6715 and Genova

Application note: A09-005A

Test of sample volume using the TrayCell

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 further in order give sufficient volume for measurement in a standard quartz cuvette. Ultra-micro quartz cuvettes are available which will allow as little as 20µl of sample to be measured directly, however these can be quite difficult to use to achieve reproducible results. An alternative, if the sample is of a sufficient concentration, is to use a TrayCell.

The TrayCell is a fibre-optic, ultra-micro cell designed for measurements of extremely small sample volumes of DNA, RNA or protein. The dimensions of the TrayCell are equivalent to a standard cuvette and so will fit in all Jenway spectrophotometers. The TrayCell is simple to use. It is first positioned in the cell holder of the spectrophotometer and the sample is then placed on an optical window on the surface of the cell. A cap containing a mirror is then fitted over the top. The type of cap used determines the path length through which the light passes.

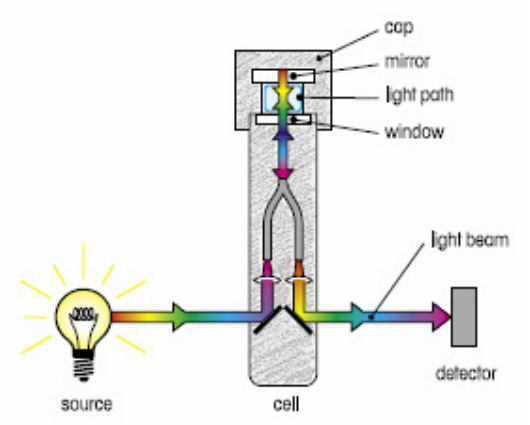

Figure 1: Schematic diagram of the light path in the TrayCell.

Light from the spectrophotometer lamp is directed to the sample in the cap by optical fibres; the light returned by the sample is reflected off the mirror in the cap and passed down a second optical fibre to the detector. Once the measurement has been made, the cap is removed and the sample can be recovered if required. The window and cap are then gently cleaned using a lint-free swap or wipe. The TrayCell remains in the cell holder during all stages; this ensures that the aperture remains in an identical position for each measurement for increased reproducibility.

There are two caps supplied with the TrayCell. Using the 1mm or 0.2mm cap creates a defined optical light path of 1mm and 0.2mm respectively. This generates virtual dilution factors of 1:10 or 1:50 in comparison to a measurement with a standard 10mm cuvette. This means that a sample giving an absorbance reading of 1.0 in a 10mm path length cuvette will give an absorbance of 0.1 using the 1mm cap and 0.02 with the 0.2mm cap. Therefore it is important that the sample to be measured using the TrayCell is sufficiently concentrated to give a reading of at least 0.05 absorbance units for reproducible measurements.

In this application note we demonstrate the sample volume requirements for using the TrayCell with the 1mm and 0.2mm caps. The required sample volume for the TrayCell with the 1mm cap is 3µl to 5µl and for the 0.2 mm cap, 0.7µl to 4µl.

Methods

A sample of egg albumin was diluted 1 in 10 in water, mixed gently by inversion and centrifuged briefly to pellet any solid material. Samples of this were measured directly using the TrayCell in the Jenway Genova and 6715 spectrophotometer. Each sample volume was measured three times for reproducibility. In the Genova the protein Direct UV mode was used. In the model 6715, the absorbance at 280 and 260nm was measured and the protein concentration for each sample calculated using the formula[1]:

$$\text{Concentration (mg/ml)} = (A_{280} \times 1.55 - A_{260} \times 0.76) \times \text{dilution factor}$$

For the 1mm cap the dilution factor was 10 and for the 0.2mm cap, 50. These factors represent the

virtual dilution of the sample due to the shorter path length.

Results

Sample volumes between 0.5 and 4µl were tested for the 0.2mm cap and volumes between 2 and 5µl for the 1mm cap. The results are presented in Figure 2 and Table 1.

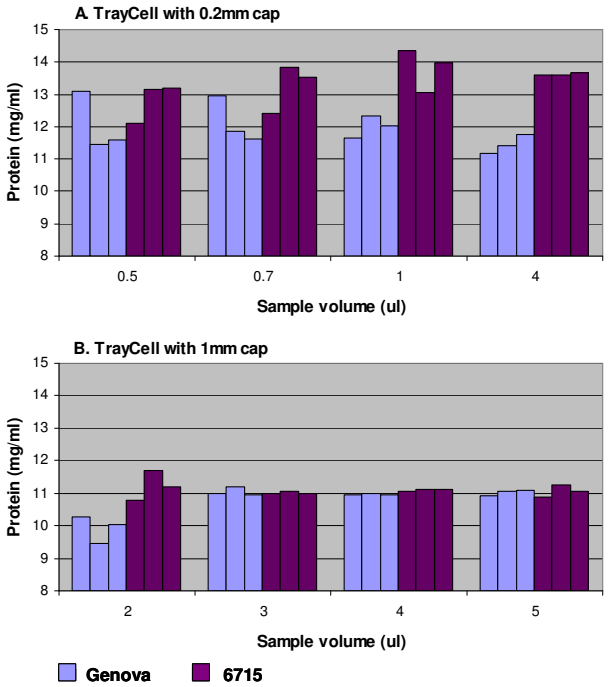

Figure 2: Protein concentration determined using various sample volumes in the TrayCell with A. the 0.2mm cap and B. the 1mm cap.

	0.2mm cap		1mm cap	
Sample Volume (µl)	Genova (mg/ml)	6715 (mg/ml)	Genova (mg/ml)	6715 (mg/ml)
0.5	12.04 ±0.90	12.81 ±0.62		
0.7	12.14 ±0.70	13.26 ±0.77		
1	12.00 ±0.35	13.80 ±0.67		
2			9.93 ±0.43	11.23 ±0.46
3			11.05 ±0.12	11.01 ±0.04
4	11.45 ±0.31	13.63 ±0.02	10.97 ±0.01	11.11 ±0.04
5			11.03 ±0.09	11.07 ±0.18

Table 1: Average protein concentration (mg/ml) and standard deviations of three replicates calculated from various sample volumes using the 0.2mm and 1mm TrayCell caps.

There were greater variations in replicates using the 0.2mm cap than with the 1mm cap and also when lower than recommended volumes were tested. The measured and calculated protein concentrations were also generally higher using the 0.2mm cap. This may be attributed to two factors. Firstly, pipetting accuracy at low volumes will not be as great as for larger volumes; if there is insufficient sample, the droplet will not fill the cap and the sample will not be of the correct path length. Secondly with a very short path length, absorbance values will be very much lower than with a standard 10mm cuvette. This means that small changes in absorbance can lead to quite large differences in concentration when the calculations are performed, due to the high dilution factor.

Conclusions

The TrayCell is a useful tool for measuring the absorbance of samples where there is limited sample available or the sample needs to be recovered after measurement without dilution. The required sample volume for the 1mm cap is 3µl to 5µl and for the 0.2mm cap, 0.7µl to 4µl. The TrayCell, especially with the 1mm cap, gave reproducible results over the range of reqired sample volumes.

When using the TrayCell it is recommended that the sample is sufficiently concentrated to give a reading of at least 0.05 absorbance units for reproducible measurements. For proteins this corresponds to a minimum concentration of approximately 0.6mg/ml for the 1mm cap and 3mg/ml for the 0.2mm cap. For nucleic acids, a minimum of 25µg/ml dsDNA is required for the 1mm cap and 125µg/ml for the 0.2mm cap.

References

1. O. Warburg, W. Christian (1941), Biochem. Z. **310**, 384 – 421.