Analytical tools used in the formulation and assessment of stability of monoclonal antibodies (mAbs)

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Analytical methods for evaluation of monoclonal antibody stability

As will be discussed in Chapter 3 there are several degradation pathways for proteins and monoclonal antibodies (mAbs). The mAb DP requires formulations that are sufficient to meet the specific TPP for the specific mAb. However, in order to do this several analytical methods are used to determine the stability of the mAb and set a shelf life for the product. There are many excellent reviews of analytics used to assess protein/mAb stability (Chang & Hershenson, 2002; Jones, 1993; Reubsaet et al., 1998a,b). Many of the analytics are used in characterization of the mAb DS that is produced. Also mass spectrometry techniques coupled with chromatography have been used to identify the alterations that occur in the mAb primary structure. Development of mAb DP formulations requires high-throughput assays since many formulations may need to be evaluated over many time points during storage. Thus, here we limit our discussion to briefly discuss the different methods used to assess stability and aid in choosing appropriate formulation excipients.

Chromatographic methods

High-performance liquid chromatography (HPLC) and recently ultra high-performance liquid chromatography have been the main workhorses for analysis of mAb chemical degradation. HPLC methods used to assess chemical degradation in proteins are usually based on change in polarity or charge of the protein. Physical degradation that results in aggregate formation is usually assessed by sizing chromatography. Some of the most common methods used for mAb analysis are as follows.

Size exclusion chromatography

Size exclusion chromatography (SEC) has been the main chromatographic method used to determine the size of proteins, and in particular the distribution of aggregates in final DP. The chromatographic matrix consists of beads with pores of a defined size, which allows for penetration of protein molecules. The greater the penetration into the pores, the greater is the residence time on the column, resulting in greater elution times. The apparent molecular weight of the applied protein sample is defined by running a set of protein molecular weight standards (Andrews, 1970; Whitaker, 1963). However, the elution of the protein is dependent only on the hydrodynamic volume so that shape plays a large

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