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Evaluation of capillary zone electrophoresis for charge heterogeneity testing of monoclonal antibodies

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ABSTRACT

Within pharmaceutical industry charge heterogeneity testing of biopharmaceuticals has to be reproducible and fast. It should pass method validation according to ICH Q2. Classical approaches for the analysis of the charge heterogeneity of biopharmaceuticals are ion exchange chromatography (IEC) and isoelectric focusing (IEF). As an alternative approach, also capillary zone electrophoresis (CZE) was expected to allow reliable charge heterogeneity profiling by separation according to the analyte's net charge and hydrodynamic radius.

Aim of this study was to assess if CZE possesses all of the required features. Therefore, beside lab internal validation of this method also an international cross company study was organized.

It was shown that CZE is applicable across a broad p*I* range between 7.4 and 9.5. The coefficient of correlation was above 0.99 which demonstrated linearity. Precision by repeatability was around 1% (maximum relative standard deviation per level) and accuracy by recovery was around 100% (mean recovery per level). Accuracy was further verified by direct comparison of IEC, IEF and CZE, which in this case showed comparable %CPA results for all three methods. However, best resolution for the investigated MAb was obtained with CZE. In dependence on sample concentration the detection limit was between 1 and 3%.

Within the intercompany study for CZE the same stressed and non-stressed samples were analyzed in each of the 11 participating labs. The finally obtained dataset contained more than 1000 separations which provided an extended dataset for further statistical evaluation. Among the different labs no significant differences between the peak profiles were observed. Mean driver for dropouts in quantitative evaluation was linked to the performance of some participating labs while the impact of the method performance was negligible. In comparison to a 50 cm capillary there was a slightly better separation of impurities and drug substance related compounds with a 30 cm capillary which demonstrates that an increased

Abbreviations: (HPMC), hydroxypropyl methyl cellulose; (EACA), epsilon amino caproic acid; (TETA), triethylenetetramine; (imaged cIEF), imaged capillary isoelectric focusing; (MAbs), monoclonal antibodies; (EOF), electro-osmotic flow; (ANOVA), analysis of variance; (IEC), ion exchange chromatography; (CPA), corrected peak area; (TOST), two one-sided tests; (ID), inner diameter.

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stability indicating potential can be combined with the increased separation velocity and high throughput capability of a shorter capillary. Separation can be performed in as little as approx. 3 min allowing high throughput applications. The intercompany study delivered precise results without explicit training of the participating labs in the method prior to the study (standard deviations in the range of 1%).
It was demonstrated that CZE is an alternative platform technology for the charge heterogeneity testing of antibodies in the pharmaceutical industry.

1. Introduction

There are different groups of modifications that influence the charge heterogeneity profile of proteins like monoclonal antibodies [1]. One of these groups causes a shift of the charge profile towards basic forms, e.g. by succinimide formation [2] or C-terminal lysine heterogeneity [1]. Another shifts the profile towards the acidic forms e.g. by deamidation [2], sialylation [3], glycation [4,5], or pyroglutamate formation [6]. In addition, fragmentation may cause a shift in both directions. Modifications like Asp isomerization do not change the net charge but can have an influence on the surface charge distribution [7]. Some of these modifications may influence potency [8]. Therefore, charge heterogeneity testing is very important for the QC testing of protein based pharmaceuticals.

Within pharmaceutical industry charge heterogeneity testing of biopharmaceutical drug products is important for control of the production process, for technical development and for release and stability testing. Therefore, the applied methodology has to be reproducible (i.e. comparable results over years) and fast (i.e. enabling of high throughput analyses). For fulfillment of underlying GMP requirements it has to pass method validation according to ICH Q2. Furthermore it should be stability indicating and be able to address compounds linked to potential critical quality attributes (CQAs).

The most common techniques currently used for charge heterogeneity testing are IEC [9], cIEF [10] and imaged cIEF [11,12]. Each method has complementary separation mechanisms. In case of cIEF and imaged cIEF analytes are focused within a pH gradient according to their apparent p*I* values, whereas in case of IEC the separation occurs by electrostatic interaction of the analytes with cationic or anionic functional groups of the stationary phase of the IEC column.

In 2011 a new CZE method for charge heterogeneity testing was published by He et al. [13]. A similar CZE method was described by Shi et al. [14]. An advantage of this method is the use of bare fused silica capillaries. Additionally, interaction of analytes with the capillary wall and any residual electro-osmotic flow (EOF) are suppressed by dynamic coating with Triethylenetetramine (TETA) and by an elevated concentration of epsilon amino caproic acid (EACA). UV detection at 214 nm enables high sensitivity. In CZE, separation is achieved based on molecular charge-to-hydrodynamic radius ratio. Investigation of capillary zone electrophoresis as an alternative approach was performed since many pharmaceutical companies consider CZE as a fast, robust, and reliable methodology for daily routine applications.

IEC, IEF and CZE have different pros and cons for different application areas or analytes. IEC which allows MS characterization of different charge species after desalting of single fractions by dialysis and/or RP-HPLC separation [15–17] is currently the most important method which is preferred for biopharmaceuticals during late stage and market supply. Due to very small amounts and MS incompatible compounds like ampholytes or running buffer components identification of CE fractions is more difficult [18] and requires indirect approaches like Rotofor [19], free flow electrophoresis (FFE) [20] offgel-fractionation [21] or complex online coupling technology [18]. In case of charge differences that are buried within the three dimensional structure IEF and CZE are expected to be advantageous whereas in case of changes of the surface charge pattern or changes without influence on the net charge IEC may be preferred [7]. Method selection has to be decided on a case by case basis.

In order to demonstrate required features a lab internal method assessment (range, precision, accuracy, sensitivity, stability indicating properties and high throughput capabilities) and an intercompany study were performed for the analysis of monoclonal antibodies by CZE. An international team of 11 laboratories from 9 independent companies/organizations in the US, Switzerland and Germany was formed to contribute to this study. As already demonstrated for capillary IEF [22] and imaged capillary IEF [23] the aim of the present CZE study was to show applicability in this context.

2. Materials and methods

2.1. CZE separation

All separations were performed on a capillary electrophoresis system capable of capillary temperature control at 20 ± 2 °C and UV detection at 210 – 220nm. Therefor a Beckman Coulter PA 800 Pharmaceutical Analysis System (PA 800 *plus* or *Enhanced*), equipped with UV detector and 214 nm filter (aperture: 800 μm, data rate: 8 Hz) was used. Separations were performed in two different bare fused silica capillaries with capillary length to detector/total length 40 cm/50 cm (ID: 50 μm; Beckman Coulter cat. no.: 338451) [variant A] and 20 cm/30 cm (ID: 40 μm; Polymicro Technologies cat. no.: TST040375) [variant B]. Before each run capillaries were rinsed with 0.1 M HCl (*acidic wash solution*) for 5 min [variant A] or for 4 min [variant B] (60 psi each). The provided MAb samples were diluted to 1 mg/ml with purified water. At least 80 μl of each sample were carefully transferred into PCR vials. After pre flushing with *separation buffer* (400 mM EACA, 2 mM TETA, 0.05% HPMC) for 10 min [variant A] or 9 min [variant B] (50 psi each) samples were injected using a pressure of 0.5 psi (10 s). Polarity was positive (capillary inlet) to negative (capillary outlet). The applied separation voltage was 30 kV. The capillary temperature was 20 °C. Separation time was 30 min [variant A] or 10 min [variant B]. Column storage for long term use: 0.1 M HCl in capillary with two ends dipped in water.

For lab internal investigation of validation characteristics same conditions as before with exception of the following points were applied: rinse with 0.1 M HCl for 1 min (60 psi); pre-flush with separation buffer for 1 min (50 psi); separation voltage: 20 kV.

2.2. Imaged cIEF and IEC

Imaged cIEF of MAb2 (p*I* approx. 8.5): after dilution sample was mixed with Pharmalytes pH 3–10 and separated with an iCE280 system (Protein Simple, Santa Clara, USA). IEC of MAb2: separation was performed in a Dionex ProPac WCX-10 column with a