The SoloVPE (Variable Pathlength Extension): A Powerful New Approach for Biological Spectroscopy

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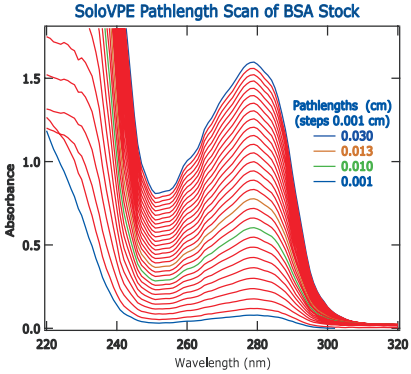
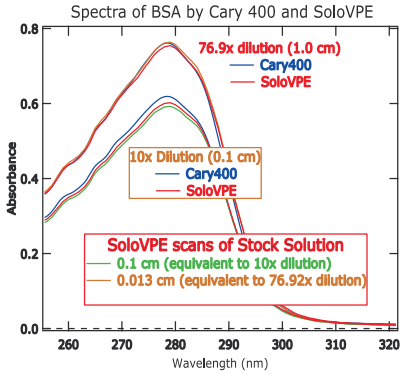
Abstract: 130

Spectroscopic Advantage of the SoloVPE

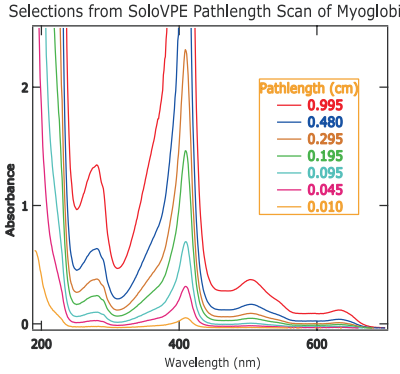
Spectra Recording with NO DILUTION Using ONE CUVETTE



Useful for Samples
- of Unknown Concentration
- from Production
- for NMR Experiments
- for Crystallography



Two dilutions of the BSA stock solution were made so that spectra could be measured on a Cary 400 using indicated cuvettes. Spectra at equivalent pathlength from the SoloVPE are shown for the dilutions.



Proteins with Multiple Extinction Coefficients
- Fluorophore labeled
- Heme containing
- Flavin containing

Beer's Law A = E c l

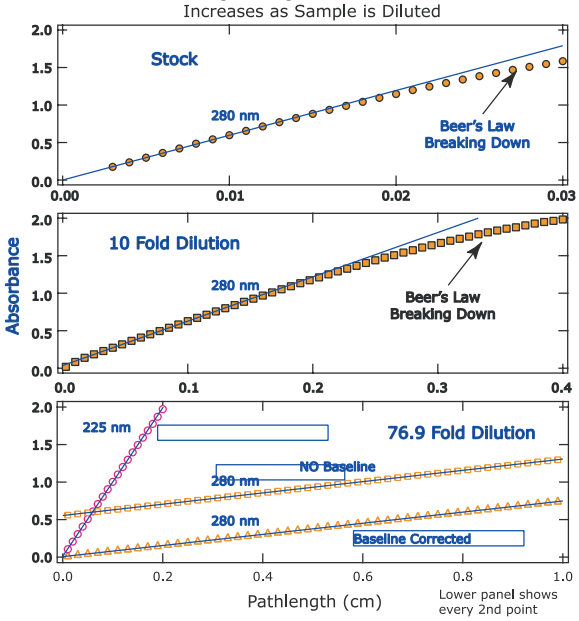
where A is the absorbance of a solution of thickness, l, containing molecules at concentration, c. E is a property of the molecule, its extinction coefficient, and has units the reciprocal of c and l. This relationship is linear in both c and l. Deviations from linearity are well known, and may arise from instrumental limitations or changes in molecular properties in solution as c increases. The SoloVPE permits quick and facile construction of a plot of A vs l (called a section plot), the slope of which is Ec.

Slope of SoloVPE Section Plot = Ec

knowledge of either E or c permits calculation of the other. The slope calculation uses multiple data points, this redundancy improves the accuracy of a computed c or E, over what would be accomplished using a single point absorbance measurement. As shown with the data here, ratios of slopes for different solutions of the same molecule provide direct measurement of concentration ratio (dilution factor). For a molecule with multiple absorptions bands, slopes of section plots at each peak provide a facile means of determining all the extinction coefficients if any one of them is known or if the concentration is known then all are determined. The large range of pathlengths available with SoloVPE, permit such studies to be carried out on a high concentration sample so that even minor bands can be characterized.

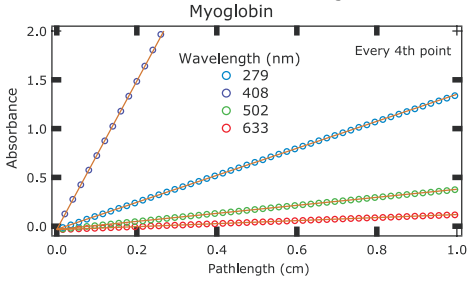
The Role of the SoloVPE in Quantitation

Accessible Pathlength Range before Beer's Law Breakdown Increases as Sample is Diluted



Lower panel shows every 2nd point

Section Plots at Various Wavelengths For Myoglobin



For BSA, an $E^{1\%}_{1\,cm}$ at 280 nm of 6.67 was used. The table shows calculated dilution factors, or alternatively the concentration of BSA in the stock and dilutions. These calculations are based on the slopes shown in the figure to the left. The column [BSA] stock uses the experimental dilution factor.

Note in the case of the 76.9x sample, the same result is obtained with or without baseline subtraction.

Data for the stock solution measured on a Cary 400 using spectra shown in the above panel.

Relative Myoglobin Extinctions

nm	Slope	$\varepsilon_{nm}/\varepsilon_{279}$
279	1.3835	1.0
408	7.6003	5.494
502	0.4110	0.297
633	0.1548	0.112

For the BSA data, the ratio of slopes at 225 nm to that at 280 nm and the $E^{1\%}_{1\,cm}$ at 280 nm, leads to an $E^{1\%}_{1\,cm}$ of 89.2 at 225

Calculations For BSA Solutions

	Section Slope	Dilution Factor	[BSA] Sample	[BSA] Stock
SoloVPE Sections				
Stock	59.69	1		89.48
10x	5.91	10.1	8.86	88.62
76.9x	0.74	80.4	1.11	85.66
76.9x*	0.75	79.4	1.13	86.69
Cary 400				
Stock	No short pathlength Available			
10x	0.61†		9.16	91.60
76.9x	0.75†		1.13	86.92

*Section plot without baseline correction
†Absorbance values

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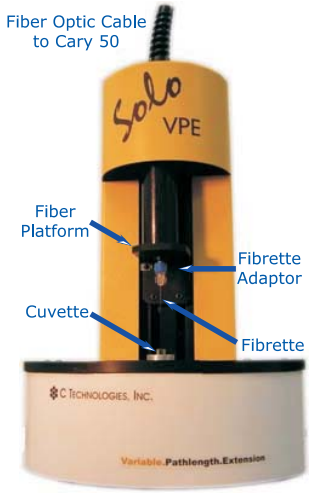
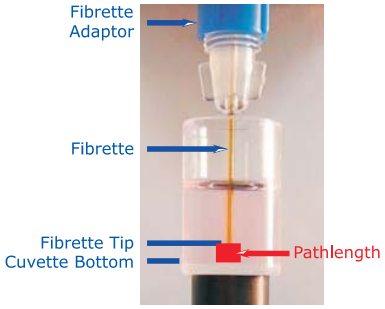
Absorption spectroscopy, often a tool used in the structural and functional characterization of biomolecules, is frequently used for both direct and indirect quantitation of biomolecules. At high concentrations, such as encountered in NMR experiments, crystallography trials, or simply in protein/nucleic production facilities, the absorbance of samples would not be readly measurable by most spectrophotometers. To measure such samples, one must either dilute the sample (yet another error source), have access to cuvettes with a variety of pathlengths (which in most laboratories is quite limited), or have access to one of the instruments specifically developed to address the measurements of high absorbances.

The SoloVPE provides a new approach to spectroscopy in which using a single cuvette a user has complete discretion in choosing the pathlength, thereby permitting measurements of dilute as well as concentrated samples. The ability to record whole spectra (or select wavelengths) as a function of pathlength permits novel applications of Beer's law not readily possible before.

Introduction to the SoloVPE



The SoloVPE operates in conjunction with a Cary 50 spectrophotometer. The Cary provides spectrally separated light to one end of a fiber optic cable which has its other end attached to a fiber platform in the SoloVPE. At the platform, the light is delivered to one end of a disposable "Fibrette" (a single piece of optical fiber) attached to the fiber platform by the Fibrette adaptor. The other end of this Fibrette is submerged within the sample in a cuvette located below the fiber platform. Light emerging from this submerged tip, passes through the sample to a detector located beneath the cuvette. The detector returns an electrical signal to the Cary 50 proportional to the light intensity impinging on it for further processing into an absorbance signal.



Pathlength for the SoloVPE is defined by the distance from the submerged tip of the Fibrette to the bottom, inside surface of the cuvette. The depth of submersion is controlled by the position of the fiber platform to which the Fibrette is attached via the Fibrette Adaptor. The vertical position of the fiber platform is itself determined by a software driven stepper motor; thereby permitting precise changes in the positioning of the Fibrette tip within the sample. This control places the pathlength used for any given measurement at the complete discretion of the user.

Some Advantages and Applications

- Eliminates errors due to dilution
- Measures dilute as well as concentrated samples
- Improves accuracy over single point absorbance measurements
- Prevents cross-contamination by use of disposable Fibrettes
- Recoverable samples
- Make additions and re-measure
- Characterize systems with multiple absorption bands with very different extinctions
- Measure extinction coefficients
- Quantify extent of dilution
- Speed

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