

Charged Aerosol Detection:

The Chromatographer's Magical Tool to Unravel the Invisible in Biopharmaceuticals

Health Inspired, Quality Driven.

Biopharmaceuticals are a pivotal weapon in the pharmaceutical arsenal, helping to treat patients suffering from critical and life-threatening diseases. They have the potential to provide a range of previously unimagined benefits and satisfy medical needs that have remained unmet thus far – but this relies upon the large-scale discovery and development of these next-generation therapeutics.

However, biopharmaceutical manufacturers must overcome several distinct challenges. Due to their inherent complexity, biopharmaceuticals require a robust manufacturing process to ensure the quality and safety profiles of the molecule, which is an incredibly time- and resource-intensive undertaking.

It currently takes on average a decade or more to bring a drug to market, costing an estimated USD 2.6 billion (including the cost of failed drug candidates).¹

It is therefore imperative to identify any potential component that may impact the quality profile of a

molecule early on in the development process.

Aggregation, often considered the Achilles' heel of many biopharmaceutical products, is a major factor that often impacts the quality profile of such molecules. Aggregation can render a drug ineffective while also potentially triggering an immune response, leaving patients with unintended clinical complications.

For example, the case of EPREX-associated Pure Red Cell Aplasia (PRCA) is a well-documented example of such an effect.²

Biopharmaceutical manufacturers can prevent and control aggregation during the product lifecycle by using non-ionic surfactants, such as poloxamers and polysorbates. These act to stabilize biopharmaceuticals by providing protection from interfacial stresses.³

References:

¹Amy Sun et al., Late-Stage Failures of Monoclonal Antibody Drugs: A Retrospective Case Study Analysis, *Pharmacology*, 105: 145-163

²Kirsty D. Ratanji et. al., Immunogenicity of therapeutic proteins: Influence of aggregation, *J Immunotoxicol*, 2014, 11(2): 99-109

³Andrea Arsiccio et. al., Surfactants as stabilizers for biopharmaceuticals: An insight into the molecular mechanisms for inhibition of protein aggregation, *Eur J Pharm Biopharm*, 28: 98-106

The power of these surfactants is substantial – they can even stabilize protein formulations that might otherwise resemble snow globes in their absence. Unfortunately, the solution to one problem often presents another entirely new challenge – in this case, for chromatographers. It's crucial to monitor these surfactants given how important they are in maintaining the quality of biopharmaceutical products. As a result, chromatographers have turned to their analytical toolbox to develop methods capable of measuring surfactant levels in biopharmaceutical formulations.

That said, chromatographic analysis of these non-ionic surfactants is not without its difficulties.

Surfactants such as poloxamers and polysorbates are polymeric and heterogeneous in nature. This means that the target analyte may be observed as a collection of peaks, making mass spectrometry (MS) detection more cumbersome and rendering the development of chromatographic conditions that much more challenging. Ultraviolet (UV) detection also proves to be challenging since these surfactants don't carry significant chromophores, leading to relatively poor sensitivity. This is where the charged aerosol detector (CAD) comes to the rescue.

Charged Aerosol Detector

The CAD is an evaporative detector that is well suited for non-volatile and semi-volatile analytes where chromophores are not present. The CAD is readily coupled with high-performance liquid chromatography (HPLC) or ultra-performance liquid chromatography (UPLC) and can be used to detect, and quantify, a variety of biological and chemical entities.

Three steps leading to detection

Nebulization: where eluent from the column is nebulized into droplets followed by evaporation to yield non-volatile particles.

Charging: where nitrogen gas from a highly charged corona needle collides with analyte molecules, resulting in charged analytes. The larger the particle, the more charge it carries.

Detection: where these particles are transferred to a collector and measured by a sensitive electrometer producing an output signal. The signal generated is in direct proportion to the quantity of analyte that is present.



Method Considerations for Poloxamers and Polysorbates

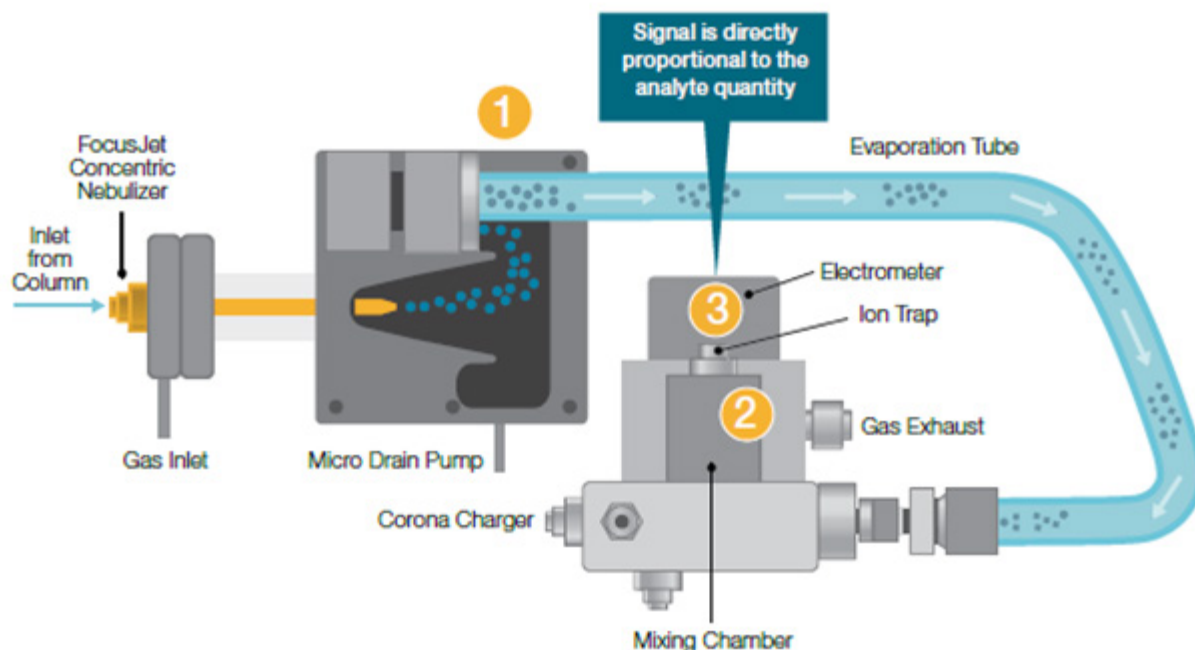


Figure 1:
Mechanism for charged aerosol detection⁴

Sample preparation is a key factor when it comes to conducting a successful CAD analysis. Depending on the formulation, pre-treatments such as solid-phase extraction or protein precipitation are often required to remove interfering excipients. The treated sample

is then analyzed using appropriate chromatographic parameters and a step gradient to produce elution in a single peak. The surfactant may then be quantified using different statistical models depending on the nature of the molecule and the assay design.

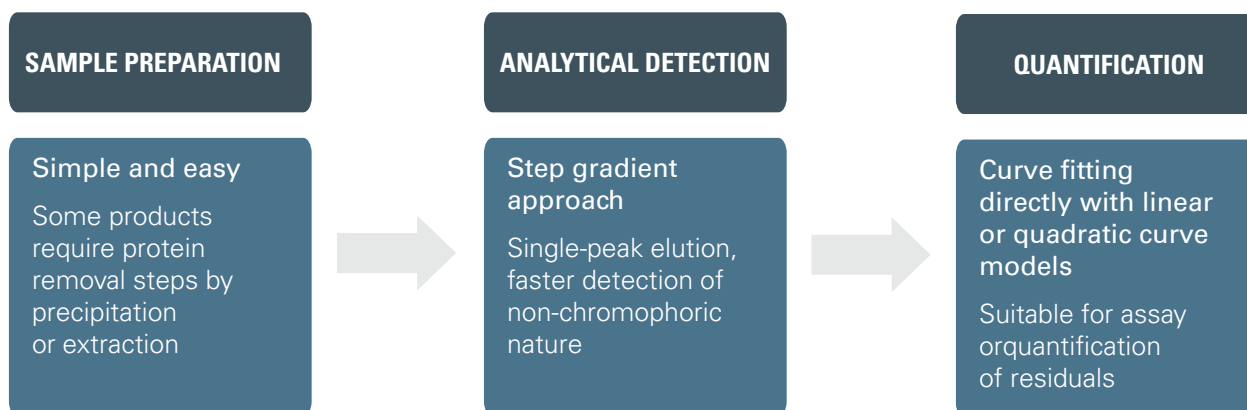


Figure 2: Analytical approach

References:

⁴HPLC Charged aerosol detection surfactants and emulsifiers application notebook, 2019. Retrieved 2019, from <https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/ai-71101-hplc-cad-surfactants-emulsifiers-ai71101-en.pdf>

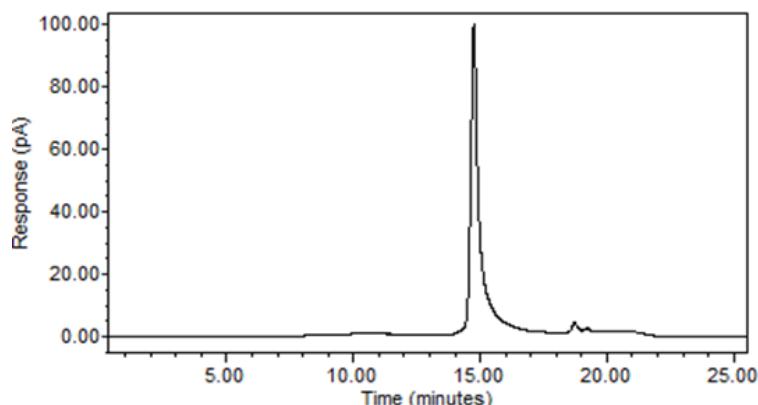


Figure 3:
Single-peak elution using reverse phase-charged aerosol detection

Figure 3 shows an example of single peak elution using reverse phase-charged aerosol detection (RP-CAD). This RP-CAD analysis is normally very robust and reproducible. This quantitative analytical methodology meets various regulatory guidelines and

In summary, there are a range of reasons why this CAD technology would provide an advantage over other, commonly used detection methods such as evaporative light scattering detection (ELSD) or MS. It offers greater performance in terms of sensitivity, precision and dynamic range when compared to ELSD, while it is much simpler to perform than MS.

is well suited for routine analytical testing, including qualification and validation involving peak identification, specificity, accuracy, linearity, robustness and precision.

In addition, and importantly, CAD offers detection independent of chemical structure. It has immense potential in the biopharmaceutical analysis space due to its applicability across a plethora of molecular types including surfactants, adjuvants, amino acids, glycans and sialic acids, to name just a few.

SGS's Biopharmaceutical Expertise

SGS provides CAD detection as part of a wide array of **biopharmaceutical testing solutions**. This includes everything from early cell bank safety assessment and product characterization to phase-appropriate method development and validation, bioanalysis and final GMP product release.

Our innovative biopharmaceutical testing, biosafety, bioanalytics and product quality control solutions add value throughout every stage of the product lifecycle. We're recognized as a true industry pioneer, with our bioanalytical team actively pursuing assay development and validation for innovative biomarkers on an ongoing basis.

SGS's team of experts are on hand to provide a range of biopharmaceutical solutions, tailored to your business's specific needs. To find out more, get in touch today.

Contact us

To discuss your early-phase clinical research and biometrics outsourcing requirements, contact us today.

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