

HTD

BIOSYSTEMS INC.

High Throughput Development



Second-derivative UV analysis (2dUV)

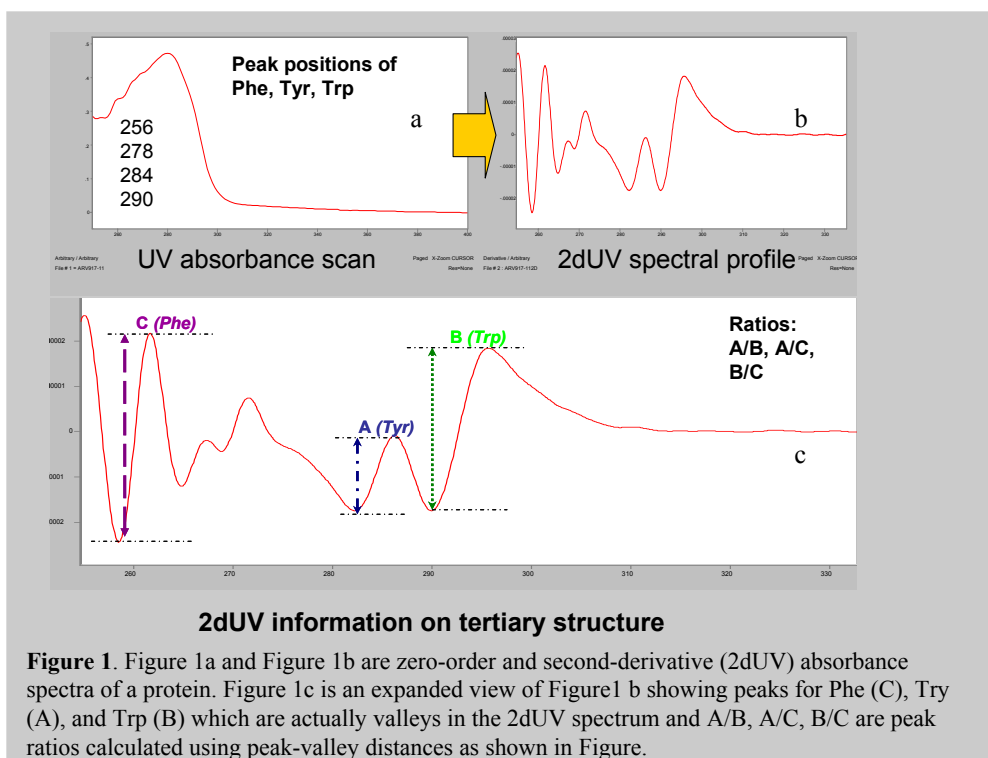


Figure 1. Figure 1a and Figure 1b are zero-order and second-derivative (2dUV) absorbance spectra of a protein. Figure 1c is an expanded view of Figure 1b showing peaks for Phe (C), Tyr (A), and Trp (B) which are actually valleys in the 2dUV spectrum and A/B, A/C, B/C are peak ratios calculated using 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.

Applications

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



Figure 2. A photodiode array UV spectrometer.



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.

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

In this example (Figure 3), a protein is stressed thermally at 50°C over time. Table 1 shows that loss in activity is correlated with changes in protein conformation (changes in A/B ratios and peak positions in Tyr and Trp).

Figure 3. The effect of thermal denaturation on a tertiary structure of a protein, monitored by 2dUV analysis. Symbols: A) 65 min at 50°C & B) 120 min at 50°C.

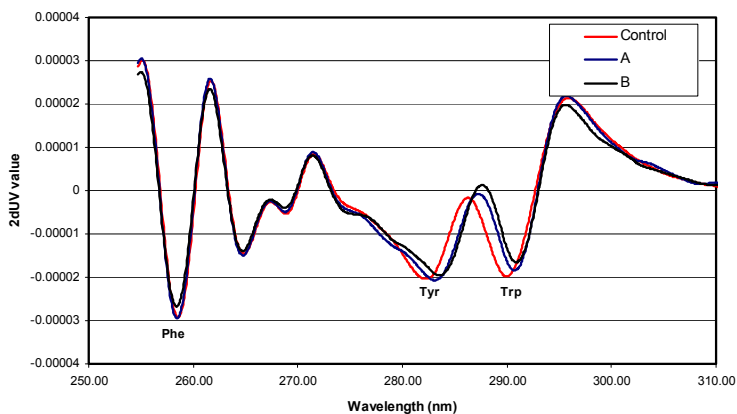


Table 1. Correlation between protein activity and conformational stability

Sample	Time/Temp.	Activity (IU/ml)	A/B ratio	Tyr peak position	Trp peak position
Control	-	100	0.454	282.47	290.27
A	65 min/50C	48	0.496	283.43	290.97
B	120 min/50C	26	0.573	283.81	291.16

Please contact HTD Biosystems for 2dUV analysis of your protein at : htd@htdcorp.com