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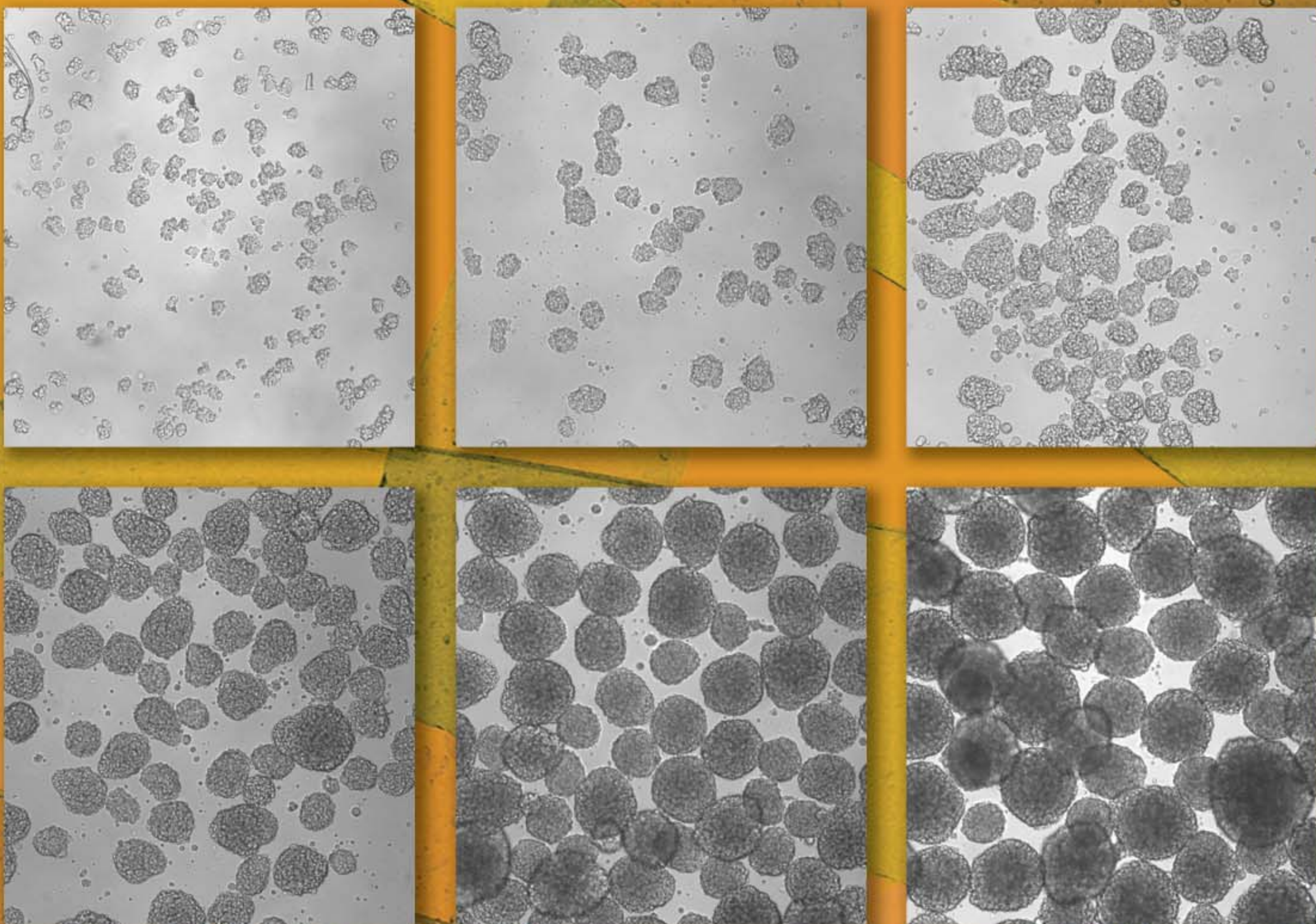

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Product Characterization of Recombinant Proteins and Monoclonal Antibodies

By KATHLEEN M. HANLEY*
and JEANNE NOVAK

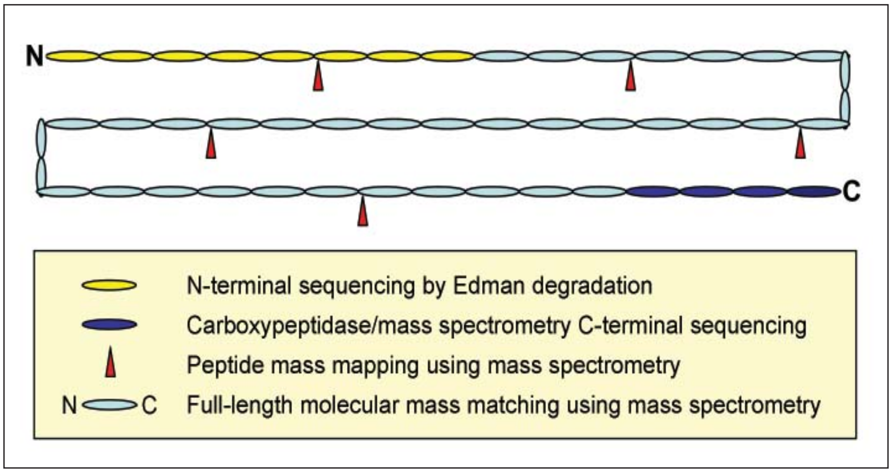

Figure 1. Different analytical methods used to determine primary amino acid sequence information.

The analytical characterization of recombinant protein therapeutic drug products has broadened to include the use of more sophisticated technologies. The expansion of technical abilities has translated into increasing the depth and breadth of our knowledge and understanding of the drug product intended for commercialization. With the availability of more precise methods, the regulatory expectations for understanding the characteristics of a protein therapeutic drug product are increasing.

A thorough understanding of a therapeutic protein's biochemical and biophysical characteristics is necessary to support investigational new drug (IND) applications and other drug regulatory filings. Regulatory agencies worldwide have approved numerous recombinant protein drug products[1-3] and now have extensive experience with methods and data appropriate for characterizing monoclonal antibodies (MAbs) and other protein therapeutics. In particular, many MAbs have been approved for use in both the US and the European Union (EU),[1-3] and several more are in clinical development (Table 1).

The first MAb approved for patient use, Orthoclone OKT3, was approved by the FDA in 1986 for treatment of organ rejection. Since that time, many of the therapeutic MAbs have been approved for oncological and immunological indications (Table 1).

Extensive knowledge and understanding of biopharmaceuticals has been developed, resulting in regulatory expectations for the types of methods to be used and product characteristics to be monitored, from initiation of manufacture through stability testing. Product characterization is not only driven by regulatory requirements, but is also steered by industry standards. Increasingly sophisticated analytical methods and instrumentation are raising the bar for what is acceptable when developing methods to characterize MAbs and other recombinant therapeutic proteins.

Analytical Method Development

Successful development and manufacture of MAbs and other protein drug products is highly dependent on the use of appropriate analytical methods for product characterization. Frequently, protein biochemical and physical attributes can be assayed using different, but complementary methods (Table 2). The use of multiple analytical methods to characterize drug products has the added benefit of supporting and confirming individual test results.[4]

Protein Structure

The primary amino acid sequence is a fundamental analytical characteristic of all protein therapeutics. Analytical tools are readily available to determine the DNA sequence of a recombinant protein therapeutic, but DNA sequence alone can not provide information on the nature of the expressed and purified protein following transcription and translation. Analytical methods have been developed to determine primary amino acid sequence information of the expressed, mature protein (Table 2, Figure 1). Edman degradation sequenc-

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Table 1. Monoclonal antibodies approved or in late-stage clinical development.			
Generic Name	**Trade Name**	**Approval Date**	**Category**
Muromonab-CD3	Orthoclone KT3	06/19/86 (US)	Immunological
Abciximab	ReoPro	12/22/94 (US)	Hemostasis
Rituximab	Rituxan	11/26/97 (US), 06/02/98 (EU)	Oncological
Daclizumab	Zenapax	12/10/97 (US), 02/26/99 (EU)	Immunological
Basiliximab	Simulect	05/12/98 (US), 10/09/98 (EU)	Immunological
Palivizumab	Synagis	06/19/98 (US), 08/13/99 (EU)	Anti-infective
Infliximab	Remicade	08/24/98 (US), 08/13/99 (EU)	Immunological
Trastuzumab	Herceptin	09/25/98 (US), 08/28/00 (EU)	Oncological
Gemtuzumab zogamicin	Mylotarg	05/17/00 (US)	Oncological
Alemtuzumab	Campath-1H	05/07/01 (US), 07/06/01 (EU)	Oncological
Ibritumomab tiuxetan	Zevalin	02/19/02 (US), 01/16/04 (EU)	Oncological
Adalimumab	Humira	12/31/02 (US), 09/1/03 (EU)	Immunological
Omalizumab	Xolair	06/20/03 (US)	Immunological
Tositumomab-I131	Bexxar	06/27/03 (US)	Oncological
Efalizumab	Raptiva	10/27/03 (US), 09/20/04 (EU)	Immunological
Cetuximab	Erbitux	02/12/04 (US), 06/29/04 (EU)	Oncological
Bevacizumab	Avastin	02/26/04 (US), 01/12/05 (EU)	Oncological
natalizumab	Tysabri	11/23/04 (US)	Immunological
Panitumumab	Vectibix	09/27/06 (US)	Oncological
Eculizumab	Soliris	03/16/07 (US), 06/23/07 (EU)	Immunological
Ranibizumab	Lucentis	(US) 01/24/07 (EU)	Therapeutic
Denosumab	NA*	Clinical Development	Therapeutic
Zanolimumab	Humax-CD4	Clinical Development	Oncological
MDX-010	NA	Clinical Development	Oncological
Anti-MRSA MAb	Aurograb	Clinical Development	Anti-infective
Pexelizumab	NA	Clinical Development	Immunological
Mepolizumab	NA	Clinical Development	Immunological
Epratuzumab	Lymphocide	Clinical Development	Oncological
Anti-RSV MAb	Numax	Clinical Development	Anti-infective
Certolizumab Pegol	Cimzia	Clinical Development	Immunological
Afelimomab	Segard	Clinical Development	Therapeutic
Catumaxomab	Removab	Clinical Development	Oncological
WX-G250	Rencarex	Clinical Development	Oncological

(NA*: Not available) Sources: PJ Carter, *Nat Rev Immun*, 2006, 6:343; JM Reichert *et al.*, *Nat Biotech*, 2005, 23:1073; <www.Clinicaltrials.gov>; <www.FDA.gov>.

Table 2. Selected analytical methods used to characterize therapeutic proteins.

Analytical Method	Protein Characteristic
SDS-PAGE (reduced)	Size, purity
Isoelectric focusing	Charge distribution, purity
Immunoblotting, ELISA, FACS	Binding affinity, avidity and immunoreactivity
HPLC	Charge, hydrophobicity, purity
Bioassay	Potency, bioactivity
Immunoassay	IgG subclass determination
Amino acid analysis	Amino acid composition
Edman degradation sequencing, MS	Amino acid sequence
Epitope mapping	Antigenicity
MS, SEC-MALS, SEC-HPLC, AUC, FFF	Relative molecular mass
CD, DSC, MS, NMR	Three-dimensional structure

Abbreviations

AUC	Analytical ultracentrifugation
CD	Circular dichroism
DSC	Differential scanning colorimetry
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
FFF	Field flow fractionation
HPLC	High pressure liquid chromatography
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
SEC-MALS	Size exclusion chromatography-multi-angle light scattering
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis

ing is commonly used to determine the N-terminal sequence of full length proteins or smaller peptide fragments, but has some limitations such as the inability to resolve certain residues, including cysteines. Additionally, this method can not be utilized if the N-terminus of the protein is acetylated. Mass spectrometry (MS) has proven to be a valuable tool for determining primary amino acid sequence information, both in support and as an alternative to the Edman degradation sequencing method.

Early in the development of this method, proteins and peptides were difficult to ionize in the MS without destroying the molecule due to their size, but the development of "soft-ionization" techniques such as matrix-assisted laser desorption time-of-flight (MALDI-TOF) MS and electrospray ionization (ESI) MS led to the successful use of MS for analytical protein chemistry.[5] This scientific advancement was recognized by awarding the 2002 Nobel Prize for Chemistry to Fenn and Tanaka for their contributions to this field. MALDI-TOF MS, ESI MS, ion trap MS, linear quadrupole ion trap (LTQ) MS, Fourier transform ion cyclotron resonance (FT-ICR) and hybrid MS/MS instruments (ESI-TOF, ESI-ion trap, TOF-TOF) are all utilized to determine the accurate molecular mass of full-length proteins.

In some instances, a comparison of the theoretical molecular mass (based on the deduced amino acid sequence) of an intact protein or peptide to the experimental mass is not appropriate due to heterogeneity from protein modifications or unknown truncations at the N- or C-termini. In such cases, enzymatic digestion of the full-length protein can be utilized to determine additional sequence information based on mass measurement of the resulting peptides. Peptide identity and identification of the specific protein modification, such as acetylation, phosphorylation, deamidation, or oxidation can be determined using MALDI-TOF MS or tandem mass spectrometers in combination with a liquid chromatographer (LC) or LC MS/MS instruments. Digested peptides are separated by reversed-phase (RP) or other LC chemistry, peptide bonds are cleaved in the MS, and accurate mass of the individual amino acids or modified amino acids is determined. This peptide mass mapping or "bottom-up" sequencing[6,7] approach is very useful for fully characterizing the primary structure of the expressed protein, but is sometimes limited by the inability to detect specific peptides that do not ionize well.

More recently, a technique termed "top-down" sequencing has been developed to obtain amino acid sequence information from full-length, undigested proteins and large peptides. Using very high resolution and high mass accuracy instruments such as an FT-ICR MS, amino acid sequence information of a full length protein can be determined. Reid and McLuckey[6] provide a comprehensive review of the use of MS for the characterization of protein and peptide mass and sequence information.

Full characterization of a biopharmaceutical should include methods to analyze higher order structure. A modified three-dimensional structure can alter the potency, immunogenicity/toxicity and stability of a protein therapeutic, and therefore, monitoring is essential.[8,9] Several different methods can be utilized to obtain structural information including circular dichroism (CD) spectroscopy, nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC), and MS. CD spectroscopy measures differences in the absorption of left-handed polarized light versus right-handed polarized light which arise due to structural asymmetry and is a common analytical method used to determine protein higher order structure.[10,11] CD spectroscopy is frequently used to monitor structural differences that may develop as a result of changes in bioprocessing methods, storage conditions, or product formulations. Structural analyses are also used for evaluating potential differences between recombinant and native proteins. Figure 2 is an example of the use of CD spectroscopy to compare the structures of a native protein to a recombinantly-expressed protein. In this case, the recombinant form of the enzyme did not have the same secondary structure as the native protein.[11]

Protein structural analysis should also include characterization of disulfide bond formation. Some recombinant proteins, and in particular, some recombinant MAbs, are susceptible to aberrant disulfide linkages. Several analytical techniques including SDS-PAGE, HPLC, and DSC have all been utilized to evaluate disulfide bond formation, although disulfide mapping using MS is the recommended method.[9] MALDI-TOF MS was used to map the abnormal disulfide bond formation in the transmembrane domain of rhodopsin mutants.[12] Previous studies had only described the abnormal disulfide linkages in the soluble, intradiscal domain of the protein.

Post-Translational Modifications

Covalent modifications to the primary amino acid backbone of recombinant protein therapeutics can occur either naturally during protein processing or as a consequence of the drug manufacturing process. Many common post-translational modifications can be detected based on their measured mass differences from the non-modified protein (Table 3).

MS is commonly used to detect many protein post-translational modifications. The Association of Biomolecular Resource Facilities (ABRF) has a very useful website that can be used to search for potential protein modifications based on mass difference (<http://www.abrf.org/index.cfm/dm.home>). LC (inline or offline), in combination with MS, is frequently used to detect and quantify protein variants. In some cases, protein characterization includes the use of forced degradation studies in order to analyze potential post-translation modifications. Figure 3 is an example of stress-induced oxidation imposed on purified recombinant aprotinin. MALDI-TOF MS analysis of the stressed-protein detected a peak of the expected molecular mass, and a second peak that was approximately +16 Da (Figure 3A). Additionally, quantitation of the stress-induced oxidized and non-oxidized forms was determined chromatographically using RP-HPLC (Figure 3B). In order to verify that the additional peak was an oxidized form of the purified protein, tryptic peptide mass mapping was performed and identified a tryptic fragment that included an oxidized methionine (data not shown). Sometimes though, characterization of protein variants requires being more of a sleuth than a scientist, as summarized in Figure 4.

Table 3. Common protein modifications and the molecular mass differences.

Modification	Mass Difference (AMU)
N-terminal acetylation	+42
Phosphorylation (Thr, Ser, Tyr)	+80
Methylation (Lys, Ser, Thr)	+12
Sulfation (Tyr)	+80
Hydroxylation (Pro, Lys, Asp, Trp)	+16
Oxidation (Met or Cys)*	**+16**
Glycosylation*	**variable**
Disulfide bond pairing*	**-2/pair**
Deamidation (Asn, Gln)*	**+1**
N-terminal pyroglutamate formation*	**-17 or -18**

*Modifications common to many monoclonal antibodies.

Antibodies are particularly susceptible to post-translation modifications (Table 3). Asparagine deamidation (conversion of asparagine to aspartate or isoaspartate) is a common post-translational modification in MAb and is facilitated by high pH, frequently occurring in asparagine-glycine dipeptides.[9] A conserved sequence (SNGQPENNYK) in the Fc portion of an IgG antibody frequently contains two deamidated asparagines (underlined), but deamidation has also been detected in other regions in both the heavy and light chains.[13] Methods to detect deamidation include ion exchange chromatography, isoelectric focusing, isoaspartate quantitation (Isoquant Detection Kit; Promega Corp., Madison, WI, USA) and enzymatic digestion followed by LC MS/MS analysis. The advantage of the MS method includes determination of site specificity.

Glycosylation is also a common post-

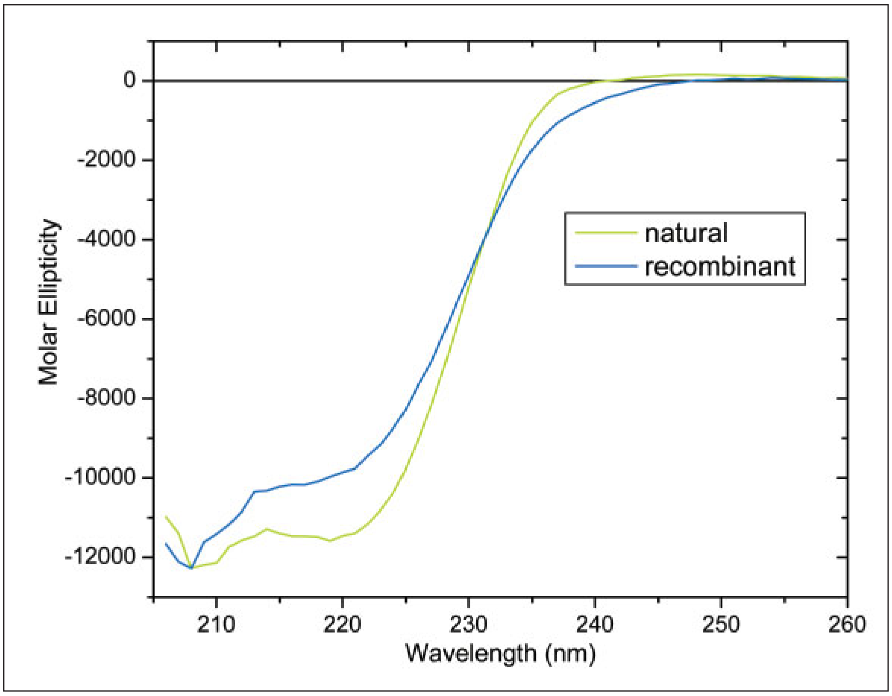


Figure 2. The use of CD spectroscopy to evaluate the secondary structures of a native and recombinant protein. CD spectroscopy in the "far-UV" spectral region (190–250 nm) was utilized to compare the secondary structures of a native and a recombinant enzyme. (Spectrum published with permission from Alliance Protein Laboratories, Thousand Oaks, CA.)

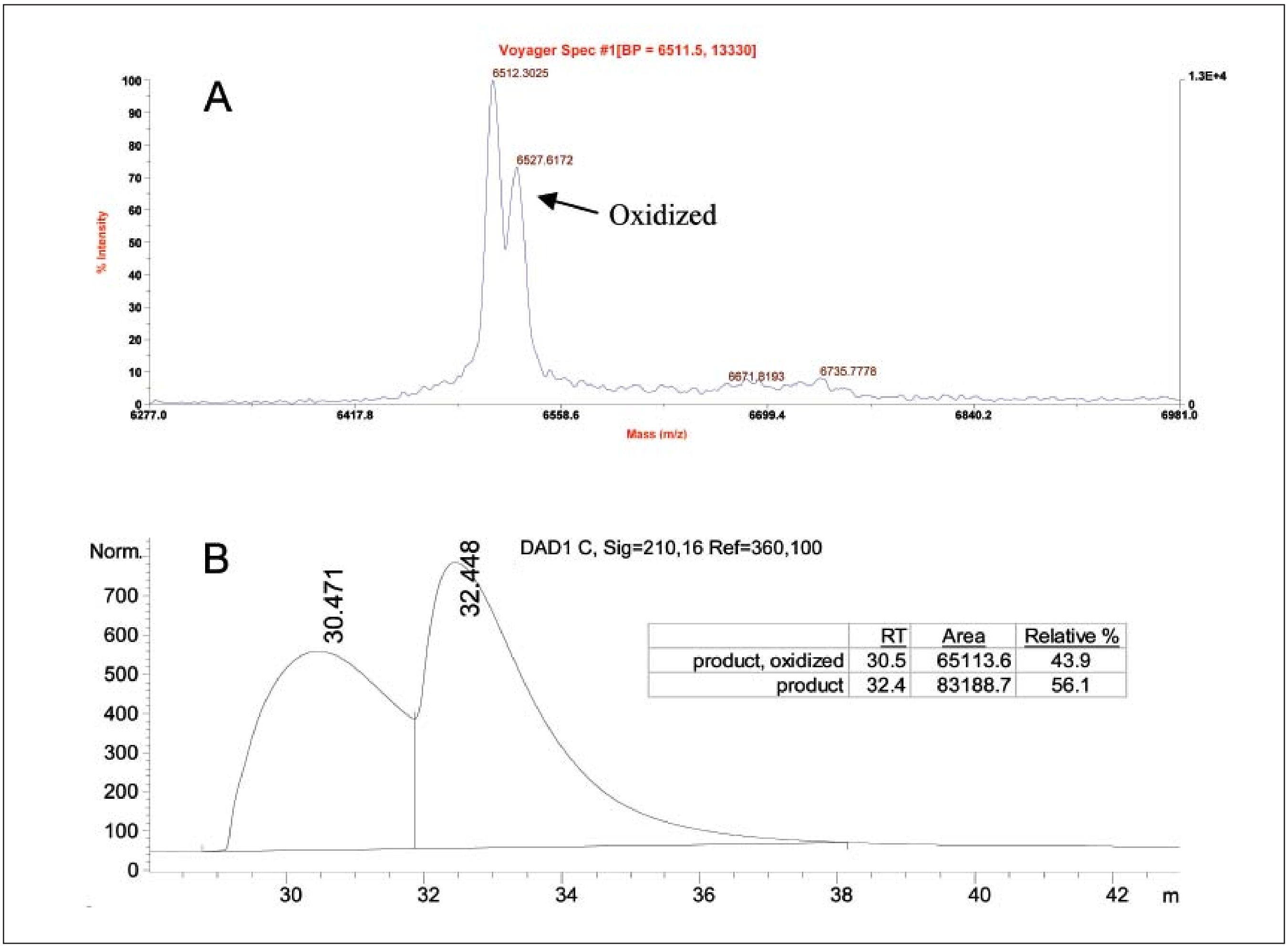


Figure 3. Detection and quantification of protein oxidation. Oxidative stress was induced on recombinant aprotinin protein and monitored using: A) MALDI-TOF MS; and B) RP-HPLC. (Source: K. Hanley and E. White, LSBC, Analytical Sciences Department.)

Product Variant: Modified Recombinant Interferon

- Theoretical MW=16,790 Da
- Experimental MW=16,806 Da by **MALDI-TOF MS** delta mass = +16 Da
- **Peptide Mass Mapping** identified +16 Da N-terminal tryptic fragment containing Met and Pro
- +16 Da variant: Oxidized Met or Hydroxyproline

Which Modification is it?

- N-terminal sequencing verified the elution of a hydroxylated proline at predicted Pro^6 position

Figure 4. The identity of a hydroxylated recombinant interferon variant was determined using mass spectrometry and Edman degradation sequencing. (Source: K. Hanley, LSBC, Analytical Sciences Department.)

Table 4. Analytical methods used to characterize protein glycosylation.

Glycan Parameter	Analytical Method
Mass	MALDI-TOF MS; LC-MS
Charge	AE-HPLC-PAD, CE, IEX-HPLC
Composition	AE-HPLC-PAD, MS
Glycan sequence	Enzymatic degradation + MALDI-TOF MS; LC-MS/MS
Sialic acids	AE-HPLC-PAD

Source: J. Windisch, *FDA/DIA Workshop Follow-on Proteins*, Feb 14, 2005.

Abbreviations	
AE-HPLC-PAD	Anion-exchange HPLC with pulsed amperometric detection
CE	Capillary electrophoresis
IEX-HPLC	Ion-exchange HPLC

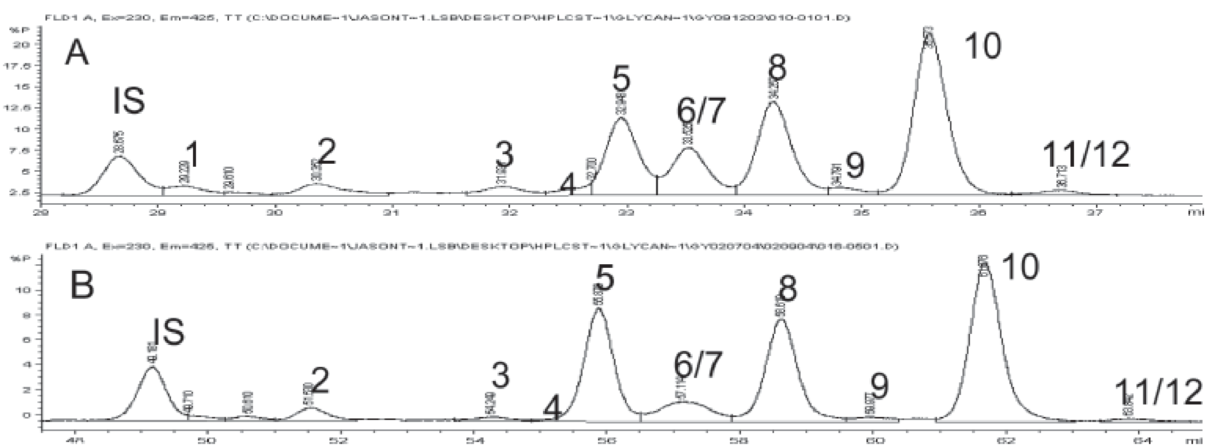
translational modification. Numerous analytical methods are utilized to characterize glycoproteins (Table 4). Protein glycosylation characterization should include methods to detect glycan composition and site occupancy.

Although glycosylation is relatively heterogeneous in nature, it is still important that characterization methods are developed to monitor changes that may occur. Glycan structures and site occupancy should be evaluated in order to determine if changes have occurred due to product storage conditions or changes to the manufacturing process. Glycan analyses should also be included when determining lot-to-lot variability during production. Figure 5 is an example of two glycan analyses that were done in order to assess glycosylation in two lots of recombinant human lysosomal acid lipase (LAL). LAL is a heavily glycosylated enzyme used in the treatment of Wolman disease and cholesteryl ester storage disease.[14] Using MS and HPLC, it was determined that the glycan structures remained similar in type and concentration between production runs.

Glycosylation should also be monitored during extended stability studies. Figure 6 is an example of a glycosylated single-chain antibody, produced as two glycoforms, that was monitored using MALDI-TOF MS. The relative ratio of the two glycoforms and the molecular mass measurements did not change significantly after 12 months of storage at –20°C. Although typically not quantitative, MALDI-TOF MS has been utilized to monitor the stability of glycoprotein vaccines in a Phase 1 clinical trial.[15]

Potency

Characterization of a protein's biological activity includes development of an appropriate assay to measure activity or potency of the purified product. Bioactivity assays are typically developed using fluorescent, chemiluminescent, or radiolabeled substrate and are used to measure protein-specific activity (moles/unit of time/unit protein). Typical potency assays to measure the activity of therapeutic MAbs are described in Table 5. *In vivo* assays in animals and *in vitro* cell-based assays are most typical, but antibody binding assays may also be developed to characterize antibody potency.[16] The potency assay should be developed to mimic as closely as possible the putative physiologic/pharmacologic activity of the product.[16]



Peak	Peak ID	Average Relative %Glycan (A)	Average Relative %Glycan (B)
1	**M2XFGlcNAc2**	1.76	ND
2	**M3XGlcNAc2**	3.04	3.45
3	**M3XGlcNAc3**	2.21	1.47
4	**M3GlcNAc4**	0.96	ND
5	**M3FXGlcNAc2**	17.81	24.57
6 & 7	**M5GlcNAc2** **M3XGlcNAc4**	11.39	7.81
8	**M3FXGlcNAc3**	22.14	23.70
9	**M3FGlcNAc4**	1.17	1.10
10	**M3XFGlcNAc4**	36.96	36.66
11 & 12	**M7GlcNAc2** **M4XGlcNAc4**	1.50	1.23

Abbreviations	
F	Fucose
GlcNAc	N-Acetylglucosamine
IS	Internal standard
M	Mannose
ND	Not detected
X	Xylose

Figure 5. Glycan analysis of two recombinant glycoproteins assessing lot-to-lot glycan variability after processing. Two lots of lysosomal acid lipase (A and B) were proteolytically digested, enzymatically deglycosylated, and isolated-glycans-quantified by HPLC. The identity of isolated glycans was determined using off-line MALDI-TOF MS. (Source: J. Thornton, E. White, and K. Hanley, LSBC, Analytical Sciences Department.)

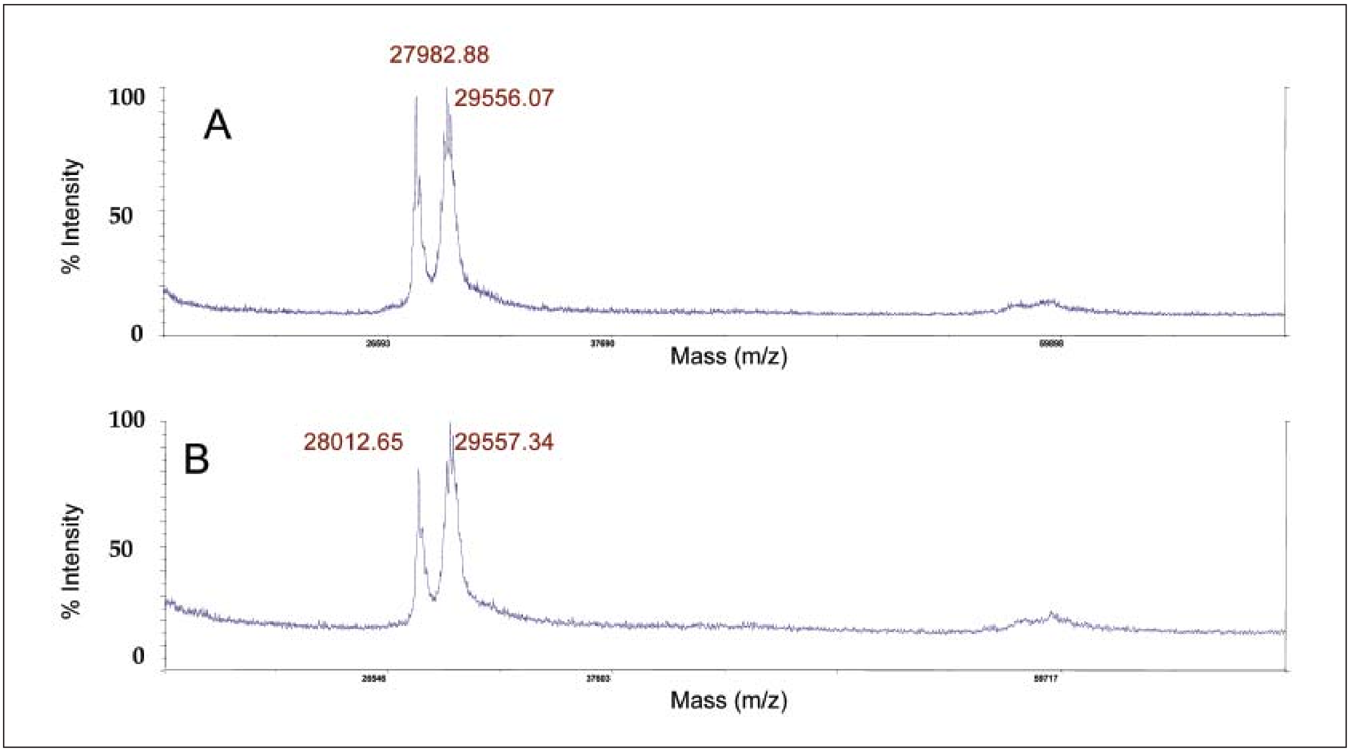


Figure 6. MALDI-TOF MS mass analysis of a recombinant single-chain antibody vaccine after storage. MALDI-TOF MS spectrum of vaccine at: A) product release; and B) after 12 months of storage at –20°C. (Source: K. Hanley, LSBC, Analytical Sciences Department.)

Product-Related Impurities

Many of the analytical methods developed to characterize proteins are also used to determine the identity of protein-derived impurities that may be included in the purified product, such as protein aggregates and truncations. Figure 7 illustrates the use of gel electrophoresis, MS and peptide mass mapping to determine the identity of an unknown protein band in an SDS-PAGE gel from a purified recombinant protein single-chain antibody vaccine product. MALDI-TOF MS peptide mass mapping analysis determined that one of the unknown protein bands was a C-terminal truncation of the purified drug product (Figure 7). The results were further substantiated using Western blot analysis and N-terminal sequencing of the truncated band (data not shown).

Protein aggregation is a major concern; therefore, analytical testing should be developed to monitor for aggregation during early protein characterization studies. Numerous methods are available to identify and quantitate protein aggregates,[17-20] although not all methods are appropriate for all laboratories and for all protein characterization.[17] Table 6 describes the advantages and disadvantages of using various methods to analyze for protein aggregation.

MALS is a technique that is based on the biomolecule's light dispersion properties[19] and is frequently used in-line with SEC-HPLC to characterize the nature of protein aggregates (SEC-MALS). Figure 8 describes the use of SEC-MALS to identify and quantitate aggregates of a 51 kDa recombinant protein.

Protein Product Characterization Applications

Product Reference Standards

In addition to analyzing the complexity of the protein therapeutic that is under investigation, the assays developed during product characterization have many different applications including their use to characterize in-house product reference standards. This is especially true when no commercial or international standard is available. Protein characterization assays are intended to fully analyze the biochemical and physical nature of the protein therapeutic, and thus, these assays are especially well suited for characterizing reference standards. Well-characterized product reference standards have many applications including assay quantitation, establishing identity of test article, method qualification/validation, and comparability testing. The appropriateness of reference standards are critical to the performance of any quality analytical method and the evaluation of data generated by that method. Regulatory guidance specifies that prior to drug submissions, a well-characterized in-house reference standard should be prepared using material that is representative of the material for use in the clinic and the final production method.[21] Additionally, in-house therapeutic antibody reference standards

Table 5. Analytical methods used to measure monoclonal antibody potency.

Potency Assay	Analytical Method
Antibody binding	ELISA, RIA, immunoprecipitation, flow cytometry
In vivo antibody response	Immunogenicity in rodents
In vitro functional assay	Cell proliferation; cytokine production

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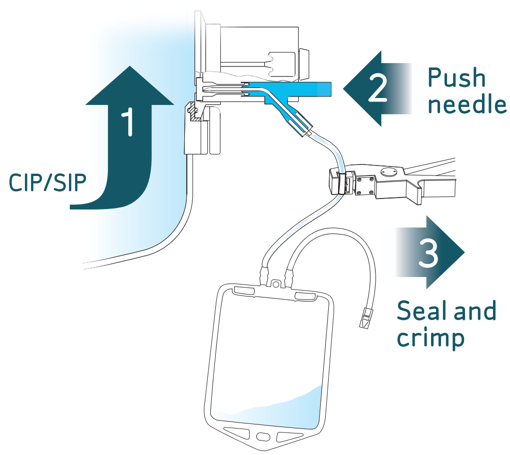

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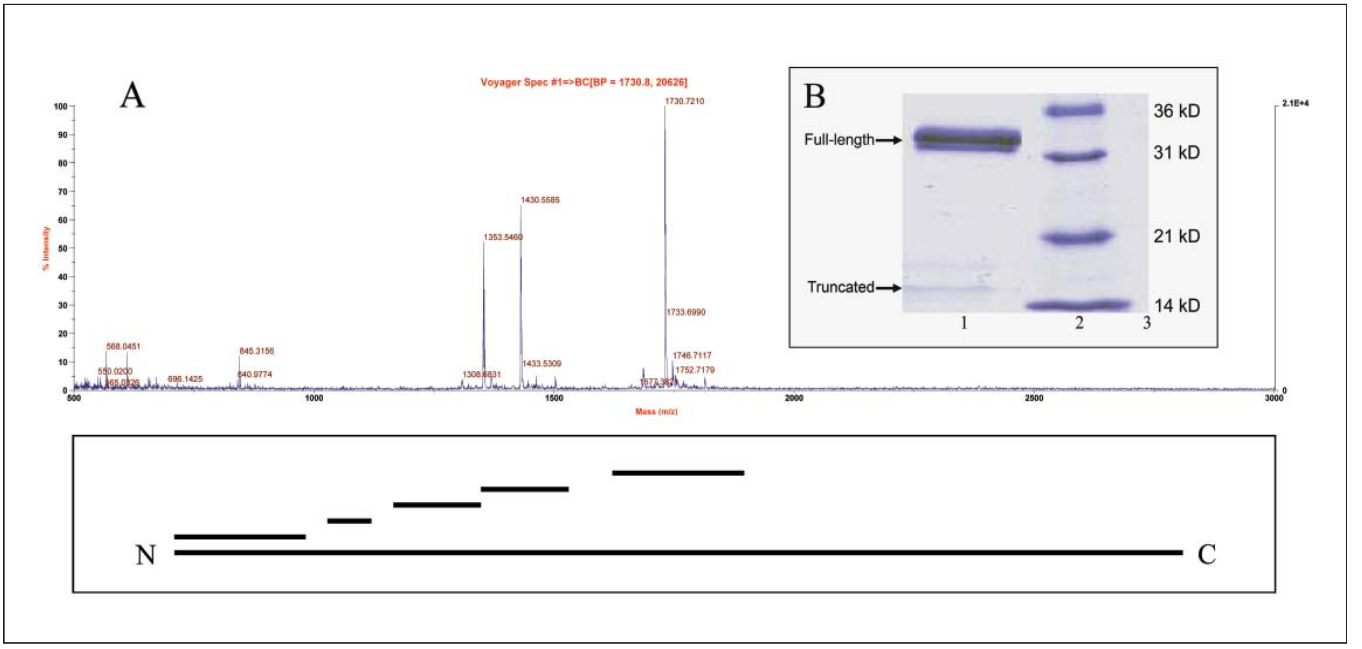

Figure 7. Identification of a product-derived, truncated impurity: A) MALDI-TOF MS analysis of in-gel tryptic digest to identify a putative truncation using peptide mass-mapping. Spectrum was acquired on a Voyager-DE STR mass spectrometer (Applied Biosystems, Foster City, CA, USA); and B) SDS-PAGE separation of purified recombinant protein and smaller molecular impurities. (Source: K. Hanley, LSBC, Analytical Sciences Department.)

Table 6. Analytical methods to assay protein aggregation.

Analytical Method	Advantages	Limitations
SEC-HPLC	• Well established • Appropriate for Characterization and QC • Ease of use and relatively inexpensive	• Possible non-specific interaction between protein and HPLC resin/hardware • Mobile phase and biopharmaceutical liquid formulation may be incompatible • SEC dilution effects may alter concentration-dependent aggregation
AUC	• Testing conducted in exact or similar sample formulation buffer • Method development is minimal • Improved data analysis software (SEDFIT, SEDPHAT) • Broad concentration range	• Instrumentation expensive ($200-400K) • Requires highly trained analyst • Not compatible with QC lab testing
MALS	• Very good dynamic range • Accurate molar mass determination (±5%) • Coupled with refractive index to quantify CHO content • HT assay development using batch mode (Dynamic LS)	• SEC-MALS similar limitations to SEC (see above)
FFF	• Minimal shear stress on sample components • Flow-based separation • Use wide range of buffer conditions • Wide dynamic range (0.001-50 µm) • No interaction with column matrix	• Requires highly trained analysts • Non-specific absorption on cross-flow membrane • Precision and accuracy for detecting low levels of aggregates is not well established

Sources: Berkowitz, *AAPS J*, 2006; 8(3):E590-605; Liu *et al.*, *AAPS J*, 2006; 8(3):E580-E589; Wen *et al.*, *Anal Biochem* 1996; 240:155-166.

are expected to be fully characterized, with known specificity and potency, and stored appropriately with periodic testing to ensure integrity.[16]

Release and Stability Testing

As previously mentioned, many of the methods developed to characterize a protein therapeutic can also be utilized for product release and stability testing. Product release testing should include development of analytical methods used to determine a protein's purity, identity, and potency. Typically, only a subset of the characterization assays are used for release testing and a still smaller set would be utilized for stability monitoring (Figure 9). This is due to the fact that many of the analytical characterization assays are developed using instrumentation that may not be easily qualified, nor the procedure easily validated for use in product release and stability testing. Additionally, in some situations, cost and time to execute and monitor assays and results for release or stability monitoring of a product is also a factor. Figure 10 describes different analytical methods that may be included when developing a Certificate of Analysis for a protein therapeutic release or stability testing.

Conclusions

Regulatory agencies are not leading the charge for how many and what types of analytical methods should be developed to fully characterize a protein therapeutic. Rather, it is the industry standards that will mandate what testing needs to be performed. This includes the use of more sophisticated analytical instrumentation for analysis and performing multiple testing. A thorough characterization of protein therapeutics will lead to the development of better assays for use in clinical investigations and ultimately, should help the drug approval process.

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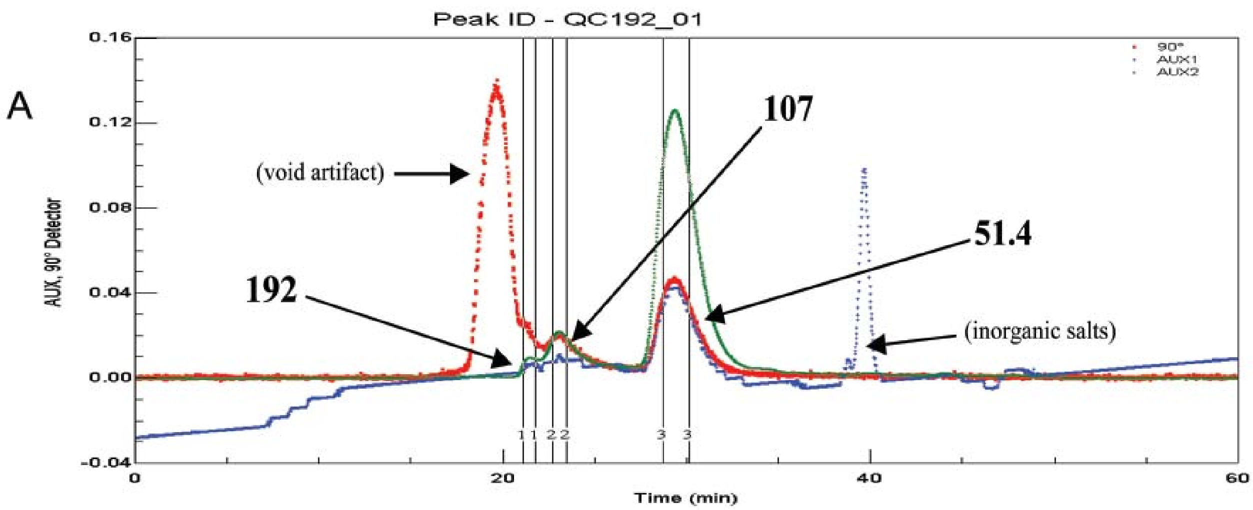

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Pk#	RT	Area	Relative %	LS MW
1	21.28	2367.20	**2.77%**	**192 kDa**
2	22.90	12427.80	**14.54%**	**107 kDa**
3	29.18	70649.50	**82.68%**	**51 kDa**
Total Area		85444.50		

Figure 8. Characterization of protein aggregates using SEC-MALS: A) SEC-MALS chromatogram monitoring UV 210 absorbance (green), light-scattering (red) and refractive index (blue) output; and B) protein monomer (51 kDa), dimer (107 kDa), and multimer (192 kDa) concentrations and approximate molecular weights were determined. (Source: E. White and K. Hanley, LSBC, Analytical Sciences Department.)

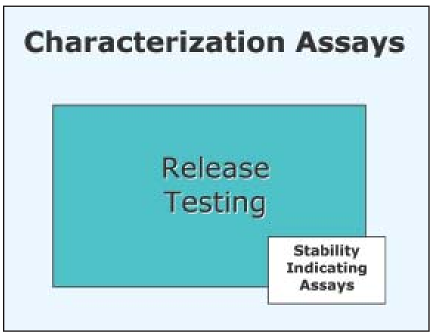

Figure 9. Use of protein characterization assays for product release and stability monitoring.

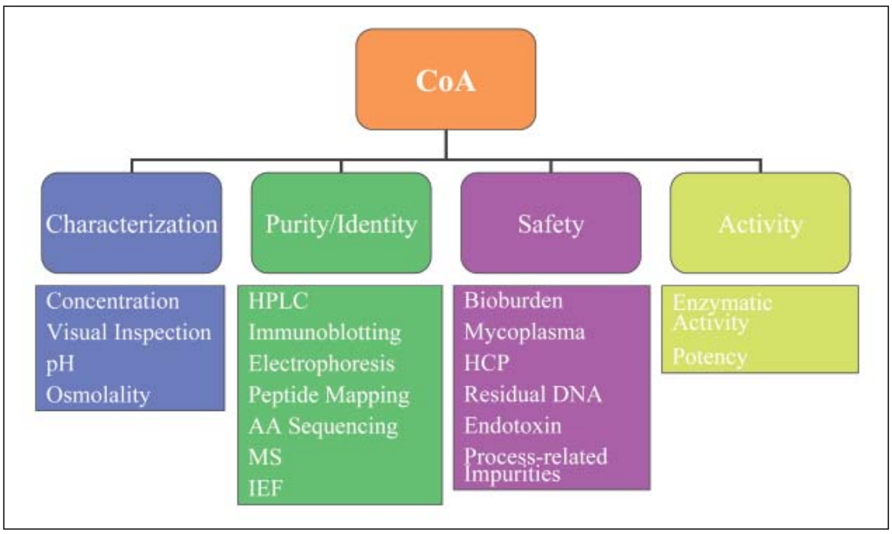

Figure 10. Analytical testing methods used to develop a Certificate of Analysis for a protein therapeutic release or stability testing.

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