Varian Cary 50 UV-Vis

Unique Optical Performance of the Varian Cary 50 UV-Vis Spectrophotometer Ideal for Analyzing Microvolume Samples

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Figure 1. Varian Cary 50 spectrophotometer

Summary

- The Hellma Traycell™ fitted to a Cary 50 UV-Vis spectrophotometer is ideal for the measurement of microlitre (<4 µL) sample volumes
- Significantly higher light throughput in the Cary 50/ Traycell system compared to a competitor's system means higher photometric range
- Removal and replacement of the Traycell for cleaning or other purpose has negligible affects on data, meaning excellent reproducibility

Aim

To evaluate the reproducibility and light throughput of a Varian Cary 50 UV-Vis spectrophotometer fitted with a Hellma Traycell™ ultra-microvolume cuvette for accurate and reproducible absorbance measurements of microvolume liquid samples.

Introduction

Given the increasing demand for simple, rapid and non-destructive methods to analyze microlitre volumes of chemical and biological samples, we have previously demonstrated that the Varian Cary 50 UV-Vis spectrophotometer fitted with a Hellma Traycell™ ultra-microvolume cuvette can accurately measure concentration values of 4 µL samples of DNA[1]. Given the occasional need to a) remove and replace the Traycell for practical purposes and b) analyze lower still concentrations of biochemical samples, in this paper we extend our initial observations to evaluate the reproducibility and light throughput of the Hellma Traycell™ when fitted to the Cary 50 UV-Vis spectrophotometer.

Figure 2. Hellma Traycell™ microvolume accessory

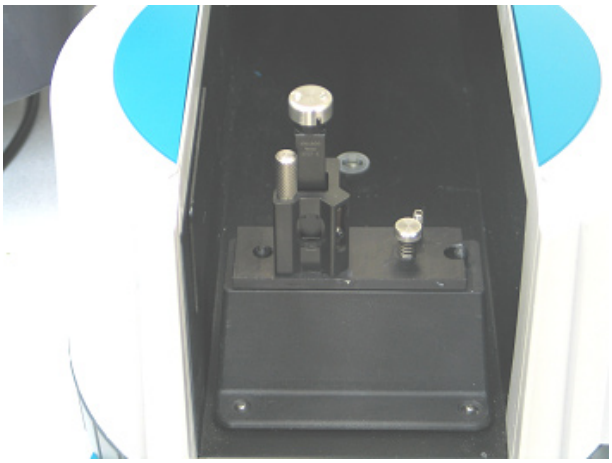

Figure 3. Varian Cary 50 Bio UV-Vis spectrophotometer sample chamber fitted with a Hellma Traycell™

Materials and Methods

A Traycell™ ultra-microvolume cuvette (Figure 2; Helma GmbH & Co., Germany) was fitted into a Varian Cary Eclipse cell holder base (standard with the Traycell Kit, part number 9910123500) in the sample chamber of a Cary 50 (Figure 3). The cell holder is optimized for the Traycell product on account of enhanced tilt and height adjustment capabilities compared to the standard Cary 50 cell holder. The Traycell was aligned vertically and horizontally to optimize light thoughput at 500 nm using the Align application module in the Varian Cary WinUV™ Bio software. 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).

Results

For comparative purposes only, data presented in Figure 4 show a single transmission scan throughout the wavelength range 250–1000 nm using a competitor's conventional UV-Vis spectrophotometer properly fitted with a Traycell. Air was initially scanned at the same wavelengths as a baseline. Data in Figure 5 show the results from the same test using the Varian Cary 50 UV-Vis spectrophomometer, however in this case the Traycell was repeatedly removed and replaced five times from the cell holder base to assess reproducibility.

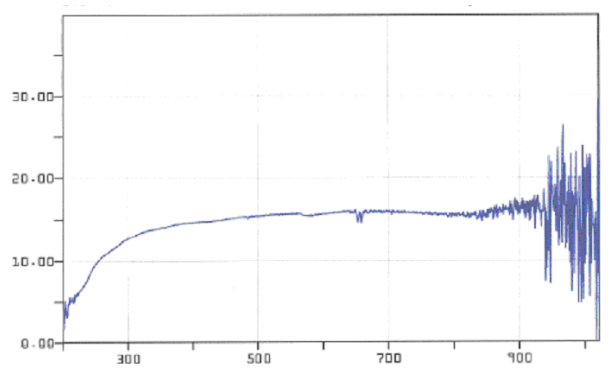

Figure 4. Reproducibility of wavelength scans of 4 µL samples of DNA, scanned over the UV region using the Traycell fitted to a conventional, competing UV-Vis spectrophotometer, not equipped with Varian's patented[2] xenon flashlamp optical design. Note noise in NIR region.

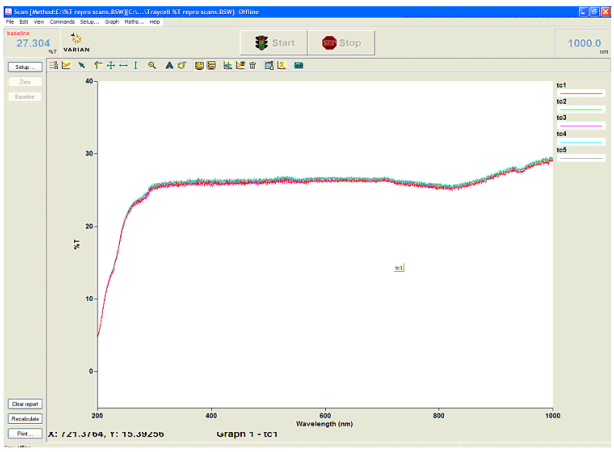

Figure 5. Reproducibility of wavelength scans of 4 µL samples of DNA, scanned over the UV region using the Hellma Traycell™ with the Cary 50 UV-Vis spectrophotometer

Table 1. Statistical data derived from raw reproducibility data given in Figure 5

No. Repeat Scans	Wavelength (nm)	Mean %T	StDev	%RSD
5	260	22.79	0.23	1.0
5	280	24.02	0.19	0.8
5	340	25.97	0.30	1.2
5	500	26.36	0.10	0.4
5	700	26.49	0.18	0.7
5	1000	29.31	0.25	0.9

Data shown in Figures 4 and 5 demonstrate that the Cary 50 fitted with the Traycell yields significantly higher %T values (>10% throughout the range 200–1000 nm) compared to %T values recorded using the Traycell fitted into a UV-Vis spectrophotometer from a competitor. Moreover, data in Table 1 show that repeatedly removing the Traycell from the cell holder and replacing it has negligible effects on the %T values recorded, thus demonstrating excellent reproducibility througout complete use of the Traycell. Comparing data in Figures 4 and 5 also clearly show that whilst there is excessive noise in the competitor's instrument in the NIR range 800–1000 nm, the Cary 50/Traycell system is well suited for sample measurements within the NIR range.

Discussion and Conclusion

Quantitation of microlitre volumes of DNA by UV-Vis spectrophotometry has a variety of benefits:

1) Low volumes - save on precious samples and reagents

2) Cleaning/purchase of quartz cuvettes or other vessels is not required

3) The method is non-destructive to samples

4) Accurate and reproducible measurements can be recorded in seconds

5) No chromophores or fluorophores are required to visualize the sample

6) No compromise in data quality compared to results recorded using larger volumes in a cuvette or microplate

In this short review, we evaluated some performance characteristics of the Hellma Traycell™ microcell fitted to the Varian Cary 50 UV-Vis spectrophotometer to confirm that a) light throughput and therefore photometric range of the Cary 50 system is far superior to that of a competitor and b) results are extremely reproducible (maximum RSD = 1.2% across the wavelength range 200–1000 nm) even when the Traycell was removed and replaced sequentially between wavelength scans.

We believe these observations indicate that the Traycell/Cary 50 system is ideal for rapid, reproducible and accurate measurements of a broad range of concentrations of microlitre volumes of DNA. It is the patented[2] optical design of the Cary 50 instrument that makes these measurements possible, since unique xenon flashlamp dynamics and a millimetre beam size maximizes the light flux through the optical system of the Traycell. We now intend to further our investigations to explore the dynamic range of this instrument system using a variety of biomolecules and samples.

Acknowledgements

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References

1. Keighley, RA and Fyfe, DJ. (2005) Simple and rapid quantitation of microlitre DNA samples using the Varian Cary 50 UV-Vis spectrophotometer. Varian application note #91; www.varianinc.com

2. Hammer, MR. (1999) Spectrophotometer. United States Patent 6,002,477.

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