AAPS Interlaboratory Study

Tier 1 Method: UV-Vis spectroscopy

Introduction

This document will briefly describe a procedure to use ultraviolet (UV-Vis) spectroscopy to measure the optical density of a protein sample. The method aims at measuring the protein content (at 280 nm) and optical density as an indicator for the turbidity of the sample (at 320 and 350 nm) of the undiluted mAb sample (ca. 1 mg/ml) in formulation buffer.

Important Notes:

1) This protocol is not a Standard Operating Protocol and assumes the required instrument is in good working order and the analysis is performed by experienced user(s). Required calibration or check standards should be run according to the manufacturer instructions. This protocol does not contain all the details of the analysis. The analyst should rely on their best judgment, routine practices, and knowledge of the technique to conduct the study; this protocol should be used as a guideline for the analysis. Samples should be analyzed immediately after thawing and preparation.

2) Many biophysical methods, such as CD, fluorescence, etc., require accurate protein concentration information. If those methods are being performed to obtain structural information, the analyst can simply follow this protocol but does **not** need to fill out the accompanying data template. The analyst will be requested to record the measured concentration on those methods' data templates.

Equipment and Materials List

- UV-Vis spectrometer
- Protein samples and formulation buffers supplied by sample originators (see Table 1)
  - Buffers, aliquots of unstressed, stir stressed, light stressed from Shipping #1
- UV-transparent cuvette (0.5-cm pathlength), well-plate, or other cell type with adjustable pathlength
- 0.1 µm filters, syringes, needles for filtering buffers

Reagents and Solutions

Table 1: The following samples and their buffers will be provided by sample originators. Buffers, aliquots of unstressed, stir stressed and light stressed from Shipping #1 should be used for this analysis. All stressed material were generated at nominally 1 mg/mL.

Proteins	Formulations	Samples
Amgen IgG2	10 mM sodium acetate, pH 5.0	Unstressed, 1 mg/mL Stir stressed, 1 mg/mL Light stressed, 1 mg/mL
NISTmAb IgG1	12.5 mM L-histidine, 12.5 mMol L-histidine HCl, pH 6.0	Unstressed, 1 mg/mL Stir stressed, 1 mg/mL Light stressed, 1 mg/mL

Procedures

Instrument selection

Select your preferred UV-Vis spectrophotometer that allows you to measure the undiluted mAb samples according to the settings specified in the Table 2 below. Because the extinction coefficients of the mAb samples range between 1.45-1.7 ml mg^{-1} cm^{-1}, the absorbance of the sample ranges up to ca. 1.7 per cm. Please measure the samples undiluted by choosing appropriate instrumentation and pathlengths (e.g., 0.5-cm cuvettes, Solo-VPE, well plate reader with pathlength correction, etc.).

Sample preparation

1. Thaw the samples from Shipping #1 and aliquot the Tier 1 vials (for the unstressed, stir, and light stressed mAb samples) as described in the instructions into smaller aliquots for the various methods and freeze until ready to use. Be sure to follow the procedure for vigorously mixing the sample prior to aliquoting.
2. For this analysis, thaw the aliquots designated for UV-Vis spectroscopy measurements (prepared and frozen in #1) by bringing them to room temperature for no more than 15 minutes.
3. Prior to analysis, mix the aliquot in the following manner: pipet with a 200 uL tip near the bottom of the sample tube 10 times in different directions but not touching the bottom or creating bubbles before dispensing the sample into the cuvette or another container.

Sample Measurement

1. Review the **UV Vis Data** Template for the requested information before beginning the measurements and input the set-up information.
2. Transfer material for UV-Vis analysis into the measurement cell, well plate, or into the measurement system by using a pipette. Avoid formation of air bubbles. Prevent evaporation of the sample by covering the sample and measuring promptly.

3. Measure the OD values of the sample as specified in Table 2 against highly purified water as blank.
4. Perform a single measurement (n = 1) of both mAb samples and placebo buffer (in parallel or subsequently).

Table 2: Measurement parameters for UV-Vis spectroscopy

Wavelength of interest	280 to 1000 nm
Cell type	UV-transparent cuvette (e.g., 0.5-cm pathlength), well plate, or other cell type with adjustable pathlength (e.g., Solo-VPE)
Blank	Highly purified water
Extinction Coefficients (ε) (mL · mg−1 · cm−1)	Amgen mAb = 1.5390; NISTmAb = 1.42
Measurement temperature	20 °C
Number of measurements per sample	1

Data Analysis

1. Use the instrument's software or manually correct the measured absorbance/OD values for the pathlength used during analysis. Use the instrument's software or manually subtract the buffer absorbance/OD values from the corresponding sample.
2. Copy and paste the pathlength-corrected (1 cm) and buffer-subtracted absorbance/OD values into the provided UV-Vis Data Reporting Template. Enter the additional requested information, including the extinction coefficient of the protein provided with the samples. The UV-Vis Data Reporting Template will automatically calculate the following results:

- OD350 nm [turbidity] (AU)
- OD280 nm [raw] (AU)
- OD280 nm [scattering-corrected] (AU)
- Protein concentration [scattering-corrected] (mg/mL)

Understanding Results

Record the various instrument parameters and the results in the **UV-Vis Data Reporting Template**. Provide the absorbance/OD values per cm (corrected for pathlength). The formulas in the UV-Vis Data Reporting Template will calculate the protein concentration based on the absorbance/OD at 280 nm, subtracted by the OD 350 nm to correct for scattered light (approximation). Furthermore, the OD values at 350 nm will be reported as a measure for the turbidity of the sample.

Troubleshooting

- Diluted samples were measured -> measure undiluted samples in cells with appropriate pathlength
- Samples were not homogenized -> make sure that the sample is properly homogenized
- Absorbance/OD above linear range of instrument -> be sure to stay within the linear range of Beer-Lambert's law during measurements by selecting the right instrument and settings (see above)
- Readouts were not corrected for the pathlength -> report pathlength-corrected absorbance values (per cm)
- Evaporation of samples during analysis -> prevent evaporation of the sample
- Artifacts from air bubbles, etc. -> prevent the formation of air bubbles, e.g., by careful and slow pipetting
- Artifacts from dirty cells -> follow cleaning procedures and/or use disposable consumables

Further Information

For any specific questions regarding this method, please contact Tim Menzen at tim.menzen@coriolis-pharma.com. Please copy aapsinterlab@nist.gov on all of your emails.