Middle down analysis of a therapeutic antibody with CZE-MS applying an in-lab designed interface

Katharina Müller[a,b], Lorenz G. Stock[a,b], and Hanno Stutz[a,b]

[a] Department of Biosciences, University of Salzburg, 5020 Salzburg, Austria
[b] Christian Doppler Laboratory for Innovative Tools for Biosimilar Characterization, 5020 Salzburg, Austria

Innovative analytical tools are required by the pharmaceutical industry to allow for a comprehensive analysis of biotherapeutics, particularly of therapeutic monoclonal antibodies (mAbs). In this context, capillary zone electrophoresis (CZE) is predestined to distinguish protein variants with subtle differences in charge and in the hydrodynamic radius. The hyphenation of CE with an Orbitrap mass spectrometer providing high mass accuracy improves the identification credibility. However, analytical challenges, which have to be tackled, comprise protein adsorption onto the separation capillary and the electrospray interface.

In the current case, a middle-down approach with preceding IdeS digest is applied for a CZE-ESI-MS characterization of $F(ab´)_2$ and Fc/2 fragments of a therapeutic antibody with an alkaline isoelectric point and pronounced adsorption propensity, i.e. rituximab (MabThera®). Protein adhesion is counteracted by physically attached successive multiple ionic polymer (SMIL) coating [1, 2] with a positively charged final layer. A discontinuous background electrolyte with pH change combines improved electrophoretic separation with appropriate analyte transfer towards the mass spectrometer. Hyphenation between a P/ACE MDQ capillary system and an Exactive mass spectrometer is realized by an in-house designed sheath-liquid interface [3]. The optimized CZE-ESI-MS method is applied in the analysis of a commercial reference product of the therapeutic antibody.

[1] M. Weinbauer, H. Stutz, *Electrophoresis* 2010, 31, 1805-1812.

[2] L.G. Stock et al., *Analytica Chmica Acta* 2017, 951, 1-15.

[3] S. Gusenkov, H. Stutz, *Electrophoresis* 2018, 39, 1190-1200.