Comparability of Protein Therapeutics: Quantitative Comparison of Second-Derivative Amide I Infrared Spectra

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ABSTRACT: Comparability determination for protein therapeutics requires an assessment of their higher order structure, usually by using spectroscopic methods. One of the most common techniques used to determine secondary structure composition of proteins is analysis of the second derivative of the amide I region of Fourier transform infrared (FTIR) spectra. A number of algorithms have been described for quantitative comparison of second-derivative amide I FTIR spectra, but no systematic evaluation has been conducted to assess these approaches. In this study, the two most common methods, spectral correlation coefficient and area of overlap (AO), are compared for their ability to determine spectral comparability of a protein as a function of changes in pH or temperature. Two other algorithms were considered as well. Recently, a QC compare similarity function found in OMNIC software has been reported as being useful in comparing amide I FTIR spectra. In addition, a new algorithm, termed modified AO, is described herein. These four methods were evaluated for their ability to determine comparability for second-derivative amide I FTIR spectra of four model proteins. The result is a framework for quantitative determination of whether any two spectra differ significantly. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:2025–2033, 2012

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INTRODUCTION

Protein therapeutics requires a native-like structure to minimize interference with the immune system and avoid adverse effects. Therefore, the assessment of higher order structure (HOS), which involves the analysis of tertiary and secondary structure of proteins, is essential to ensure a consistent quality of therapeutic products HOS attributes. The US Food and Drug Administration has recently suggested that the biopharmaceutical industry should invest more effort into the HOS characterization of biological products as early as possible in their development stage. It is well known that the structural stability of proteins can be significantly affected by changes in process or formulation,[1,2] which, in turn, can pose a major concern regarding the safety of a biopharmaceutical product. Hence, when there is a modification in the formulation, delivery, or manufacturing process, the resulting protein samples must be comparable to its reference standard. Comparability studies are then carried out to assess product stability and batch-to-batch variability to ensure quality and safety of protein therapeutics. Consequently, comparability studies must consider HOS as part of the overall package.

Changes in the secondary structure composition of protein therapeutics can potentially lead to aggregation and adversely impact the drug activity.[3,4] Furthermore, a detailed analysis of secondary structure composition as a function of formulation conditions could serve as a guide for predicting stability. Thus, the establishment of biophysical tools that are both effective and sensitive is pivotal for the detection of subtle changes of HOS of protein

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therapeutics, such as secondary structure, during biopharmaceutical development. Fourier transform infrared (FTIR) spectroscopy is a well known technology to provide information about the secondary structure composition of proteins in a variety of physical states.[5–7] In particular, the correlation between protein structure and IR band frequencies of the amide I region (1700–1600 cm^{-1}) is well established.[8] As a result, FTIR spectroscopy has been successfully employed in the assessment of the structure and stability of protein samples, as well as assisting in the choice of an optimal formulation for biopharmaceutical products.[9–12] Amide I spectra are usually somewhat featureless because of the overlap of the broad underlying components of protein secondary structure. Therefore, the use of amide I spectra for both quantification of secondary structure components and comparability studies requires the application of resolution-enhancement methods, such as second-derivative analysis, to resolve the underlying individual components.

Various methods have been proposed for the quantitative comparison of amide I FTIR spectra. The spectral correlation coefficient (SCC) method has been used to quantitatively compare the second derivative of amide IR spectra as described by Prestrelski et al.[12] SCC calculations yield *r* values to diagnose the spectral similarity, where lower *r* values indicate a reduced similarity to a reference spectrum. As such, the SCC method has been used extensively for many researchers for determining comparability of IR spectra.[13–15] However, Kendrick et al.[16] discovered during the analysis of second-derivative FTIR spectra of many proteins that, in some cases, the calculated *r* value did not agree with the visual inspection. This discrepancy was ascribed to an offset for the baseline of the amide I spectra. Moreover, the SCC method can yield unreasonably high *r* values even after baseline correction for spectra with high similarity for peak position, but with significant differences in peak intensity. In response to these problems, Kendrick et al.[16] proposed the area of overlap (AO) algorithm for comparison of FTIR spectra. This method involves baseline correction of the amide I second-derivative spectra, followed by the normalization of the area under the curve to a value of 1.0. Then, the percentage of the overlapping area between the two spectra can be calculated. The AO methodology recommended by Kendrick et al.[16] has been adopted by many researchers to estimate the overall spectral similarity.[17–19] Despite these efforts, there is still no consensus on the most appropriate method to use to demonstrate comparability of amide I FTIR spectra. For example, Griebenow and Klibanov[20] argued that the use of second derivative instead of Fourier self-deconvolution (FSD) spectra as the starting point for AO calculations could be problematic because of the lack of preservation of the relative integrated band intensities, a view previously stated by Jackson and Mantsch.[21] More recently, a study contradicting this view has been published.[22] Moreover, in some cases, the baseline correction of the amide I second-derivative spectra does not seem to be performed properly as the baseline slope and absorbance intensity was not adjusted to zero as evidence by the lack of linearity in between both ends of the spectral region.[14,23] This lack of consistency for baseline correction could contribute to a misleading result. Alternatively, van de Weert et al.[24] proposed that the original absorbance profile rather than resolution-enhanced bands from FSD or second-derivative analysis are more adequate for AO calculations. This approach claims that unresolved components contain the same structural information and would require less mathematical modifications such as baseline correction that could bias the analysis. Finally, qualification of a method for use in quantitative comparison of FTIR spectra using the QC compare similarity (QCCS) algorithm found in OMNIC software, Thermo Scientific, Madison–Wisconsin has been recently described.[23] The same QCCS algorithm has been used to compare circular dichroism spectra of proteins as well.[25]

In this study, an extensive and comprehensive design comparing various mathematical approaches is described for the quantitative comparison of second-derivative amide I FTIR spectra. In addition to previously reported methods, a modified AO (MAO) method is introduced in an effort to improve the sensitivity of the original AO approach by giving greater weight to the peak maxima over portions of the spectrum closer to the baseline. These methods (SCC, AO, and MAO) are evaluated along with the QCCS algorithm found in OMNIC software.

To investigate whether the sensitivity of these methods are protein secondary structure dependent, four model proteins were selected. They include (1) myoglobin (Mb), which is predominantly α-helical in structure; (2) immunoglobulin (IgG), which contains predominantly β-sheet structure; (3) casein, which is mostly random coil or unordered structure; and (4) lysozyme, which contains a mixture of α-helix, β-sheet, and random coil structures. Thus, this work sought to demonstrate that these methodologies could be employed with proteins from different structural classes. To obtain a wide range of conformations, these studies were conducted at different temperatures and pH values.

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