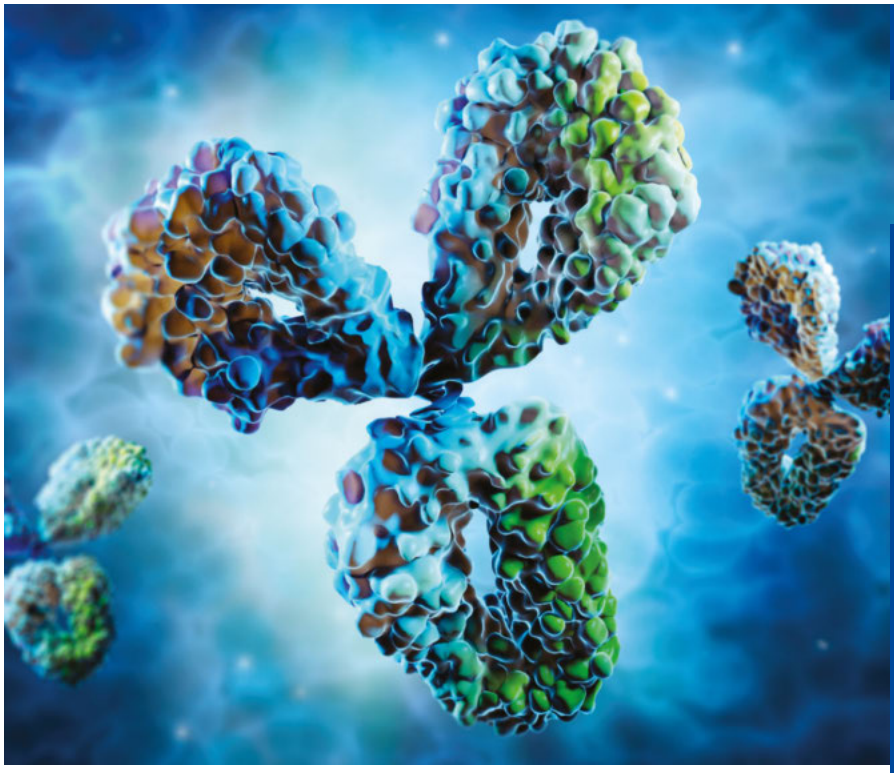

APPLICATION BRIEF

UV/Vis Spectroscopy

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Determination of Monoclonal Antibody Aggregation using UV/Vis Spectroscopy

Introduction

Monoclonal antibodies are antibodies produced by a cloned cell line and consist of identical antibody molecules. As a biopharmaceutical product, they are becoming more widespread as treatments for a variety of diseases including osteoporosis and various cancers.[1] Furthermore, monoclonal antibodies have received emergency authorization for use as treatment for SARS-CoV-2, or COVID-19, from the FDA under the drug names casirivimab and imdevimab.[2]

Aggregation of antibodies may take place due to unwanted folding of the protein structure and can be influenced by a wide variety of factors including pH, presence of other free bases and, most importantly for this application, temperature.[3] Aggregation index provides a semi-quantitative view of the extent of aggregation in antibodies and can be determined by simply measuring the UV/Visible spectrum of the material. In order to determine the aggregation index, the following equation is used:[4]

$$AI = \left[\frac{A_{340}}{A_{265} - A_{340}}\right] \times 100$$

It is generally accepted that an aggregation index of below 10 for samples containing large molecules (such as antibodies) corresponds to solutions with insignificant numbers of soluble aggregates present.[4]

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Experimental

NIST standard humanized IgG_{1K} monoclonal antibody was purchased from Merck and diluted to 1 mg/L in deionised water. Two types of stress were introduced; freeze-thaw stress and thermal stress. Freeze-thaw stress was introduced by holding a vial containing an aliquot of antibody solution in liquid nitrogen for 10 seconds, then in 50 °C water for 10 seconds, repeated 10 times. Thermal stress was introduced by heating a vial containing an aliquot of antibody solution in a water bath at 65 °C for 20 minutes.

UV-Visible spectra were collected using the PerkinElmer LAMBDA® 465 diode-array UV/Vis spectrometer. Blank-corrected spectra were collected between 250 and 1000 nm. The absorbance values at 265 and 340 nm were used with the aforementioned equation in order to determine aggregation index.

Results

The spectra of the three antibody samples subjected to different conditions are shown in Figure 1.

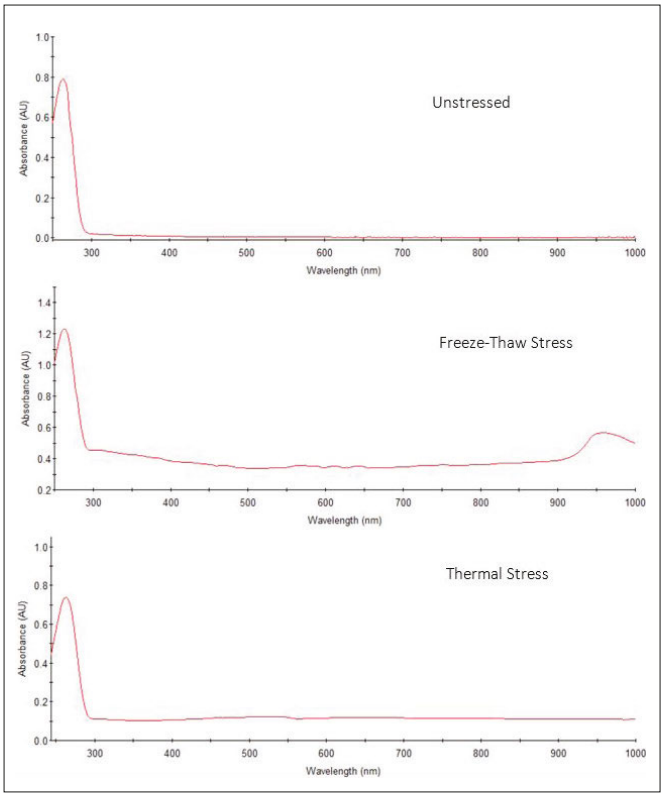

Figure 1. UV/Vis Spectra of Antibody Samples.

The calculated aggregation index for each sample is shown in Table 1.

Table 1. Calculated aggregation index for each antibody sample.

Sample	Calculated Aggregation Index
Unstressed	1.70
Freeze-thaw stress	52.94
Thermal stress	16.29

It can be seen from these results that the antibodies in the unstressed sample show no significant aggregation whereas the stressed samples have aggregated. This provides an accelerated view of what can occur over time to antibody-based medications when not stored correctly.

Summary

UV/Visible spectroscopy provides a fast method for estimation of the extent to which antibodies have become aggregated. The PerkinElmer LAMBDA 465 with UVLab™ software allows users to measure samples in less than one minute.

References

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4. Katayama DS, Nayar R, Chou DK, Campos J, Cooper J, Vander Velde DG, Villarete L, Liu CP, Cornell Manning M. Solution behavior of a novel type 1 interferon, interferon-tau. J Pharm Sci. 2005, 94(12), 2703-15.

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