Neutralization Triggers Aggregation after Low pH Viral Inactivation in Therapeutic Antibody Manufacturing

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Over the past decades, monoclonal antibodies and related constructs have become an important class of active pharmaceutical ingredients [1]. Yet owed to the high molecular complexity of those therapeutic proteins, challenges in their manufacturing remain [2]. Formation of protein aggregates is a commonly encountered route of physical degradation. Aggregates are a safety issue, since they have been linked with immunogenicity in patients upon administration of protein-based drugs [3]. During chemical viral inactivation following affinity capture, the protein temporarily exposed to an acidic environment, which often leads to protein aggregation [4,5]. Therefore, improved understanding of the principles behind antibody aggregation under those conditions could allow for improved design of that unit operation.

The supra-molecular association state of an antibody during incubation at low pH was monitored by in-situ dynamic light scattering (DLS). Further, aggregate formation after neutralization was investigated by means of size-exclusion chromatography (SEC) coupled with multi-angle light scattering (MALS) as well as DLS. Specific care was put into control of the solution conditions, i.e. pH and ionic strength, since those were presumed to play a key role in the aggregation process.

Under conditions of low pH and low ionic strength (i.e. mirroring viral inactivation [6]), formation of protein aggregates could not be detected. Only upon raising solution pH as done at the end of viral inactivation, aggregation was observed. Below a certain threshold pH value, incubation time until neutralization as well as pH itself had no impact on aggregation outcome. Only above that threshold, incubation time and solution pH affected the aggregation process.

Lack of detectable aggregate formation under low pH and ionic strength as encountered during viral inactivation can be explained by sufficient electrostatic repulsion between protein molecules, which suppresses supra-molecular association. Nevertheless, incubation under acidic conditions induces conformational changes in the protein that lead to exposure of hydrophobic residues otherwise buried inside the folded protein. Upon increase of solution pH, electrostatic repulsion is substantially reduced, which results in coagulation of the (partially) denatured protein molecules. These insights into the aggregation mechanism encountered during and after viral inactivation are highly valuable for rational selection of conditions that minimize the extent of protein aggregate formation during downstream processing of therapeutic antibodies.

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