Optimize Non-Reduced CE-SDS Analysis with the SCIEX Low pH SDS Sample Buffer

Low pH SDS sample buffer CE-SDS analysis

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Introduction

In the biopharma industry, capillary electrophoresis sodium dodecyl sulfate (CE-SDS) is applied at all stages of the pharmaceutical development process. The technique’s main use is monitoring variations in low-level fragments and impurities. In addition, CE-SDS can reveal valuable information for process development, glycosylation site occupancy, and molecular size variant analysis for lot characterization and release.[1,2,3]
To support this range of testing, a fully optimized and robust method is required.

Given the prevalence of kit-based methods and commercially available reagents, CE-SDS method development is typically focused around optimizing a given molecule’s sample preparation. Typical sample preparation includes dilution of a molecule to a set concentration in an SDS-containing matrix, addition of a reducing or alkylating agent, and incubation of the preparation for a set amount of time at a set temperature.

The goal of sample preparation optimization is to minimize any method induced degradation of the molecule. While all proteins are prone to thermally induced degradation, antibodies are especially vulnerable to fragmentation due to the fragile nature of the hinge region. This occurrence is commonly attributed to a disulfide-bond reduction and exchange reaction and can significantly alter the true representation of the size heterogeneity of a protein.[2] Additionally, it can increase the variability of quantitative CE-SDS methodologies in non-reduced analyses. As most applications seek to quantitatively determine a molecule’s size distribution for purity/stability, achieving reproducible results with minimal artifact creation is key to method development.

One frequently overlooked optimization area is sample buffer pH. Non-reduced sample analysis commonly involves the use of a sample buffer consisting of 100 mM Tris-HCl buffer at pH 9.0. This basic pH sample buffer favors both alkylation and disulfide bond formation and thiol–disulfide exchange.

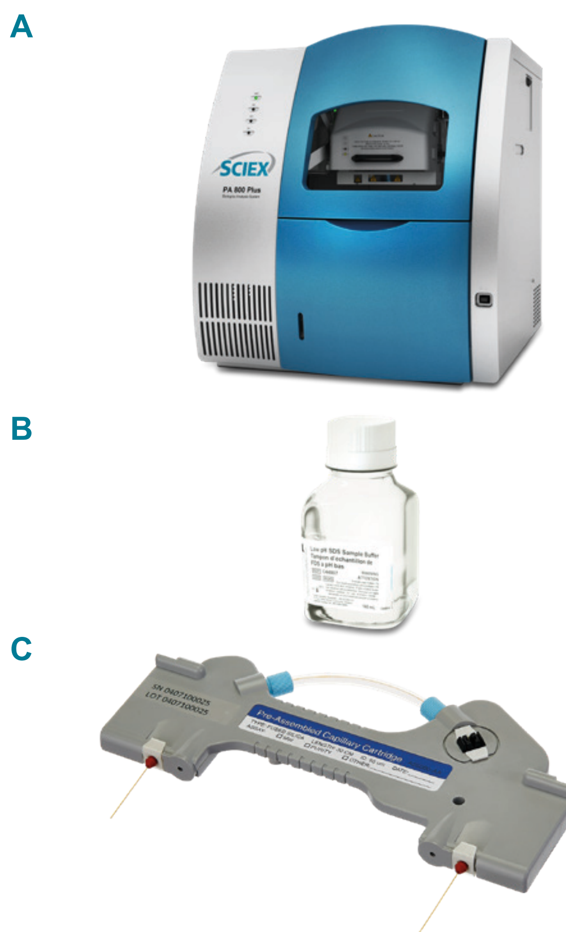

Figure 1. (A) The PA 800 Plus Pharmaceutical Analysis System, (B) the Low pH SDS Sample Buffer (P/N C44807) and (C) pre-assembled cartridge (P/N A55625).

Optimize Non-Reduced CE-SDS Analysis

- Improve method robustness
- Decrease method induced fragmentation
- Greater agreement with orthogonal purity methods such as Size-Exclusion Chromatography (SEC)[4]

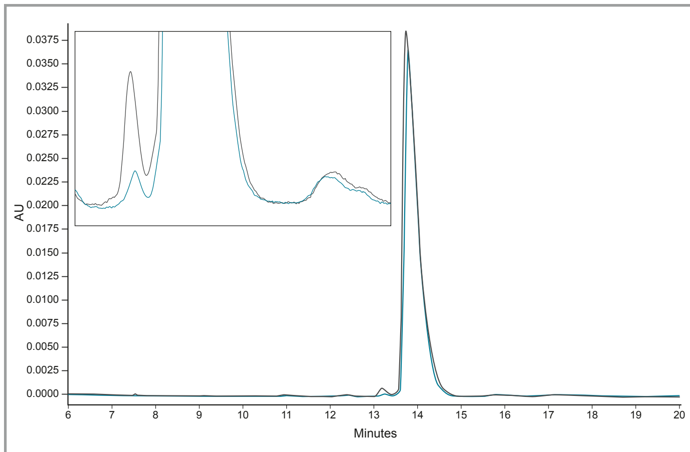

Figure 2. Fullview and zoomed electropherogram of adalimumab biosimilar prepared with SDS-MW Sample Buffer pH 9 (black trace) and Low pH SDS-MW Sample Buffer pH 6.8 (blue trace).

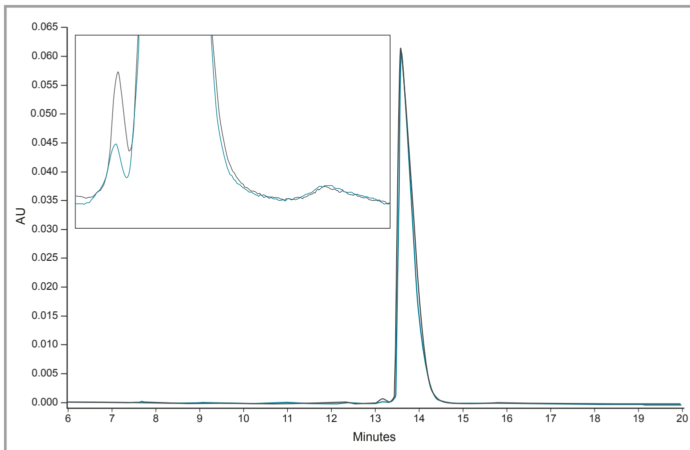

Figure 3. Fullview and zoomed electropherogram of infliximab biosimilar prepared with SDS-MW Sample Buffer pH 9 (black trace) and Low pH SDS-MW Sample Buffer pH 6.8 (blue trace).

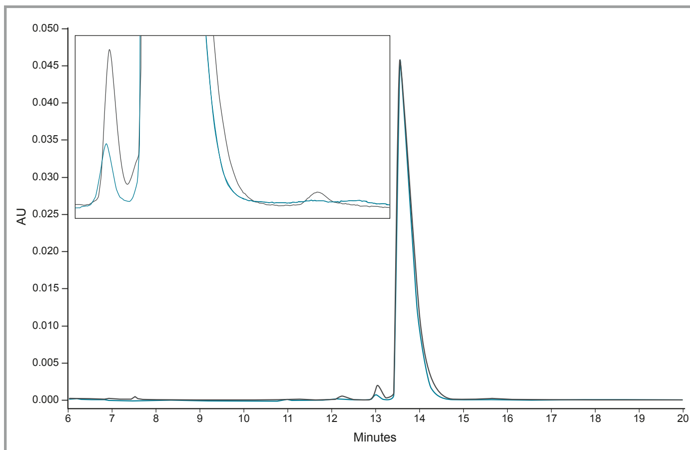

Figure 4. Fullview and zoomed electropherogram of drozitumab biosimilar prepared with SDS-MW Sample Buffer pH 9 (black trace) and Low pH SDS-MW Sample Buffer pH 6.8 (blue trace).

However, an acidic sample buffer may help prevent disulfide bond formation and thiol–disulfide exchange.[5] Beyond pH, certain buffer salts, such as phosphate, have been shown to increase levels of protein fragmentation at higher concentrations.[6] Others, such as citrate, have been found to cause precipitation at low sample storage temperatures.

To demonstrate the effect of sample buffer pH on monomeric purity, three commercially available monoclonal antibody biosimilars were prepared and analyzed using the following buffers: (1) 100 mM Tris–HCl, pH 9.0 (SCIEX SDS-MW Sample Buffer) and (2) 100 mM Tris–HCl, pH 6.8 (SCIEX Low pH SDS Sample Buffer). The samples were prepared as described in the sample preparation section and instrument conditions used are described in the separation and analysis section. The average results of triplicate injections are shown in Tables 1-3.
By decreasing the sample buffer pH from 9.0 to 6.8, the Corrected Area Main Peak % was increased between 1.3 and 2.1%. The main increase in purity primarily came from a decrease in the Heavy-Heavy-Light Chain (HHL) fragment peak, the fragment migrating prior to the Intact Antibody Main Peak.[7]

Methods

Instrumentation

All experiments were performed on the PA 800 Plus Pharmaceutical Analysis System (SCIEX). Pre-assembled bare fused silica cartridges of 50 µm ID × 10 cm to detection were used for the separation.

Reagents

SDS-MW Gel buffer, Acidic Wash Solution, Basic Wash Solution, SDS-MW Sample Buffer and Low pH SDS-MW Sample Buffer were all manufactured by SCIEX (Carlsbad, CA). Lodoacetamide was purchased from Millipore-Sigma (St. Louis, MO). The biosimilar therapeutic proteins of adalimumab, drozitumab, and infliximab were purchased from Absolute Antibody (Redcar, United Kingdom).

The SDS-MW Gel Buffer creates an entangled polymer network for separation of the SDS-protein complexes. The SDS-MW Gel Buffer comprises a proprietary polymer buffer formulation (at pH 8.0) with 0.2% SDS. The SDS-MW Sample Buffer and Low pH SDS-MW Sample Buffer (P/N C44807) are used to prepare the SDS-protein complex for the IgG Purity assay. The acidic wash solution is a reagent comprised of 0.1 N HCl. The basic wash solution is a reagent composed of 0.1 N NaOH.

Preparation of Samples

All samples were prepared in triplicate to a final concentration of 0.5 mg/mL per the following procedure: 50 µL of the sample (1 mg/mL) was combined with 5 µL of 500 mM IAM and 45 µL of the specified SDS-MW sample buffer to a total volume of 100 µL. Samples were mixed well, centrifuged and heated at 70° C for 5 minutes. Each mixture was cooled to room temperature, centrifuged and transferred to a separate sample vial for injection.

Separation and Analysis

An optimized separation method and sequence were set up for batch analysis of 24 samples at a time. For each separation cycle, the capillary was first preconditioned with 0.1 N NaOH, 0.1 N HCl, deionized water, and SDS-MW Gel buffer. A water plug was introduced prior to sample injection by injecting for 0.5 minutes at 5 PSI. Samples were introduced by applying 5 PSI of pressure for 65 seconds. Pressure injections were used to normalize the sample load due to different ionic strengths of the buffers. A voltage of 15 kV (normal polarity) was applied during electrophoretic separations with the capillary maintained at 25° C using recirculating liquid coolant. The system was programmed to automatically replenish all reagents through an increment of the buffer array after every eight cycles.

	% Total Fragments	% Main Peak Purity	% Total HMW* Species	% HHL
Adalimumab pH 6.8	2.0	96.9	1.1	0.5
Adalimumab pH 9.0	3.6	94.8	1.6	1.3

Table 1. Average of triplicate injections of adalimumab biosimilar prepared using SDS-MW Sample Buffer pH 9.0 and Low pH SDS-MW Sample Buffer pH 6.8.

	% Total Fragments	% Main Peak Purity	% Total HMW* Species	% HHL
Infliximab pH 6.8	3.1	96.7	0.2	0.9
Infliximab pH 9.0	4.2	95.4	0.4	2.2

Table 2. Average of triplicate injections of infliximab biosimilar prepared using SDS-MW Sample Buffer pH 9.0 and Low pH SDS-MW Sample Buffer pH 6.8.

	% Total Fragments	% Main Peak Purity	% Total HMW* Species	% HHL
Drozitumab pH 6.8	2.5	97.1	0.4	0.9
Drozitumab pH 9.0	4.3	95.1	0.6	2.2

Table 3. Average of triplicate injections of drozitumab biosimilar prepared using SDS-MW Sample Buffer pH 9.0 and Low pH SDS-MW Sample Buffer pH 6.8.

* High Molecular Weight

Conclusions

While sample incubation time and temperature play an important role in non-reduced CE-SDS method optimization, sample buffer pH should also be considered to help prevent disulfide bond scrambling. It was observed that the SCIEX Low pH SDS-MW Sample Buffer (P/N C44807) significantly decreased fragmentation of several commercially available antibodies under non-reducing conditions. Obtaining the optimal sample buffer for a molecule can lead to increased method robustness and greater correlation with orthogonal purity methods.

References

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