Solo VPE
Variable.Pathlength.Extension

The Effect of Inadequate Sample Mixing on UV Concentration Measurements

Summary / Abstract:

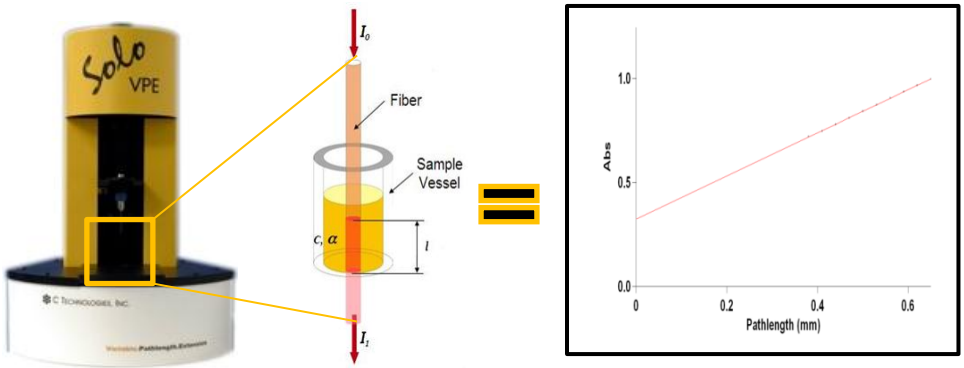
Frequently, structural changes within protein samples can take place that may alter experimental measurement results. Many problems can arise from the improper handling of these types of samples. Improperly mixed samples can cause results to be skewed and inconsistent. However, there is a simple step can reduce or eliminate this variability saving time and money by avoiding repeated measurement and investigations of unexpected results.

Vortex mixers are quite common in most laboratories. In biologics and analytical laboratories they may be used to mix the reagents of an assay or to mix protein samples. Proper sample vortexing helps ensure accurate measurement results.

A manual alternative to the electric vortex mixer is the "*finger vortex*" technique, which achieves mixing through repeated rocking of the sample while held between the thumb and finger. Though simple, this technique can take longer and will be less effective.

The data reported here clearly demonstrate the impact and importance of proper sample mixing when executing concentration measurements protocols.

Apparatus / Equipment



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Method & Results

The SoloVPE and Slope Spectroscopy offer a new type UV-Vis measurement method, specifically *Slope* measurements for rapid, accurate and reproducible concentration results. No longer are scientists bound to dilution factors and fixed path lengths. The SoloVPE precisely varies the measurement pathlength create a *Section* (Absorbance vs. Pathlength) plot that complies with Beer's Law. The slope of this data is directly is directly proportional to the sample concentration. This method combined with SoloVPE's pathlength resolution of 0.005 mm allows the system to make measurements of even the most highly concentrated samples without dilution and frequently without baseline correction.

For this application note, the concentration measurements of protein samples were made with the *SoloVPE Variable Pathlength* system. As is the case for most SoloVPE methods, the sample volume required is related to the concentration. Higher concentration samples require less volume than those that are more dilute. This constraint exists because the light travels vertically through the sample when using the SoloVPE and the height of the liquid in the sample vessel must exceed the maximum pathlength to be measured. The SoloVPE System accommodates a variety of vessel styles, including disposable UV plastic vessels. The different vessels allow users to minimize the sample volume required.

Analysis

Buffer/Baseline Correction: Baseline Correction is not required when the absorbance of the buffer does not display significant pathlength dependence. The way to determine whether baseline correction is required is to perform a Quick Slope measurement on your buffer media with no active in it. A Quick Slope result close to zero suggests that Baseline Correction is likely not required. The samples tested for this study did not require baseline correction.

Sample Preparation: Testing was performed on several sets of identical aliquots, all frozen for several days and then thawed on the bench top for approximately 2 hours.

- Bench top VWR "Vortex Genie 2" vortexer fitted with a rubber pad, set at setting 7 out of 12. Mixing as follows: 4-5 seconds vortexing , tip sample upside-down and "flick" once, tip sample right-side-up and "flick" once
- Used aliquot of 100 µl for each sample
- Note: Adequate mixing of higher volumes such as 350 µl in a 500 µl tube may require more than a short burst on the vortexer.

Sample Measurement: Traditional absorbance measurements rely on a single value measured through a fixed 10 mm cell which may have been diluted to be within the linear range of the spectrophotometer. This technique requires additional measurement/prep time, additional consumable costs and introduces dilution error since the sample has been changed to fit the measurement device. In contrast, the SoloVPE is capable of measuring the neat sample to accurately verify the concentration. The accuracy of the measurement is inherently verified based upon the quality of the regression which is reported as the R^2 value of the Slope regression line.

Dataset Name	Dataset Type	Wavelength (nm)	Slope (Abs/mm)	R2 Value	EC Value	Concentration	Concentration Units	Deviation
Sample 1 UNmixed Top Edge	Quick Slope	280	2.568829	0.999993	0.67	38.34074	mg/ml	21.9%
Sample 1 UNmixed Center	Quick Slope	280	2.259425	0.999993	0.67	33.72276	mg/ml	
Sample 1 UNmixed Bottom Edge	Quick Slope	280	3.421033	0.999994	0.67	51.06019	mg/ml	
Sample 1 mixed-1	Quick Slope	280	2.582346	0.999994	0.67	38.54248	mg/ml	0.1%
Sample 1 mixed-2	Quick Slope	280	2.580091	0.999989	0.67	38.50882	mg/ml	
Sample 1 mixed-3	Quick Slope	280	2.587551	0.999991	0.67	38.62017	mg/ml	
Sample 2 UNmixed Top Edge	Quick Slope	280	2.621776	0.999996	0.67	39.13098	mg/ml	21.3%
Sample 2 UNmixed Center	Quick Slope	280	2.807533	0.999981	0.67	41.90348	mg/ml	
Sample 2 UNmixed Bottom Edge	Quick Slope	280	3.549977	0.999375	0.67	52.98473	mg/ml	
Sample 2 mixed 1	Quick Slope	280	2.838958	0.999998	0.67	42.37251	mg/ml	0.2%
Sample 2 mixed 2	Quick Slope	280	2.830274	0.999987	0.67	42.2429	mg/ml	
Sample 2 mixed 3	Quick Slope	280	2.830891	0.999997	0.67	42.25211	mg/ml	
Sample 3 UNmixed Top Edge	Quick Slope	280	1.754107	0.999985	0.67	26.1807	mg/ml	19.6%
Sample 3 UNmixed Center	Quick Slope	280	2.415959	0.999988	0.67	36.05909	mg/ml	
Sample 3 UNmixed Bottom Edge	Quick Slope	280	2.591133	0.999994	0.67	38.67362	mg/ml	
Sample 3 mixed 1	Quick Slope	280	2.067957	0.999986	0.67	30.86502	mg/ml	0.3%
Sample 3 mixed 2	Quick Slope	280	2.0778	0.999982	0.67	31.01194	mg/ml	
Sample 3 mixed 3	Quick Slope	280	2.079516	0.999994	0.67	31.03755	mg/ml	
Sample 4 UNmixed Top Edge	Quick Slope	280	1.535014	0.999981	0.67	22.91065	mg/ml	31.2%
Sample 4 UNmixed Center	Quick Slope	280	1.94376	0.999995	0.67	29.01134	mg/ml	
Sample 4 UNmixed Bottom Edge	Quick Slope	280	2.817321	0.999997	0.67	42.04957	mg/ml	
Sample 4 mixed 1	Quick Slope	280	1.989722	0.999993	0.67	29.69734	mg/ml	0.4%
Sample 4 mixed 2	Quick Slope	280	1.999203	0.999986	0.67	29.83885	mg/ml	
Sample 4 mixed 3	Quick Slope	280	2.007156	0.999983	0.67	29.95755	mg/ml	
Sample 5 UNmixed Top Edge	Quick Slope	280	1.605388	0.999991	0.67	23.96102	mg/ml	27.3%
Sample 5 UNmixed Center	Quick Slope	280	2.359506	0.999993	0.67	35.2165	mg/ml	
Sample 5 UNmixed Bottom Edge	Quick Slope	280	2.833253	0.999996	0.67	42.28735	mg/ml	
Sample 5 mixed 1	Quick Slope	280	2.007208	0.999989	0.67	29.95833	mg/ml	0.5%
Sample 5 mixed 2	Quick Slope	280	2.002145	0.999994	0.67	29.88276	mg/ml	
Sample 5 mixed 3	Quick Slope	280	2.023209	0.999986	0.67	30.19714	mg/ml	

Conclusion

The SoloVPE is capable of make accurate measurements of highly concentrated protein samples using the unique Slope Spectroscopy method that dynamically characterizes the sample to achieve compliance with Beer's Law. A high R^2 value confirms a strong correlation result which is generally a strong indicator of an accurate measurement; however, the SoloVPE can only characterize the sample that is loaded into the system and in the absence of proper mixing, variability associated with the sample may mistakenly be attributed to the measurement system, which is why it is critical to include proper mixing techniques in the sample preparation section of the test protocol. Proper execution of this simple step will help ensure the accuracy of the Quick Slope results and in doing so save time and money by avoiding repeated measurements and analysis of unexpected results.