

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

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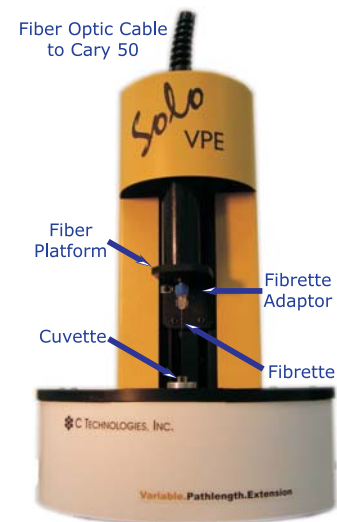
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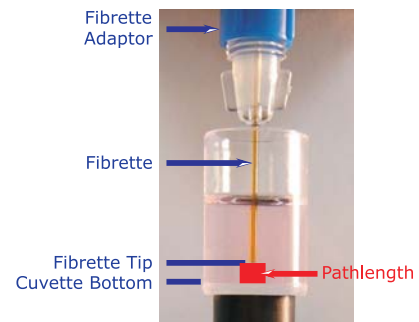
Absorption spectroscopy, often a tool used in the structural and functional characterization of biomolecules, is frequently used for both direct and indirect quantitation of biomolecules. At high concentrations, such as encountered in NMR experiments, crystallography trials, or simply in protein/nucleic production facilities, the absorbance of samples would not be readily 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.



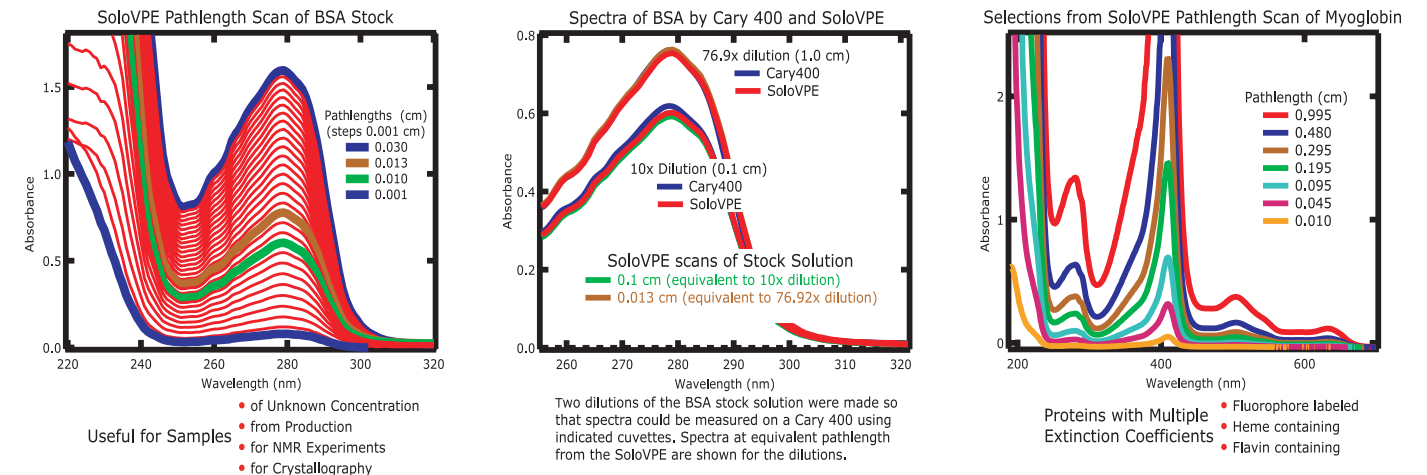
Pathlength for the SoloVPE is defined by the distance from the submerged tip of the Fibrette to the bottom, inside surface of the cuvette. The depth of submersion is controlled by the position of the fiber platform to which the Fibrette is attached via the Fibrette Adaptor. The vertical position of the 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

Spectroscopic Advantage of the SoloVPE

Spectra Recording with NO DILUTION Using ONE CUVETTE



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

