Sample Analysis Report
Number 50

Precision Microvolume Spectrophotometry of Lysosyme at 100mg/ml

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Introduction

The unavailability of instruments able to provide accurate measurement of the absorbance characteristics of proteins in highly concentrated solutions has presented a significant barrier to research. The development of a new type of Microvolume cuvette which can be installed in a standard Cary 4000/5000 or 6000i spectrophotometer and yet deliver performance in 1ul (0.2mm pathlength) similar to the performance of a top quality research-grade spectrophotometer in a volume a thousand times greater permits a new level of investigation at concentrations which were previously impossible to measure.

The limits to resolution of spectrophotometry are generally set by the photometric noise level and stray light performance of the instrument. Thus a 50 mg/ml Protein sample that would have an absorbance of approximately 100 Abs in a centimeter pathlength can be measured in a 1/50 cm pathlength, typical of 1ul sample capacity instrumentation, with an absorbance of 2 Abs. This will probably be close to the limits of most budget instruments and precision will be poor. A sample with 1mg/ml could be expected to have a 1/50 cm pathlength absorbance of 0.002 Abs, which is already well below the noise threshold of most instruments, even those designed for small volume samples. This presents a problem for researchers who have small volumes of High or low concentration samples.

By coupling the powerful performance of the Cary 4000 reference grade spectrophotometer with the small sample capability and sample path precision of the Hellma Traycell the problem of quantitation of small quantities of Protein in small volumes is solved. Noise levels as low as 0.0002 Abs with samples of 1ul mean those protein concentrations of as low as 100ug/ml can be measured in a single microlitre. (Or down to approx 4 ng DNA.) The instrumentation also offers the significant advantage of being able to take a scan of the sample at this concentration, which can verify purity. If 4ul of sample can be spared, the increase in path-length from 0.2 to 1mm means that as good or better results can be obtained with samples containing as little as 20ug/ml

The dynamic range of the Cary 4000, to in excess of 9 Abs in normal cuvettes (see sample Analysis report Number 37) or about 7 Abs in the TrayCell, allows Protein concentrations from about 20ug/ml-100,000 ug/ml to be measured. (Rear beam attenuation is required with readings above 4A)

Instrumentation

The Cary 6000i spectrophotometer (below) fitted with manual and automated rear beam attenuators and a 1 cm cell holder modified to provide maximum precision with a Hellma Traycell.

Materials and Reagents

Lysosyme manufactured by Sigma Aldrich was diluted in distilled deionised water to 100mg/ml

Conditions

The Cary 6000i was used for this experiment but in the UV/Vis region where the same results could be expected from a Cary 4000, between 200 and 380 nm. The Traycell was mounted and aligned to 3.8%T using the precision cell holder. A bandwidth of 2 nm, scan speed of 60 nm/min with an averaging time of 2 seconds and data interval of 2 nm were used. Rear beam attenuation to 1.3 Abs was used to offset the internal attenuation of the Traycell

Discussion

The sample of lysosyme studied did not behave as initially expected. The scans were repeated to show that the instrument was measuring a precisely reproducible phenomena, but the expected protein peak at 280 nm was replaced by a 'plateau' region at 2 abs (equivalent to about 40 Abs) whereas a peak of intensity up to 200Abs was expected at 280 by simple extrapolation of 1 cm path measurements of dilutions of the sample.

This observation indicates that highly concentrated solutions are behaving in an unusual way with respect to absorbance measurements. This observation may well be indicative of molecular association bonding or of bond distortion.

Conclusion

Although this is a very simple experiment, the unexpected results show that there is a physical phenomena taking place in the concentrated solution which is radically different from an expected extrapolation of dilute sample behavior. This observation should be confirmed by precision dilution studies and experiments with different pathlengths.

Graphics and Tables

Figure 1 Lysosyme, 100mg/ml, scanned in a 0.2 mm pathlength Hellma Traycell

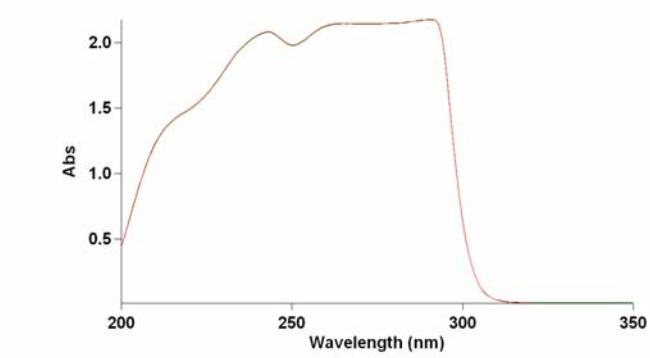


Figure 2 Detail from baseline at 315nm showing precision and flatness of baseline (+- 0.0006 Abs)

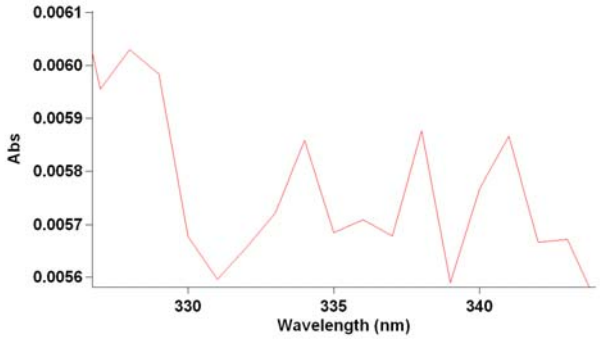
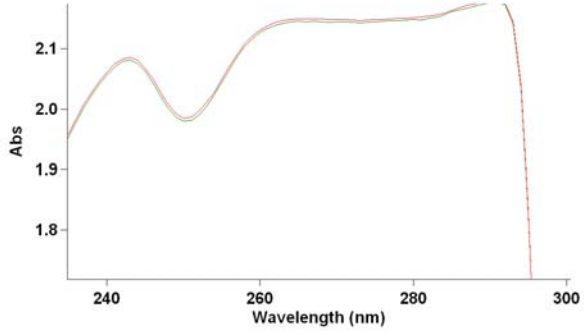


Figure 3 *Reproducibility of scans with 12 minute delay between readings*



These data were observed using standard Varian Cary UV instruments on 28/09/2005
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