Are you measuring your protein correctly?
Comparison of microvolume spectrophotometers

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Introduction

Spectroscopy is a technique used to measure biomolecules due to their interaction with electromagnetic radiation. Proteins have maximal absorbance of electromagnetic radiation in the form of light at 280 nm. By applying the Beer-Lambert law, where absorbance depends linearly on the concentration, protein concentrations can be quantified[1]. Microvolume spectrophotometers are commonly used to measure the concentrations of nucleic acids. However, due to unreliability with sample column breakage with detergent-based samples [2], they are not routinely accepted for protein analysis. A market leader was compared with a new instrument, the DeNovix DS-11+ for accuracy and reproducibility of protein concentrations.

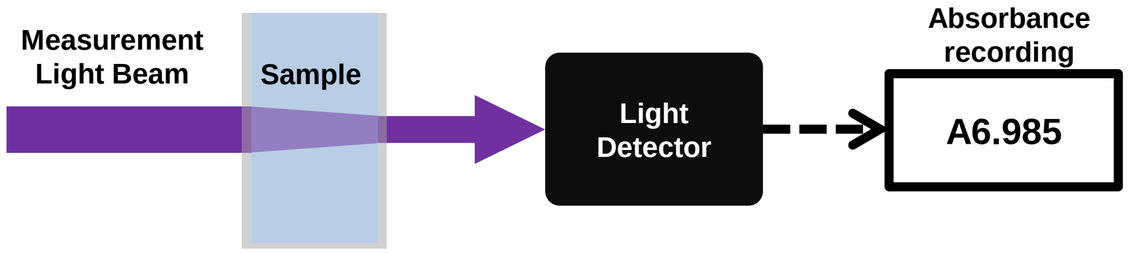

Figure 1: Measurement principle for solution samples.

Quantification of purified M2-1 protein in salt-based buffer

Table 1: Bovine M2-1 protein quantification using a spectrophotometer, DS-11+ and NanoDrop 2000. n/N=3/9.

Method	Spectrophoto-meter (mg/mL)	DS-11+ (mg/mL)	NanoDrop 2000 (mg/mL)
Sample A	12.30	14.24	13.05
Sample B	13.80	13.74	5.36
Sample C	14.50	14.93	13.67
Mean±SEM	13.5 ± 0.65	14.30 ± 0.35	10.69 ± 2.67

Purified bovine M2-1 protein was divided into 3 samples: A, B and C. Bovine M2-1 was suspended in a buffer containing 20 mM Tris/HCl pH 7.5, 5 % glycerol, 1 mM DTT and 600 mM NaCl. Quantification of each sample was carried out using a spectrophotometer (10 mm path length; 1 in 10 dilution) as an independent, reference concentration, the DeNovix DS-11+ and NanoDrop 2000. The DS-11+ produced concentrations with a small range between each sample which were close to the reference concentrations measured with the spectrophotometer. The NanoDrop 2000 had a large range of concentrations measured due to failure to measure protein sample B correctly.

Quantification of BSA in a range of Triton X-100 concentrations

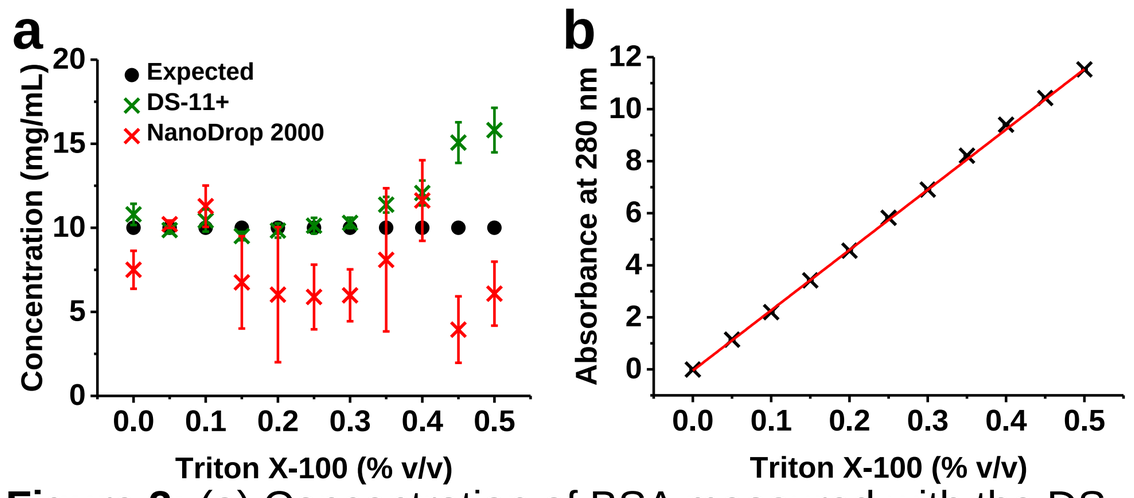

Figure 2: (**a**) Concentration of BSA measured with the DS-11+ and NanoDrop 2000 with an increasing Triton X-100 concentration. BSA samples were made to 10 mg/mL (expected) in H_2O. n/N=3/9. (**b**) Absorbance of Triton X-100 at 280 nm. n/N=1/3.

The DS-11+ measured BSA concentrations consistently closer to the expected value up to 0.3 % v/v Triton X-100. This may be attributed to the absorbance of Triton X-100 at 280 nm (Fig 2b). The NanoDrop 2000 performed poorly at Triton X-100 concentrations higher that 0.1 % v/v due to the sample column breaking. The sample column breakage occurs due to reduced surface tension in detergent containing solutions. Sample column breakage did not occur with the DS-11+.

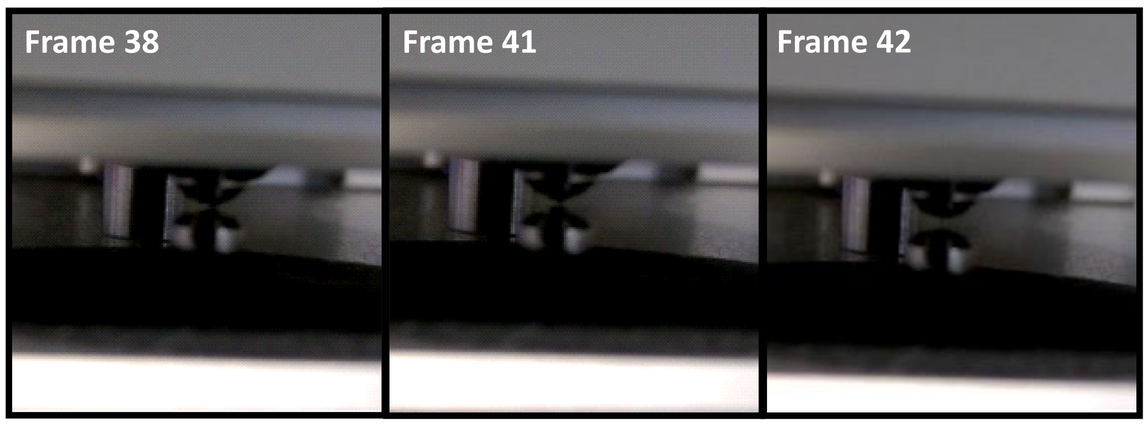

Figure 3: Frames from video of sample column breakage on the NanoDrop 2000 at 0.25 % v/v Triton X-100.

Conclusions

- The DS-11+ was more reliable for measuring proteins in both salt-based buffer and with Triton X-100 compared to the NanoDrop 2000.
- Column breakage occurred with both salt-based and Triton containing buffers on the NanoDrop 2000 resulting in poor reliability of protein quantification.
- The DS-11+ worked reliably up to 0.3 % v/v Triton X-100 whereas the NanoDrop 2000 only worked up 0.10 % v/v.

References

1. Schmid, Franz-Xaver(Apr 2001) Biological Macromolecules: UV-visible Spectrophotometry. In: eLS. John Wiley & Sons Ltd, Chichester. http://www.els.net [doi: 10.1038/npg.els.0003142]
2. Thermo Scientific (2009) *T044-TECHNICAL BULLETIN: Sample Reproducibility*, USA. www.thermoscientific.com

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