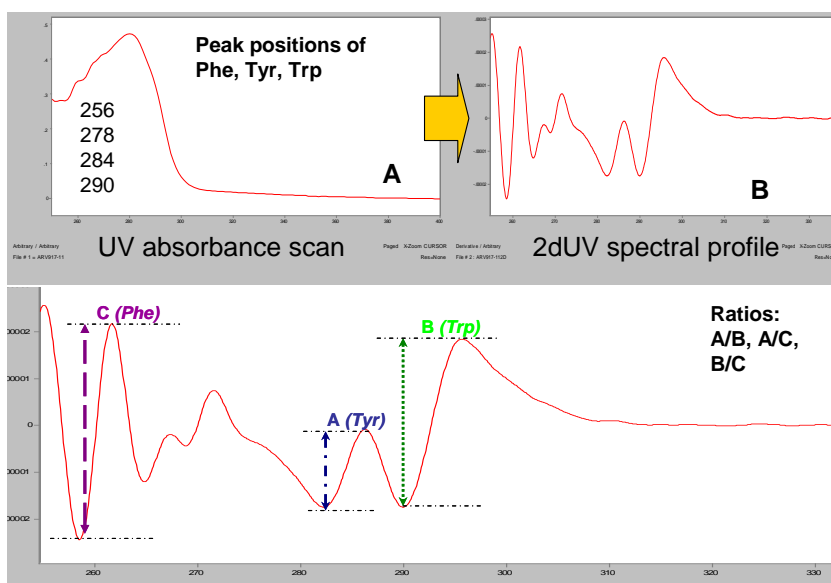


Second-derivative UV analysis (2dUV)



Accelerating Development for Optimal Results

Second-derivative UV analysis (2dUV)



2dUV information on tertiary structure

Figure A and Figure B are zero-order and second-derivative (2dUV) absorbance spectra of a protein. Figure C is an expanded view of Figure B showing peaks for Phe (C), Try (A), and Trp (B) which are actually valleys in the 2dUV spectrum and A/B, A/C, B/C are peak ratios calculated using peak-valley distances as shown in Figure.

Principle of 2dUV Spectroscopy

The derivative spectra in the 250-300 nm absorbance region of the three aromatic amino acids (Phe, Tyr, Trp) can be employed to monitor subtle structural changes in the tertiary protein conformation through changes in peak positions and peak ratios. The changes are directly correlated to changes in the microenvironments around the aromatic amino acids. Highly reproducible and accurate measurements of amino acid derivative peaks can be made using of a photodiode array UV instrument.



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BIOSYSTEMS INC.
High Throughput Development

1061 Serpentine Lane, Suite E, Pleasanton CA 94566
Tel. 510.367.0528, www.htdcorp.com



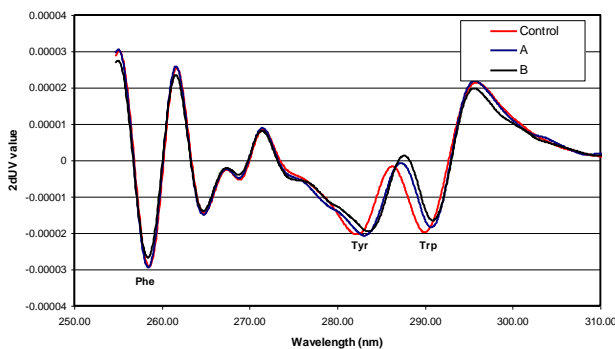
Advantages of using 2dUV in Protein Formulation Studies

1. Provides early information on subtle conformational changes that can precede loss of bioactivity as well as chemical and physical degradation (see example below).
2. This method can determine the effect of various formulation variables (pH, ionic strength, stabilizers) on protein stability by monitoring changes in tertiary protein conformations.
3. In contrast to fluorescence spectroscopy which only reports structural information on Tyr, 2dUV analysis provides average microenvironment information on three reporter amino acids (Phe, Tyr, Trp).
4. High-throughput assay requiring small sample volume over a wide protein concentration (0.1 – 200 mg/ml) obtained by UV measurements on a diode-array spectrophotometer.
5. Highly sensitive, reproducible and non-destructive.

Applications of 2dUV spectroscopic analysis

- Tertiary structural conformation analysis
- Melting/Denaturation curves
- Protein quantification in presence of preservatives

Example of 2dUV in Protein Formulation studies: Correlation between bioactivity and tertiary conformation.



- A protein is stressed thermally at 50C over time and protein activity is correlated with protein conformational stability using 2dUV spectroscopy.
- Loss in activity is correlated with changes in protein conformation (changes in A/B ratios and peak positions in Tyr and Trp).