Application Note

Keywords

- DNA
- RNA
- Protein
- Biomolecules
- Life sciences

Techniques

- UV absorbance spectroscopy

Applications

- DNA quantification
- RNA quantification
- Protein quantification
- DNA purity

Measuring DNA Absorbance with the STS-UV Microspectrometer

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Biological molecules are routinely measured using UV absorbance to obtain critical information on concentration and sample purity. With a wavelength range of 190 to 650 nm and approximately 1.5 nm optical resolution, the STS-UV microspectrometer is ideal for these measurements. The STS-UV packs tremendous performance into a compact, powerful microspectrometer to provide high quality UV absorbance data over a wide range of sample concentrations. In this application note, we demonstrate this incredible performance through absorbance measurements of DNA samples ranging from 0.15 to 150 µg/mL.

Introduction

DNA measurements typically involve samples at the extremes of the concentration range with DNA suspended in solution at either very high or very low concentrations. While dilution can be done with concentrated DNA solutions, low concentration samples present more of a challenge. Without the benefit of an adjustable pathlength spectrometer or small volume sampler, the cuvette pathlength itself must be adjusted to enable measurement of a wide range of concentrations. The high dynamic range and signal to noise ratio of the STS-UV enable DNA measurements over a wide range of concentrations without the need for changing pathlength to accommodate absorbance levels ranging from 0.005 to over 1.5 absorbance units (AU).

Experiment Details

Various concentrations of Salmon DNA (Sigma D-1626) were prepared in deionized water. DNA absorbance was measured in a 1 cm path-length cuvette with an STS-UV and the deuterium lamp of a

DH-2000-BAL balanced, deuterium and tungsten halogen light source. Measurements were made with both the deuterium and tungsten halogen lamps and with just the deuterium lamp to show the impact of out of band light on the maximum absorbance level achieved with the spectrometer.

Results

The absorbance spectra and absorbance at 260 nm (OD260) measured with STS-UV for low concentrations of DNA (0.15 to 2.5 µg/mL DNA) are shown in Figures 1 and 2. Replicate measurements at these concentrations showed variability of less than 0.0004 AU (error bars fall within the data marker). The linearity of the low concentration data is demonstrated by a correlation coefficient value of 0.9999 for a linear least squares fit of these data points. As shown in Figure 1, STS-UV provides repeatable, high quality DNA absorbance data even with absorbance values as low as 0.005 AU.

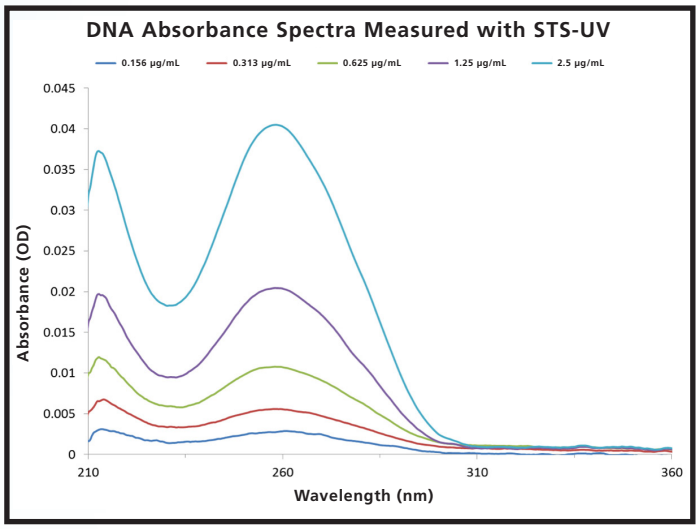

Figure 1: Low concentration (0.15 to 2.5 µg/mL) DNA solution absorbance measured with STS-UV

The linearity and wide dynamic range of the STS-UV are further demonstrated in Figure 3 for DNA concentrations ranging from 0.15 to 110 µg/mL. The linearity of the data over this wide concentration and absorbance range is illustrated by an R2 value of 0.9996. There is a slight flattening or roll-off in absorbance at 110 ug/mL suggesting that absorbance is linear to somewhere between 100 and 110 µg/mL at approximately 1.6 AU.

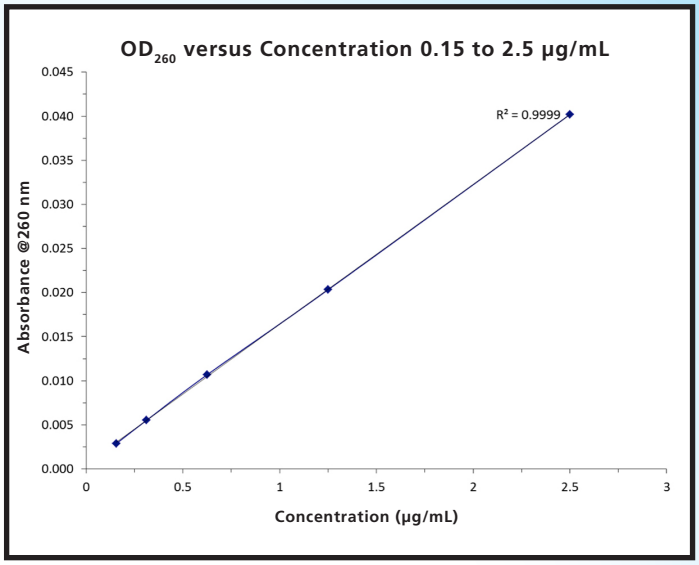

Figure 2: OD260 versus DNA concentration for concentrations ranging from 0.15 to 2.5 µg/mL DNA in water

The maximum absorbance measured with STS-UV is shown in Figure 4. Absorbance values as high as approximately 2.1 AU were measured. As shown by the data points in red, the OD260 of DNA with concentration is very linear to approximately 1.6 AU between 100 and 110 µg/mL DNA. At the higher absorbance value measured for DNA concentrations above 100 µg/mL, the flattening or roll-off in the graph suggests that the measurements are near the stray light limit for these measurements.

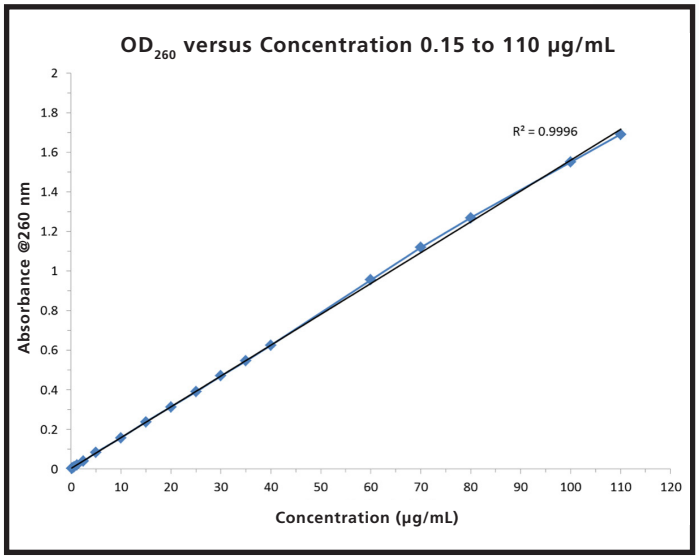

Figure 3: OD260 versus DNA concentration for concentrations ranging from 0.15 to 110 µg/mL DNA in water

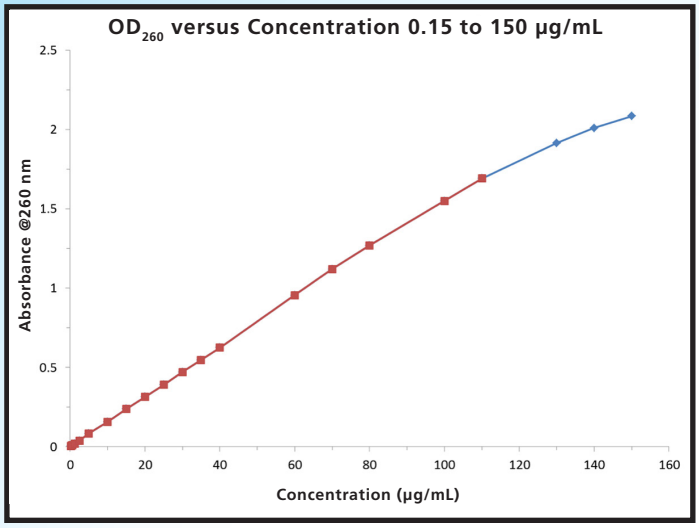

Figure 4: OD260 versus DNA concentration for concentrations ranging from 0.15 to 150 µg/mL DNA in water

It is important to note that with STS-UV, this entire concentration range was measured using a 1 cm pathlength cuvette. No pathlength adjustment was required to measure DNA absorbance at 260 nm ranging from 0.005 to 2.1 AU.

Conclusion

With a dynamic range of 4600:1 and signal to noise ratio of more than 1500:1, the STS-UV packs tremendous performance into a compact, powerful microspectrometer. Ideal for DNA, RNA and protein determination, STS-UV covers the entire range from 190 to 650 nm with approximately 1.5 nm optical resolution. As shown for these DNA samples ranging from 0.15 to 150 µg/mL, STS-UV enables measurements over a wide absorbance range from 0.005 to 2.1 AU. STS-UV simplifies UV absorbance measurements by enabling measurements over a wide concentration and absorbance range without the need for adjustable sampling accessories.

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