Automating “Flash Characterization” of Antibodies via Microdroplet Reactions in an Unmodified Agilent Jet Stream Source (AJS)

Abstract

Antibody characterization with IdeS enzyme digestion, reduction with tris(2-carboxyethyl)phosphine (TCEP), triethylphosphine (TEP), or dithiothreitol (DTT) typically requires a minimum of 30 minutes using a bulk solution in order to observe significant reaction products. Recently, attention has been drawn to the use of microdroplet reactions for antibody analysis (Gunawardena, et al., Anal. Chem. 2023, 95, 3340-3348). The microdroplet reactions are attractive due to the rapid reaction rate (microseconds) and high reaction yield achieved in the AJS ESI spray chamber. The NIST IgG1 mAb was used to optimize microdroplet reaction conditions on two reactions, IdeS cleavage and disulfide bond reduction, using the Agilent Jet Stream ESI source. Optimized conditions were applied to several commercial antibodies to test robustness and broad applicability of the conditions. The experiments demonstrated exceptional results and in addition to the time savings, the cost of analysis is dramatically lowered due to the reduction in enzyme and antibody consumption for the characterization reactions. We call the optimized workflow “Flash Characterization”.

The results demonstrate:

- Automated Microdroplet reaction with 85% efficiency is achieved with an unmodified JetStream electrospray spray source
- Ultrafast Microdroplet enables “Flash Characterization” of mAbs
- Microdroplet reaction leads to dramatic time savings and cost reduction on enzyme and reagents usage.

Speaker: Tobias Weimer, Product Specialist LC/MS, Agilent Technologies GmbH