Measuring the purity of low volumes of DNA at 4 °C using the Agilent Cary 60 UV-Vis spectrophotometer with fiber optics microprobe

Application Note

Pharma/Biotech

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Summary

The Agilent Cary 60 UV-Vis spectrophotometer is the ideal instrument for measuring small amounts of biological samples directly from their storage environment, e.g., a refrigerator. This application note demonstrates how the Cary 60 is used to measure the purity of DNA at 4 °C using the micro fiber optics accessory – resulting in significant time and cost savings without compromise in the accuracy and reproducibility of data quality.

- No more need for cuvettes
- Measure samples directly from the refrigerator at 4 °C
- Save time and money per analysis

Introduction

Measurement of DNA purity and concentration by UV-Vis spectrophotometry has been a valuable tool in the field of biotechnology for many years, since the methodology was originally developed by Warburg and Christian in 1942[1], and later optimized for simple and rapid measurements in the laboratory by Sambrook et al in 1989[2]. Key benefits of the spectrophotometric approach mainly involve the facts that measurements are 1) simple 2) accurate and 3) non-destructive to the sample. This saves time without any compromise on the quality of data generated, as well as providing the financial benefit of not having to use large volumes of samples or additional consumables to makes such measurements.

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Previously, we have shown that the purity of microvolumes of DNA can be accurately and reproducibly measured at ambient temperatures in a Cary 50 UV-Vis spectrophotometer fitted with a ultra-microvolume cuvette[3]. In the present study, we extend our observations to the new Cary 60 UV-Vis using fiber optics, measuring small volume of samples at 4 °C under conditions of normal laboratory fluorescent ambient lighting. This approach allows users to take the instrument to the sample, rather than the conventional approach in spectroscopy in which the sample is presented to the instrument, thereby allowing the user to complete their analysis in a fraction of the time. The unique, optical configuration of the Cary 60 makes this possible mainly by virtue of the unique, high-intensity xenon flash lamp combined with the latest electronics enabling the system to effectively monitor small changes in absorbance without any effect of ambient light. Key benefits of this approach are discussed further in this document.

Apparatus and materials

Part Number	Description
G6860AA	Cary 60 UV-Vis with WinUV software and PC
7910035600	Fiber optic microprobe
G6866A	Fiber optic probe coupler
6610000800	Quartz cuvettes (1 cm pathlength; pack of two)
D-4764 (Sigma Chemical Company)	Calf Thymus DNA; 1 unit

Methods and results

Varying concentrations of 0–50 µg/mL calf thymus DNA were prepared by dissolving in purified (MilliQ) water.

The Cary 60 instrument was fitted with the fiber optics coupler and microprobe as shown in Figure 1. Blank readings of 40 µl purified (MilliQ) in Eppendorf tubes were taken.

Since molecular biology reagents are commonly stored under refrigeration, all samples were taken and analyzed directly from the refrigerator at 4 °C.

Figure 1. The Cary 60 instrument fitted with the Fiber Optics Accessory taking a reading of 50 µl DNA in an Eppendorf tube at 4 °C

Initially, scans were taken from 420 to 310 nm using the WinUV 'Scan' software module to confirm the maximum absorbance peak and the authenticity of the purchased DNA sample at a 50:50 dilution (Figure 2).

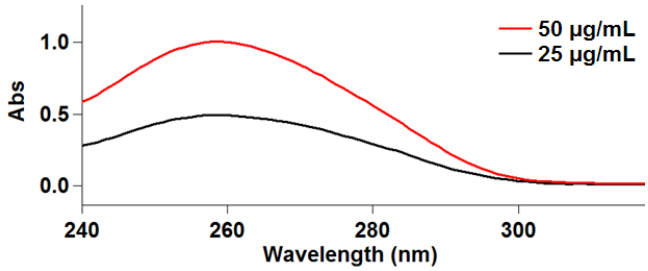

Figure 2. Scans of 50 µl samples of DNA at 4 °C at two concentrations showing the characteristic absorbance peak at 260 nm. Note peak absorbance of 1.0 absorbance units for 50 µg/mL DNA versus peak absorbance of 0.5 absorbance units for 25 µg/mL DNA, demonstrating adherence to the Beer–Lambert Law ($A = \epsilon cl$)

In order to confirm the purity of the DNA, 260/280 ratio measurements were made using the dedicated WinUV software module 'RNA/DNA' which automatically calculates the purity of the DNA sample. This application is extremely simple to use, as shown in Figure 3, and the analyses were completed in less than a minute.

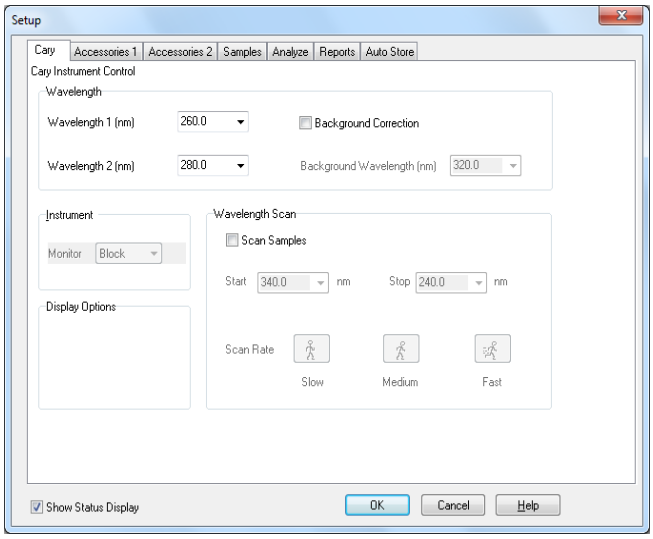

Figure 3. Simple, dedicated Cary WinUV automated calculations for DNA purity, authenticity and concentration

Finally, a concentration curve was plotted by the WinUV 'Concentration' software module to assess the linearity and reproducibility of readings of varying concentrations of the DNA samples under identical laboratory conditions (Figure 4). The microprobe was washed with MilliQ water in-between DNA sample readings. The linearity of the concentration curve demonstrates that carryover from the previous samples was undetectable.

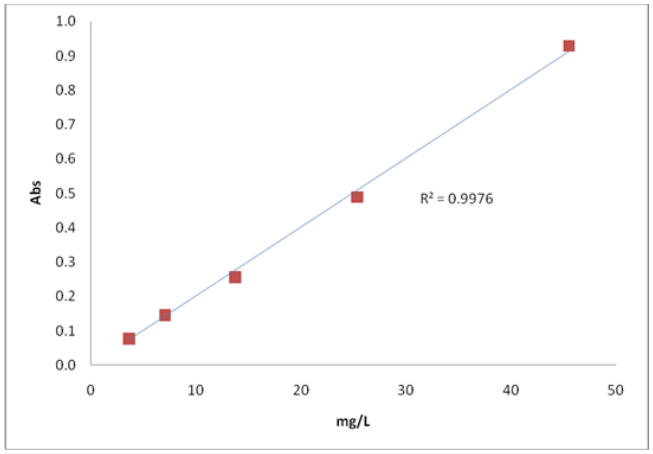

Figure 4. Concentration curve for DNA samples (0–50 µg/mL) using 50 µL samples at 4 °C in Eppendorf tubes measured under conditions of normal fluorescent laboratory lighting with the microprobe fiber optics accessory. Calibration eqn: Abs = 0.02004*Conc; Correlation Coefficient = 0.9976.

Discussion

The time taken to read all five samples was less than two minutes. Conventional methods would use a micro-cuvette, which would involve waiting for the sample to warm up to ambient temperature, and cleaning the micro-cuvette between measurements. All in all, the time of analysis when using the Cary 60 fiber optics system is significantly reduced by over an estimated 80%, and in addition, the fiber optic technique is not prone to potential contamination due to sample handling. The accuracy in the data, along with the significant time saved in reading cold samples with the microprobe demonstrates that the Cary 60 provides a simple and rapid, cost-effective system for the measurement of biological samples such as DNA. This approach can easily and directly be extended to other sample types such as protein measurements at 280 nm for example.

Conclusion

The Cary 60 UV-Vis is the ideal instrument for using fiber optics sampling due to its unique, high-intensity xenon flash lamp which provides superior sensitivity and reproducibility. The speed of analysis, reproducibility in results, and ease of use in measuring the DNA samples as shown in this application note demonstrates these benefits.

Other key benefits of using the Cary 60 with fiber optics to analyze biological samples include:

- Hot and cold samples (4 –110 °C) can be read with no concern for condensation, as observed in cuvettes at these temperatures which results in incorrect and irreproducible results
- No limit on sample volumes – can be as low as 40 µL or infinitely large
- No need for purchase of expensive quartz micro-cuvettes or time-consuming cleaning of cuvettes
- Solids, powders or gel-based samples can also be analyzed using reflectance probes

1. Warburg, O. and Christian, W. (1942) *Biochem Z.,* **310**; 384.
2. Sambrook, J., Fritsch, EF. and Manniatis, T. (1989) Molecular Cloning. A Laboratory Manual. Second Edition, Cold Spring Haror Laboratory Press, Cold Spring Harbor.
3. Keighley, B. and Fyfe, D. (1995) *Application Note* **#91**. www.agilent.com

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