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ADDRESSING PARTICULATE MATTER IN PROTEIN THERAPEUTICS

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Purpose

Protein aggregation in therapeutics can be accelerated by transportation, storage and presence of contaminants (e.g. silicone). Irreversible aggregation of proteins is detrimental not only in reducing the concentration and biological availability of the active protein, but also in triggering immunogenic reactions in patients.

Physical and chemical characterization of particulates are an integral part of drug product quality control to ensure patient safety.

A better understanding of the particles in protein therapeutics allows continuous improvement by ensuring in-depth knowledge, control of the process and identification of critical steps.

Objectives

In this study, we show how a combination of various orthogonal methods for physical and chemical in-depth characterization of inherent (aggregates), intrinsic particles (from the manufacturing process) and extrinsic particulates (contaminants) found in protein therapeutic products can be used for the quantification and characterization of particles from the visible to the sub-visible range.

Methods

Stress study:

Forced degradation study: IgG1-type monoclonal antibody (Infliximab) spiked with 0.5% silicone oil

Mechanical stress: shaking at 20 min -1 and 60 min-1 for variable time periods (0-1020 min)

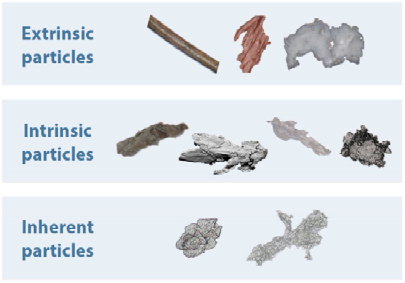
Particulate characterization:

Visible particles:

- classified using light microscopy
- characterized using FT-IR and/or SEM-EDX
- aggregates of proteinaceous nature further identified using LC-MS/MS peptide mapping

Sub-visible particles: characterized using MFI and FlowCam Nano

Results

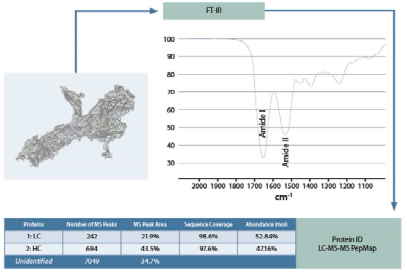


Light microscopy images and further classification of particles from the FT-IR and SEM-EDX characterization results.

The study first conducted visual inspection and chemical characterization of contaminants. Visual inspection of the vials was performed to assess the presence of particles in the visible range (>100µm). Subsequently, isolation techniques were employed, followed by light microscopy, to facilitate visual assessment and broad categorization of particles.

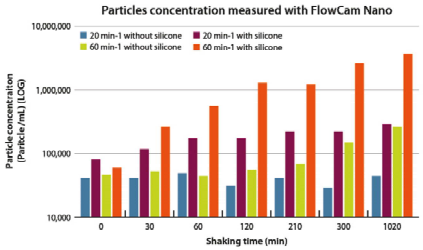
FT-IR and SEM-EDX analysis were then employed for chemical characterization, depending on the nature of the particle. The contaminants identified primarily originated from single-use equipment, PPE, and the production process.

PepMap LC-MS/MS was used for further investigation of proteinaceous particles to determine the amino acid sequence of the aggregate. Comparison with the amino acid sequence of Infliximab confirmed that the aggregates were formed by the antibody.

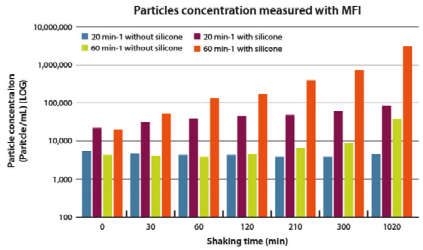


Protein	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)	
1: LC	242	21.9%	98.6%	52.84%	Protein ID LC-MS-MS PepMap
2: HC	694	43.5%	97.6%	47.16%	
Unidentified	7049	34.7%			

Characterization of proteinaceous particles by FT-IR and Protein ID by LC-MS-MS PepMap.



Total particle concentration per time measured with the FlowCam Nano with and without silicone.

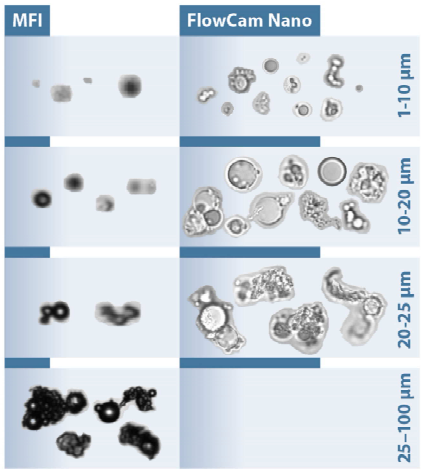


Total particle concentration per time measured with the MFI with and without silicone.

Sub-visible particles were examined using MFI and FlowCam Nano. While MFI consistently reported lower particle concentrations compared to FlowCam Nano, direct comparisons are challenging due to their different size measurement capabilities.

The combined use of these two measurement techniques, given their distinct measurement capabilities and ranges, produces a comprehensive understanding of sub-visible particle populations across a wider size spectrum, ultimately contributing to a more accurate assessment of particle content and distribution.

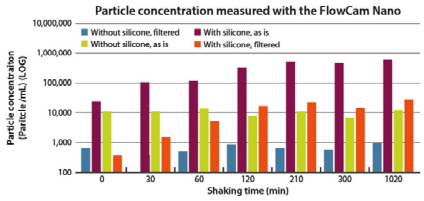
Two clear trends emerged: mechanical stress promotes particle formation, and the samples containing silicone exhibit a higher particle count compared to silicone-free samples.



Comparison of FlowCam nano and MFI images at different scales.

Additionally, a dataset was generated by applying the same shaking speed (20 min⁻¹) with and without silicone, and it was morphologically filtered.

FlowCam Nano data were filtered to eliminate silicone droplets, allowing a focused comparison of protein aggregates in stress samples. Notably, both filtered and unfiltered data showed a consistent increase in particle concentration over time, indicating that the rise in particles was not solely attributed to finely distributed silicone droplets but also indicated a genuine increase in protein aggregates due to mechanical stress in the presence of silicone.



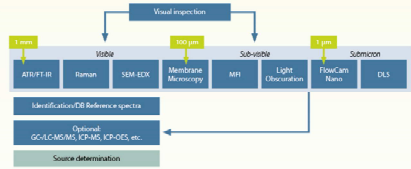
Total particle concentration per time measured with FlowCam Nano with and without image analysis.

Conclusions

In summary, the presence of particles holds critical importance in ensuring the quality of pharmaceutical products. Characterizing these particles not only aids in pinpointing their source of origin but also paves the way for continual improvement by enhancing our understanding, process control, and identification of critical stages.

The study effectively employed a multi-faceted approach, combining visual inspection, non-destructive spectroscopic methods, and advanced analytical techniques to unveil the diverse origins of contaminants, with a notable emphasis on single-use equipment, personal protective gear, and the production process. Furthermore, sophisticated data analysis software enabled the extraction of protein aggregate concentrations while excluding contributions from foreign particles, silicone droplets, and air bubbles, enhancing accuracy.

Finally, this study demonstrates the critical importance of employing a fast and structured approach for the comprehensive physical and chemical characterization of particulates in protein therapeutic products using targeted, iterative and orthogonal methods of analysis.



Workflow for the in-depth characterization and identification of particulates.

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