Higher Order Structure (HOS) with Advanced Vibrational Spectroscopy

PROTA-3S™

FT-IR Protein Structure Analyzer

Speed. **S**ensitivity. **S**implicity.

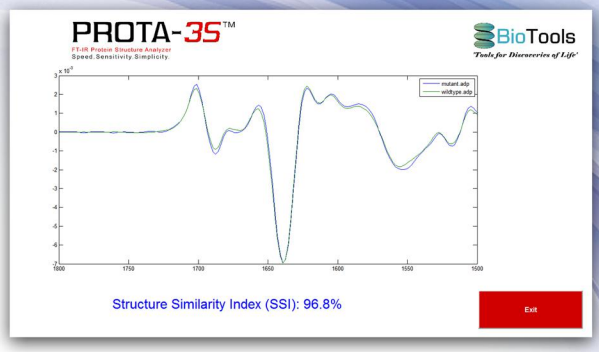
- Incredibly fast measurements
- Concentration as low as 0.1mg/ml
- No limit on high concentration
- *New* independent software including:
  - structure analysis
  - comparability algorithm
  - shadow plots

Advantages of PROTA-*3S*

Study your sample at its final formulation with no dilution required

In Any Formulation...
Liquids, Solids, Patch, Membrane, Chip, Excipients, Nanoparticles, Microspheres

Fast measurements!
From Low to High Concentration!



PROTA-*3S* Software Screenshot

Industry & Academic Leaders Choose PROTA for Their Lab's Protein, R & D, Formulation and Quality Control (selected list):

Abbott/Abbvie
AstraZeneca
Biogen
Bristol-Myers Squibb
Eli Lilly
Genzyme
GlaxoSmithKline
Hospira
KBI
National Cancer Institute
Novartis
Pfizer
Shire
Takeda
Teva Pharma
Synageva BioPharma
University of Colorado
USDA-NCAUR

BioTools

For more information call 561.625.0133
info@btools.com, www.btools.com - Florida, USA

Higher Order Structure (HOS) with Advanced Vibrational Spectroscopy

ChiralRAMAN-2X™

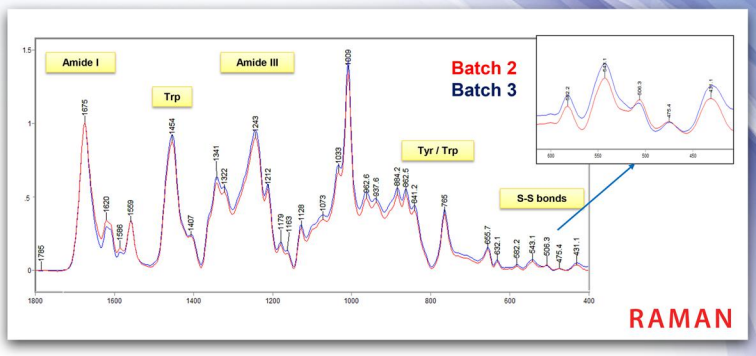
Raman Optical Activity (ROA)

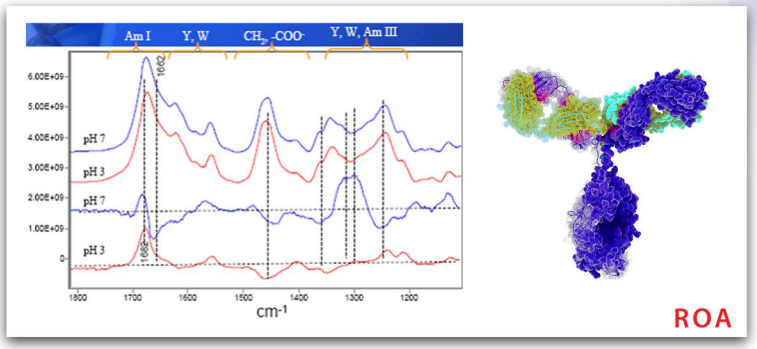
- Most sensitive spectroscopic technique for elucidation of HOS
- Secondary and tertiary structure (including conformation of Tyr, Trp, Phe, SS bonds)
- Prediction of aggregation/denaturation
- Ideal for antibodies and ADCs
- One measurement gets two spectra: Raman and ROA

Advantages of ChiralRAMAN-2X™

- Complete vibrational spectrum from 100 to 2000 cm^{-1} is accessible in one measurement.
- Water, an excellent media for studies of biomolecules, is not excluded as a solvent.
- Only micrograms – milligrams of sample required.
- Extremely fast collection Raman spectra, as fast as 150 msec.

Two Spectra from One Measurement:

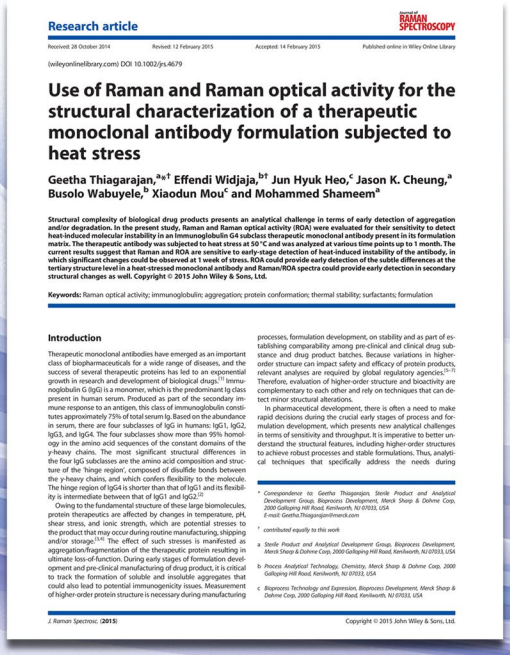




BioTools

For more information call 561.625.0133
info@btools.com, www.btools.com - Florida, USA

Recent Publications/Presentations From Leaders in the Field



Research article

Journal of RAMAN SPECTROSCOPY

Received: 28 October 2014 Revised: 12 February 2015 Accepted: 14 February 2015 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jrs.4679

Use of Raman and Raman optical activity for the structural characterization of a therapeutic monoclonal antibody formulation subjected to heat stress

Geetha Thiagarajan,[a*†] Effendi Widjaja,[b†] Jun Hyuk Heo,[c] Jason K. Cheung,[a] Busolo Wabuyele,[b] Xiaodun Mou[c] and Mohammed Shameem[a]

Structural complexity of biological drug products presents an analytical challenge in terms of early detection of aggregation and/or degradation. In the present study, Raman and Raman optical activity (ROA) were evaluated for their sensitivity to detect heat-induced molecular instability in an Immunoglobulin G4 subclass therapeutic monoclonal antibody present in its formulation matrix. The therapeutic antibody was subjected to heat stress at 50 °C and was analyzed at various time points up to 1 month. The current results suggest that Raman and ROA are sensitive to early-stage detection of heat-induced instability of the antibody, in which significant changes could be observed at 1 week of stress. ROA could provide early detection of the subtle differences at the tertiary structure level in a heat-stressed monoclonal antibody and Raman/ROA spectra could provide early detection in secondary structural changes as well. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: Raman optical activity; immunoglobulin; aggregation; protein conformation; thermal stability; surfactants; formulation

Introduction

Therapeutic monoclonal antibodies have emerged as an important class of biopharmaceuticals for a wide range of diseases, and the success of several therapeutic proteins has led to an exponential growth in research and development of biological drugs.[1] Immunoglobulin G (IgG) is a monomer, which is the predominant Ig class present in human serum. Produced as part of the secondary immune response to an antigen, this class of immunoglobulin constitutes approximately 75% of total serum Ig. Based on the abundance in serum, there are four subclasses of IgG in humans: IgG1, IgG2, IgG3, and IgG4. The four subclasses show more than 95% homology in the amino acid sequences of the constant domains of the γ-heavy chains. The most significant structural differences in the four IgG subclasses are the amino acid composition and structure of the 'hinge region', composed of disulfide bonds between the γ-heavy chains, and which confers flexibility to the molecule. The hinge region of IgG4 is shorter than that of IgG1 and its flexibility is intermediate between that of IgG1 and IgG2.[2]

Owing to the fundamental structure of these large biomolecules, protein therapeutics are affected by changes in temperature, pH, shear stress, and ionic strength, which are potential stresses to the product that may occur during routine manufacturing, shipping and/or storage.[3,4] The effect of such stresses is manifested as aggregation/fragmentation of the therapeutic protein resulting in ultimate loss-of-function. During early stages of formulation development and pre-clinical manufacturing of drug product, it is critical to track the formation of soluble and insoluble aggregates that could also lead to potential immunogenicity issues. Measurement of higher-order protein structure is necessary during manufacturing processes, formulation development, on stability and as part of establishing comparability among pre-clinical and clinical drug substance and drug product batches. Because variations in higher-order structure can impact safety and efficacy of protein products, relevant analyses are required by global regulatory agencies.[5–7] Therefore, evaluation of higher-order structure and bioactivity are complementary to each other and rely on techniques that can detect minor structural alterations.

In pharmaceutical development, there is often a need to make rapid decisions during the crucial early stages of process and formulation development, which presents new analytical challenges in terms of sensitivity and throughput. It is imperative to better understand the structural features, including higher-order structures to achieve robust processes and stable formulations. Thus, analytical techniques that specifically address the needs during

* Correspondence to: Geetha Thiagarajan, Sterile Product and Analytical Development Group, Bioprocess Development, Merck Sharp & Dohme Corp, 2000 Galloping Hill Road, Kenilworth, NJ 07033, USA
E-mail: Geetha.Thiagarajan@merck.com

† contributed equally to this work

a Sterile Product and Analytical Development Group, Bioprocess Development, Merck Sharp & Dohme Corp, 2000 Galloping Hill Road, Kenilworth, NJ 07033, USA

b Process Analytical Technology, Chemistry, Merck Sharp & Dohme Corp, 2000 Galloping Hill Road, Kenilworth, NJ 07033, USA

c Bioprocess Technology and Expression, Bioprocess Development, Merck Sharp & Dohme Corp, 2000 Galloping Hill Road, Kenilworth, NJ 07033, USA

Merck Paper

Current Pharmaceutical Biotechnology, 2009, 10, 391-399 391

Application of Vibrational Spectroscopy to the Structural Characterization of Monoclonal Antibody and its Aggregate

Cynthia H. Li[a] and Tiansheng Li*

[a]*Department of Formulation and Analytical Resources, Amgen Inc., One Amgen Center Dr., MS 30E-0-B, Thousand Oaks, CA 91320, USA;* [b]*HTL Biosolutions, Inc., 77 University Dr., 2nd Floor, Camarillo, CA 93012, USA*

Abstract: Aggregation is often the major issue during formulation and manufacturing development of therapeutic proteins, in particular human monoclonal antibody. Currently, there is a lack of structural information of aggregates of such large protein as human antibodies, due to the large molecular sizes of the aggregates. In this article, we shall discuss the application of vibrational spectroscopies including FT-IR, Raman and Raman Optical Activity (ROA), to characterize the structures of various types of monoclonal antibody aggregates formed under different stresses. Two different classes of human monoclonal antibodies, namely IgG1 and IgG2, have been subjected to this structural investigation. The common stresses leading to antibody aggregation, mis-folding or unfolding during manufacturing and formulation include exposure to acidic pHs, heat and shear stress. The effect of different types of stresses on the structure and aggregate formation of human monoclonal antibodies has been investigated by employing vibrational spectroscopy. While data present only monoclonal antibody, the same technology can be used for any protein aggregates.

INTRODUCTION

In recent years, therapeutic proteins, in particular monoclonal antibodies, have emerged as an important class of protein therapeutics for various types of diseases. During the pre-clinical development, including manufacturing and formulation processes of human monoclonal antibodies, aggregation and mis-folding of the proteins are often observed [1-7]. There are a number of factors, including temperature, shear stress, pH, ionic strength and surface exposure that can lead to aggregation of monoclonal antibodies in solution. Exposure to agitation and other mechanical stresses during the manufacturing process can cause antibody aggregation [8-10]. Particle formation in solutions of monoclonal antibodies usually results from the formation of insoluble aggregates. However, due to the large molecular weight and complex structure of monoclonal antibodies, there are only limited biophysical techniques available to study the structures of antibody aggregates and mis-folded antibodies. In recent years, light scattering techniques and analytical ultracentrifugation have been employed quite extensively to investigate the molecular mechanism of protein aggregation, particularly the size and shape of protein aggregates [11]. However, with the exception of vibrational spectroscopic techniques, there is virtually no other biophysical technique available to characterize the structures of insoluble protein aggregates. One of the major advantages of vibrational spectroscopy, including FT-IR and Raman, is that the technique is not limited by the physical states of the analyzed samples [12-17]. Proteins in essentially all physical states such as the liquid, gel, colloidal and solid states can be analyzed by using FT-IR and/or Raman spectroscopy. FT-IR and Raman spectroscopies have been widely used to characterize the structures of proteins [18-20]. Raman optical activity spectroscopy (ROA) has been used to study the heat and acid induced denaturation of proteins [21, 22] and the conformation of proteins as well as virus assemblies [23]. It has been shown that overall signature bands in the ROA spectra of proteins can be used to detect different tertiary structure folds and are sensitive to changes in protein tertiary structures [24]. Spectral analysis and band assignments of FT-IR and Raman techniques have been constantly updated and published [25-35]. There are a number of articles focused on the application of FT-IR to the characterization of protein aggregates [36-39]. The accumulation of FT-IR and Raman data-bases for proteins has made it possible to characterize structures of protein therapeutics under various formulation and process conditions.

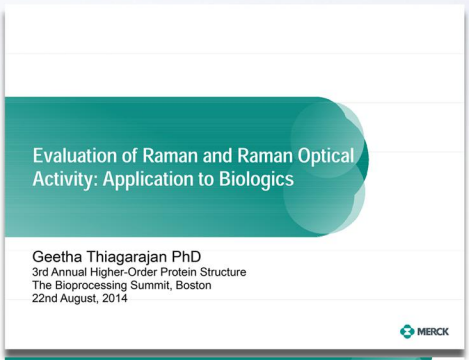
Vibrational spectroscopy coupled with microscopy is one of the most powerful techniques for identifying visible particles that are isolated from drug product solutions. While the underlying theory of the techniques is the same, the application is specific to visible and sub-visible particles, and this will be discussed in Chapter 3.2.

In this article, we shall discuss the use of vibrational spectroscopies, including FT-IR, Raman and Raman Optical Activity (ROA), to investigate the structures of various types of monoclonal antibody aggregates and unfolded species formed under different stresses. Two different classes of human monoclonal antibodies, namely IgG1 and IgG2, have been subjected to this structural investigation. Common stress conditions which lead to antibody aggregation, mis-folding or unfolding during manufacturing and formulation including exposure to acidic pHs, heat and sheer stress have been evaluated. Molecular conformations of protein side chains such as Trp, Tyr and disulfide bonds of antibody aggregates, have been characterized by using Raman and ROA spectroscopies. The structural characterizations of mono-

*Address correspondence to this author at the HTL Biosolutions, Inc., 77 University Dr., 2nd Floor, Camarillo, CA 93012, USA; Tel: (805) 807-1685; E-mail: dli@htlbio.com

1389-2010/09 $55.00+.00

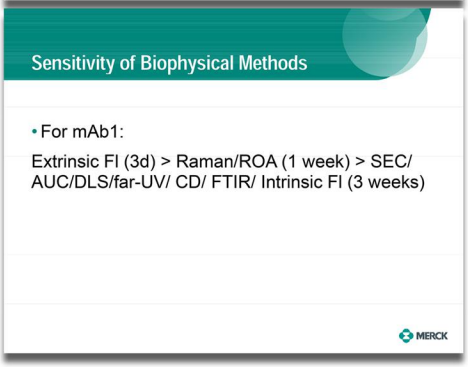
Amgen Paper



Evaluation of Raman and Raman Optical Activity: Application to Biologics

Geetha Thiagarajan PhD
3rd Annual Higher-Order Protein Structure
The Bioprocessing Summit, Boston
22nd August, 2014

MERCK



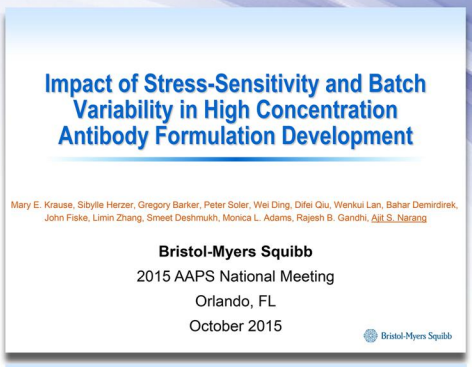
Sensitivity of Biophysical Methods

- For mAb1:

Extrinsic FI (3d) > Raman/ROA (1 week) > SEC/ AUC/DLS/far-UV/ CD/ FTIR/ Intrinsic FI (3 weeks)

MERCK

Merck Presentation



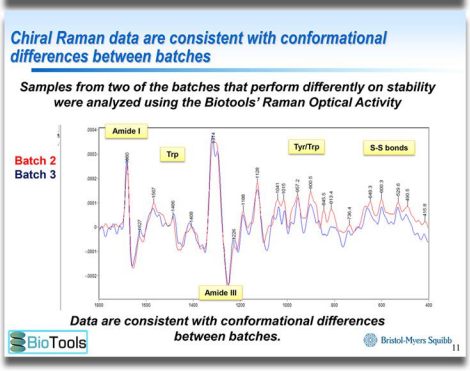
Impact of Stress-Sensitivity and Batch Variability in High Concentration Antibody Formulation Development

Mary E. Krause, Sibylle Herzer, Gregory Barker, Peter Soler, Wei Ding, Difei Qiu, Wenkui Lan, Bahar Demirdirek, John Fiske, Limin Zhang, Smeet Deshmukh, Monica L. Adams, Rajesh B. Gandhi, Ajit S. Narang

Bristol-Myers Squibb

2015 AAPS National Meeting
Orlando, FL
October 2015

Bristol-Myers Squibb



Chiral Raman data are consistent with conformational differences between batches

Samples from two of the batches that perform differently on stability were analyzed using the Biotools' Raman Optical Activity

Batch 2
Batch 3

Amide I
Trp
Tyr/Trp
S-S bonds
Amide III

Data are consistent with conformational differences between batches.

BioTools

Bristol-Myers Squibb 11

BMS Presentation

BioTools

For more information call 561.625.0133
info@btools.com, www.btools.com - Florida, USA

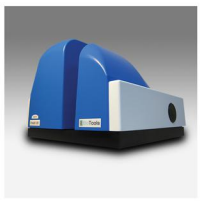
BioTools, Inc. | **Product Highlights**

RamTest™
HANDHELD RAMAN IDENTIFIER

A Handheld Raman That Measures What Others Can't!
@ 532 nm: - increased sensitivity (~5-10x) - full range 100-4000 cm^{-1} - fluorescence automatically removed
Optimizied for a variety of fields! Add your own spectra, measure through packages/containers, true portability

BioTools Mobile µRaman™ and µ-BioRAMAN™
Revolutionary Portable Raman Microscope

Introducing Raman-on-the-Go! The World's First Portable Raman Microscope!
- Measure anywhere with true portability
- Piezo-stage for mapping
- SameSpot optical system
- Versatile sampling options



ChiralIR-2X™

Combining the sensitivity of FT-IR with stereo-specificity of CD into a powerful new technique: Vibrational Circular Dichroism (VCD). For chiral analysis such as absolute configuration, enantiomeric purity and reaction monitoring. #1 selling and #1 published VCD instrument in the world!

MANTIS™
DUALPEM VCD ACCESSORY

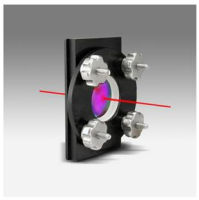
For the first time ever existing FT-IR spectrometers can be upgraded to VCD.

PROTA-3S™
FT-IR Protein Structure Analyzer
Speed.Sensitivity.Simplicity.

Flagship for protein analysis.
3S offers speed, sensitivity to handle low concentrations, and turn-key simplicity.

ChiralRAMAN-2X™

Raman Optical Actvity (ROA) - a marriage of Raman spectroscopy and optical activity is being touted as one of the most sensitive techniques for the determination of higher order structure of proteins. BioTools is the first and only company in the world to offer a dedicated commercial spectrometer for its measurement. Generation II is now available, fully integrated with research-grade microscopy.



Accessories and Software

Unique accessories, including µ-Rim™ Drop Coated Deposition Raman (DCDR) slides, UniverSERS™ SERS substrates and the world's #1 cell for FT-IR of aqueous solutions.
ComputeVOA™ and CompareVOA™: exclusive, one of a kind software for the calculation of VCD and ROA.

Lab Services

Structure characterization for chiral and biological molecules; all applications of Raman and FT-IR.

17546 Bee Line Hwy | Jupiter, FL 33458 USA
info@btools.com | btools.com

BioTools