Simple and rapid quantitation of microliter DNA samples using the Agilent Cary 50/60 UV-Vis

Application Note

Biotech

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Introduction

Conventional UV-Vis spectroscopy typically involves the analysis of milliliter sample volumes (1-3 mL). However, there is an increasing need for the miniaturization of sample volumes as the demand for sample throughput increases and costs per analysis are driven down. The optics of the Agilent Cary 50 and Cary 60 UV-Vis spectrophotometers were designed to deliver highest quality data from a broad range of different UV-Vis applications, including those utilizing extremely small sample volumes. In this example, we demonstrate that the unique, small beam geometry of the Cary 50/60, coupled with a high intensity xenon flash lamp and <1.5nm spectral bandwidth slits, provide an optical design ideally suited for absorbance measurements on microliter sample volumes with no compromise in the quality of the data generated. Specifically, we apply this application to the measurement of DNA concentration by determination of the ratio of absorbance of nucleic acid in samples at 260 nm versus the absorbance of the same samples at 280 nm as originally described by Warburg and Christian in 1942[1].

Aim

To demonstrate the capability of a Cary 50/60 UV-Vis fitted with a ultra-microvolume cuvette for accurate and reproducible concentration measurements of microvolume samples of DNA.

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Instrumentation

A ultra-microvolume cuvette was placed in a Cary 50 UV-Vis and aligned to optimize the light throughput. (This experiment can also be performed in a Cary 60 UV-Vis). Adjustments were made in the cuvette holder in order to obtain light transmission values greater than 25% at 500 nm, relative to transmission readings taken at 100% T (with air at ambient laboratory temperature in the sample beam).

Materials and reagents

DNA from the bacterial vector pBUDCE4.1 (Invitrogen) was purified from an overnight bacterial culture using an EndoFree Plasmid Giga Kit (Qiagen). Following extraction using the kit, DNA samples were eluted from the column in 1.5 mL Endotoxin free water (Sigma Chemical).

DNA samples (4 μL) were pipetted to the sample well on top of the ultra-microvolume cuvette (fitted with the 1.0 mm pathlength cap). Readings were taken by the Cary 50 UV-Vis using the DNA/RNA module of the Cary WinUV software. This software module also permits wavelength scans to be performed to evaluate purity, reproducibility and validity.

Conditions

The operating parameters used are shown in Table 1.

Table 1. Operating parameters

Parameter	Setting
Wavelength 1 (nm)	260.0
Wavelength 2 (nm)	280.0
Background correction	On
Background wavelength (nm)	320.0
Scan samples	On
Start (nm)	400
Stop (nm)	200
Display options	Overlay data
Scan rate	Fast

Results

The data presented in Figure 1 show superimposed wavelengths scans from five repeats of 4 μL undiluted samples of DNA following removal and replacement of the ultra-microvolume cuvette before each successive scan.

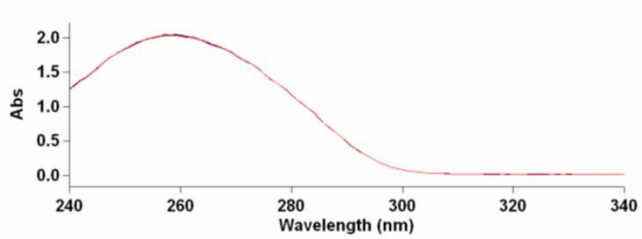

Figure 1. Reproducibility of wavelength scans of 4 μL samples of DNA, scanned over the UV region using the Cell with the Cary 50 UV-Vis spectrophotometer

The data (260:280 nm absorbance ratios) from Figure 1 were used by the software to determine the concentration of DNA within the samples used. Results for the five repeats of the experiment are shown in Table 2.

Table 2. Results for determination of DNA concentration in the DNA samples using the Cell

Sample	A(260)	A(280)	Bg(320)	Ratio	Nucleic acid (μg/mL)
1	2.0321	1.1709	0.0188	1.7474	1224.8799
2	2.0413	1.1698	0.0210	1.7586	1229.4436
3	2.0313	1.1718	0.0212	1.7469	1222.9269
4	2.0374	1.1730	0.0216	1.7509	1226.4993
5	2.0255	1.1730	0.0220	1.7407	1218.7515

The statistical variation of the repeat readings was 1224.5 ± 3.6 μg/mL DNA thus demonstrating an overall reproducibility of the concentration measurements to within ± 0.29% RSD.

In order to compare the reproducibility of measurements of DNA concentration measured in the ultra-microvolume cuvette, with those using a conventional 1 cm pathlength cuvette, the measurements in Table 1 were repeated using a different sample of DNA n a volume of approximately 400 μL in a 1 cm pathlength low-volume cuvette. Absorbance spectra from this experiment are shown in Figure 2.

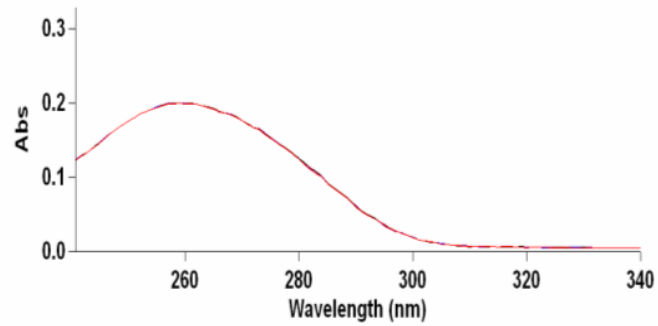

Figure 2. Reproducibility of wavelength scans of 400 µL samples of DNA (diluted 100-fold), scanned over the UV region using a 1 cm pathlength, low-volume cuvette with the Cary 50 instrument system

The corresponding reproducibility values for DNA concentration were calculated using the data in Figure 2. The data in Table 3 shows that the calculated DNA concentration in this instance was 117.8 ± 0.2 µg/mL. This represents an overall variation of 0.17% RSD throughout the five repeats in a 1 cm pathlength sample vessel.

Table 3. Results for determination of DNA concentration in the DNA samples using a 1 cm pathlength, low-volume cuvette

Sample	A(260)	A(280)	Bg(320)	Ratio	Nucleic acid (µg/mL)
1	0.2001	0.1252	0.0058	1.6270	117.9586
2	0.1998	0.1243	0.0059	1.6376	117.6966
3	0.2001	0.1248	0.0057	1.6321	117.9866
4	0.1994	0.1241	0.0055	1.6340	117.6978
5	0.1989	0.1242	0.0055	1.6302	117.4164

Conclusion

Quantitation of DNA by UV-Vis spectrophotometry is a valuable tool and has the merits of being simple, accurate and non-destructive to the sample. However, recent demands for increasingly smaller sample volumes and higher sample throughput can limit the use of cuvettes which can be costly and require frequent and thorough cleaning if quartz. This Application note demonstrates that the Cary 50 and Cary 60 UV-Vis spectrophotometers, when used with the ultra-microvolume cuvette, provides a research tool capable of reproducibly measuring the concentration of samples of DNA 4 µL in volume whilst providing a high precision wavelength scan to verify the sample purity. Analyses can take as little as two seconds including scan and data calculation and we show that the reproducibility of calculated DNA concentration values is less than 0.3% (RSD). Moreover, since the pathlength of the ultra-microvolume cuvette can be as small as 0.2 mm the photometric range of the Cary 50 and Cary 60 can be extended to absorbances over 150 Abs. This means that biological samples can be used undiluted, or diluted to a much smaller degree than is often required for measurements taken using a 1 cm pathlength.

It is the patented design of the Agilent Cary 50/60 instrument with its unique xenon flash lamp coupled with a small beam geometry and optimized light throughput that allows these measurements to be possible. Such ultra-low volume measurements can also be extended to protein as well as other biological samples.

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References

1. Warburg, O. and Christian, W. (1942) Biochem Z., 310, 384.

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