Practical Tips for Monomer/Aggregate and Fragment Content Monitoring of monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs)

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Product characterization is the essential foundation for successful biological drug development. Understanding the chemistry, structure, and biological activities ensures the quality control will measure critical product safety, purity, and potency attributes, as per ICH Q6B. Furthermore, in May 2019, the Food and Drug Administration (FDA) published guidance titled *Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations*. In the guidance, aggregation as a critical quality attribute is specifically mentioned. In this article, we will explore tips to improving the method of Size Exclusion Chromatography (SEC) which is often used to measure monomer/aggregate and fragment content of mAbs and ADCs.

Size exclusion chromatography (SEC) is a mode of liquid chromatography that separates molecules according to their hydrodynamic radius (size). The stationary phase in the SEC column consists of spherical, porous particles with a carefully controlled pore size through which molecules diffuse, based on their size difference, using an aqueous buffer as the mobile phase. SEC is an entropically-controlled separation process in which the analytes are "filtered" rather than through chemical interactions between the stationary phase and the analyte. It is crucial that residual silanol interactions, from silica-based stationary phases, are minimized as this interaction results in tailing analyte peaks. This problem is magnified when dealing with larger biomacromolecules.

There has been a major trend in the chromatography field, in recent years, to develop methods that are optimized for high throughput. High throughput is especially important in the pharmaceutical industry, where hundreds of samples are analyzed daily in quality control (QC) laboratories. Longer run times cost the prospective company money in terms of mobile phase consumption, instrument time, and energy usage. Generally, there are three ways to decrease the run time in a chromatographic method: increase the flow rate, increase the column temperature, or decrease the particle size of the column packing material. For SEC of biomolecules, increasing the flow rate will result in peak broadening along with the concomitant increase in back pressure.

Another way to decrease the method run time is to increase the column temperature. For biomolecules,

the main challenge in obtaining high-speed and high-resolution separations in SEC is the slow mass transfer of the analytes between the interstitial space of the column and the pore space of the stationary phase. By increasing the temperature, the mobile phase viscosity decreases and the kinetics of the analytes increase, thus increasing the mass transfer rate of the analytes. However, elevated temperatures can cause aggregation and fragmentation of proteins, especially proteins with flexible regions in their tertiary structure.

Recently, advances in column technology have enabled the production of columns packed with smaller particles of 2 µm, or less, in diameter. Chromatographic theory portends that smaller particle size leads to narrower peaks due to better column efficiency. The limiting factor with using small particle size columns is the increase in back pressure that accompanies these columns. In addition, one study has suggested that, because of the high pressures generated by these columns, frictional heating may cause on-column aggregation or denaturation of temperature sensitive proteins, though this has not been widely reported in the literature.

The three considerations mentioned above, flow rate, temperature, and particle size, all play a role in the separation performance of a SEC experiment. In addition, column length and pore size are important parameters although not specifically discussed in this article. A non-optimized flow rate can lead to band broadening and loss of resolution of critical pairs. Temperature can be used to speed up a separation; however, it does come at the risk of sample degradation. Smaller particle sizes can aid in maximizing the number of theoretical plates in the separation, albeit with an increase in back pressure. All five of these variables should be carefully chosen and monitored to optimize performance and ensure a robust, compliant method.

Explore tips to improving the method of Size Exclusion Chromatography (SEC) to measure monomer/aggregate and fragment content of mAbs and ADCs.