

The Effect of Spectral Bandwidth on the Determination of Nucleic Acid Quantity and Purity

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Key Words

- DNA
- RNA
- Spectral Bandwidth
- UV-Visible

Differences in the absorption spectra of DNA and proteins can be exploited to determine the purity of nucleic acids in solution by using the $A_{260/280}$ Ratio method. The $A_{260/280}$ Ratio method, derived from an assay originally developed by Warburg and Christian for the determination of nucleic acid impurities in protein, has become a standard method for estimating the purity of nucleic acid samples.¹

In this application note, we show that a spectral bandwidth (SBW) of less than 2.0 nm yields improved results for the determination of both the actual concentration of DNA and the purity of the nucleic acid solution using the $A_{260/280}$ Ratio method. Thermo Fisher Scientific offers the following instruments with a SBW of less than 2.0 nm:

- Thermo Scientific Evolution 300 and Evolution 600
- Thermo Scientific Evolution 60S
- Thermo Scientific BioMate 3S
- Thermo Scientific GENESYS 10S UV-Vis Bio

Background

The sum of the individual absorption spectra of the four nucleic acid bases of DNA exhibits a peak maximum at approximately 260 nm as shown in Figure 1. The UV absorption spectrum of proteins has a peak maximum at approximately 280 nm. Thus, the purity of nucleic acid samples in the presence of protein contamination can be determined using the ratio of the UV absorptions of the sample at 260 and 280 nm, referred to as the $A_{260/280}$ Ratio. As the protein concentration in the sample increases, the $A_{260/280}$ ratio decreases due to the increase in absorption at 280 nm. A pure sample of DNA typically exhibits a $A_{260/280}$ ratio of 1.7 to 2.0.²

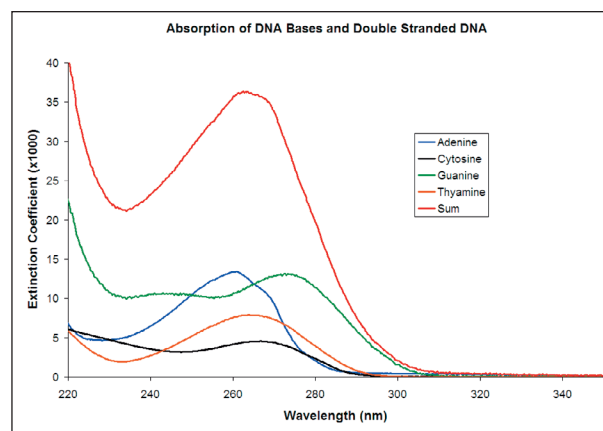


Figure 1: The UV absorption spectra of the individual DNA bases: adenine, cytosine, guanine, and thymine. The sum of the four bases, representing the UV absorption of a fragment of DNA.

The absorption spectrum of DNA is broad, with a full width at half maximum (FWHM) value of approximately 45 nm. Typically, a spectrophotometer with a spectral bandwidth (SBW) of one-tenth the FWHM of the sample peak is required for optimal resolution, in this case a SBW of less than or equal to 4.5 nm. To illustrate the effect of spectral bandwidth on the measured absorbance value, the absorption spectrum of calf thymus DNA acquired at a SBW of 0.5 nm and 4.0 nm is shown in Figure 2.

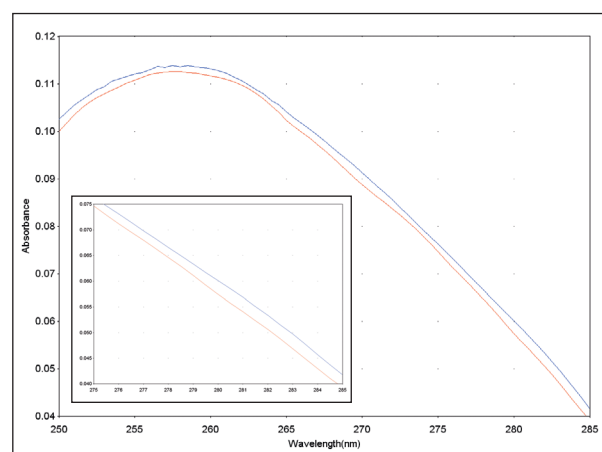


Figure 2: The absorption spectrum of calf thymus DNA acquired at spectral bandwidths of 0.5 nm (blue) and 4.0 nm (red)

Experimental Methods

A 2 mg/mL stock solution of high molecular weight calf thymus DNA was prepared by dissolving the lyophilized DNA into SSC buffer (0.15 M NaCl, 0.015 sodium citrate, pH 7.0). The solution was stirred overnight to ensure complete dissolution. Serial dilutions of 200 to 0.6 ng/ μ L of the stock solution were prepared as presented in the results and discussion section below. The product information sheet supplied with the lyophilized DNA indicated that the $A_{260/280}$ ratio was determined to be 1.9; no information on the method used to make this determination was supplied in the specification.

A Thermo Scientific UV-Visible spectrophotometer with a xenon lamp was used for all of the experiments. Measurements were performed using SBWs of 0.5, 1.0, 2.0 and 4.0 nm with an integration time of 2 seconds. All measurements were made in 4.0 mm semi-micro quartz cuvettes. Five replicate measurements were taken for each condition and the average result and standard deviation were calculated and reported.

The actual DNA concentrations reported were determined using an extinction coefficient of $0.0124 \mu\text{L ng}^{-1} \text{cm}^{-1}$. This extinction coefficient was determined using a second independent set of five DNA solutions with concentrations ranging from 1.0 to 300 ng/ μL .

Results and Discussion

Quantity of DNA

The effect of SBW on the quantitation of DNA at each concentration level is depicted in Table 1. From these results it is observed that the calculated concentration consistently underestimates the actual concentration of the DNA present in the solution as the actual value decreases. As expected, the percent deviation of the calculated value increases monotonically as the actual value in solution decreases. For example, at a SBW of 0.5 nm the deviation for the calculated value of the concentration of DNA in solution is:

- <5% for solutions 50 ng/ μL or greater
- 11 – 15% for solutions 10 – 1.25 ng/ μL
- nearly 20% for solutions <1.0 ng/ μL

An increase in percent deviation becomes even more evident as SBW increases. At a SBW of 4.0 nm, the deviation increases from:

- <5% to 8.2% for solutions 50 ng/ μL or greater
- 16% to 27.5% for solutions 10 – 1.25 ng/ μL
- nearly 20% to >30% for solutions <1.0 ng/ μL

This data clearly indicates that the accuracy of the concentration measurement decreases as SBW increases.

The error in the DNA concentration results from the resolution of the absorption spectrum at the measurement wavelength. As mentioned previously, a general rule for the minimum resolution required to resolve the peak is 1/10 of the FWHM of the peak. Here the influence of the

width of the SBW is obvious. The increase in deviation between the actual and the calculated solution concentrations results from a corresponding increase in the amount of the absorption spectrum that was integrated. At a SBW of 0.5 nm, the approximate measurement region at 260 nm is 259.5-260.5 nm. However, at a SBW of 4.0 nm, the approximate measurement region at 260 nm is 256-264 nm. *The overall increase in the standard and total deviations of the measurements is a clear indication of the effect of increasing bandwidth on the overall quality of the measurements.*

Purity of DNA

The effect of SBW on the analysis of DNA is most evident in the determination of the nucleic acid purity from the $A_{260/280}$ ratio method at each concentration level. The results from the $A_{260/280}$ measurements on a pure DNA sample, as described in the experimental section above, are given in Table 2. As expected, the absorption data for each solution shows a *decrease* in the measured absorption as SBW *increases*. These differences are expected to be eliminated by the ratio measurement of the DNA purity; however, the data shows that this is not the case. For example, at a SBW of 0.5 nm the average $A_{260/280}$ ratio from the eight solutions is 1.83, but at a SBW of 4.0 nm the ratio value is much higher at 1.95. The dependence of concentration on the accuracy of the $A_{260/280}$ ratio is shown in Table 2. At concentrations below 2.5 ng/ μL , the values obtained at a SBW greater than 1.0 nm show a slightly larger deviation. It is also important to note that the variation in the observed $A_{260/280}$ ratio is nearly identical for a SBW of 0.5-2.0 nm. *This clearly demonstrates that the standard deviation and range observed for purity measurements also increases as the SBW is increased and that the accuracy and precision of the measurement of dilute DNA solutions begins to decline above a SBW of 2.0 nm.*

Table 1: Actual and Calculated Concentrations of DNA Solutions as a Function of Spectral Bandwidth (SBW)

Solution	Actual Concentration (ng/ μL)	Calculated Concentration (ng/ μL)				Deviation (ng/ μL)				% Error			
		SBW (nm)				SBW (nm)				SBW (nm)			
		0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0
1	199.75	202.93	201.96	203.66	203.32	3.18	2.21	3.91	3.57	1.6	1.1	1.9	1.8
2	99.88	95.98	95.14	95.24	94.98	3.90	4.74	4.63	4.90	3.9	4.9	4.9	5.1
3	49.94	47.64	47.01	46.58	46.11	2.30	2.93	3.36	3.83	4.6	6.2	7.2	8.2
4	9.99	8.87	8.88	8.69	8.61	1.12	1.11	1.30	1.38	11.2	12.5	14.6	15.9
5	5.00	4.43	4.44	4.33	4.29	0.56	0.56	0.67	0.71	11.3	12.6	15.1	16.4
6	2.50	2.11	2.17	2.07	2.02	0.39	0.33	0.43	0.48	15.4	15.6	20.1	23.4
7	1.25	1.06	1.11	0.97	0.98	0.19	0.14	0.28	0.27	15.4	13.0	25.4	27.5
8	0.63	0.50	0.51	0.50	0.46	0.12	0.12	0.13	0.17	19.2	23.2	25.4	33.5
Total Deviation						11.76	12.13	14.72	15.31	82.7	89.1	114.5	131.8
Average Deviation						1.47	1.52	1.84	1.91				

Table 2: A_{260/280} Ratios of DNA Solutions as a Function of Spectral Bandwidth (SBW)

Solution	DNA Concentration (ng/μL)	A _{260/280} Ratio				
		SBW (nm)				
		0.5	1.0	2.0	4.0	5.0
1	199.75	1.82	1.80	1.82	1.85	2.03
2	99.88	1.85	1.85	1.86	1.88	2.04
3	49.94	1.82	1.82	1.83	1.85	2.00
4	9.99	1.86	1.86	1.84	1.88	1.90
5	5.00	1.86	1.86	1.83	1.91	1.92
6	2.50	1.82	1.84	1.78	1.92	2.33
7	1.25	1.81	1.86	2.05	2.00	2.40
8	0.63	1.82	1.80	1.79	2.33	2.33
Average A_{260/280} Ratio		1.83	1.84	1.85	1.95	2.12
Standard Deviation		0.02	0.03	0.09	0.16	0.20
Range		0.05	0.06	0.28	0.48	0.50

Pre-programmed Nucleic Acid Tests

We offer a variety of spectrophotometers dedicated to the life science laboratory. These instruments include built-in assay methods for the analysis of nucleic acid samples that may be customized for the individual needs of your laboratory. Customized assays can be stored to internal memory and recalled for later usage.

As shown in Figure 3, a method for determining the concentration and purity of nucleic acids using the A_{260/280} ratio assay is just one of the methods available. Also included are pre-programmed assays for measuring both A_{260/280} and A_{260/230} ratios with scanning. This feature allows the user to visually inspect the purity of the sample and determine the wavelength of maximum DNA and protein absorption for more accurate ratios.

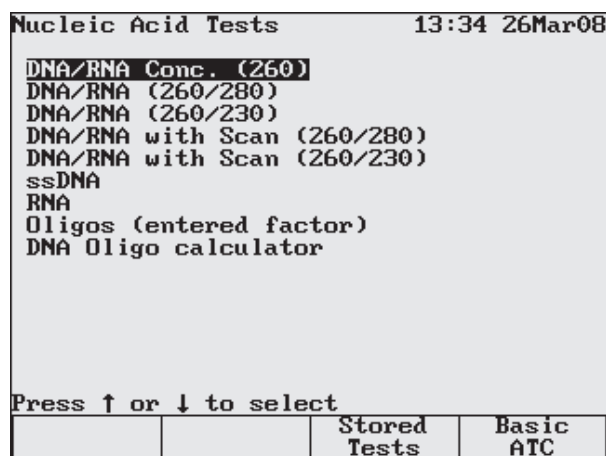


Figure 3: The Evolution™ 60S local control software displays the pre-programmed test methods available for nucleic acid analysis assays. These pre-programmed test methods are also available in the local control software of the BioMate™ series and Evolution 300/600 instruments.

Summary

The data presented here clearly indicates the influence of spectral bandwidth on the accuracy of concentration and purity measurements of nucleic acid samples. This application note also illustrates that the deviation between the actual concentration and the calculated concentration of DNA increases as a function of SBW. *The nucleic acid purity data clearly indicate that when dilute solutions of nucleic acids are examined, a SBW less than 2.0 nm yields improved results.*

References

- Warburg, O.; Christian, W.Z. *Biochem.*, 1942, 310, 384-421.
- Maniatis, T.; Fritsch, E.F.; Sambrook, J; *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1982, Cold Spring Harbor, NY.

Ordering Information

Recommended Systems for Nucleic Acid Analysis

Description	Part Number
BioMate 3S UV-Vis, US line cord	840-208300
BioMate 3S UV-Vis, Europlug & UK line cords	840-209900
GENESYS 10S Bio UV-Vis, US line cord	840-207700
GENESYS 10S Bio UV-Vis, Europlug & UK line cords	840-209300
Evolution 60S UV-Vis, US line cord	840-208500
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