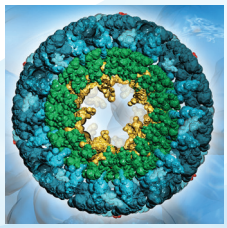
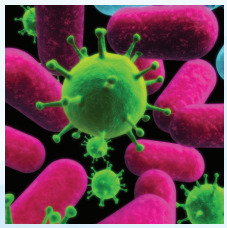
HORIBA Scientific

Absorbance

Protein A280 for Protein Concentration





Application Note
Life Sciences

Figure 1: Duetta 2-in-1 fluorescence and absorbance spectrometer

Introduction

The new HORIBA Duetta fluorescence and absorbance spectrometer offers many unique benefits for molecular spectroscopy. It is primarily thought of as a spectrofluorometer that combines absorbance and fluorescence spectroscopy to correct the fluorescence fingerprint for concentration-related effects. To learn more about this unique inner-filter Effect (IFE) correction, refer to our Duetta Applications Note entitled: "Automatic Correction of Fluorescence Spectra for Primary and Secondary Inner-filter Effects (IFE) with Duetta™ ". However, it is important to note that Duetta can also be used as a precise absorbance spectrometer. This application note explores the use of Duetta for a common absorbance application, the Protein A280 application.

Protein A280

Knowing the protein concentration is commonly required in studies of protein biochemistry and molecular biology. The concentration of a protein can be acquired by measuring the absorbance at 280 nm and then using the Beer-Lambert law (Equation 1):

Equation 1: Beer-Lambert law

$$A = \epsilon * b * c$$

where A is absorbance, ϵ is molar absorptivity in $M^{-1}cm^{-1}$, b is cell path length in cm, and c is concentration in M (mol/L). To get the molar absorptivity of a protein at 280 nm, (ϵ_{280}) the Edelhoch method is used, where the number of tryptophan, tyrosine, and cysteine residues in the protein are used to calculate the ϵ_{280} with Equation 2. (Edelhoch, 1967) (Gill, 1989) (C. Nick Pace, 1995)

Equation 2: Protein Absorptivity at 280 nm calculated from the number of tryptophan, tyrosine, and cysteine amino acid residues in the protein sequence

$$\epsilon_{280\ nm} = (\#Trp * 5500) + (\#Tyr * 1490) + (\#Cys * 125)$$

Duetta 2-in-1 fluorescence and absorbance spectrometer provides a software application called Protein A280 that will measure the absorbance of a protein solution and calculate the concentration of that protein based on knowledge of the amino acid. This easy-to-use App in EzSpec software makes protein determination quick and hassle-free.

Equation 3: Beer-Lambert law applied to calculate protein concentration from Absorbance at 280 nm (A280), cell path length (b), and calculated molar absorptivity ($\epsilon_{280\ nm}$)

$$[protein] = A280/(b * \epsilon_{280\ nm})$$

Experiment and Results

Absorbance spectra were measured for serial dilutions of bovine serum albumin (BSA) protein in water. The structure of BSA is shown in Figure 2.

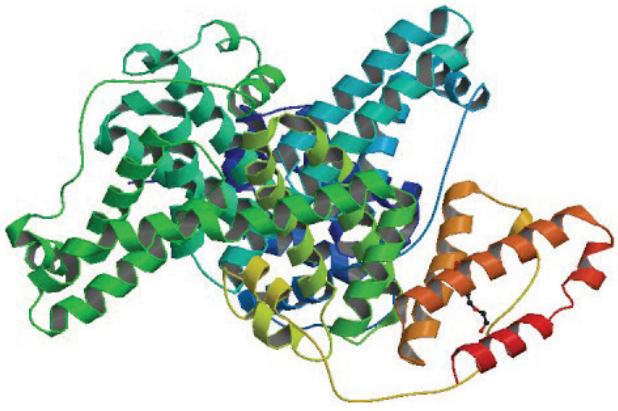


Figure 2: Structure of bovine serum albumin protein (Bujacz, 2012)

The concentration of each solution was determined using the Protein A280 application, with the given parameters of 3nm band pass, 0.1 sec integration time, and scanned absorbance from 250nm to 450nm with 1nm step increment. *A priori* information on the number of tryptophan, tyrosine, and cysteine residues is necessary for the Protein A280 application to calculate the total combined molar absorptivity of BSA and the concentration. This method icon is shown in Figure 3.

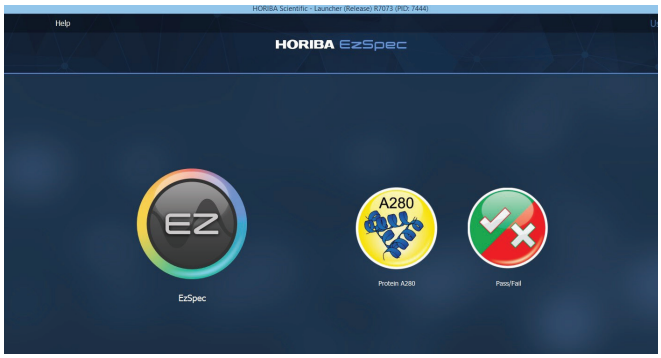


Figure 3: Duetta's EzSpec software launcher screen displays the Protein A280 App that opens a dedicated applications program

BSA is known to have 3 tryptophan residues, 21 tyrosine residues, and 35 cysteine residues. This information was input into the Protein A280 software app. The structure of BSA was obtained from the protein data bank entry from (Bujacz, 2012).

Protein A280

Sample Info

	Sample Name	# of Tryptophans(W)	# of Tyrosines(Y)	# of Cysteines(C)
1	BSA 1	3	21	35
2	BSA 2	3	21	35
3	BSA 3	3	21	35
4	BSA 4	3	21	35
5	BSA 5	3	21	35

Figure 4: Method window for the Protein A280 App, with sample information input for absorbance measurement of bovine serum albumin solutions

The absorbance spectrum of the BSA dilution series, having a peak around 280 nm, is shown by Figure 4. From the absorbance at 280 nm, and the number of tryptophan, of BSA and the combined molar absorptivity values are calculated for each solution, and displayed in the table shown in Figure 5.

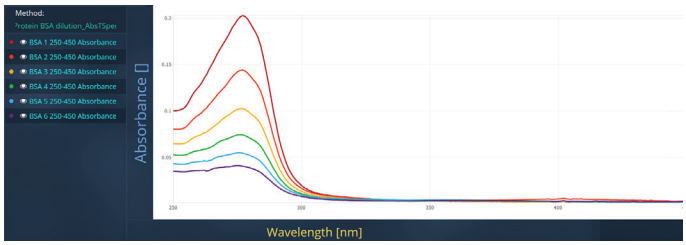


Figure 5: Spectra measured for BSA on Duetta in Protein A280 Application

	Sample Name	A(280 nm)	# Trp	# Tyr	# Cys	Molar absorptivity (1/M*cm)	Cell path length (cm)	Concentration(mol/L)
1	BSA1	0.194475	3	21	35	228165	1	8.52342e-07
2	BSA2	0.137518	3	21	35	228165	1	6.02713e-07
3	BSA3	0.0971861	3	21	35	228165	1	4.25947e-07
4	BSA4	0.0701678	3	21	35	228165	1	3.07531e-07
5	BSA5	0.0515468	3	21	35	228165	1	2.25919e-07
6	BSA6	0.0382164	3	21	35	228165	1	1.67494e-07

Figure 6: Protein A280 molar absorptivity and concentration results for six solutions of BSA protein

Summary

The Protein A280 application in EzSpec software allows a user to determine the concentration of a protein solution quickly and easily. The concentrations of six solutions of BSA were determined in the range of 0.7 to 3.7 µM in this experiment. The Duetta has the capability of measuring this same protein in the range of 10 nM to approximately 20 µM concentration (absorbance peak range of 0.005 to >1.0). The range of concentrations for other proteins will vary based on the molar absorptivity. Duetta 2-in-1 fluorescence and absorbance spectrometer is designed to conveniently measure protein absorbance and concentration..

References

Bujacz, A. (2012). Structures of bovine, equine and leporine serum albumin. Acta Crystallographica Section D, 1278-1289. Retrieved from https://www.rcsb.org/structure/4f5s

C. Nick Pace, F. V. (1995). How to measure and predict the molar absorption coefficient of a protein. Protein Science, 2411-2423.

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Gill, S. a. (1989). Calculation of protein extinction coefficients from amino acid sequence data. Analytical Biochemistry, 319-326.

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