KN11

Biopharmaceuticals by Capillary Electrophoresis: Mass Spectrometry, Affinity, Isoelectric Focusing, Process Analysis

Hermann Wätzig[1], Christin Scheller[1], Finja Krebs[1,2], Susanne Dörks[2], Udo Burger[2], Markus Kramer[3], Marc Hoffstedt[1], Robert Minkner[1], Sophie Hartung[1], Yannick Wilke[1], Antonia Wittmann[1], Debbie van den Burg[4,5], Cari Sänger-van de Grient[4]

[1]Institute of Medicinal and Pharmaceutical Chemistry, Technische Universität Braunschweig, Germany, h.waetzig@tu-braunschweig.de

[2]ProteinSimple, a Bio-Techne brand, Wiesbaden-Nordenstadt, Germany

[3]CureVac AG, Tübingen, Germany

[4]Kantisto BV, Baarn, The Netherlands

[5]MPYA Science&Technology, Stockholm, Sweden

Summary

In order to contribute to the scientific research on the SARS-CoV-2, we developed two CIEF imaging methods to characterize the quality and stability of messenger ribonucleic acid (mRNA) vaccines, particularly mRNA encapsulated in lipid nanoparticles (LNPs). A variety of stressed and lipid composition altered samples were measured. Results were supported by data from an encapsulation assay and particle sizing. A method using 9 M urea as an additive showed two broad and jagged peaks, with the peak shape providing detailed information. The summed peak area of both peaks showed RSDs ranging from 2 to 8% when measured in triplicate, and appears to depend on the size of the LNPs. In the second method, a combination of 5.5 M urea and 2 M N-ethylurea was used. This method is characterized by high reproducibility of the pI value (< 0.5%). The reproducible peak area (RSD of 2-7%) correlates linearly with the mRNA content. This is also true for the first method. Stress is evident from the change in pI and peak area. In addition, experiments were performed with the addition of a fluorescent dye, which greatly increased the sensitivity of the methods. Both methods can be used to characterize LNP stability, e.g., in studying different storage times at different temperatures and freeze-thaw cycles, as well as the ability of the methods to distinguish lipid compositions and measure batch-to-batch variability [1].

Collagen is a very important and highly abundant structural protein but hard to analyse due to its insolubility. In order to investigate collagen in a liquid environment and to maintain its biological function, we milled and suspended collagen in a phosphate buffer pH 7.4, 12.5 mM using a dual zentrifuge (ZentriMix 380R) to obtain particles with a size below 5 µm. Using these small collagen particles, affinity CE (ACE) was performed to study binding properties of Human Serum Albumin, Human Fibronectin and Collagenase Type I from Clostridium histolyticum.

Antibody self-interaction including aggregation has been correlated to hydrophobic patches as well as the electrostatic potential distribution on a protein surface. These approaches rely on 3-D structures which may not always be available but can be predicted with an increasing precision from the sequence.

A capillary zone electrophoresis (CZE) method was developed for the monitoring of the mAb concentration during cell culture processes. CZE method development rules are outlined, particularly discussing various capillary coatings, such as a neutral covalent polyvinyl alcohol (PVA) coating, a dynamic successive multiple ionic-

polymer (SMIL) coating, and dynamic coatings using BGE additives such as triethanolamine (T-EthA) and triethylamine (TEA). The dynamic T-EthA coating resulted in most stable electro-osmotic flows (EOFs) and most efficient peak shapes [2]. A general update on method development and validation in CE can be found in [3].

CE-MS has been around for a long time and has recently evolved into a mature technique [4]. We demonstrate excellent structural information with the MauriceFlex system for the Waters mAb, the NIST mAb and matuzumab. This new system did not show a single failure for technical reasons during two months of use.

References

[1] F. Krebs, U. Burger, S. Dörks, M. Kramer, H. Wätzig, Two quality and stability indicating imaged CIEF methods for mRNA vaccines, ELECTROPHORESIS 43 (2022) 1971–1983.
[2] D. van der Burg, H. Wätzig, C.E. Sänger-van de Griend, Method development for quantitative monitoring of monoclonal antibodies in upstream cell-culture process samples with limited sample preparation – An evaluation of various capillary coatings, ELECTROPHORESIS 44 (2023) 96–106.
[3] F. Krebs, H. Zagst, M. Stein, R. Ratih, R. Minkner, M. Olabi, S. Hartung, C. Scheller, B.H. Lapizco-Encinas, C. Sänger-van de Griend, C.D. García, H. Wätzig, Strategies for capillary electrophoresis: Method development and validation for pharmaceutical and biological applications—Updated and completely revised edition, ELECTROPHORESIS 44 (2023) 1279–1341.
[4] S. Hartung, R. Minkner, M. Olabi, H. Wätzig, Performance of capillary electrophoresis instruments – State of the art and outlook, TrAC Trends Anal. Chem. 163 (2023) 117056.