

Therapeutic Antibody Aggregation Analysis using CX300 and ParaySelect™

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INTRODUCTION

Monoclonal antibodies (mAbs) are important protein-based biopharmaceuticals with applications in a wide range of treatments from rheumatoid arthritis to cancer.

As with all protein therapeutics, mAbs may become degraded at any of the stages between production and administration, including protein self-assembly or aggregation. Aggregation is to be avoided because it results in loss of function, decreased solubility, and, most importantly, enhances unwanted immunogenicity. Process monitoring and quality control systems for measuring aggregation include dynamic light scattering or turbidimetry, which require high sample concentrations and relatively large sample volumes for data acquisition.

Herceptin™ is an important biopharmaceutical used to treat early and advanced Her2⁺ cancers including breast, stomach and oesophageal cancer, either alone or as an antibody-drug conjugate.

In this Technical Note, we demonstrate a method for monitoring levels of aggregation in preparations of Herceptin™ using ParaySelect™ rapid separation technology integrated with CX300 particle analyses.

METHOD

Herceptin™ was isolated from tissue culture supernatant derived from HEK293 cells transfected with Herceptin expression plasmids, by affinity chromatography on human anti-Fc-Sepharose. Eluted Herceptin™ (0.3 µg/µL in 10 mM histidine, pH 6.0) was diluted 1:8 either in 10 mM histidine, pH 6.0 or PBS, with antibody levels checked by immunoblotting (Fig. 1), prior to tagging using 0.05 µg/µL FITC-labelled Fab anti-Herceptin fragment. Fluorescently-tagged material (100 µL) was applied to a ParaySelect™ cartridge equilibrated in either histidine buffer or PBS as above and subsequently 30 µL was analysed using CX300 (Fig. 2) with data collection for 60 seconds.

RESULTS

Fig. 3 shows the CX300 analysis output. At a concentration equivalent to that used in patient treatment, Herceptin™ after 60 min in PBS displays substantial aggregation, approximately 300 times greater than the same concentration in histidine buffer, which shows almost none. These data emphasise the importance of selecting the correct buffer for formulation and functional analysis of biotherapeutics. The combination of ParaySelect™ with CX300 demonstrates the ease with which protein self-assembly or aggregation may be analysed.

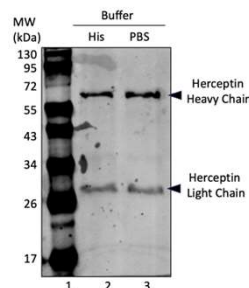


Fig. 1 Immunoblotting of Herceptin™ in either histidine buffer or PBS prior to aggregation analysis.

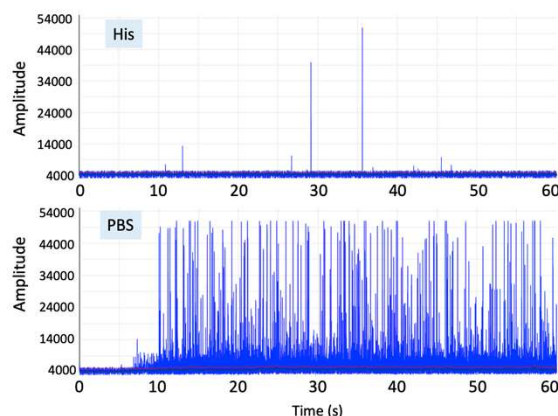


Fig. 2 CX-300 profiles of aggregated Herceptin™ species in indicated buffers.

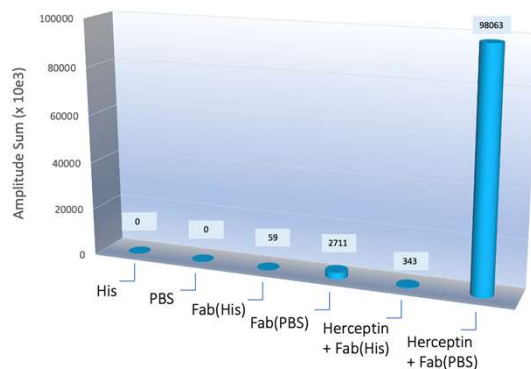


Fig. 3 Data summary of samples analysed.

CONCLUSIONS

CX-300 is a platform for the rapid and sensitive analysis of protein aggregation.

- Fast analysis - 60 seconds
- Small sample requirement - 30 µL
- Wide dynamic range - >3 log