The Effect of Inadequate Sample Mixing on UV Concentration Measurements

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Abstract

Structural changes within protein samples can occur, especially over extended periods of time. These changes, in conjunction with improper sample handling, can cause results to be skewed and inconsistent. Proper mixing techniques can reduce or eliminate this variability, saving time and money by avoiding repeated investigations of unexpected results.

Vortex mixers are the most effective tools in achieving sample homogeneity. They are quite common in most laboratories and they may be used to mix the reagents of an assay or to mix protein samples. Proper sample vortexing is imperative as it helps to ensure accurate results.

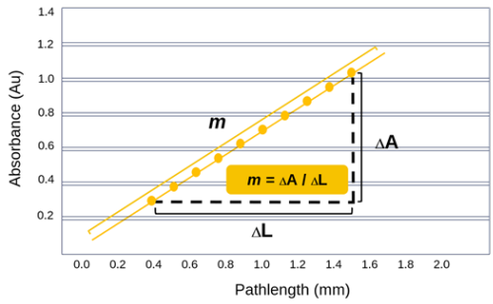
A manual alternative to the electric vortex mixer is the inversion technique. This technique achieves mixing through repeated rocking of the sample while held between the thumb and a finger. Though simple, this technique can take longer and will be less effective.

The data reported here clearly demonstrates the impact and importance of proper sample mixing when measuring samples.

Method and Results

The Slope Spectroscopy® method offers a new ultraviolet-visible (UV-Vis) method in which slope-based measurements can achieve rapid, accurate, and reproducible concentration results. Scientists are no longer bound to dilution factors and fixed-pathlength measurements. The CTech™ SoloVPE® System precisely varies the measurement pathlength creating a section (absorbance vs. pathlength) plot that complies with the Beer-Lambert law (Figure 1). The slope is required to have a coefficient of determination (R^2) ≥0.999, resulting in the absorbance varying

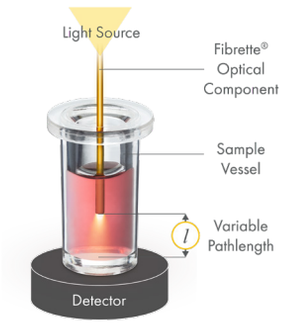
Figure 1. Variable pathlength UV-Vis absorbance readings



proportionally with respect to the changes in pathlength. This method, combined with its pathlength resolution of 0.005 mm, allows the system to measure even the most highly concentrated samples, without dilution and frequently without baseline correction.

For this application note, the concentration measurements of multiple protein samples were analyzed. As is the case for most applications in which the SoloVPE System is used, 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, using Variable Pathlength Technology, travels vertically through the sample and the height of the liquid in the sample vessel must exceed the maximum pathlength to be measured (Figure 2). The SoloVPE System accommodates a variety of vessel styles, including disposable UV plastic vessels provided by Repligen. The different vessels allow users to minimize the sample volume required.

Figure 2. Variable Pathlength Technology (VPT)



Analysis

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

- Benchtop VWR "Vortex Genie 2" vortexer fitted with a rubber pad, set at 7 (out of 12 settings). Mixing as follows:
  1. Four to five seconds vortexing the entire vial.
  2. Tip sample upside down and flick once.
  3. Tip sample right side up and flick once.
- Used aliquot of 120 µl for each sample benchtop.
  - The samples were only aliquoted when they were ready to be measured.
- No bubbles were present.
  - If there were bubbles, they would be removed by tapping the side of the vessel or by pipetting them out.
  - Micro bubbles are negligible.
- The time between mixing and data acquisition was minimal.
  - The sample is never left to sit.
  - Mix the sample again if there has been an extended period of time between replicates.
  - Adequate mixing of higher volumes (more than 200 µl) may require more time on the vortexer.

Sample Measurement: Traditional absorbance measurements rely on a single value measured through a fixed 10 mm cell, which is generally diluted to be within the linear range of the spectrophotometer. This technique requires additional preparation time, increases consumable costs, and introduces dilution error to fit the linear range of the device. In contrast, the SoloVPE System measures neat samples to accurately verify the concentration. The accuracy of the measurement is inherently verified by the quality of the regression, which is reported as the R^2 value of the slope.

Conclusion

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

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Table 1. Unmixed vs. mixed sample data

Dataset Name	Dataset Type	Wavelength (nm)	Slope (Abs/mm)	R^2 Value	EC Value	Concentration (mg/ml)	Deviation
Sample 1 Unmixed Top Edge	Quick Slope	280	2.568829	0.999993	0.67	38.34074	21.9%
Sample 1 Unmixed Center	Quick Slope	280	2.259425	0.999993	0.67	33.72276	
Sample 1 Unmixed Bottom Edge	Quick Slope	280	3.421033	0.999994	0.67	51.06019	
Sample 1 Mixed-1	Quick Slope	280	2.582346	0.999994	0.67	38.54248	0.1%
Sample 1 Mixed-2	Quick Slope	280	2.580091	0.999989	0.67	38.50882	
Sample 1 Mixed-3	Quick Slope	280	2.587551	0.999991	0.67	38.62017	
Sample 2 Unmixed Top Edge	Quick Slope	280	2.621776	0.999996	0.67	39.13098	21.3%
Sample 2 Unmixed Center	Quick Slope	280	2.807533	0.999981	0.67	41.90348	
Sample 2 Unmixed Bottom Edge	Quick Slope	280	3.549977	0.999375	0.67	52.98473	
Sample 2 Mixed-1	Quick Slope	280	2.838958	0.999998	0.67	42.37251	0.2%
Sample 2 Mixed-2	Quick Slope	280	2.830274	0.999987	0.67	42.2429	
Sample 2 Mixed-3	Quick Slope	280	2.830891	0.999997	0.67	42.25211	
Sample 3 Unmixed Top Edge	Quick Slope	280	1.754107	0.999985	0.67	26.1807	19.6%
Sample 3 Unmixed Center	Quick Slope	280	2.415959	0.999988	0.67	36.05909	
Sample 3 Unmixed Bottom Edge	Quick Slope	280	2.591133	0.999994	0.67	38.67362	
Sample 3 Mixed-1	Quick Slope	280	2.067957	0.999986	0.67	30.86502	0.3%
Sample 3 Mixed-2	Quick Slope	280	2.0778	0.999982	0.67	31.01194	
Sample 3 Mixed-3	Quick Slope	280	2.079516	0.999994	0.67	31.03755	
Sample 4 Unmixed Top Edge	Quick Slope	280	1.535014	0.999981	0.67	22.91065	31.2%
Sample 4 Unmixed Center	Quick Slope	280	1.94376	0.999995	0.67	29.01134	
Sample 4 Unmixed Bottom Edge	Quick Slope	280	2.817321	0.999997	0.67	42.04957	
Sample 4 Mixed-1	Quick Slope	280	1.989722	0.999993	0.67	29.69734	0.4%
Sample 4 Mixed-2	Quick Slope	280	1.999203	0.999986	0.67	29.83885	
Sample 4 Mixed-3	Quick Slope	280	2.007156	0.999983	0.67	29.95755	
Sample 5 Unmixed Top Edge	Quick Slope	280	1.605388	0.999991	0.67	23.96102	27.3%
Sample 5 Unmixed Center	Quick Slope	280	2.359506	0.999993	0.67	35.2165	
Sample 5 Unmixed Bottom Edge	Quick Slope	280	2.83323	0.999996	0.67	42.28735	
Sample 5 Mixed-1	Quick Slope	280	2.007208	0.999989	0.67	29.95833	0.5%
Sample 5 Mixed-2	Quick Slope	280	2.002145	0.999994	0.67	29.88276	
Sample 5 Mixed-3	Quick Slope	280	2.023209	0.999986	0.67	30.19714	