Introduction

The Cary 3500 is a double beam spectrophotometer that simultaneously measures up to four independent temperature experiments across eight cuvette positions. This is made possible with fiber optics and xenon flash lamp light source eliminating warm up times.

The instrument allows for high throughput and low sample volumes, perfect for valuable biological samples where quantity is a concern. Equipped with the Cary Workstation software, methods for wavelength reads, scanning, concentration, kinetics, and temperature measurements are streamlined and effortless.

Features of the Agilent Cary3500 UV-Vis Spectrophotometer

- Control temperature accurately and quickly from 0 °C to 110 °C with an air-cooled Peltier system consisting of 4 independent zones across 8 cuvette positions. Option for non temperature control also available.
- Control UV-Vis thermal ramp measurements with the aid of solid state digital Cary temperature probes, with optional monitoring from within the cuvette.
- Analytical accuracy for small-volume measurements with a highly-collimated and uniform beam of < 1.5 mm width, coupled with permanent optical alignment of the stationary cell

Light Source	Xenon flash lamp 250 Hz
Scanning Speed	150,000 nm/min
Thermal Ramp Rate	0.1 - 40 °C/min
Spectral Bandwidth	0.1 - 5.0 nm
Wavelength Range	190 - 1100 nm
Photometric Range	4 Abs
Min Sample Volume	50 µL

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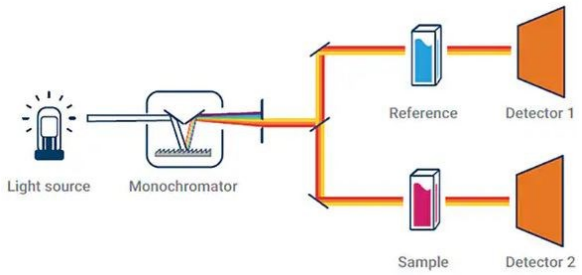
Agilent Cary 3500 UV-Vis Spectrophotometer

- Perform UV-Vis wavelength reads, absorbance concentration, kinetics, and temperature ramp experiments.
- High sample throughput (8 cuvettes) and low samples volume (50 uL) capability.
- Simultaneously collect data across 8 cuvette positions in a single experiment.
- Integrated air-cooled Peltier system can customize 4 independent temperature controlled zones over 8 cuvette positions simultaneously.

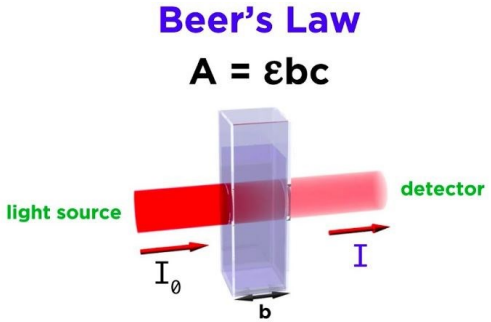
Agilent Cary 3500 UV-Vis Spec - Thermal Melt Analysis of RNA Oligo

Theoretical Background

Spectroscopy allows the study of how matter interacts with or emits electromagnetic radiation. When radiation interacts with matter, several processes can occur, including reflection, scattering, absorbance, fluorescence/phosphorescence (absorption and re-emission), and photochemical reactions (absorbance and bond breaking).



When light passes through or is reflected from a sample, the amount of light absorbed is the difference between the incident radiation (I_O) and the transmitted radiation (I). The amount of light absorbed is expressed as absorbance (A). For most applications, absorbance values are used since the relationship between absorbance and both concentration (C) and path length (b) is linear at dilute concentrations. This relationship is known as Beer-Lambert Law ($A = \varepsilon bC$) where (ε) is the molar absorptivity and is a sample dependent constant.



Thermal Melt of RNA Oligo by UV-Vis Absorbance Measurements @ 260nm

Sample: RNA Oligo

Concentration: 75 µM

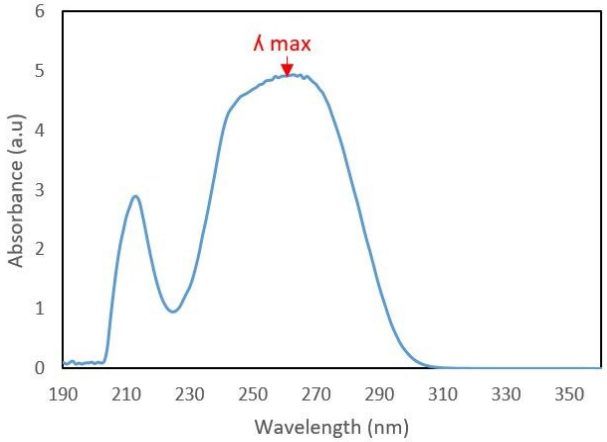
Buffer: 1M NaCl, 10mM Na_2HPO_4, 1mM Na-EDTA

Temperature Range: 10 °C - 95 °C

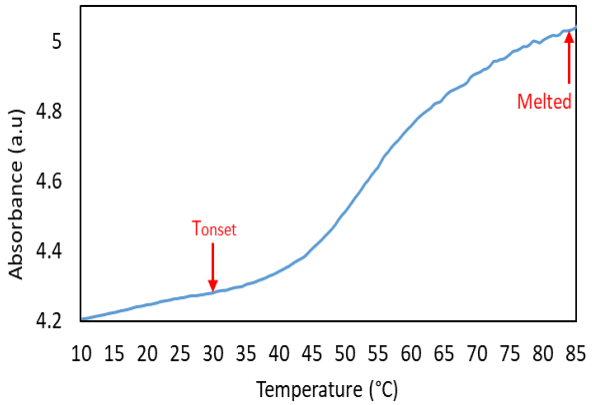
Temperature Ramp: 1 °C/min

Data Interval: 0.5 °C/data point

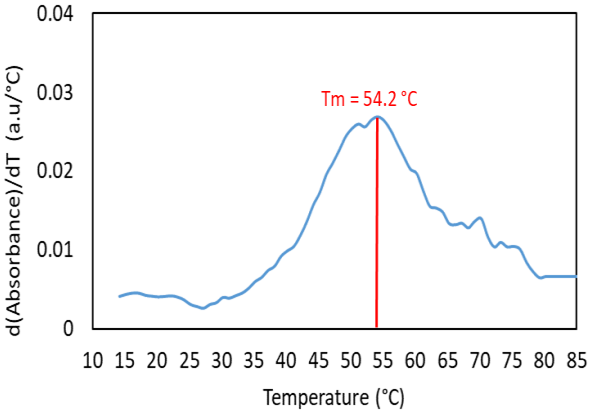
A 70 uL sample of 75 µM RNA duplex was loaded into a 1 cm path length cuvette along with a buffer blank for background correction. A wavelength scan to determine the wavelength of maximum absorption (260 nm) was performed over the range 190 nm - 360 nm and is displayed in the figure below.



A thermal melt experiment was programmed to run on the Cary3500 over the temperature range from 10 °C to 95 °C. The wavelength selected was 260 nm, the ramp rate was set to 1 °C/min, the data interval was set to 0.5 °C/min, and the bandwidth was set to 2 nm. The result is shown in the following figure.



The data shows that the sample begins to unfold at around 30 °C (T_{onset}) and is fully unfolded at around 80°C. To further investigate the thermal melting curve and obtain the melting temperature of the sample (T_m), the first order derivative of absorbance with respect to temperature can be calculated by the software. The first order derivative plot is displayed below.



By looking at the first order derivative plot, we are analyzing the rates of change in absorbance with respect to temperature. The temperature at which the greatest rate change in absorbance with respect to temperature occurs is the melting temperature. The melting temperature was determined to be 54.2 °C.