



Biopharmaceutical
Production Series

Informa Life Sciences' 2nd Annual

Forced Degradation for Biologics

Hear new data from the **leading industry experts** on how to get ahead in your forced degradation studies- including presentations from **Genentech, Roche, Sanofi pasteur & MedImmune**

Discover the latest methods and techniques in forced degradation studies, and hear solutions to common implementation pitfalls

12-13 April 2011, Danubius Regent's Park Hotel, London, UK

Join the forced degradation experts shaping the industry

- Dr Y. John Wang, *Principal Scientist*, **Genentech**, USA
- Dr Joerg Hoernschemeyer, *Laboratory Head Pharmaceutical and Analytical R&D Biologics*, **F. Hoffmann-La Roche**, Switzerland
- Dr Kevin Harper, *Director of Formulation and Stability*, **Sanofi Pasteur**, North America
- Dr Patrick Garidel, *Head of Pharmaceutical Basic Development*, **Boehringer Ingelheim**, Germany
- Dr Sabine Boeckle, *Group Head Analytical Development and QC*, **F. Hoffmann-La Roche**, Switzerland
- Dr Nicolas Moniotte, *Scientist*, **GlaxoSmithKline Biologicals**, Belgium
- Christopher Gee, *Senior Scientist*, **MedImmune**, UK
- Dr Nadine Ritter, *Senior CMC Consultant*, **Biologics Consulting Group**, USA
- Professor Pauline Rudd, *Principal Investigator, NIBRT, Professor of Glycobiology*, **University of Dublin**, Ireland

5 reasons why you should attend this conference:

1. Learn the **latest analytical techniques** in place for optimising **biophysical characterisation**- Boehringer Ingelheim, University of Dublin, London School of Pharmacy & University of Geneva share their latest data
2. **Benchmark your FD study** by hearing the **latest in developments in stress tests**. Do not miss industry case study from **Sanofi Pasteur** on **automated methods**
3. **Overcome the common pitfalls** concerning study implementation to improve the success of your forced degradation study
4. Gain a thorough understanding of the **degradation pathways of aggregation, oxidation and deamidation**, from **Roche, MedImmune** and **Merck Serono**
5. **Dr Nadine Ritter** discusses the importance of **extractables** and **leachables** in the packaging of products

PLUS DON'T MISS

Monday 11th April 2011: **Pre-Conference Workshop W**

Aggregation: Delving into the Mechanism

Led by: **Dr Jennifer McManus**, National University of Ireland Maynooth and NIBRT, Ireland

Tuesday 12th April 2011: **Evening Seminar X**

The Growth and Characterisation of Sub-visible Particulates

Led by: **Professor Tudor Arvinte**, Therapeomic Inc. and **University of Geneva**, Switzerland
Dr Kevin Harper, Sanofi Pasteur, North America

Thursday 14th April 2011: **Post-Conference Workshop Y**

Validation of Stability-Indicating Methods for Biotechnology and Biosimilar Products – How and When to Use Forced Degradation Studies

Led by: **Dr Nadine Ritter**, Biologics Consulting Group. USA

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PRE-CONFERENCE WORKSHOP • MONDAY 11 APRIL 2011

Aggregation: Delving into the Mechanism

09.30 Registration • 10.00 Start • 16.30 End of Workshop

Lunch, morning and afternoon refreshments provided

Aggregation is a problem often encountered during the production of therapeutic proteins. There are many ways to induce protein aggregation, but any specific method may determine the mechanism by which this process progresses. This workshop will explore various mechanisms leading to protein aggregation and will review current and emerging techniques and tools for detecting protein aggregates. Topics to be discussed include:

- Molecular detail of the mechanism behind this pathway

- Insight into the conditions that induce aggregation
- What role does protein structure play?
- How best to detect aggregation- potential new tools
- Important factors when designing correct experiments
- How to collect the correct data

Dr Jennifer McManus, Stokes Lecturer, National University of Ireland Maynooth, Head of Protein Aggregation Group, NIBRT, Ireland

CONFERENCE DAY ONE: TUESDAY 12 APRIL 2011

08:00 Registration

09:00 Chairman's welcome and opening remarks

PRACTICAL EXPERIENCE OF THE LATEST STRESS TESTS IN FORCED DEGRADATION STUDIES AND FUTURE DEVELOPMENTS

09:05 **Predictive or not predictive? That is the question - The value of forced degradation studies for biologics**

Forced degradation studies can facilitate the understanding of the stability profiles and the degradation pathways for biologics products. In order to extrapolate the value of these studies, it is critical to ensure the predictability of the methods. In this presentation, the predictiveness of various methods will be discussed and evaluated.
Dr Judy Chou, Vice President, Research and Development, Tanvex Biologics, Inc., USA

09:50 **Degradation pathway of the photo-oxidation of a vaccine protein candidate**

The photochemical oxidation of a vaccine protein candidate was investigated by screening excipients known for their anti-oxidant properties. The screening was performed readily in 96-well plates and degradation products were characterised by capillary electrophoresis (CE), dynamic light scattering (DLS), intrinsic fluorescence emission (IF) and UV-vis absorbance (UV-vis). The protein was found to form aggregates upon light irradiation. When ascorbic acid was added to the formulation, the degradation was even faster. Only thiol-containing excipients could keep the protein stable from photo-oxidation. The combination of four analytical tools was the key to draw hypothesis on the degradation pathway, and to propose an effective formulation mixture to keep the protein from irreversible aggregation.
Dr Nicolas Moniotte, Scientist, GlaxoSmithKline Biologicals, Belgium

10:30 Morning coffee and networking

11:00 **Forced degradation laboratory studies for high concentration formulations: An approach for understanding protein sensitivity to process steps in manufacturing**
Manufacturing of protein biologics routinely involves process steps that expose the active drug to stress factors such as mechanical shear, air-liquid interface, irradiation, heat, freeze-thaw, and surface interactions. Laboratory stress models are a useful approach to evaluate the sensitivity of the protein drug to specific stress factors. The protein instability observed and measured using these forced protein degradation studies assist in establishing limits for manufacturing process parameters. Specific laboratory stress models and associated data will be presented for high concentration antibody solutions.
Dr Don Eisenhauer, Senior Pharmaceutical Scientist, Abbott Laboratories, USA

11:40 **Panel discussion: Real life regulatory submissions and common misunderstandings**
• Forced degradation study timeline
• Regulations concerning 'bridging study' implementation
Led by **Dr Nadine Ritter, Senior CMC Consultant, Biologics Consulting Group, USA** and a panel of speakers from the day

12:20 Lunch and networking

PRESENT AND FUTURE ANALYTICAL TECHNIQUES FOR THE BIOPHYSICAL CHARACTERISATION OF BIOLOGICS

14:00 **Automated methods used in forced degradation studies**
High throughput screening of sensitive biologics can be designed to provide information on optimal formulations, by submitting them to a variety of degradation/aggregation stresses. These can be selected by examination of putative pathways as discerned from the structure of the molecule itself. However the large amounts of data generated, as well as the large number of manipulations involved, for example dilutions into differing buffer systems, requires automation strategies to truly be comprehensive. Accordingly, this talk will attempt to bring into focus a variety of automation technologies to assist in that process.
Dr Kevin Harper, Director of Formulation and Stability, Sanofi Pasteur, North America

14:40 **Rapid biophysical screening and characterisation of biologics**
This presentation discusses the common challenges that industry have when working with biologics, and potential methods to overcome them. There are several tools available for improvement, however, this discussion will talk about those needed for the all important task of rapid stability screening. With the use of case studies, this talk with further give examples of orthogonal methods, giving insight into the advantages and disadvantages and how forced degradation studies can be improved.
Dr Patrick Garidel, Head of Pharmaceutical Basic Development, Boehringer Ingelheim, Germany

15:20 **SPOTLIGHT PRESENTATION**
These presentations are hosted by leading service providers who operate in the field of forced degradation studies for biologics and offer an opportunity to learn about the latest developments and technological advancements in the industry. If you would like to host a spotlight presentation please contact Christopher Handsley, Business Development Manager:
Email: christopher.handsley@informa.com Tel: +44 (0) 20 7017 7278

15:50 Afternoon tea and networking

16:30 **Differentiation between unfolded, native or aggregated proteins**
Challenges to assess the native, denatured or aggregated protein structure integrity of the primary sequence of a protein is the most important to ensure the correct protein folding and the overall protein structure. However, also secondary, tertiary or even quaternary structures should be addressed during development of biologics since they may be important for potency or safety of the molecule. Different biophysical methods will be presented including examples and limits of these methods. Moreover, examples using alternative methods will be given that can differentiate between the native, denatured or aggregated protein structure.
Dr Sabine Boeckle, Group Head Analytical Development and QC Biotech Products, F. Hoffmann-La Roche, Switzerland

17:10 **Biophysical characterisation of vaccines: present and future analytical techniques**
Our focus is to develop effective vaccines against different diseases caused by pathogens such as HIV, Influenza, Chikungunya, Ebola, Marburg and others. Several state of the art analytical tools are available for the characterisation of vaccines such as Circular Dichroism (CD) Spectral Analysis, Tryptophanyl Fluorescence Emission, Differential Scanning Calorimetric (DSC) Analysis, Carbohydrate Profiling and Sequencing Analysis, Capillary Electrophoresis (CE) and high resolution Mass Spectrometric Analysis. For the immunological characterization one may use surface Plasmon resonance technique, or Octet system. These assays may help in performing in-depth biophysical characterisation of vaccines. Some of these assays may be used for screening optimal formulation conditions for a given vaccine. We will discuss data related biophysical characterisation of HIV vaccines.
Dr Indresh K. Srivastava, Director, Purification and Analytical Development, Vaccine Research Centre, National Institute of Allergies and Infectious Diseases, USA

17:50 Closing remarks by the chairperson and end of Day One

"An interesting conference that brought together people with different backgrounds to deliver an interesting agenda"

O'Hara, UCB, 2010 speaker

EVENING SEMINAR • TUESDAY 12 APRIL 2011

The Growth and Characterisation of Sub-visible Particulates

Registration 18.15 • Start 18.30 • Finish 21.30
Dinner and refreshments will be provided

Sub-visible particles detected in biopharmaceuticals may have different origins: they may be pure protein aggregates, glass particles, leachables such as Tungsten, silicon oil, metals and also complexes of these leachables with proteins and protein aggregates. Based on case studies different mechanisms of protein aggregation will be discussed as well as methods that permit the detection of sub-visible particulates in formulations in conditions as near as possible to those in which the drug is applied in vivo. A new method will be presented that can detect sub-visible particles in liquid formulations inside unopened vials or prefilled syringes.

- Definition of sub-visible particulates
- Methods of detection and prediction of aggregation onset
- Advantages and disadvantages of different characterisation methods
- Future developments for routine analysis
- Dinner and refreshments will be provided

Dr Kevin Harper, Director of Formulation and Stability, Sanofi Pasteur, North America
Professor Tudor Arvinte, Chairman, CEO, Therapeomic Inc., School of Pharmacy, University of Geneva, Switzerland

Discover latest methods to detect sub-visible particulates

CONFERENCE DAY TWO: WEDNESDAY 13 APRIL 2011

09:00 Chairman's welcome and opening remarks

THE LATEST FORCED DEGRADATION TECHNIQUES AND METHODS FOR INCREASING STABILITY

09:05 **Glycosylation: Insight into the growing importance**
Glycosylation is a parameter which determines the efficacy, pharmacokinetics and safety of most biological drugs including erythropoietin, the pituitary hormones and monoclonal antibodies. Glycans play a role in maintaining the structure of proteins and may thus protect against aggregation. Controlling, monitoring and analysing in detail the glycosylation of recombinant proteins is a major challenge which will also be addressed.
Professor Pauline Rudd, Principal Investigator, NIBRT, Professor of Glycobiology, University of Dublin, Ireland

09:45 **The use of PEGylation for biologic stability**
The covalent conjugation of poly(ethylene glycol) (PEG) to therapeutic proteins is a clinically proven method to extend their circulation half-life and to minimise potential immunogenicity. While there are other half-life strategies that have been explored, protein PEGylation is currently used on at least 9 registered products with at least double that number currently in the clinical pipeline. PEGylation has the advantage that it can decrease protein aggregation resulting in final dosage forms that are more easy to administer in the clinic. PEG is a hydrophilic, random coiled polymer that when conjugated to a protein provides steric shielding effects to decrease the propensity of the protein to aggregate.
Professor Steve Brocchini, Professor of Chemical Pharmaceutics, London School of Pharmacy, UK

10:25 Morning coffee and networking

MECHANISMS OF THE COMMON DEGRADATION PATHWAYS

11:00 **The key use of orthogonal techniques in the study of protein aggregation**
Proof and understanding of protein aggregation should be derived through a convergence of evidence from numerous lines of inquiry, independent inductions, all of which point to an unmistakable conclusion. Based on case studies, different orthogonal methods will be presented such as absorption, fluorescence intensity, fluorescence lifetime, fluorescence anisotropy, Nile red spectroscopy, light microscopy, electron microscopy, analytical ultracentrifugation, FFF, CD. No single result from these methods denotes a general, absolute proof of the aggregation state, but together they point to unmistakable conclusions and reveal a broad picture on the protein aggregation states.
Professor Tudor Arvinte, Chairman, CEO, Therapeutic Inc., School of Pharmacy, University of Geneva, Switzerland

11:40 **Developing specific deamidation assays for biopharmaceuticals**
This talk presents case studies of the development and validation of deamidation assays for biopharmaceuticals to monitor in-process samples as well as for routine QC for release and stability testing. The methods developed are based on different approaches. Positive controls were generated by subjecting the target molecule to deamidation-inducing conditions and by fully characterising them employing mass spectrometry approaches.
Dr Horst Bierau, Scientific Advisor and Relation Manager, Analytical Development Biotech Products, Technical Operations, Merck Serono SpA, Italy

12:20 **SPOTLIGHT PRESENTATION**
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Email: christopher.handsley@informa.com
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12:50 Lunch and networking

14:00 **Protein oxidation - The good, the bad and the ugly**
Determination of protein oxidation plays an important role in drug development of biologics as it may affect protein function as well as its stability. For the use of such molecules in therapeutics a good comprehension of their degradation pathways is indispensable. This presentation summarises the key degradation pathways and the formulation approaches allowing a satisfactory shelf-life, ensuring both efficiency and safety of such biotherapeutics.
Dr Joerg Hoernschemeyer, Laboratory Head, Pharmaceutical and Analytical R&D Biologics, F. Hoffmann-La Roche Ltd, Switzerland

14:40 **Assessment of deamidation sites in antibody products**
This presentation features a discussion of an analytical strategy for assessment of deamidation, as a potential critical quality attribute in relation to post-translation modifications to support antibody product development; Description of the methods used to identify and measure deamidation propensity at specific sites within the CDR of novel antibody products using peptide mapping LC-MS, and determination of the effect on product potency and structure; Review of the hydrophobic and steric nature of residues surrounding deamidation motifs, and presenting a case study on the effect of adjacent site-specific amino acid substitutions on the rate of deamidation, alongside assessment of any changes to structure and potency.
Christopher Gee, Senior Scientist, MedImmune, UK

15:20 Afternoon tea and networking

15:50 **The pathway through succinimide, from Asn deamidation and Asp dehydration**
This presentation will discuss the variety of stress tests that cause both deamidation and dehydration (isomerisation). It is important to understand exactly what effect the deamidation or dehydration can have on the biologics property, which is why methods of quantification are vital. This presentation will look into the different methods of quantification and different stability and predictability techniques.
Dr Y. John Wang, Principal Scientist, Genentech, USA

IMPLICATIONS OF BIOLOGICAL ASSAYS IN FORCED DEGRADATION STUDIES

16:30 **Bioassays and forced degradation studies**
Bioassays are required throughout the biopharmaceutical drug development process and for product release. Forced degradation samples can demonstrate that the bioassay is stability-indicating and permit assessment of the effects of potential product-related impurities. The bioassay may be sensitive to a range of structural changes but interpretation of changes in bioassay response can be complicated. Recent technological developments offer new possibilities in bioassay use.
Dr Jane Robinson, Principal Scientist, National Institute for Biological Standards & Control, UK

FORCED DEGRADATION STUDIES AND IMPORTANCE IN PACKAGE DESIGN

17:10 **Current expectations for extractable/ leachable studies with biotechnology/biosimilar products**
A concern among regulators is the potential impact on patient safety of compounds derived from container/closure systems. While material compatibility and extractable/leachable studies have long been a part of pharmaceutical product development requirements, there have been new, unpredicted interactions when those materials are used for protein therapeutics. Prior historical data on the safety profile of extractable/leachable compounds from many common materials does not seem to be fully predictive of their impact on biopharmaceutical products. This presentation will give an overview of the risk assessments being applied to extractable/leachable issues for biotech products, and discuss experimental strategies being employed by sponsors for conducting these studies during product development in alignment with current regulatory expectations.
Dr Nadine Ritter, Senior CMC Consultant, Biologics Consulting Group, USA

17:50 Chairman's closing remarks and end of conference

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POST-CONFERENCE WORKSHOP • THURSDAY 14 APRIL 2011

Validation of Stability-Indicating Methods for Biotechnology and Biosimilar Products – How and When to Use Forced Degradation Studies

09.00 Registration • 09.30 Start • 16.30 End of Workshop
Lunch, morning and afternoon refreshments provided

While there is ICH guidance on the validation of a test method to assure it is suitable for its intended use, there is minimal guidance on how to validate the stability-indicating capability of test methods used in stability protocols. Yet these methods are required by regulation to be validated, and measuring product degradation is clearly their intended use. Currently, the inadequate experimental validation of stability-indicating methods is a major observation in regulatory reviews of BLA/MAA dossiers and in compliance inspections, particularly for biotechnology and biosimilar products. This workshop will provide attendees with:

- Overview of why, when and how stability-indicating methods should be qualified, then validated, for their intended use.
- Design and execution of comprehensive, systematic forced degradation studies is one of the major elements of method validation for stability programs.
- Appropriate experimental designs will be presented, practical points on how to conduct the studies will be shared.
- Strategies for the use of the data throughout the product's lifecycle will be discussed.

Dr Nadine Ritter, Senior CMC Consultant, Biologics Consulting Group, USA

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