MOSBRI Molecular-Scale Biophysics Research Infrastructure Basic Level School

UV/Visible Spectroscopy SOP

Simple protocol for a UV spectrum acquired with a double beamline spectrophotometer

Description:

Protein quantification at 280 nm is monitored by recording a full spectrum between 240 and 340 nm. Measurements are performed with 60 µL of buffer and sample at 20°C in a 1 cm quartz cell, reference 105.202-QS.10 (Hellma, France) using a JASCO V-750 spectrophotometer (JASCO Corporation, Japan). Baseline subtraction at 340 nm is performed with Spekwin32 software[1] to accurately calculate protein concentration. The cuvettes should be cleaned with 2 % Hellmanex / water / 70 % ethanol (3 times).

Protocol:

1. Switch on the instrument and allow it to warm up for 20 min.
2. Insert a 1 cm quartz cuvette and record a spectrum from 240 - 340 nm of the empty cuvette in order to identify any possible absorption of the cuvette itself or from contaminants at the required wavelengths.
3. Record a blank spectrum with the buffer alone between 240 and 340 nm. The absorbance will go up at low wavelengths as carbonyl and amide bonds as well as salts absorb below 240nm; DTT and β-mercaptoethanol absorb at even higher wavelengths. Make sure that the transmittance signal is sufficient in the range of analysis. Usually, below 240 nm, the transmittance is close to 0%. Make sure that the buffer is transparent at the required wavelengths or, if there is some absorbance, make sure that it is negligible and still below the specified limit of the instrument In most spectrophotometers, the measurement range of the instrument is limited. As a rule of thumb, if you do not have the manufacturer specifications available, you should not measure absorbances below 0.1 or above 1.0. Importantly, this upper limit is for the total sample, i.e. including the absorbance of the buffer and cuvette. Check the instrument manual for the high absorbance limit). If not, use a less absorbent buffer.
4. Record a spectrum of the protein solution and subtract the blank spectrum. Visually inspect the spectrum shape: check that the maximum absorption is below the specified limit of the instrument. If this is not the case, dilute the protein solution and repeat the measurement.
5. Calculate the aggregation index (*AI*, equation 1) and the A_{260}/A_{280} ratio. If there is
6. no sign of aggregation ($AI < 2$) or contaminants, use the A_{280}, A_{214}, or A_{205} value and the corresponding molar extinction coefficient to calculate the concentration of the protein solution in the cuvette (equation 2).
7. Bear in mind that both the measurement and the molar extinction coefficient bear uncertainties and that the A_{260}/A_{280} ratio should be below 0.6 if the protein contains tryptophan(s).

[1] *F. Menges "Spekwin32 - optical spectroscopy software", version 1.72.2, 2016, http://www.effemm2.de/spekwin/*

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Equations:

$$AI = \frac{100 \cdot A_{340}}{A_{280} - A_{340}} \quad (1)$$

$$A_i = \varepsilon_i \cdot c \cdot l \quad (2)$$

Institut Pasteur PFBMI Plateforme de Biophysique des Macromolécules et de leurs Interactions