Understanding and Overcoming the Impact of CE-SDS Failure in QC Testing

Abstract

Quality control (QC) is a vital step in the biotherapeutic workflow that covers both in-process and batch release product analyses. QC depends on analyst self-assurance and instrument reliability, both of which are pivotal for meeting regulatory requirements. Analytical systems applied in a QC environment should ensure the continuous quality of test results produced and reported, as biologics are highly sensitive to changes in the development and manufacturing processes.

The identity, stability and purity of biotherapeutic proteins are routinely evaluated using capillary electrophoresis (CE) technology in QC laboratories. When properly implemented, automated CE systems provide a powerful solution to the growing need for maximizing the efficiency of both staff and instruments while reducing cost and improving quality. As with any laboratory instrument, failure modes should be carefully and promptly examined due to their adverse impact on time, resources and capital associated with the QC workflow. Failed injection as a result of air bubbles, size marker misalignment or a low-resolution profile may occur when using CE-sodium dodecyl sulfate (CE-SDS) for biotherapeutic QC analysis. In this white paper, we review CE-SDS technology and associated failure rates in the context of QC testing and explore considerations for workflow and recovery implementation.

CE Technology Overview

CE for the separation of molecules based on their differential migration by size or charge is a technique that began to take off in the 1980s[1]. During a CE-SDS run, SDS-coated proteins are introduced into one end of narrow-bore capillaries via pressure, vacuum or voltage, whereas the other end remains immersed in buffer. Once an electric field is established between the electrodes from a power source, the proteins migrate through a sieving matrix made of either linear or partially-branched polymers. Direct detection happens on-column, from which accompanying software displays the output in the form of an electropherogram for further analysis. Compared with traditional slab gel approaches, which are labor-intensive and semiquantitative at best, CE-SDS affords a multitude of advantages which have facilitated its evolution to a standard technology employed for the satisfaction of reproducible and quantitative QC criteria[2,3]. First, CE is exceptionally sensitive in its detection capabilities, requiring very small sample volumes. Second, the high-resolution capability translates to highly quantitative results. Third, separation time is shorter, in the order of minutes versus hours. Finally, CE can be automated, enabling quick and simple method development, reproducible results, assay flexibility and overall increased efficiency. Indeed, cross-laboratory studies have scrutinized the reliability and robustness of CE-SDS and attest to its application in this setting[4,5]. In 2010, guidance from the International Conference on Harmonization began to recommend CE as a suitable and acceptable method for protein analysis in regulated environments[6]. Since then, many biopharmaceutical companies have adopted CE-SDS and its associated compliant software for QC workflows, particularly for release testing where SDS-PAGE has traditionally been used.

However, the small sample size required for analysis by CE also mandates accurate and precise injection. Samples are mainly injected by pressure or applying voltage, and each method depends on the capillary diameter and length. Therefore, troubleshooting has focused on controlling differences in the time of a flow created by either mode of injection by adjusting assay parameters (e.g., sample, buffer, voltage) and the instrument's injection settings[7,8]. An injection failure, which can be simply caused by air bubbles, can block the capillary, break the current and result in system downtime leading to costs in both time and capital[7]. Injection failure rates are an often overlooked cost of QC. Thus, there is much to gain from having technology in place that is easy to use, robust and reliable.

The Hidden Cost of Failure Rates

Documented and straightforward standard operating procedures (SOPs) are fundamental to the proper investigation and follow-up action for a system or process failure. Otherwise, the entire QC process is squandered. The obvious impact of a failure mode is acute, and on the QC workflow, but the chronic cost of failure rates from a business perspective is often less obvious to appreciate.

Not all failures are instrument-related, and each should be investigated to determine the specific cause. In **Figure 1**, we outline a representative workflow of actions taken when a system injection failure is identified. Note time and capital costs increase with the number of steps required to requalify your system. An instrument requiring less manual operation and fewer procedural steps can simplify your investigation into the cause of failure, saving your company both time and money.

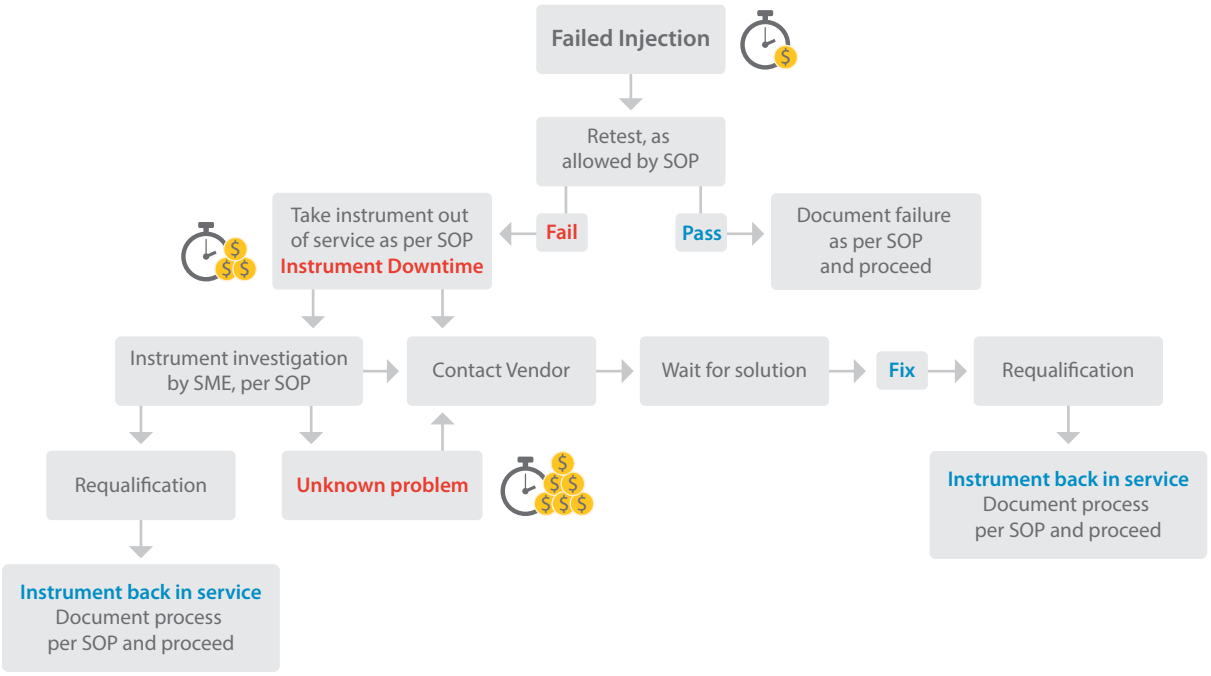

FIGURE 1. The impact of an instrument injection failure on your workflow. Note that a sample retest action will depend on auto-sampler stability and your QC lab guidelines, as not all compounds are stable for 24 hours.
SOP, standard operating procedures; *SME*, subject matter expert.

If an instrument is down, the ramifications are often felt in both the QC in-process early phases of development and late-phase batch release testing. Especially costly to your business is an unforeseen disqualification that interrupts product release and goal fulfillment. Also, your other ongoing research projects and instrument(s) availability may be impacted as you decide whether to risk or protect their use, or even delay the maintenance of one over another more urgent matter. Therefore, it's important to address just how often your workflow and available in-house resources can accommodate failures. In **Table 1** we estimate the impact instrument failure rates have in terms of downtime over a one-year period.

Failure Rate	1%	5%	10%	15%	20%	25%
Downtime (days)	3.65	18.25	36.5	54.75	73	91.25

TABLE 1. The time lost in days as a function of the failure rate over a one-year period. The number of downtime days was calculated by multiplying 365 (days per year) by the percent failure rate

In a given QC lab, a system failure rate of 25% could translate to a whopping 91 days of lost time and requalification procedures as stated in your SOP. The total loss is likely to be extended due to the time it takes to bring in vendor support, complete deviation documentation and then requalify the instrument, culminating in potentially delayed product release. As a result, you'll likely need to incorporate additional strategies and backup instruments to support continuous quality of test results—expenses that add up quickly.

Are All CE-SDS Workflows Created Equal?

Many companies differentiate themselves by offering various all-inclusive suite of CE technology solutions. However, in the context of sample injection, the available CE platforms are largely divided by either hydrodynamic or electrokinetic injection principles, with some offering both in addition to vacuum. The electrokinetic mode is more feasible and, thus, far more common in the application of CE-SDS for protein separation, specifically due to the viscous nature of the sieving gel matrix within the capillary.

The workflow for system setup and sample injection, though, varies greatly among the automated technologies available; some require numerous manual steps that introduce opportunities for human error and, thus, increase injection failure rates. The workflow presented in **Figure 2** is representative of a conventional CE-SDS system, which is complex and error prone. Simply put, as the number of steps increases, so does the probability of introducing errors and subsequent troubleshooting of slowdowns, failures and other problems. Moreover, these multistep, complicated protocols need to be meticulously performed and maintained to avoid contamination, corrosion and/or leaks within the system.

Preferably, for biotherapeutic in-process and batch release QC testing, where instrument reliability and precision are paramount, the system you chose should enhance ease-of-use and reduce operator-dependent variability.

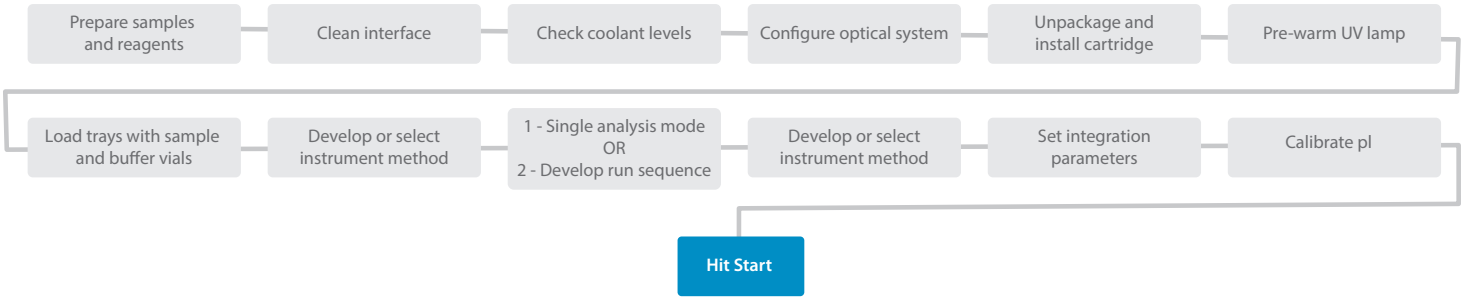

FIGURE 2. Typical system setup and analytical operation workflow for various automated CE-SDS technologies.

"CEing Your Sample" With Maurice

Maurice™ performs size-based CE-SDS on up to 48 samples per batch, guaranteeing 100 injections per cartridge. The pre-qualified, ready-to-use cartridge design spares researchers the laborious capillary assembly, maintenance and optimization required with other CE-based methods. There's no manual setup or upkeep required, and contamination concerns are removed by cartridge design. If you get a failure, you often just need to replace the cartridge rather than disassemble the instrument for cleaning and maintenance. Maurice is a fully-automated system with an easy-to-follow workflow for CE-SDS analysis: just pop in one of the ready-to-go cartridges, drop in your sample vials or a 96-well plate and hit Start (**Figure 3**). This simplified workflow certainly reduces the chance of analyst error.

FIGURE 3. CE-SDS Workflow using Maurice. It takes less than 10 minutes to start a run once your samples and reagents are prepared.

Samples are electrokinetically injected into the cartridge capillary based on their defined location in the batch and subsequently electrophoresed within. The peaks are directly detected via UV absorbance at 220 nm and plotted on an electropherogram.

Maurice provides highly reproducible peak profiles (coefficient of variance, ≤2%) and relative migration times for each peak within a wide molecular weight range (10–270 kDa). Reduced IgG samples can be analyzed with superior baseline peak resolution (≥1.5) for non-glycosylated heavy chain-to-heavy chain composition. Moreover, you can preprogram batch and method parameters, monitor your run in real-time and analyze data using Compass for iCE software that is compliant with the Food and Drug Administration's Title 21 Code of Federal Regulations Part 11 (21 CFR 11). Compass software has many tools to ensure data authenticity and integrity, including but not limited to restricted access, secure computer-generated time-stamped audit trails, e-signatures and compliant exporting or importing into third-party software like Chromeleon and Empower. These features together with rapid analysis and platform methods make Maurice a valuable system for biopharmaceutical QC testing.

Summary

CE-SDS is a rational step forward beyond the traditionally used SDS-PAGE methods in biopharmaceutical QC labs. Its cross-examination by multiple laboratories and acceptance as a suitable analytical technique by regulatory agencies verifies its use in this setting.

Several automated CE-SDS systems are commercially available. However, the conventionally used systems are error-prone due to their complicated designs and laborious workflows. The true cost of instrument injection failures in a QC laboratory is sometimes overlooked but can be minimized with a system that reduces the number of operational steps required, is mechanically simple and straightforward to troubleshoot. Delays inherent in system retesting, instrument out of service and/or external technical support required add up in downtime, which can impair ongoing research, delay product release and disrupt other business operations. All in all, it's critical to appreciate and understand the hidden costs associated with a failed QC test before you decide on the CE-SDS system to adopt in your laboratory.

Advantages of Maurice Over Other CE-SDS Platforms for QC laboratory

- ✓ Simplified Workflow
  - Reduced system maintenance
  - Preassembled ready-to-use cartridge
- ✓ Higher Throughput
- ✓ Intelligent System Design
  - Automatic cartridge qualification
  - Active current monitoring and injection recovery
- ✓ Better Molecular Weight Quantitation

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