data interval. No pre-alignment of the system was necessary.

To demonstrate reproducibility, 20 repeated measurements of the 3.0 mg/mL BSA solution were performed, using an ultra-microvolume cuvette (70 µL), with an identical ultra-microvolume cuvette containing PBS buffer in the reference position. Another set of 10 repeated measurements of the same solution, using a standard 3.5 mL, 10 mm optical pathlength, quartz cuvette were also done. In this case, an identical cuvette containing PBS buffer was used as the reference. These measurements were all performed at 278 nm with signal averaging time of 1 second and spectral bandwidth of 2 nm.

Results

Simultaneous measurements

The blank and five BSA samples were measured simultaneously in the Cary 3500 Multicell UV-Vis. Ultra-microvolume cuvettes (2 x 2.5 mm aperture with a 10 mm pathlength) were used. A wavelength scan was performed from 250 to 350 nm to allow for qualitative sample analysis (Figure 2). This data was then used to assess the linearity of the instrument (see the Linearity in microcells section, following).

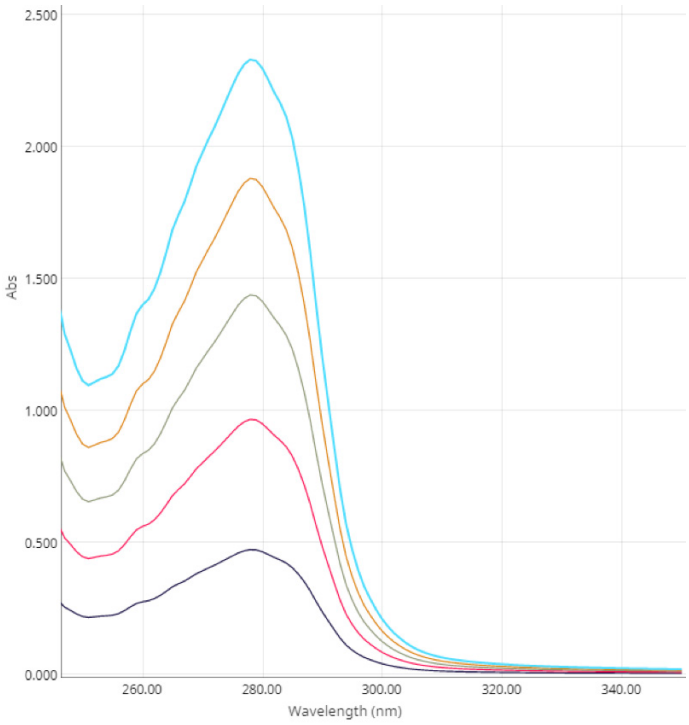

Figure 2. Simultaneously collected wavelength scans of five BSA samples in 70 µL ultra-microvolume cuvettes.

Protein purity

The absorbance intensity at 350 nm can be used to quantify and correct for the presence of any contaminant in a protein sample. As can be seen on Figure 2, the absorbance at 350 nm in the samples examined here is 0 Abs. Thus, the concentration of BSA in these samples can be accurately determined as directly proportional to the intensity of the peak maximum on the spectrum. No impurity correction is required.

Linearity in microcells

The peak maximum evident on each BSA wavelength scan is located at 278 nm (Figure 2). The absorbance intensity at 278 nm peak was extracted for each of the samples, using Cary UV workstation software, and plotted against the protein concentration in each of the samples measured (Figure 3). These data clearly demonstrate the linearity of the Cary 3500 system (Figure 3). The data show that the linear range extends to nearly 2.5 Abs—even when used with the small aperture ultra-microvolume cuvettes.

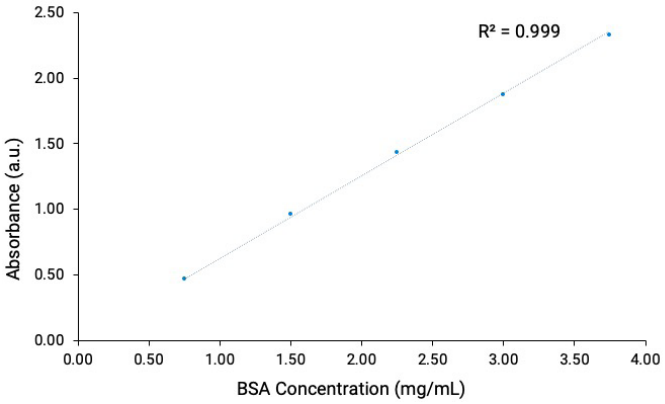

Figure 3. Linearity of the Cary 3500 Multicell UV-Vis demonstrated by plotting the absorbance at 278 nm and concentration of five BSA samples.

Reproducibility

To demonstrate reproducibility, 20 repeated measurements of the 3.0 mg/mL BSA sample were performed in either a 70 µL, 2 x 2.5 mm aperture ultra-microvolume cuvette, or a 3.5 mL standard cuvette. Both cuvettes had a 10 mm optical pathlength. The reproducibility (standard deviation) of the measurements was not impacted by the reduced size of the aperture window of the ultra-microvolume cuvette, as shown in Figure 4. The standard deviation of the repeated measurements is 0.00042 for the 70 µL ultra-microvolume cuvette and 0.00029 for the standard 3.5 mL cuvette. The average of the measurements using the ultra-microvolume cuvette was 1.863 Abs. The average of the measurements using the standard 3.5 mL cuvette was 1.858 Abs. The difference between the two was within the measurement uncertainty of the instrument.

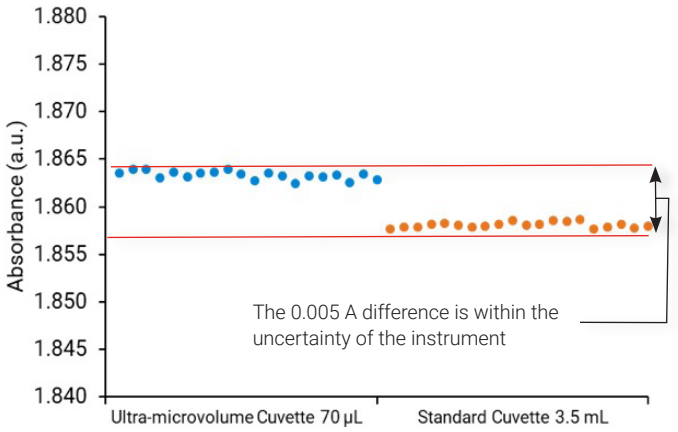

Figure 4. The absorbance of 20 repeated measurements for a 70 µL ultra-microvolume cuvette, compared to a standard 3.5 mL cuvette.

A risk of using low volume cuvettes is reduced light throughput, due to a smaller aperture cuvette window, that is commonly part of the cuvette design. Reduced light throughput contributes to both decreased linear absorbance range and decreased measurement reproducibility. The Cary 3500 has a highly focused beam that ensures maximum light through the sample and reproducibility as good as a standard 3.5 mL cuvette (Figure 4).

Discussion

Quantitative measurements can be made via the intensity of the absorbance maxima on a UV-Vis spectrum if the absorption coefficient is known. For samples such as the BSA measured here, this absorption coefficient is known. Where the absorption coefficient is not known, it must be calculated from the slope of a calibration curve, created by measuring multiple standards. This can be a time-consuming process and one that can lead to measurement errors that might not be identified until the end of the experiment.

Analysts may choose to improve confidence in their data by including samples with known concentrations within an analytical sequence. These samples act as internal controls. The concern with this type of measurement is that samples and standards are not measured at the same time, so variables may have changed between measurements. By measuring samples and calibration standards at a single point in time as demonstrated in this note (Figure 2), other

environmental variables, or bias that may influence sample preparation can be greatly reduced.

When measuring samples that may be unstable, the one-at-a-time method potentially introduces errors. As the samples sit on the bench, waiting their turn for measurement, their absorbance may shift. This shift may be due to temperature changes, the effect of light hitting the solution or due to chemical changes in the solution. This may introduce systemic errors for the quantification measurement. By measuring all cuvette positions simultaneously, the Cary 3500 Multicell UV-Vis spectrophotometer removes these unwanted variables and increases confidence in the results that are generated. It also saves significant measurement time.

Conclusions

The Cary 3500 UV-Vis Multicell spectrophotometer creates new possibilities for quantitative and qualitative protein analysis. The system is designed for productivity and reproducibility, with its unique integrated nonmoving multicell holder. By measuring all eight cuvette positions simultaneously, it effectively reduces any unwanted variables that may arise during subsequent measurements. Together these features provide a powerful analytical UV-Vis system.

References

1. Practical Handbook of Biochemistry and Molecular Biology, Fasman, D.G., Ed. (1992). CRC Press, Boston.

www.agilent.com/chem/cary3500uv-vis

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