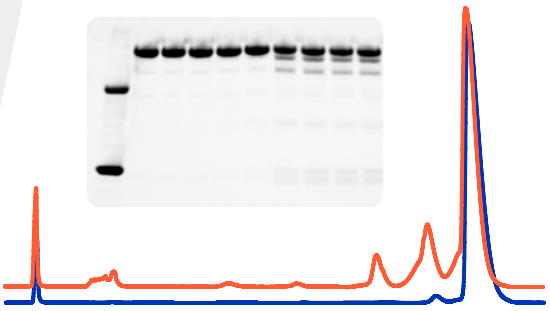
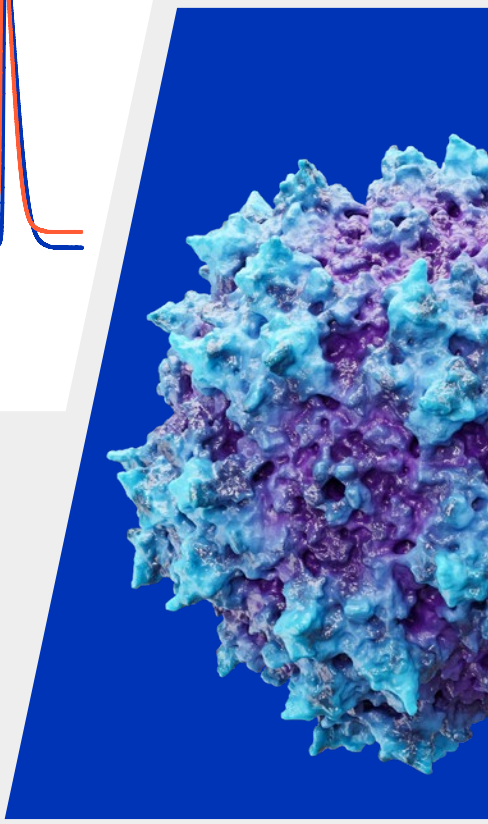
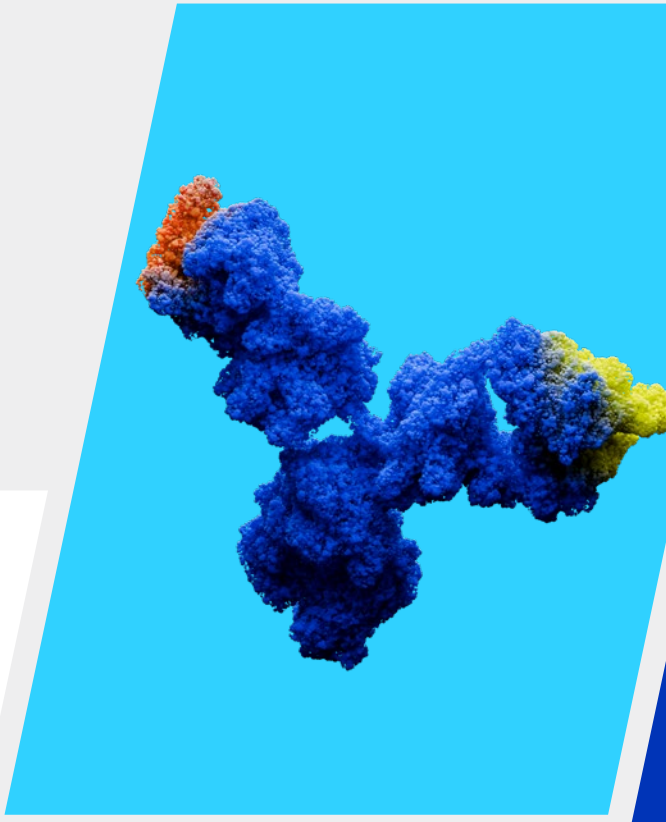
R&D systems™
by bio-techne

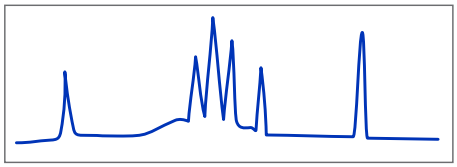




Advancing the Analysis of *Next Generation Biotherapeutics*

Protein Charge and Size Characterization Using Capillary Electrophoresis





Contents

Introduction

Biologics have transformed modern medicine, but their journey from concept to clinic has never been simple. Early biotherapeutics such as proteins and monoclonal antibodies often take years or even decades to develop, with validation and method transfer being a major challenge. Historically, scientists relied on manual, labor-intensive techniques such as SDS-PAGE gels, ELISA, and ultracentrifugation, which provided valuable insights but lacked speed, reproducibility, and scalability.

The evolution of analytical tools has significantly changed that landscape. Advances like capillary electrophoresis (CE), high-resolution mass spectrometry, and automated imaging systems have accelerated workflows, improved sensitivity, and enabled deeper characterization of complex molecules. For example, CE-SDS replaced traditional gels with automated, high-throughput analysis of protein identity and purity, while imaged capillary isoelectric focusing (icIEF) brought higher precision compared to ion exchange chromatography (IEX) to charge variant profiling—critical for ensuring product quality and stability.

Today, as we enter an era of novel biotherapeutics that includes antibody-drug conjugates (ADCs), bispecific antibodies, fusion proteins, and therapeutics utilizing viral vectors like LNPs and AAVs as delivery vehicles, the need for rapid, reliable analytical tools is greater than ever. These modalities introduce unique challenges: heterogeneity, stability concerns, and intricate critical quality attributes (CQAs). Modern tools such as the Maurice™ and MauriceFlex™ systems meet these challenges head-on, combining multiple CE-based assays—CE-SDS, icIEF, and icIEF fractionation—on a single system to deliver fast, reproducible, and insightful data. This integration not only accelerates development timelines but also ensures confidence in product quality from early research through commercial manufacturing.

As we look ahead, the future of bioanalytics will continue to shape the speed and success of biotherapeutic innovation. By leveraging advanced technologies like CE, LC-MS & UHPLCs we can shorten development cycles, reduce costs, and ultimately bring life-changing therapies to patients faster, where they are needed most.

Antibody-Drug Conjugates

Antibody-drug conjugates (ADCs) are a class of biologics that combine a mAb with a chemotherapy drug via a stable linker molecule. When the antibody binds to the cancer cell, it releases the drug inside the cell to kill it. This targeted approach delivers the toxic drug directly to cancer cells, minimizing the damage to healthy cells and reducing the side effects of traditional chemotherapy. There are several commercially available biotherapeutic ADCs, such as Adcetris® (brentuximab vedotin), Kadcyla® (trastuzumab emtansine), Trodelvy® (sacituzumab govitecan), and many more, for the treatment of various cancers.

Because of their structural complexity, ADCs require advanced analytical techniques to characterize their Critical Quality Attributes (CQAs) that are important for their safety, efficacy, stability and clearance.

Advantages of the Maurice System for ADC Analysis

The Maurice system enables high-resolution charge heterogeneity analysis using icIEF and size/purity analysis using CE-SDS. For ADC analysis, the Maurice system is particularly well-suited because:

- ✓ On-Board Mixing is a feature of the Maurice system that automatically mixes reagents with samples right before analysis, thus reducing hands-on time and minimizing the risk of sample degradation.
- ✓ icIEF is less affected by linker-drug interactions compared to ion exchange chromatography (IEX), making it ideal for ADC charge heterogeneity analysis.
- ✓ The On-Board Mixing (OBM) capability minimizes sample handling and prevents degradation of pH-labile ADCs like Enhertu® during analysis.
- ✓ OBM improves reproducibility and stability, especially for unstable ADCs, as demonstrated with Enhertu.

Key Distinctions of the Method

The ampholyte mixture is enhanced using acetic acid, and the temperature of the autosampler is reduced to 4°C to minimize degradation.

The option to use the in-built 458 nm filter enables selective fluorescence detection, which is useful for ADCs like Enhertu that have a fluorescently active linker or payload.

Results

icIEF analysis revealed charge heterogeneity and stability differences between native and stressed samples of Herceptin, Kadcyla, and Enhertu. OBM and ADC-optimized icIEF methods significantly improved reproducibility and minimized degradation for pH-labile ADCs like Enhertu. CE-SDS analysis confirmed structural profiles and identified variants, including high molecular weight species in stressed samples.

FIGURE 1A.

Herceptin icIEF Analysis

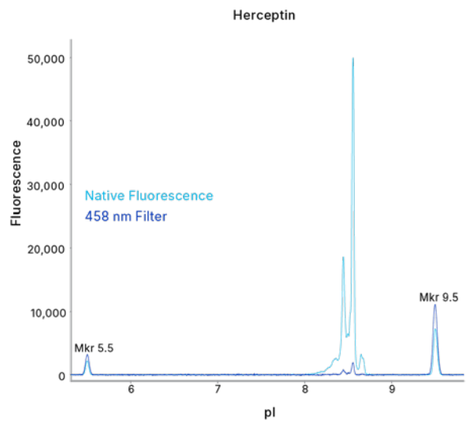


FIGURE 1B.

Enhertu icIEF Analysis

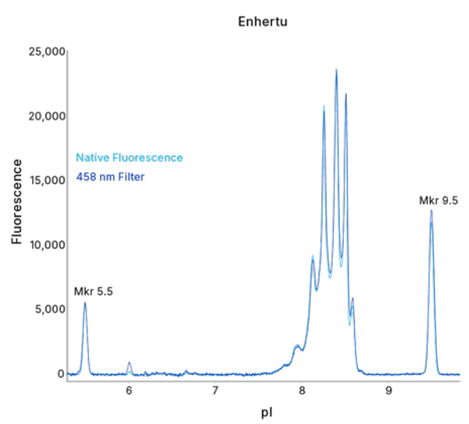


Figure 1. icIEF analysis of Herceptin (A) and Enhertu (B) using the 458 nm filter in Maurice. An overlay of profiles of each molecule analyzed with native fluorescence (280 nm) and the 458 nm filter are shown. Herceptin does not emit fluorescence at 458 nm, whereas Enhertu shows highs fluorescence at that wavelength.

FIGURE 2A.

Non-Reduced CE-SDS Analysis

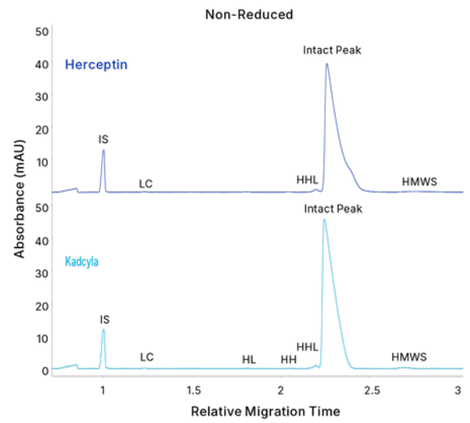


FIGURE 2B.

Reduced CE-SDS Analysis

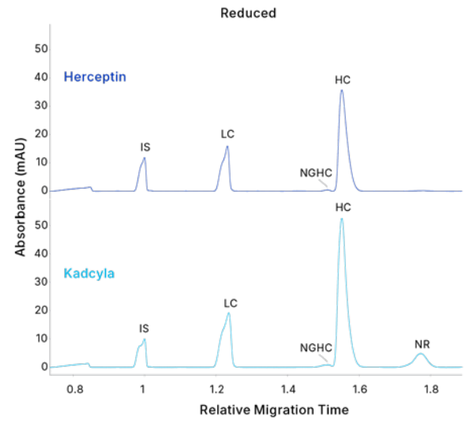


Figure 2. CE-SDS analysis of Kadcyla under non-reduced (A) and reduced conditions (B) on Maurice. For Herceptin and Kadcyla, the intact peak is seen under non-reduced conditions along with other minor peaks, while the LC, HC, and NGHC are detected under reduced conditions. (HMWS: high molecular weight species; NR: non-reduced peak).


biotechne / protein simple

Application Note

Unraveling Structural Complexities of ADCs with Maurice Platforms

Introduction

Unraveling Structural Complexities of ADCs with Maurice Platforms

This application note demonstrates how the Maurice™ system enables robust characterization of antibody-drug conjugates (ADCs) through charge and size-based analysis.

Explore App Note

Scan the QR Code or Visit:
rndsystems.com/an-adc-maurice

Expert Insights: Pioneering ADC Development & Analysis

In this interview, Jana Stockel, PhD, Manager of Bioprocess and Analytical Development, and David Richards, PhD, Scientist at MilliporeSigma, discuss their analytical approach to advancing ADC development. They share how their teams use technologies icIEF and icIEF fractionation on the Maurice and MauriceFlex systems to troubleshoot charge heterogeneity, deepen product characterization, and navigate the growing complexity of modern bioconjugates.

Jana Stockel, PhD
Manager, MilliporeSigma

David Richards, PhD
Scientist, MilliporeSigma

> “The primary advantage of MauriceFlex from the perspective of LC-MS analysis is the sample compatibility. The charge variants of interest are fractionated within an ammonium acetate matrix. This reduces the sample preparation time compared to other charge variant separation techniques such as ion exchange chromatography, where buffer exchange is often required due to the salt content present from the mobile phase.

- David Richards, PhD, Scientist, MilliporeSigma

1. What are your roles and responsibilities at MilliporeSigma, and how do you work together? [Jana]

I lead the bioanalytical team in the Process and Analytical Development group at our Cherokee site in St Louis. I oversee the bioanalytical work to support ADC development while remaining partially active in the lab. My hands-on focus is on charge-based characterization using icIEF, including method development, troubleshooting, and charge variant fractionation on the MauriceFlex. In my group we support ADC development using a range of complementary techniques including CE-SDS, ELISA, SPR, and LC-MS. David is focused on LC-MS characterization, where he works at the molecular level to identify the root cause behind the heterogeneity we observe. We use icIEF to define and separate the charge variants and LC-MS to further characterize those differences.

2. Tell us how you are using the MauriceFlex system for your analysis work [Jana]

At this stage, we primarily use the MauriceFlex for troubleshooting and deeper investigation of charge heterogeneity. When we observe changes in a charge profile during process development of an ADC, we can fractionate specific variants and further characterize them to better understand how those changes relate to the product and process.

3. Can you comment on downstream LC-MS methods you are using with the charge variant fractions, and what additional insights these methods are providing? [David]

The primary advantage of MauriceFlex from the perspective of LC-MS analysis is the sample compatibility. The charge variants of interest are fractionated within an ammonium acetate matrix. This reduces the sample preparation time compared to other charge variant separation techniques such as ion exchange chromatography, where buffer exchange is often required due to the salt content present from the mobile phase. Intact and subunit LC-MS analysis can therefore be readily performed to identify and characterize mass differences that correspond to various charge variations.

Read Full Interview
Scan the QR Code or Visit:
bio-techne.com/fyp-adc-maurice

Technical Tips: Optimizing ADC Analysis using Maurice Platforms

Dr. Monica Tyagi discusses key steps for method development and optimization of ADC analysis using icIEF and CE-SDS methods on Maurice Systems.

Watch Video
Scan the QR Code or Visit:
youtu.be/fb7ZhM9ER34

Advancing Charge Variant Characterization of ADCs and mAbs using icIEF Fractionation and LC-MS

This webinar from industry experts describes how fractionation of ADCs on MauriceFlex and in-depth characterization of charge fractions with mass spec provides novel insights.

Watch Webinar
Scan the QR Code or Visit:
bio-techne.com/wbr-adc-mauriceflex

Fusion Proteins

A fusion protein is a single, engineered protein made by combining two or more different proteins. By joining together different proteins that have different beneficial qualities, the potency, stability, and specificity of fusion proteins can be greatly enhanced compared with naturally occurring proteins. A bispecific antibody (next section) is a specific type of fusion protein or hybrid molecule designed to bind to two different antigens at once. Therefore, a bispecific antibody is a type of fusion protein, but not all fusion proteins are bispecific antibodies. Examples of commercially available fusion proteins include Etanercept (Enbrel®) and Abatacept (Orencia®) for rheumatoid arthritis, Aflibercept (Eylea®) for wet age-related macular degeneration, and Rilonacept (Arcalyst®) for treating inflammatory conditions.

Rapid and High-Quality Analysis of Therapeutic Fusion Proteins with Maurice Turbo CE-SDS

Etanercept (Enbrel®) is used to treat autoimmune conditions such as rheumatoid arthritis and psoriasis. Because it is a heavily glycosylated molecule with a complex structure, it is challenging to analyze during its development and manufacturing. This application note describes a simple, rapid CE-SDS method to analyze an Etanercept biosimilar using the Turbo CE-SDS™ cartridge on the Maurice system, offering a high throughput alternative to HPLC for analyzing the size and purity of fusion proteins or biosimilars.

Advantages of the Maurice System for Fusion Protein Analysis

- ✓ Fast and high throughput CE-SDS analysis
- ✓ Non-reduced analysis in 8 minutes per sample
- ✓ Reduced analysis in 5.5 minutes per sample

Results

Representative electropherograms of the Etanercept biosimilar are shown below. The method resulted in excellent linearity (R2 > 0.99) and reproducibility (%RSD <0.6) while delivering high quality data between 8-12 minutes.

Conclusion

The Turbo CE-SDS cartridge has been specifically designed for high throughput applications while maintaining data quality. Furthermore, the Maurice platform offers other cartridge options as well for biotherapeutic analysis: CE-SDS PLUS cartridge provides even higher-resolution CE-SDS data for QC and GMP release, and the cIEF (200) and 400 icIEF cartridges enable charge heterogeneity analysis with imaged capillary isoelectric focusing (icIEF). This versatile system simplifies workflows and ensures robust analytical performance throughout drug development and manufacturing processes.

FIGURE 3A.

Non-Reduced Conditions

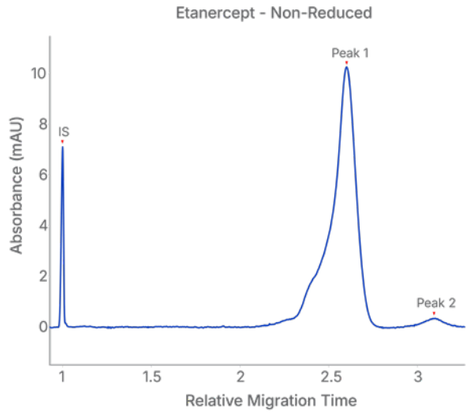


FIGURE 3B.

Reduced Conditions

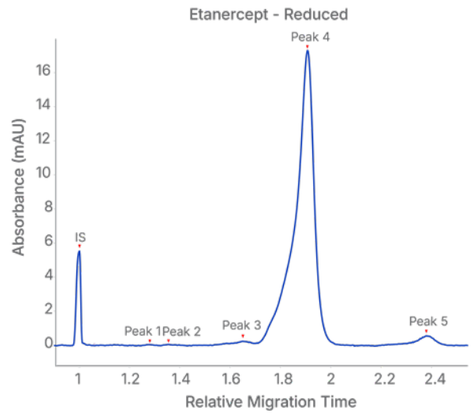


Figure 3. Analysis of Etanercept with Maurice Turbo CE-SDS. Profile of Etanercept under A. non-reduced conditions, showing a broad, major peak, and B. reduced conditions, with smaller peaks corresponding to smaller fragments after reduction except Peak 5, which likely corresponds to the intact fusion protein.

Rapid and High-Quality Analysis of Therapeutic Fusion Proteins with Maurice Turbo CE-SDS™

This application note describes the Turbo CE-SDS method, a fast, reliable, and label-free approach for analyzing fusion proteins like Etanercept, with excellent linearity and reproducibility, facilitating high-throughput applications while ensuring data quality.

Download App Note

Scan the QR Code or Visit:
rndsystems.com/an-fusion-turbo

Addressing the Challenges of Size and Purity Analysis for Fusion Proteins, ADCs, and AAVs Using CE-SDS

This webinar from Caroline Daniels (Shattuck Labs) describes a routine workflow to separate structurally similar molecules based on the number of N-linked glycosylation sites using the Maurice system and the Turbo CE-SDS cartridges that supports process development, GMP release, and stability testing.

Watch Webinar

Scan the QR Code or Visit:
bio-techne.com/wbr-fusion-turbo

Bispecific Antibodies (bsAbs)

A bispecific antibody (bsAb) is genetically engineered to bind to two different antigens at the same time, unlike a native antibody that only binds to a single antigen. For example, this dual function allows bispecific antibodies to act as a bridge by linking a cancer cell to a T cell to help the immune system find and destroy cancer. This type of therapy is being used and studied for various cancers, including lymphoma, leukemia, multiple myeloma, and non-small cell lung cancer. Some commercially available biotherapeutic bsAbs are Blincyto (Blinatumomab), Rybrevant (Amivantamab), and Lunsumio (Mosunetuzumab).

Unlike other immunotherapies that require individual patient engineering, bispecific antibodies can be used directly, allowing for a faster start to treatment. However, because they activate the immune system, bsAb therapies can cause serious side effects like cytokine release syndrome and neurotoxicity. There is research underway to develop bispecific antibodies that target multiple pathways on cancer cells to more effectively inhibit tumor growth.

Advantages of the MauriceFlex System for Analyzing Bispecific Antibodies

- ✓ icIEF, CE-SDS, and icIEF fraction collection capabilities, all in one system.
- ✓ icIEF fraction collection enables downstream analysis with a variety of mass spectrometry and photometry methods and binding analysis with surface plasmon resonance (SPR).

Comparing Innovator and Biosimilar BsAbs with icIEF Fractionation and LC-MS

The MauriceFlex System enables icIEF-fractionation to collect charge variants of the Mosunetuzumab innovator and its biosimilar. The FabRICATOR® Digestion enzyme (Genovis) is utilized to cleave these bsAbs for subunit mass analysis using a Waters BioAccord LC-MS.

Both the innovator and its biosimilar showed comparable charge profiles but differed in structural details. The Innovator displayed correct assembly and glycation in acidic fractions. The biosimilar exhibited mispaired species (light chain mismatch) and retained C-terminal lysine in basic variants.

Combining icIEF-fractionation and Mass Photometry to Provide Insights on Bispecfic Charge Variants and Aggregation

This webinar demonstrates how combining the MauriceFlex system and the TwoMP mass photometry streamlines charge–size variant characterization for bispecific charge variants. This workflow enables the direct tracking of LMWS and HMWS across acidic, main, and basic peaks under control and forced degradation, providing insights into aggregation based on different conditions.

Watch Tech Tips Video

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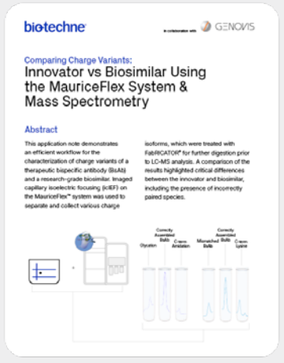
bio-techne.com/resources/webinars/maurice flex-mp



Comparing Charge Variants: Innovator vs Biosimilar Using the MauriceFlex System & Mass Spectrometry

This application note provides details on MauriceFlex fractionation methods, antibody digestion with FabRICATOR, and a complete analysis of the innovator and biosimilar.

Explore App Note

Scan the QR Code or Visit:
rndsystems.com/an-subunit-ms

FIGURE 4.

Innovator vs. Biosimilar BsAbs

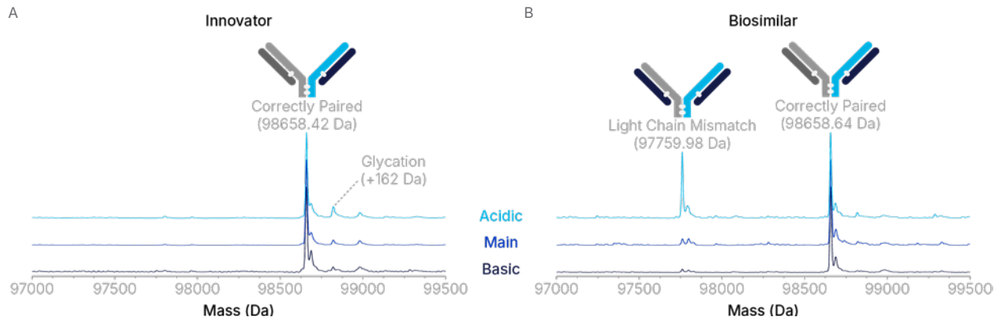


Figure 4. Subunit mass analysis using LC-MS of the Fab'2 subunit shows critical differences between the innovator and biosimilar. The innovator shows correctly paired BsAbs along with evidence of glycation, especially in its acidic variants (A), while the biosimilar clearly shows the presence of mismatched species in its acidic peaks in addition to the correctly assembled BsAb (B).

Results

FIGURE 5.

Comparing Innovator and Biosimilar BsAbs with icIEF Fractionation and Mass Photometry.

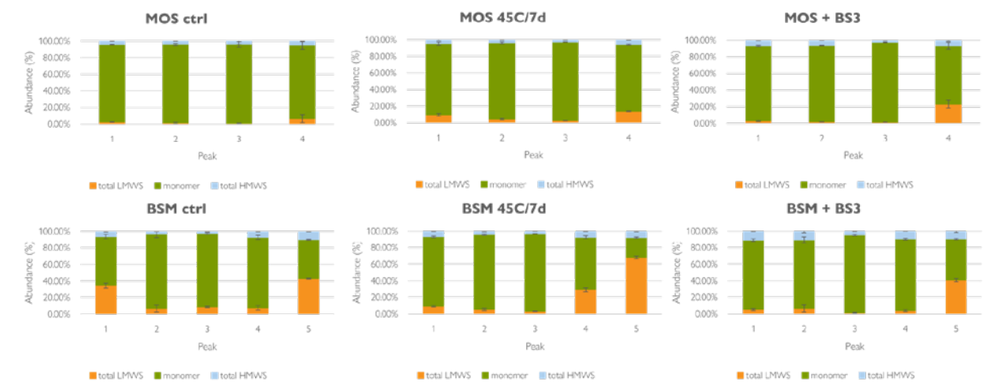


Figure 5. Charge variants of Mosunetuzumab (innovator and biosimilar) were separated and collected with the MauriceFlex system and further characterized with mass photometry, without buffer exchange or re-optimization, on the TwoMP system (Refeyn) to understand the distribution of size variants within each collected charge variant.

Comparing Innovator and Biosimilar BsAbs with icIEF Fractionation and SPR

Charge isoforms of Mosunetuzumab (innovator and biosimilar) were separated and collected on the MauriceFlex system. The acidic, main, and basic fractions were analyzed for their binding with the ligands CD3 and CD20 on the Alto™ System (Nicoya), which conducts SPR measurements.

FIGURE 6.

Comparative Analysis of Binding Kinetics

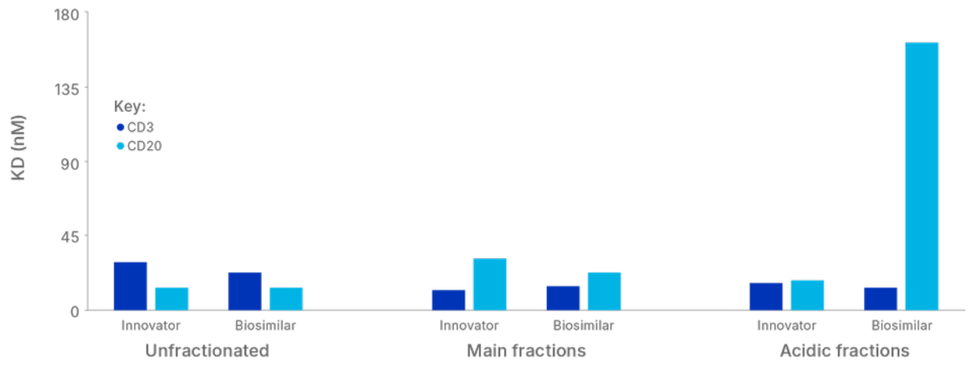


Figure 6. A comparative analysis of binding kinetics (KD) between different charge variant fractions and the CD3 epsilon and CD20 antigens. Data are shown for unfractionated, main fractions, and acidic fractions of innovator and biosimilar samples binding with each antigen.

Conclusion

Charge variants in antibodies can lead to reduced binding affinity and potency, making thorough characterization essential for product quality. This study demonstrates a streamlined workflow combining icIEF-based fractionation with SPR analysis to evaluate charge variants. Using MauriceFlex and Alto, high-purity fractions of Mosunetuzumab and a biosimilar revealed notable binding difference, particularly in the biosimilar's acidic species. These results highlight the value of advanced analytical techniques for ensuring biotherapeutic quality and consistency.

Assessing Changes in Binding Affinity of Biomolecular Charge Variants with icIEF Fractionation & SPR

This webinar and appllication note discuss how icIEF and SPR combine to uncover critical charge heterogeneity and binding differences in therapeutic antibodies.

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bio-techne.com/wbr-spr-mauriceflex

Read App Note
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rndsystems.com/an-spr

From Your Peers: Quality Assistance: Utilizing Maurice Platforms to Help Companies Bring New Biotherapeutics to Patients in Need

Quality Assistance, a Belgium-based contract research organization, provides GMP- and GLP-compliant analytical services from the end of the preclinical phase through marketing in CMC and bioanalysis. Specializing in innovative products, the company supports clients in bringing therapies to market for patients with unmet medical needs, prioritizing patient well-being in all its work. We spoke with Dr. Arnaud Delobel, who is the Director of R&D and Innovation at Quality Assistance, to learn about how Maurice platforms are helping the team achieve their goals. Here is an excerpt from the From Your Peers interview:

Arnaud Delobel, PhD,
Director, R&D and Innovation, Quality Assistance

> "For charge variant analysis, we have largely replaced ion exchange chromatography with the icIEF on the Maurice system.... Once we brought it in-house, it was the ideal platform for us - the icIEF fractionation capability enabled us to get further insights into the charge variants.
>
> **- Arnaud Delobel, PhD,**
> Director, R&D and Innovation, Quality Assistance

What are the new trends/therapeutic modalities that you are most excited about?

We work a lot on Biologics, primarily on monoclonal antibodies (mAbs). However, we are working more and more on antibody drug conjugates (ADCs), which have more challenges in terms of analytical characterization. The icIEF profile of an ADC is even more complex than that of a regular mAb. We also see a lot of bispecific antibodies as well as AAVs. AAVs today are quite tricky because there are a lot of safety issues. Many labs are still developing AAVs for rare diseases, and the characterization of AAVs is very complex and can be done at different levels: at the intact capsid level to quantify the empty/full particles, for example, but also at the VP protein level, to get separation between VP1, VP2, VP3, and their potential impurities. We work a lot on these kinds of modalities as well as mRNA and lipid nanoparticles. The Maurice and MauriceFlex systems help us in this field.

What drove your decision to bring the Maurice/MauriceFlex systems in-house?

For charge variant analysis, we have largely replaced ion exchange chromatography with the icIEF on the Maurice system. The issue we had for quite some time was the ability to identify the charge variants that we detected using icIEF. Before the launch of the MauriceFlex, we had to switch to ion exchange chromatography and collect the fractions or couple it directly with MS. Once we brought it in-house, it was the ideal platform for us - the icIEF fractionation capability enabled us to get further insights into the charge variants. For these charge variant fractions, we mainly use intact mass spectrometry analysis to obtain good identification of the peaks, but if we need further characterization, we do peptide mapping using LC-MS. The latter may require us to collect and pool fractions from multiple runs to obtain the required sensitivity to get more information on the post-translational modification, such as deamidation or oxidation. In addition, we also use the Maurice CE-SDS capabilities for protein size analysis.

Read Full Interview
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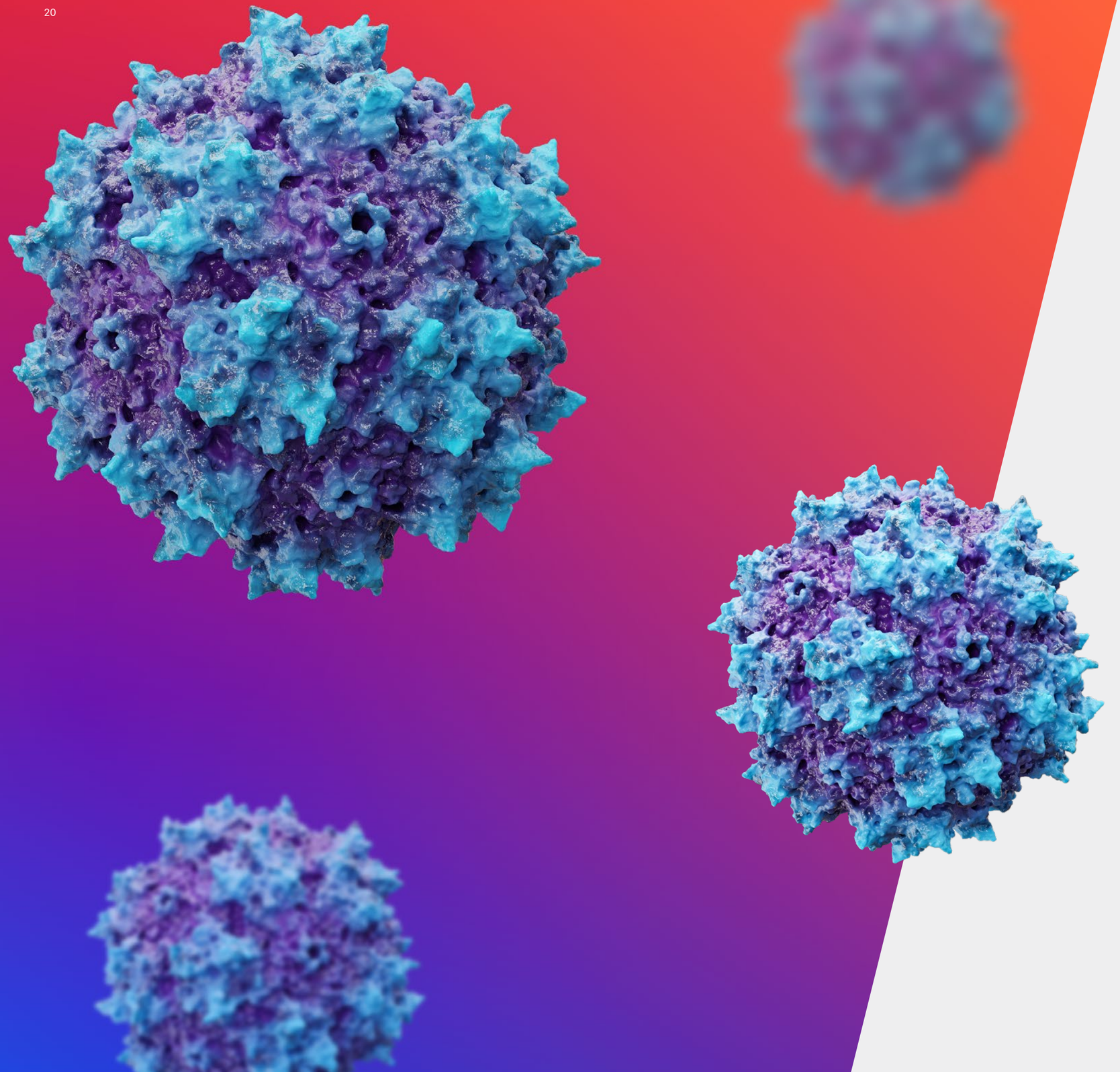
bio-techne.com/fyp-qa-maurice



AAV Viral Vectors

An AAV vector is a modified adeno-associated virus (AAV) used in gene therapy to efficiently deliver its therapeutic gene payload without integrating into the host's DNA. These vectors were developed by removing all viral genes from the virus, leaving only the essential inverted terminal repeats (ITRs) to hold the therapeutic DNA. Once inside the cell, the gene can be transcribed and translated, allowing the cell to produce a therapeutic protein or RNA to correct a genetic defect.

Some commercially available AAV-based gene therapies are Luxturna (voretigene neparvovec-rzyl) for Leber congenital amaurosis (LCA), Zolgensma (onasemnogene abeparvovec-xioi) for spinal muscular atrophy (SMA), Hemgenix (etranacogene dezaparvovec) for hemophilia B, and Roctavian (valoctocogene roxaparvovec) for hemophilia A.

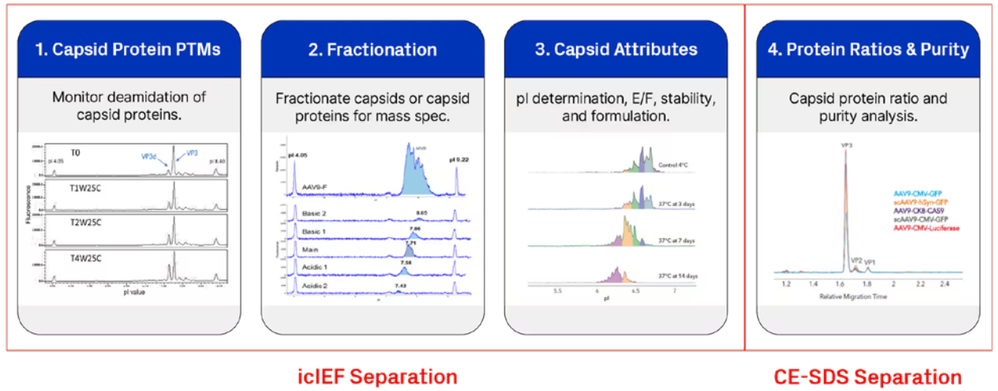
Advantages of the MauriceFlex System for Analyzing AAVs

- ✓ icIEF, CE-SDS, and icIEF fraction collection capabilities, all in one system.
- ✓ Analysis of capsid protein deamidation, providing insights into AAV potency.
- ✓ Analysis of other Critical Quality Attributes, including identity, purity, capsid protein ratio, and capsid content (empty full).

> “While a quantitative peptide map or MAM, as in the data we presented, is a powerful tool to monitor capsid deamidation, a more rapid and suitably GMP-compliant mehtod benefits routine product testing.
>
> **- Xiaoping He et al., 2023, in reference to icIEF for AAV**

FIGURE 7.

MauriceFlex System Advantages



Maurice CE-SDS for AAV Analysis

CE-SDS on the Maurice/MauriceFlex system can be easily implemented across the AAV development pipeline. Two cartridges, the Turbo CE-SDS and CE-SDS PLUS, are both designed to generate high quality data that provides insights into AAV identity, purity, and capsid ratios. The Turbo CE-SDS cartridge is for high throughput applications and provides data in 5.5-8 minutes, while the CE-SDS PLUS provides superior resolution in 25-35 minutes, making it suitable for QC applications. A summary of how the two cartridges compare for the analysis of AAVs is provided below.

Results

FIGURE 8A.

Turbo CE-SDS

FIGURE 8B.

CE-SDS Plus

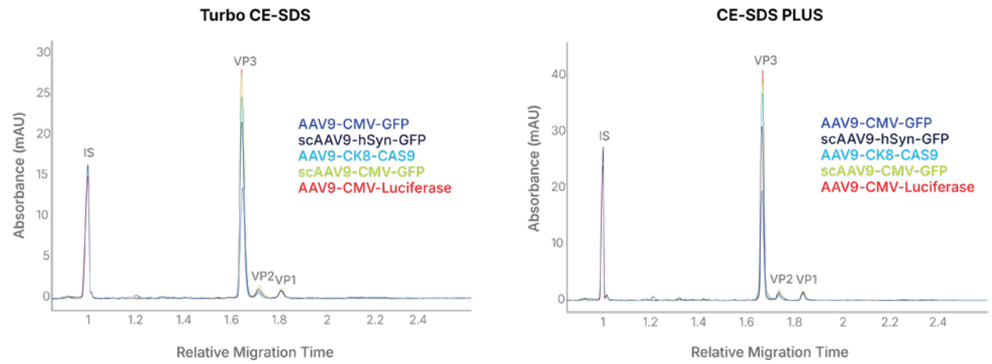


Figure 8. Measuring the capsid protein ratio of AAVs with Maurice CE-SDS. Five AAV samples with different transgenes were analyzed with A. Turbo CE-SDS and B. CE-SDS PLUS. Different sample profiles are clearly captured by both methods, from which the respective capsid protein ratios were measured.

TABLE 1.

Capsid Protein Ratios for Five Different AAV Samples Determined with Maurice Turbo CE-SDS & CE-SDS PLUS. VP1 Not Listed as Ratios Presented are Set with VP1 = 1

Capsid Protein Ratios

	VP3		VP2	
	PLUS	TURBO	PLUS	TURBO
AAV9-CMV-GFP	16.09	15.10	0.87	0.84
scAAV9-hSyn-GFP	20.73	17.96	1.49	1.34
AAV9-CK8-CAS9	27.31	27.28	1.40	1.36
scAAV9-CMV-GFP	21.40	21.01	1.64	1.80
AAV9-CMV-Luciferase	26.98	26.22	1.40	1.30

Conclusion

Fast, reliable analytics are critical for viral vector development. This study demonstrates how Maurice CE-SDS enables two complementary assays—Turbo CE-SDS and CE-SDS PLUS—for rapid, reproducible characterization of AAV identity, purity, capsid ratios, and impurities. Both methods delivered consistent results across serotypes and stages of development. With automation, easy cartridge switching, and icIEF capability on the same platform, Maurice streamlines workflows, reduces time and cost, and maximizes value for viral vector analysis.

Assessing AAV Product Quality

icIEF on the Maurice system provides a powerful approach for AAV characterization. This technique enables rapid, reproducible profiling of empty, intermediate, and full capsids under native and denatured conditions. Leveraging dual detection modes of absorbance and native fluorescence, icIEF delivers critical insights into capsid DNA content and charge heterogeneity, helping assess product quality and stability with minimal sample and short run times. icIEF also serves as a stability-indicating assay, thus providing valuable information on the potency and efficacy of these viral vectors.

Results

FIGURE 9A.

UV Absorbance

FIGURE 9B.

Native Fluorescence

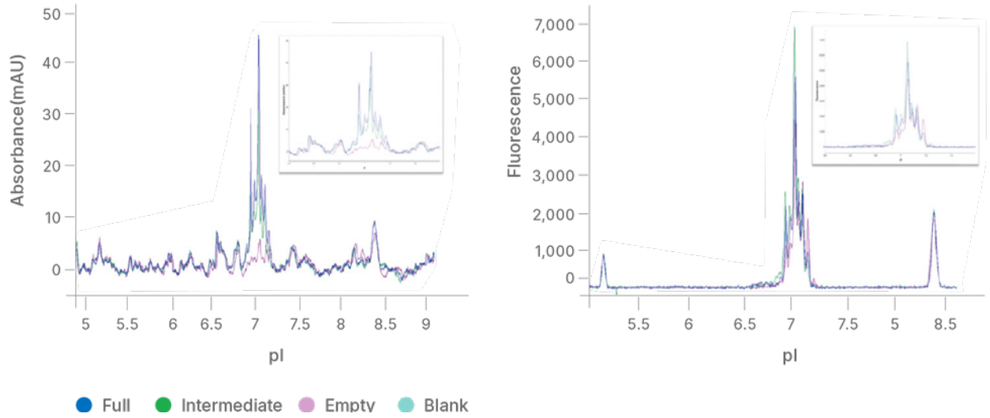


Figure 9. Comparison of UV absorbance (A) and native fluorescence (B) for empty, intermediate, and full AAV8 samples. Significantly lower absorbance is observed in the empty AAV sample (purple). All three samples have similar native fluorescence signals.

Technical Tips: Optimizing AAV Analysis Using Maurice Platforms

Dr. Monica Tyagi discusses key steps for method development and optimization of AAV analysis using icIEF, icIEF-fractionation and CE-SDS methods on Maurice Systems.

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FIGURE 10.

Results from Chemical Stress Studies

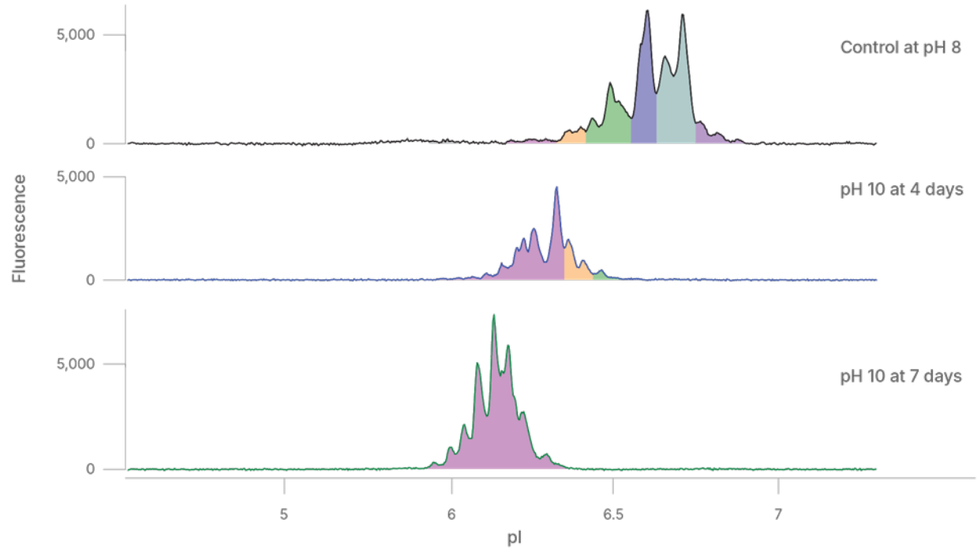

Figure 10. Incubation at high pH under 37°C induces significant changes in AAV8 DS. The AAV8 DS sample was exchanged into a pH 10 buffer and incubated for either 4 days or 7 days prior to analysis using the intact method, resulting in a dramatic increase in acidic variants.

Conclusion

icIEF on the Maurice system enables the analysis of several AAV CQAs. High resolution charge separation reveals shifts in acidic, main, and basic peaks that point to the identity, post translational modifications, and overall stability of the molecule. Furthermore, because DNA does absorb significant UV energy at 280 nm (although its ideal UV absorbance is 260 nm), empty, partially full, and full AAV capsids can be detected through icIEF using the absorbance mode. Because of its versatile applications for AAV analysis, the Maurice system is a highly useful analytical platform for gene therapy development workflows.

From Your Peers:
The Benefits of Maurice for AAV Characterization

What is Viralgen's mission, and how is its work impacting human health?

Viralgen is a CDMO specializing in the manufacture of gene therapy products for the treatment of rare diseases such as Juvenile Parkinson's or SPG50. Our mission is to support our clients from the developmental stage of the project to commercial manufacturing of gene therapies, ensuring that the highest quality standards are met under GMP conditions.

What is your role at Viralgen?

I work as an Analytical Development Scientist. My job consists of developing new analytical techniques to characterize our viral vector products and to ensure that they meet the GMP standards. It's important to mention that for analytical development under GMP conditions, a method needs to be robust, highly reproducible, and meet several requirements in terms of linearity, accuracy, and precision.

How does the Maurice instrument fit into your daily work?

The Maurice instrument is a really versatile and user-friendly platform. Currently, we use CE-SDS to perform a purity assessment of our drug products, and we have also developed a method to detect the impurities that may appear in our biotherapeutic, to determine if its source is protein or DNA. Recently, we have started developing icIEF methods to monitor the stability of our samples over time, as well as to assess if our capsids have developed post translational modifications.

We are using icIEF to assess the identity of our samples. AAVs consist of several stereotypes from 1 to 10, and each of them has different characteristics and target tissues. It's very convenient in gene therapy manufacturing to use a simple icIEF method to distinguish between the stereotypes and unequivocally determine the serotype of our sample.

Dr. Ana Carreras González
Analytical Development Department, Viralgen

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AAV Characterization in Gene Therapy Manufacturing

This webinar discusses how CE-SDS and icIEF on Maurice enable rAAV purity, impurity detection, and batch stability analysis to meet GMP standards.

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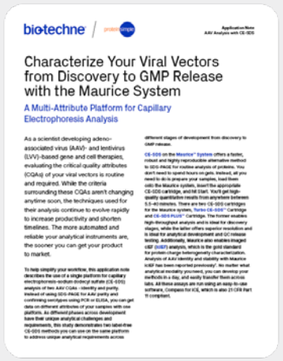

Characterize Your Viral Vectors from Discovery to GMP Release with the Maurice System

This application note illustrates how Turbo CE-SDS and CE-SDS PLUS assays can help characterize your AAVs.

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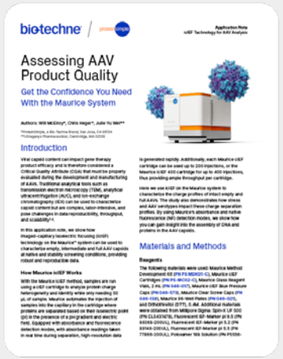

Assessing Your AAV Product Quality? Get the Confidence You Need With Maurice

This application note describes icIEF methods for assessing the stability and capsid content of AAVs.

Explore AAV App Note
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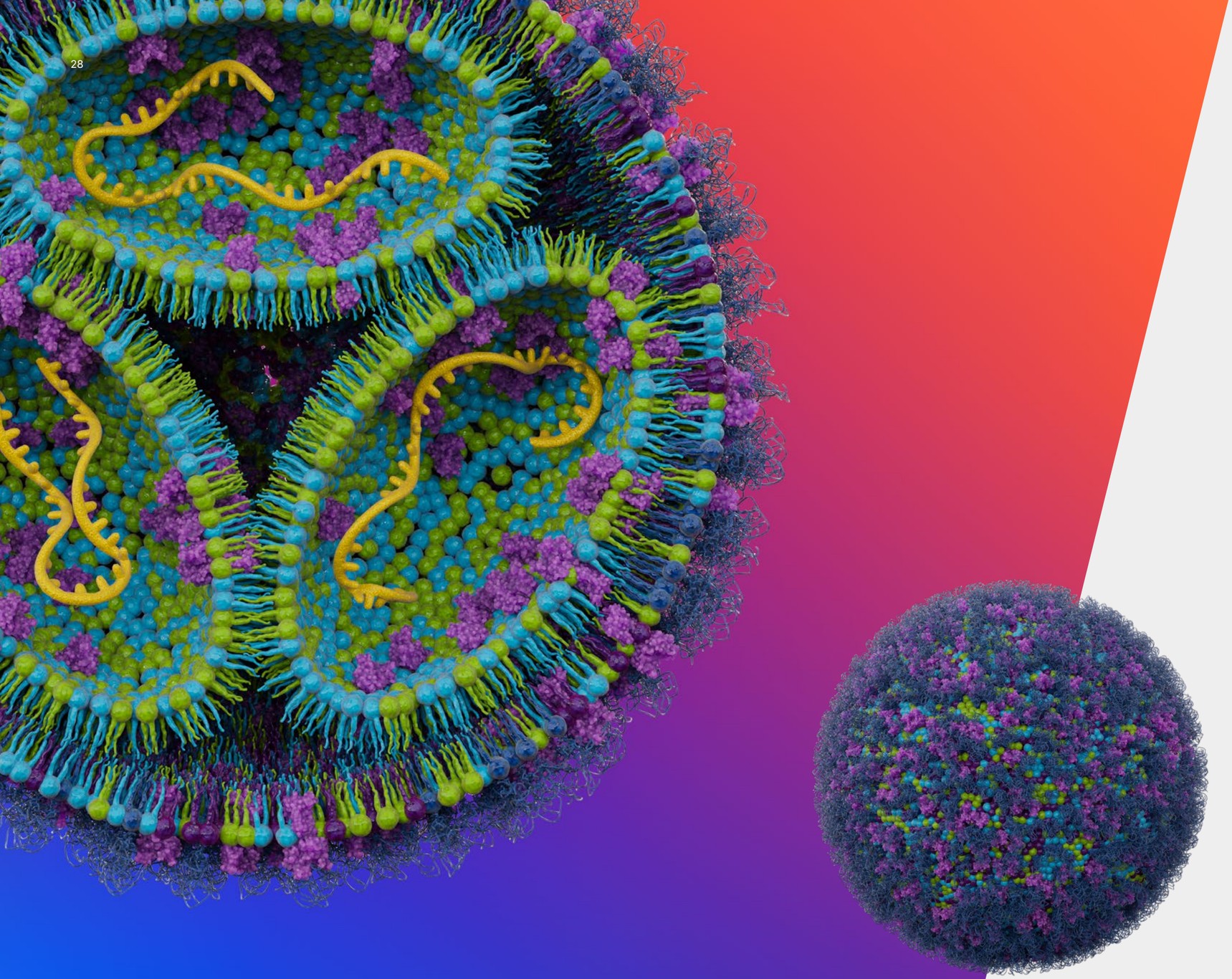


mRNA LNPs

Another class of modern drugs that has enormous therapeutic potential is messenger RNA, more commonly known as mRNA. mRNAs are natural single-stranded ribonucleic acids produced in the human body during transcription, and their main role is to facilitate protein synthesis by providing critical genetic coding information. Understanding this process inspired research in the field of medicine, exploring its potential in delivering therapeutic proteins to host cells upon transfection. There are several advantages of mRNA drugs, including better safety and their ability to trigger long-term protein expression.

A long-standing challenge of mRNA therapeutics has been their successful delivery into host cells, which is where LNPs, or lipid nanoparticles, made a foray. As drug delivery agents, LNPs offer specific advantages to mRNAs, such as protection from degradation by host enzymes, increased bioavailability, and targeted delivery. The most notable mRNA therapeutics in LNPs have been the two commercially available COVID-19 vaccines by Pfizer and Moderna, with over 235 mRNA drug candidates under development.

LNP Analysis with the Maurice System

- Characterize the stability of mRNA-loaded LNPs for various storage times at different temperatures and freeze–thaw cycles
- Distinguish lipid compositions to help measure batch-to-batch variability.
- Detect changes in surface charge heterogeneity.
- Analyze packaging efficiency with packaged mRNA detection and quantitation.

FIGURE 11A.

Absorption

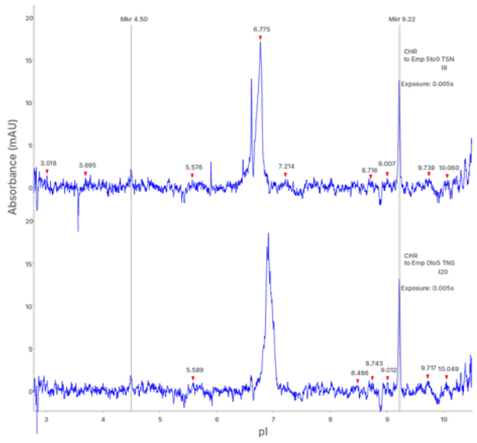


FIGURE 11B.

TNS Dye

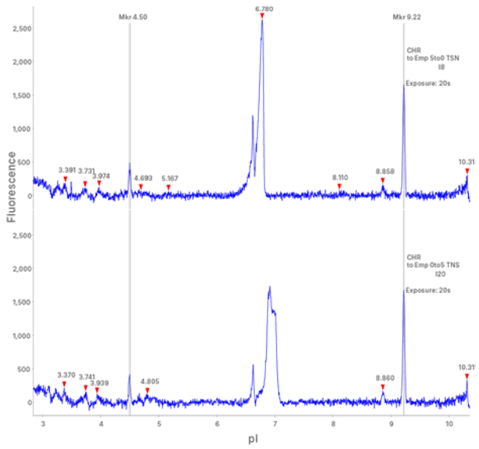


FIGURE 11C.

RNA Dye

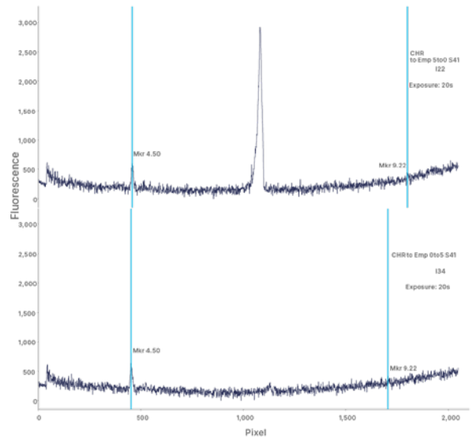
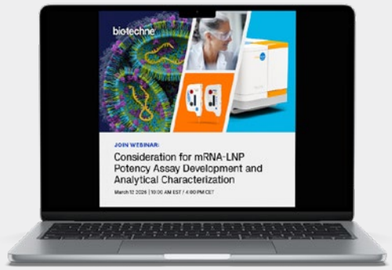


Figure 11. Comparison of lipid nanoparticles (LNPs) with and without packaged mCherry mRNA. The top panel shows LNPs containing mCherry mRNA, while the bottom panel shows empty LNPs without mRNA. Differences between packaged and unpackaged particles are visualized using both absorbance and native fluorescence detection modes.

Conclusion

Maurice provides a powerful platform for LNP analysis by enabling direct detection of packaged mRNA, assessing packaging efficiency, and monitoring surface charge heterogeneity. Its dual detection capabilities allow differentiation between loaded and empty particles, evaluation of lipid composition, and stability analysis under various conditions, making it an ideal solution for ensuring quality and consistency in mRNA-LNP therapeutics.



Considerations for mRNA-LNP Potency Assay Development for Gene Editing Products

This webinar discusses key considerations for potency assay development and analytical characterization of mRNA-LNPs for gene editing products.

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Explore our recently updated brochure to learn more about the Maurice family of instruments, including the powerful MauriceFlex, a one-stop system for automated cIEF and CE-SDS.

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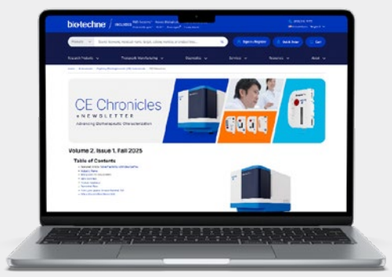

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Read our quarterly CE Chronicles eNewsletter to get in-depth access to the latest resources related to biotherapeutic charge and size characterization CE. Each issue has a focused theme and related content, including AAV analysis, CE-SDS, and more!

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Peer-Reviewed Publications

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1. Liu, F., Song, W., Jia, S., Wang, G., Zhang, X., Lyu, C., Xing, D., Qu, Y., Liu, B., Yu, R., Li, S., Gao, R., & Zhang, J. (2026). Insight on photosensitivity of bispecific antibody-drug conjugates: ROS-mediated degradation and payload conjugation-induced structure alterations. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences, 216, 107374. **https://doi.org/10.1016/j.ejps.2025.107374**

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6. Wen, X., Liu, A. P., Song, J., Leng, C., Wang, J., Russo, B., Thiagarajan, G., Wang, H., Dow, X. Y., Hua, X., Ao, X., Mittal, S., Gennaro, L., & Gunawan, R. (2025). Enzymatic Desialylation Enables Reliable Charge Variant Characterization of Highly Glycosylated and Sialylated Fc Fusion Proteins. ACS pharmacology & translational science, 8(2), 394–408. **https://doi.org/10.1021/acsptsci.4c00460**

7. Wang, L., Wu, H., Cao, T., Li, H., Shen, P., Lu, L., & Zhang, Z. (2024). Identification of structural origins of complex charge heterogeneity in therapeutic ACE2Fc fusion protein facilitated by free-flow isoelectric focusing. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V, 198, 114248. **https://doi.org/10.1016/j.ejpb.2024.114248**

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8. Reyda, M. R., Ji, Q., Huang, M., Sokolowska, I., Yan, Q., Mulholland, J., Mo, J., & Hu, P. (2026). Systematic characterization of lysine glucuronidation in a bispecific antibody. mAbs, 18(1), 2612471. **https://doi.org/10.1080/19420862.2025.2612471**

9. Chen, H., Ji, N., Zhou, M., & Bu, Y. (2026). Study on the hole and knob related impurities in the purification of bispecific antibodies. Protein expression and purification, 238, 106836. **https://doi.org/10.1016/j.pep.2025.106836**

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AAV Peer-Reviewed Publications

12. Hoyle, B., Hill, J., & Bello, D. (2025). Quantifying the Full-to-Empty Adeno-Associated Virus (AAV) Capsid Ratios and Their Impact on Transduction Efficiency in vitro. The AAPS journal, 28(1), 36. **https://doi.org/10.1208/s12248-025-01194-8**

13. McElroy, W., Huang, S., He, X., Zhou, C., Heger, C. D., Powers, T. W., Anderson, M. M., Sloan, C., & Lerch, T. F. (2025). Enabling icIEF Peak Identification of AAV Capsid Proteins by Fractionation on MauriceFlex and Subsequent Analysis by LC-MS. Electrophoresis, 46(1-2), 22–33. **https://doi.org/10.1002/elps.202400201**

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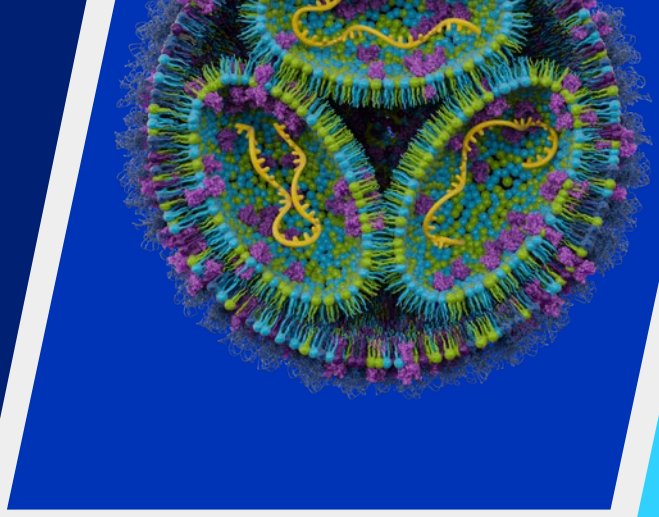
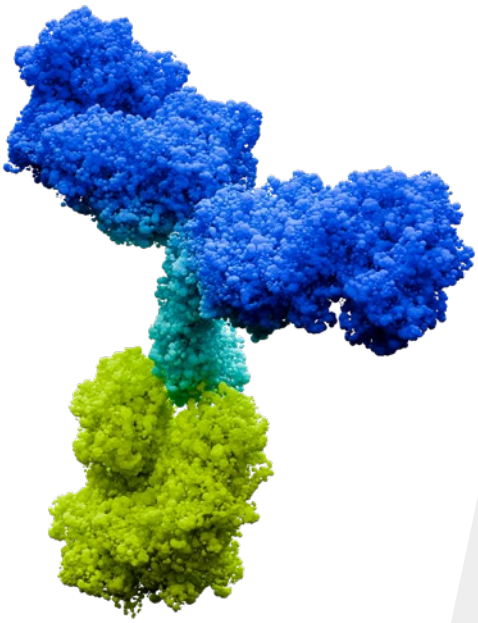
mRNA LNPs Peer-Reviewed Publications

16. Krebs, F., Burger, U., Dörks, S., Kramer, M., & Wätzig, H. (2022). Two quality and stability indicating imaged CIEF methods for mRNA vaccines. Electrophoresis, 43(20), 1971–1983. **https://doi.org/10.1002/elps.202200123**

17. Loughney, J. W., Minsker, K., Ha, S., & Rustandi, R. R. (2019). Development of an imaged capillary isoelectric focusing method for characterizing the surface charge of mRNA lipid nanoparticle vaccines. Electrophoresis, 40(18-19), 2602–2609. **https://doi.org/10.1002/elps.201900063**





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