

DNA/RNA Quantification Using DIPUV-Mini and a Tidas Spectrometer

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Author: Alexander Dickson

Abstract:

Concentrations of DNA in solution (31µg/mL and 688µg/mL) were measured with a spectrometer and UV/Vis light source in a DIPUV-Mini. Due to the 2mm pathlength, use of a DIPUV-Mini does not require a pre-measurement dilution within this concentration range, thus a potential source of error was eliminated.

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 687.6µg/mL.

Measurements were taken in triplicate using a DIPUV-Mini. The DIPUV-Mini was connected 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	-0.0017
30.83	0.1116
56.59	0.1945
85.49	0.3012
115.66	0.3821
143.94	0.4551
281.63	0.8399
428.91	1.2409
561.15	1.5639
687.61	1.7340

Table 1: DNA Concentrations and Resultant Absorbance Values

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

$$A = \epsilon lc$$

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.

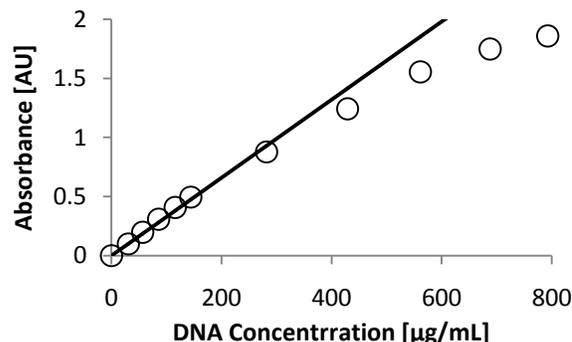


Figure 1: Measured versus Theoretical Absorbance

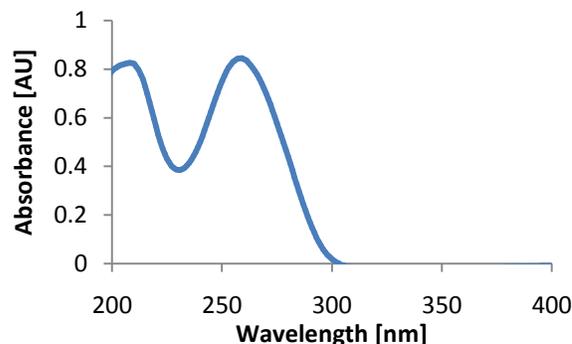


Figure 2: Typical DNA Measurement (281.6µg/mL)