GENOVA and 6715 Application note: A09-007A

Use of the microcuvette holder with plastic microcuvettes

Introduction

Common applications of UV/Visible spectrophotometers include direct measurement of nucleic acids and proteins to determine their concentration. Often there is very little sample available and the researcher may not wish to dilute it to give sufficient volume for a standard cuvette. In addition, if there are numerous samples to measure, the use of quartz cuvettes requires washing between samples and may lead to cross contamination if the sample needs to be recovered. In these situations, disposable UV plastic cuvettes requiring a small sample volume are often the cuvettes of choice.

Jenway UV plastic cuvettes, part code 035 143 require only 70µl of sample and can be used at wavelengths from around 230nm, so they are suitable for direct measurements of both nucleic acids and proteins. However since the cell holder aperture is larger than the sample "window" in the cuvette, it is possible for light to pass through the side walls and cause scattering, leading to inaccurate and inconsistent results. For this reason, a microcuvette holder, part code 630 304, has been designed for use in Jenway spectrophotometers with this cuvette.

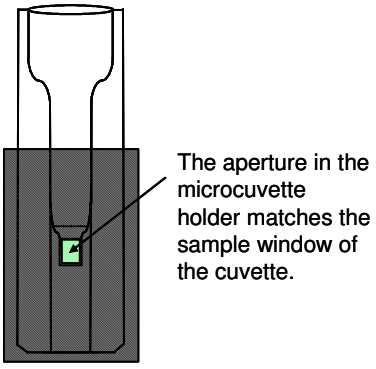

Figure 1: Schematic diagram showing the aperture of the microcuvette holder in relation to the cuvette 035 143.

In this application note we demonstrate how using the microcuvette holder improves transmission through the cuvette and how when it is not used, inaccurate results are obtained when determining DNA concentrations.

Methods

The % transmission of the cuvette both in the standard single cell and microcuvette holders was measured across the UV range. In the Genova the purity scan mode was used; in the model 6715 the spectrum mode was used with the plot interval set to 1nm. A background scan was first performed in air between 200 and 300nm with the appropriate cell holder installed. 0.5ml of water was then placed in the cuvette and scanned using each cell holder in turn. The results from the Genova were recorded using the Dataway software, transferred to Microsoft Excel and the % transmission (%T) values calculated from the absorbance values using the formula:

$$\%T = 100 \times (1/10^{Abs})$$

The results from the model 6715 were saved on an SD card and copied from the 67 series software into Excel.

For DNA determinations, a 100µg/ml solution of genomic DNA was diluted 1 in 4 with 10mM Tris, 1mM EDTA solution, pH 8.0 (TE buffer) to give a 25µg/ml solution. 70µl of this solution was pipetted into the cuvette and was scanned in both instruments between 220 and 310nm both in the microcuvette holder and in the standard single cell holder using TE buffer as a blank.

Results

Figure 2 shows the %T scans for a sample of water in the 035 143 cuvette using both the standard and microcuvette holders.

The transmission results demonstrate that using the standard cuvette holder with this cuvette can reduce transmission levels by up to approximately 20% in the UV region. This means that samples will have a higher background absorbance and readings will not be as sensitive due to light from the source scattering through the unshielded sides of the cuvette.

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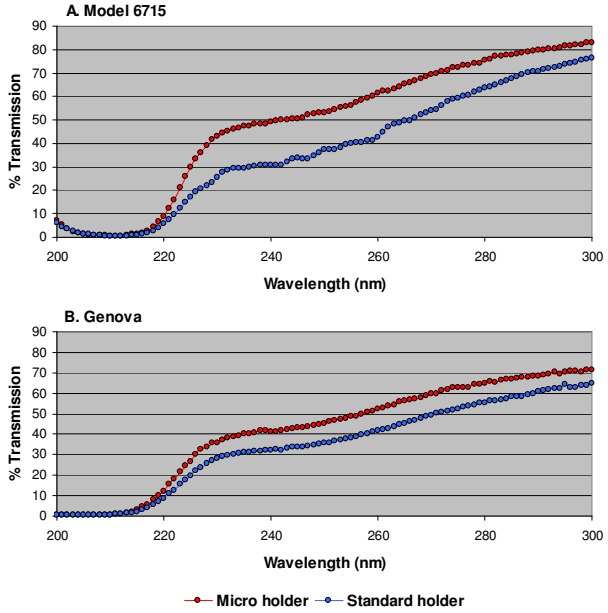

Figure 2: Transmission plots of cuvette 035 143 with the microcuvette and standard cuvette holders. A. results from model 6715; B. results from the Genova.

A 50µg/ml solution of dsDNA gives an absorbance at 260nm of 1.0 and the A_{260}/A_{280} ratio should be of the order of 1.8 to 2.0. Table 1 shows the absorbance values for the DNA solution at 260 and 280nm measured in 035 143 using both types of cuvette holder. It can be seen that using the standard cuvette holder underestimates the DNA concentration by at least 50% and gives a lower A_{260}/A_{280} ratio. These differences are demonstrated more clearly in the spectra of the DNA shown in Figure 3.

	Model 6715		Genova	
	Micro holder	**Standard holder**	**Micro holder**	**Standard holder**
DNA (µg/ml)	25	25	25	25
Expected A_{260}	0.500	0.500	0.500	0.500
Actual A_{260}	0.508	0.246	0.500	0.196
Calculated DNA (µg/ml)	25.4	12.3	25.0	9.8
Actual A_{280}	0.290	0.152	0.269	0.120
A_{260}/A_{280}	1.75	1.62	1.86	1.63

Table 1: Expected and measured absorbance values of a dsDNA solution using cuvette 035 143 in the standard and microcuvette holders.

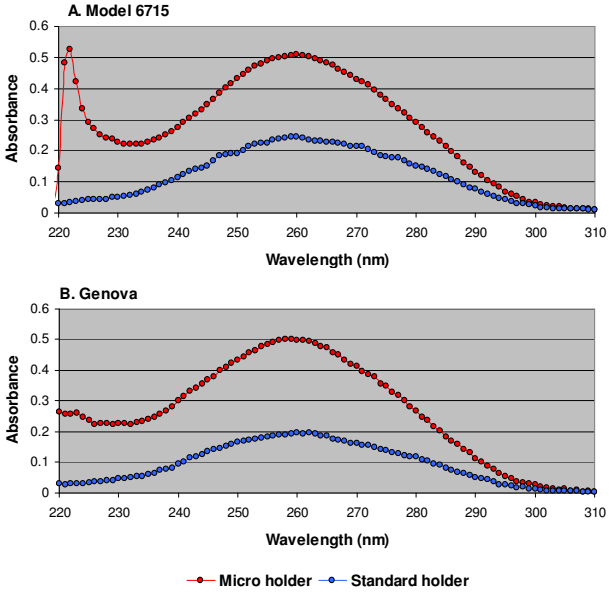

Figure 3: Spectra of the dsDNA solution using cuvette 035 143 in the standard and microcuvette holders. A. results from model 6715; B. results from the Genova.

Conclusions

For the use of ultra micro cuvettes such as 035 143 where the walls of the cuvette are not shielded or black, it is important to use a microcuvette holder in order to reduce the aperture to match that of the sample window. This ensures that the incident light passes through the sample only and is not scattered by the walls of the cuvette.

The beam height, or z dimension of all Jenway spectrophotometers is 15mm and the aperture in the microcuvette cell holder is set at this height. Different beam height cuvettes are available, so it is important that cuvettes with the correct beam height are used.

From the results presented here, we have demonstrated that the microcuvette holder both improves transmission of light through the sample and prevents underestimation of absorbance readings, which, in direct assay methods such as nucleic acid and protein determinations, may lead to an underestimation of concentration.

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