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Original Article

A Comparative Study of SDS-PAGE, SEC-UV, and SEC-MALS in Assessing Monoclonal Antibody Aggregation

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ABSTRACT

Objectives: Monoclonal antibodies are prone to aggregate formation at elevated temperatures due to the disruption of their native conformation resulting in protein denaturation, disulphide linkage disruptions and eventually resulting in the loss of their key biological functions. Detection of protein aggregation is a key quality attribute which ensures biological stability, potency and dosage determination. Bulk biological manufacturing, logistics and supply chain, storage, patient delivery are the transition points of any clinical product Lifecyle. A wide variety of advanced analytical methods are used in the biopharma industry for ensuring high standard product quality and safety aspects for the product to meet its desired clinical outcome. Thermal kinetics-based studies provide an insight into molecular folding and aggregate formation as they forcibly induce protein aggregation at an accelerated pace. Forced thermal aggregation studies mimic the temperature excursion influence on the therapeutic monoclonal antibodies during their product lifecycle. **Materials and Methods:** In the current study, we evaluated protein aggregation of IgG1 Cetuximab mAb sample using advanced analytical techniques including Sodium dodecyl Sulphate-Poly Acryl Amide Gel Electrophoresis (SDS-PAGE), Size Exclusion Chromatography (SEC), and multi-angle light Scattering (SEC-MALS), and cetuximab sample was subjected to thermal kinetics to evaluate aggregation at an elevated temperature of 70° centigrade at multiple time points T_0, T_1 and T_2 etc., **Results and Discussion:** Resulting thermal aggregation products are analysed using traditional SDS PAGE and advanced SEC-UV and SEC-MALS methods. We observed dimer formation because of protein aggregation in a time of 30 min at 70°C. Further increment in temperature resulted in an overall increase in the quantity of the dimer product and additionally resulted in the formation of higher order trimer species. **Conclusion:** Analytical results from three different platforms have indicated protein aggregation. SDS-PAGE is a simple cost-effective tool for preliminary aggregation assessment. Advanced techniques provide a robust, high-throughput and accurate quantified results for the estimation of protein aggregates.

Keywords: SDS-PAGE, SEC-UV, SEC-MALS, Monoclonal Antibody.

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INTRODUCTION

Monoclonal Antibodies (mAbs) represent a revolutionary advancement in therapeutic modalities, demonstrating remarkable specificity and efficacy in targeting various pathological conditions, including cancer and autoimmune diseases. Rapid advancements have emerged in recent years on manufacturing and testing of the mAbs. Biosimilar mAb's are gaining traction as cheaper alternates to their innovator counterparts. Quality and stability of mAbs poses significant challenges, as they can be susceptible to aggregation during formulation, storage, logistics and clinical administration, potentially leading to reduced efficacy and adverse effects (Basle *et al*., 2019). Cetuximab is a chimeric antibody and is commercially available for the treatment of colorectal cancer and head and neck cancer. It is an https://en.wikipedia.org/wiki/Epidermal_growth_factor_receptor (EGFR) inhibitor.

Various analytical techniques are employed to assess mAb aggregation, among which Sodium dodecyl Sulphate-Poly Acryl Amide Gel Electrophoresis (SDS-PAGE), Size Exclusion Chromatography (SEC), and Multi-Angle Light Scattering (SEC-MALS) stand out due to their ability to provide detailed insights into protein stability and heterogeneity (Şahin and Roberts, 2012). HOS studies including SV-AUC and NMR are orthogonal techniques which analyse the samples in their native state provide an in-depth molecular understanding, however it is beyond the scope of current work.

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SDS-PAGE serves as a foundational tool for separating mAb aggregates by size, enabling the visualization of monomers and higher-order oligomers. SDS-PAGE is a widely used technique for analyzing protein size and purity. Proteins gets separated based on their electrophoretic mobility, which is a function polypeptide chain length. This method is effective for detecting high molecular weight aggregates and can provide qualitative information about the presence of aggregates in mAb samples (Sreenivasan *et al*., 2024). It is also cost friendly and requires minimal scientific training as compared to any other advanced analytical techniques.

In contrast, SEC offers a more refined approach, separating mAbs based on hydrodynamic volume, which is crucial for accurately determining the aggregation profile in complex formulations. Moreover, the integration of SEC with MALS enhances the capacity to characterize the absolute molecular weight of mAb species for their aggregation profiles, addressing the limitations of existing methodologies highlighted in previous studies (Yoshino *et al*., 2011). This comprehensive analysis facilitates the development of more stable mAb formulations. Regulatory agencies such as the FDA and EMA mandate stringent monitoring of aggregate formation due to its potential impact on therapeutic efficacy and patient safety. Therefore, robust analytical methodologies capable of detecting, characterizing, and quantifying aggregation events are essential for ensuring process robustness, batch consistency, and regulatory compliance in large-scale mAb production (Bhirde *et al*., 2020).

SEC is a widely used technique for characterizing monoclonal antibodies (mAbs) by distinguishing various aggregate forms, including dimers and trimers. It plays a crucial role in assessing mAb stability under different stress conditions. However, one limitation of SEC is its reduced ability to detect exceptionally large or insoluble aggregates, which may affect the comprehensiveness of certain analyses (Yoshino *et al*., 2011). Size-Exclusion Chromatography (SEC) becomes significantly more effective for molecular weight determination when integrated with a Multi-Angle Light Scattering (MALS) detector. This detector assesses the intensity of light scattered by an analyte at multiple angles relative to the incident laser beam. The combination, referred to as SEC-MALS, enables molecular weight measurement independent of elution time. In this setup, the SEC column functions solely to separate different molecular species in solution, allowing them to reach the MALS and concentration detectors individually (McIntosh *et al*., 2021). The retention time itself is not crucial for analysis, aside from its role in ensuring adequate protein separation. SEC-MALS is particularly useful for distinguishing between covalent and non-covalent aggregates, providing a comprehensive view of the aggregation state (Bana *et al*., 2023). Since the calibration of these instruments is independent of the column and does not require reference standards, SEC-MALS is regarded as an absolute technique for molecular weight determination (Some *et al*., 2019).

Ultimately, understanding the behaviour of mAbs in solution conditions is crucial for optimizing their pharmaceutical applications. Monoclonal Antibodies (mAbs) represent a significant advancement in therapeutic modalities, offering high specificity and efficacy in treating cancer, autoimmune diseases, and other pathological conditions (Kűkrer *et al.*, 2010). However, their stability remains a major challenge, as mAbs are prone to aggregation during formulation, storage, and administration. Aggregation can compromise therapeutic efficacy and increase the risk of adverse effects due to associated immunogenicity aspects (Basle *et al*., 2019). To assess mAb aggregation, various analytical techniques are employed, e.g: SDS-PAGE, Size Exclusion Chromatography (SEC), and Multi-Angle Light Scattering (SEC-MALS). Standing out for their ability to provide detailed insights into protein stability and heterogeneity (Kaur, 2021).

SDS-PAGE is a fundamental tool for analysing protein size and purity. It separates proteins based on their electrophoretic mobility, enabling the detection of high molecular weight aggregates and visualization of monomers and oligomers. This method offers qualitative insights into the presence of aggregates in mAb samples (Sreenivasan *et al*., 2024). The method has a key advantage of providing a dynamic range and the technology is affordable, simple yet a powerful analytical skill making it a preferred choice for most academic fraternity and serves as a preliminary screening tool in the biopharma industrial domain (Liu *et al.*, 2007).

SEC-UV provides a more refined and advanced approach, separating mAbs based on hydrodynamic volume to accurately determine the aggregation profile in complex formulations. It is widely used for distinguishing aggregate forms such as dimers and trimers and plays a crucial role in evaluating mAb stability under stress conditions. It has gained a wide industrial reputation and regulatory acceptance due to its robustness and accuracy. However, SEC like any analytical technology, has its limitations in-terms of detecting exceptionally large or insoluble aggregates, which may affect the comprehensiveness of some analyses (Yoshino *et al*., 2011).

SEC-MALS Size-Exclusion Chromatography coupled with Multi-Angle Light Scattering enhances SEC's capability by integrating a Multi-Angle Light Scattering (MALS) detector, which measures light scattering intensity at multiple angles to determine absolute molecular weight independent of elution time. Unlike traditional SEC, SEC-MALS does not rely on column calibration or reference standards, making it an absolute convenient and reliable technique for molecular weight determination Daniel Some *et al*., Waters corporation). It is particularly effective in distinguishing between covalent and

non-covalent aggregates, providing a comprehensive view of the aggregation state (Bana *et al.*, 2023, Folta-Stogniew and Williams, 1999). It is commercially available in two angles and eight angles for light scattering measurements. Two fixed angles-typically a low angle (e.g., 90°) and a high angle (e.g., 15°–30°) and an eight angle measures between 10° and 150°.

MATERIALS AND METHODS

Bovine Serum Albumin (BSA) obtained from Sigma(A7030) was used as a reference protein standard. Sodium phosphate monobasic, Sodium phosphate dibasic and Sodium chloride were purchased from Sigma. 0.2 µm membrane filter was purchased from Millipore Corporation. Commercially available mAb IgG1 Cetuximab sample (Alkem laboratories) of 5 mg/mL was used in the current study. Inhouse Type 1 water was used for dilution of samples. Pre-cast SDS PAGE gels of 4-12% was obtained from thermofisher scientific (Invitrogen-NP0322BOX). NUPAGE sample reducing agent (NP004), NUPAGE MES SDS running buffer (NP002), Novex Prestained Protein standard and LC6060 Simply blue safe stain (465034) were procured from Thermofisher scientific for SDS PAGE analysis. YMC PACK-DIOL200 300*8.0MM I.D 5 um, 20 nm column (DL20S05-3008WT) was used for SEC analysis (Alhazmi and Albratty, 2023; D'Atri *et al.*, 2024).

Thermal degradation conditions

Cetuximab sample 5.0 mg/mL was diluted to achieve a final concentration of 1.0 mg/mL using type 1 water. Three aliquots of 1.0 mg/mL sample were prepared and subjected to varying and time dependent temperature stress conditions. One aliquot of sample was incubated at 70°C for 1 hr. After 30 min from initiation of incubation of first sample, second aliquot was kept for incubation at 70°C. Third aliquot serves as control sample with out any treatment. Step wise incubation leads to two treated samples with different times of incubation (30 and 60 min). Time course-based aggregation approach was followed to understand the effect of temperature and its potential influence in the quantitative aggregate assessment with respect to time function. It also evaluates the capability and sensitivity of the various analytical methods protein aggregate determination (Chan, 2018).

SDS PAGE Analysis

Analysis was performed using a pre-cast 4-12% SDS PAGE gel. A 5.0 µg of non-reduced sample was loaded on to the gel in specific lanes. Control non-treated sample was loaded in the initial well followed by samples treated at 70°C for 30 min and 60 min (Figure 1). Thermal kinetics study was designed to ensure T0, T1 and T2 samples were loaded at the same time onto the SDS-PAGE as they were parallelly subjected to induced thermal stress. Post thermal stress incubation, test samples for analysis are prepared by diluting the samples in a NR dye and type -1 water to ensure an equal load of 5.0 ug. Pre-stained protein markers ranging from 3.5 KDa to 260 KDa were procured from Biorad and were loaded to determine the approximate molecular weight of the proteins separated in the gel. All samples (20 uL volume) were loaded into the gel using gel loading tips. 1X NUPAGE SDS running buffer was used as a running buffer. Gel was run at a constant voltage of 150 V for 1 hr. Run was aborted when dye front reached the end of the gel. Gel was removed from the plates and stained with simply blue safe stain solution for protein band detection. Destaining was performed using a type 1 water until visible bands appear in the gel with clear background (Cernosek *et al.*, 2024; Zhu *et al.*, 2013).

SEC-UV analysis coupled with (Multi Wavelength Detector) MWD and (Multi Angle Light Scattering) MALS Detector

SEC-UV based separation was performed on Shimadzu HPLC (model no.LC-2030C Plus) using YMC PACK-DIOL200 300*8.0MM I.D 5 um, 20 nm column and 50 mM Sodium Phosphate + 150mM NaCl pH 7.2 buffer as mobile phase in isocratic mode. The column was equilibrated with mobile phase for 1 hr. Post equilibration mobile phase was injected on to the column to ensure a stable baseline devoid of interfering peaks. BSA standard (50 uL and 50 µg conc.) was injected onto the HPLC-SEC column (Boer *et al.*, 2021). Control mAb IgG1 cetuximab sample (50 µg) was injected initially in the sequence followed by sample treated at 70°C for 30 min and 60 min respectively. A total amount of 50 µg of sample was injected onto the column. IgG1 cetuximab samples were maintained at a temperature of 2-8 °C during analysis in the autosampler. Column temperature was maintained at 25°C using an inbuilt thermostatic column compartment. Elution profile was recorded at a wavelength of 280 nm. Sample outlets post UV detection was connected to a three angle MALS detector (Some *et al.*, 2019; Hong *et al.*, 2012). Spectral raw data was recorded and processed using Astra software.

RESULTS

SDS PAGE analysis

A major prominent band was observed at ~150 kDa in the control sample lane #3 along with lower molecular weight impurities of approx. 110 to 60 kDa. A prominent band at ~150 kDa along with higher molecular and lower molecular weight separation bands were observed in the temperature treated samples. Intensity of high and low molecular weight protein bands showed a visual difference between samples of varying incubation time periods. Sample loading details are indicated below.

SEC and SEC MALS

BSA protein standard protein consisting of a prominent main peak along with higher molecular weight species. This demonstrates

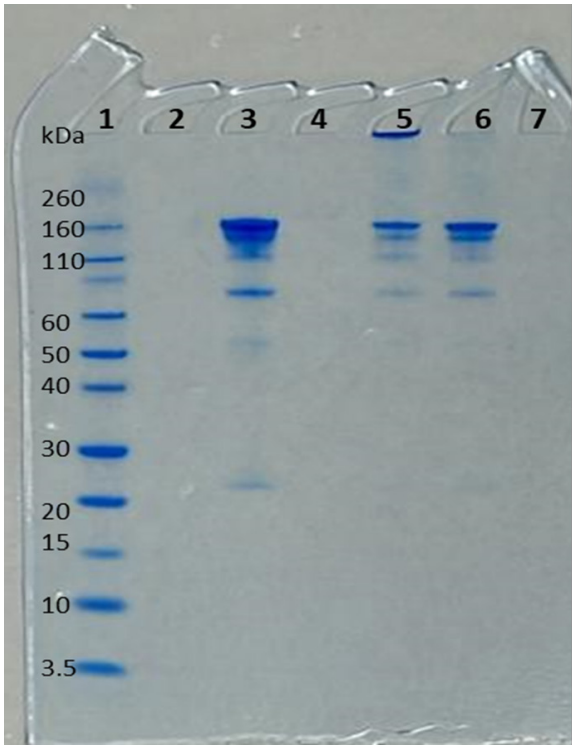
the analytical technique capability and the column separation efficiency in resolving different molecular weight species. In line with BSA sample, thermal stress IgG1 cetuximab samples have shown a main peak along with higher and lower molecular weight species. Intensity of the higher molecular weight species was found to be increasing in the sample subjected to heat treatment with longer incubation times. Decrease in the relative percentage of the main peak, low molecular weight species and increase in the relative percentage of high molecular weight species was observed with increase in incubation time (Gabrielson *et al.*, 2006).

MALS data for BSA standards was processed using Astra software, main peak BSA sample has shown a molecular weight of ~65.0 kDa as expected. Control samples have shown main peak with a molecular weight of ~150 kDa for IgG1 Cetuximab sample. IgG1 cetuximab sample treated for 30 min at 70°C has shown a main peak of ~147.0 kDa along with a high molecular weight peak of molecular weight ~ 350 kDa indicating dimeric form of the protein. For sample treated for 60 min at 70°C has shown main peak of ~450 kDa along with high molecular weight peak indicating potential trimeric form of the protein.

DISCUSSION

Protein aggregation during commercial-scale biopharmaceutical manufacturing, storage, and distribution presents a critical challenge, as it can profoundly impact the biological potency, immunogenicity, and pharmacokinetic profile of the therapeutic molecule. Consequently, aggregation and degradation are subjected to rigorous monitoring throughout the product development lifecycle, spanning early-stage formulation studies to full-scale manufacturing and lot release testing. Accelerated thermal stress studies serve as a pivotal tool for evaluating the thermodynamic stability and aggregation propensity of biologics by simulating real-world stress conditions encountered during manufacturing, transport, and long-term storage. These studies provide predictive insights into the conformational integrity, aggregation kinetics, and structural resilience of protein therapeutics upon exposure to elevated temperatures. Implementing robust In-Process Quality Control (IPQC) strategies at critical junctures ensures process consistency, regulatory compliance, and clinical efficacy of the final biopharmaceutical product. Advanced analytical tools serve a major purpose in the detection of aggregates. These assays also serve as gold standards as lot release assays and are heavily scrutinized by QC teams as the data is used for regulatory submissions.

Advanced analytical methodologies with high sensitivity, resolution, and reproducibility are essential for precise aggregate characterization. In this study, traditional SDS-PAGE was utilized and correlated with data acquired from state-of-the-art chromatographic techniques, SEC-UV and SEC-MALS, to assess aggregate formation under forced thermal stress conditions. The inherent advantages of SDS-PAGE, including its cost-effectiveness, high-throughput capability, and well-established applicability, make it an indispensable preliminary screening tool for assessing protein integrity in a short turnaround time. Results from



Lane 1	Marker
Lane 2	Blank
Lane 3	Control sample
Lane 4	Blank
Lane 5	Sample treated at 70°C for 60 mins
Lane 6	Sample treated at 70°C for 30 mins
Lane 7	Blank

Figure 1: SDS PAGE data showing main band along with aggregates in control samples and samples treated for different time points.

SDS-PAGE indicated a prominent protein band at approximately 150 kDa (Figure 1) for the untreated control monoclonal antibody (mAb) IgG1 sample, consistent with its expected molecular weight. Upon subjecting the mAb sample to thermal stress at 70°C for 30 min (Lane 6, Figure 1), the primary band at ~150 kDa persisted, albeit with reduced intensity, suggesting thermally induced protein degradation over time. A decrease in lower molecular weight impurities was observed relative to the control, alongside the emergence of a faint band above 150 kDa, indicative of early-stage aggregate formation. Further exposure to 70°C for 60 min (Lane 5, Figure 1) resulted in a further reduction in band intensity at ~150 kDa, signifying progressive degradation. Compared to both the control and the 30-min heat-stressed sample, the high-molecular-weight aggregate band became more prominent, confirming a time-dependent increase in aggregation. These findings underscore SDS-PAGE's capability to detect aggregate formation, albeit in a semi-quantitative manner.

Comparative Analysis Using SEC-UV

SEC-UV analysis provided qualitative and quantitative insights into protein aggregation, demonstrating a high degree of concordance with SDS-PAGE results. Analysis of the BSA control sample exhibited a purity of 92.93% (Table 1), with clear resolution between the main peak and high-molecular-weight species in the UV 280 profile (Figure 2), highlighting the method's efficacy.

For cetuximab control sample, SEC-UV detected a purity of 90.18% (Table 2), with 4.83% high-molecular-weight species and 4.99% low-molecular-weight species. The UV 280 profile (Figure 3) demonstrated a well-resolved main peak, distinctly separated from high- and low-molecular-weight variants. Upon thermal stress at 70°C for 30 min, the main peak intensity decreased (Figure 4), accompanied by a notable increase in high- and low-molecular-weight species. The percentage purity of the main peak declined to 85.58% (Table 3), with a concurrent increase in high-molecular-weight species (9.12%), while low-molecular-weight species decreased slightly to 4.4%. Following 60 min of incubation at 70°C, a further reduction in main peak intensity (Figure 4) was observed, along with a continued increase in high-molecular-weight species. The percentage purity of the main peak dropped to 78.52% (Table 4), with high-molecular-weight species increasing to 17.36%, whereas low-molecular-weight species remained relatively stable at 4.11%. Overlaid UV profiles of control and heat-stressed samples clearly demonstrated time-dependent aggregation, reinforcing SEC-UV's sensitivity in detecting aggregation and degradation species. Comparative data was presented in Table 5 indicating reduction in % main peak and increase in %HMMS. Compared to SDS-PAGE, SEC-UV analysis offered faster turnaround times and quantitative insights, making it more practical for real-time monitoring. Additionally for the sample

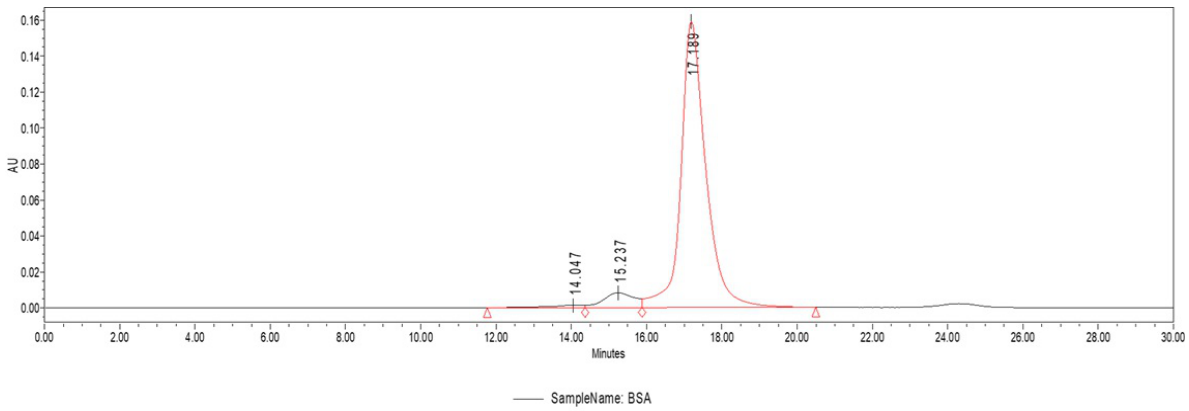


Figure 2: SEC UV Profile obtained for BSA standard sample.

Table 1: Purity data for BSA Sample.

Retention Time (min)	Area	% Area	Peak Details
14.047	86880	1.11	HMMS 2
15.237	468639	5.96	HMMS 1
17.189	7305330	92.93	Main Peak

Table 2: Purity data for control sample.

Retention Time (min)	Area	% Area	Peak Details
14.543	398044	4.83	HMMS 1
15.604	7423840	90.18	Main Peak
16.848	357477	4.34	LMMS 1
19.237	53315	0.65	LMMS 2

Table 3: Purity data for sample treated at 70°C for 30 min.

Retention Time (min)	Area	% Area	Peak Details
12.072	519943	6.58	HMMS 2
14.345	271512	3.44	HMMS 1
15.596	6760464	85.58	Main Peak
16.825	321487	4.07	LMMS 1
19.232	25835	0.33	LMMS 2

Table 4: Purity data for sample treated at 70°C for 60 min.

Retention Time (min)	Area	% Area	Peak Details
12.024	1300275	14.34	HMMS 2
14.525	273620	3.02	HMMS 1
15.597	7118292	78.52	Main Peak
16.895	334868	3.69	LMMS 1
19.202	37968	0.42	LMMS 2

Table 5: Comparative Purity data obtained for control sample and stressed samples.

Sample Details	%HMMS	%Monomer	%LMMS
Control sample	4.83	90.18	4.99
Sample treated at 70°C for 30 min	10.02	85.58	4.4
Sample treated at 70°C for 60 min	17.36	78.52	4.11

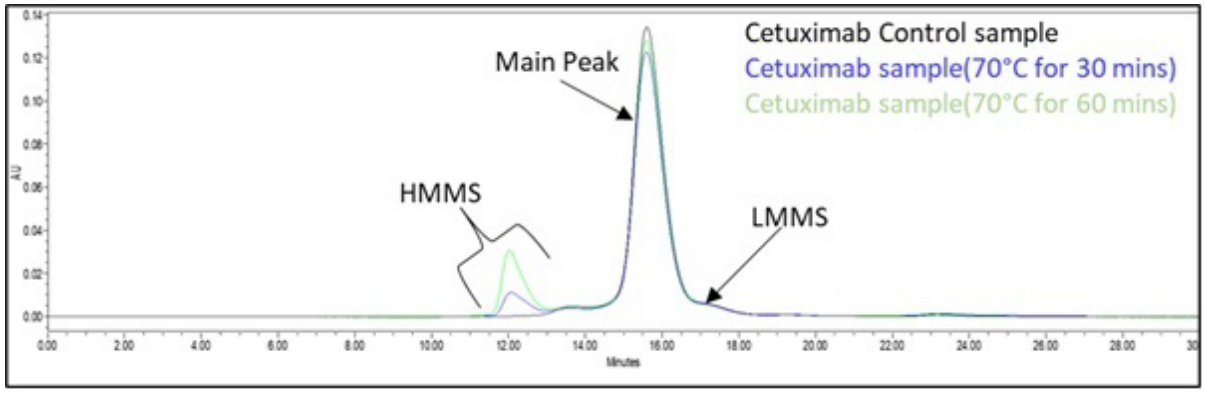


Figure 3: Over laid SEC UV profile of Cetuximab control sample along with sample treated at 70°C for 30 min and 60 min.

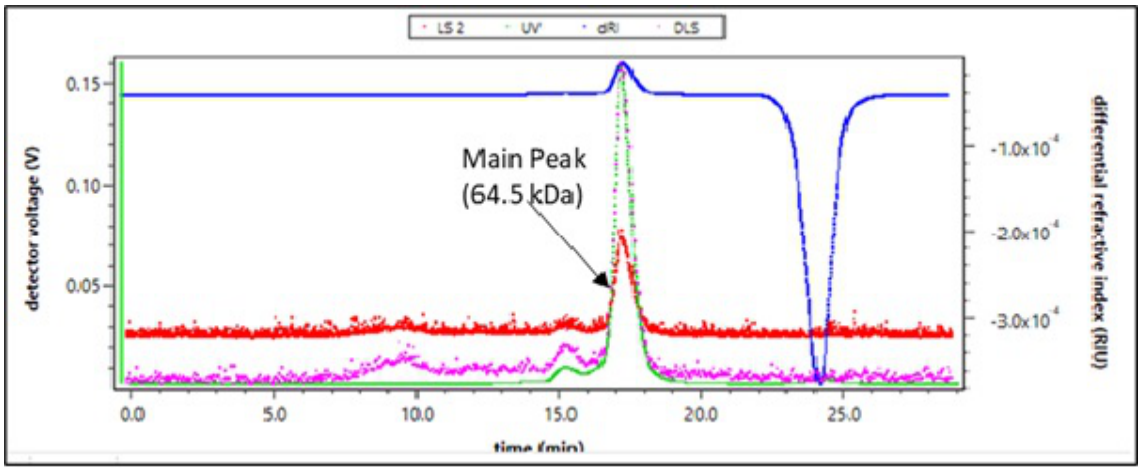


Figure 4: SEC MALS Profile obtained for BSA standard sample.

treated at 70°C for 60 min (Lane 5 of Figure 1) a prominent main band at approx. 150 kDa was observed but with reduced intensity compared to control sample and sample treated at 70°C for 30 min indicating the degradation of the protein. Low molecular weight impurities were found to be reduced compared to the control sample and sample treated at 70°C for 30 min and a prominent band at molecular weight more than 150 kDa was observed indicating the increased aggregation with time of incubation. SDS PAGE was found to be capable of detecting the aggregates formed in the samples (Shrivastava *et al.*, 2023).

SEC-MALS for Molecular Weight Determination and Aggregate Characterization

When SEC-UV was coupled with MALS detection, absolute molecular weight measurements of monomeric and aggregated species were obtained, further enhancing the characterization of protein aggregation. The BSA standard control exhibited a molecular mass of 64.5 kDa (Figure 4), aligning with expected values. The cetuximab control sample showed a primary peak corresponding to 150.3 kDa (Figure 5). The 30-min heat-stressed sample retained a main peak at 147.4 kDa, with a distinct dimeric species (~335.7 kDa) emerging, signifying early-stage aggregation under thermal stress (Figure 6). The 60-min heat-stressed sample exhibited a main peak at 148.6 kDa, but with the presence of a trimeric species (~438.6 kDa), indicating a progressive increase in aggregation with extended incubation (Figure 7).

Comparative Assessment of Analytical Techniques

Upon evaluating all three analytical techniques, results were found to be highly consistent in detecting protein aggregation. However, differences in their capabilities were evident: SDS-PAGE, while effective as a preliminary screening tool, provided only semi-quantitative data and required longer analysis times. SEC-UV enabled rapid quantification of aggregates with superior resolution, making it an efficient method for real-time quality control. SEC-MALS, through absolute molecular weight determination, provided the most detailed characterization of aggregation, distinguishing between monomers, dimers, and trimers with high precision. Heat-induced aggregation is often mediated by hydrophobic exposure, intermolecular β-sheet

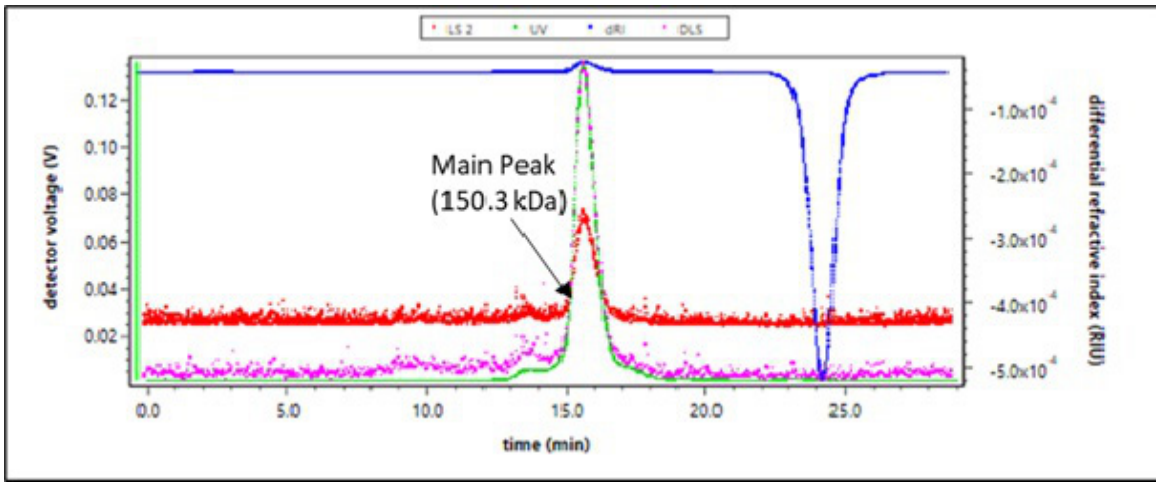


Figure 5: SEC MALS Profile obtained for Cetuximab control sample.

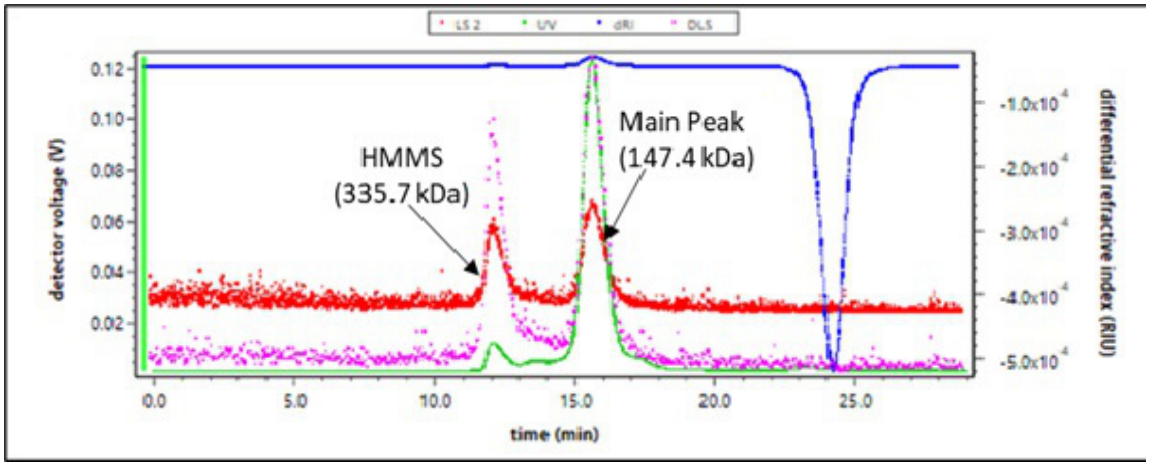


Figure 6: SEC MALS Profile obtained for Cetuximab sample treated for 30 min at 70°C showing dimeric high molecular mass aggregate.

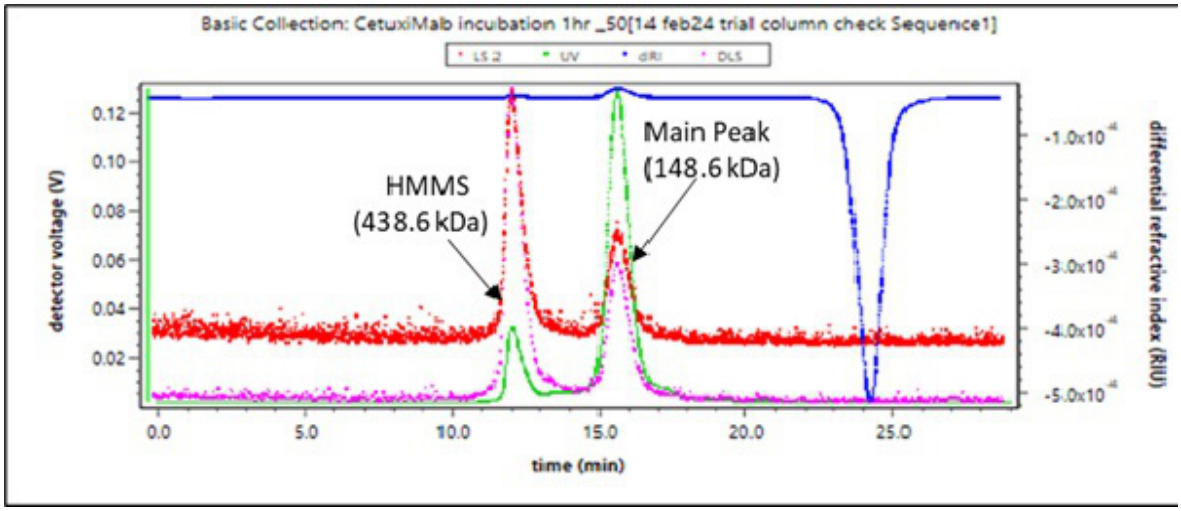


Figure 7: SEC MALS Profile obtained for Cetuximab sample treated for 60 min at 70ºC showing trimeric high molecular mass aggregates.

formation, and non-covalent or covalent crosslinking, resulting in the formation of soluble oligomers, higher-order aggregates, and potential insoluble particulates. These thermally stressed samples were analysed alongside an untreated control, allowing for a comparative assessment of each analytical technique's sensitivity, resolution, and quantification capability. In a commercial biopharmaceutical manufacturing environment, where large-scale production necessitates rapid and precise quality assessments, SEC-UV and SEC-MALS offer superior analytical advantages. Their ability to quantify aggregates, reduce analysis time, and ensure batch integrity makes them indispensable tools for real-time process optimization and aggregation monitoring, minimizing batch failure risks and ensuring product consistency (Vlasak and Ionescu, 2011).

CONCLUSION

The objective of this study was to evaluate traditional analytical techniques like SDS-PAGE based separation and additional complementary advanced analytical techniques SEC-UV and SEC-MALS, in detecting and quantifying protein aggregation in monoclonal antibody (mAb) formulations. Protein aggregation is a Critical Quality Attribute (CQA) in biopharmaceutical manufacturing, as it can lead to reduced bioactivity, increased immunogenicity, and compromised product stability and regulatory approvals.

While all three techniques deployed in the current research study demonstrated utility in detecting mAb aggregation, SEC-UV and SEC-MALS exhibited superior analytical performance due to their higher sensitivity, shorter analysis time, and enhanced quantification capabilities. SDS-PAGE remains a cost-effective, preliminary screening tool, but its semi-quantitative nature, limited resolution, and inability to provide absolute molecular weight measurements restrict its use in high-resolution aggregate characterization. SEC-UV enables rapid quantification of aggregates, making it suitable for in-process monitoring and stability testing, while SEC-MALS provides absolute molecular weight determination and structural insights, making it an indispensable tool for comprehensive aggregation profiling, regulatory submissions, and formulation optimization.

In the context of large-scale biopharmaceutical manufacturing, where real-time process control and batch consistency are paramount, SEC-UV and SEC-MALS emerge as preferred methodologies for ensuring regulatory compliance, quality assurance, and enhanced process robustness.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SDS-PAGE: Sodium dodecyl sulphate-Poly acryl amide gel electrophoresis; **SEC:** Size exclusion chromatography; **SEC-MALS:** Size exclusion chromatography- multi-angle light scattering; **MABS:** Monoclonal antibodies; **EGFR:** Epidermal Growth Factor Receptor; **FDA:** Food drug administration; **SEC-UV:** Size-exclusion with UV absorbance detection; **BSA:** Bovine Serum Albumin; **HPLC:** High Performance Liquid Chromatography; **kDa:** Kilo Dalton; **CQA:** Critical quality attribute.

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