Capillary electrophoresis; one technique to completely characterize biopharmaceuticals
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Biopharmaceuticals – therapeutically active biomolecules – have become an important class of drugs. Over 260 variants are on the market nowadays. They have proven to be efficient in the treatment against many diseases, such as cancer, diabetes, autoimmune disorders, and anemia. Moreover, they show an increased efficacy, specificity, and less side effects compared to low-molecular weight therapeutics. As a result of expression, purification, and storage processes, biopharmaceuticals can undergo post-translational modifications, degradation, and aggregation, which can highly affect the efficacy and stability of the product. Therefore, the product must be subjected to characterization analyses in order to monitor the physical, chemical, biological, or microbiological properties of the product. Additionally, biosimilars – which are developed in order to function equally compared to the structure and clinical properties of the original product – need to be extensively checked before they can enter the market. This review describes the ability of capillary electrophoresis (CE) to completely characterize a biopharmaceutical in terms of their structural and physicochemical properties, based on literature from the last two years. We describe the following characterization analysis categories ordered from intact to amino acid level: size variants, charge variants, aggregates, N-linked glycosylation, O-linked glycosylation, and amino acid sequence. For the analysis of charge and size-based variants, CE is extensively developed allowing even more successful separations compared to chromatographic techniques. The same applies for analyzing N-glycans and O-glycans. The following CE approaches have been performed regarding these characterization analyses: capillary isoelectric focusing (CIEF), capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), ultra-high voltage capillary electrophoresis (UHVCE), open-tubular capillary electrochromatography (OT-CEC), CIEF-CZE, and CZE-CZE. Moreover, CZE and CGE were also accomplished in microfluidic setup. The CE-based separation techniques have been coupled to either UV/vis, laser-induced fluorescence, or MS detectors. The main reason for the wide application of CE in this field is its resolving power based on charge and/or size, which has proven to be essential for post-translational modifications that often affect the electrophoretic mobility of a compound. Determination of aggregates and amino acid sequencing is mainly performed using other techniques than CE, although CE could also be applied for these analyses. Thus, CE can be considered as a suitable technique to completely characterize biopharmaceuticals.