GENOVA Application note: A09-006A

Protein direct UV measurements 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. There are ultra-micro quartz cuvettes available which will allow as little as 20µl of sample to be measured directly, however they 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 extremely easy 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.

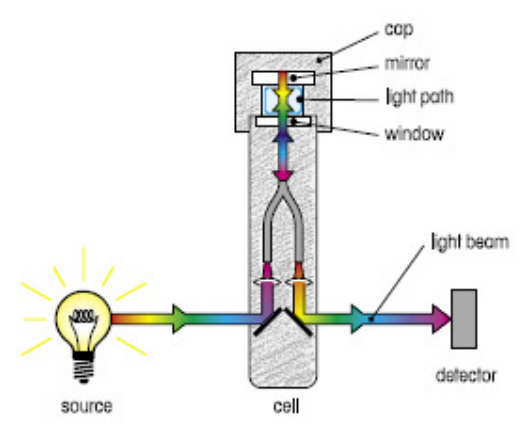

Figure 1: Schematic diagram of the light path in the TrayCell.

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 protein concentration measurements using the TrayCell in the Jenway Genova using the Direct UV mode and compare the results with those from a standard 10mm path length cuvette.

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. Initial measurements indicated that this solution was approximately 10mg/ml. This was further diluted in water 1:1 (0.5), 1 in 5 (0.2), 1 in 10 (0.1), 1 in 20 (0.05), 1 in 50 (0.02) and 1 in 100 (0.01). Samples of these dilutions were measured directly using the TrayCell with both the 1mm and 0.2mm caps and a 100µl volume quartz cuvette (035 138) in the Jenway Genova.

In the Genova the protein Direct UV mode was used. The software calculates the protein concentration according to the formula[1]:

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

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For the 1mm cap the dilution factor is 10 and for the 0.2mm cap, 50. These factors represent the "virtual" dilution of the sample due to the shorter path length. These were entered in the SETUP menu under DILUTION as follows: for the 10mm path length cuvette the dilution was set to 0001+0000µl (no dilution); for the 1mm cap it was set to 0001+0009µl (1 in 10 dilution) and for the 0.2mm cap it was set to 0001+0049µl (1 in 50 dilution). Concentrations were recorded as mg/ml.

Results

Protein concentrations for each dilution were calculated by the Genova software. The values obtained are displayed in Figure 2 and summarised in Table 1.

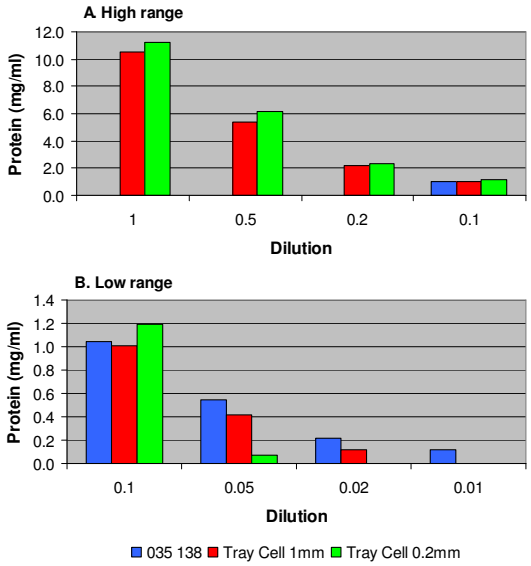

Figure 2: Protein concentrations of each dilution as measured in cuvettes with a 10mm, 1mm or 0.2mm path length. A. shows the higher concentrations and B. the lower concentrations.

	Protein concentration (mg/ml)		
Sample dilution	035 138	Tray Cell 1mm	Tray Cell 0.2mm
1	-	10.55±0.09	11.23±0.36
0.5	-	5.35±0.07	6.15±0.59
0.2	-	2.16±0.01	2.32±0.28
0.1	1.04±.03	1.01±0.03	1.19±0.20
0.05	0.54	0.42±0.003	0.08±0.30
0.02	0.21	0.11±0.04	-
0.01	0.11	0.001±0.04	-

Table 1: Protein concentrations of each sample dilution as shown in Figure 2. Measurements in the TrayCell were performed in triplicate.

The results show that the TrayCell with both the 1mm and 0.2mm caps gave similar values in the high range of protein concentrations. These are also comparable to the samples measured in a conventional 10mm path length cuvette, where the absorbance range allowed.

If the absorbance data is plotted against the sample dilution, all three cuvettes demonstrate a linear standard curve (Figure 3). The 0.2mm cap gave a higher variation between samples due to the lower absorbance levels.

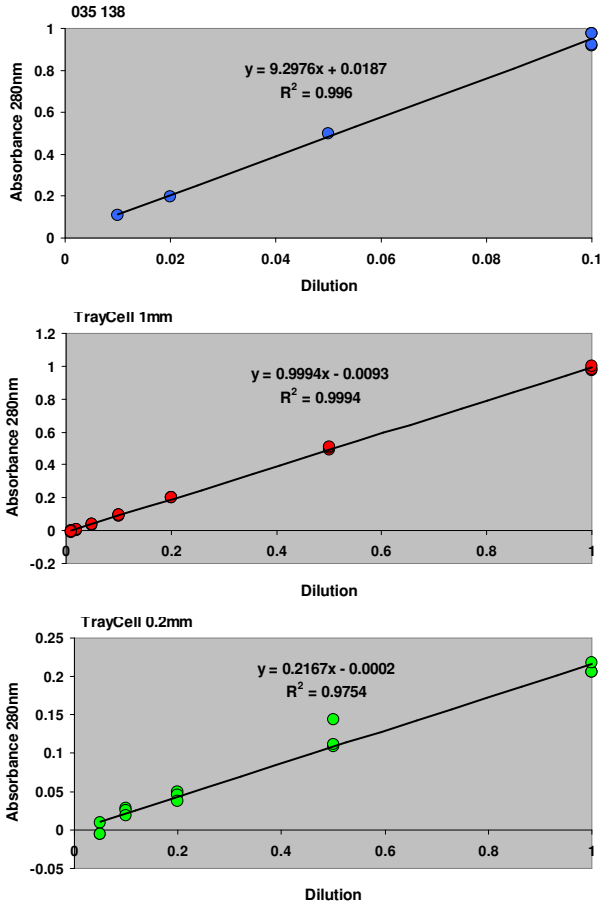

Figure 3: Standard curve plots for the sample dilutions measured at each path length.

Considering an absorbance value of 0.05 to be the lowest practical reading for reproducible results, the results here indicate that the realistic limits for protein detection with the TrayCell are approximately 2mg/ml with the 0.2mm cap and 0.5mg/ml with the 1mm cap. The lowest concentration with a 10mm path length cuvette is approximately 0.05mg/ml.

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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. Therefore if the sample is sufficiently concentrated, only a very small volume is required. Concentration values obtained with the TrayCell are comparable to those obtained with standard 10mm path length cuvettes.

Due to the virtual dilution factor of the caps, it is possible to measure protein of a much higher concentration (greater than 10mg/ml) without diluting the sample. The direct UV method using the TrayCell is rapid and requires very little sample which can be recovered after measurement if required.

Applications where the TrayCell might be used could include:

1. Measurement of protein stocks/standards for concentration verification.
2. Rapid measurement of protein concentration after extraction.
3. Determination of protein concentrations produced in expression systems.
4. Other applications where absorbance levels are too high to be read in conventional 10mm path length cuvettes.

References

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