

Compact Spectrometer for UV Absorbance Measurements

Application Note



KEYWORDS

- Proteins
- UV response
- DNA

TECHNIQUES

- Absorbance

APPLICATIONS

- Pharmaceuticals analysis
- Protein concentration measurement
- DNA analysis

The SR6 is a compact spectrometer with excellent UV response. Its high signal to noise ratio (SNR) performance and excellent thermal stability make SR6 a desirable choice for applications including pharmaceuticals analysis, protein concentration measurements and UV plasma monitoring. In this application note, we evaluate the SR6 as an option for UV absorbance measurements.

The SR6 is a multipurpose spectrometer, suitable as a setup for the lab or as a customized system integrated into high-volume industrial and OEM applications. The spectrometer is available in models covering wavelength ranges within ~185-1100 nm, and connects to light sources, optical fibers and sampling optics to optimize spectral setups for various applications.

One of the distinguishing features of the SR6 is its response in the UV region, especially the range from 200-280 nm. This UV response is primarily a function of the detector and can best be taken advantage of by pairing it with optical bench components – gratings and the like – designed for the UV. Adding a UV light source and sampling optics that transmit UV light provides the optimum configuration for the application.

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To observe the UV response and absorbance linearity of the SR6 spectrometer, we measured the absorbance of various concentration levels of DNA samples in water and of NIST-traceable potassium dichromate standards.

UV Absorbance of DNA Samples

For the DNA measurements, our samples were deoxyribonucleic acid (free acid crude oligonucleotides) measured in a 1 cm pathlength quartz cuvette. Samples were diluted by removing 0.5 mL per sample, adding 0.5 mL natural spring water and then mixing the contents with a disposable pipette. The cuvette was left undisturbed during the dilution process to prevent errors from sample contamination or improper cuvette fitment.

Our setup comprised the SR6 spectrometer, a deuterium-tungsten halogen lamp (using only the deuterium bulb for UV illumination), a cuvette holder with 1 cm quartz cuvette, and solarization-resistant optical fibers. The spectrometer and light source were warmed up for 30 minutes prior to use, and a fiber optic variable attenuator was used, as needed, to control the light from the deuterium source to the spectrometer.

We measured nearly 30 concentration levels of oligo DNA from 0.5-70 µg/mL, with the resulting spectra demonstrating strong signal quality in the UV and consistency across spectra (Figure 1).

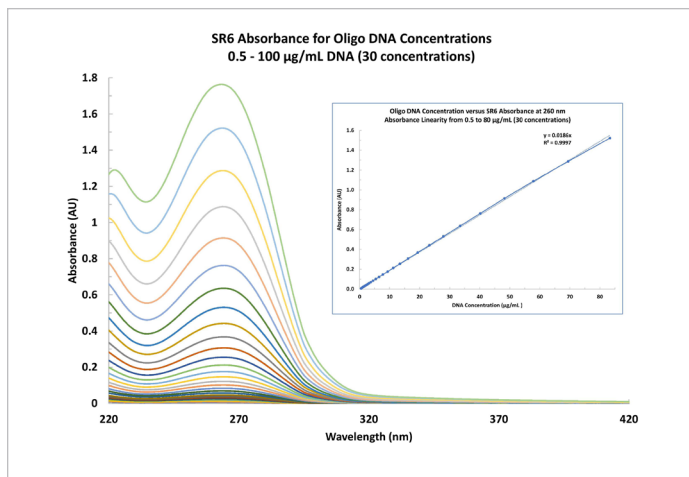


Figure 1. With excellent response in the UV, the SR6 is a versatile spectrometer for measurement of biological macromolecules such as proteins and DNA.

UV Absorbance Linearity of Potassium Dichromate

To evaluate the absorbance linearity of the SR6, we measured the absorbance of potassium dichromate solutions at 257 nm, using a 265 nm LED for illumination. Various concentrations of the standard were measured with high absorbance linearity (Figure 2).

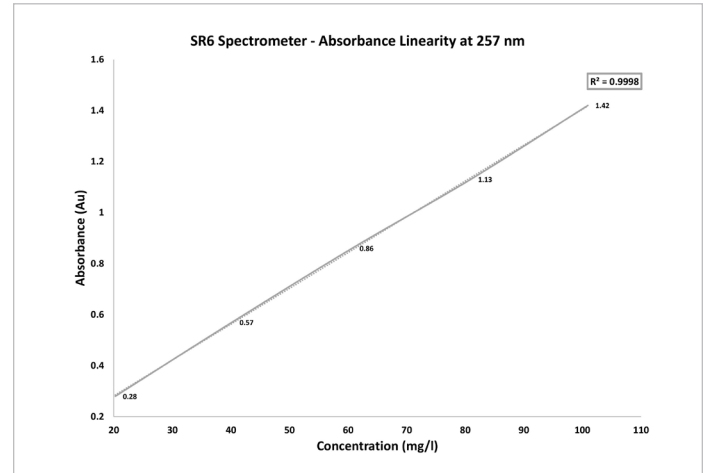


Figure 2. The SR6 demonstrates strong absorbance linearity in the UV, with additional measurements revealing maximum linear absorbance levels of approximately 2 AU.

With a high absorbance linearity spectrometer, users can measure a wide range of sample concentration levels accurately and with minimal sample preparation between measurements. This can be critical for applications where very precise measurement accuracy is desired or where you have a limited amount of sample to work with.

Signal to Noise Ratio Enhancement in the SR6

SR6 spectrometers operate with OceanView spectroscopy software and include OceanDirect, a powerful, cross-platform Software Developers Kit with an Application Programming Interface.

OceanDirect allows users to write custom software solutions for their spectrometer, to optimize spectrometer performance and to access critical data for analysis. OceanDirect also enables High Speed Averaging Mode (HSAM), a hardware-accelerated signal averaging tool that markedly enhances spectrometer SNR per unit time by eliminating the readout time for each spectra.

Typically, to improve SNR users turn to signal averaging, which is achieved by averaging spectra in software on the host computer. Compared with the HSAM hardware-accelerated averaging, this requires more time to compute the equivalent number of averages. As a result, in a given period of time the SR6 will perform significantly more spectral averages and yield a far superior SNR per unit time – as high as 3500:1. This is important for real-time applications, where decisions must be made very quickly and with high accuracy.

Additional Functionality with the SR6

SR6 has trigger mode options that enable actions such as synchronizing spectral acquisition to an external event (e.g., pulsing of a lamp) or timing spectral acquisition to meet certain sampling conditions. Triggering provides accurate timing and synchronization between the SR6 spectrometer and other devices, adding another layer of versatility to your spectral measurements.

