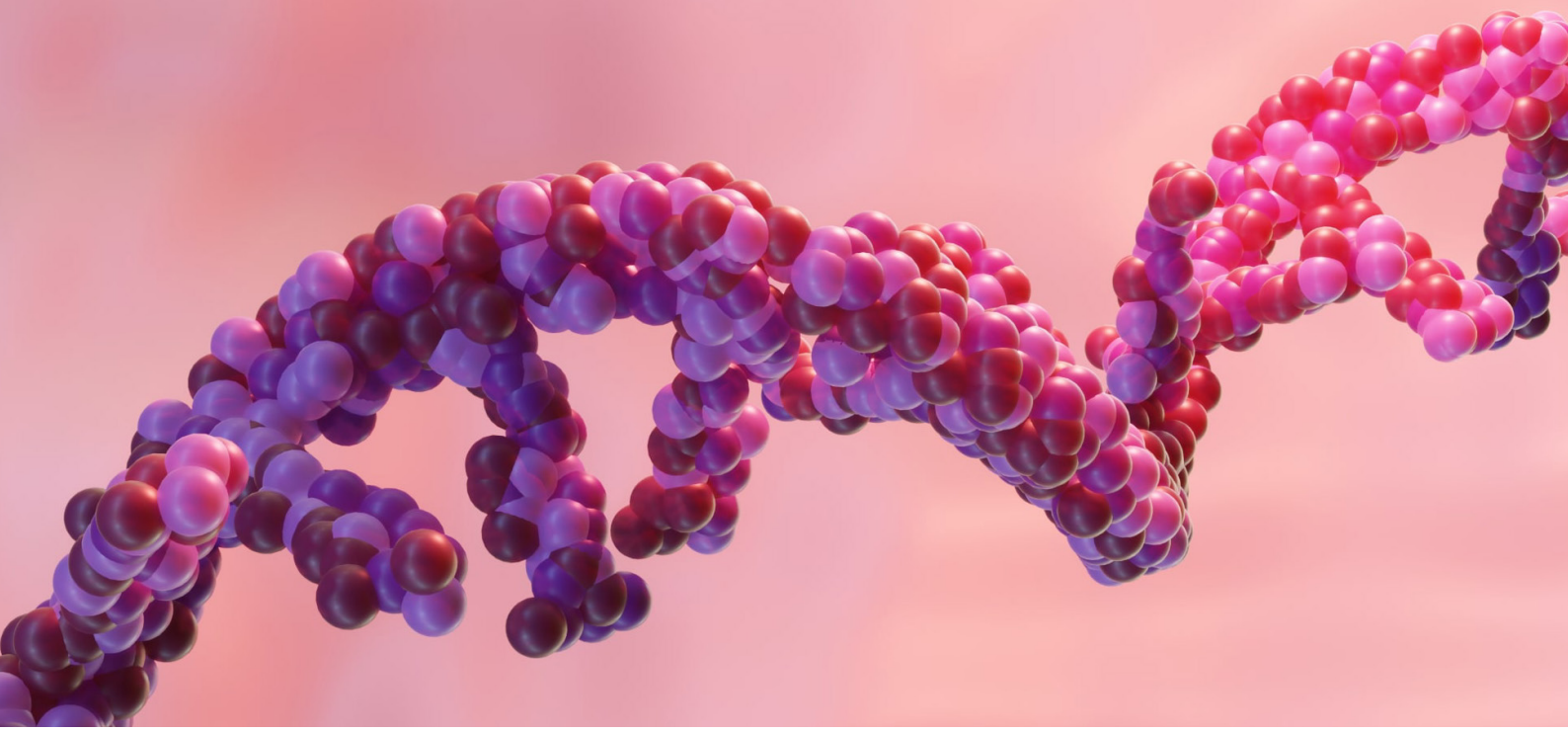
Technical note



Accurate Oligonucleotide Quantification with NanoDrop Spectrophotometers

Introduction

Quantification of nucleic acids is traditionally performed by spectrophotometric absorbance measurements at 260 nm. Researchers will often use absorbance measurements as a quality control step for various applications involving nucleic acid molecules such as oligonucleotides, dsDNA, and RNA. When quantifying oligonucleotides with Thermo Scientific™ NanoDrop™ Microvolume UV-Vis Spectrophotometers, special considerations are needed to ensure the most accurate results. These considerations relate directly to the unique structural characteristics of oligonucleotides and the specific measurement parameters for quantification with NanoDrop instruments.

Absorbance quantification of oligonucleotides

The UV spectrum of nucleic acids has distinct features, including a prominent peak at 260 nm and a trough at 230 nm (Figure 1).

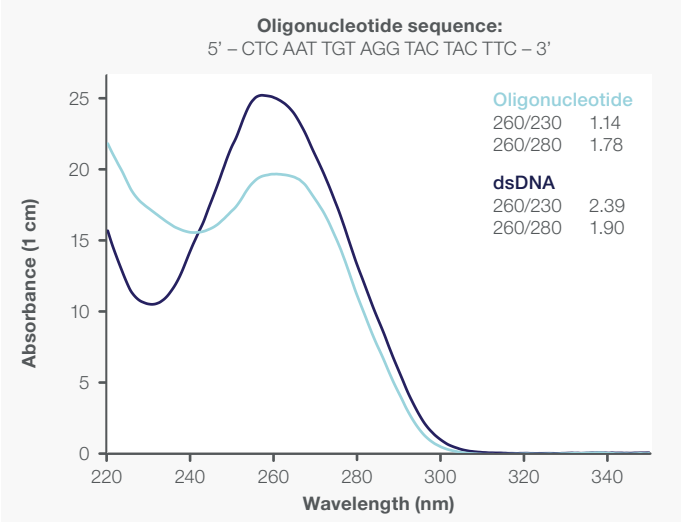


Figure 1. The UV spectrum of a pure oligonucleotide (light blue) and dsDNA (dark blue). As shown in the insert, the purity ratios can deviate significantly from the accepted values for large nucleic acid molecules.

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Oligo Sequence (5' - 3')	Oligo-specific concentration factor (ng-cm/µL)	A260	Concentration using general ssDNA concentration factor 33 (ng/µL)	Concentration using oligo-specific concentration factor (ng/µL)	% Difference
AAA AAA AAA AAA AAA AAA	25.41	21.20	699.60	538.69	26.0
/56-FAM/CCC CCT TTT CCC CCT CCC TTT CCC CCT CCC TTT CCC CCT TTT CCC	38.18	33.89	1118.37	1293.92	14.6
CTC AAT TGT AGG TAC TAC TTC	32.19	19.89	656.37	640.26	2.5

Table 1. Demonstration of effect of using incorrect factors to calculate the concentration of various oligonucleotides. The most accurate concentration is calculated from an oligo-specific concentration factor.

However, each individual nucleotide has its own unique absorbance profile, where the λ_{max} for all bases is between 255 nm and 280 nm. The classic nucleic acid UV spectrum is the combined spectra of each individual nucleotide contained therein. The UV spectra and extinction coefficients of synthetic oligonucleotides can vary from those exhibited by other nucleic acid molecules; because oligonucleotides are short, single-stranded molecules, their UV spectra and extinction coefficients will be dependent on base composition and sequence rather than that of large DNA or RNA molecules. Therefore, to ensure the most accurate quantification results for oligonucleotides, it is critical to use an oligo-specific concentration factor rather than the general ssDNA factor of 33 ng-cm/µL (Table 1). This concentration factor can be calculated by typing the sequence in the Oligo DNA and Oligo RNA applications in the NanoDrop Ultra and NanoDrop Eight software packages and can subsequently be used in the Custom Factor or Microarray applications. (Microarray application is not available for the NanoDrop Lite Plus spectrophotometer.)

Various modifications, such as fluorophores, are often placed on either the 5' or 3' ends of oligonucleotides. Many of these modifications will absorb light in the UV or visible regions of the spectrum and can affect the quantification result. To get the most accurate quantification result, it is important to consider the A260 correction factor for the modification. The correction factor, as well as the oligo-specific concentration factor, are typically found on product literature provided by the manufacturer. Otherwise, the A260 correction factor can be determined by calculating the ratio $A_{260}/A_{\text{dye wavelength}}$ after making a UV-Vis measurement of the pure dye. The A260 correction factor can be added to the NanoDrop software via the Dye Editor under software settings. The Microarray application will ensure the modification's A260 correction factor is automatically applied to the oligonucleotide concentration result.

The traditional purity ratios (A260/A280 and A260/A230), which are used as an indication of the presence of contaminants in nucleic acid samples, do not apply for oligonucleotides because the shapes of their UV spectra are highly dependent on their base compositions (Figure 1 insert).

NanoDrop-specific measurement considerations

NanoDrop instruments can measure a wide concentration range because of their capability to read samples using multiple pathlengths: 1.0 mm to 0.1 mm for the NanoDrop Eight instrument, and 1.0 mm to 0.03 mm for the NanoDrop Ultra instrument. The instrument's acceptable error increases as the pathlength is shortened. In many instances, the concentration of an oligonucleotide stock will be high enough for the instrument to use the shorter pathlengths. It is recommended to make dilutions to ensure measurements are being made with the 1.0 mm or 0.2 mm pathlength for the NanoDrop Ultra instrument and the 1.0 mm pathlength for the NanoDrop Eight instrument. The 1.0 mm or 0.2 mm optical pathlengths have much smaller error tolerances, 3% and 5% respectively. The error for the 0.2 mm pathlength on the NanoDrop Eight is 10%, making the 1.0 mm pathlength the more accurate option.

To determine the approximate absorbance of the oligonucleotide stock, perform a calculation by using the Beer-Lambert equation shown below (Equation 1).
To ensure that the absorbance is being measured by using either the 1 mm or 0.2 mm pathlength, it is important to avoid measuring over 62.5A at 260 nm.

$$A = \varepsilon bc$$

Equation 1.

Where:

A = Absorbance (1 cm, normalized with NanoDrop spectrophotometers)

ε = Molar extinction coefficient (L/(mol·cm))

b = Pathlength

c = Concentration (M, mol/L)

The Beer-Lambert equation can be used to determine the theoretical absorbance of an oligonucleotide stock. Most oligonucleotide manufacturers will provide the molar extinction coefficient, which can be used in this equation.

Example calculation: How to use the Beer-Lambert equation to determine absorbance of a 100 µM oligonucleotide stock.

Oligonucleotide Extinction Coefficient = 227200 (L/(mol cm))

Oligonucleotide Stock Concentration = 100 µM

Enter in the Beer-Lambert equation:

A260 = 227200 * (1 cm) * (0.0001 M)

A260 = 22.72

Another issue to consider is baseline correction. The NanoDrop software has a default baseline correction set at 340 nm. In most cases, it is important to have this correction performed because it corrects for light scattering events that may skew the results. In other cases, a more accurate result will be obtained when the baseline correction is turned off. For example, if an oligonucleotide has a modification that will absorb light at 340 nm, the baseline correction should be turned off, otherwise the 340 nm absorbance would be incorrectly subtracted from the 260 nm absorbance.

Conclusion

In molecular biology laboratories, UV-Vis spectrophotometry is a common tool for analyzing various types of nucleic acids including oligonucleotides, dsDNA, and RNA. For NanoDrop instruments, there are specific considerations when measuring the concentration of oligonucleotides. To ensure the most accurate quantification result, the following precautions should be taken: 1) Use an oligo-specific conversion factor rather than the general ssDNA conversion factor of 33 ng-cm/µL; 2) Use the Microarray application of the NanoDrop software if the oligonucleotide has a fluorophore modification so the necessary corrections are automatically performed; 3) Make dilutions to ensure that measurements are being made with the 1.0 mm or 0.2 mm pathlengths; 4) Turn off the default baseline correction if an oligonucleotide has a modification that absorbs light at 340 nm. Following these suggestions with a NanoDrop spectrophotometer will support accurate and reproducible quantification of oligonucleotides.

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