DNA/RNA Quantification Using 2mm Cuvette and a Tidas Spectrometer
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Abstract:

Concentrations of DNA in solution (31µg/mL and 561µg/mL) were measured with a spectrometer and UV/Vis light source in a cuvette. A 2mm pathlength cuvette does not require a pre-measurement dilution within this concentration range, thus a potential source of error was eliminated. Although a 2mm cuvette has a total internal volume of 0.7mL, only 350µL is required to obtain an accurate measurement.

Experimental Procedure:

Standard solutions of DNA (Sigma D1626) were prepared gravimetrically using 18.2MΩ/cm ultrapurified water as a solvent. Solutions were prepared between 0.0µg/mL and 561.1µg/mL.

Measurements were taken in triplicate using a 2mm cuvette (PN CUV2102-1) in a standard cuvette holder (PN 89340). An appropriate cuvette spacer (PN 89342) allowed for consistent placement of the cuvette within the holder. The cuvette holder was attached via two 500µm SMA-terminated fiber optic cables (PN FO-500SMA-1M) to a Tidas I spectrometer (PN TIDAS-I) and a UV/Vis light source (PN D4H).

Data were collected in 1nm increments across the full range of the instrument (190nm-720nm). The instrument was configured such that reference measurements yielded an 80% total intensity. All measurements utilized 18.2MΩ/cm ultrapurified water as a reference solution.

Results:

Experimental results are presented in Table 1:

DNA [µg/mL]	Absorbance @260nm [AU]
0.00	0.002 ± 0.001
30.83	0.101 ± 0.009
56.59	0.200 ± 0.004
85.49	0.311 ± 0.005
115.66	0.409 ± 0.003
143.94	0.497 ± 0.009
281.63	0.879 ± 0.010
428.91	1.243 ± 0.012
561.15	1.555 ± 0.003

Table 1: DNA Concentrations and Resultant Absorbance Values

Since absorbance with respect to concentration follows the Beer-Lambert Law,

$$A = \varepsilon l c$$

expected absorbance values were calculated from the DNA solution concentrations. Literature values of ε for dsDNA are listed as 0.020µg/ml*cm.

Experimental data can be found in Figure 1 with the calculated absorbance measurements indicated by a solid line. Absorbance measurements are expressed at a 260nm wavelength. Deviation from the theoretical value at higher absorbance values is a result of stray light interference within the spectrometer.

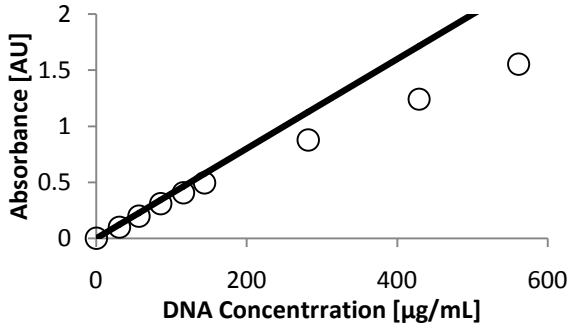

Figure 1: Measured versus Theoretical Absorbance

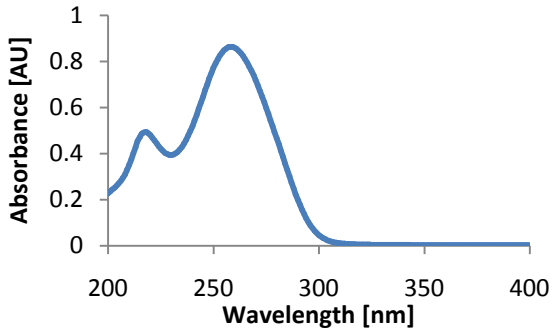

Figure 2: Typical DNA Measurement (281.6µg/mL)