Assessment of statistical significance of minor changes in HOS using circular dichroism – a new approach

D. Marshall Ph.D., Applied Photophysics Limited, Surrey, U.K.

Introduction

During development of biosimilars and innovator biotherapeutics the importance of detecting minor changes in higher order structure (HOS) is well recognized. Increasing demands from regulatory authorities for objective, statistically-validatable data presents a challenge for certain data-types including circular dichroism (CD) data. Here we present results from subjecting an IgG_1 to a range of degradation conditions, comparing the resulting CD spectra followed by a rigorous statistical analysis. The significance of the differences between the datasets can be objectively evaluated leading to stronger regulatory submissions.

Methods

Sample preparation and CD analysis

Samples of IgG_1 were subjected to a range of degradation conditions as shown in the table below. Aliquots were then loaded into 96-well microplates, alternating buffer-sample-buffer-sample etc.. Using a Chirascan Q100 to generate high quality CD spectra and raw data suitable for rigorous statistical analysis, four independent replicates of each sample were analyzed as follows:

- Secondary structure: far-UV (190 to 250 nm, 0.1 mm pathlength flow cell)
- Tertiary structure: near-UV (250 to 350 nm, 10 mm pathlength flow cell)

Sample pretreatment	Expected effect
0.3% H_2O_2, 20°C, 3 hours	Oxidation
pH 8.5, 40°C, 1 week	Asn deamidation/Asp isomerization
2 M glucose, 40°C, 1 week	Glycation
Control: dialysis only	No effect
Reference: no treatment	No effect
Buffer	Phosphate buffered saline, pH7.4

Fully integrated system for HOS analysis

$$WSD = \sqrt{\sum_{i=1}^{n}\left[\left(\frac{1}{n}\right)\left(\frac{|y_{Ai}|}{|y_A|_{ave.}}\right)(y_{Ai}-y_{Bi})^2\right]}$$

From spectra to numerical data

Set up
- Approximately 30 minute set-up
- Prepare 96-well plate
- Select experimental conditions

Run
- Unattended operation
- Run up to 48 buffer-sample pairs in 24 hours

Analyze data
- Inspect raw data
- Automatically average/baseline correct
- Statistical analysis for HOS comparison

Data interpretation

Absorbance-normalized data were compared using the weighted spectral difference (WSD) method to generate a quality attribute for statistical analysis[1]. This attribute was analyzed with a quality range approach with +/-2SD acceptance criteria as recommended for intermediate (tier 2) risk ranking[2].

[1] *Dinh et al., Anal. Biochem. 464 (2014):60-62*

[2] *Statistical approaches to evaluate analytical similarity; Guidance for Industry; CDER/CBER/FDA*

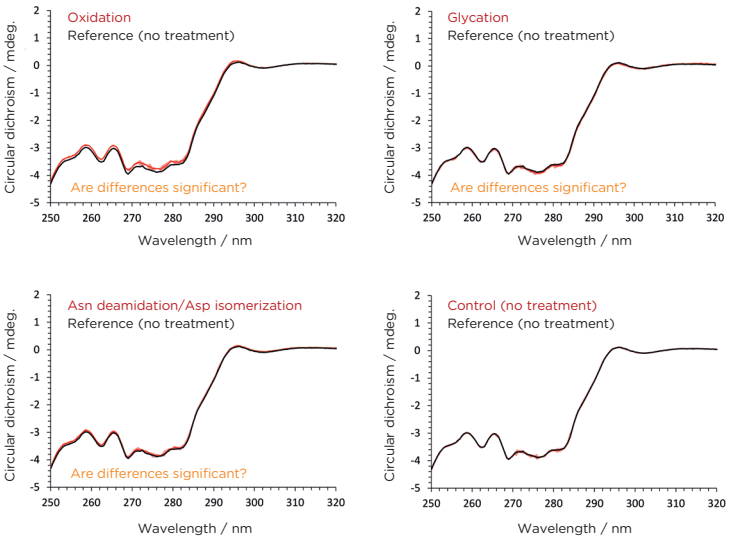
Results: HOS comparisons

Secondary structure CD analysis

Statistical analysis provided objective confirmation of the visual comparison of CD spectra i.e. there were no significant changes in secondary structure of IgG_1 samples subjected to stressed conditions (data not shown).

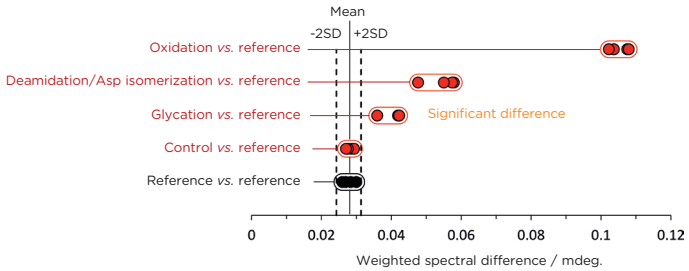
Tertiary structure CD analysis

High sensitivity CD analysis generated near-UV spectra that, upon visual inspection, suggested minor variations in tertiary structure when compared to untreated (reference) samples (see below).



Data analysis – minor differences in tertiary structure are statistically significant

Results indicate that all treatments affected the local environment of aromatic side chains (tertiary structure).



Tier 2 quality range approach applied +/-2SD acceptance criteria.
Differences in tertiary structure significant using +/-2SD criteria.

Conclusion

Objective, quantifiable comparison and quantification of differences or similarities in HOS throughout biotherapeutic development will:

- **enable informed decision-making**
- **enable definition of an acceptable range for HOS variability within a control strategy**
- **provide objective monitoring for HOS changes throughout development and scale-up**
- **strengthen totality of evidence for regulatory submissions**

AppliedPhotophysics

www.photophysics.com

CS2004 v.2.00