毛细管电泳 (CE)

—— 蛋白类药物应用文集



SCIEX

The power of precision

毛细管电泳（Capillary Electrophoresis, CE）作为一种高分辨率、高灵敏度的分离分析技术，近年来在生物药分析领域得到广泛应用。随着生物制药行业的快速发展，特别是单克隆抗体（mAb）、抗体偶联药物（ADC）、疫苗产品以及细胞与基因治疗（CGT）等创新药物的不断涌现，对质量控制技术提出了更高要求。

生物药具有结构复杂、修饰类型多样、质量属性高度关联等特点，传统分析手段在部分关键质量属性（CQA）的表征与监控中仍面临分辨率、通量或方法适配性的挑战。毛细管电泳技术凭借高分辨率、高灵敏度、自动化程度高及方法重复性良好等优势，已在生物药研发、工艺表征和质量控制中发挥重要作用，尤其适用于以下分析方向：

- 纯度与片段分析（CGE / CE-SDS），用于完整性、降解片段及杂质水平评估；
- 电荷异质性分析（cIEF），用于主峰、酸性峰和碱性峰等电荷变异体的分离与定量；
- 分子大小与聚集体相关分析（CZE 等），用于分离不同组分并辅助评估样品均一性；
- 核酸完整性及片段分析，用于 mRNA、寡核苷酸及其他核酸类样品的长度分布和完整性评价；
- 病毒载体结构分析（如 AAV、慢病毒），用于衣壳蛋白组成、核酸载荷及相关质量属性研究。

SCIEX 提供的毛细管电泳平台（如 PA800 Plus、BioPhase 8800 及 Intabio ZT）已在研发与质量控制（QC）环节广泛应用。

本应用文集基于近年行业发展趋势，筛选生物药领域中高市场需求、高技术价值的热点应用场景，按照应用类型进行系统整理，旨在为用户提供全面的应用参考及解决方案思路。

蛋白类药物

毛细管电泳在蛋白类药物分析中处于核心地位，尤其在电荷变异体分析和纯度控制方面，已成为行业标准方法之一。药典及 ICH 指南均明确要求蛋白药物需进行电荷异质性分析，CE 技术凭借高分辨率和定量能力，为抗体药物开发与放行提供关键支持。

目录

1. Multi-capillary gel electrophoresis to increase throughput for the purity analysis of biotherapeutic proteins 5
2. Enhancing purity and heterogeneity assessment: Seamless method transfer from PA 800 Plus system to BioPhase 8800 system 11
3. Unleashing the Power of Speed: Accelerating time to answer with CE-SDS and Laser-Induced Fluorescence detection in under 12 Minutes 16
4. Efficient transfer of the high-speed CE-SDS method from the PA 800 Plus system to the BioPhase 8800 system ... 23
5. BioPhase 8800 系统进行高通量 CE-SDS 方法学验证 28
6. A comparison between IgG HR CE-SDS on the PA 800 Plus system and the equivalent assay on the BioPhase 8800 system. 32
7. Using dithiothreitol as reducing agent in CE-SDS analysis with Native Fluorescence detection 39
8. Parallel charge variant analysis of conventional and new modality therapeutic proteins by multi-capillary isoelectric focusing. 43
9. BioPhase™ 8800 系统进行 EPO 电荷变异体的高通量分析 48
10. High-throughput released N-glycan identification using CE and a customizable glycan library 51
11. N-linked glycan analysis of NISTmAb, etanercept and trastuzumab 56
12. 毛细管区带电泳（CZE）对单克隆抗体的电荷异质性分析 61
13. 毛细管区带电泳用于半胱氨酸偶联 ADC 样品的电荷异质性分析 68

14. Rapid charge variant analysis of NANOBODY® using capillary zone electrophoresis (CZE) 71

15. Capillary zone electrophoresis (CZE) separation of charge isoforms of acidic NANOBODY® samples 74

16. Analysis of co-formulated monoclonal antibodies by capillary zone electrophoresis 78

17. Rapid charge heterogeneity analysis of new therapeutic protein modalities 83

18. 利用毛细管区带电泳和高分辨质谱技术对小核酸抗体偶联药物 (AOC) 各组分的分离及结构确认 87

19. 高通量 BioPhase™ 8800 系统对抗体偶联药物的纯度和电荷异质性分析 91

20. 十六烷基磺酸钠 - 毛细管凝胶电泳法用于双抗的纯度分析 96

21. CE-SDS 方法对多肽药物的纯度分析 101

22. Subunit analysis of NISTmAb by capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) and capillary isoelectric focusing (cIEF) 103

23. 毛细管区带电泳方法进行生长激素（GH）电荷异构体的分析 108

24. 毛细管电泳法用于重组人血清白蛋白分子大小变异体和等电点分析 110

25. 毛细管等电聚焦电泳对胰岛素类产品的等电点分析 113

26. 高灵敏度 CE-SDS-LIF 方法用于 T7 RNA 聚合酶纯度分析 116

27. CE-SDS-LIF 方法对单抗药物的纯度分析（5-TAMRA 标记） 119

Multi-capillary gel electrophoresis to increase throughput for the purity analysis of biotherapeutic proteins

Theresa Merlino, Robert Yocum, Liming Zhao, Christian Milbrandt, Neil Hershey, and Colleen Oakes
Janssen, USA

This technical note demonstrates a high throughput purity analysis utilizing the multi-capillary BioPhase 8800 system as an alternative to PA 800 Plus for early-phase CE-SDS testing to accommodate the increasing analytical demands. In the biotherapeutic life-cycle, the process analytical sciences (PAS) group at Janssen is at the beginning of the development process of new biotherapeutics and receives test samples from both upstream and downstream API (active pharmaceutical ingredient) development groups as well as the formulations development group (Figure 1). Per year, approximately 40,000 samples come to PAS for various analyses. The upstream samples come from process optimization for cell growth, such as bioreactor optimization. The downstream samples come from protein purification development. The drug product development group conducts experiments and sends samples for buffer formulation, stability, and degradation studies.

Key features of the automated CE workflows

- **Time to answer:** the pre-injection conditioning times in the CE-SDS lightning method are 60% shorter compared to the traditional method, reducing the analysis time of a 96-well plate to only 10 hours whereas the traditional method would run a plate in 12 hours
- **Comparable performance to legendary assay:** the purity assessment of multiple molecule types was similar between the PA 800 Plus system and the BioPhase 8800 system
- **Ideal linearity:** An R^2 of 0.99 was achieved using 5 concentration points for all 3 compounds analyzed
- **Equivalency:** The BioPhase 8800 system was deemed equivalent analytical platform to the PA 800 Plus system to the traditional method

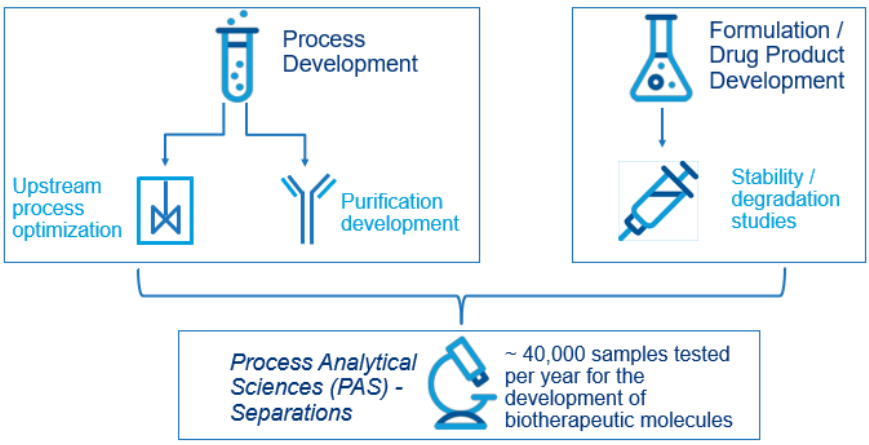


Figure 1. Diagram showing the relationship between process development in upstream and downstream API group, formulation and drug product development group, and the process analytical sciences – separations group

Introduction

Out of the 40,000 samples, over 11,000 are analyzed using capillary electrophoresis sodium dodecyl sulfate (CE-SDS) assay alone. Therefore, quality-driven, high throughput techniques are critical for developing new drugs, enabling scientists to make quick and informed decisions based on accurate data to meet demanding timelines.

CE-SDS is the most common assay to determine the purity and fragmentation of biotherapeutic proteins in drug development, with over 11,000 samples analyzed per year. In the past, the PA 800 Plus system, a single capillary platform, has been the instrument of choice. However, it takes approximately 24 hours to analyse 24 reduced or non-reduced samples. Consequently, smaller batch analysis and longer overall cycle times on PA 800 Plus system leads to limited instrument availability and longer turnover time to get data to customers, thus becoming a bottleneck in the development process. Therefore, the multi-capillary BioPhase 8800 system was evaluated as an alternative to the PA 800 Plus system for early-phase CE-SDS testing to accommodate the increasing high throughput demands.

Methods

Samples: The process development samples, purified monoclonal (mAb), bispecific and trispecific antibodies, and a vaccine product are proprietary samples of Janssen.

Sample preparation: The purified protein samples were analyzed under reduced and non-reducing conditions according to the protocol described elsewhere.[1,2] The bioprocessing samples were diluted in water to a target concentration and then combined with SDS buffer, 10 kDa standard, and n-ethylmaleimide (NEM) for Non-Reduced (NR) conditions or β-mercaptoethanol (BME) for Reduced (R) conditions and then heat denatured for a defined time and temperature.

CE-SDS reagents: BioPhase CE-SDS Protein Analysis Kit (PN C30085) was from SCIEX (Framingham, MA).

The β-mercaptoethanol (Part # M3148-25ML) and N-Ethylmaleimide (34115-M) were from Sigma Aldrich (St. Louis, MO, USA).

Capillary cartridges: CE-SDS analysis was performed using a BFS capillary cartridge – 8 x 30 cm (P/N: 5080121) on the BioPhase 8800 system and the pre-assembled cartridge (P/N A11147) on PA 800 Plus system, both from SCIEX (Framingham, MA).

Capillary electrophoresis instrument and consumables: The BioPhase 8800 system (P/N: 5083590F) and a starter kit of 4 sample plates, 4 reagent plates and 8 outlet plates (P/N: 5080311) were from SCIEX. CE-SDS analysis was performed using UV detection with 220 nm filters.

The PA 800 Plus system (P/N: A74603) was equipped with a PDA detector. Universal vials (P/N: A62251) and caps (P/N: A62250) were from SCIEX (Framingham, MA).

Software: BioPhase 8800 software, version 1.2 e-license was used to create methods and sequences for data acquisition. The data was converted using the data converting tool and analyzed using Empower™ software.

Evaluation of BioPhase 8800 system for early development

4 conditions were studied to assess the performance equivalence between the BioPhase 8800 system and the PA 800 Plus system for reduced and non-reduced CE-SDS samples:

- Comparison of purity assessment across multiple modalities
- Reproducibility and consistency assessment between the 8 capillaries on the BioPhase 8800 cartridge
- Linear dynamic range across multiple protein concentrations
- Comparison of purity assessment for various process development samples

Percentage purity assessment comparison across multiple modalities

A purified mAb molecule, bispecific, trispecific and a vaccine product were separated using the PA 800 Plus system and the BioPhase 8800 systems for the purity assessment. On the BioPhase 8800 system, 2 separation methods were tested: the "traditional" and lighting methods. The significant difference between "traditional" and lightning CE-SDS methods is the pre-injection capillary conditioning steps (Table 1).

Table 1: Time frame for the pre-injection capillary conditioning steps in this study's traditional and lighting methods

Pre-injection capillary conditioning steps:	
Traditional	Lightning
Total time: 25 minutes	Total time: 10 minutes
Base: 80 PSI, 2 min	Base: 80 PSI, 3 min
Base: 20 PSI, 5 min	
Acid: 20 PSI, 5 min	Acid: 50 PSI, 2 min
Water: 20 PSI, 3 min	Water: 50 PSI, 1 min
Gel: 80 PSI, 10 min	Gel: 80 PSI, 4 min

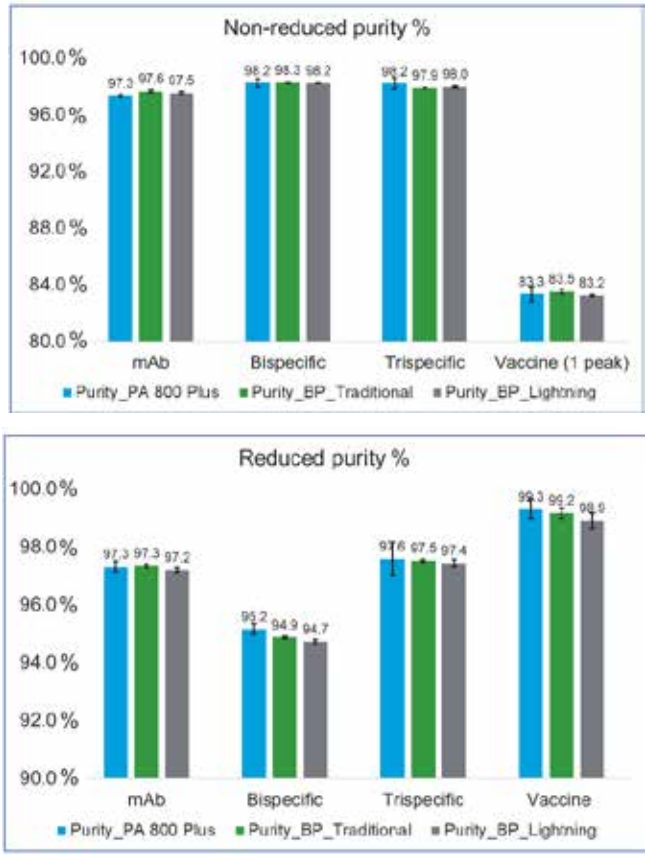


Figure2: Purity percentage comparison assessment between PA 800 Plus and the BioPhase 8800 system of multiple molecule types under non-reduced (top) and reduced (bottom) conditions.

Figure 2 shows a remarkable similarity of purity values across different molecule types. The error bars on the PA 800 Plus represent the standard deviation of n=6 runs across multiple instruments and analyst preparations. The error bars on the BioPhase 8000 system (BP) using traditional method represent the standard deviation of n=6 within a single sequence run. The error bars on the BP using lightning method also represents the standard deviation of n=6 within a single sequence run.

Purity comparison between the two platforms was found to be well within the benchmark set at Janssen.

Reproducibility and consistency assessment between the 8 capillaries on the BioPhase 8800 cartridge

A bispecific mAb sample was reduced and separated using the BioPhase 8800 system. Figure 3 shows the intra-capillary reproducibility of the 8 CE-SDS separations obtained in capillaries A through H. Table 2 reveals the consistency between the capillaries in both total corrected peak area and purity percentage.

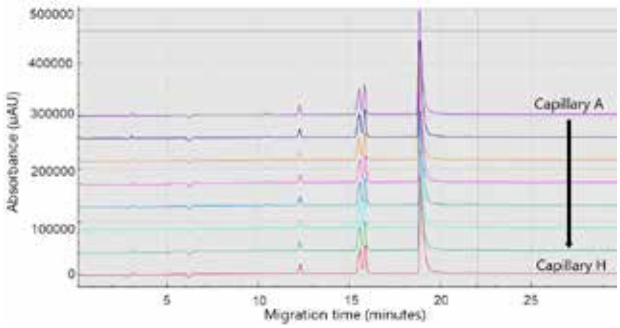


Figure 3: Overlay of 8 CE-SDS separations of a bispecific mAb under reducing conditions on the BioPhase 8800 system.

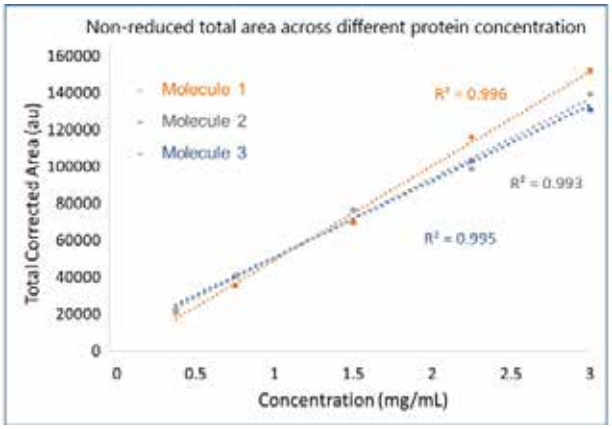


Figure 4. Linear curve of 3 molecules with different protein concentrations.

Table 2: Percentage of purity and total corrected peak area inter-capillary comparison on the BioPhase 8800 system.

Capillary	% Purity	Total Corrected Area
A	99.00	70536
B	98.94	67061
C	98.94	65531
D	98.87	65024
E	98.93	65769
F	98.98	69608
G	98.96	70238
H	98.94	67397

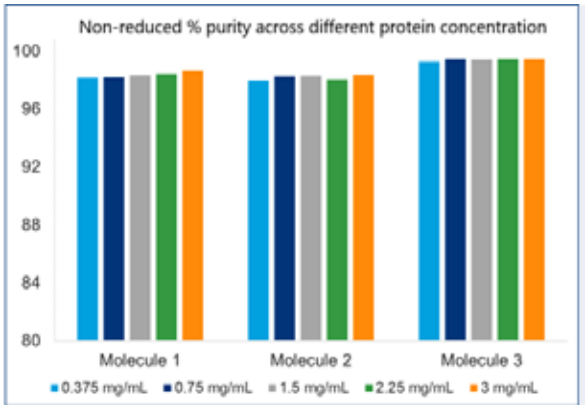


Figure 5: Consistency of percentage purity obtained for the 3 molecules studied at 5 different target concentrations.

Linear dynamic range across multiple proteins at different concentrations on the BioPhase 8800 system

Three molecules were investigated, ranging from 0.375 to 3 mg/mL, to determine the linear response of the BioPhase 8800 system. Figure 4 shows the response between the total corrected peak area as a function of final protein concentration injected in the instrument. The R^2 was > 0.99 for all 3 molecules, satisfying our requirements for linear response in the desired concentration range.

Figure 5 shows the consistency in the percentage purity obtained across the different protein concentrations for all 3 proteins demonstrating the consistency of the detector's response on the BioPhase 8800 system.

Comparison of purity for various process development samples

The process development sample type is the most challenging sample type to determine the equivalency between PA 800 and the BioPhase 8800 system. Process development samples vary significantly depending on the excipients used, different bioreactor conditions, and purification or formulation conditions.

Figure 6 shows a graph where the left Y-axis is the non-reduced percentage purity, and the right Y-axis is the difference in percentage purity of 21 process development samples. The green line shows the trend of the difference observed, and the red line across all graphs represents the passing threshold. The light blue

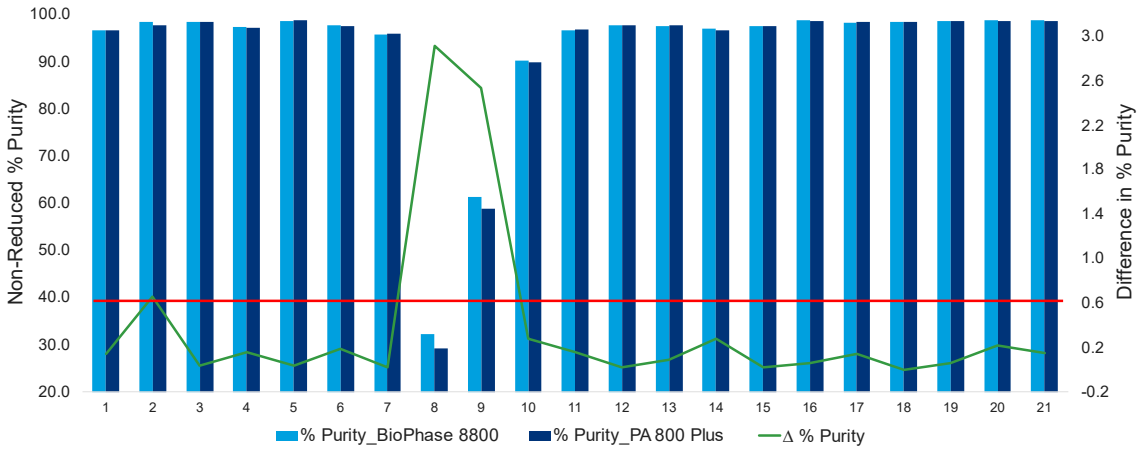


Figure 6: Twenty-One (21) Process development sample comparison between PA 800 Plus and the BioPhase 8800 systems. Light blue bars represent the purity values observed using the BioPhase 8800 and the navy blue represents the values obtained using the PA 800 Plus system.

bar is the data obtained using the BioPhase 8800 system, and the navy blue bar is the PA 800 Plus system.

Most samples were observed to be within the passing threshold margin set between the two systems. However, samples 8 and 9 were deemed outliers due to the level of degradation observed.

Based on the data shown in Figure 6 it was determined that the BioPhase 8800 system performance is equivalent to the PA 800 Plus system.

Direct comparison between electrophoretic profiles

Figure 7 directly compares the PA 800 Plus and BioPhase 8800 systems. The data is a separation of a traditional mAb sample under non-reduced conditions. The electropherogram on the top shows the full view of the separation and circled is the difference in peak height between the IgG peaks on both platforms.

The peak height on the BioPhase 8800 system is considerably higher compared to the PA 800 Plus. The difference in peak height and area is due to the innovative stray light control engineered into the BioPhase 8800 system, which increases the sensitivity of the system.

The zoomed-in view shows the overall peak shape for the smaller peaks to be similar. Even though the total corrected area counts differ, the relative percentage and peak shapes are similar between the 2 instruments.

Similarly, Figure 8 shows the separation of the same molecule under reduced conditions. It is notable that the peaks are more intense on the BioPhase 8800 system compared to the PA 800 Plus. Consequently, the ratio between the heavy and light chains differs between the 2 platforms, but since the overall purities remained similar, the results were determined to be equivalent.

Based on this extensive study, the BioPhase 8800 system was deemed a suitable alternative to PA 800 Plus for early-phase CE-SDS testing.

Conclusions

- The reduction in pre-injection conditioning time in the "Lightning" method decreases the cycle time by 60% compared to the “Traditional” method
- Comparison of multiple modalities measured similar purity results for all molecules investigated. The BioPhase 8800 system also demonstrated great inter-capillary reproducibility for profile and purity analysis
- Comparison between PA 800 Plus and BioPhase 8800 systems measured similar purity for 21 process development samples
- The R^2 for linearity of the BioPhase 8800 was better than 0.99% for the tested final protein

concentrations ranging from 0.375 mg/mL to 3.000 mg/mL

- The BioPhase8800 system was deemed equivalent and a suitable alternative to the PA 800 Plus system

References

1. IgG Purity and Heterogeneity Assay Kit for the PA 800 Plus Pharmaceutical Analysis System.
2. CE-SDS protein analysis kit for the BioPhase 8800 system.

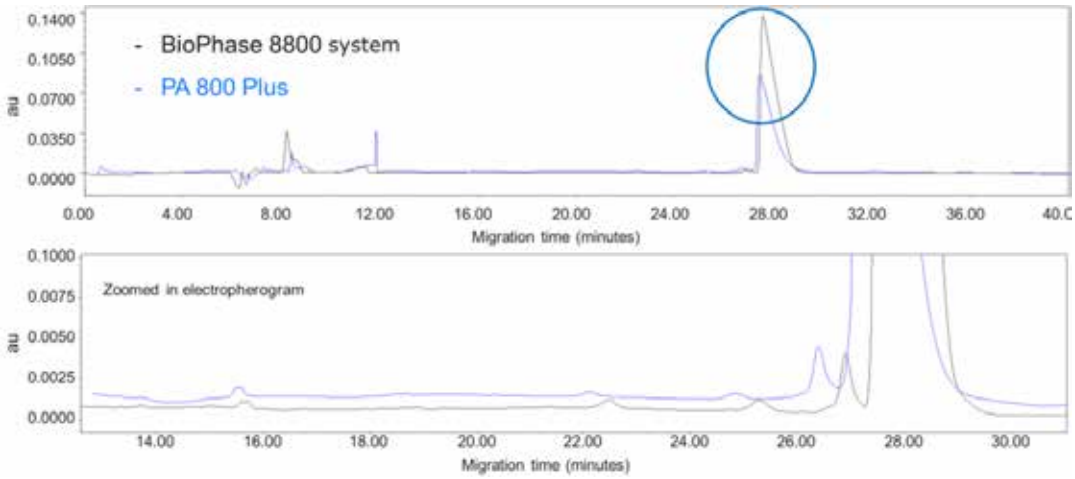


Figure 7. Direct comparison between PA 800 plus and the BioPhase 8800 systems of a mAb sample under non-reduced conditions

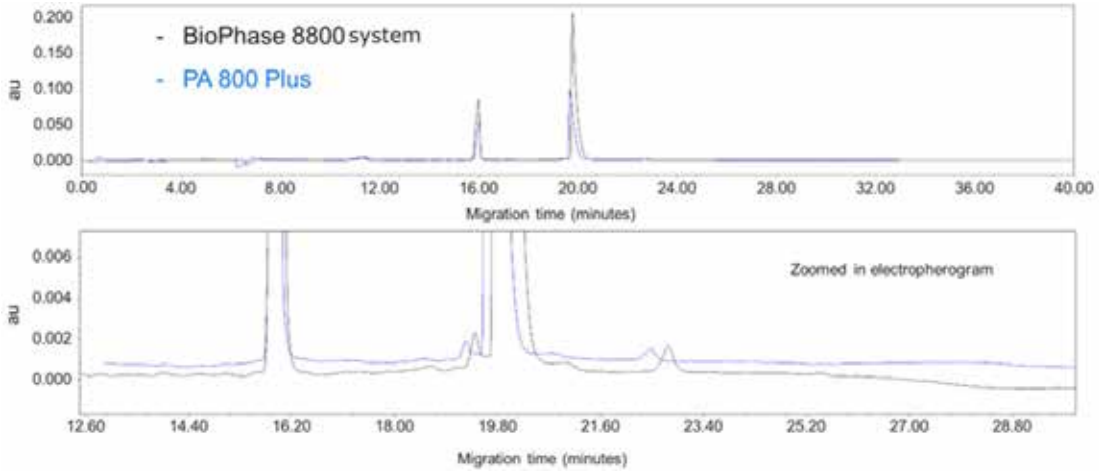


Figure 8. Direct comparison between PA 800 Plus and the BioPhase 8800 systems of a mAb sample under reduced conditions

Enhancing purity and heterogeneity assessment: Seamless method transfer from PA 800 Plus system to BioPhase 8800 system

Marcia Santos
SCIEX, USA

Analytical teams in drug development face increasing requests for higher throughput to keep up with growing drug pipelines. Adopting higher throughput technologies requires a successful transfer of methods for validated assays to new technologies.

Biotherapeutic protein purity and fragmentation analysis are commonly determined using the capillary sodium dodecyl sulfate (CE-SDS) assay. This assay is validated and supported on the PA 800 Plus system using a photodiode array detector (PDA) at 220 nm or 214 nm.

Here, we discuss the development and use of a 214 nm filter to facilitate seamless method transfer from the single-capillary PA 800 Plus system to the 8-capillary BioPhase 8800 system. Comparative experiments were designed to evaluate the performance of the CE-SDS application of the BioPhase 8800 system equipped with the newly developed proprietary 214nm filter with the PA 800 plus system using a 214 nm band pass filter.

This work uses the NIST Monoclonal Antibody Reference Material 8671 (NIST mAb). Critical quality attributes assessed in this work include heavy chain (HC) and light chain (LC) ratios under reduced conditions and monomeric purity determination under non-reduced conditions (Figure 1).

Key benefits of the new 214 nm filter on the BioPhase 8800 system

- **Enable scale-up** of core analytical assays from validated single capillary systems with easy method transfer
- **Enhanced sensitivity:** Innovative filter design enhances sensitivity on the BioPhase 8800 system
- **Superior performance**: Achieve lower baseline noise with a 2 Hz data acquisition rate and 8 s for peak width at half height
- **Excellent purity analysis data consistency**: Monomeric purity calculated from non-reduced NIST is comparable between the 2 platforms and to the NIST investigation report

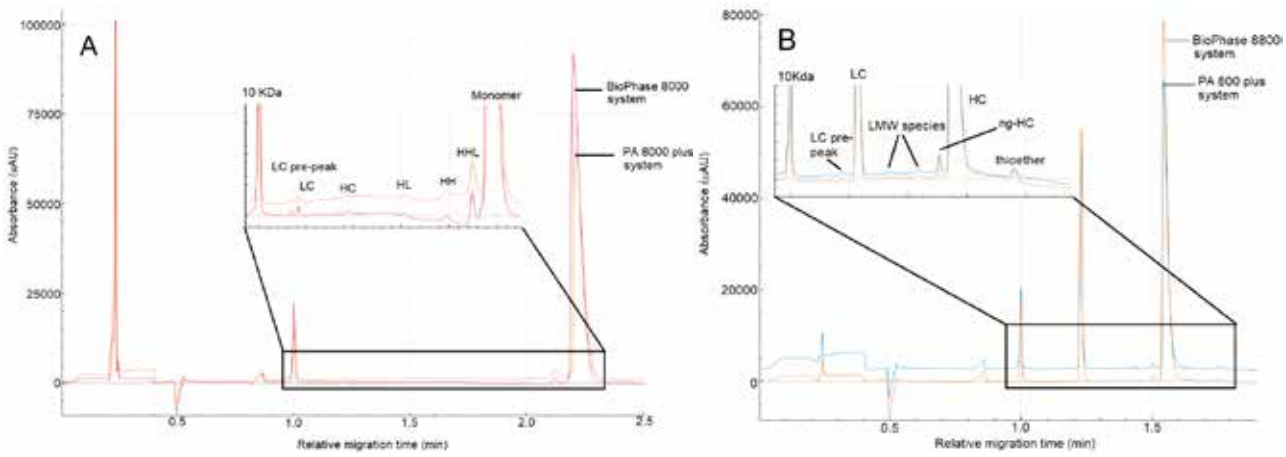


Figure 1. Comparison of CE-SDS application between BioPhase 8800 system and PA 800 Plus system. Panel A indicates NIST mAb under non-reducing conditions. Panel B indicates NIST mAb under reducing conditions. The peak assignment was primarily based on the NIST report of investigation reference material 8671 of 2023.[1]

Introduction

Historically, the detection wavelength used to analyze or quantify proteins and peptides falls within the range of 200 and 214 nm due to the strong absorbance signal of a $\pi \rightarrow \pi^*$ transition.[4] However, alternative wavelengths, such as 220 and 280 nm, can also be utilized. The specific wavelength depends mainly on the amino acid sequence of the protein and the nature of the solvent. For example, proteins containing many amino acids with aromatic groups, such as tryptophan, tyrosine and phenylalanine, have a higher absorbance at 280 nm than proteins lacking these amino acids.

Therefore, to determine protein concentration via a spectrophotometer or by separation technologies such as liquid chromatography and capillary electrophoresis, the standard wavelength of 214 nm or 220 nm is typically employed.[4]

In this study, the objective is to facilitate a seamless method transfer from the PA 800 Plus system using a 214 nm bandpass filter to the BioPhase 8800 system, which incorporates a new proprietary filter design.

Methods

Sample: NIST Monoclonal Antibody Reference Material 8671 was purchased from the National Institute of Standards and Technology (NIST).

Sample preparation: The NIST mAb samples were prepared under reduced (R) and non-reducing (NR) conditions according to the protocol described elsewhere.[2,3] An enough sample was prepared to run on both instruments to reduce bias due to variation in sample preparation. The stressed sample was prepared by exchanging buffer into 25mM Tris HCl pH 8 with a final 5 mg/mL concentration and incubated at 40°C for 5 days.

CE-SDS reagents: BioPhase CE-SDS Protein Analysis Kit (P/N C30085) was from SCIEX (Framingham, MA). The β-mercaptoethanol (P/N M3148-25ML) and iodoacetamide (P/N A3221) were from Sigma Aldrich (St. Louis, MO, USA). The Tris-HCl pH 8 was prepared from Trizma base (P/N 93352) and acidified with 0.1M HCl (P/N 2104) to pH 8. 0.1M HCl and Trizma base were from Sigma Aldrich (St. Louis, MO, USA).

Capillary cartridges: CE-SDS analysis was performed using a BFS capillary cartridge – 8 x 30 cm (P/N: 5080121) on the BioPhase 8800 system and the pre-assembled cartridge (P/N A11147) on the PA 800 Plus system, both from SCIEX (Framingham, MA).

Capillary electrophoresis instrument and consumables: The BioPhase 8800 system (P/N: 5083590F) and a starter kit of 4 sample plates, 4 reagent plates and 8 outlet plates (P/N: 5080311) were from SCIEX (Framingham, MA). CE-SDS analysis was performed using UV detection with the new 214 nm filter.

The PA 800 Plus system (P/N: A74603) had an ultra-violet single point detector (UV detector) and a 214 nm band pass filter. Universal vials (P/N: A62251) and caps (P/N: A62250) were from SCIEX (Framingham, MA). A 200 µm cartridge aperture was used.

Software: The BioPhase 8800 system and the BioPhase 8800 software, version 1.2 e-license, were used to create methods and sequences for data acquisition and analysis. The PA 800 Plus system was controlled, and the data was analyzed using 32 Karat, version 10. For easier comparison between the PA 800 Plus system and the BioPhase 8800 system, the data from PA 800 Plus system was converted to .cdf.

Parameters used to compare the 214 nm filter between PA 800 Plus and the BioPhase 8800 systems

A few parameters were used to assess the comparability of the data acquired at 214 nm using filters on the PA 800 Plus system and the BioPhase 8800 system with the new 214 nm filter:

1. The baseline noise
2. Purity of the monomer
3. The ratio of heavy chain to light chain
4. Case study: stressed NIST sample comparison

Description of the new 214 nm filter

The filter used on the PA 800 Plus system is a bandpass filter. Bandpass filters are designed to filter out a specific range of wavelengths from a light source. In the case of the PA 800 Plus system, the 214 filter with a range of +/- 10 nm allows only a specific range of wavelength to pass through the capillary window. The rest of the spectrum from the Deuterium lamp – the light source – is blocked.[5]

The filter on the BioPhase 8800 system is a proprietary assembly designed to allow a higher transmittance of light compared to the bandpass filter used on the PA 800 Plus system. Therefore, the new filter design enhances detection sensitivity at 214 nm on the BioPhase 8800 system.

The baseline noise

In general, the CE-SDS method uses a data rate of 2 Hz, and the digital filter is set to normal on the PA 800 Plus system. The root mean square (RMS) noise observed from the reduced and non-reduced NIST samples on PA 800 Plus was approximately 5. The RMS noise observed on the BioPhase 8800 system closely matches the noise on the PA 800 Plus system when using a data rate of 2 Hz and peak width at half height (PWHH) of 8 s. The RMS noise observed on the BioPhase 8800 system was 3.5.

Table 1 summarizes the RMS noise found on both systems.

Table 1. The RMS noise found on the 2 systems.

System	RMS noise	
	Non-reduced	Reduced
PA 800 Plus system	4.86	5.33
BioPhase 8800 system	3.51	3.47

Therefore, the data rate used in this work was set at 2 Hz, and PWHH was set at 8 s.

Monomeric purity

The monomeric purity was calculated using the equation below, based on the corrected peak area (CA) of the non-reduced NIST sample. The monomeric purity on the 2 systems was calculated by each CE platform's respective software.

$$Monomeric\ purity\ (\%) = \frac{CA\ monomer}{CA\ monomer + \sum CA\ fragments} \times 100\%$$

Table 2 shows the CA of the monomer, the summed CA of the fragments for both systems and the calculated purity compared to the NIST report published in 2023.[3]

It is worth noting that the reported peak area for the monomer on the BioPhase 8800 system is nearly 30% higher than that on the PA 800 Plus system. This difference is attributed to the enhanced sensitivity of the innovative design of the 214 nm filter.

The results for monomeric purity of NIST reported by PA 800 Plus system and BioPhase 8800 system are comparable.

Table 2. Monomeric purity comparison between PA 800 Plus system and BioPhase 8800 system.

System	Non-reduced NIST mAb	
	PA 800 Plus system	BioPhase 8800 system
CA of monomer	27212.577	37850.7
Summed CA of the fragments	1146.604	849.160
Purity*	95.96	97.81

*The purity of the monomer reported by NIST is 96.63%.

The ratio of the heavy chain and light chain

The ratio of the heavy chain to light chain ratio (HC/LC) was calculated by dividing the CA of the heavy chain (HC) by that of the light chain (LC). The CA was determined using the respective software of the 2 CE platforms. Table 3 displays the HC/LC ratio reported by both instruments.

Due to the higher sensitivity of the BioPhase 8800 system, the ratio HC/LC is slightly higher than that of the PA 800 Plus system.

Table 3. HC/LC ratio reported on the PA 800 Plus system and BioPhase 8800 system.

System	Reduced NIST mAb	
	PA 800 Plus system	BioPhase 8800 system
CA LC	8190.587	9646.813
CA HC	18183.006	23313.174
HC/LC	2.22	2.42

Figure 2 demonstrates the overlay of the CE-SDS electropherogram for the stressed NIST sample, showing good similarity between the 2 separations. Notably, the BioPhase 8800 system produced a higher peak intensity in the separation and superior resolution compared to the PA 800 Plus system.

Table 4 illustrates the relative migration time (RMT) and corrected peak area percentage (CA%) for both PA 800 Plus system and the BioPhase 8800 system. The peaks identified were LC, HC, the intact monomer and the following fragments: heavy-light chain (HL), heavy-heavy (HH), heavy-heavy-light (HHL) and high molecular weight species (HMW).

Table 4. Comparison of the relative migration time and corrected peak area percentage for stressed NIST between the PA 800 Plus system and BioPhase 8800 system.

	RMT		CA%	
Peak name	PA 800 Plus system	BioPhase 8800 system	PA 800 Plus system	BioPhase 8800 system
LC	1.21	1.21	4.53	4.35
HC	1.48	1.47	1.61	1.87
HL	1.76	1.77	0.83	0.82
HH	1.99	2	5.40	5.63
HHL	2.13	2.13	7.42	5.44
monomer	2.24	2.23	65.52	71.57
HMW	2.56	2.56	14.70	10.33

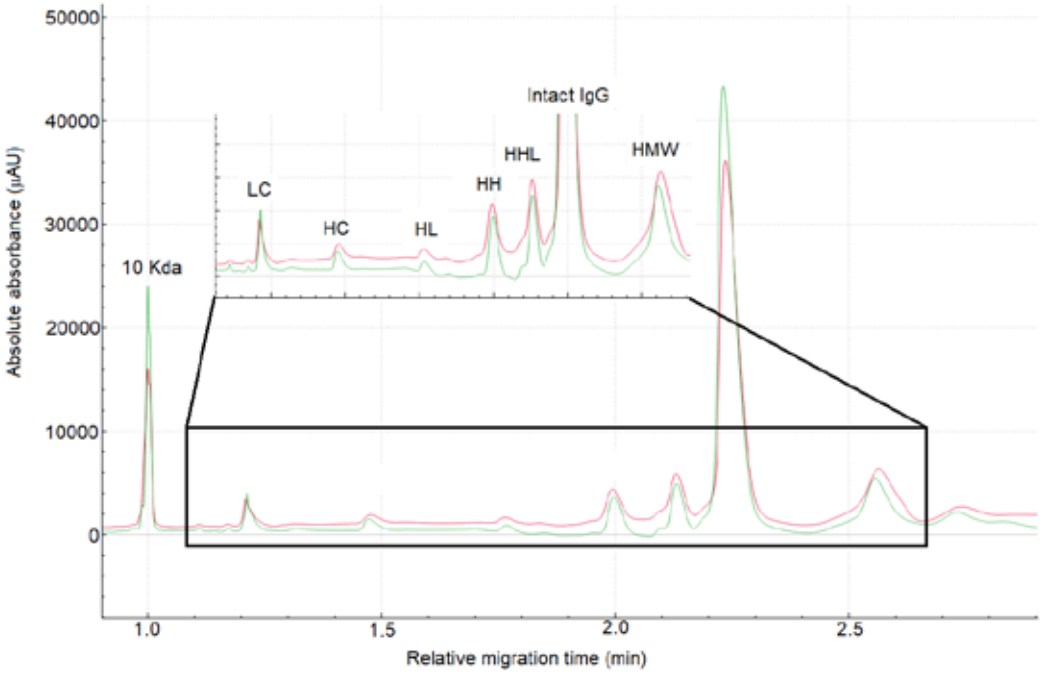


Figure 2. Overlay of CE-SDS electropherogram for stressed NIST using the PA 800 Plus system and the BioPhase 8800 system under non-reduced conditions. The red trace represents the PA 800 Plus system, and the green represents the BioPhase 8800 system.

Conclusions

- The remarkable data consistency between the PA 800 Plus system and BioPhase 8800 system allows for seamless method transfer
- The enhanced sensitivity of the BioPhase system allows for better resolution and higher intensity peaks while maintaining comparable corrected %peak area. Therefore, similar HC/LC ratios and monomeric purity were found between the 2 platforms.
- The flexible detector settings on the BioPhase 8800 system allow the user to fine-tune the separation and achieve a baseline noise comparable to the PA 800 Plus system. This work demonstrated that the data rate of 2 Hz and PWHH of 8s offer low baseline noise comparable to PA 800 plus system.
- Excellent comparability of CA% between the PA 800 system and BioPhase 8800 system for the stressed NIST sample was demonstrated

References

1. Report of investigation reference material 8671 NISTmAb, humanized IgGκ Monoclonal Antibody, 2023.
2. IgG Purity and Heterogeneity Assay Kit for the PA 800 Plus Pharmaceutical Analysis System.
3. CE-SDS protein analysis kit for the BioPhase 8800 system.
4. B.J.H. Kuipers and H. Gruppen, Prediction of molar extinction coefficients of proteins and peptides using UV absorption of the constituent amino acids at 214 nm to enable quantitative reverse-phase high-performance liquid chromatography-mass spectrometry analysis, J. Agric. Food Chem. 2007, 55, 5445-5451.
5. L. P. Huelsman. Analog Electrical Filters. Encyclopedia of Physical Science and Technology, Third edition,2003, 519 – 530.

Unleashing the Power of Speed: Accelerating time to answer with CE-SDS and Laser-Induced Fluorescence detection in under 12 Minutes

Marcia Santos
SCIEX, USA

This technical note demonstrates a strategy to accelerate capillary sodium dodecyl sulfate (CE-SDS) separation methods by using optimized instrument settings to reduce analysis time while maintaining the robustness and resolution of CE-SDS. The applicability of this approach extends to the analysis of monoclonal antibodies (mAb) and next-generation biotherapeutic molecules, making it ideal for high-throughput screening.

Upstream bioprocessing is a fast-paced environment where thousands of samples are screened yearly, and in bioprocessing, the demand for sample analysis throughput is high. To meet the throughput requirement and achieve a fast time to answer, well-established analytical technologies, such as conventional CE-SDS or SDS-PAGE, may face challenges due to their traditionally low throughput nature.[1,2]

Key features of the ultra fast CE-SDS workflow

- **High throughput and fast time to answer:** By enabling 1.8 min/sample or 96 samples in 4.3 hrs (Figure 1)
- **Reduced separation time:** Achieve a well resolved separation in under 12 minutes by applying unique instrument settings
- **Increased sensitivity with LIF detection**: Chromeo P503 dye enables highly sensitive detection
- **One CE platform for the product's entire lifecycle:** The BioPhase 8800 system can bridge the gap between upstream bioprocessing and quality control (QC)
- **Carryover:** No carryover was observed in the region of interest of the electropherogram.

Introduction

In the biopharma industry, CE-SDS is the gold standard analytical technology for addressing characterization in areas

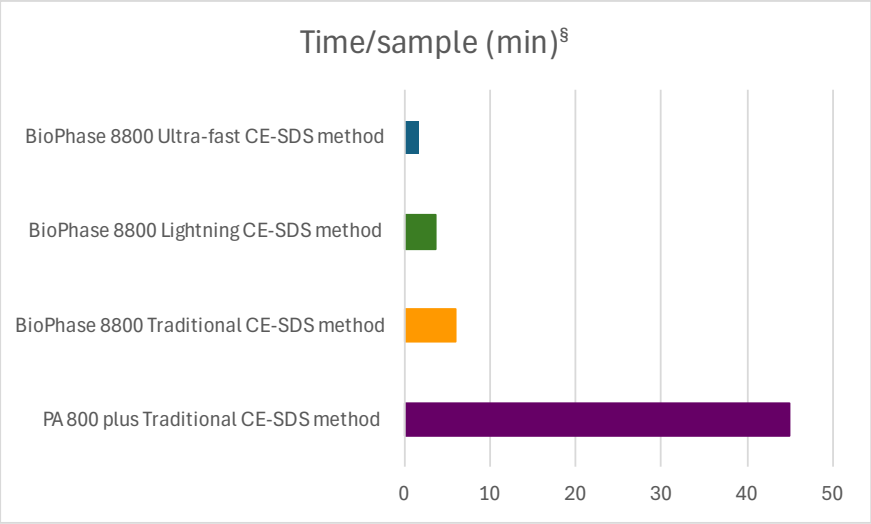


Figure 1: Timeline comparison among the high-resolution CE-SDS method on PA 800 Plus, the methods on the BioPhase 8800 systems traditional, lightning and the Ultra speed CE-SDS separation for a high-throughput bioprocessing environment. § Time per sample is total cycle time divided by 8 (BioPhase 8800 - only)

such as protein purity, fragmentation, stability, glycan site occupancy, and aggregation.[3] Although CE-SDS instruments are automated and a more reliable alternative to SDS-PAGE, they historically have a low throughput and can become a bottleneck during bioprocess development.

Traditionally, microchip technology is the technique of choice when high throughput is needed. However, microchips may suffer from premature failure due to chip clogging and a lack of robustness and reproducibility.[4] Further, cell culture samples are generally a complex mixture of salts, buffers, amino acids, and enzymes, all working synergistically to provide an environment to help cell growth and protein expression.[1,2,5] Clogging can be quite challenging when using chip-based technology to analyze cell culture samples directly. Therefore, although utilizing the same analytical platform, chemistry, and software to characterize and determine critical quality attributes from the bioprocessing environment to commercial quality control is highly desirable, as this approach can help eliminate the technology transfer barrier from the early development phase to QC but it is also highly challenging.

This work presents an enhanced CE-SDS workflow designed to empower bioprocessing development scientists to semi-quantify expressed antibodies and other biotherapeutic molecules from upstream bioprocessing samples and cell culture optimization.

This innovative strategy leverages the BioPhase 8800 system's multi-capillary environment, enabling the screening of 96 samples in half a working day. Utilizing the same reliable and robust CE platform, chemistry, and software (BioPhase 8800 software or Empower) throughout the biopharma continuum significantly reduces reagent usage and minimizes plate waste.

Methods

Sample: IgG control standard (P/N391734) was from SCIEX (Carlsbad-CA).

Reagents: BioPhase CE-SDS Protein Analysis Kit (PN C30085) was from SCIEX (Framingham, MA). The β-mercaptoethanol (β -ME) (P/N M3148-25ML), Dimethylsulfoxide (DMSO) (P/N D8418) were from Sigma-Aldrich (St. Louis – MO). The Chromeo P503 dye (P/N 15106) was from Active Motif (Carlsbad-CA).

Capillary cartridges: CE-SDS analysis was performed using a BFS capillary cartridge – 8 x 30 cm (P/N: 5080121) from SCIEX (Framingham, MA).

Capillary electrophoresis instrument and consumables: The BioPhase 8800 system (P/N: 5083590F) and a starter kit of 4 sample plates, 4 reagent plates and 8 outlet plates (P/N: 5080311) were from SCIEX. CE-SDS analysis was performed using LIF detection with an excitation wavelength of 488 nm solid-state laser and emission wavelength of 600 nm/80 nm bandpass filter FWHM 12.5 mm (P/N 65736) from Edmund Optics (Barrington, NJ).

Software: BioPhase 8800 software, version 1.2 e-license, was used to create methods and sequences for data acquisition.

Preparation of Chromeo P503 dye stock solution: 200 µL of DMSO was mixed with the lyophilized Chromeo P503 dye until complete dissolution, followed by the addition of 200 µL of double-distilled and de-ionized water (DDI water), making a 2.5 mg/mL solution. This solution should be in the dark at 2-8°C for up to 2 weeks.

Preparation of Chromeo P503 dye working solution: dilute the 2.5 mg/mL stock solution to 0.5 mg/mL with DDI water.

Sample preparation for CE-SDS-LIF analysis under reducing conditions: All samples were diluted in SDS sample buffer pH 9 to a final concentration of 10 µg/mL in a 100 µL volume. Then, it was heat-denatured and reduced by adding 5 µL of β -ME and heated at 70°C for 10 minutes.

Labeling procedure: After the samples were reduced and heat denatured, add 5 µL of (0.5 mg/mL) P503 dye working solution, mix well and incubate at 70°C for 10 min. The colors will transition from a deep blue to a purple-reddish color. The samples were transferred to the sample plate and placed into the BioPhase 8800 system for analysis.

Results and discussion

This study evaluated various system parameters, including separation temperature and voltage, capillary conditioning, and pre-injection conditioning methods, to enhance throughput without compromising reproducibility and resolution or introducing carryover. The investigation's outcome enables analysis of the mAb samples using CE-SDS in 1.5 min per reduced sample, equivalent to 96 samples within 3 hours,

significantly advancing high-throughput capability. The high-throughput can be further enhanced by automating sample preparation with a liquid handler such as the Biomek i5.[6]

Screening separation voltage and temperature conditions

In capillary electrophoresis (CE), 3 factors can directly and effectively impact the separation time: capillary length, electric field (voltage), and separation temperature.[7]

Common strategies to decrease separation time often involve reducing the capillary length, increasing the separation temperature, or elevating the electric field individually or in combination. However, these parameters may negatively impact peak resolution. Therefore, considering and optimizing these parameters is critical to balancing reduced separation time and peak resolution.

It is desirable to increase the throughput of CE-SDS to support bioprocess development samples while retaining optimal analytical performance, such as the resolution between the light chain and possible clipped or fragmented pieces of antibody, as well as the non-glycosylated (ng-HC) and glycosylated heavy chain (HC). Figure 2 shows the impact of the temperature and voltage on the separation time compared to the standard conditions (-15kV at 25°C). While the standard separation condition offers exceptional resolution between the ng-HC and HC peaks, the separation time is relatively long, 22 min.

However, by adjusting the voltage, as shown in the middle panel of Figure 2, the separation time can be reduced from 22 min (traditional method) to only 9 minutes at -30kV. Similarly,

Table 1: Resolution between ng-HC and HC under various separation conditions

Conditions	Resolution ng-HC and HC
25C_20kV	1.49
25C_25kV	1.39
25C_30kV	1.16
30C_20kV	1.38
30C_25kV	1.33
30C_30kV	0.46

the separation time can be further reduced when the temperature is increased to 30°C (bottom panel). The increased separation speed may compromise peak resolution. Table 1 illustrates the impact of these separation conditions on the resolution between the ng-HC and HC. Ideally, a resolution of

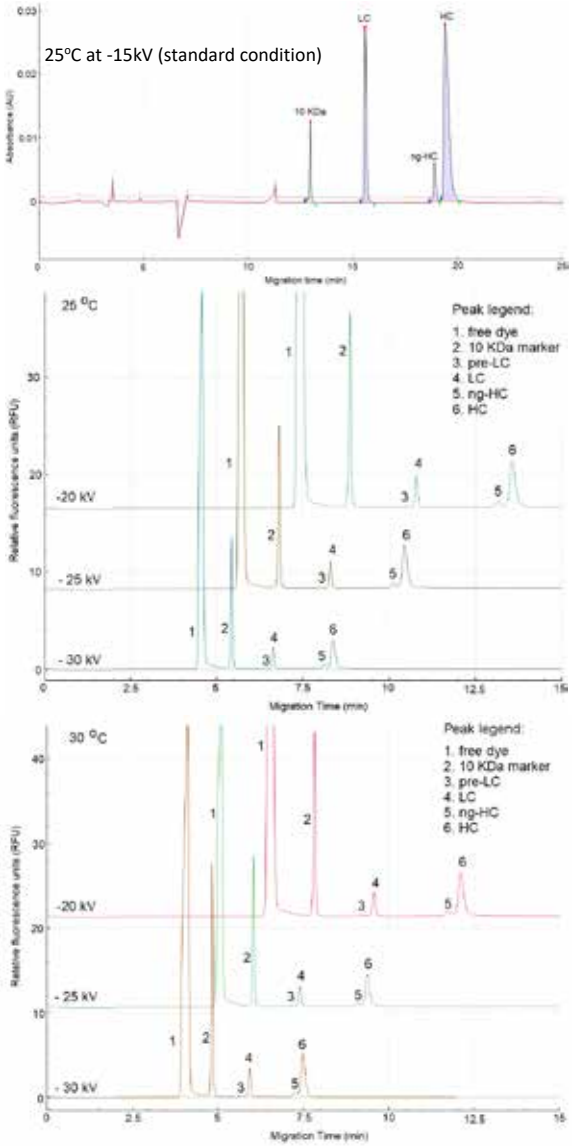


Figure 2: Overlay of the CE-SDS separations of IgG control standard at 25 °C and 30°C at various voltage conditions.

1.5 is desired, but a minimum resolution of 1.0 is required for reliable peak integration.[7]

Based on the data collected, 25kV at 30°C or 25°C is optimal to maintain the separation time below 12 minutes for reduced samples while maintaining near baseline resolution between ng-HC and HC.

Adapting capillary conditioning parameters and methods

In typical CE-SDS separation method, a considerable amount of time comes from pre-injection capillary conditioning steps, which take at least 9 minutes. In this work, the strategy was implemented to remove the pre-injection capillary conditioning steps (5 minutes in total) except for gel rinse, which was reduced from 4 to 3 minutes.

However, reducing capillary conditioning steps could induce migration time inconsistencies and peak shape degradation due to capillary surface modification over time and carryover. Therefore, a new capillary treatment approach was tested by introducing the shorted capillary conditioning method every 6 injections or at the half-sample plate mark. Figure 3 demonstrates the new sequence setup used in this study.

The sequence was set up with a standard conditioning method, as described in the application guide[8], as the first step, followed by 6 modified separation methods containing only a gel rinse step (Figure 4).

Sequence Summary

	Run #	Column	Method Name	Rep. #	Error Recovery
	1	0	LIF600_Zeus_cond_30C_2minNaOH80psi	1	☐
⊞	2	1	LIF600_Zeus_30C_25kV_12min	1	☐
⊞	3	2	LIF600_Zeus_30C_25kV_12min	1	☐
⊞	4	1	LIF600_Zeus_30C_25kV_12min	2	☐
⊞	5	2	LIF600_Zeus_30C_25kV_12min	2	☐
⊞	6	1	LIF600_Zeus_30C_25kV_12min	3	☐
⊞	7	2	LIF600_Zeus_30C_25kV_12min	3	☐
	8	0	LIF600_Zeus_inter injec cond_30C	1	☐
⊞	9	1	LIF600_Zeus_30C_25kV_12min	4	☐
⊞	10	2	LIF600_Zeus_30C_25kV_12min	4	☐
⊞	11	1	LIF600_Zeus_30C_25kV_12min	5	☐
⊞	12	2	LIF600_Zeus_30C_25kV_12min	5	☐
⊞	13	1	LIF600_Zeus_30C_25kV_12min	6	☐
⊞	14	2	LIF600_Zeus_30C_25kV_12min	6	☐
	15	0	LIF600_Zeus_inter injec cond_30C	1	☐
⊞	16	1	LIF600_Zeus_30C_25kV_12min	7	☐
⊞	17	2	LIF600_Zeus_30C_25kV_12min	7	☐
⊞	18	1	LIF600_Zeus_30C_25kV_12min	8	☐
⊞	19	2	LIF600_Zeus_30C_25kV_12min	8	☐
⊞	20	1	LIF600_Zeus_30C_25kV_12min	9	☐
⊞	21	2	LIF600_Zeus_30C_25kV_12min	9	☐
	22	0	LIF600_Zeus_inter injec cond_30C	1	☐
⊞	23	1	LIF600_Zeus_30C_25kV_12min	10	☐
⊞	24	2	LIF600_Zeus_30C_25kV_12min	10	☐
⊞	25	1	LIF600_Zeus_30C_25kV_12min	11	☐
⊞	26	2	LIF600_Zeus_30C_25kV_12min	11	☐
⊞	27	1	LIF600_Zeus_30C_25kV_12min	12	☐
⊞	28	2	LIF600_Zeus_30C_25kV_12min	12	☐
	29	0	LIF600_Zeus_Shutdown_30C	1	☑

Figure 3: Sequence set up for high throughput CE-SDS analysis.



Figure 4: Proposed separation method for ultra-high throughput. The separation method contains only one gel rinse prior to injection.

Capillary surface cleaning is critical for robust separation. Therefore, a mid-plate complete capillary conditioning treatment (Figure. 5) was introduced to minimize surface contamination and ensure separation consistency.

With this setup, the user can separate 192 samples in 8.5 hrs, equivalent to 1.8 min/sample.



Figure 5: mid-plate complete capillary conditioning treatment, dubbed LIF600_Zeus_inter inject cond_30C in Figure 3.

Evaluation of the performance of the ultra fast CE-SDS assay

The first important aspect to consider in assay performance is always the inter and intra-capillary reproducibility. The second consideration is carryover.

Inter and intra-capillary reproducibility

Figure 6 shows the overlay of electropherograms from a reduced IgG control standard sample of 48 separations from capillaries A, C, E and G. The reproducibility of each separation is notable. To substantiate the observation in Figure 6, Table 2 shows the calculated inter-capillary average migration time and %corrected peak area for the 4 peaks observed for the IgG control standard under reducing conditions.

Figure. 7 illustrates the consistency across 48 injections for migration time (panel A) and corrected peak area percentage (CPA%), demonstrating the robustness of this assay.

Carryover considerations

Laser-induced fluorescence detection is an advantageous and sensitive technique in CE to analyze materials in low concentrations, such as proteins expressed in cell cultures.[7]

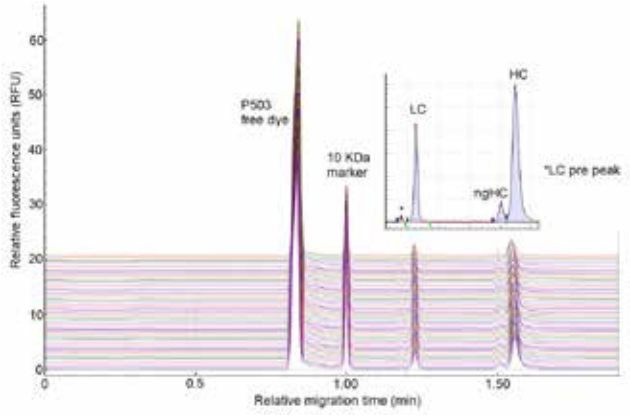


Figure 6: Overlay of reduced P503 labeled IgG control standard. Conditions: 12 minutes separation time, 1000V/cm and 20°C cartridge temperature.

However, the high sensitivity of this detection method also poses the risk of interference from carryover. Separations towards the end of the sequence are more susceptible to carryover due to the accumulation of free dye.

Sample buffer was injected as blank injections in the same capillary after sample injections to investigate the presence of carryover. Figure 8 shows the sample plate setup for this evaluation.

Table 2: The average migration time and %RSD for intercapillary precision.

Inter capillary n=48				
Inter-capillary average (n=48)				
	pre-LC	LC	ng-HC	HC
Average migration time	7.22	7.52	9.24	9.52
Average corrected peak area %	1.40	27.86	6.82	63.92
Resolution ngHC/HC	N/A	N/A	N/A	1.32
Inter-capillary %RSD / standard deviation (n=48)				
	pre-LC	LC	ng-HC	HC
Migration time	1.17/0.084	1.18/0.089	1.21/0.112	1.23/0.117
Corrected peak area %	6.47/0.090	2.14/0.596	5.07/0.346	0.71/0.451
Resolution ngHC/HC	N/A	N/A	N/A	2.98

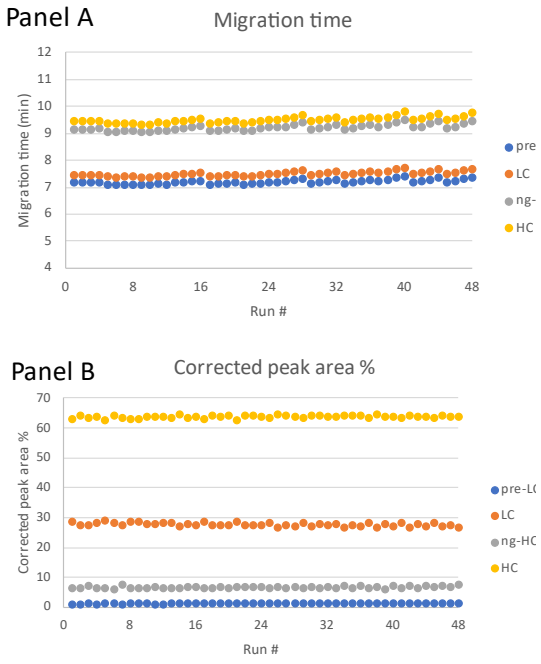


Figure 7: Assay reproducibility graph. (A)migration time consistency. (B) corrected peak area % consistency, n=48

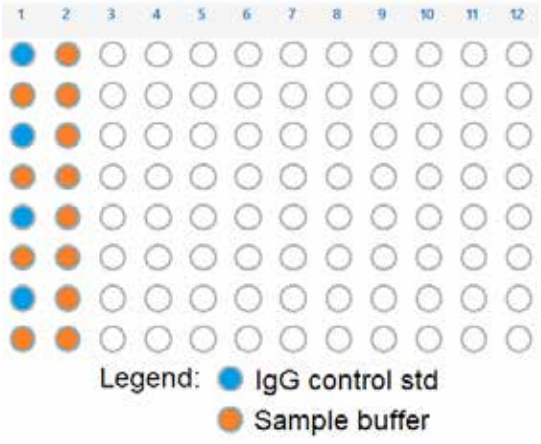


Figure 8: Sample plate setup to investigate carryover.

Figure 9 presents the 12th (last) injection of IgG control standard separation on capillaries A, C, E and G, followed by a blank (sample buffer) injection on the same capillaries. The figure indicates a minor carryover present, where the free dye of P503 is migrating. The minor carryover occurred in the region before the 10 kDa marker on the electropherogram. Therefore, it did not interfere with the migration of the protein of interest, as the separation window of interest is after the 10KDa marker, which exhibits a clean baseline without any visible carryover.

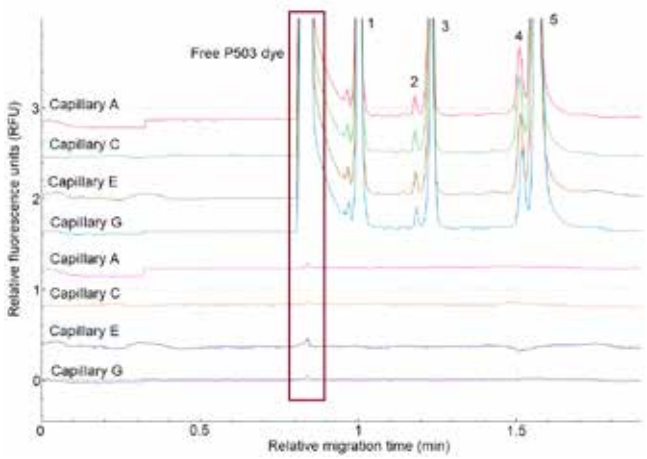


Figure 9: Carryover assessment using blank runs after sample separation. Legend: 1. 10 Kda, 2. pre-LC, 3. LC, 4. ng-HC, 5. HC. The rectangle highlights the Free P503 dye.

With this setup, the user can separate 192 samples in 8.5 hrs, equivalent to 1.8 min/sample, while maintaining migration time consistencies.

Conclusions

- Analysis time was reduced to 1.8 min per sample from 6.25 min per sample, equivalent to 96 samples per half working day with the ultra-fast CE-SDS method
- Increased throughput of the CE-SDS with LIF detection was successfully demonstrated for the semi-quantitative assessment of antibody samples
- The reproducibility of the corrected peak area percentage is below 6.5% for the pre-LC peak – the smallest species in the electropherogram
- The baseline resolution between ng-HC and HC was comparable to the longer traditional method
- Minor carryover of free P503 peak is restricted to a non-relevant region of the electropherogram, enabling confident semi-quantitation of the sample peaks

Reference

1. M. Kornecki, F. Mestmäcker, S. Zobel-Roos, L. H. de Figueiredo,H. Schlüter and J. Strube; Host Cell Proteins in Biologics Manufacturing:The Good, the Bad, and the Ugly; Antibodies 2017, 6, 13.
2. F. Li, N. Vijayasankaran, A. (Yijuan) Shen, R. Kiss and A. Amanullah; Cell culture processes for monoclonal antibody production; mAbs 2:5, 466-477(2010).
3. A Series of Collaborations Between Various Pharmaceutical Companies and Regulatory Authorities Concerning the Analysis of Biomolecules Using Capillary Electrophoresis; B. Nunnally et al.; Chromatographia 2006, 64, 359 - 368.
4. S. D. Noblitt, C. S. Henry; Overcoming Challenges in Using Microchip Electrophoresis for Extended Monitoring Applications; Capillary Electrophoresis and Microchip Capillary Electrophoresis: Principles, Applications, and Limitations, 2013.
5. Petra Gronemeyer, Reinhard Ditz and Jochen Strube; Trends in Upstream and Downstream Process Development for Antibody Manufacturing; Bioengineering 2014, 1, 188-212.
6. High throughput CE-SDS protein analysis using fully automated sample and reagent preparation workflow; Technical note, 2022.
7. J. P. Landers, 1997, Handbook of Capillary Electrophoresis, second edition.
8. CE-SDS protein analysis kit for the BioPhase 8800 system.

Efficient transfer of the high-speed CE-SDS method from the PA 800 Plus system to the BioPhase 8800 system

Marcia Santos
SCIEX, USA

This technical note introduces a new capillary electrophoresis sodium dodecyl sulfate (CE-SDS) method on the BioPhase 8800 system, equivalent to the gold standard high-speed (HS) method on the PA 800 Plus system. The new method enables a seamless method transfer from the PA 800 Plus system to the BioPhase 8800 system, offering higher throughput with 8 parallel capillaries(Figure 1).

The introduction of the PA 800 Plus system and the IgG Heterogeneity and Purity Assay has made the CE-SDS method an essential analytical tool for characterizing therapeutic biomolecules. In the fast-paced environment of process development and support, where thousands of samples are screened annually, there is a demand for quick, reliable sample analysis method that can be efficiently transferred throughout the pipeline. To support this demand for even higher throughput, the IgG HS method is easily transferrable from the PA 800 Plus system to the 8-capillary BioPhase 8800 system.

Key features of the HS CE-SDS method

- **Seamless transition from the to the BioPhase 8800 system:** By taking advantage of the flexibility of the hardware settings on BioPhase software, an equivalent IgG HS CE-SDS method was developed
- **Same outstanding performance:** Consistency between runs and cartridges demonstrates the method's reproducibility. Inter-capillary reproducibility over 96 samples is <5% for light chain (LC), glycosylated (HC) and non-glycosylated heavy chains (ng-HC).
- **Similar migration time as original PA 800 Plus method**: Migration time of the 10 kDa marker on the BioPhase 8800 system high-speed method is within 1 minute (min) window compared to the migration time on the PA 800 Plus system.
- **The MW estimation** for lysozyme and carbonic andrydrase was better than 16%.

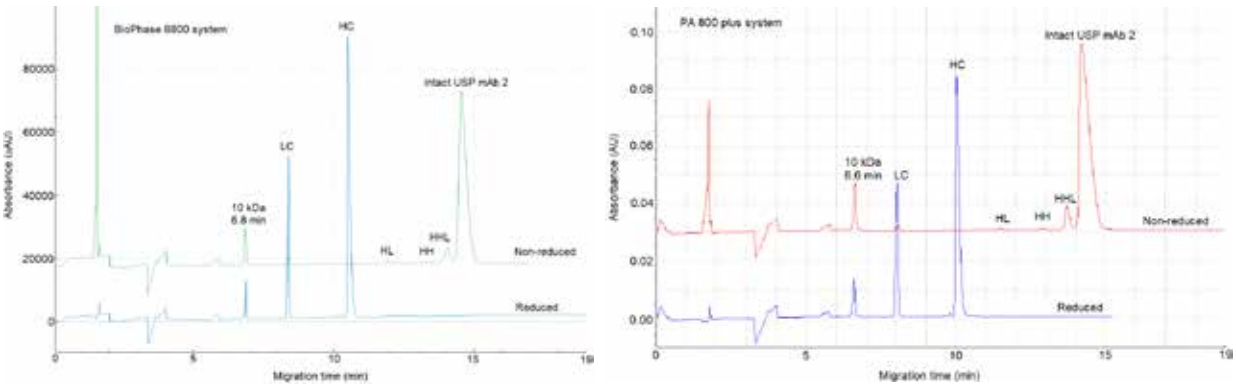


Figure 1: Separation of USP mAb 002 under reducing and non-reducing conditions on the BioPhase 8000 system (left) and on PA 800 Plus (right) using the HS CE-SDS method. Separation conditions are in the methods section. Migration time of 10 kDa marker on the BioPhase 8800 system using the HS CE-SDS method is within 1 min window compared to the migration time on the PA 800 Plus system.

Introduction

CE-SDS is widely used in biotherapeutic analyses for lot release, stability testing, formulation-buffer screening, process development, cell line development and product characterization. There are 2 versions of the CE-SDS separation method available on the PA 800 Plus system: the IgG high-resolution (IgG HR) and IgG HS. The IgG HR method was created for the high resolution between the ng-HC and the HC. Both methods have been adopted, validated, transferred and used in analytical development and quality control for numerous commercial molecules. However, only the IgG HR separation method was previously available on the BioPhase 8800 system. The IgG HS separation method on the PA 800 Plus system was created to reduce the total analysis time by 15 minutes, without compromising the data quality compared to the IgG HR method. The effective separation length is the fundamental difference between the IgG HR and HS methods. Effective length is the distance the molecule travels from introduction to detection. In the IgG HS method, the effective length is 10 cm. This distance is much shorter than the traditional IgG HR, where the effective length is 20 cm with a 30 – 40 min separation time. Consequently, the separation is considerably quicker.

This study proposes a new CE-SDS-based method for the BioPhase 8800 system that matches the performance of the IgG HS CE-SDS method on the PA 800 Plus system.

Methods

Samples: USP mAb 002, Monoclonal IgG1 - United States Pharmacopeia (USP) Reference Standard (P/N: 1445547-2MG) was from USP (Rockville, MD). NISTmAb, Humanized IgG1k Monoclonal Antibody (P/N: 8671) was from NIST (Gaithersburg, MD). IgG control standard (P/N: 391734) was from SCIEX (Framingham, MA).

Reagents: The BioPhase CE-SDS Protein Analysis Kit (P/N: C30085) was from SCIEX. The β-mercaptoethanol (β -ME) (P/N: M3148-25ML) and iodoacetamide (IAM) (P/N: I6125) were from Sigma-Aldrich (St. Louis, MO).

Capillary cartridges: For the BioPhase 8800 system, the CE-SDS analysis was performed using a BFS capillary cartridge – 8 x 30 cm (P/N: 5080121) from SCIEX. For the PA 800 Plus system, the CE-SDS analysis was performed using a BFS pre-assembled capillary cartridge (P/N: A55625) from SCIEX.

Capillary electrophoresis instrument and consumables: The BioPhase 8800 system (P/N: 5083590F) and a starter kit of 4 sample plates, 4 reagent plates and 8 outlet plates (P/N: 5080311) were from SCIEX. CE-SDS analysis was performed using UV detection at 220 nm.

The PA 800 Plus system (P/N: A51963AE) was equipped with a PDA detector. The detection wavelength was 220 nm.

Instrument methods: On the PA 800 Plus system, the capillary conditioning method comprises 0.1M NaOH rinse at 20 psi for 10 min, 0.1M HCl rinse at 20 psi for 5 min, ddi H2O rinse at 20 psi for 2 min,SDS-MW gel buffer rinse for 10 min at 70 psi and a voltage separation at 15 kV for 10 min. The separation method consists of 0.1M NaOH rinse at 70 psi for 3 min, 0.1M HCl rinse at 70 psi for 1 min, ddi H2O rinse at 70 psi for 1 min and SDS-MW gel buffer rinse for 10 min at 70 psi, 2 water dips, an injection at 5 kV for 20 seconds, and the separation at 15 kV for 20 min

The separation method used on the BioPhase 8800 system is shown in Figure 2.

Software: BioPhase software, version 1.2 e-license, was used to create methods and sequences for data acquisition and data analysis on the BioPhase 8800 system. The 32 Karat (version 10.1) software package was used for data acquisition and processing.



Figure 2: Proposed HS CE-SDS separation method for the BioPhase 8800 system.

Sample preparation for CE-SDS analysis under reducing and non-reducing conditions: The USP mA 2 IgG sample was used without any previous treatment. 10 µL of the USP mAb 2 IgG sample was mixed with 2 µL of 10 kDa marker, 83 µL of SDS sample buffer pH 9 and 5 µL of β -ME or 250mM IAM for reduced and non-reduced conditions, respectively. Then, the mixture was heat-denatured at 70°C for 10 min before CE analysis.

Results and discussion

Selecting 10 kDa as the internal mobility marker and defining a migration time window for 10 kDa

In CE, using an internal mobility marker is crucial. This is due to the consistent relative position between the marker and the sample peaks, which guarantees reproducibility for different runs, capillaries, chemistry lots, instruments and users.

The first step in developing the method was to set a migration time requirement. Although, any peak could be used as a marker, the migration time for a mAb molecule peak under reduced or non-reduced conditions can vary due to differences in the antibody hydrodynamic size and primary sequence. These differences can affect the hydrophobicity of the mAb. Consequently, using a mAb peak as a reference can impact assay accuracy and reproducibility, as it may change from molecule to molecule, making the method non-viable as a platform method.[1] Figure 3 illustrates the difference between

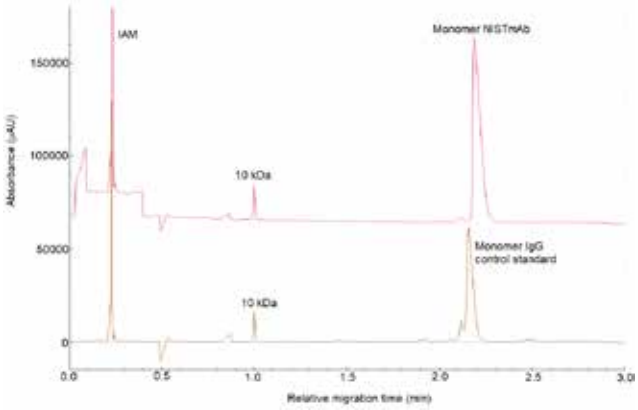


Figure 3: Migration time difference of 2 different mAbs under non-reducing conditions. The top trace is from NISTmAb, and the bottom trace is from the IgG control standard under non-reducing conditions using the IgG standard method on the BioPhase 8800 system (-15 kV/25°C).

the migration time of the monomer peak for NISTmAb and SCIEX's IgG control standard mAbs.

Defining a migration time window for the 10 kDa internal marker is more reliable than using a mAb peak, as this molecule exhibits a consistent migration pattern regardless of the antibody sample and the sample preparation conditions (reducing and non-reducing). Figure 1 shows that the migration time of the 10 kDa internal marker using the IgG HS method was around 6.6 minutes on the PA 800 Plus system under reducing and non-reducing conditions. Therefore, the 10 kDa molecule was used as a marker to investigate other instrument settings in this study. Arbitrarily, a requirement for the migration time of the 10 kDa on the BioPhase 8800 system was set at 6.6? +/- 1 min. Migration time of 10 kDa marker on the BioPhase 8800 system using the HS CE-SDS method was within 1 min window compared to the migration time on the PA 800 Plus system, demonstrating excellent data consistency between the two systems.

Investigating the separation temperature and electric field strength

It is well known that 3 main factors can impact the separation time: capillary length, electric field (voltage), and separation temperature.[2]

On the BioPhase 8800 system, the total capillary length is fixed at 30 cm with 20 cm of effective length. Thus, the electric field and separation temperature are the only variables that can be adjusted in a method.

Figure 4 shows the separation of reduced USP mAb 002 under various voltages and temperature conditions.

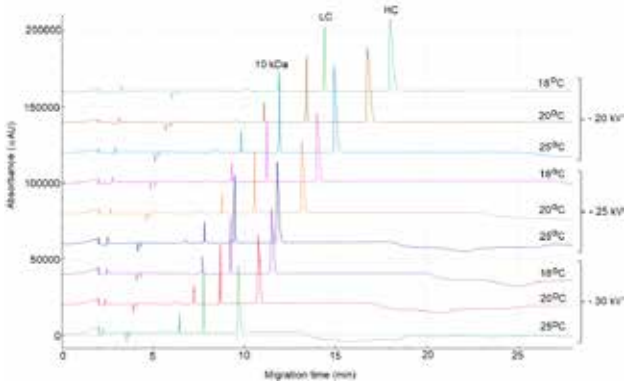


Figure 4: Overlay of the CE-SDS separation traces of USP mAb 002 at various separation voltages and temperatures.

As expected, the higher the electric field and temperature, the faster the migration time. However, as the peaks of interest migrated faster, the baseline fluctuation from the separation

gel buffer became more significant and could potentially interfere with peak integration. The background signal from the separation gel buffer was influenced by temperature and electric field. Figure 4 shows that the baseline noise was less significant at lower temperatures, i.e., 18°C, even at higher electric fields.

Even though the baseline interference was mitigated at 18°C, the migration time of the 10 kDa marker at the highest electric field (-30 kV) was 7.69 min, which falls outside the target range of 6.5 +/- 1 min. Therefore, further method optimization was carried out as described below.

Optimizing separation ramp time

Another method setting that is often overlooked is the ramp time. Ramp time is when the instrument's high voltage power supply goes from 0 kV to the set separation voltage. The ramp time setting is crucial because it directly impacts the reproducibility of the separation. When the voltage is applied, the electrode generates a sudden heat wave that propagates to the surrounding areas, affecting the capillary.[3-4] The temperature gradient may induce dispersion and reduce separation efficiency. However, theoretical models of the thermal gradient, as explained by Guiochon et al.,[5] indicate that this effect is negligible for capillaries with an inner diameter of 100 µm. The inner diameter of the capillary used in this application was only 50 µm.

Typically, in CE-SDS-based separation, the BioPhase 8800 and PA 800 Plus systems have a pre-set ramp time of 2 minutes. Figure 5 shows the influence of the ramp time on the separation of USP mAb 002 under reducing conditions. In the lower trace with a ramp time of 2 min, the 10 kDa marker migrated at 7.6 min. The upper trace shows a separation using a ramp time of 0.5 min, where the 10 kDa marker migrated at 6.8 min, which falls within the required migration time window. Therefore, 0.5 min was selected as the optimal ramp time. The final optimized HS CE-SDS method comprised an 18°C separation temperature and a-30 kV separation voltage with 0.5 min ramp time.

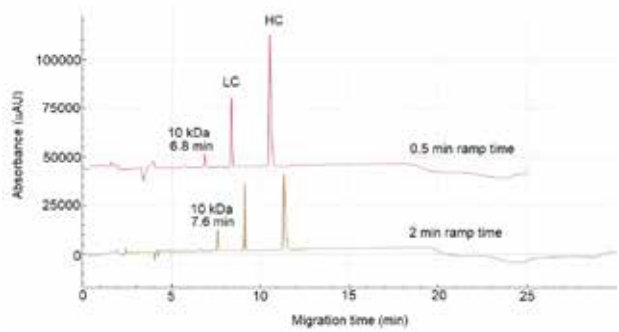


Figure 5: Overlay of electropherograms obtained with NISTmAb and USP mAb002 under non-reducing conditions using the IgG standard method from PA 800 Plus system (-30 kV/18°C).

Reproducibility of the method

To ensure the decrease in ramp time did not compromise the method's reproducibility, a discrete precision study was conducted using 2 cartridges. Figure 6 shows the average migration time values for the 10 kDa marker obtained from 2 cartridges. The error bar is the standard deviation from 96 separations per cartridge. The RSD for the 10 kDa migration time with both cartridges was better than 1.6%, demonstrating excellent assay reproducibility.

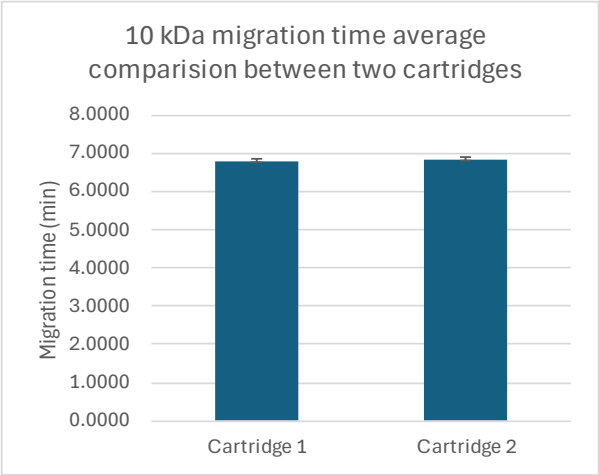


Figure 6: Average migration time values for the 10 kDa marker obtained from 2 cartridges with 96 samples per cartridge.

This method was also tested with the intact USP mAb under non-reducing conditions, and the CPA %RSD was 4.3%, indicating high assay reproducibility.

Molecular weight estimation

A simple experiment was conducted to estimate the molecular weight of carbonic anhydrase and lysozyme, aiming to determine whether the change in separation temperature adversely affected the estimation. Figure 7 shows an overlay of the molecular weight size standards (bottom, blue trace), lysozyme (middle, green trace) and carbonic anhydrase (top, red trace). The X-axis represents the molecular weight, while the Y-axis shows absorbance units.Table 1 presents the

corrected peak area average and the percentage of relative standard deviation for the LC, HC and ng-HC of the USP mAb 002 reference standard under reducing conditions. The consistency of these values, with a %RSD of less than 5% for both cartridges demonstrates the excellent reproducibility of the method.

Table 1. Average values and reproducibility of USP mAb 002 under reducing conditions

cartridge	Average values for corrected peak area		
	LC	ng-HC	HC
#1	7746.29	120.73	19107.87
#2	7105.25	110.04	17447.68
cartridge	%RSD for corrected peak area		
	LC	ng-HC	HC
#1	3.80	4.82	3.76
#2	3.41	4.76	3.26

Using the BioPhase software, the estimated molecular weight of lysozyme and carbonic anhydrase were determined to be 14.3 and 30.7 kDa, respectively, based on the separation of the molecular weight size ladder and point-to-point curve fitting. These values are in excellent agreement with the values listed in the UniProt database[6], with an accuracy value of 12% and 16% for lysozyme and carbonic anhydrase, respectively.

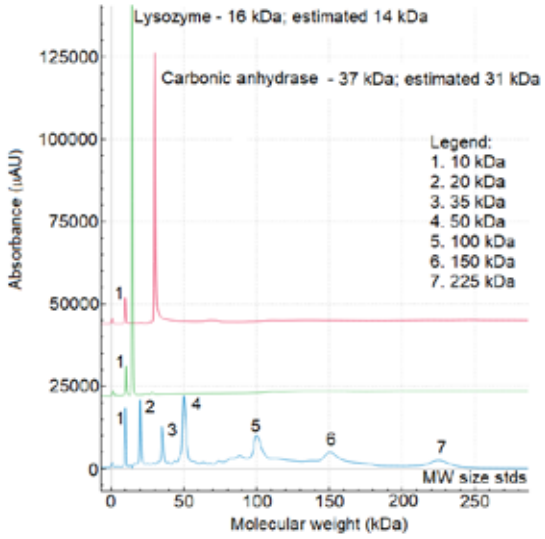


Figure 7: Overlay of electropherograms obtained with the molecular weight size standards (stds), lysozyme and carbonic anhydrase.

Conclusions

- Seamless transfer of the HS CE SDS method from the PA 800 Plus system to the BioPhase 8800 system with a higher throughput was accomplished
- Accurate molecular weight estimation with accuracy better than 16% was achieved
- Excellent assay reproducibility was demonstrated with %RSD of CPA < 5%
- A successful method transfer was achieved using 18 °C for separation temperature and 30 kV for separation voltage with a 0.5 min for ramp time

Reference

1. Nunnally B. et al. A series of collaborations between various pharmaceutical companies and regulatory authorities concerning the analysis of biomolecules using capillary electrophoresis. Chromatographia 2006 64:359 - 368.
2. Noblitt SD andHenry CS. Overcoming challenges in using microchip electrophoresis for extended monitoring applications; Capillary electrophoresis and microchip capillary electrophoresis: principles, applications, and limitations. 2013
3. Palonen S et al.Effect of initial voltage ramp on separation efficiency in non-aqueous capillary electrophoresis with ethanol as background electrolyte solvent. J. Chrom A 2005 1068 (1):107-114.
4. Xuan X et al.Joule heating effects on separation efficiency in capillary zone electrophoresis with an initial voltage ramp. Electrophoresis 2006 27: 3171–3180.
5. Dose EV and Guiochon G Timescales of transient processes in capillary electrophoresis. J. Chrom. A 1993 652(1):263-275.
6. UniProt: the universal protein knowledgebase

BioPhase 8800 系统进行高通量CE-SDS方法学验证

The method validation of CE-SDS method on high-throughput BioPhase 8800 system

刘冬科，张晓霞，高铁，陈泓序，刘冰洁

Liu Dongke, Zhang Xiaoxia, Gao Tie, Chen Hongxu, Liu Bingjie

SCIEX，中国

SCIEX, China

关键词： BioPhase 8800 system；CE-SDS；方法学验证

1. 前言

十二烷基硫酸钠毛细管电泳法（CE-SDS）是目前生物制药企业进行蛋白药物研发和质控中纯度测定的标准方法。2006年、2012年先后在PA 800 Plus仪器上完成了CE-SDS方法的全球多实验室联合验证及中国实验室的联合验证[1-2]，获得了优异的实验结果，并将该方法收录于中国药典2015版第三部[3]。

SCIEX于2022年推出了BioPhase 8800高通量毛细管电泳分析系统，上市以来受到企业的广泛关注。该系统通过8根平行的毛细管同时进行检测分析，以8倍的通量获得与PA 800 Plus同质量的数据，在保证分析效果和准确性的前提下，提高大量样本的分析检测效率[4-5]。

为了进一步验证BioPhase 8800系统CE-SDS蛋白纯度分析的稳定性与可靠性，本文提出了多通道毛细管电泳技术的方法学验证的方案，并在BioPhase 8800上进行CE-SDS方法学验证。该方案下，方法验证数据均获得了满意的结果，整个验证工作24小时内即可完成，在保证实验稳定可靠的前提下充分发挥了BioPhase 8800系统在CE-SDS高通量分析中的优势。

2. 仪器及方法

BioPhase 8800系统，配备紫外检测器（UV，波长220 nm），BioPhase软件1.2.20（SCIEX）。用于BioPhase 8800系统的熔融石英毛细管卡盒 8×30 cm（SCIEX, PN 5080121），内径50 µm，20/30.2 cm 有效/总长；采集频率：4 Hz；样品室温度：25 ℃；毛细管温度：25 ℃。

毛细管针间冲洗和胶填充：0.1 mol/L氢氧化钠溶液在 80 psi压力下冲洗2 min，0.1 mol/L 盐酸溶液在 50 psi 压力下冲洗2 min，CE级水在50 psi 压力下冲洗1 min。 SDS 凝胶缓冲液在80 psi 压力下冲洗4 min。进样：-5 kv，20 s。分离：-15 kv，35 min（非还原）；-15 kv，25 min（还原）。

3. 试剂及样品

3.1. 试剂

CE-SDS蛋白分析试剂盒（SCIEX, PN C30085），包含：凝胶缓冲液，样品缓冲液，10 kDa内标，碱性冲洗液，酸性冲洗液，CE级水。BioPhase 8800系统启动盘套装（SCIEX, PN 5080311），包含：4块样品盘、4块缓冲液盘、8块出口盘。低pH磷酸盐样品缓冲液（SCIEX, PN C57805, pH 6.5）； 2- 巯基乙醇（Sigma-Aldrich PN M7154）；碘乙酰胺（Sigma-Aldrich PN I1149）。

3.2. 样品溶液制备

用低pH磷酸盐样品缓冲液将样品稀释至1 mg/ml，以相同稀释倍数稀释制剂缓冲液，为空白试剂。

还原（R）样品制备：取单抗样品溶液（1 mg/mL）95 µL，加入2-巯基乙醇5 µL，涡旋混匀； 70 ℃加热 15 min，冷却至室温后6000 rpm 离心 1 min。取 100 µL 转移至96孔的样品盘中，立即进行分析。

表1. CE-SDS方法验证的样品入口盘布局图（样品孔中数字单位为mg/mL）

Channel	1	2	3	4	5	6	7	8	9	10
A	Blank-NR	SST-NR	Blank-NR	Blank-NR	Sample-NR	Blank-R	SST-R	Blank-R	Blank-R	Sample-R
B	Blank-NR	SST-NR	SST-NR	0.50-NR	Sample-NR	Blank-R	SST-R	1.50-R	0.50-R	Sample-R
C	Blank-NR	SST-NR	1.50-NR	SST-NR	Sample-NR	Blank-R	SST-R	1.25-R	0.25-R	Sample-R
D	Blank-NR	SST-NR	1.25-NR	0.25-NR	SST-NR	Blank-R	SST-R	1.00-R	0.125-R	Sample-R
E	Blank-NR	SST-NR	1.00-NR	0.125-NR	Sample-NR	Blank-R	SST-R	SST-R	0.0625-R	Sample-R
F	Blank-NR	SST-NR	0.75-NR	0.0625-NR	Sample-NR	Blank-R	SST-R	0.75-R	SST-R	Sample-R
G	Blank-NR	SST-NR	0.50-NR	0.03125-NR	Sample-NR	Blank-R	SST-R	0.50-R	0.03125-R	SST-R
H	Blank-NR	SST-NR	Blank-NR	Blank-NR	Blank-NR	Blank-R	SST-R	Blank-R	Blank-R	Blank-R

非还原（NR）样品制备：取样品溶液（1 mg/ml）95 µL，加入 0.8 mol/ L 碘乙酰胺 5 µL，涡旋混匀；68-72 ℃孵育 5 min，冷却至室温后 6000 rpm 离心 1 min。取 100 µL转移至96孔的样品盘中，立即进行分析。

还原/非还原空白试剂的制备：95 µL空白试剂代替样品溶液进行相应处理。

4. 样品盘布局及方法验证方案

CE-SDS方法验证的样品入口盘布局见表1，左5列为NR样品，右5列为R样品。若一列有剩余样品孔，则加入相应的Blank。第3、8列线性样品及第4、9列定量限样品均重复进样3次，第5、10列重复性样品重复进样6次。

系统适用性测试样品（System Suitability Test，SST）推荐如下摆放：1）在96孔样品盘的任意一列加入SST，作为第一针运行，考察8根毛细管间一致性；2）在放置样品的一列随机位置加入一个SST，随该列样品一同检测，考察单次进样是否正常；3）所有样品列运行结束后，最后再运行一次SST列，考察检测全过程迁移时间是否稳定。

5. CE-SDS方法验证结果

5.1 特异性

将 A-H 8个通道的Blank、Sample电泳图叠加对比，发现8个通道样品出峰处均无干扰，典型结果如图2。NR样品中除了IgG主峰外，还可检测到到P1-P7等杂质。R样品可检测到LC、ngHC、HC的峰。

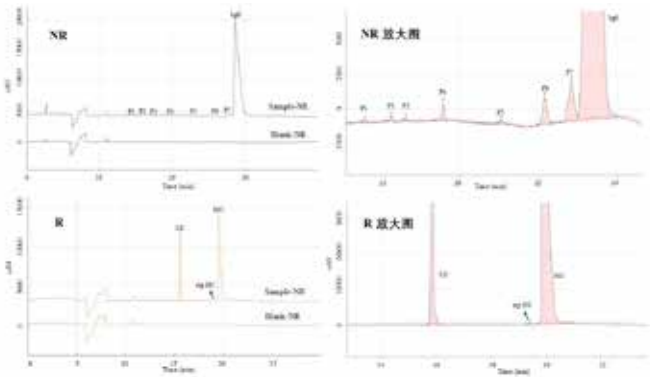


图1. CE-SDS非还原、还原样品特异性验证电泳图

5.2 线性、定量限（LOQ）与检出限（LOD）

将线性样品列的5个浓度点进行线性回归，LC、HC、LC +HC +ngHC、IgG线性系数R^2 > 0.99（图2），方法在0.5 mg/mL -1.5 mg/mL间具有良好的线性。

利用定量限样品列的5个浓度点进行线性回归，根据响应值的标准偏差（σ）和校正曲线的斜率（s）计算定量限（10 σ/s）、检出限（3.3 σ/s）。还原LOQ为0. 8 µg/mL，LOD为0. 3 µg/mL。非还原LOQ为0. 4 µg/mL，LOD为0. 1 µg/mL。

5.3 回收率

分别将各峰的5个浓度点带入相应的标准曲线计算回收率，回收率范围在93.80 %-104.18 %（表2）。方法回收率高、准确度好。

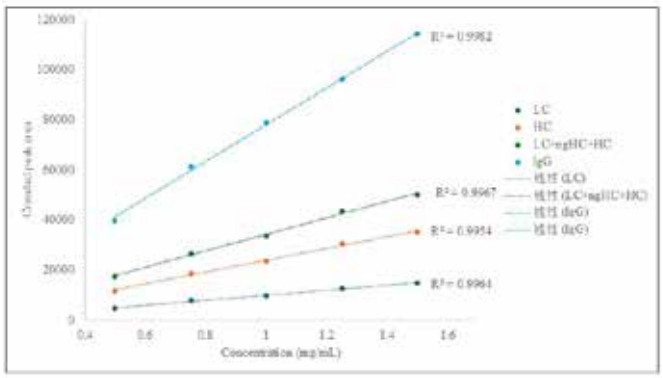


图2. CE-SDS线性结果统计

表2. 回收率结果统计

Conc（mg/mL）	Recovery% (n=3)			
	LC	HC	LC +ngHC+HC	IgG
0.50	95.76	95.93	95.89	93.80
0.75	103.18	103.11	103.13	101.95
1.00	99.49	99.00	99.14	104.18
1.25	101.99	102.62	102.43	100.48
1.50	98.52	98.30	98.37	98.00

5.4 精密度

5.4.1制备重复性

NR和R样品各平行制备6份，按表1摆放在样品盘的第5列和第10列的6个通道中。制备重复性结果统计如下表3， Injection Rep 1-6表示分别统计了第1-6次进样时6个通道的制备重复性。LC、HC、IgG的迁移时间（MT）RSD值为0.15 %- 1.50 %，校正峰面积百分比（CAP %）的RSD值为0.08 %-0.42 %，表明样品制备重复性良好，也表明通道间具有良好的一致性。

5.4.2. 进样重复性

进样重复性结果统计如下表4，Channel B-G表示分别统计了每个通道的6次重复进样的结果。各通道6次重复进样LC、HC、IgG主峰的迁移时间RSD为0.32 %-1.53 %，校正峰面积百分比的RSD为0.10 %-0.37 %，表明BioPhase 8800多通道毛细管电泳进样重复性良好。

表3. 制备重复性结果统计（n=6个通道）

Injection Rep	%RSD (LC)		%RSD (HC)		%RSD (IgG)	
	MT	CAP%	MT	CAP%	MT	CAP%
1	0.55	0.37	0.57	0.16	1.50	0.09
2	0.60	0.41	0.66	0.20	1.32	0.15
3	0.52	0.42	0.59	0.39	1.51	0.08
4	0.55	0.32	0.32	0.18	1.28	0.11
5	0.76	0.36	0.15	0.18	1.01	0.17
6	0.43	0.38	0.21	0.15	0.98	0.08

表4. 进样重复性结果统计（n=6次重复）

Channel	%RSD (LC)		%RSD (HC)		%RSD (IgG)	
	MT	CAP %	MT	CAP %	MT	CAP %
B	0.37	0.36	0.39	0.15	1.53	0.10
C	0.50	0.37	0.55	0.24	1.01	0.11
D	0.56	0.33	0.58	0.35	0.98	0.19
E	0.44	0.21	0.54	0.21	0.86	0.15
F	0.48	0.16	0.32	0.35	1.43	0.22
G	0.62	0.30	0.29	0.20	1.26	0.18

5.5 迁移时间稳定性

计算各通道第一针和最后一针进样的SST（样品进样的前、后）主峰、轻链、重链的迁移时间偏差，结果见表5。所有峰进样前后的迁移时间的差值均 < 1 min。表明BioPhase 8800在序列分析过程中迁移时间稳定性良好。

6. 结论

本文提出了多通道技术的方法学验证方案，并在BioPhase 8800 高通量毛细管电泳分析系统上进行了CE-SDS方法验证工作：方法特异性好，样品制备重复性、进样重复性各峰迁移时间RSD值均 < 2 %，校正峰面积百分比RSD值均 < 0.5 %。各峰在浓度0.5 mg/mL-1.5 mg/mL之间表现出较好的线性， $R^2 > 0.99$，准确度及回收率在93.80 %-104.18 %，定量限结果低至0.0004 mg/mL。

表5. 各通道第一针、最后一针SST迁移时间偏差统计

Channel	MT偏差（min）		
	LC	HC	IgG
A	0.50	0.64	0.49
B	0.55	0.71	0.34
C	0.65	0.62	0.41
D	0.60	0.78	0.56
E	0.53	0.73	0.66
F	0.63	0.68	0.65
G	0.65	0.71	0.58
H	0.60	0.63	0.66

上述实验24小时内即可完成，在保证实验结果稳定可靠的前提下充分发挥了多通道设备的优势，表明BioPhase 8800具备优异的CE-SDS蛋白纯度分析能力，可广泛用于生物制药企业蛋白药物的高通量分析。

7.参考文献

[1] Nunnally, B et al. A Series of Collaborations Between Various Pharmaceutical Companies and Regulatory Authorities Concerning the Analysis of Biomolecules Using Capillary Electrophoresis. Chroma 64, 359–368 (2006).

[2] Li, Meng et al. Interlaboratory method validation of capillary electrophoresis sodium dodecyl sulfate (CE-SDS) methodology for analysis of mAbs. Electrophoresis vol. 42,19 (2021): 1900-1913.

[3] 国家药典委员会. 中华人民共和国药典：三部[S]. 北京：中国医药科技出版社，2015：296-297.

[4] SCIEX Tech Note: High-throughput multi-capillary SDS gel electrophoresis of proteins. RUO-MKT-02-13394-A.

[5] SCIEX Tech Note: Lightning capillary electrophoresis sodium dodecyl sulfate (CE-SDS) workflow for high-throughput analysis of biotherapeutics. RUO-MKT-02-14803-A.

A comparison between IgG HR CE-SDS on the PA 800 Plus system and the equivalent assay on the BioPhase 8800 system

Marcia Santos, Zoe Zhang, SCIEX, USA

Introduction

This technical note shows the side-by-side comparison between the CE-SDS IgG high resolution (HR) separation method on the PA 800 Plus system and the CE-SDS method on the BioPhase 8800 system for reduced and non-reduced monoclonal antibody (mAb) samples. Sample preparation protocols and electropherograms using UV, laser-induced fluorescence (LIF) and native fluorescence (NF) detection are compared.

Key learning points

- The CE-SDS separation method on the BioPhase 8800 system is equivalent to the IgG HR separation on the PA 800 Plus system. Same reagents and capillary chemistry enable equivalent performance and facilitate method transfer between systems.
- Both methods are compatible with all detection types available on the systems, allowing for an intuitive setup and flexibility to meet different sensitivity needs.
- NF and LIF detection enable smoother baselines, facilitating integration and accurate quantitation of low-abundance analyte peaks. NF has the additional benefit of no sample labeling compared to LIF.

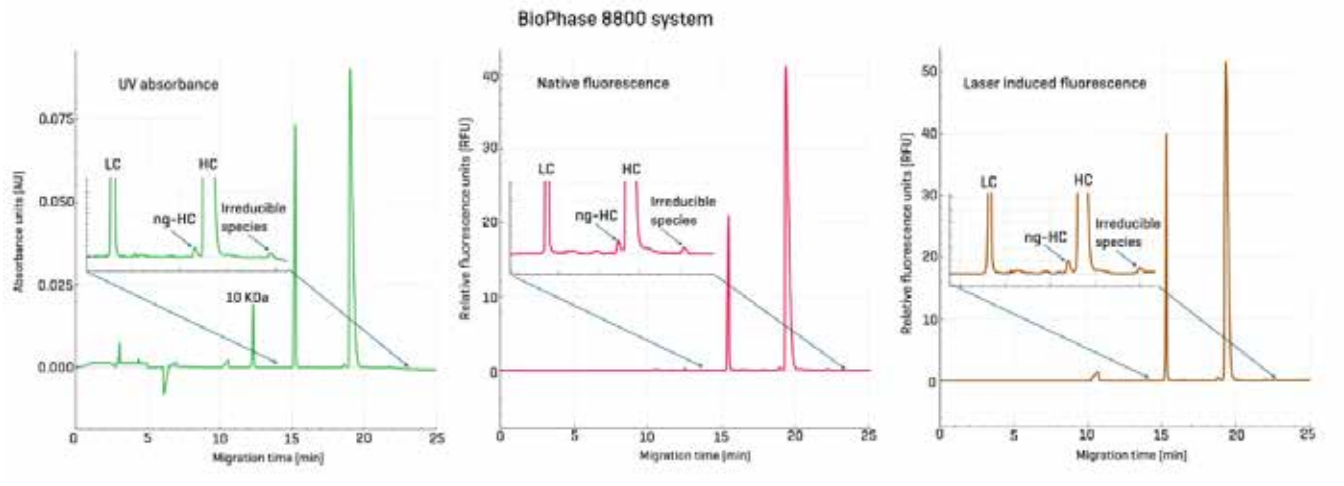


Figure 1 CE-SDS separation profiles of reduced infliximab using the BioPhase 8800 system using 3 detection modes: UV (left), NF (center) and LIF (right). Methods and detector conditions are in the materials section. Legend: LC: light chain; ng-HC: non-glycosylated heavy chain; HC: heavy chain.

Materials

The BioPhase CE-SDS Protein Analysis Kit (P/N: C30085), the BioPhase BFS capillary cartridge - 8 x 30 cm (P/N 5080121), BioPhase sample and reagent plates (4,4,8) (P/N 5080311), NISTmAb (P/N 5089359) and the pre-assembled bare-fused silica capillary cartridge (P/N A55625) were from SCIEX (Marlborough, MA). The β-mercaptoethanol (β-ME) (P/N: M3148-25ML), Iodoacetamide (IAM) (P/N A3221) and Chromeo P503 dye (P/N 30693) were obtained from Sigma-Millipore. Infliximab was purchased from Myonex (Horsham, PA).

Sample preparation for UV or NF detection:

Take 10 µL of a 10 mg/mL NISTmAb or infliximab and mix with 90 µL of sample buffer, add 2 µL of the 10 kDa Internal Standard and 5 µL of β-ME for reduced condition or 5 µL of 250 mM of iodoacetamide (IAM) for non-reduced condition. The mixture was incubated at 70 °C for 10 minutes.

Sample preparation for LIF detection:

Chromeo P503 dye stock solution preparation:

Add 200 µl reagent grade methanol to lyophilized Chromeo P503 (1 mg) to make a 5 mg/ml stock solution. Make 10 µL aliquot and store at -20°C.

Chromeo P503 dye working solution preparation:

Take one 5 mg/mL aliquot from the freezer and dilute in water to make a 1 mg/mL solution and keep in the dark until use.

Preparation of reduced mAb:

Take 10 µL of a 10 mg/mL NISTmAb or infliximab and mix with 90 µL of sample buffer and 5 µL of β-ME. Incubate at 70°C for 10 minutes. To the reduced sample add 2 µL of Chromeo P503 dye working solution and incubate for 10 min at 70°C. Take 10 µL of the reduced sample and add 45 µL of ddi water and incubate in the dark for 5 minutes to stop the reaction. Finally, add 45 µL of sample buffer prior to CE separation.

Capillary electrophoresis instruments: The PA 800 Plus system (P/N A66528) equipped with a photodiode array detector and LIF detector was from SCIEX (Marlborough, MA). The data acquisition was performed using 32 Karat software v. 10, and analysis was performed using the BioPhase 8800 system software V1.5. The BioPhase 8800 system (P/N: 5314860) equipped with UV, LIF and NF was from SCIEX (Marlborough, MA). The BioPhase 8800 system and the BioPhase 8800 software, version 1.5 e-license, were used to create methods and sequences for data acquisition and analysis.

Instrument methods: CE-SDS separation for the BioPhase 8800 system and the IgG HR separation method for the PA 800 Plus system were used, as described in the respective application guide, which can be downloaded at SCIEX.com/support.

Discussion

Instrument methods and conditions used in UV, NF, and LIF detection modes

The CE-SDS methodology involves heat denaturing of a specified concentration of protein in the presence of SDS. After denaturation, the sample components are separated by size into a capillary containing a replaceable SDS-polymer matrix. The matrix provides the sieving selectivity for the separation, with a focus on high-resolution or baseline separation between the non-glycosylated heavy chain (ng-HC) and the glycosylated heavy chain (HC).

This separation is routinely performed using the PA 800 Plus system, a single capillary platform capable of separating 24 samples in approximately 18 hours of uninterrupted operation.

The BioPhase 8800 system, a multi-capillary platform, can deliver the analysis of a 96-well plate in a similar time frame, significantly increasing the throughput.

Both systems are equipped with UV absorbance and LIF detection capabilities, whereas NF is only available on the BioPhase 8800 system. The separation methods on the BioPhase 8800 system and the PA 800 Plus system are very similar and share the exact same chemistry and sample preparation protocols.

Table 1 summarizes the key parameters for high-resolution IgG separations, which also apply to other antibody modalities such as ADCs, bi-/tri-specifics, nanobodies, and AOCs. The PA 800 Plus system and BioPhase 8800 system use identical separation voltage, injection settings, and UV/LIF wavelengths. NF detection is the only differentiating feature, available exclusively on the BioPhase 8800 system. Table 2 displays the sample preparation conditions, which are identical for both systems.

Table 1. Critical separation parameters comparison between PA 800 Plus system and BioPhase 8800 system.

Critical separation parameters	PA 800 Plus system	BioPhase 8800 system
Method name	IgG HR* separation	CE-SDS separation
Capillary type	Bare fused silica	
Capillary dimensions	Effective length: 20 cm; total length: 30 cm	
Capillary temperature	25°C	
Separation voltage	15 kV (cathodic)	
Injection voltage	5 kV (cathodic)	
Separation duration	30 min (reduced); 40 m in (non-reduced)	
Detection type and wavelength	220 nm (PDA or UV filter)	220 nm UV filter
	LIF (520 nm excitation and 600 nm emission)	LIF (520 nm excitation and 600 nm emission)
	Not applicable	NF (280 nm excitation and 350 nm emission)

*High-resolution

Table 2. Sample preparation for CE-SDS separation.

Critical sample preparation parameters	PA 800 Plus system	BioPhase 8800 system
Sample type	Reduced; non-reduced	
Internal marker	10 kDa (2 µL per sample preparation – 100 µL)	
Sample concentration	UV and NF: 1 µg /µL; LIF: approximately 0.01 µg/µL (after final dilution)	
Compatible reducing agents	β-ME (5 µL/100 mg of protein); 0.5 M of dithiothreitol (5 µL/100 µg of protein)	
Heat denaturation temperature and duration	70°C / 10 minutes	

Separation profile comparability of reduced infliximab and non-reduced NISTmAb using UV, NF, and LIF detection modes.

Figure 2 and 3 showcase typical CE-SDS of reduced infliximab and non-reduced NISTmAb profiles. While the separation profiles show great comparability, upon close inspection, the insets show how the baseline changes with each detection mode. From the 3 detection modes on the BioPhase 8800 system, the LIF and NF consistently showed a lower baseline root mean square (RMS) noise of 0.02, while UV has an RMS noise of 6.99. The smoother baseline enables more reliable integration for the quantitation of low abundant species.

Table 3 demonstrates that the corrected peak area (CPA) percentages for the key analytes—light chain (LC), non-glycosylated heavy chain (ng-HC), and heavy chain (HC)—are highly consistent between the two platforms when analyzed using UV detection. Comparable agreement is also observed for CPA% values obtained using LIF detection. The observed differences across detection modalities arise from the

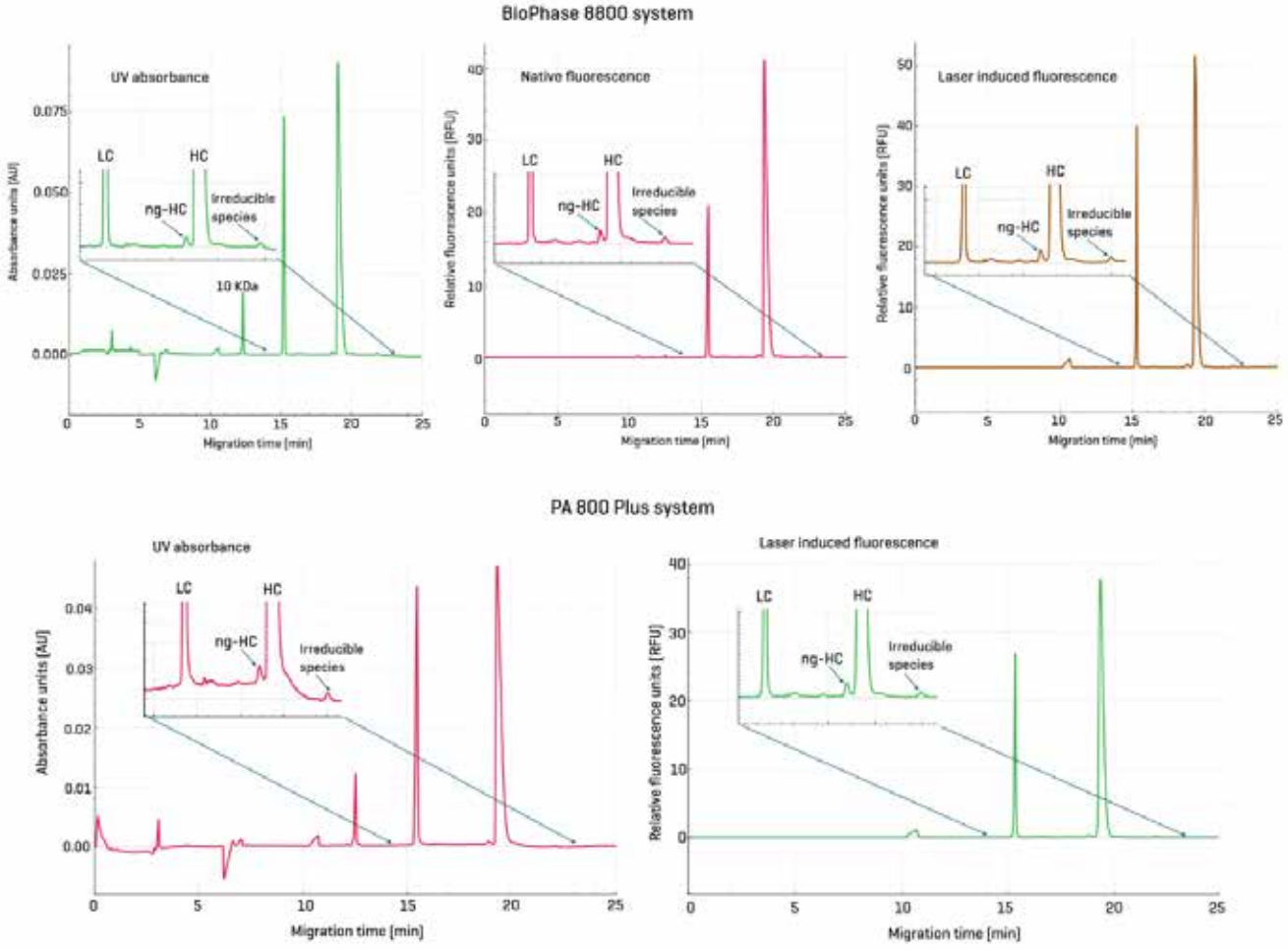


Figure 2. Top panel: CE-SDS separation profiles of reduced infliximab using the BioPhase 8800 system using 3 detection modes: UV (left), NF (center) and LIF (right). Bottom panel: CE-SDS separation profiles of non-reduced NISTmAb using the PA 800 Plus system. Methods and detector conditions are in the materials section. Legend: LC: light chain; ng-HC: non-glycosylated heavy chain; HC: heavy chain.

fundamental physicochemical principles governing each technique.

UV absorbance at 220 nm primarily reflects the strong absorbance of peptide bonds, which are common to all protein species. In contrast, LIF signal intensity is influenced by the fluorescent dye's quantum yield and the availability of accessible primary amines for labeling, which can vary among protein fragments. NF detection is driven largely by the intrinsic fluorescence of aromatic amino acids, particularly tryptophan.

Table 3: HC/LC and CPA% for reduced infliximab using the IgG HR method (PA 800 Plus system) and the equivalent method on the BioPhase 8800 system. *Corrected peak area percentage

	PA 800 Plus system		BioPhase 8800 system		
Average values (*n*=6)	UV	LIF	UV	LIF	NF
CPA* % LC	32.87	22.11	30.25	22.35	21.39
CPA % ng-HC	0.44	0.50	0.45	0.58	0.54
CPA % HC	66.42	76.71	68.68	76.44	77.33
HC/LC	2.02	3.47	2.27	3.42	3.61

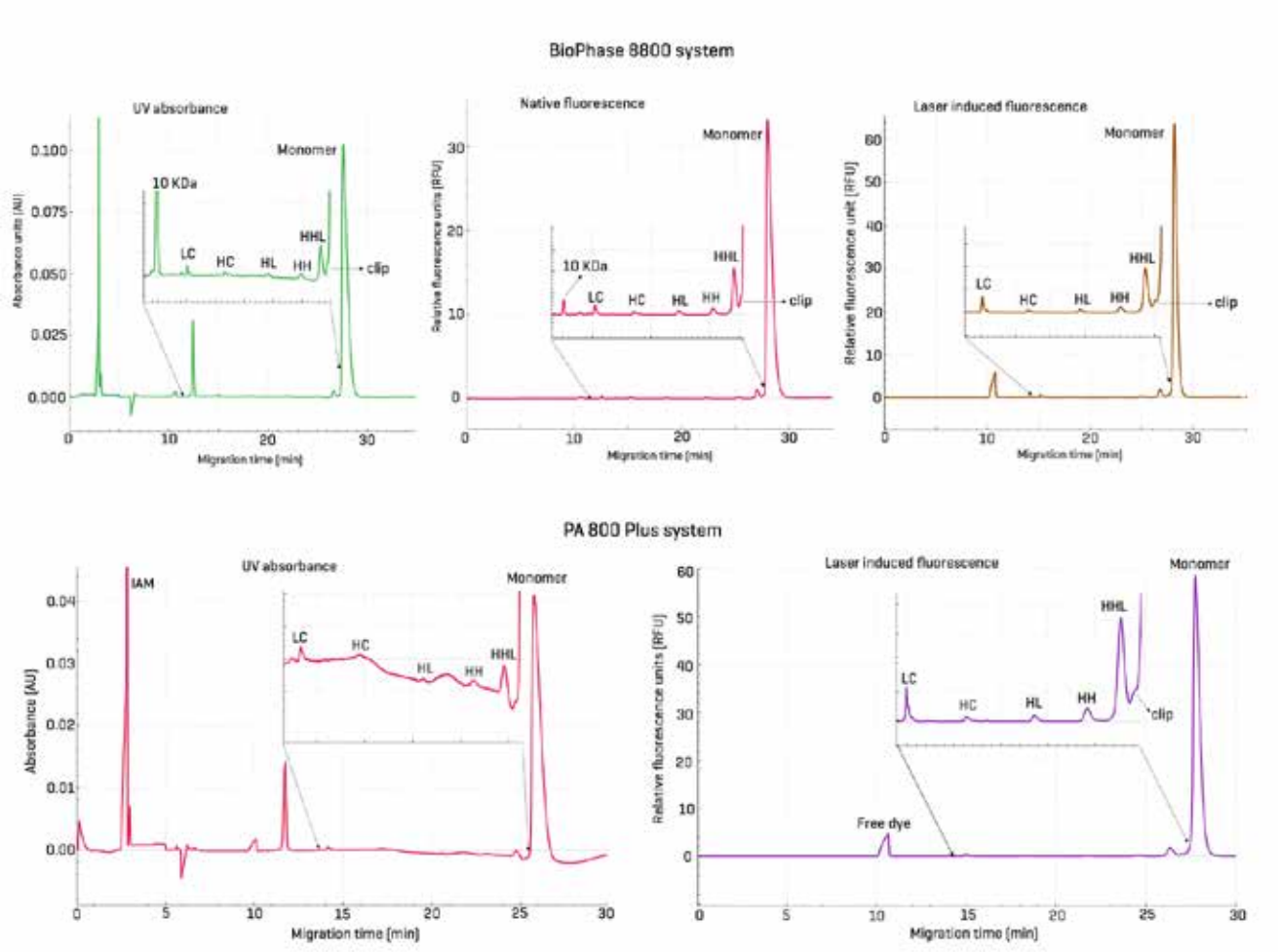


Figure 3. Top panel: CE-SDS separation profiles of non-reduced NISTmAb using the BioPhase 8800 system, using 3 detection modes, UV (left), NF (center) and LIF (right). Bottom panel: CE-SDS separation profiles of non-reduced NISTmAb using the PA 800 Plus system. Methods and detector conditions are in the materials section. Legend: LC: light chain; ng-HC: non-glycosylated heavy chain; HC: heavy chain, HL: heavy-light chain, HH: heavy-heavy chain, HHL: heavy-heavy-light chain.

In summary, regardless of the detection mode used, the species found using the popular UV detection was also found in LIF and NF detection modes.

Signal normalization to correlate detector response

A strategy to normalize the HC/LC ratio differences is to normalize the corrected peak area obtained from each detection mode by the relevant number of Trp for NF, Lyz for LIF, and number of amino acids (aa) for UV detection.[3]

Table 4 shows the number of Trp, Lys, and aa, which theoretically are responsible for the detector's response.

Table 4: Number of Trp, Lyz, and aa in infliximab

Infliximab	# Trp	# Lys	# aa
LC	3	10	214
HC	9	33	450

Table 5 summarizes the HC/LC ratios before and after detector-specific normalization. Because the expected molar HC-to-LC ratio is 1:1, normalized values should ideally approach one. After normalization, the ratios converge toward this value; however, slight deviations remain—approximately 1.1 for UV and NF and 1.0–1.2 for LIF—reflecting inherent differences in detection mechanisms rather than true compositional variation.

Table 5: HC/LC ratios comparison between UV, NF, and LIF detection modes before and after normalization

	HC/LC	Normalized HC/LC
UV	2.3	1.1
NF	3.6	1.1
LIF	3.4	1.0

Purity analysis

The %Purity values show strong agreement between the two CE platforms. On the PA 800 Plus system, purity measured by UV detection (n=6) was 97.57%, while LIF detection (n=6) produced a slightly lower value of 95.45. On the BioPhase 8800 system, UV detection (n=6) yielded a comparable purity of 97.60%, with LIF (n=3) at 95.70% and NF detection (n=6) at 96.70%. The NIST reported purity is 97.87%. As expected, the monomer purity decreases slightly due to the higher sensitivity of the LIF and NF detection schemes.

NF detection mode enables better sensitivity and peak integration

Figure 4 compares the UV (bottom) and NF (top) baselines for the non-reduced NISTmAb separation from 18-35 minutes, where low-abundance fragments typically appear. The high molecular weight (HMW) species migrating at ~ 33 minutes is clearly resolved with a flat baseline in NF detection, compared to baseline waviness in UV detection, making accurate integration difficult.

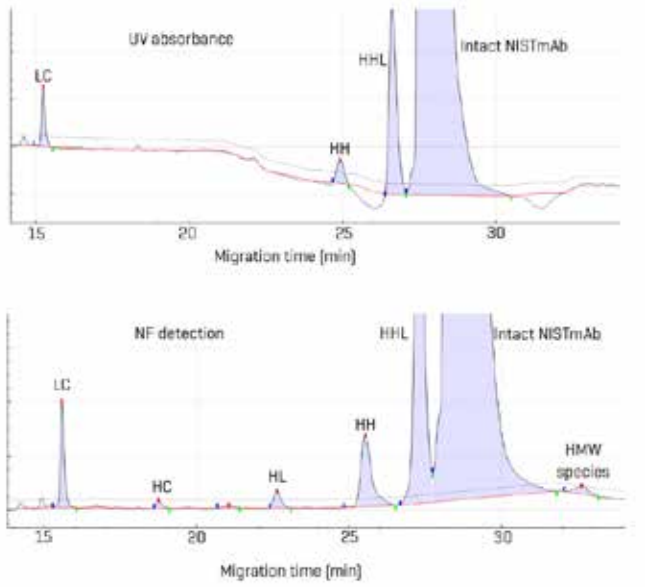


Figure 4. CE-SDS separation of NISTmAb under non reducing conditions. Top panel: UV detection; Bottom panel: NF detection

Conclusions

- Overall, both platforms deliver equivalent CE-SDS performance, enabling interchangeable use for analytical characterization workflows
- All three detection modes—UV, NF, and LIF—produce consistent electropherogram patterns across platforms, confirming cross-platform method equivalency
    - Strong alignment in HC/LC ratios and CPA%, across detection modes and CE platforms
    - The monomer purity is also highly comparable, with minor differences arising from detection sensitivity rather than separation differences. Results that corroborate NIST's finding.
- The increased sensitivity of NF and baseline stability without time consuming sample labeling enables the detection and reliable integration of low abundance species

References

1. Z. Yang, M. Santos, T. Li, M. Pulido, J. Luo and S. Mollah; Lightning capillary electrophoresis sodium dodecyl sulfate (CE-SDS) workflow for high-throughput analysis of biotherapeutics, RUO-MKT-02-14803-A.

2. A. Turner, K. Yandrofski, S. Telikepalli, J. King, A. Heckert, J. Filliben, D. Ripple and J. E. Schiel; Development of orthogonal NISTmAb size heterogeneity control methods; Analytical and Bioanalytical Chemistry, 410(2018)2095–2110.

3. T. Li and S. Mollah, Enhanced AAV capsid protein analysis using CE-SDS with Native Fluorescence (NF) detection on the BioPhase 8800 System, 2025, MKT-36129-A

Using dithiothreitol as reducing agent in CE-SDS analysis with Native Fluorescence detection

Marcia Santos, Tingting Li, SCIEX, USA

Capillary electrophoresis–sodium dodecyl sulfate (CE-SDS), performed under both reducing and non-reducing conditions, is a widely used analytical technique in biopharmaceutical workflows, including upstream bioprocessing, analytical development, and quality control. The reduction of disulfide bonds facilitates complete denaturation and dissociation of higher-order structures. β-Mercaptoethanol (BME) is a commonly used reducing agent for this purpose, effectively converting disulfide linkages into free sulfhydryl groups and disrupting tertiary and quaternary protein structures.

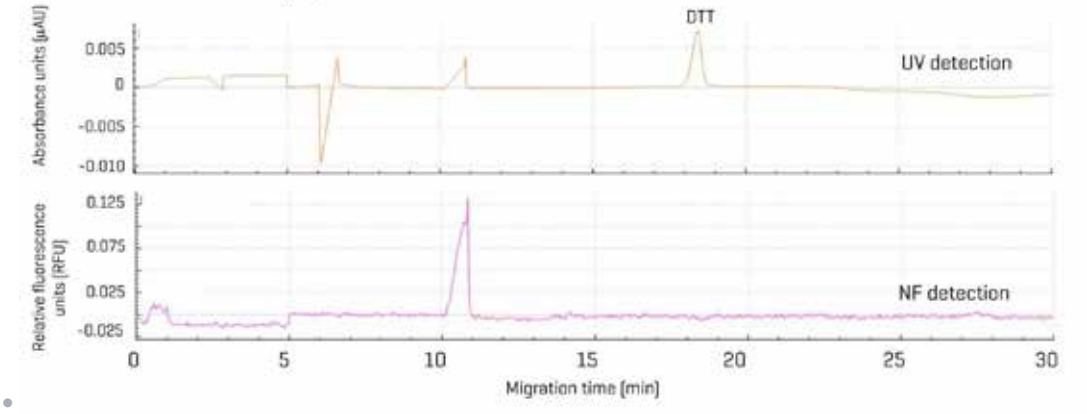
Despite its effectiveness, the use of BME is associated with significant safety concerns due to its strong odor and toxicity, necessitating handling in a chemical fume hood. Consequently, many laboratories restrict or prohibit the use of BME in both manual and automated sample preparation workflows.

Dithiothreitol (DTT), also known as Cleland's reagent, is frequently used as an alternative reducing agent. However, DTT exhibits strong ultraviolet (UV) absorbance at 220 nm (Figure 1, top trace) which can result in a prominent interfering peak in CE-SDS electropherograms when UV detection is used. This interference complicates data interpretation and has limited the adoption of DTT in CE-SDS workflows relying on UV detection.

This technical note demonstrates that, despite its UV absorbance, DTT does not interfere with CE-SDS analysis when native fluorescence (NF) (Figure 1, bottom trace) detection is employed. By leveraging NF detection, the use of DTT as a reducing agent in CE-SDS becomes viable, enabling safer and more flexible sample preparation without compromising analytical performance.

Key learning points

- **Enable safer CE-SDS workflows** by replacing BME with low-odor, automation-friendly DTT.
- **Eliminate DTT-related interference** by pairing DTT reduction with native fluorescence (NF) detection.
- **Maintain analytical confidence,** as DTT delivers CE-SDS-NF results comparable to the BME gold standard, with <1% CPA difference for major species (LC and HC).
- **Enables easy automation setup:** no fume hood required
- **Simplify method adoption** with a robust, high-throughput-ready CE-SDS solution without compromising CQA assessment



- Figure 1. CE-SDS overlay of sample buffer pH 9 and DTT using UV (top) and NF (bottom) detection.

Introduction

DTT functions primarily as a reducing agent that cleaves disulfide bonds in proteins and peptides, thereby preventing the formation of both intramolecular and intermolecular disulfide linkages between cysteine residues.[1,2] By disrupting these covalent bonds, DTT promotes protein denaturation and facilitates effective separation under reducing CE-SDS conditions. When comparing DTT with BME, each reducing agent presents distinct advantages and limitations. DTT is a stronger and more effective reducing agent; however, it is considerably less stable than BME and must be prepared fresh. In contrast, BME is more chemically stable but is highly volatile and characterized by a strong, unpleasant odor. DTT's significantly reduced odor improves laboratory handling conditions and overall user safety. Consequently, DTT is often the reducing agent of choice for use with liquid handling systems in high-throughput sample preparation workflows.

The reducing action of DTT occurs via thiol–disulfide exchange reactions. DTT contains two thiol (–SH) groups that donate electrons to disulfide (S–S) bonds within proteins, converting them into free thiol groups. This mechanism typically proceeds through two sequential steps: the formation of a mixed disulfide intermediate between DTT and the target disulfide bond, followed by intramolecular cyclization of DTT to form a stable six-membered ring and the subsequent release of the reduced protein.[1,2]

The reducing efficiency of DTT is pH-dependent, with optimal activity observed at pH values above 7. In contrast, BME maintains effective reducing capability over a broader pH range, which may influence reducing agent selection depending on assay conditions.

Materials

The BioPhase CE-SDS Protein Analysis Kit (P/N: C30085), the BioPhase BFS capillary cartridge - 8 x 30 cm (P/N: 5080121), BioPhase sample and reagent plates (4,4,8) (P/N: 5080311), NISTmAb (P/N 5089359) were from SCIEX (Marlborough, MA). The BME (P/N: M3148-25 mL), DTT (P/N 20290) were obtained from Sigma-Millipore (St Luis, MO).

Preparation of 0.5M DTT solution: Using an analytical balance, weigh 77 mg of DTT and mix with 1 mL of distilled and de-ionized (ddi) water. This solution must be prepared fresh and discarded after use.

Preparation of sample blanks: Take 95 µL of sample buffer pH 9 and mix with 5 µL of 0.5M DTT solution, mix well. Incubate for 10 minutes at 70 °C. Let the sample cool to room temperature before CE experiment.

Sample preparation for UV or NF detection: Mix well 10 µL of NISTmAb with 90 µL of sample buffer pH 9 and 5 µL of the 10 kDa internal standard and 5 µL of BME or DTT. Incubate for 10 minutes at 70˚C. Let the sample cool to room temperature before the CE experiment.

Capillary electrophoresis instrument: The BioPhase 8800 system (P/N: 5314860) equipped with UV, LIF, and NF was from SCIEX. The BioPhase 8800 system and the BioPhase 8800 software, version 1.5 e-license, were used to create methods and sequences for data acquisition and analysis.

Instrument methods: This work used the lightning CE-SDS separation method for the BioPhase 8800 system, which can be downloaded at SCIEX.com/support.

Discussions

DTT interfering peak using UV detection

Figure 2 compares the CE-SDS-UV electropherogram of NISTmAb reduced with DTT (top trace) to that of a sample blank with DTT (bottom trace). The results clearly show that DTT migrates in a region typically occupied by several minor product-related impurity species. Notably, the DTT peak migrates in close proximity to the non-glycosylated heavy-chain (ng-HC) species, which is an important critical quality attribute (CQA) due to its classification as a product impurity.[3] Consequently, despite the advantages of DTT as a reducing agent, its co-migration with critical impurity species renders it unsuitable for reduced CE-SDS analysis when UV detection is employed.

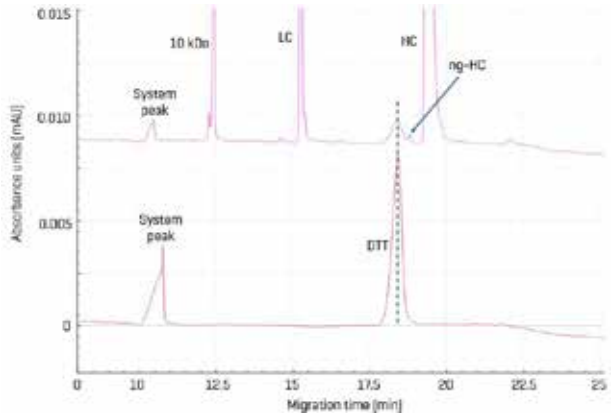


Figure 2. CE-SDS-UV (220nm) overlay of NISTmAb reduced with DTT and sample blank with DTT.

DTT and NF detection

Although DTT is not compatible with reduced CE-SDS analysis when UV detection is used, a different outcome is observed when native fluorescence (NF) detection is used. Figure 1 presents an overlay of CE-SDS separations of the sample blank (containing only DTT) acquired using UV detection (top trace) and NF detection (bottom trace). While a prominent DTT peak is evident in the UV electropherogram, no corresponding signal is observed under NF detection conditions.

The absence of a detectable DTT signal with NF detection demonstrates that DTT does not cause detectable interference and is therefore compatible with reduced CE-SDS analysis using NF detection.

Comparison: DTT and BME using NF detection

BME has traditionally been the reducing agent of choice for CE-SDS-based separations.[4] Therefore, a direct comparison of CE-SDS separation profiles of NISTmAb reduced with DTT and BME is essential to assess the suitability of DTT as an alternative reducing agent. Figure 3 presents an overlay of CE-SDS-NF separations of NISTmAb reduced with DTT (top trace) and BME (bottom trace), demonstrating a high degree of similarity between the two profiles.

To confirm this observation, table 1 summarizes the corrected peak area percentages (CPA%) obtained from the separation profiles in Figure 3.

To simplify the data analysis, the minor species peaks were grouped as follows: the minor species before the LC were integrated together as group 1, while species migrating between the heavy chain (HC) and LC were assigned to Groups 2, 3, and 4.

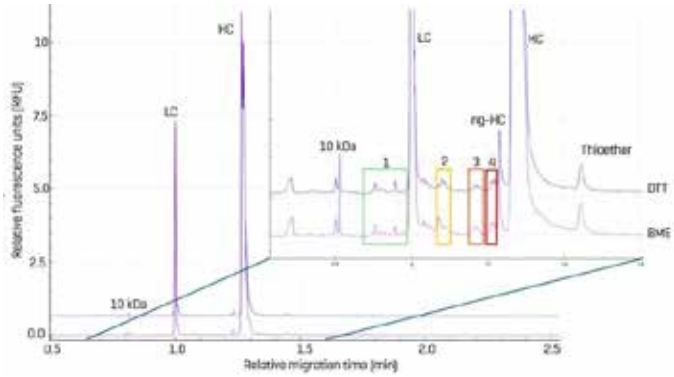


Figure 3. Overlay of reduced CE-SDS-NF separations of NISTmAb using DTT (top) and BME (bottom). The minor peaks inside the rectangles labelled 1, 2, 3 and 4 were grouped for simplification purposes only.

The results shown in Table 1 confirm the similarity in performance between DTT and BME. The difference between the CPA% for major species such as LC and HC are 0.84% and 0.28% respectively, and identical normalized HC/LC to the number of tryptophan residues (Trp).[5] This work demonstrates that DTT can safely and reliably be used for reduced CE-SDS analysis using NF detection.

Table 1. CPA% of the NISTmAb reduced species found in CE-SDS-NF from Figure 3 (n=3).

Peak group name	CPA% (BME)	CPA% (DTT)
1	0.26	0.28
LC	22.15	22.34
2	0.31	0.35
3	0.11	0.11
4	0.16	0.15
ng-HC	0.59	0.50
HC	76.01	75.80
Thioether	0.41	0.47
HC/LC (normalized to the # of Trp)	1.1	1.1

Conclusions

- DTT does not show interference in CE-SDS-NF analysis
- BME and DTT exhibit comparable performance in reduced CE-SDS-NF analysis of NISTmAb, with similar separation profiles, purity (CPA%), and HC/LC ratios (Table 1)
- DTT is associated with reduced odor and lower toxicity relative to BME , enabling safer and more convenient handling
- The lower volatility of DTT may facilitate liquid handling and support high-throughput workflows by reducing the need for chemical fume hoods

References

1. I. B. Santarino, S. C. B. Oliveira, A. M. Oliveira-Brett; Protein reducing agents dithiothreitol and tris(2-carboxyethyl) phosphine anodic oxidation; Electrochemistry communications 23(2012)114-117.
2. M. Dadouch, Y. Ladner and C. Perrin; Analysis of Monoclonal Antibodies by capillary electrophoresis: sample preparation, separation, and detection; Separations 8(2021)4.
3. Liu GY, Kim JK, Tang S, Yan Y, Hopkins M, Laredo D, Yang TC, Mutino J, Kamen DE, Graham KS, Shameem M, Wang S, Li N. The non-glycosylated variant in therapeutic monoclonal antibodies preferentially forms large aggregates under typical thermal stresses used in forced degradation studies. Mabs 17-1(2025)2543768.
4. A. Guo, G. Camblin, M. Han, C. Meert and S. Park; Role of CE in biopharmaceutical development and quality control; Capillary electrophoresis methods for pharmaceutical analysis, Elsivier, Amsterdam 2008, pp.357-400.
5. M. Santos and Z. Zhang, The CE-SDS lightning separation method using the BioPhase 8800 system with UV, LIF and NF detection; SCIEX technical note.

Parallel charge variant analysis of conventional and new modality therapeutic proteins by multi-capillary isoelectric focusing

Fang Wang, Sahana Mollah
SCIEX, USA

Introduction

As biologics pipelines continue to grow, resulting in larger numbers of samples requiring characterization, the necessity for high throughput charge variant analysis is increasing in the biopharmaceutical industry. While existing capillary electrophoresis (CE) techniques offer rapid separation times with high reproducibility, they have historically have had a limited throughput capability due to a single capillary format. Because of this linear sampling approach, an assay like capillary isoelectric focusing, often used as a stability release assay, can be quite lengthy for even small sample sets, potentially requiring selective sampling strategies. The BioPhase 8800 system (Figure 2) employs a multi-capillary separation approach, enabling parallel CE analysis for up to eight samples simultaneously, potentially accelerating and expanding the analysis.

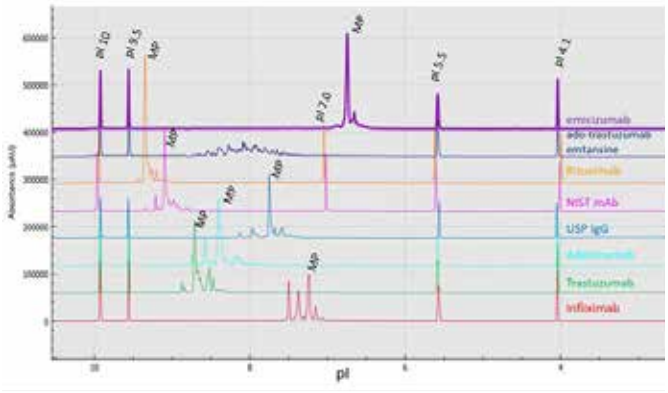


Fig. 1. Overlay of a single cIEF separation of six biotherapeutic molecules and two reference standards (NIST mAb and USP IgG). Separations were performed using the BioPhase 8800 system and an 8-capillary cartridge. The X-axis represents calibrated isoelectric point (pI) values identified using the following pI markers which were co-injected with each of the samples: 10.0, 9.5, 7.0, 5.5, and 4.1, MP indicates the main charge isoform peak for each sample. Separation conditions: each capillary in the multi-capillary cartridge had a Neutral coating to minimize electro-osmotic flow, 50 μm I.D., 30 cm Length total (20 cm Length effective); focusing was performed at 25 kV for 15 min; mobilization was performed at 30 kV for 30 min; applied voltage was normal polarity; capillary temperature was controlled at 20oC



Fig. 2. The BioPhase 8800 system

Monoclonal antibodies (mAbs) and related new therapeutic modalities are the focus of current biopharmaceutical development [1] in the treatment of numerous diseases including cancer, autoimmune disorders [2], and even SARS-CoV-2.[3] During manufacturing, unpredictable heterogeneity due to co- and post-translational modifications, aggregation, or degradation,[4] caused by improper formulation or storage conditions,[5] can lead to decreased clinical efficacy or immunogenicity. In order to monitor protein stability, a high resolution, robust bioanalytical method is critical to assess charge isoform variation

Key features

- Versatile multi-capillary system, supporting parallel charge variant analysis
- Assay capable of assessing protein therapeutic stability through charge isoform separation
- Streamlined workflow including reagents, lab supplies, and pre-built 8-capillary cartridges
- Intuitive, user-friendly software enables fast batch data analysis with minimum user intervention

Capillary isoelectric focusing (cIEF) is one of the most effective techniques for charge heterogeneity characterization of monoclonal antibodies (mAbs) [6] and other therapeutic modalities such as antibody-drug conjugates (ADC).[7] This automated technique can effectively identify isoelectric points of therapeutic biomolecules and their charge variants. cIEF is applicable to release testing and is capable of providing quantitative information regarding peak area distribution in addition to pI values for the various charge isoform species. These data can be used not only in a quality control setting but also to inform manufacturing processes. [8]

In this technical note, a multi-capillary isoelectric focusing technique was used to analyze a wide range of protein therapeutics, which includes two IgG reference standards (NIST and USP), four therapeutic monoclonal antibodies (trastuzumab, rituximab, infliximab and adalimumab), a bispecific antibody (emicizumab) and finally an antibody-drug conjugate (ado-trastuzumab emtansine). Rapid characterization of forced degradation based stability testing products is also demonstrated using the BioPhase 8800 system.

Material and methods

Chemicals: The NIST mAb (RM 8671) reference material 8671 was purchased from NIST (Gaithersburg, MD) and the USP IgG (P/N 1445550) was from Sigma (St. Louis, MO). The four monoclonal antibody therapeutics of trastuzumab, rituximab, infliximab and adalimumab, the bispecific antibody emicizumab and an antibody drug conjugate ado-trastuzumab emtansine were all from Myonex (Norristown, PA). The BioPhase cIEF kit (P/N C30101) was from SCIEX. The cIEF kit includes: CE grade water, cathodic stabilizer, anodic stabilizer, cIEF anolyte, cIEF catholyte, cIEF chemical mobilizer, cIEF Urea, cIEF gel, cIEF neutral capillary conditioning solution, and cIEF formamide. BioPhase sample and reagent plate kits (P/N 5080311) containing 4 sample plate; 4 reagent plates and 8 outlet plates was from SCIEX (Framingham, MA). The carrier ampholyte mixture was Pharmalyte (P/N 17-0456-01) from Cytiva (Marlborough, MA) purchased from VWR. pI marker kit (P/N A58481) was from SCIEX (Framingham, MA).

Forced degradation conditions: NIST mAb, trastuzumab, rituximab, and infliximab, and ado-trastuzumab emtansine were separately buffer exchanged into 25 mM TrisHCl, pH 8 to a final concentration of 5 mg/mL and incubated at 40°C. Sample aliquots were taken out at day one and day five and immediately frozen at -80°C.

Sample preparation: Sample preparation reagents were provided in the BioPhase cIEF kit. The cathodic and anodic stabilizer solutions were prepared by adding DDI water to the pre-weighted reagent supplied in the cIEF kit. The samples were diluted to 5 mg/mL with CE grade water. For each sample, a master mix containing 200 µL urea-cIEF gel, 25 µL cathodic stabilizer, 3 µL anodic stabilizer, 12 µL Pharmalyte, and 2 µL of pI markers 10.0, 9.5, 7.0, 5.5, and 4.1 according to their neutral or basic characteristics (shown in Figure 1), were added and mixed thoroughly at room temperature. 100 µL of each sample were dispensed in wells A1 through H1 of a BioPhase sample plate.

Multi-capillary isoelectric focusing: The multi-capillary cIEF separations utilized the BioPhase 8800 system (P/N 5089278, SCIEX) equipped with UV and LIF detection. The cIEF separation was performed using a 8-capillary neutral coated cartridge (P/N 5080119). The instrument methods for cIEF conditioning, separation and shutdown are pre-loaded in the software and were used without modification. The BioPhase software was used for data processing.

Results and discussion

The versatility of the BioPhase 8800 system

Figure 1 shows the multi-capillary isoelectric focusing of 2 immunoglobulin reference standards and 6 commercial therapeutic biologics: NIST mAb and USP IgG molecules, trastuzumab, rituximab, infliximab and adalimumab, emicizumab, and ado-trastuzumab emtansine. In order to properly calibrate pI of the separation gradient, at least 3 pI markers were co-injected based on the estimated pI value of the sample peaks, e.g., in addition to the 10.0, 5.5, and 4.1 standards, the pI 9.5 marker was co-injected with the neutral species and the pI 7.0 with the basic species.

The pI values of the investigated protein samples covered the range of 6.5 to 9.5, with emicizumab being the most acidic (main peak pI=6.75) and rituximab the most basic (main peak pI=9.35), and the rest of them in between. Therefore, because of the basic character of the NIST mAb and rituximab, the pI 7.0 marker was co-injected with these samples instead of the pI 9.5 marker. In contrast, the opposite was administered for the rest of the samples since they were more acidic, or else the pI 7.0 marker would have interfered with the resulting profiles. Most notably, Figure 2 also highlights both the simplicity and complexity of the charge variant profiles, such as rituximab being the simplest while infliximab and ado-trastuzumab emtansine had the most complex profiles. The complexity of ado-trastuzumab emtansine profile is likely due to heterogeneity of the sample consisting of 2-8 conjugated small drug emtansine conjugation with the antibody. Table 1 lists the pI and corrected peak area% composition values for all

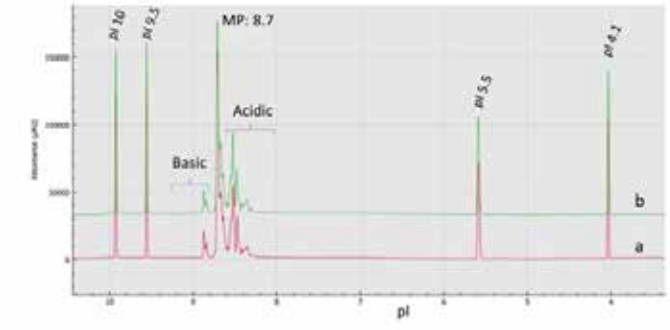


Figure 3. Evaluation of long-term storage at 4°C on the charge variant profile of trastuzumab. Experimental conditions are the same as Figure 1.

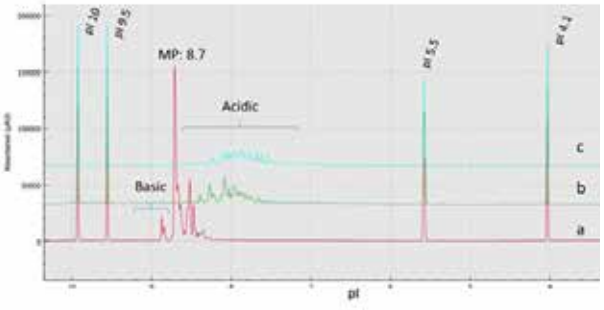


Figure 4. Forced degradation study of the trastuzumab. Trace a shows the typical profile of the unstressed sample. Trace b and c refer to the trastuzumab exposed to pH 8 buffer storage and 40**°C** for 1, and five days, respectively. Experimental conditions are the same as Figure 1.

investigated molecule basic, main, and acidic components. The %RSD from triplicate injections were RSD<1.2% and 0.03 pI unit precision. Due to the numerous multi-conjugated forms, it was challenging to pick out specific peaks. Hence statistical analysis for ado-trastuzumab emtansine is not reported.

Storage and forced degradation studies

Figure 3 compares the charge heterogeneity profiles of trastuzumab before (trace a) and after long-term (6 months) While the long-term storage of trastuzumab at 4°C did not reveal any changes in the charge variant profile (Figure 3), the forced degradation study of the same molecule kept at 40°C and in pH 8 storage buffer shows rapid decomposition (Figure 4). After one day of forced degradation, all sample isoforms shifted towards a more acidic pI (trace b).

In addition, significant degradation of the main isoform was observed, with peaks appearing in the acidic region possibly indicating deamidation, C-terminal lys cleavage or other modifications which causes the molecule to become more acidic. Orthogonal techniques such as middle down MS may help elucidate the degradation products. Further degradation and shift to a more acidic pI can be observed after five days of pH 8 and 40°C treatment (trace c). Comparable behavior was observed during the forced degradation of the other molecules investigated. Figure 5 shows the degradation profiles of NIST mAb (Panel A), rituximab (Panel B), infliximab (Panel C), and ado-trastuzumab emtansine (Panel D). Generally, trace a in each pane represents the unstressed sample. Interestingly, the degradation happened at different rates. Rituximab (Panel B) and ado-trastuzumab emtansine (Panel D) samples quickly degraded, for example, even the main component was not detectable after one day of stress (traces b). The NIST mAb (Panel A) and the infliximab (Panel C), on the other hand, showed slower degradation kinetics. These molecules only showed significant changes in the charge heterogeneity profile after five days under forced degradation stress conditions (traces c).

Table 1. Corrected peak area and corrected peak area composition (%), and pI values (of the highest peak) for the basic, main, and acidic groups for the molecules investigated in this study (n=3), CA (corrected area).

Molecule	Total CA	Basic pI	Main pI	Acidic pI	Basic CA%	Main CA%	Acidic CA%
Trastuzumab	23983	8.87	*8.71*	*8.52*	*6.00*	*60.5*	*33.5*
Rituzimab	26178	*9.44*	*9.35*	*9.26*	*2.30*	*77.9*	*19.8*
Infliximab	*16841*	*7.51*	*7.25*	*7.16*	*48.7*	*38.1*	*13.2*
Adalimumab	*23835*	*8.58*	*8.40*	*8.15*	*23.4*	*56.9*	*19.7*
Emicizumab	*17235*	*6.88*	*6.75*	*6.66*	*4.80*	*71.7*	*23.5*
NIST mAb	*19269*	*9.08*	*9.08*	*8.98*	*11.6*	*69.6*	*18.8*
USP IgG	*20981*	*7.95*	*7.77*	*7.70*	*21.3*	*50.7*	*28.0*

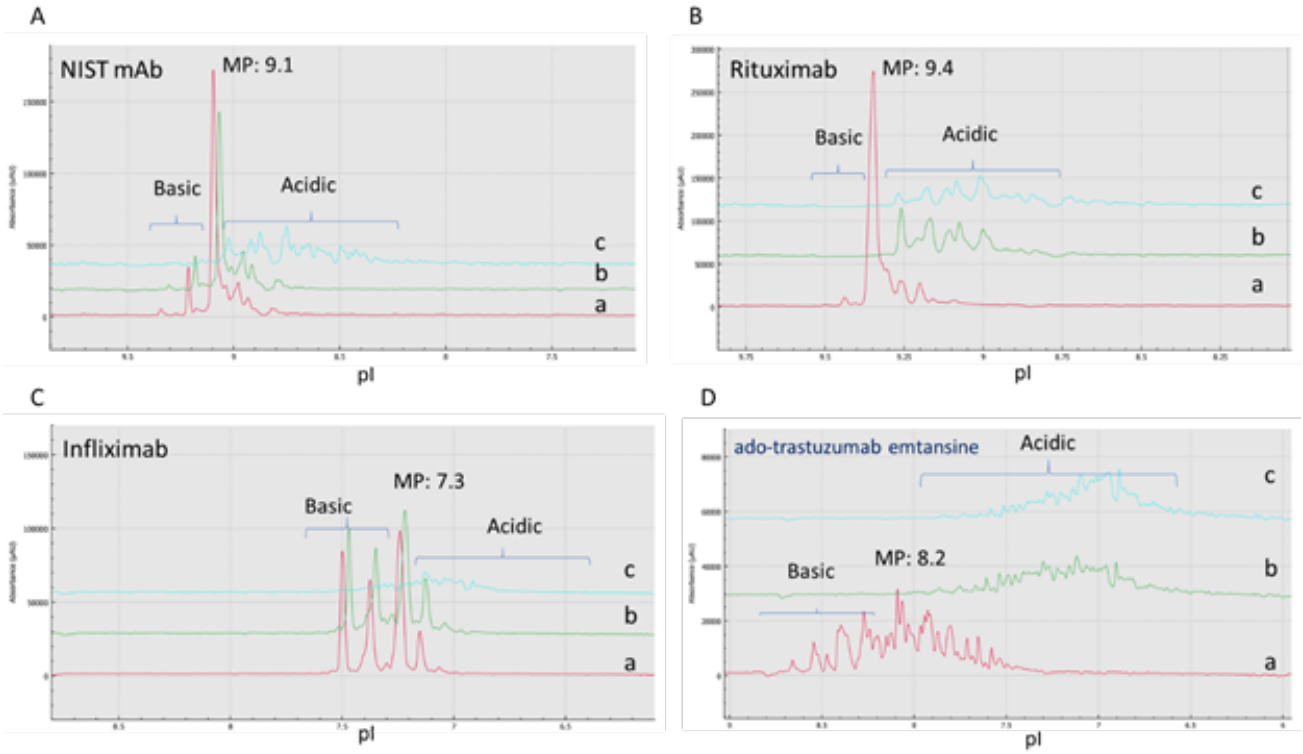


Figure 5. Detailed view of the cIEF traces before and after force degradation treatment of the NIST mAb (Panel A), the rituximab (Panel B), the infliximab (Panel C) and the ado-trastuzumab emtansine (Panel D) samples. Samples were stressed at 40°C in pH 8 storage buffer: one day (trace b) and five days (trace c). Trace a represents the unstressed sample in all panels. MP is the main component of the proteins investigated. Conditions for the BioPhase 8800 system were the same as in Figure 1.

Conclusions

- This technical note highlighted the multiplexing capability of the BioPhase 8800 system by analyzing six commercial biologics, including one bi-specific, one ADC, and two antibody reference standard products in only one hour.
- BioPhase 8800 system can deliver high-resolution charge heterogeneity analysis based on isoelectric focusing separation in a multi-capillary environment.
- The BioPhase 8800 system enables accelerated characterization and stability monitoring of biologics, such as shelf-life and forced degradation studies of different protein therapeutics in one batch. This study revealed a variable degradation kinetics the multi-capillary system was able to easily profile.

References

1. Strohl, W.R., Current progress in innovative engineered antibodies. Protein Cell, 2018. 9(1): p. 86-120.
2. Singh, S., et al., Monoclonal Antibodies: A Review. Curr Clin Pharmacol, 2018. 13(2): p. 85-99.
3. Toniati, P., et al., Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: A single center study of 100 patients in Brescia, Italy. Autoimmun Rev, 2020. 19(7): p. 102568.
4. Ogle, J.M. and V. Ramakrishnan, Structural insights into translational fidelity. Annu Rev Biochem, 2005. 74: p. 129-77.
5. Wang, W., et al., Antibody structure, instability, and formulation. J Pharm Sci, 2007. 96 (1): p. 1-26.
6. Salas-Solano, O., et al., Robustness of iCIEF methodology for the analysis of monoclonal antibodies: an interlaboratory study. J Sep Sci, 2012. 35(22): p. 3124-9.
7. Lechner, A., et al., Insights from capillary electrophoresis approaches for characterization of monoclonal antibodies and antibody drug conjugates in the period 2016-2018. J Chromatogr B Analyt Technol Biomed Life Sci, 2019. 1122-1123: p. 1-17.

8. Rogers, R.S., et al., A View on the Importance of "Multi-Attribute Method" for Measuring Purity of Biopharmaceuticals and Improving Overall Control Strategy. AAPS J., 2017. 20(1): p. 7.

BioPhase™ 8800 系统进行EPO电荷变异体的高通量分析

High-throughput analysis of EPO charge variants on BioPhase™ 8800 system

刘冬科，高铁，陈泓序，郭立海
Liu Dongke, Gao Tie, Chen Hongxu, Guo Lihai

SCIEX，中国
SCIEX, China

关键词： BioPhase™ 8800系统；cIEF；促红细胞生成素；等电点；电荷变异体；

1. 前言

促红细胞生成素（Erythropoietin, EPO）是一种高度糖基化的糖蛋白，携带不同的电荷变异体，分子具有微观不均一性。糖基化对稳定EPO的体内生物活性具有重要作用，因此电荷变异体的种类和含量是EPO产品质量控制的重要参数。 cIEF的方法可实现EPO电荷变异体的分离，变异体数目、峰面积百分比与欧洲药典CZE方法较一致[1]。同时，cIEF方法可获得每个变异体的精确等电点，在不同来源或不同批次EPO样品的比较中，等电点数据是不同变异体匹配和指认的重要信息。

作为高通量的毛细管电泳分析系统，BioPhase™ 8800系统通过8根平行的毛细管同时进行检测分析，以8倍的通量获得与PA 800 Plus同质量的数据，在保证分析效果和准确性的前提下，提高大量样本的分析检测效率。方法具有以下特点：

1. 同一样品电荷变异体的等电点、峰面积百分比、分离度在BioPhase™ 8800与PA 800 Plus上结果一致；
2. 方法通量高，速度快，平均每个样品仅用时4.5 min，显著提高了大量样本的分析检测效率。
3. 同一样品8通道间一致性高，各变异体pI值RSD < 0.2%，峰面积百分比RSD < 6.5%，分离度RSD < 5.5%。
4. 单通道连续6针进样重复性好，各变异体pI值RSD < 0.9%，峰面积百分比RSD < 5.5%，分离度RSD < 3.5%。

2. 仪器及方法

BioPhase™ 8800系统，配备紫外检测器（UV，波长280 nm），BioPhase软件1.2.20（SCIEX）。用于BioPhase™ 8800系统的中性涂层毛细管卡盒8 × 30 cm（SCIEX PN 5080119）；BioPhase™ 8800系统启动盘套装（SCIEX PN 5080311），包含4块96孔样品盘、4块缓冲液盘、8块出口盘。

BioPhase™ 8800 系统进行 EPO电荷变异体分析的cIEF分离方法：进样：25 psi，99 s；聚焦：-25 kV，6 分钟；化学迁移：用100 mM氨水来代替阳极液，-30 kV，30 分钟。数据采集频率：4 Hz；样品室温度：10 ℃；毛细管温度：25 ℃；检测波长：280 nm；

新卡盒预冲洗方法：CE 级水，70 psi 压力冲洗10 分钟；CE 级水，70 psi 压力冲洗10 分钟；中性毛细管冲洗液，70 psi 压力冲洗20 分钟。

预冲洗及关机冲洗方法：中性毛细管冲洗液，70 psi 压力冲洗5 分钟。

3. 试剂及样品

用于BioPhase™ 8800系统的毛细管等电聚焦（cIEF）试剂盒（SCIEX PN C30101），包含cIEF 胶，阳极液，阴极液，阳极稳定剂，阴极稳定剂，化学迁移剂，中性毛细管冲洗液，甲酰胺，尿素和CE 级水，按照试剂盒说明书制备相应浓度的试剂。pI 3.21 Marker、pI 5.91 Marker、pI 6.61 Marker（AES）。两性电解质（GE Healthcare）。Ultracel YM-10 滤膜（Millipore）。

欧洲药典EPO标准品（Eur，购自EDQM），用Ultracel YM-10 滤膜对其进行超滤浓缩处理，最终浓度为4 mg/mL。

Master Mix的配制：每份样品需要：90 µL 6 M 尿素-cIEF 胶、6.0 µL of 3-10 两性电解质、15.0 µL阳极稳定剂、各0.5 µL的pI Marker（3.21、5.91、6.61）。

最后，以20 µL样品与110 µL Master Mix比例进行混合，涡旋混匀。取100 µL加入样品盘一列的8个样品孔中，样品摆放见图1。

此时8个样品孔中的样品相同， 使用 BioPhase™ 8800仪器及BioPhase软件采集分析，考察单次进样8通道一致性，单通道连续进样6次的重复性。

另取100 µL样品，使用PA 800 Plus仪器及32Karat软件采集分析，比较同一样品在BioPhase™ 8800及PA 800 Plus两个仪器上的电泳结果。

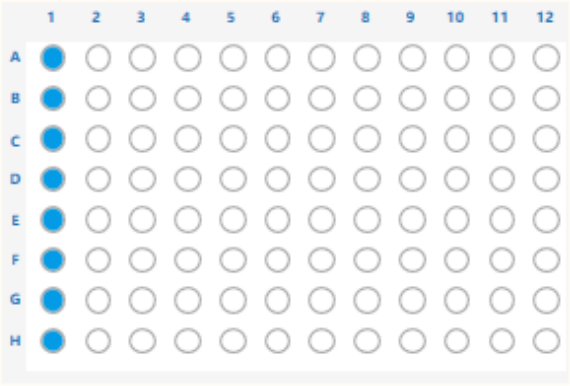


图1. BioPhase™ 8800样品盘摆放

4. 结果与讨论

4.1 同一样品使用 BioPhase™ 8800与PA 800 Plus采集结果分析

对于同一EPO样品，使用BioPhase™ 8800进行CIEF分析的电泳图见图2a，使用PA 800 Plus进行CIEF分析电泳图见图2b，两个仪器上均检测到EPO的8个电荷变异体，各变异体的pI值、峰面积百分比（Area%）、分离度（R）结果对比见表1，同一样品在两个仪器上的结果较一致。

4.2 BioPhase™ 8800多通道重复性结果分析

对8通道的样品同时连续进样6次，考察单次进样8通道间的一致性，以及单通道连续进样6针的重复性。

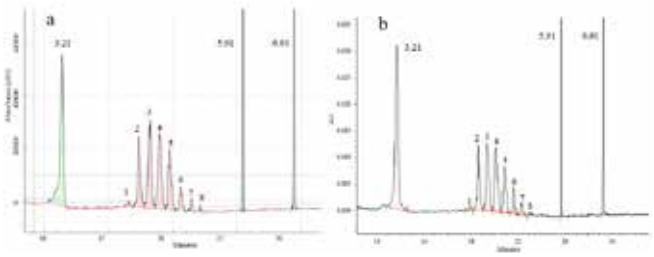


图2. 同一样品在不同仪器及软件中进行电荷变异体分析的CIEF电泳图（a：BioPhase™ 8800仪器，BioPhase软件，b：PA 800 Plus仪器，32Karat软件）

表1. 同一EPO样品在BioPhase™ 8800 与PA 800 Plus上电荷变异体分析结果的平均值（n=3）

Peak	pI		Area%		R	
	Biophase™ 8800	PA 800 Plus	Biophase™ 8800	PA 800 Plus	Biophase™ 8800	PA 800 Plus
1	4.25	4.25	1.37	1.41	14.77	15.39
2	4.39	4.39	19.14	19.51	1.81	1.80
3	4.57	4.57	28.36	28.51	2.12	2.06
4	4.71	4.70	25.75	25.51	1.57	1.46
5	4.85	4.85	16.86	16.96	1.51	1.66
6	5.01	5.01	6.07	5.76	1.87	1.98
7	5.17	5.17	1.87	1.79	2.62	2.71
8	5.29	5.29	0.59	0.56	2.72	2.76

单次进样8通道间表现出良好的一致性，电泳图见图3a，统计结果见表2。各变异体pI值RSD < 0.2%，峰面积百分比RSD < 6.5%，分离度RSD < 5.5%。

单通道6针进样重复性好，电泳图见图3b，统计结果见表3。各变异体pI值RSD < 0.9%，峰面积百分比RSD < 5.5%，分离度RSD < 3.5%。

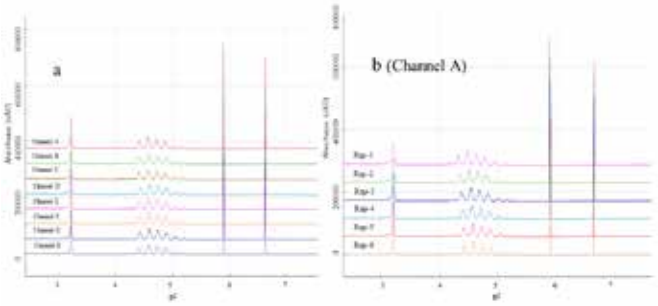


图3. Biophase™ 8800系统中使用CIEF方法进行EPO电荷变异体分析的电泳图（a）8通道A-H；（b）A通道重复6针

5. 结论

本文使用BioPhase™ 8800 系统进行EPO电荷变异体分析，结论如下：

1. 同一样品各电荷变异体的等电点、峰面积百分比、分离度在BioPhase™ 8800与PA 800 Plus上结果一致；
2. 同一样品8通道间一致性高；
3. 单通道连续6针进样重复性好；
4. 方法通量高，速度快，显著提高了大量样本的分析检测效率，当面对不同批次、来源等大量EPO样品检测时，BioPhase™ 8800系统可在保证分析效果和准确性的前提下，实现大量样品的快速分析，提高检测分析效率。

6. 参考文献

[1]. SCIEX Tech Note：cIEF方法对EPO变异体的分析（RUO-MKT-02-9621-ZH-A）

表2. BioPhase™ 8800 EPO电荷变异体分析的单次进样8通道一致性结果（n=8）

Peak	pI		Area%		R	
	Mean	RSD	Mean	RSD	Mean	RSD
1	4.26	0.17%	1.64	4.26%	/	/
2	4.41	0.19%	20.60	1.32%	1.86	2.33%
3	4.59	0.18%	28.89	0.86%	2.08	1.91%
4	4.73	0.20%	25.16	0.73%	1.62	4.44%
5	4.87	0.14%	16.18	1.00%	1.57	5.19%
6	5.03	0.16%	5.32	1.98%	1.94	4.95%
7	5.18	0.12%	1.64	1.69%	2.65	3.80%
8	5.31	0.16%	0.57	6.05%	2.96	4.49%

表3. BioPhase™ 8800 EPO电荷变异体分析的单（A）通道6针重复性结果（n=6）

Peak	pI		Area%		R	
	Mean	RSD	Mean	RSD	Mean	RSD
1	4.23	0.63%	1.63	5.11%	/	/
2	4.37	0.77%	19.85	2.26%	1.89	1.09%
3	4.54	0.85%	28.90	0.59%	2.10	0.86%
4	4.69	0.81%	25.31	0.98%	1.67	1.03%
5	4.83	0.79%	16.44	1.92%	1.50	1.88%
6	4.99	0.70%	5.55	1.61%	1.89	2.01%
7	5.15	0.52%	1.71	3.30%	2.60	1.86%
8	5.28	0.52%	0.61	4.59%	2.88	3.31%

High-throughput released N-glycan identification using CE and a customizable glycan library

Featuring the BioPhase 8800 software and the BioPhase Fast Glycan Labeling and Analysis kit

Fang Wang, Roy Estrada and Greg Manley
SCIEX, USA

This technical note demonstrates the procedures for a high-throughput N-linked glycan identification using the new BioPhase Fast Glycan Labeling and Analysis kit with the BioPhase 8800 system. This kit allows customers to achieve large-scale N-glycan profiling with streamlined sample preparation. The user-friendly BioPhase 8800 software empowers customers to customize molecule-specific glycan libraries, which can be employed in a platform separation method.

The early stages of biotherapeutics development require thousands of samples to be screened monthly. High-throughput assays are therefore needed to shorten the development time, while providing reliable data to comprehensively characterize and monitor critical attributes, such as protein purity, charge variant distribution and glycan profiling. Glycosylation plays a significant role in the stability, safety and efficacy of protein therapeutics and is often considered a critical quality attribute (CQA). Therefore, it is necessary to closely monitor the glycan profile of a biotherapeutic molecule throughout the entire development process.

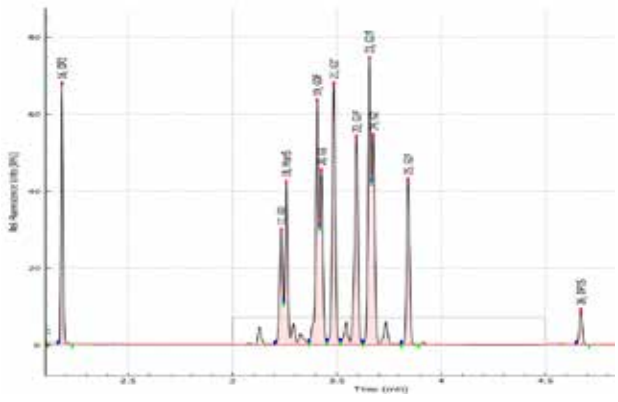


Figure 1. Standard glycan panels containing 9 glycan standards identified with a customized glycan library using the Fast Glycan Analysis module in the BioPhase 8800 software.



The SCIEX solution for glycan analysis on the next-generation multi-channel BioPhase 8800 system.

In 2017, SCIEX launched a Fast Glycan Labeling and Analysis kit that can be used on the PA800 Plus system. The kit provides fast sample preparation with a separation cycle time less than 9 minutes for N-linked glycans. But when such technique (now using the BioPhase Fast Glycan Labeling and Analysis kit) is coupled with the parallel capabilities of the 8 capillaries on the BioPhase 8800 system, it allows glycan analysis to be performed at rates as fast as 1 minute per sample. Further, the newly released BioPhase 8800 software allows customers to modify the N-glycan library based on a specific molecule, which provides flexibility in data analysis and increases confidence in the identification.

Key features of the glycan analysis workflow

- **High-throughput glycan analysis workflow:** The workflow can achieve a 1-minute per sample analysis rate
- **Flexible data analysis software:** The BioPhase 8800 software provides automatic peak identification for common N-glycans and allows customers to customize their glycan libraries based on molecule type

Methods

Materials: The BioPhase Fast Glycan Labeling and Analysis kit (P/N: C30098) was from SCIEX (Framingham, MA). A test mix containing 9 glycan standards was provided as a gift from the SCIEX research and development group. The test mix includes APTS-labeled G0, G0F, G1, G1F, G2, G2F, Man5, Man6 and Man7 at concentrations similar to a typical monoclonal antibody-released glycan mixture (Figure 1).

Instruments: The BioPhase 8800 system (P/N: 5083590F), BioPhase BFS capillary cartridge - 8 x 30 cm (P/N: 5080121), and BioPhase sample and reagent plates (4,4,8) (P/N: 5080311) were from SCIEX. The BioPhase 8800 system was equipped with a laser-induced fluorescence (LIF) detector. The excitation and emission wavelengths of the LIF detector were 488 and 520 nm, respectively.

Software: The BioPhase 8800 software, version 1.2 was used to create the instrument method and sequence and for data acquisition and processing.

Glycan standard preparation: To make a 9-glycan standard sample for testing, 10 µL of the glycan standard mixture stock was mixed with 50 µL of CE-grade water. The sample mixture was briefly spun down to remove bubbles and collect all liquid at the bottom of the tube. A 55 µL aliquot of the solution was transferred into a sample well.



Figure 2. The reagent profile. A) Inlet reagent profile and B) outlet reagent profile.

Bracket standards (BST) preparation: The BST was prepared following the fast glycan application guide.[1] Specifically, 200 µL of CE-grade water was added to the BST vial in the kit and mixed using a vortex mixer. The resulting solution was dispensed into vials in 80 or 40 µL aliquots. To prepare enough samples for all 8 capillaries, 760 µL of CE-grade water was added to an 80 µL aliquot of the reconstituted BST and then mixed using a vortex mixer. A 100 µL sample of the diluted BST solution was transferred to row #1 of each testing column. BST was injected into the capillary for each analysis before the sample injection.



Figure 3. Instrument method settings. A) Conditioning method, B) separation method and C) shutdown method.

Methods and sequence: Figure 2 shows the inlet reagent (Figure 2A) and outlet reagent profiles (Figure 2B). Figure 3 shows the conditioning (Figure 3A), separation (Figure 3B) and shutdown (Figure 3C) methods. A typical sequence starts with the conditioning method, then the separation method is used to analyze standards and unknown samples. Finally, the sequence ends with the shutdown method. The reagent files, method files

and a sample sequence can be downloaded from the SCIEX website.[1]

Instrument setup: Reagents and samples were loaded following the reagent plate map for the corresponding sequence. Multiple sequences were performed with 2 different cartridges, 3 different reagent lots and triplicate injections of each condition. Data were collected and processed following the steps described below.

Data processing: Data processing was achieved using the BioPhase 8800 software with the Fast Glycan Analysis module turned on. Detailed settings can be found in the results and discussion section.

Results and discussion

This technical note demonstrates a streamlined workflow to customize a molecule-specific glycan library with mixtures of glycan standards. In this workflow, glucose unit (GU value), a calibrated value based on the migration time of the APTS-labeled glycan peaks relative to the GU ladder, was used for peak identification. The APTS-labeled glycan standards were first analyzed using the instrument method shown in Figure 3B. Data from multiple sequences were collected and used to demonstrate the robustness of the identification method and the process of building or modifying glycan species in the GU table for future analysis. Figure 4 shows a high-level workflow for this procedure.

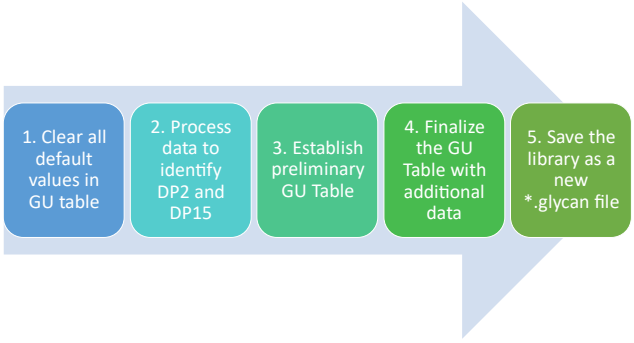


Figure 4. Summary of the workflow to build the fast glycan identification library using standards.

Step 1: Load the data into the processing module and enable the Fast Glycan Analysis module in the Post Analysis tab. In the Fast Glycan Analysis pop up window, check the Enable box and clear the GU table at the bottom, while keeping all contents in the Glucose Ladder section (Figure 5B). Choose an example data profile to be analyzed. Click Analyze to process the data. If the default parameters are not suitable for the results, proceed to step 2.

Step 2: Adjust the parameters in the Integration tab to allow APTS, DP2, DP15 and all major glycan standards to be integrated. Non-glycan peaks should not be integrated. Adjust the APTS and DP2 minimum height and DP2/APTS MT ratio to allow these 3 peaks to be identified (Figure 5). Specifically, if the height of DP2 in the electropherogram is smaller than the value shown in the APTS and DP2 minimum height box, lower the minimum height value. If another labeling artifact present between the APTS and DP2 peaks is mistakenly identified as DP2, increase the minimum height value and/or reduce the Tol value in theDP2 / APTS MT ratio (Figure 5, insert B). Use parameters such as the positive threshold, minimum cluster distance, straight baseline and suspend integration to optimize the peak integration (Figure 5, insert A).

***Step 3*:** In the results table under the electropherogram, keep the peak number, name and GU value columns active and uncheck the remaining columns (Figure 5, insert C). Copy the results into a Microsoft Excel spreadsheet and convert the pasted text to columns, as shown in Figures 6A and 6B. Add an additional column called Tolerance. Add a value of 0.05 for tolerance for each row (Figure 6C). Names of the glycan standards can be added based on sample information. Table 1 shows the preliminary GU table corresponding to the electropherogram shown in Figure 5. After the preliminary GU table is built, the values are manually pasted into the GU Table module of the Fast Glycan Analysis module (Figure 7). Click the Analyze button to process the data. Apply the processing method and analyze all the loaded data.

Step 4: Check all data and adjust the Integration parameters if necessary to ensure the standard runs are processed properly. DP2 and DP15 should be identified and more than 90% of the selected data files should have all standard peaks identified and labeled. Using the overlay option, export the GU values of all the data files. Calculate the tolerance of the GU table by multiplying the standard deviation (SD) in the summary table by 5 for each identified glycan species. Update the preliminary GU table with the new tolerance value and update the GU value with the mean of the processed data (Table 1). Process all data again and confirm the robustness of the final processing method.

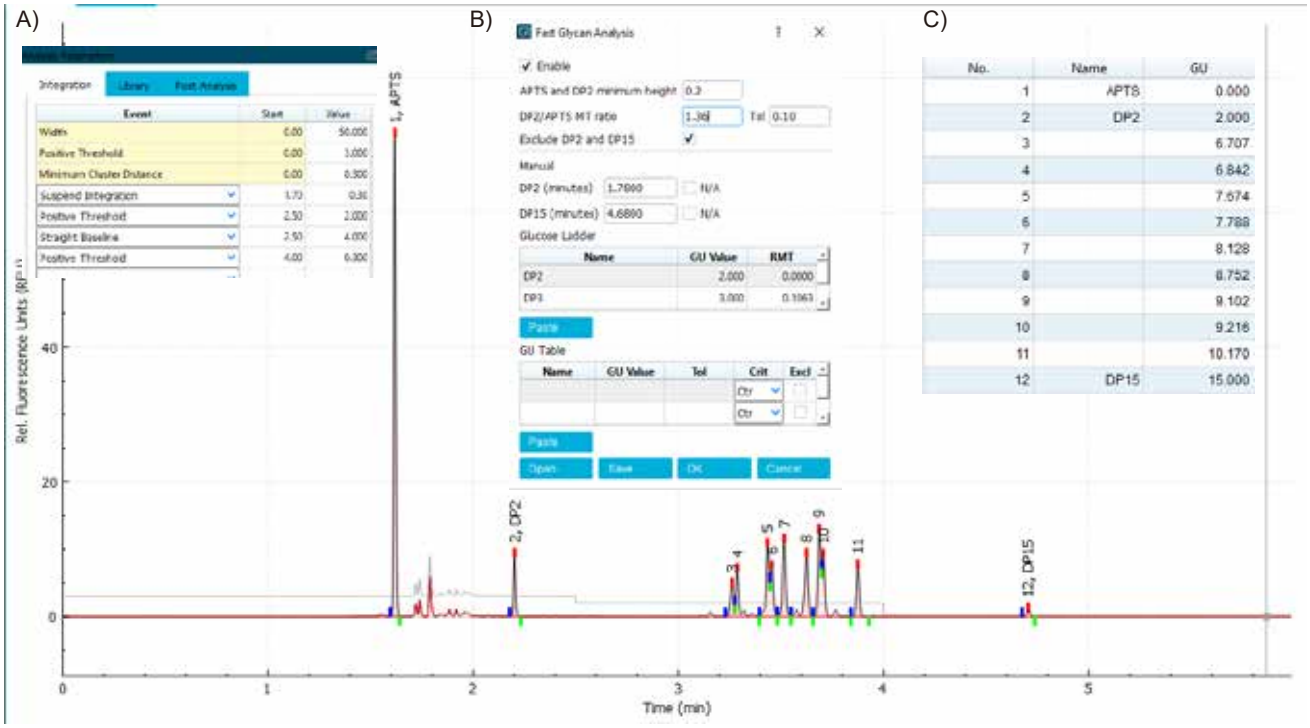


Figure 5. Electropherogram of glycan standard mixture with key marker peaks identified. The electropherogram of the glycan standard mixture was analyzed with the Integration parameters shown in insert A and Fast Glycan Analysis parameters shown in insert B. This method allowed the integration and identification of the marker peaks for APTS, DP2 and DP15. Inset C shows the results table with No. Name and GU column selected.

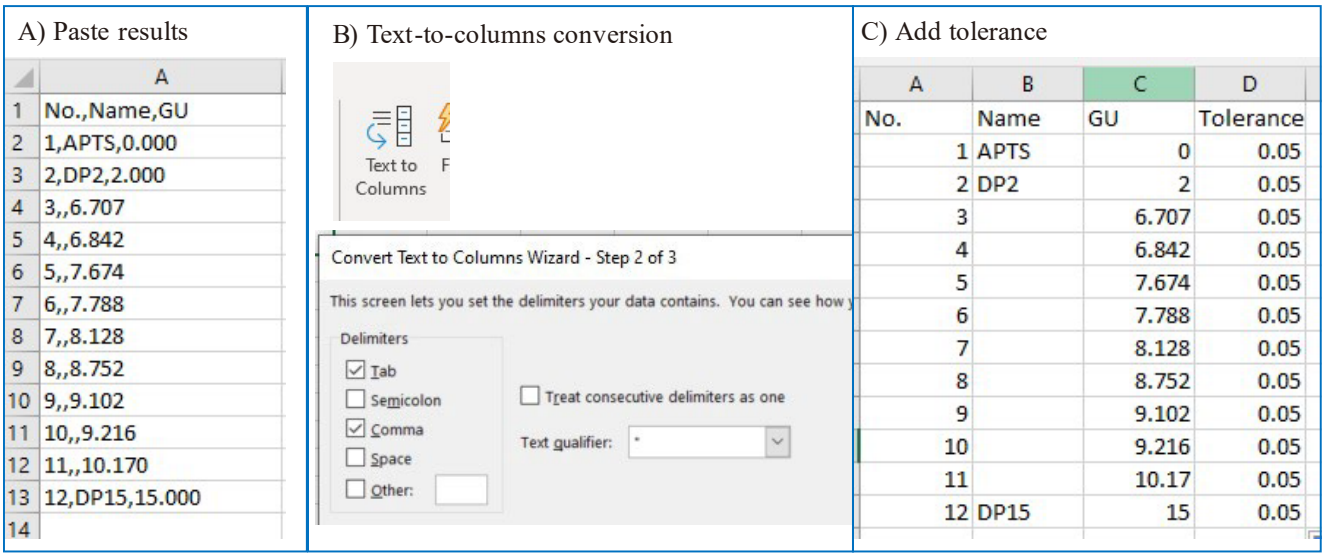


Figure 6. The process to convert the results table from an electropherogram into a preliminary GU table.

Step 5. Use the Save button on the bottom of the page to save the Fast Glycan Analysis method with the standards to the glycan library file for future use (Figure 7).

Table 1. GU value and tolerance for the preliminary and final GU tables in the Fast Glycan Analysis module.

#	Name	GU	Tolerance	Final GU	Final tolerance
3	*G0*	*6.702*	*0.05*	*6.7001*	*0.026*
4	*Man5*	*6.828*	*0.05*	*6.833*	*0.031*
5	*G0F*	*7.669*	*0.05*	*7.6685*	*0.028*
6	*G1*	*7.783*	*0.05*	*7.7814*	*0.028*
7	*G1'*	*8.118*	*0.05*	*8.1193*	*0.033*
8	*G1F*	*8.738*	*0.05*	*8.7421*	*0.033*
9	*G1'F*	*9.092*	*0.05*	*9.0955*	*0.027*
10	*G2*	*9.197*	*0.05*	*9.2028*	*0.036*
11	*G2F*	*10.151*	*0.05*	*10.1559*	*0.033*

GU and tolerance are values from the preliminary GU table based on a single injection. Final GU and tolerance values were calculated based on a total of 108 data files from 6 injections of the standard mixture using 3 lots of reagents. The Tolerance values specify the window of the GU value for peak identification.

Conclusions

- The BioPhase Fast Glycan Labeling and Analysis kit provides all-inclusive sample preparation and high-throughput glycan analysis workflow
- The analysis time is 1 minute per sample with a cycle time of 8 minutes per injection
- The BioPhase 8800 software provides flexibility to add, remove and edit the glycan library to include glycans of interest or recalibrate GU tables
- Improved data processing automation and batch process capability

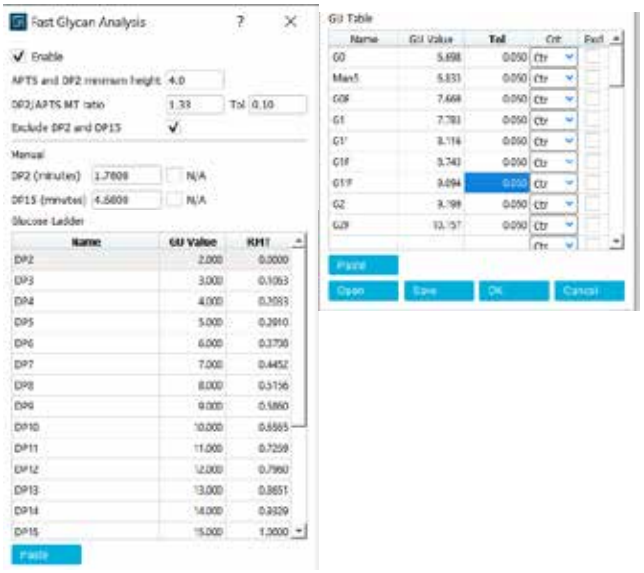


Figure 7. An example of the Fast Glycan Analysis module with an initial GU table and other settings.

References

1. BioPhase Fast Glycan Labeling and Analysis Kit (For the BioPhase 8800 System), Application Guide, SCIEX, RUO-IDV-05-8648-A
2. BioPhase 8800 System Operation Guide, RUO-IDV-05-8643-D

N-linked glycan analysis of NISTmAb, etanercept and trastuzumab

Featuring the BioPhase 8800 system and the Fast Glycan Labeling and Analysis kit

Marcia Santos and Fang Wang
SCIEX, USA

Introduction

This technical note demonstrates the high-throughput N-linked glycan analysis of the NIST monoclonal antibody (NISTmAb), trastuzumab and etanercept (Figure 1) utilizing the Fast Glycan Labeling and Analysis kit for the BioPhase 8800 system. It describes the entire workflow—from sample preparation (denaturation, digestion, labeling and cleanup) to data analysis—and shows the accuracy and reliability of the workflow.

In early therapeutics development, the ability to quickly screen many clones and cell culture expression conditions is critical, while in later stages, extensive characterization becomes essential. Since glycosylation plays a significant role in the stability, safety and efficacy of protein therapeutics, glycan expression profiling is a critical quality attribute (CQA) that must be monitored closely. Therefore, a streamlined, accurate and high-throughput glycan analysis assay is necessary for all phases of biopharmaceutical development.

The BioPhase 8800 system is a multi-capillary platform that consists of eight capillaries, enabling users to process five 96-well plates (or 480 samples) in one working day using the Fast Glycan Labeling and Analysis kit. The streamlined data analysis utilizes predefined integration parameters. The automatic glycan peak assignment is based on glucose units and uses a fully customizable glycan library, which enables accurate identification.

Key features of released N-linked glycan analysis

- **High-throughput glycan analysis workflow:** The workflow can achieve a 1-minute-per-sample analysis rate.
- **Intuitive and flexible data analysis software:** BioPhase software provides automatic peak identification for common N-glycans. It also allows customers to customize their glycan libraries based on molecule type.

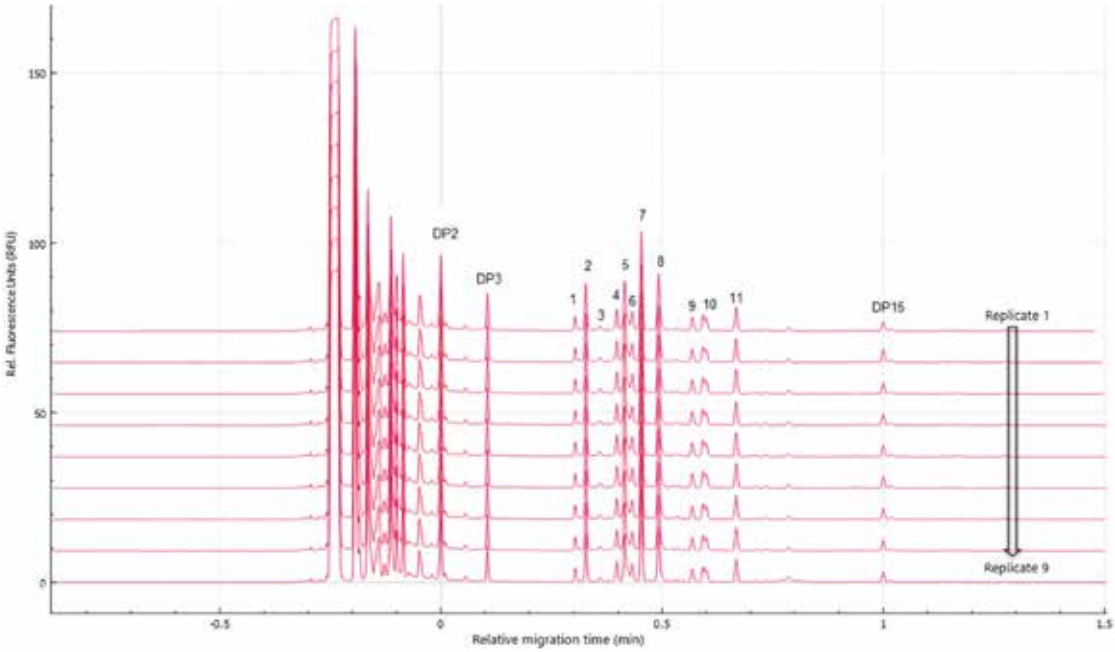


Figure 1. Overlay of nine replicates of etanercept separation. The overlay demonstrates highly resolved and reproducible separation achieved with the Fast Glycan Labeling and Analysis kit. Peak legend: 1) FA2BG2S2, 2) A2(6)G1S1, 3) FA2(6)G1S1, 4) A2G2S1, 5) A2BG2S1, 6) FA2G2S1/M5, 7)FA2BG2S1, 8)M6, 9) M7[D3]/M7[D1]/FA2(6)G1, 10) FA2(3)G1/A2G2/ FA2B(6)G1, 11) FA2G2

- **All-inclusive sample preparation in one kit:** The Fast Glycan Labeling and Analysis kit provides all the chemicals and enzymes needed for N-glycan analysis.

Methods

Materials: The Fast Glycan Labeling and Analysis kit (P/N: C30098) was from SCIEX. The NISTmAb, Humanized IgG1κ Monoclonal Antibody, was from NIST reference material (RM) 8671. The adalimumab and trastuzumab were purchased from the local pharmacy.

Instrument: The BioPhase 8800 system (P/N: 5083590F), a BFS capillary cartridge – 8 x 30 cm (P/N: 5080121) and a starter kit of four sample plates, four reagent plates and eight outlet plates (P/N: 5080311) were from SCIEX. The BioPhase 8800 system was equipped with a laser-induced fluorescence (LIF) detector and 520 nm emission filter (P/N: 144940).

Software: BioPhase software 1.2 was used to create the instrument method and sequence for data acquisition and processing.

Methods and sequence: Figure 2 shows the A) conditioning method, B) separation method and C) shutdown method for glycan analysis. A typical fast glycan analysis sequence starts with the conditioning method, progresses to the separation method for analyzing standards and unknown samples and then ends with the shutdown method.

Instrument setup: Reagents and sample plates were loaded according to the reagent plate map for the corresponding sequence. Multiple sequences were performed using two instruments, two cartridges from different lots and reagent kit from three different lots.

Superior fast glycan sample preparation

Figure 3 shows the magnetic bead-mediated sample preparation workflow, which consists of five steps: A) sample denaturation and digestion, B) glycan capture C) APTS labeling, D) cleanup and E) elution with water. The detailed steps for sample preparation can be found in the application guide for the Fast Glycan Labeling and Analysis kit.[1]

This magnetic bead-mediated sample preparation shows great benefits. First, the sample is prepared with an open tube, so better labeling efficiency can be achieved. Second, this method does not require centrifugation, and is therefore amenable to automation. In addition, the throughput is much higher: eight samples can be prepared simultaneously within 70 minutes.



Figure 2. Instrument method settings. A) Conditioning method, B) separation method and C) shutdown method.

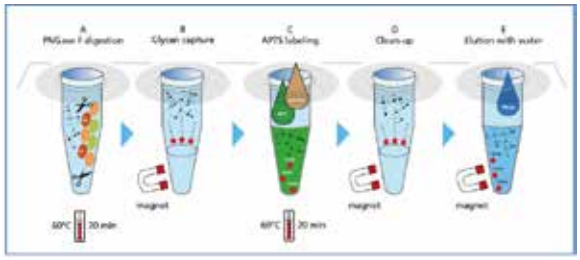


Figure 3. Magnetic bead-mediated sample preparation for N-linked glycan labeling and cleanup.

High-resolution glycan separation and streamlined data analysis

Preloaded separation methods and sequence templates for glycan analysis are available in the BioPhase software, facilitating usability.

A bracketing standard with DP2 and DP15 is integrated in the first sample row as anchors to ensure correct glucose unit calculation and peak assignment for later data analysis.

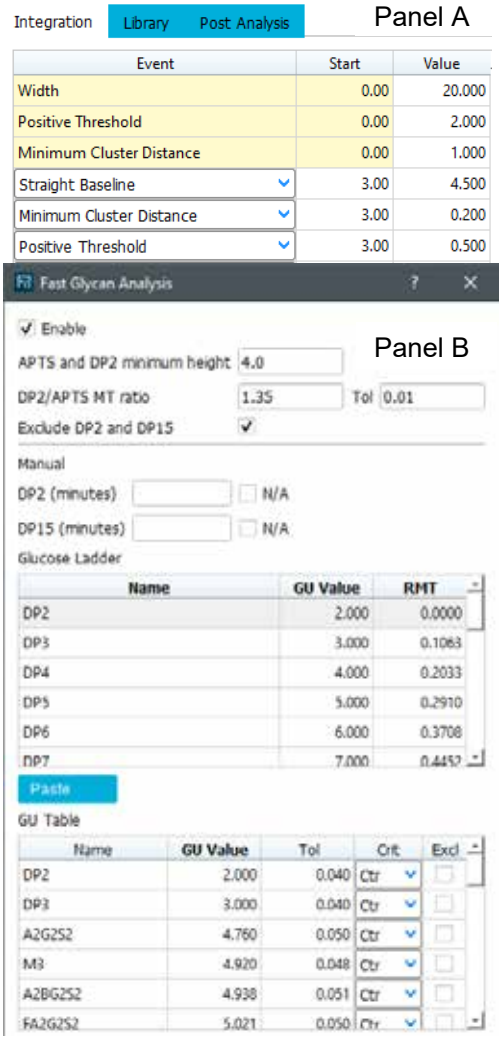


Figure 4. The embedded fast glycan data analysis parameters in .dana files. Panel A: integration events**,** Panel B: GU tables**.**

Predefined integration parameters (.dana file) and a glycan library (.glycan file) are also preloaded for data analysis. All parameters needed for data integration, glucose unit tables for glucose ladder and N-linked glycan species (Figure 4) are embedded in these files.

As all parameters will directly impact the identification, it must be customized based on different modalities during data analysis.

The integration process is described in a previous technical note[2] and is beyond the scope of the discussion here.

Figure 1, Figure 5 and Figure 6 show the overlays of nine replicates of N-linked glycan analysis from etanercept, trastuzumab and NISTmAb, demonstrating a high level of reproducibility. Four major N-linked glycans were detected in trastuzumab, including FA2, FA2(6)G1, FA2(3)G1 and FA2G2 (Figure 5). In addition to the four glycans detected in trastuzumab, three more glycans were observed in the NISTmAb electropherogram: FA2B, M7[D1] and A2BG2 (Figure 6). A more complex glycan profiling was achieved by employing the Fast Glycan Labeling and Analysis kit. The data (Figure 1) from etanercept demonstrates the ability of the kit to complex fusion proteins. Eleven N-linked glycans were separated and identified, including high mannose species and complex glycans. Sialylated glycans are the dominant species observed in the profile, including bi- and tri-antennary glycans. All of the glycan assignments are consistent with the findings detailed in previous publications[3,4], highlighting the accuracy and reliability of the automatic software characterization.

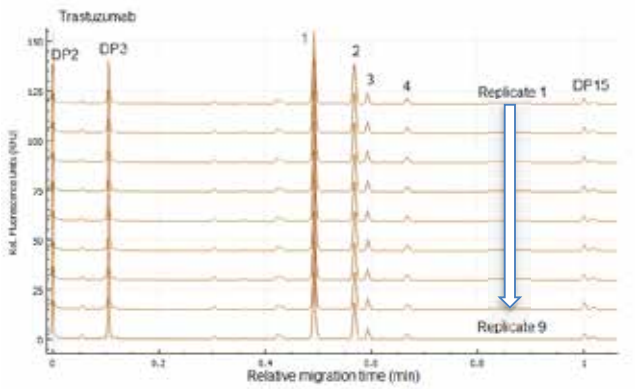


Figure 5. Overlay of nine replicates of trastuzumab glycan separation using the Fast Glycan Labeling and Analysis kit. Peak legend: 1. FA2, 2. FA2(6)G1, 3. FA2(3)G1, 4. FA2G2.

To evaluate the reproducibility of this workflow, a statistical analysis was performed on the nine replicates of N-linked glycan analysis from trastuzumab, NISTmAb and etanercept. The results are separately presented in Table 1, Table 2 and Table 3. The tables include migration time (MT), relative migration time (RMT), corrected peak area (CPA), corrected peak area percentage (CPA%) and glucose units (GU).

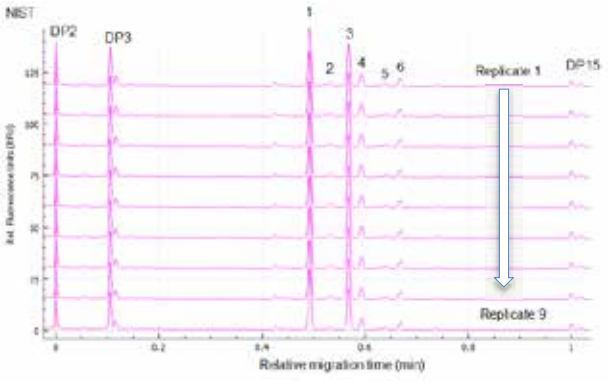


Figure 6. Overlay of nine replicates of NISTmAb glycan separation using the Fast Glycan Labeling and Analysis kit. Peak legend: 1. FA2, 2. FA2B, 3. M7[D1] / FA2(6)G1, 4. FA2(3)G1, 5. A2BG2, 6. FA2G2.

Table 1. Statistical results to demonstrate the reproducibility of trastuzumab glycan analysis. The percentage of the square root of standard deviation (%RSD) for MT, RMT, CPA, CPA% and GU are presented.

%RSD	FA2	FA2(6)G1	FA2(3)G1	FA2G2
MT	0.37	0.37	0.37	0.36
RMT	0.07	0.05	0.04	0.05
CPA	2.92	2.80	2.89	2.99
CPA%	0.70	0.69	0.56	0.73
GU	0.06	0.05	0.04	0.05

Table 2. Statistical results to demonstrate the reproducibility of NISTmAb glycan analysis. The percentage of the square root of standard deviation (%RSD) for MT, RMT, CPA, CPA% and GU are presented.

%RSD	FA2	FA2B	M7[D1]/ FA2(6)G1	FA2(3)G1	A2BG2	FA2G2
MT	0.28	0.28	0.27	0.27	0.27	0.27
RMT	0.06	0.05	0.05	0.00	0.05	0.04
CPA	3.03	3.89	3.21	3.54	5.06	3.85
CPA%	1.35	2.17	1.49	1.52	2.90	2.04
GU	0.05	0.05	0.04	0.04	0.04	0.04

Table 3. Statistical results to demonstrate the reproducibility of etanercept glycan analysis. The percentage of the square root of standard deviation (%RSD) for MT, RMT, CPA, CPA% and GU are presented.

%RSD	FA2BG2S2	A2(6)G1S1	FA2(6)G1S1	A2G2S1	A2BG2S1	FA2G2S1/ M5	FA2BG2S1	M6	M7[D3]/ M7/ FA2(6)G1	FA2(3)G1/ A2G2/ FA2B(6)G1	FA2G2
MT	0.38	0.37	0.37	0.36	0.36	0.37	0.36	0.36	0.36	0.35	0.35
RMT	0.08	0.07	0.05	0.06	0.07	0.06	0.06	0.05	0.04	0.03	0.04
CPA	2.51	2.47	4.86	2.53	2.29	2.16	2.26	2.12	2.29	6.56	2.82
CPA%	0.67	0.70	4.11	0.86	0.79	1.72	1.02	1.23	1.04	7.88	2.64
GU	0.06	0.05	0.04	0.05	0.06	0.05	0.06	0.05	0.04	0.02	0.04

Conclusions

- The Fast Glycan Labeling and Analysis kit for the BioPhase 8800 system provides all-inclusive sample preparation and a high-throughput glycan analysis workflow for mAbs and complex glycan profiling
- BioPhase software performed reliable glycan peak identifications with average GU %RSD values lower than 0.06% for all three proteins
- The customizable library allows for streamlined and reliable data analysis
- The workflow can achieve a 1-minute-per-sample analysis rate

References

1. Fast Glycan Labeling and Analysis kit for the BioPhase 8800 system. SCIEX application guide, RUO-IDV-05-8648-A.
2. Wang, F. et al. High-throughput released N-glycan identification using CE and a customizable glycan library. SCIEX technical note, MKT-26425-A.
3. Giorgetti, J. et al. Monoclonal antibody N-glycosylation profiling using capillary electrophoresis – Mass spectrometry: Assessment and method validation. Talanta. 2018, 178: 530–537. doi: 10.1016/j.talanta.2017.09.083.
4. Tan, Q. et al. Characterization and comparison of commercially available TNF receptor 2-Fc fusion protein products; mAbs 2012, 4(6): 761–774. doi: 10.4161/mabs.22276.

毛细管区带电泳（CZE）对单克隆抗体的电荷异质性分析

CMarcia R Santos

Global Tactical Marketing, Beckman Coulter Life Sciences, Brea, CA USA

前言

电荷异质性分析对于单克隆抗体的表征是非常重要的，因为它可提供有关产品的质量和稳定性的重要信息。异质性可源于分子层面的改变，如C-末端赖氨酸修饰、脱酰胺化和翻译后修饰。毛细管等电聚焦（cIEF）是一种可以根据蛋白各亚型等电点的变化分离电荷异构体的方法。cIEF需要在涂层毛细管中进行电泳分离，涂层毛细管可以抑制电渗流（EOF）并且可以防止蛋白在管壁表面的吸附，但需要较长的分析时间周期和复杂的样品制备。

本文展示了通过使用一个简单的分离缓冲体系和非涂层毛细管，可以在12分钟内对单克隆抗体的电荷异质性进行分析，得到高分辨率和高重现性的分离结果。在本研究中，使用有效长度为20 cm的短毛细管即可获得较高分辨率，若使用有效长度为40 cm的毛细管还可以揭示单克隆抗体的更精细的结构。

实验条件

毛细管区带电泳

分离缓冲液的配制：

0.05% HPMC , 380 mM EACA, 1.9 mM TETA

400 mM EACA + 2 mM TETA（ pH =5.7）缓冲液的配制

在含有95 mL去离子水的100 mL烧杯中加入5.25 g的EACA（Sigma, cat. no. A-7824）和30 µL TETA（三乙烯四胺，Sigma, cat. no. 90460）。充分溶解后，用冰醋酸调节pH至5.7 ± 0.05。将上述溶液转移至100 mL容量瓶中，并用去离子水定容。所得溶液用0.2 µm 滤膜过滤。

1% HPMC溶液的配制

将1g HPMC（Sigma, cat. no. H-7509）加入到含有100 mL去离子水的烧杯中，室温过夜使其充分溶解。

分离缓冲液的配制

移取400 mM EACA + 2 mM TETA（pH = 5.7）缓冲液8.55 mL至 15 mL离心管中，加入1% HPMC溶液450 µL，充分混合。每次使用前新鲜配制。这里准备的分离缓冲液量足够运行9次分离。

毛细管冲洗液

0.1 N HCl 溶液（Fluka, part no. 94015）。

样品

单抗X是一种具有代表性的治疗性单克隆抗体。原液为20 mg/mL的单抗 X，用去离子水稀释至1 mg/mL，并用于cIEF和CZE的分离。

毛细管

规格：熔融石英，50 µm内径，360 µm外径。

用于高分辨率分离方法的毛细管总长度为50 cm，有效长度为40cm；用于快速分离方法的毛细管总长度为30cm，有效长度为20cm。

仪器

本次实验使用的仪器是PA 800 plus生物制药分析系统配备UV或PDA检测器。使用UV检测器时，在CZE实验中配置214 nm的滤光片并且数据采集频率设置为4 Hz，而在cIEF实验中配置280 nm的滤光片。PDA检测器则用于重复CZE实验用于之后和UV/vis的数据进行比较。样品储藏温度和卡盒的温度分别为20 ℃和25 ℃。

CZE方法

CZE分离方法参数

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	5.00 min	BI:E1	BO:E1	forward, In / Out vial inc 9	Rinse with 0.1 N HCl
2		Rinse - Pressure	50.0 psi	5.00 min	BI:B1	BO:B1	forward, In / Out vial inc 9	Rinse with EACA/HPMC buffer
3		Inject - Pressure	0.5 psi	10.0 sec	SI:A1	BO:A1	Override, forward	Sample injection
4	0.00	Separate - Voltage	30.0 KV	15.00 min	BI:A1	BO:A1	0.17 Min ramp, normal polarity	Separation
5	15.00	End						

CZE毛细管活化方法参数

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	5.00 min	BI:F6	BO:F6	forward	Rinse with 0.1 N HCl
2		Rinse - Pressure	50.0 psi	5.00 min	BI:A6	BO:A6	forward	Rinse with buffer
3		Inject - Pressure	0.5 psi	10.0 sec	SI:A1	BO:B6	Override, forward	
4	0.00	Separate - Voltage	30.0 KV	30.00 min	BI:B6	BO:B6	0.17 Min ramp, normal polarity	
5	30.00	End						

CZE关机方法参数

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1	0.00	Separate - Pressure	50.0 psi	5.00 min	BI:F6	BO:F6	forward	Rinse with 0.1 N HCl
2	5.00	Wait		0.00 min	BI:C1	BO:C1		Capillary ends in water
3	5.00	Lamp - Off						Turn off lamp

CZE高分辨率分离方法

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	5.00 min	BI:E1	BO:E1	forward	Rinse with 0.1 N HCl
2		Rinse - Pressure	50.0 psi	10.00 min	BI:B1	BO:B1	forward	Rinse with EACA/HPMC buffer
3		Inject - Pressure	0.5 psi	10.0 sec	SI:A1	BO:A1	Override, forward	Sample injection
4	0.00	Separate - Voltage	30.0 KV	40.00 min	BI:A1	BO:A1	0.17 Min ramp, normal polarity	Separation
5	40.00	End						

使用5-8窄范围两性电解质的毛细管等电聚焦的实验

cIEF实验用到的试剂，样品制备和分离方法参考之前的技术说明[2]。

结果与讨论

分别运用cIEF和CZE的方法对单抗X电荷异质性进行了分析。

典型单抗X等电聚焦的分离说明其具有复杂的电荷异构体，等电点范围从pI 6.3到pI 6.91（图1）。在pH = 5.7的缓冲体系下，由于可能存在潜在蛋白溶解度问题[1]，我们并不能确认使用CZE分离是否能够对此样品进行有效的分离。然而，实验结果表明，虽然该单抗是弱酸性的，且其等电点与分离缓冲液的pH值相差不到1个pH单位，但CZE方法在12分钟内就可以获得完全的高分辨的分离（图2）。

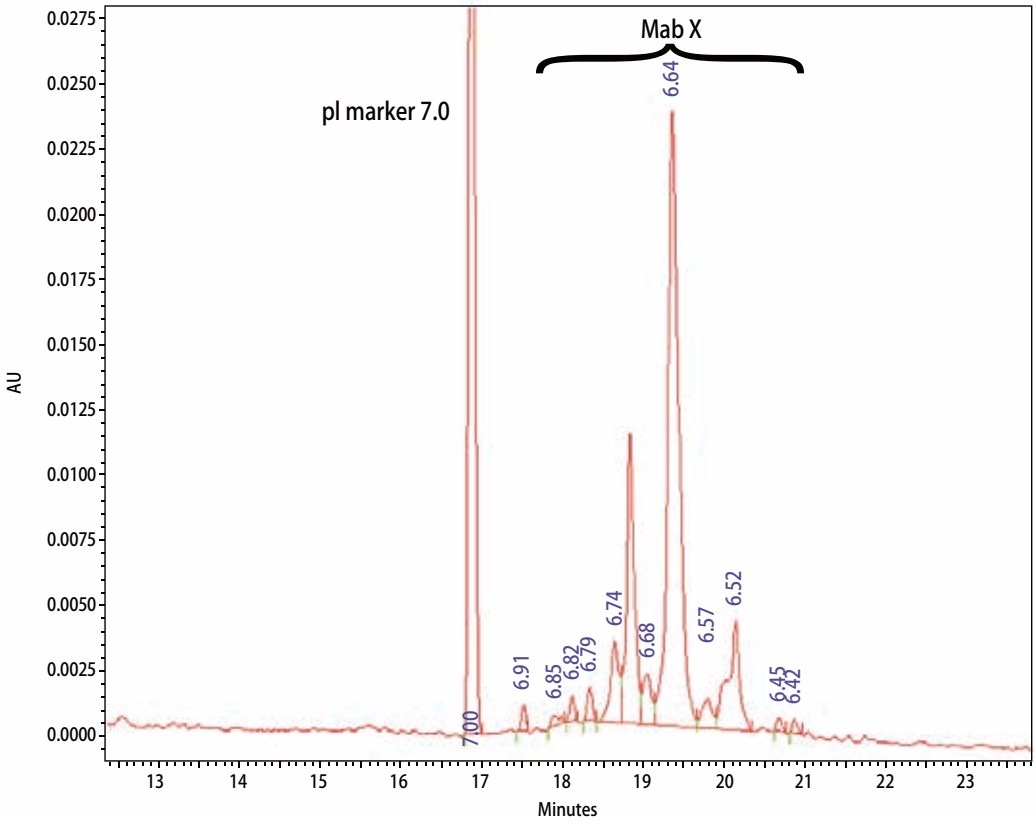


图1. 单抗X的cIEF电泳图，单抗的浓度为1 mg/mL。

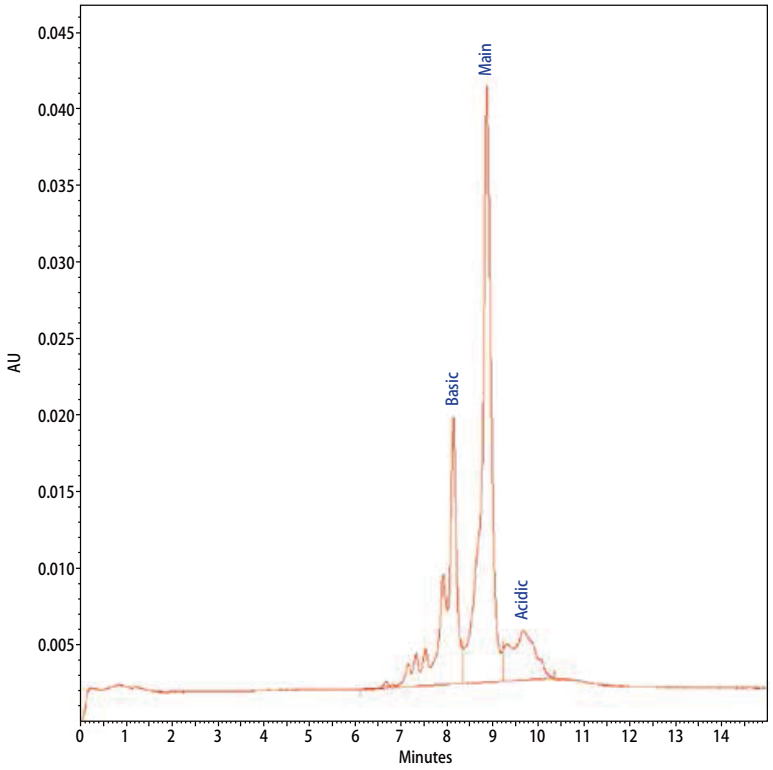


图2. 单抗X在CZE条件下的电泳图和积分。电荷异构峰分别归类为碱性峰，主峰和酸性峰。CZE条件：单抗X 1 mg/mL；分离缓冲液：0.05% HPMC，380 mM EACA（氨基己酸），1.9 mM TETA；熔融石英毛细管，30 kV分离电压。

结果表明，CZE的分离方法可以实现高分辨率的分离（图3）。此外，在碱性峰区域的分辨率优于cIEF。CZE的一个重要的优势是不仅仅分辨率高，而且分析速度快。得到完整的电荷异质性分离分析，CZE只需要12分钟而cIEF则需要23分钟。

实验的重复性是十分重要的，在这里进行了连续多次的CZE运行来说明其分离重现性（图4）。按照图2的方式对这些数据进行积分（图5），得到碱性峰、主峰和酸性峰群。

连续多针运行结果表明CZE分离具有杰出的重现性，其酸性峰、主峰和碱性峰的校正峰面积RSD小于3%，迁移时间RSD小于0.55%（表1）。

表1. CZE分析单抗X的原始数据。碱性、中性和酸性样品峰的矫正峰面积和迁移时间的重现性。

CZE of Mab X	Corrected Area			Migration Time		
Run #	Basic	Main	Acidic	Basic	Main	Acidic
Mab X run #1	12899.79	21950.23	4363.77	8.24	8.99	9.81
Mab X run #2	12382.87	21373.5	4155.31	8.13	8.87	9.67
Mab X run #3	12572.88	21441.31	4333.81	8.18	8.91	9.73
Mab X run #4	12483.24	21438.48	4383.71	8.22	8.97	9.78
Mab X run #5	13410.81	22744.52	4581.26	8.15	8.89	9.7
Mab X run #6	12900.86	21703.76	4418.44	8.23	8.98	9.8
Mab X run #7	12906.9	21945.95	4437.96	8.23	8.98	9.8
Mab X run #8	13118.91	22094.07	4569.69	8.23	8.98	9.8
Mab X run #9	13046.59	21908.14	4501.46	8.24	8.99	9.82
Mean:	12858.09	21844.44	4416.16	8.21	8.95	9.77
Std Dev:	328.39	428.21	130.96	0.04	0.05	0.05
%RSD:	2.55	1.96	2.97	0.51	0.53	0.55

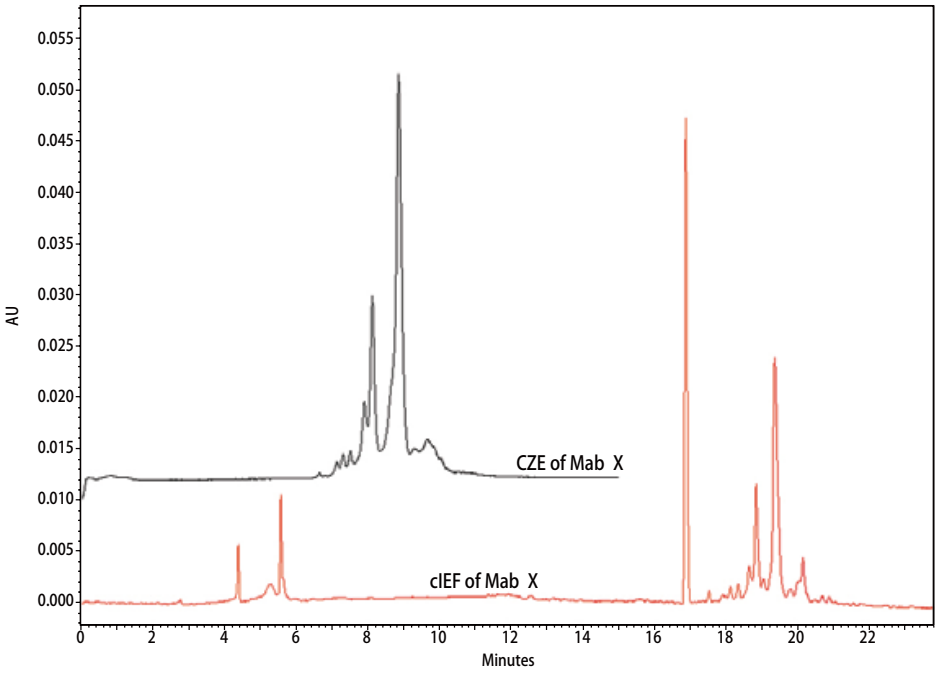


图3. 单抗X的cIEF和CZE分析方法对比。

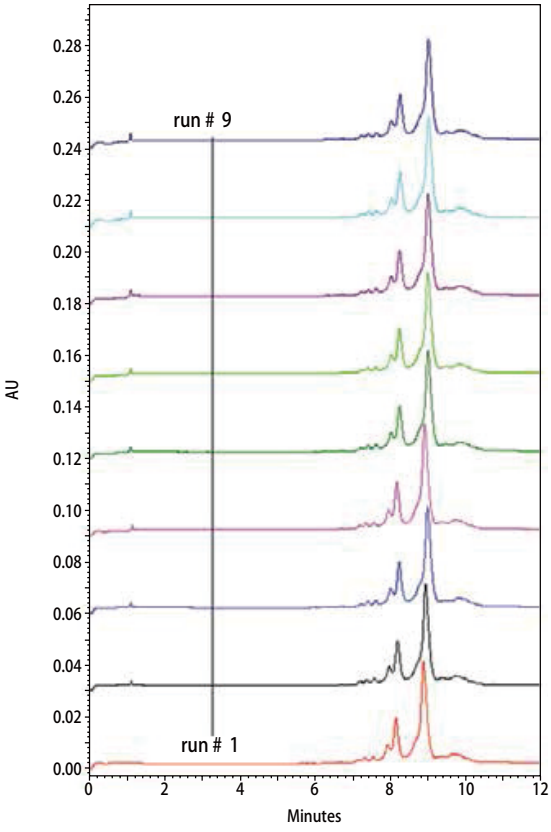


图4. 单抗X的CZE分离。9针连续的分离均使用UV检测器。单抗X的浓度为1 mg/mL；缓冲液：0.05% HPMC，380 mM EACA，1.9 mM TETA；熔融石英毛细管，30 kV分离电压。

#		Event	Start Time	Stop Time	Value
1	☑	Width	0.000	0.000	1
2	☑	Threshold	0.000	0.000	200
3	☑	Shoulder Sensitivity	0.000	0.000	1e+006
4	☑	Integration Off	0.000	6.000	0
5	☑	Minimum Cluster Distance	0.000	0.000	25

图5. 分析单抗X 的32 Karat软件积分参数。

CZE还具有很大的灵活性，不同检测器的类型都能提供相同的性能。使用CZE的方法无论配置二极管阵列检测器或是紫外检测器对单抗X进行分离都可以获得相同的分辨率。此外，使用这两种检测器还可得到相同的组分面积百分比（图6）。

若希望获得更高的分辨率，可通过增加毛细管的有效长度，能得到比快速分离的方法更精细的分离。如图7所示，使用有效长度为40 cm的毛细管对单抗X进行分离，尽管总分离时间有所增加，但高分辨率有了明显的提高。

更高的分辨率有利于对单抗X的降解状态（如暴露在60 ℃ 48个小时）进行分析。然而，只有增加毛细管的有效长度，才有可能看到因为热不稳定性形成的酸性峰和碱性峰的精细结构。

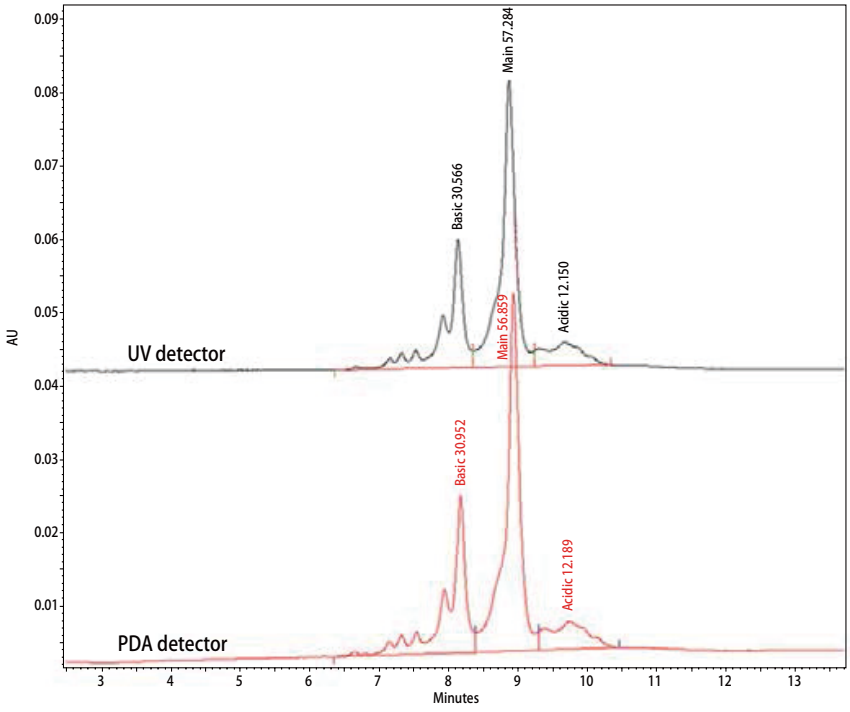


图6. 使用UV和PDA检测器进行CZE分离的对比。UV检测器（红色），PDA检测器（黑色）。分离条件均如图1所述。

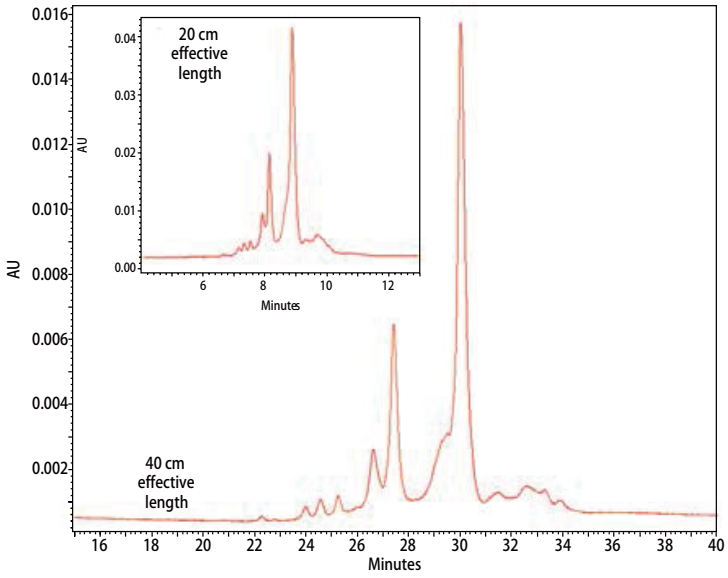


图7. 不同毛细管有效长度的结果对比。CZE分离条件如前所述。40 cm有效长度（下图），20 cm有效长度（插图）。

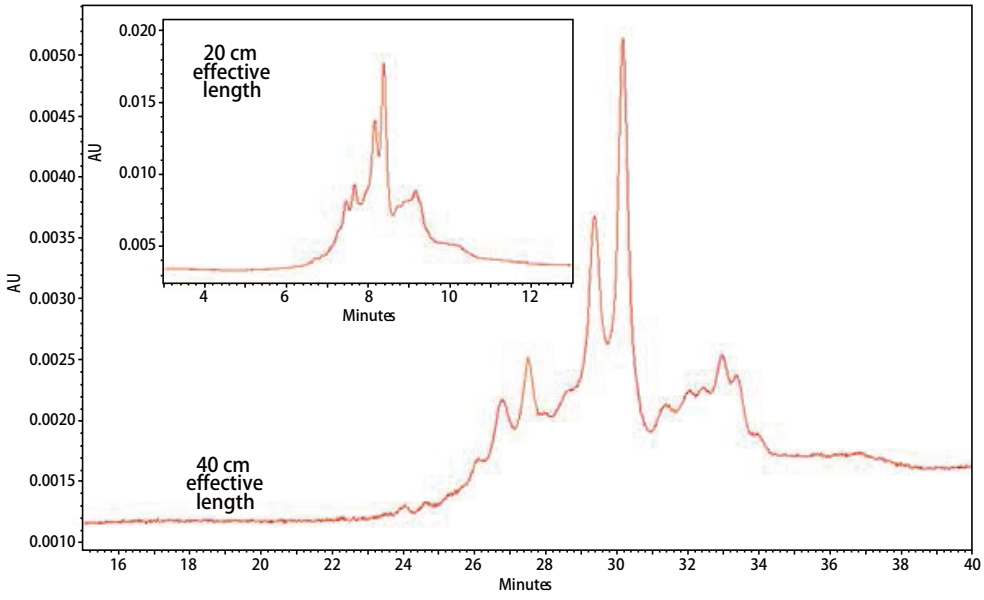


图8. 单抗X在高温下（60℃）暴露5天降解后的分析。分别使用40 cm毛细管有效长度（下图）和20 cm毛细管有效长度（插图）进行CZE的分离。

结论

使用CZE和cIEF的方法都可以很容易的检测蛋白质电荷异质性。cIEF通常用于获得电荷异质性和等电点的信息，而本文介绍的CZE方法具有对典型单抗快速及高重现性分离的能力。该CZE方法可以通过增加毛细管的有效长度进行提高峰分辨率的优化。CZE分离的性能不受检测器的影响，CZE可以在碱性峰、主峰和酸性峰分离中表现出很好的重现性。基于上述优势可见，CZE是一种可以替代cIEF的快速分析方法，在保证分离效果和重现性的同时，为高通量分析提供了可能。

REFERENCES

1. Yan He, Colleen Isele, Weiying Hou, Margaret Ruesch. 2011. Rapid analysis of charge variants of monoclonal antibodies with capillary zone electrophoresis in dynamically coated fused-silica capillary. Journal of Separation Science, vol. 34: pages 548-555.
2. Ingrid D. Cruzado-Park, Scott Mack and Chitra K. Ratnayake, A Robust cIEF Method: Intermediate Precision for the pH 5-7 Range, PN A-12015

毛细管区带电泳用于半胱氨酸偶联ADC样品的电荷异质性分析

Analysis of Charge Heterogeneity in Cysteine Coupled ADC Samples by Capillary Zone Electrophoresis

唐红梅，王文涛，陈泓序，罗继，郭立海

Tang Hongmei, Wang Wentao, Chen Hongxu, Luo Ji, Guo Lihai

SCIEX, 中国;

SCIEX, China;

Key words: CZE, ADC, Charge heterogeneity

1. 前言

电荷异质性分析对于单抗以及抗体偶联药物(ADC)的表征都是非常重要的，因为它可提供有关产品的质量和稳定性的重要信息。对于单抗，中国药典中提到可采用毛细管区带电泳(CZE)、离子交换色谱(IEX-HPLC) 、毛细管等电聚焦电泳(cIEF)或成像毛细管等电聚焦电(icIEF)等适当方法测定电荷变异体。单抗本身就具有复杂的异质性(如糖基化和其他翻译后修饰等)，单抗本身的异质性及偶联造成的异质性的叠加会导致ADC的复杂程度大幅提高。对于具有高度异质性的ADC，需采用具有高分离度的可靠分析方法进行全面分析，并且根据小分子药物和连接子的化学性质、偶联方式(氨基偶联、巯基偶联、定点偶联等)以及产品的复杂性选择合适的特性分析方法。使用IEX-HPLC方法时，由于小分子药物多位点连接的可能性以及分析物与色谱柱固定相之间潜在的非特异性相互作用，会导致样品分离度降低；而cIEF或icIEF方法中需要使用预混液，预混液中存在一些其他试剂可能会对ADC样品有影响导致分离重复性不稳定。而毛细管区带电泳(CZE)方法根据电荷与体积之比的差异对样品进行分离，具有分离度高、前处理简单、对样品影响小等特点，可用于半胱氨酸偶联的ADC样品电荷异质性分析。

本文通过使用CZE试剂盒方法，可以快速的对半胱氨酸偶联的ADC样品电荷异质性进行有效分析，并对CZE方法的稳定性进行了考察，得到了高分离度和高重现性的分离结果。

2. 实验部分

2.1 实验试剂和样品信息

实验试剂：CZE试剂盒(SCIEX, PN C44790)，包含0.1M HCl，CE Grade Water (SCIEX, PN C48034)，CZE Rapid Charge Variant Separation buffer，蛋白质测试混合物；按照试剂盒说明书制备相应浓度的试剂。

样品：半胱氨酸偶联的ADC样品(Enhertu)由某生物制药公司提供，DAR值为8，PI 7.9，浓度20.0 mg/mL；

2.2 样品前处理

Enhertu样品溶液制备：用CE Grade Water将样品稀释至1 mg/mL，取100 µL放入到PCR管中，作为待测溶液。

2.3 仪器及方法

CZE方法实验条件：采用是PA 800 plus生物制药分析系统，UV检测器，采集频率：4 Hz，采集波长214 nm；非涂层毛细管，50 µm内径，50/ 60.2 cm(有效长度/总长度)；毛细管温度：25 ℃；样品室温度：15 ℃；进样条件：0.5 psi，20 s；分离条件：25 kV，35 min。

毛细管预冲洗：序列运行前毛细管先用0.1 N HCl进行50 psi冲洗5 min，再用CZE buffer进行50 psi冲洗5 min。每次运行开始时，0.1 N HCl进行30 psi冲洗1 min，再用CZE buffer进行30 psi冲洗2 min。

3. 结果与讨论

3.1 Enhertu样品CZE方法分离结果

本文采用Enhertu样品进行了电荷异质性分析，毛细管电泳分离结果如下图1所示，通过谱图可以看出样品电荷异质性较强，其中PK#3,4是主峰，主峰校正峰面积比例(CAP)为67.68 %，PK#1,2是碱性峰，校正峰面积比例为8.49%，Pk5,6,7为酸性峰，校正峰面积比例为23.83 %。

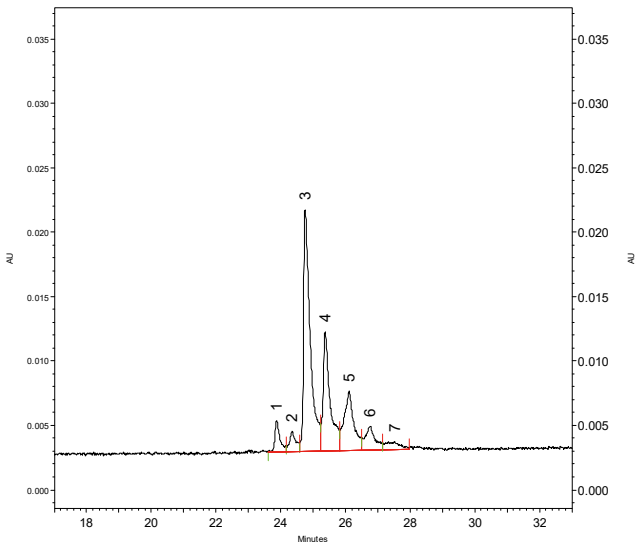


图1. Enhertu样品CZE电荷异质性分析的电泳图

3.2 Enhertu样品CZE方法重复性考察

对同一样品管的Enhertu样品进行连续6针重复，统计每1针中主峰，碱性峰，酸性峰的迁移时间和校正峰面积，考察样品连续进样6针的重复性，实验结果电泳图见图2；主峰，酸性峰，碱性峰CAP的RSD值< 5.0 %，并且主峰迁移时间(MT) 的RSD值< 0.5 %，统计结果见表1；实验结果表明半胱氨酸偶联的ADC样品连续6针进样重复性良好，CZE方法可用于半胱氨酸偶联的ADC样品电荷异质性的分析。

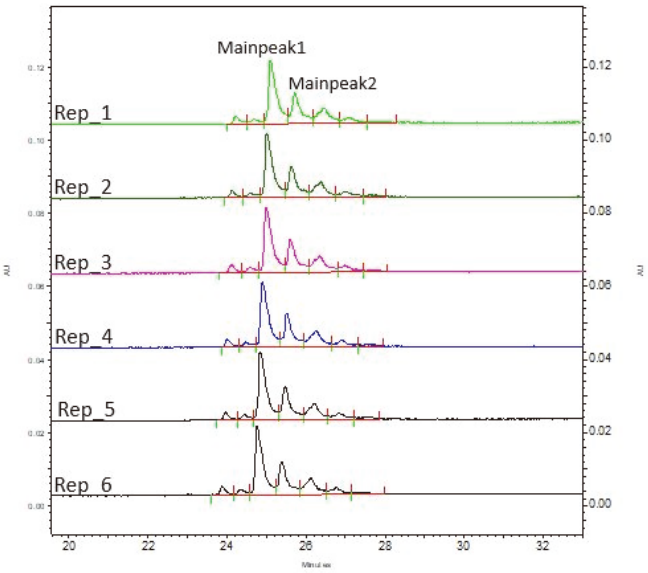


图2. Enhertu样品6针重复性电泳图

表1. Enhertu样品连续6针进样重复性结果(n=6)

序号	碱性峰 CAP %	主峰CAP %	主峰1 MT (min)	主峰2 MT (min)	酸性峰 CAP %
Rep-1	8.49	67.68	24.75	25.38	23.83
Rep-2	8.23	66.95	24.84	25.46	24.83
Rep-3	8.06	68.72	24.89	25.50	23.22
Rep-4	8.54	68.27	24.98	25.60	23.19
Rep-5	8.31	69.16	25.00	25.62	21.35
Rep-6	8.41	68.52	25.09	25.71	23.07
RSD %	2.13	1.15	0.49	0.47	4.89

4. 结论

本文借助于PA 800 plus生物制药分析系统以及CZE试剂盒方法对半胱氨酸偶联的Enhertu样品电荷异质性进行了分析，主要结论如下：

1. CZE方法分离度高，能够把样品中碱性峰、主峰、酸性峰明显分离，适用用于半胱氨酸偶联的ADC样品电荷异质性的分析；
2. CZE方法重复性良好，适合用于半胱氨酸偶联的ADC样品电荷异质性分析；同一样品连续6针进样重复性好，主峰迁移时间的RSD值均＜0.5％，主峰，酸性峰，碱性峰CAP的RSD值＜5.0％；

Rapid charge variant analysis of NANOBODY® using capillary zone electrophoresis (CZE)

Dries Dejaegere[1], Gustavo Rivera[1] and Stephen Lock[2]
[1]Sanofi Large Molecules Research, Belgium, [2]SCIEX; United Kingdom.

This technical note demonstrates the development of a rapid CZE method for charge variant analysis of NANOBODY samples. With a minimum method optimization using SCIEX Rapid Charge Variant Analysis kit, the developed CZE assay has been evaluated against conventional methods, such as capillary isoelectric focusing (cIEF) and cation exchange chromatography (CEX).

Nanobody molecules are derived from a type of antibody that contains only heavy chains, rather than an antibody with both heavy and light chains. A single nanobody domain is approximately 10% of a standard monoclonal antibody (mAb) size.[1,2] By connecting individual nanobody domains together, multivalent nanobody molecules are produced that can bind to multiple targets, making this type of molecule a promising drug modality for complex disease pathology.

Charge variants are important product quality attributes for biological molecules, as they can influence the stability and biological activity of the biotherapeutics. Characterizing the charge heterogeneity of protein therapeutics is important for critical quality attribute (CQA) assessment to ensure drug safety, efficacy and potency.[3] Differences in charge variants are often observed due to post-translational modifications, such as chemical modifications to select amino acid sites like deamidation, glycosylation, etc.[4]

The rapid CZE assay can achieve the separation of parent intact species from deamidated impurities in ess than 12 minutes, significantly increase the throughput comparing to conventional cIEF and CEX methodology.

Key features of rapid CZE analysis of NANOBODY samples

- **Minimum method development:** using SCIEX Rapid Charge Variant Analysis kit, limited method optimization was needed to develop a rapid CZE assay
- **Effective charge variant separation:** The method is capable of separating charge variants found in a stressed sample using a separation time of less than 12 minutes (Figure 1)
- **Rapid analysis:** Analysis times were faster using CZE than conventional methods. The CZE method achieved separations in 12 minutes, compared to 26 minutes for cIEF and 35 minutes for CEX
- **Seamless data process:** Compatible with Chromeleon™ chromatography data software (CDS)

Methods

Sample preparation: 10 mg/mL NANOBODY samples (45–75 kDa), in formulation buffer (pH 6–7.4), were diluted 10-fold with 3

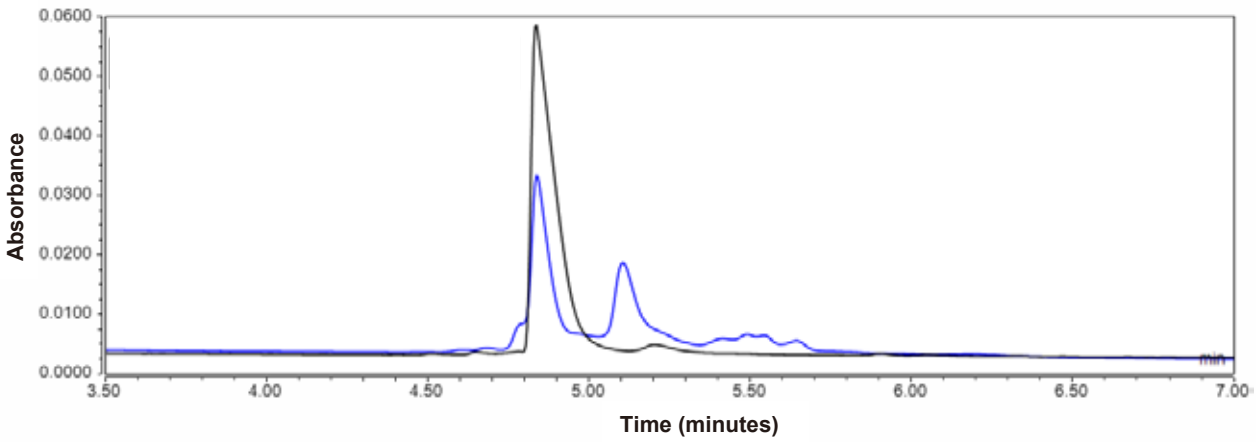


Figure 1. **CZE separation of intact and deamidated NANOBODY samples.** A comparison of an intact NANOBODY sample (black) overlayed with the same sample that had undergone a forced deamidation stress (blue). The figure highlights how a generic CZE method can separate charge isoforms of a NANOBODY sample with limited method optimization. The separation time was 12 minutes.

different types of buffers: 1) Milli-Q water, 2) CZE kit buffer 3) 20:80 (v/v) CZE buffer: Milli-Q water. Then, the prepared samples were injected onto mass spectrometry or stored in the autosampler (at 12°C) during a batch analysis for a maximum of 1 day.

CZE separation method: Samples were analyzed using a PA 800 Plus system (SCIEX). A bare fused silica capillary (length 30.2 cm, effective length 20 cm, 50 µm ID, SCIEX P/N: 338451) was conditioned with 0.1M HCl (5 min, 50 psi) and CZE buffer solution from the kit (5 min, 50 psi). Then, a voltage conditioning step was applied (30 min, 30 kV, normal polarity, 0.17-min ramp).

Samples were analyzed using the CZE Rapid Charge Variant Analysis kit (SCIEX P/N: C44790). The method began with a capillary equilibration step by rinsing with 0.1M HCl (5 min, 50 psi) and CZE buffer solution (3 min, 40 psi). The sample was then pressure injected (10 sec, 0.7 psi), followed by a post-injection of water (10 sec, 0.1 psi). The sample was separated using normal polarity (16 min, 30 kV, 1-min ramp) at 25°C.

At the end of a batch, a shutdown method was run. This method included a rinse with 0.1M HCl (5 min, 50 psi) and water (5 min, 50psi), followed by an additional water rinse (10 min, 100 psi).

CE detection: Detection was performed by UV absorbance at 214 nm at a data sampling rate of 4 Hz.

CE data processing: Data were collected using the PA 800 Plus system and 32 Karat software (SCIEX). The data were exported and processed in Thermo Scientific™ Chromeleon CDS.

CEX data were obtained using an in-house method developed on an Agilent 1200 HPLC system using a BioPro IEX SF column. The cIEF profile was obtained by cIEF analysis on a PA 800 Plus system. The same PA 800 Plus system was used following previously reported methods.[4] The master mixture was prepared by mixing the sample with urea (3M), cIEF gel, Pharmalytes 3–10, cathodic stabilizer (L-arginine) and anodic stabilizer (iminodiacetic acid).

Results and discussion

This study aimed to achieve a rapid charge variant analysis for NANOBODY samples. A 12-minute CZE method was developed, significantly increasing the sample throughput compared to classical in-house approaches using CEX and cIEF, which require approximately 45 minutes to complete the data acquisition.

The separation as evaluated in the initial tests using 2 different NANOBODY molecules. The isoelectric point (pI) of sample B was 9.2 and the pI of sample C was 10.0. A series of 5 samples were then analyzed to test the desired throughput using a 16-minute method and 4 Hz sampling rate. Finally, the effect of the sample buffer was evaluated. Figure 2 compares the same nanobody molecule (sample B) diluted with 1) CZE buffer solution, 2) Milli-Q water and 3) 20:80(v/v) CZE buffer: Milli-Q water. Dilution with Milli-Q water yielded the best peak shape. This result was attributed to improved field amplified sample stacking compared to the other sample solvents[5].

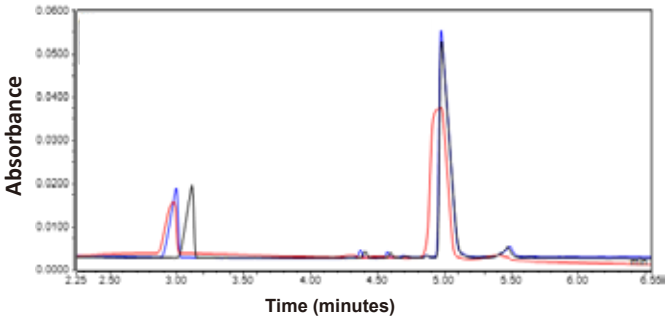


Figure 2. **The effect of the sample buffer on the CZE electropherograms.** Samples were diluted in CZE buffer (red), a mixture of CZE buffer and Milli-Q water (blue) or Milli-Q water (black).

To evaluate the reproducibility of the CZE assay, NANOBODY sample B was injected in 5 different days. The overlaid electropherograms demonstrate consistent results across the days (Figure 3).

Finally, this CZE method was compared against traditional in-house cIEF and CEX approaches. An example of Nanobody sample C analyzed using the 3 methods is shown in Figure 4. This comparison (Figure 4) shows that the CZE method can separate and detect charge variants (for example, deamidated species) of a Nanobody sample in less than half the analysis time

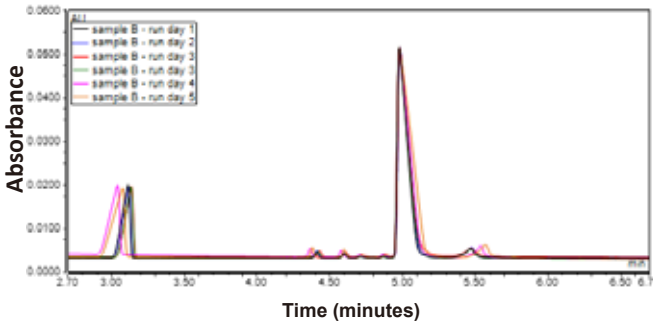


Figure 3. **5-day reproducibility study of NANOBODY CZE analysis**

of the other 2 conventional techniques with very little up-front development time.

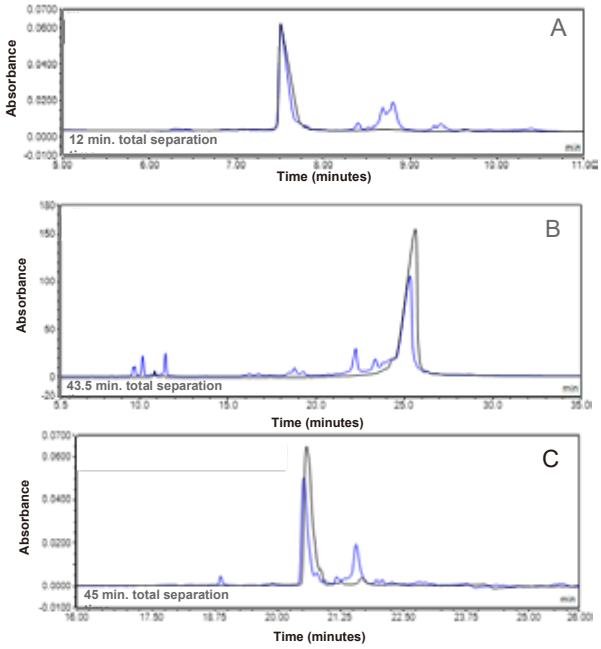


Figure 4. **Comparison of intact Nanobody (black) and stressed NANOBODY (blue) samples separated using 3 methods.** Samples were analyzed by CZE (A), a classic CEX method (B) and standard cIEF method (C) without optimization[6].

Conclusions

- With limited method development and optimization time a rapid CZE method for the separation and detection of charge isoforms present in Nanobody samples was achieved using the SCIEX CZE Rapid Charge Variant Analysis kit
- Different dilution buffers were evaluated to achieve the optimal peak shape and separation in the electrophoretic separation. Milli-Q water demonstrates the best peak shape.
- Performance of this assay over 5 days illustrates consistent data quality and separation profile using the rapid 12-minute CZE method we developed. The throughput is twice that of current inhouse CEX and cIEF methods.
- Seamless data processing using Chromeleon CDS facilitates data integrity.

References

1. Jovčevska I and Muyldermans S (2020),The Therapeutic Potential of Nanobodies. BioDrugs 34:11–26.
2. Ingram JR, Schmidt FI, Ploegh HL (2018), Exploiting Nanobodies' Singular Traits. Ann Rev Immunol 36:695–715.
3. Liu H, et al. (2014). In vitro and in vivo modifications of recombinant and human IgG antibodies. MAbs. 2014;6(5):1145-54.
4. Moritz, B. et al. (2015). Evaluation of capillary zone electrophoresis for charge heterogeneity testing of monoclonal antibodies, J. Chromatogr. B., 983-985, 101-110.
5. Zarad, W. et. Al (2021). Field amplified sample stacking and in-capillary derivatization for forensic analysis of morphine and morphine-6-glucuronide in human urine by capillary electrophoresis. Talanta Open 3, 100041.
6. Ratnayake, C. et al. (2018). Achieving Robust cIEF Analysis of Monoclonal Antibodies While Increasing Capillary Run-Life and Maintaining High Resolution and Reproducibility. Sciex technical note RUO-MKT-02-7480-A.

Capillary zone electrophoresis (CZE) separation of charge isoforms of acidic NANOBODY® samples

Dries Dejaegere[1] and Stephen Lock[2]
[1]Sanofi Large Molecules Research, Belgium, [2]SCIEX; United Kingdom.

NANOBODY® molecules, due to their small size and simple structure, are currently of interest as potential candidates for cancer drugs and in the treatment of autoimmune diseases.[1] Multivalent NANOBODY®[2,3] molecules are generated by linking individual NANOBODY® building blocks together, resulting in structures that are smaller than a classical monoclonal antibody (mAb).

Multivalent NANOBODY® molecules can exhibit a wide range of isoelectric point (pI) values due to the diversity of building blocks used in the formatting. Like mAb molecules, NANOBODY® molecules can be prone to chemical modification during their manufacturing process or storage, such as, deamidation or pyroglutamate formations resulting in acidic charge variants, whereas succinimides form basic isoforms. The assessment of these critical quality attributes (CQAs) is important, as these chemical modifications can impact the overall charge of the protein, ultimately reducing its stability and potency.[4]

In classical CZE separation, the protein pI should always be higher than the pH of the CZE buffer so that the target proteins carry a charge and can move along the capillary towards the detector window when an electric field is applied. Normally, the pH of CZE buffer is at 5.7, which is designed to separate more basic protein molecules (above pI 7) in the classical separation. In this technical note, reversing the polarity of the applied field and using CZE to analyze acidic targets under the same classical buffer conditions was tested. The application of this approach to different NANOBODY® molecules which have been treated under various conditions to generate chemical modifications was studied. Additionally, the work also compares this approach with alternative separation modes, for example anion exchange separation (AEX) as shown in Figure 1.

Key features of CZE separation of acidic NANOBODY® molecules using a conventional buffer system

- **Detection of PTMs on acidic proteins** after thermal or chemical stress using the same CZE buffer used for classic separation
- **Full examination of CZE separation mechanism** of NANOBODY® molecules highlighted that the method could analyse acidic proteins whose pI was below 5.2 by simply reversing the polarity of separation using a classical CZE buffer without any modification.
- **CZE provides faster and better resolving power** than a platform AEX method for a NANOBODY® with pI < 5.2

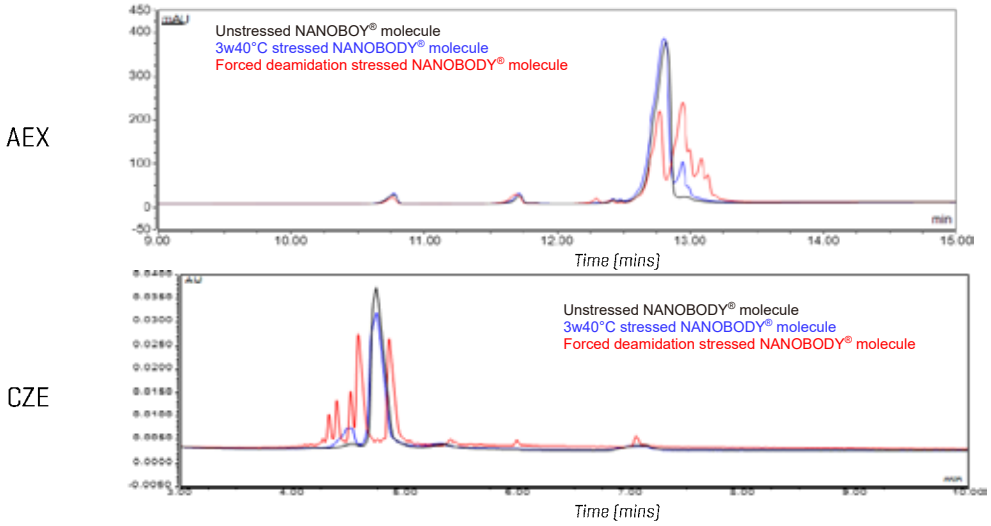


Figure 1. A comparison of the analysis of a NANOBODY® molecule with pI 4.4 by both CZE and AEX. In each technique, the separation trace of the untreated NANOBODY® is overlayed with a corresponding sample treated under thermal and forced deamination stressed conditions.

Introduction

CZE is a widely adopted technique for separating and detecting charge variants in biopharmaceuticals. CZE was originally developed in 2011 by He et al.[4] and subsequently subjected to industry ring trials in 2015.[5] CZE is a robust method suitable for implementation in a GMP environment. CZE has been traditionally applied mainly to neutral or basic targets[5,6] as the isoelectric point of the targets had to be above the pH of the CZE buffer which was traditionally set at 5.7. With the advent of more diverse protein structures the application of CZE to more acidic proteins are required. In this technical note the use of CZE buffers with pH 5.7 in the analysis of acidic NANOBODY® molecules was investigated.

Methods

Sample preparation: NANOBODY® molecules (45–75 kDa), originally at 10 mg/mL in formulation buffer containing amino acids (pH 4.4 –7.0), were diluted 10-fold with Milli-Q water before injection. Samples were stored in the autosampler during a batch analysis at 12°C for a maximum of 1 day.

CZE separation method: Samples were separated using a PA 800 Plus system (SCIEX). Before samples were tested, a bare fused silica capillary (length 30.2 cm, effective length 20 cm, 50 µm ID, SCIEX P/N: 338451) was conditioned with 0.1M HCl (5 min, 50 psi) and voltage conditioned (30 min, 30kV) after a CZE buffer rinse (5 min, 50 psi) using a commercially available buffer from the CZE Rapid Charge Variant Analysis kit (SCIEX, P/N: C44790)

Samples were then analyzed using a method that initially applies capillary equilibration by rinsing with 0.1M HCl (2 min, 40 psi) and the commercial CZE separation buffer (2 min, 40 psi). The sample was pressure injected (10 sec, 0.7 psi), followed by a post injection of water (10 sec, 0.1 psi). The sample was separated using reverse polarity (16 min, 30 kV, 1-min ramp) at 25°C.

At the end of a batch, a shutdown method was run that applied a rinse with 0.1M HCl (5 min, 50 psi) and water (5 min, 50 psi). An additional water rinse (10 min, 100 psi) was then performed.

CE detection: NANOBODY® molecule peaks were detected by UV adsorption at 214 nm using a 200 µm x 100 µm aperture and a data sampling rate of 4 Hz.

CE data processing: Data were collected using the PA 800 Plus system in 32 Karat software (SCIEX) and transferred in AIA format and processed in Thermo Scientific™ Chromeleon CDS (7.2.10).

Results and discussion

This investigation explored the use of a commercial CZE buffer, which was initially designed to analyze basic mAbs, for the analysis of more acidic NANOBODY® molecules. In classical CZE separation the buffer's pH needs to be different from the pI of the target protein for it to have charge and move under an applied electric field. When the proteins have a lower pI than the buffer pH and a normal or positive voltage is applied to the capillary, the proteins will move in the reverse direction away from the detector window and will not be detected. For acidic proteins, a reverse polarity or negative field needs to be applied, which pushes the negatively charged proteins towards the detector window.

In Figure 2 the effect of the pI of the protein on the CZE separation on a series of different NANOBODY® molecules under the same conditions and using a commercial CZE buffer was studied. It was found that when the pI was above 5.35 no peaks were observed. This was expected as the pI of the NANOBODY® molecule was close to the pH of the separation buffer, preventing proteins from moving towards the detection window. As the pI of the proteins drops, separation improves and migration of the protein speeds up, producing good separation of thermally and forced deaminated stressed samples. When the pI of the NANOBODY® molecule was 5.35, only peaks associated with a thermally stressed sample were detected. This was because the protein's pI was 0.4 pH units below that of the buffer.

When the same proteins were analyzed by an in-house AEX method, as shown in Figure 3, separations of the NANOBODY® molecules at pI 5.35 and 5.4 were obtained. However, no separation of the NANOBODY® molecule at pI 7 was observed as the protein did not interact with the AEX stationary phase and eluted very early in the dead volume of the column (< 1 minute).

Direct comparison between the CZE and AEX separation of an acidic NANOBODY® molecule with a pI of less than 5.0, as highlighted in Figure 1, shows that CZE provides both faster separation (except for the pI 5.2 NANOBODY® molecule) and better resolution than AEX.

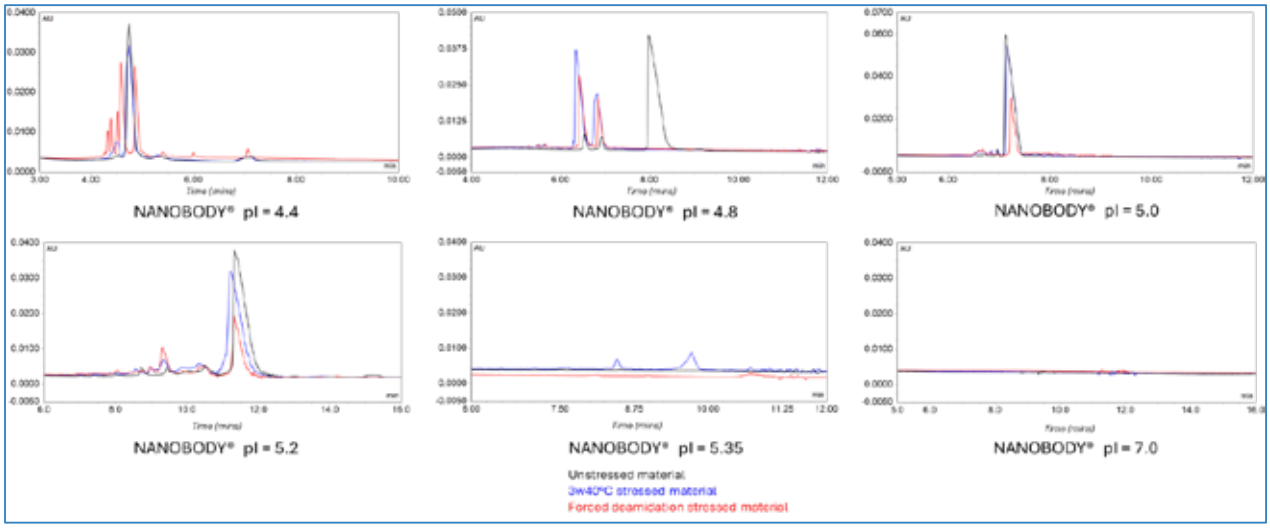


Figure 2. The effect of the pI of a NANOBODY® molecule on the CZE separation of untreated, forced chemically modified and thermally stressed NANOBODY® samples.

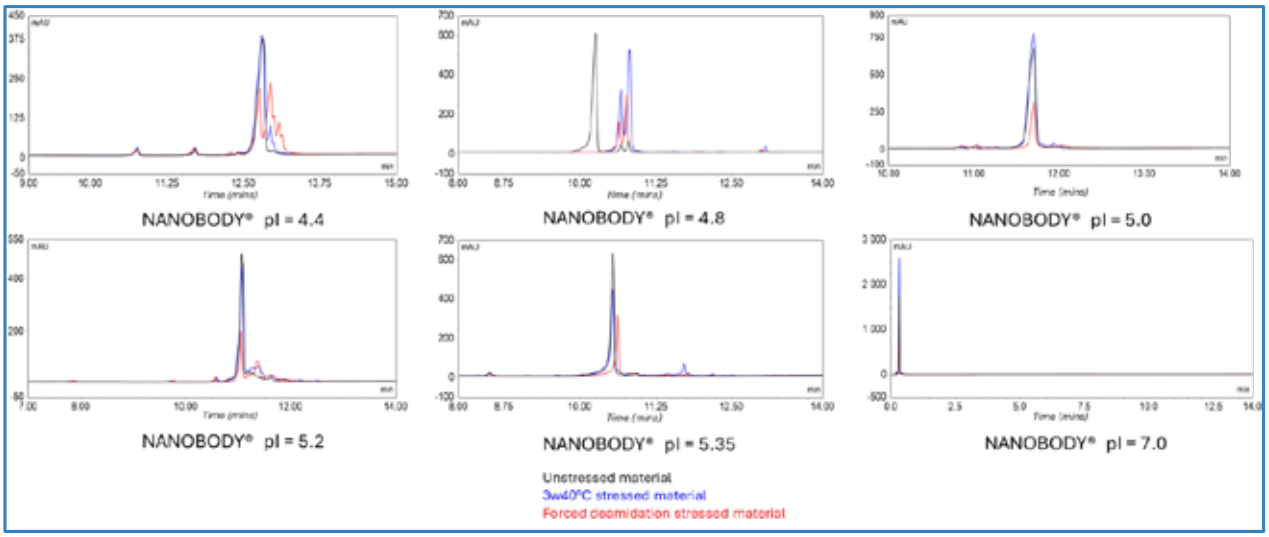


Figure 3. The effect of the pI of a NANOBODY® molecule on the AEX separation of untreated and forced chemically modified and thermally stressed NANOBODY® samples

Conclusions

- Compared to AEX for NANOBODY® molecules with pI <5.2, CZE was found to be faster and more resolutive than the corresponding in-house AEX method.
- Analysis of acidic NANOBODY® molecules (pI below 5.2) is optimally performed under reverse polarity conditions utilizing the commercial CZE buffer from SCIEX .
- To achieve optimal separation when pI is close to the pH of the buffer (pH range 5.2- 6.2) it is recommended to adjust the pH of the CZE buffer to maintain a minimum differential of 0.5 units from the expected pI of the protein.
- For proteins higher in pI (> 6.2), it is recommended to use the classical separation (applicable for basic proteins) as previously described[7].

References

1. Jin B-k., et al (2023). NANOBODIES®: A review of generation, diagnostics and Therapeutics. Int. J. Mol. Sci. 2023, 24, 5994, 1-22.
2. Jovčevska I and Muyldermans S (2020),The Therapeutic Potential of Nanobodies. BioDrugs 34:11–26.
3. Ingram JR, Schmidt FI, Ploegh HL (2018), Exploiting Nanobodies' Singular Traits. Ann Rev Immunol 36:695–715.
4. He, Y., et al. (2011), Rapid analysis of charge variants of monoclonal antibodies with capillary zone electrophoresis in dynamically coated fused-silica capillary, J. Sep. Sci., 34 (5), p.548-555.
5. Moritz, B. et al. (2015). Evaluation of capillary zone electrophoresis for charge heterogeneity testing of monoclonal antibodies, J. Chromatogr. B., 983-985, 101-110.
6. Moritz, B. et al. (2017). Optimization of capillary zone electrophoresis for charge heterogeneity testing of biopharmaceuticals using enhanced method development principles. Electrophoresis 38 (24), 3136-3146.
7. Dejaegere, D. et al. (2023). Rapid charge variant analysis of NANOBODY® using capillary zone electrophoresis (CZE). Sciex application note document number MKT-29481-A.

Analysis of co-formulated monoclonal antibodies by capillary zone electrophoresis

Marcia Santos

SCIEX, USA

While co-formulated drugs have been around for some time, the remarkable success of biologics has propelled co-formulated antibodies into the spotlight, especially following the COVID-19 pandemic.[1] This technical note demonstrates a simple and reliable 7-minute (min) capillary zone electrophoresis (CZE) separation method for assessing the charge heterogeneity of co-formulated monoclonal antibodies (mAb), which could serve as a potential assay for lot release.

The effective combination of antibodies is a powerful strategy employed in various drugs, leveraging the synergistic properties of each component. Examples of co-formulated therapies include polyvalent vaccines, such as the poliovirus vaccine, and combinations of fast- and intermediate-acting insulin.[2]

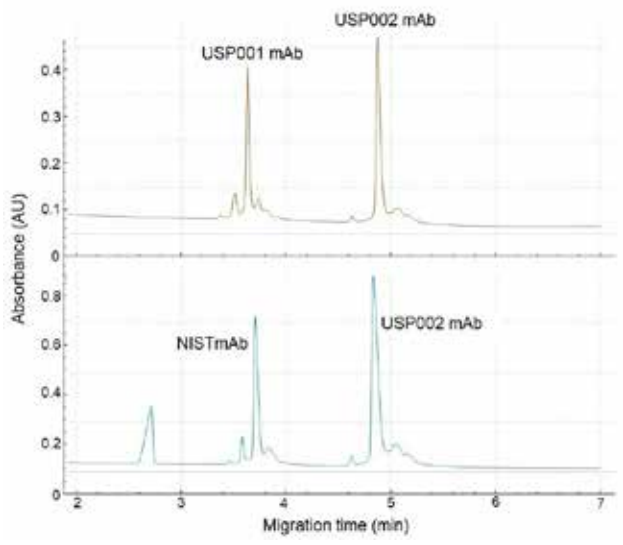


Figure 1: Comparison of the CZE results for co-formulated USP001 and USP002 mAbs (top trace) with those for NISTmAb and USP002 mAb samples (bottom trace).

Chromatography-based technologies, including size exclusion, ion exchange, and reversed-phase liquid chromatography, are invaluable for analyzing these complex mixtures.[3] However, the characterization landscape is only complete with orthogonal techniques to charge heterogeneity such as capillary isoelectric focusing (cIEF) and CZE. CZE and cIEF are charge heterogeneity-based separation methods, sensitive enough to detect degradation and post-translational modifications. (PTMs).[4]

This technical note presents a straightforward and robust method for quickly assessing the charge heterogeneity of co-formulated antibodies using CZE (Figure 1).

Key features of co-formulated monoclonal antibodies by CZE

- **Fast separation method:** A less than 7-min separation was achieved for charge heterogeneity of co-formulated antibodies.
- **Straightforward workflow:**
  - **Simple sample preparation** – Antibody samples are diluted in water and injected without the need for buffer exchange
  - **Commercially pre-assembled cartridge:** Consistent capillary length ensures reproducibility and comparability of data.
- **Quantitative assay:** good linearity of the calibration curve, with an R^2 of 0.999

Introduction

In a clinical setting, co-formulated antibodies provide several benefits, such as enhanced therapeutic efficacy through cooperative effects between multiple antibodies, improved patient convenience with fewer required treatments, reduced risk of dosing errors, and potentially better patient compliance than administering individual antibodies separately. From a regulatory perspective, co-formulated antibodies are classified as new drugs. Therefore, factors such as stability, protein interactions, protein concentrations, excipient types, and pH must be rigorously investigated.[2]

Therefore, a variety of analytical technologies are necessary to characterize these complex mixtures, including size exclusion chromatography (SEC), ion exchange chromatography (IEC), and dynamic light scattering (DLS).[5] Jihong et al.[6] reported that charge-based separations like cIEF and IEC are particularly useful due to their sensitivity to modifications such as protein interactions, pH, stability, etc.

However, cIEF sample preparation can be complex and challenging due to the numerous reagents required for preparation and separation, making it less desirable in a quality control setting. He et al.[4] reported a simple yet robust CZE method for separating monoclonal antibodies, requiring minimal sample preparation. Moritz et al.[7] published a landmark paper discussing an intercompany collaborative effort using CZE to analyze mAbs in detail. This study successfully demonstrated that the CZE for charge heterogeneity profiling of mAbs is precise, accurate, robust, linear, and sensitive across sites and users with no prior training on the application. Therefore, the straightforward implementation of this assay makes it ideal for a regulated GMP environment.

In this work, mAb reference standards from NIST and USP were used as surrogate molecules to emulate co-formulated antibody mixtures. The feasibility and quantitation of this assay were conducted using the CZE rapid charge variant analysis kit.

Methods

Samples: NISTmAb, Humanized IgG1k Monoclonal Antibody (P/N: 8671) was from NIST (Gaithersburg, MD). USP mAb 001, Monoclonal IgG1 (P/N 1445539) and USP mAb 002, Monoclonal IgG1 (P/N 1445547) were from USP, Frederick, MD. Before CZE analysis, these samples were diluted to 1mg/mL in water.

Reagents: The CZE Rapid Charge Variant Analysis kit (P/N: **C44790**) and cIEF Charge Heterogeneity analysis kit (P/NC30101) were from SCIEX (Framingham, MA).

Capillaries and capillary cartridges: The pre-assembled bare fused silica capillary cartridge (P/N A55625) for CZE experiments and the neutral capillary used in cIEF experiments (P/N 477441) were from SCIEX.

Capillary electrophoresis instrument: PA 800 plus system equipped with a UV detector was from SCIEX. Data acquisition for CZE and cIEF experiments was carried out at 214 nm and 280 nm, respectively.

Instrument methods: The CZE and cIEF methods used in this study were as described in the respective application guides.[8,9]

Software: 32 Karat software, Version 10, was the acquisition software and data analysis was performed with the BioPhase software, version 1.2.

Results and discussion

CZE vs. cIEF

CZE is the simplest mode of capillary electrophoresis (CE) separation, where sample molecules are separated based on their size-to-charge ratio. Essentially, molecules with smaller hydrodynamic volumes and higher charges move faster under the applied electric field.[10]

In contrast, cIEF is a sophisticated, high-resolution separation mechanism where the protein molecules are separated based on their isoelectric point (pI)[10]. cIEF sample mixture is a complex combination of:

1. ampholytes - species that promote the separation based on pI
2. cathodic and anodic stabilizers – compounds that maintain the pH gradient stable inside the capillary
3. pI markers – to aid in the pI assignment
4. the protein of interest

The CZE application for the separation of mAbs to elucidate their charge heterogeneity was developed by He et al., using a bare fused silica capillary. The buffer system consists of ε-aminocaproic acid (EACA) and triethylenetetramine (TETA).

TETA, a polyamine, interacts with the negatively charged silica surface, thereby enhancing the resolution of basic variants.[7]

Figure 2 compares the CZE and cIEF separations of NISTmAb. Besides the inherent high-resolution capability of cIEF, the overall profiles of CZE and cIEF are comparable. Although CZE cannot determine pI information, it is equally sensitive to charge heterogeneity changes resulting from sample stability, PTMs, protein-to-protein interactions, to name a few.[6]

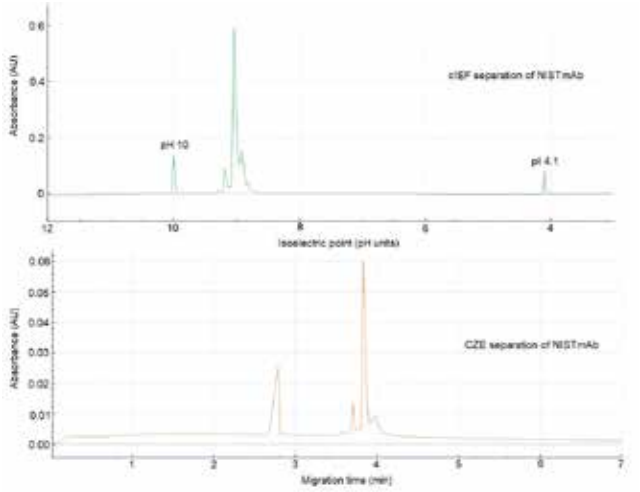


Figure 2: Comparison between cIEF (top trace) and CZE (bottom trace)separations of NISTmAb.

Feasibility of CZE to characterize co-formulated antibodies

Figure 1 shows 2 electropherograms (e-grams). The top e-gram displays the CZE separation of USP001 and USP002, each at a concentration of 1μg/μL. Similarly, the bottom e-gram shows the profile separation of NISTmAb and USP002. These mixtures were specifically chosen because the pI of the mAbs (main peak) differ by approximately 1 pH unit. Therefore, Figure 3 demonstrates excellent separation between the 2 antibodies, indicating the suitability of CZE for assessing the charge heterogeneity of these co-formulated samples.

It is important to note that if the physicochemical properties of the antibodies are similar, such as having closely aligned pI values, the profiles will inevitably overlap. In such cases, the use of this assay is not recommended.

Linearity of the method

In antibody co-formulation, challenges include antibody interactions with excipients, stability in formulation buffer and pH, and concentration disparities between the antibodies. Figure 3 shows a stacked view of the NISTmAb and USP002 mAb to demonstrate the linearity of this assay when applied to the concentration disparity. In this experiment, the concentration of NISTmAb was kept constant at 1.0 μg/μL. Meanwhile, the concentration of USP002 increased from 0 to 1.0 μg/μL by increments of 0.2 μg/μL.

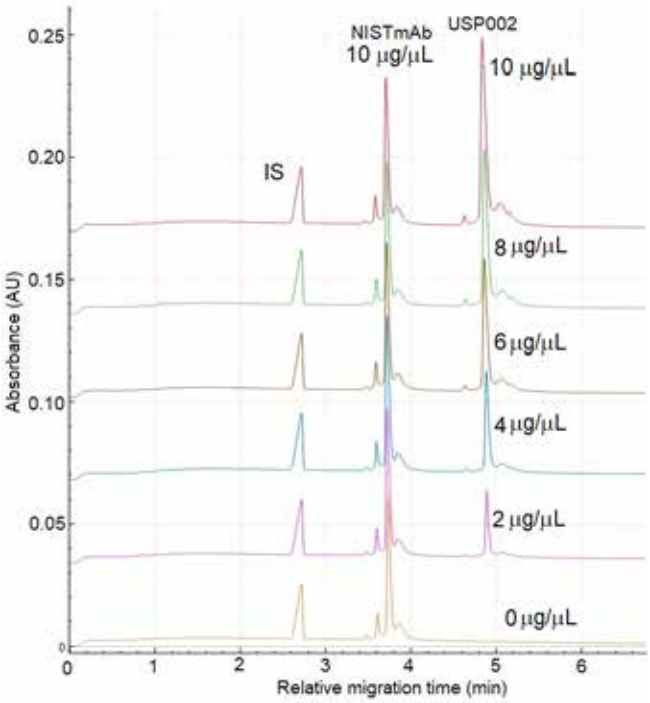


Figure 3: CZE electropherograms of co-formulated NISTmAb and USP002 mAb. The concentration of NISTmAb was kept constant at 10 μg/μL and the USP002 mAb varied from 0 to 10 μg/μL at 0.2 μg/μL increments.

Typically, in capillary electrophoresis, to eliminate any variations from the sample injection, a calibration curve is done using an internal or external standard. The internal standard refers to a compound spiked into the sample at a known concentration. In this work, the internal standard (IS) is the peak migrating at approximately 2.7 min in the NISTmAb separation in Figure 3. This peak is attributed to L-histidine in the formulation buffer,[11] at a concentration of 25 mM. Figure 4 shows the calibration curve with the amount ratio on the Y-axis and the area ratio on the X-axis. The amount ratio is calculated

by dividing the amount of the compound of interest at various concentrations and the amount of the IS in µg/µL. Likewise, the X-axis is calculated by dividing the corrected peak areas (CPA) of the compound of interest at various concentration levels by the corrected peak area of the IS.

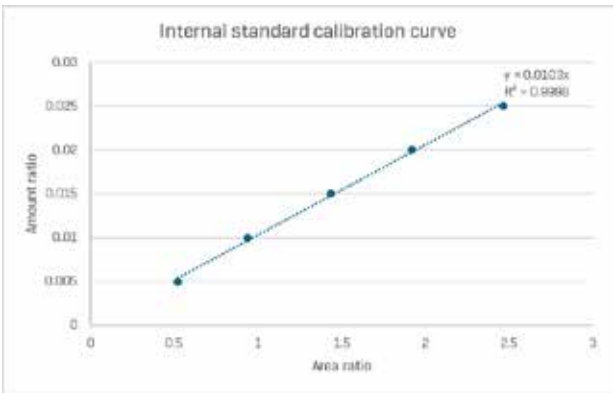


Figure 5: USP002 mAb calibration curve, using the IS in the NISTmAb. The area ratio was calculated by dividing the area under the curve of the USP002 mAb by the area of the histidine peak. The amount ratio was calculated by dividing the amount in ug/uL the USP002 mAb by the amount of the histidine in ug/uL.

In this work, the corrected peak area for USP002mAb is the sum of the basic, main, and acidic variants. To validate the quantitative assay, a mixture of 1.0 µg/µL NISTmAb and 0.50 µg/µL USP002 was analysed using this assay.

The concentration of the USP002 mAb was calculated based on CPA, using the calibration curve shown in Figure 5, revealing a concentration of 0.51 µg/µL for USP002, demonstrating a recovery of 102%.

Overall, the CZE assay utilizing the SCIEX CZE rapid charge variant analysis kit shows potential as a lot-release assay for co-formulated mAbs, offering high-speed separation in 7 minutes, along with high resolution and quantitation capabilities.

Conclusions

- **A streamlined workflow with a simple sample preparation and fast separation was developed for charge heterogeneity analysis of co-formulated mAbs.**
    - **Simple sample preparation:** requires only sample dilution in water
    - **Fast separation:** co-formulated antibodies can be separated by charge heterogeneity in less than 7 minutes
    - **Ease of use**: A commercial kit combines chemistry and pre-assembled cartridge.
- **Quantitative assay:** The assay demonstrated good linearity and provided accurate quantitation results.

References

1. S. Liu, Y. Yan, C. M. Secor, Z. R. Oberholtzer, D. J. Skow, Mu. Sheikh, Y. Moon, Y. Fu, C. Sandu, S. Wang, N. Li, J. B. Nguyen, M. P. Rosconi & E. A. Pyles (2024) Enrichment strategy and initial characterization of heterodimers enriched from a co-formulated cocktail of therapeutic antibodies against SARS-COV-2, mAbs, 16:1, 2338301
2. V. M. Chauhana, H. Zhang, P. A. Dalby, J. W. Aylotta, (2020) Advancements in the co-formulation of biologic therapeutics, Journal of controlled release Volume 327, Pages 397-405
3. F. Kuhne, K. Heinrich, M. Winter, J. Fichtl, G. Hoffmann, F. Zähringer, K. Spitzauer, M. Meier, T. A. Khan, L. Bonnington, K. Wagner, J. O. Stracke, D. Reusch, H. Wegele, M. Mormann, and P. Bulau, (2023), Identification of Hetero-aggregates in Antibody Co-formulations by Multi-dimensional Liquid Chromatography Coupled to Mass Spectrometry, Anal. Chem. 95, 2203−2212
4. Y. He, C. Isele, W. Hou, M. Ruesch, (2011) Rapid analysis of charge variants of monoclonal antibodies with capillary zone electrophoresis in dynamically coated fused-silica capillary, J Sep Sci., 34(5):548-55.
5. Y. He, C. Isele, W. Hou, M. Ruesch, (2011) Rapid analysis of charge variants of monoclonal antibodies with capillary zone electrophoresis in dynamically coated fused-silica capillary, J Sep Sci., 34(5):548-55.
6. J. Kim, Y. J. Kim, M. Cao, N. De Mel, K. Miller, J. S. Bee, J. Wang, X. Wang and M. Albarghouthi (2020) Analytical characterization of coformulated antibodies as combination therapy, mAbs, 12:1, 1738691.
7. M. Cao, N. De Mel, A. Shannon, M. Prophet, C. Wang, W. Xu, B. Niu, J. Kim, M. Albarghouthi, D. Liu, E. Meinke, S.

Lin, X. Wang & J. Wang (2019) Charge variants characterization and release assay development for co-formulated antibodies as a combination therapy, mAbs, 11:3, 489-499.

8. B. Moritz, V. Schnaible, S. Kiessig, A. Heyne, M. Wild, C. Finkler, S. Christians, K. Mueller, L. Zhang, K. Furuyad, M. Hassel, M. Hamm, R. Rustandi, Y. He, O. S. Solano, C. Whitmore, S. Ae. Park, D. Hansen, M. Santos, M. Lies, (2015) Evaluation of capillary zone electrophoresis for charge heterogeneity testing of monoclonal antibodies, Journal of Chromatography B, 983–984, 101-110.
9. CZE rapid charge variant analysis kit for the PA 800 Plus Pharmaceutical Analysis System, application guide.
10. Capillary isoelectric focusing (cIEF) kit for the PA 800 Plus Pharmaceutical Analysis System application guide.
11. J. P. Landers, (1997), Handbook of capillary electrophoresis, second edition.
12. A. Turner and J. Schiel (2018), Qualification of NISTmAb charge heterogeneity control assays, Analytical and Bioanalytical Chemistry, 410:2079–2093.

Rapid charge heterogeneity analysis of new therapeutic protein modalities

Zoltan Szabo and Andras Guttman
SCIEX, Brea and Redwood City, CA 92130, USA

Introduction

This technical note reports on the use of the SCIEX CZE Rapid Charge Variant Analysis Kit (P/N C44790) for rapid high resolution charge variant analysis of two bispecific antibodies. Comprehensive charge heterogeneity study of these test articles required < 10 minute total CZE analysis time with excellent migration time (0.2 %RSD) and corrected peak area distribution (<3.5% RSD) reproducibility. The method was also applied to forced degradation based stability testing, especially focusing on deamidation, i.e., generating acidic charge variant shifts.

Charge heterogeneity analysis of new therapeutic protein modalities, such as multispecific antibodies, fusion proteins and nanobodies, is of high importance during product development, production, stability and release testing.[1] Therefore, rapid and reproducible high throughput process analytical technology methods are of high value to biopharma. Analysis of charge variants is also important during forced degradation studies for quality assessment.[2] Charge heterogeneity of these new generation biotherapeutics arises from deamidation, methionine oxidation, C-terminal lysine variants, N-terminal pyroglutamate formation and glycosylation, just to list the most frequent ones.[2-4]

Traditionally used methods for protein charge variant analysis are ion exchange chromatography [5] and various forms of isoelectric focusing [6, 7]. However, both chromatographic and isoelectrophoretic methods are relatively slow, requiring up to 1 hour of analysis time. To alleviate this issue, capillary zone electrophoresis (CZE) was introduced a decade ago for charge heterogeneity analysis of protein therapeutics [8]. In CZE, the differential electromigration of the sample components is based on their hydrodynamic volume to charge ratio, i.e., can be readily optimized for high resolution separation of charge variants. CZE also represents a versatile separation platform to analyze product related attributes with efficient sample preparation, also supporting high throughput screening processes. With the use of high concentration zwitterionic buffer components such as epsilon amino caproic acid and specific capillary coating additives like triethylenetetramine [9], the electroosmotic flow can be minimized during capillary electrophoresis. In this way, bare fused silica capillary columns can be readily employed to obtain rapid analysis of the charge variants of therapeutic proteins.

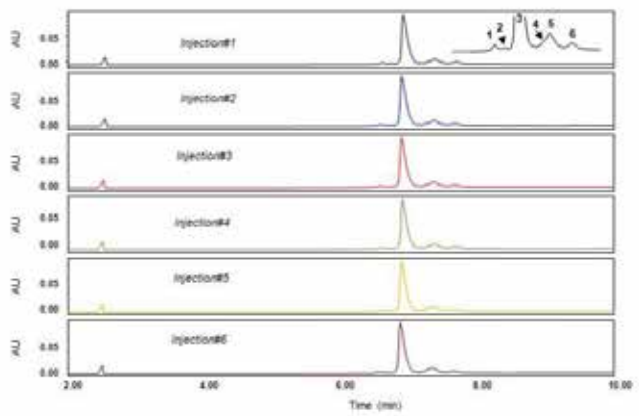


Figure 1. CZE charge heterogeneity analysis and reproducibility study of the bispecific mAb-1 test article. Peaks 1 and 2: fast migrating charge variants, Peak 3: main component, Peaks 4-6: slow migrating charge variants. The inset depicts the enlarged part of the electropherogram with the peaks numbered. Conditions: 30 cm total capillary length (20 cm effective, 50 µm ID), applied electric field strength: 1000 V/cm, Separation temperature: 25°C. Pressure injection at 5 psi for 10 sec.

Rapid charge variant analysis of two bispecific antibodies was demonstrated revealing highly reproducible separations in less than 10 minutes with excellent migration time and corrected peak area distribution reproducibility. The method was readily applied for forced deamidation monitoring.

Key features

- Rapid analysis times (<10 minutes) of the native forms of new modality protein therapeutics
- Excellent migration time (0.2 %RSD) and corrected peak area (1.55 %RSD) reproducibility
- Easy sample preparation protocol for low concentration samples
- High throughput analysis option for stability testing

Experimental

Chemicals

TRIS base and hydrochloric acid (37%) were purchased from Sigma Aldrich (St. Louis, MO, USA). The bispecific monoclonal antibody test articles of mAb-1 and mAb-2 were from Genentech (South San Francisco, CA) and Amgen (Thousand Oaks, CA), respectively. To perform the charge heterogeneity separation of new modalities, the CZE Rapid Charge Variant Analysis Kit (SCIEX, Brea, CA, part#C44790) was used consisting of CZE Rapid Charge Variant Separation Buffer, Acid Wash/Regenerating Solution, CE Grade Water and Protein Test Mix.

Sample preparation

Test article mAb-1 (30 mg/mL) was diluted with water to 1 mg/mL concentration and analyzed by CZE. Test article mAb-2 had significantly lower concentration of (0.2 mg/mL) thus to avoid matrix related disturbances, it was buffer exchanged prior to CZE analysis by washing the therapeutic protein three times with 100 µL of water through a 10 kDa cut-off centrifugation filter (Millipore, Billerica, MA) at 14 000 x g. The purified protein was recovered in the same volume as the volume dispensed onto the filter in the formulation buffer.

In order to perform forced deamidation mAb-1 sample, Tris-HCl buffer was prepared at 1 M concentration by carefully adding concentrated hydrochloric acid solution to TRIS to adjust the pH at 8.7. The solution of test article mAb-1 was diluted with water and 1 M Tris-HCl buffer (pH 8.7) to obtain a 1 mg/mL protein solution in 100 mM Tris-HCl buffer. The diluted sample solution of the therapeutic protein was incubated in a heating block at 45 ºC and samples were taken for CZE separation after 1, 2 and 3 days of incubation times and buffer exchanged on 10 kDa cut-off filters prior to analysis.

Capillary zone electrophoresis

All separations were carried out in a PA 800 Plus Pharmaceutical Analysis System (SCIEX) using the EZ-CE Pre-Assembled Capillary Cartridge (SCIEX, part# A55625) with 30 cm total capillary length (20 cm effective length, 50 µm ID). The separation voltage was 30 kV at 25ºC. Injection: 5 psi for 10 sec for mAb-1 and 20 sec for mAb-2. All data was acquired and processed by the 32Karat ver10.1 software package (SCIEX).

Results and discussion

The charge heterogeneity of two bispecific antibody test articles was assessed by CZE with special attention to migration time and corrected peak area reproducibility. Forced degradation of the samples were also accomplished.

The CZE charge variant analysis of test article mAb-1 was accomplished in less than 8 minutes, featuring the separation of 6 peaks as shown in Figure 1. The inset depicts the enlarged part of the electropherogram with the peaks numbered. Peaks 1 and 2 were faster migrating possible basic species, Peak 3 the main component and Peaks 4-6 were slower migrating, possible acidic species. To verify the acidic and/or basic feature of these components, capillary isoelectric focusing analysis is required, which was outside of the scope of this exercise. The 6 consecutive runs of the test article mAb-1 resulted in the average migration time and corrected peak area % reproducibility of 0.2 %RSD (Table 1) and 1.55 %RSD (Table 2), respectively.

Next the therapeutic bispecific antibody test article mAb-2 was analyzed the same way but, in this instance, sample injection without appropriate preparation did not result in evaluable electropherogram (Figure 2, trace A). Removal of the sample formulation matrix, on the other hand, lead to the separation of 5 components as shown in Figure 2, Trace B in less than 6 minutes. Peak 1 was a faster migrating possibly basic ingredient, Peak 2 was the main component and Peaks 3- 5 were slower migrating possibly acidic species. The migration time and corrected peak area % reproducibility were evaluated similarly to those discussed above and were found as 0.28 %RSD and 1.58 %RSD, respectively.

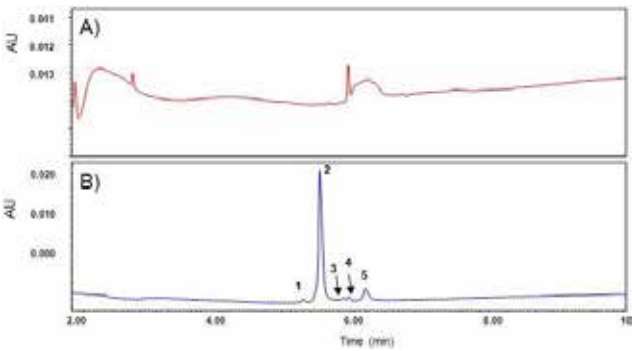


Figure 2. Effect of sample preparation on the charge heterogeneity. Analysis of the mAb-2 test article without (A) and with (B) formulation matrix removal. Separation conditions were the same as in Figure 1, except the injection parameters: 5 psi/20 sec.

Monitoring deamidation of therapeutic proteins provides crucial information about the efficacy of the product. To show the applicability of the rapid capillary zone electrophoresis to address this significant issue, a forced degradation study was performed at pH 8.7 to promote accelerated deamidation using the mAb-1 test article sample. In the time course study, samples were taken at 0 (control), 1-day, 2-day and 3-day time points and analyzed by the CZE method described above. Figure 3 depicts the resulting quantitative data of the separated species. Please note that with increasing incubation time, the shoulder Peak 4 slowly merged into Peak 5, thus, during quantitative evaluation these were considered as a single value (Figure 3, column 4+5). As one can observe, the areas of fast migrating Peak 1 and the main component (Peak 3) decreased, while Peaks 4+5 and 6 increased over the time course of the study. As a first approximation we consider this effect was caused by temperature induced deamidation under high pH conditions, resulting in the increase of the slower migrating, probably acidic components. While this treatment caused deamidation of both Peaks 1 and 3, Peak 2 did not change during this process.

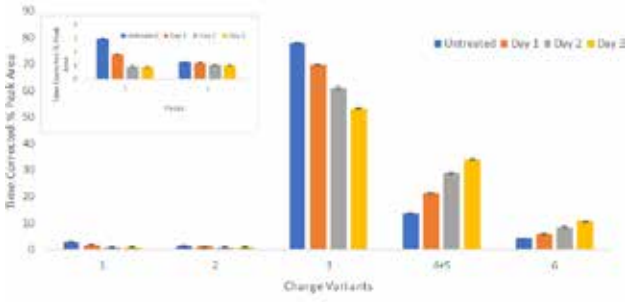


Figure 3. Quantitative CZE analysis of the forced deamidation. Charge variant products of mAb-1 test article in 100 mM Tris buffer (pH 8.7) after 1 (red bar), 2 (gray bar) and 3 (yellow bar) days of treatment in comparison to the untreated (blue bar) sample. Charge variant peaks 1 and 2: slower migrating components, peak 3: main component, Peaks 4+5 and 6: slower migrating components. The inset depicts the enlarged part of the changes in peaks 1 and 2.

Table 1. Migration time reproducibility of the separated charge variants of test article mAb-1 in Figure 1.

Migration time (min)									
Peaks	Injection#1	Injection#2	Injection#3	Injection#4	Injection#5	Injection#6	Average	STDEV	% RSD
1	6.60	6.58	6.58	6.57	6.57	6.55	6.58	0.01	0.22
2	6.73	6.71	6.71	6.70	6.70	6.68	6.71	0.02	0.26
3	6.91	6.89	6.90	6.89	6.88	6.87	6.89	0.01	0.18
4	7.31	7.29	7.30	7.29	7.29	7.27	7.29	0.01	0.19
5	7.37	7.35	7.37	7.35	7.35	7.33	7.35	0.01	0.17
6	7.68	7.66	7.67	7.66	7.65	7.63	7.66	0.02	0.20

Average: 0.20 %

Table 2 Corrected peak area reproducibility values of the separated charge variants of test article mAb-1 in Figure 1.

Time corrected % peak area									
Peaks	Injection#1	Injection#2	Injection#3	Injection#4	Injection#5	Injection#6	Average	STDEV	% RSD
1	2.94	2.94	2.97	3.02	2.96	2.99	2.97	0.03	0.94
2	1.21	1.23	1.22	1.25	1.28	1.29	1.25	0.03	2.35
3	77.74	77.79	77.84	77.90	77.75	77.92	77.82	0.07	0.09
4	3.54	3.37	3.39	3.39	3.65	3.31	3.44	0.12	3.35
5	10.21	10.37	10.30	10.28	10.09	10.32	10.26	0.09	0.88
6	4.36	4.29	4.27	4.16	4.26	4.17	4.25	0.07	1.66

Average: 1.55%

Conclusions

- Rapid charge variant analysis of two new modality protein test articles was demonstrated.
- Highly reproducible separations were obtained for both bispecific antibodies in less than 8 minutes with excellent migration time (0.2% RSD) and corrected peak area distribution (<3.5% RSD) reproducibility.
- The importance of sample preparation by removing the formulation matrix was apparently important for the lower concentration mAb-2 test article.
- Charge variant changes were systematically monitored after forced deamidation of the mAb-1 test article in a 3-day time course revealing significant decrease in the quantity of the main components towards the slower migrating, probably acidic species.
- The CZE technique reported here can be readily applied for high throughput charge heterogeneity analysis of new therapeutic modalities.

Acknowledgment

The stimulating discussions with Elliott Jones and Sahana Mollah are greatly appreciated..

References

1. Singh, S.K., G. Narula, and A.S. Rathore, Should charge variants of monoclonal antibody therapeutics be considered critical quality attributes? Electrophoresis, 2016. 37(17-18): p. 2338-46.
2. Hsu, Y.R., et al., Selective deamidation of recombinant human stem cell factor during in vitro aging: isolation and characterization of the aspartyl and isoaspartyl homodimers and heterodimers. Biochemistry, 1998. 37(8): p. 2251-62.
3. Harris, R.J., Processing of C-terminal lysine and arginine residues of proteins isolated from mammalian cell culture. J Chromatogr A, 1995. 705(1): p. 129-34.
4. Moorhouse, K.G., et al., Validation of an HPLC method for the analysis of the charge heterogeneity of the recombinant monoclonal antibody IDEC-C2B8 after papain digestion. J Pharm Biomed Anal, 1997. 16(4): p. 593-603.
5. Stoll, D., et al., Characterization of therapeutic antibodies and related products by two-dimensional liquid chromatography coupled with UV absorbance and mass spectrometric detection. J Chromatogr B Analyt Technol Biomed Life Sci, 2016. 1032: p. 51-60.
6. Righetti, P.G., Determination of the isoelectric point of proteins by capillary isoelectric focusing. J Chromatogr A, 2004. 1037(1-2): p. 491-9.
7. Sosic, Z., et al., Application of imaging capillary IEF for characterization and quantitative analysis of recombinant protein charge heterogeneity. Electrophoresis, 2008. 29(21): p. 4368-76.
8. He, Y., et al., Analysis of identity, charge variants, and disulfide isomers of monoclonal antibodies with capillary zone electrophoresis in an uncoated capillary column. Anal Chem, 2010. 82(8): p. 3222-30.
9. Moritz, B., et al., Evaluation of capillary zone electrophoresis for charge heterogeneity testing of monoclonal antibodies. J Chromatogr B Analyt Technol Biomed Life Sci, 2015. 983-984: p. 101-10.

利用毛细管区带电泳和高分辨质谱技术对小核酸抗体偶联药物(AOC)各组分的分离及结构确认

Application of Capillary Zone Electrophoresis (CZE) and High resolution mass spectrometry (HRMS)in the components separation and structures confirmation of Antibody-oligonucleotide conjugates (AOC)

张晓霞[1]，刘冬科[1]，郭琳洁[2]，罗继[1]，陈泓序[1]，郭立海[1]
Zhang Xiaoxia[1], Liu Dongke[1], Guo Linjie[2], Luo Ji[1], Chen Hongxu[1], Guo Lihai[1]

[1] *SCIEX，中国；[2] 上海大学*
[1] *SCIEX, China;[2] Shanghai University*

Keywords: AOC, CZE, PA 800 Plus, HRMS, X500B QTOF system, Components Separation, Structure confirmation

1. 前言

小核酸药物行业发展迅速，至今已有15款药物获批，成为继小分子和抗体药物后的第三波创新药浪潮。然而受限于递送手段的限制，目前已上市的小核酸药物仅能针对肝脏靶点。小核酸抗体偶联药物（Antibody-oligonucleotide conjugates, AOC）利用抗体将治疗性寡核苷酸递送至特定细胞或组织，将抗体的组织特异性优势，与小核酸的靶点特异性优势相结合，拓展了寡核苷酸药物的组织/细胞靶向性。近年来，诸多公司将注意力和资源集中在开发基于AOC技术的先进药物上，2021年11月，AOC先驱Avidity Biosciences公司的小核酸抗体偶联物AOC1001的I期临床试验完成首例患者给药，标志着全球首款AOC药物进入临床试验。

AOC药物作为具有巨大潜力的新型生物药物，其药物质量的控制对于安全性和有效性至关重要。一方面，每个部分包括抗体的纯度、特异性、亲和力等，小核酸的纯度、稳定性，连接子的稳定性、细胞毒性等需要检测；另一方面，完整AOC药物本身的质量包括抗体和小核酸的偶联比例、游离抗体、游离小核酸的比例等都需要严格监测。

毛细管电泳技术是抗体和小核酸等极性大分子药物分析的经典技术，高分辨质谱(HRMS)也是一种抗体和小核酸表征的利器，两种技术在抗体药物和小核酸药物结构表征和质量控制中均有广泛的应用。本文着重介绍毛细管电泳技术和高分辨质谱技术在AOC药物组分分离及结构确认中的应用。

本实验展示了CZE和HRMS两种技术联合使用在AOC药物杂质分离、纯度分析以及各组分结构确认中的应用。样品为一款单抗偶联寡核苷酸的AOC药物，通过CZE方法分离出了3个单抗偶联寡核苷酸相关的峰，推测是由于单抗上连接了不同数量的寡核苷酸产生的分离。通过HRMS一级精确分子量，进一步确认了CZE分离的出的3个单抗偶联寡核苷酸相关峰为单抗连了1个，2个和3个分子的寡核苷酸。同时，两种技术计算的各组分比例结果也非常一致。

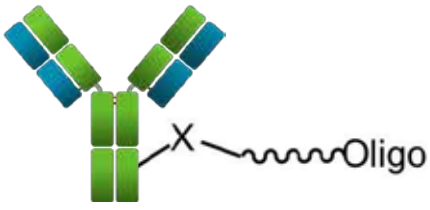


图1. AOC药物的示意图

2. 材料和方法

2.1试剂和样品

样品：本实验使用的样品为一种单抗偶联寡核苷酸的AOC药物，浓度为40 mg/mL。IgG的理论分子量为147244 Da，寡核苷酸(oligodeoxynucleotide, ODN)的理论分子量为6581Da，与IgG连接前需先对ODN进行修饰，将ODN的5'-端磷酸骨架与HS-C6结构反应，形成HS-C6修饰的ODN(分子量6777Da)。

IgG和ODN的连接方式为Sulfo-SMCC偶联方式，偶联过程见图2，整个偶联过程分为两步，第一步IgG的游离-NH_2与SMCC反应形成马来酰亚胺激活抗体(maleimide-activated IgG)，之后再与连接在HS-C6的ODN反应，得到最终的偶联产物。

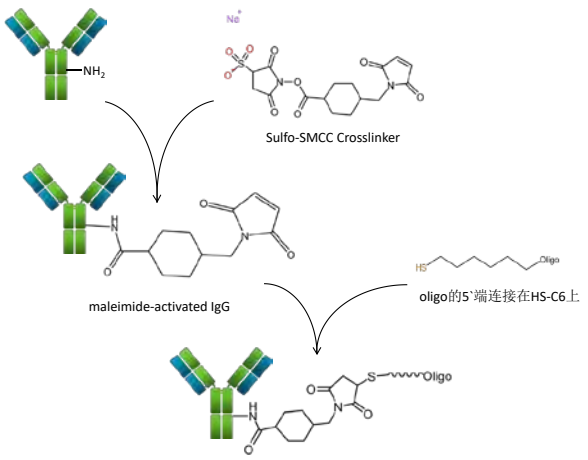


图2. IgG和ODN偶联过程示意图

样品前处理：

CZE方法：将AOC样品及IgG标准品分别用超纯水稀释至1 mg/ml，ODN标准品用超纯水稀释至0.1mg/ml，上机检测，超纯水作为空白对照。

LC-MS方法：将AOC样品和IgG标准品分别用50 mM NH_4HCO_3溶液稀释至200 ng/μL，上机检测，AOC样品进样2 μL，IgG标准品进样1 μL。

试剂：

CZE分离缓冲液：含50mM $Na_2B_4O_7$，25mM β-环糊精和10mM SDS的缓冲液，pH约为9.0。

配制方法：配制200 mM硼砂， 100 mM β-环糊精（20%乙腈溶解），100 mM SDS 储备液。分别依次取上述溶液 2.5 mL、2.5 mL、1 mL，加水至总体积10 mL，混合均匀。

液相流动相：A相，2%乙腈/水(含0.1%甲酸)，B相，98%乙腈/水(含0.1%甲酸)。

2.2 仪器及方法

毛细管电泳条件：

采用SCIEX PA 800 Plus药物分析系统，匹配二极管阵列检测器，全波长扫描，采集频率： 2 Hz，窗口狭缝： 100 × 200 μm；熔融石英毛细管，50 μm内径，50/ 60.2 cm (有效长度/总长度)；进样条件：0.5 psi, 5 s样品, 1 psi, 5s分离缓冲液；分离电压：10 kV, 40 min；毛细管温度：25 ℃，样品室温度：15 ℃。

毛细管针间冲洗： 0.1 mol/L氢氧化钠溶液在50 psi压力下冲洗3 min，再用去离子水在50 psi压力下冲洗2 min，最后用CZE分离缓冲液在50 psi压力下冲洗3 min。

色谱条件：

采用Exion LC™系统，色谱柱：Waters BEH200 SEC，150 × 4.6 mm, 1.7 μm，洗脱程序：35%的B相等度洗脱，流速：0.22 ml/min，柱温箱：40 ℃。

质谱条件：

采用SCIEX X500B QTOF系统，TOFMS扫描，质谱参数见表1。

表1. 质谱参数

参数	值
Curtain gas (psi):	35
Collision Gas(psi):	7
IonSpray Voltage(V):	5000
Temperature(℃):	450
Declustering Potential(V):	275
Ion Source Gas1(psi):	45
Ion Source Gas2(psi):	45
TOF start mass (m/z):	600
TOF stop mass (m/z):	5000
Accumulation time (s):	1

3. 结果与讨论

3.1 CZE结果分析

在CZE条件下可有效分离游离IgG，AOC及游离ODN分子。标准品和样品的分离结果见图3。

在CZE中，样品的分离基于分子在电场作用下荷质比的差异。在碱性缓冲液条件下，由于样品中的IgG、ODN以及AOC都带负电荷，在正电场下荷质比越大的分子迁移速度越慢。荷质比主要由电荷数和分子量决定，游离IgG分子量最大，带的负电荷数量比较少，荷质比小，因此会最先出峰。游离ODN由于分子量最小，电荷数最高，因此荷质比最大，会最后出峰。对于AOC来说，其分子量与IgG相比变化不大，而电荷数随着ODN的偶联而增大，因此迁移速度会慢于IgG，且偶联的OND数目越多，电荷增加越多，迁移速度越慢。迁移时间顺序为，IgG < IgG-(n)ODN <ODN。在本次实验中，IgG标准品的迁移时间在6.25 min，ODN标准品的迁移时间在20.95 min，与上述理论推导出峰顺序一致。

AOC样品各峰指认：峰1迁移时间与IgG标准品的一致，推测为游离IgG的峰。峰2,3,4迁移时间6.97-8.52 min，在IgG和ODN之间，根据前述理论推导，推测为IgG上偶联了不同数量ODN分子的峰，且峰2偶联的ODN分子数量最少，峰4偶联的ODN分子数量最多。峰7迁移时间与ODN标准品的一致，推测为游离ODN的峰。峰5和峰6迁移时间比峰7略早，但是紫外吸收光谱图与ODN标准品的一致，推测分别为HS-C6修饰的ODN及其二聚体。

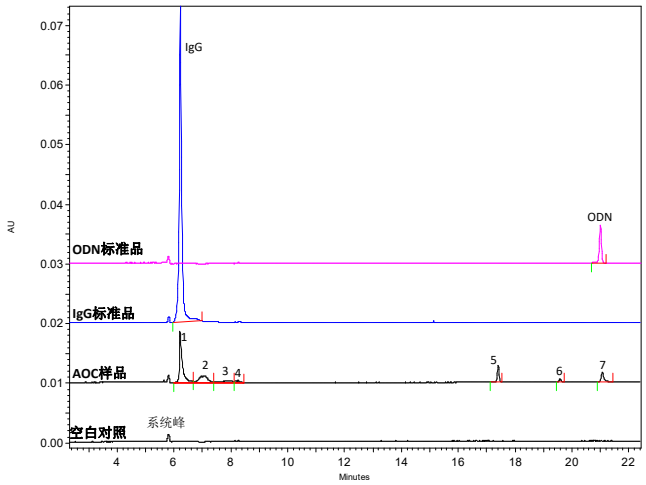


图3. CZE条件下空白对照、AOC样品，IgG标准品及ODN标准品的电泳图。

3.2 LC-MS结果分析

为了验证CZE中推断的峰2、3、4是否为IgG偶联了不同数量ODN的峰，并确认偶联的数量，我们尝试了HRMS的方法。通过HRMS分别检测了IgG标准品和AOC样品， MS结果见图4。在SCIEX OS软件中对IgG标准品TOF MS图谱进行解卷积，确认IgG的分子量为147244.2Da，在AOC样品TOF MS图谱的解卷积结果中也检测到分子量为147246.3Da的一簇峰，推断是游离IgG的峰，与IgG标准品相比多的2Da可能是由于AOC样品异质性较强导致的解卷积后分子量差异。在AOC解卷积图谱中还检测到比游离IgG分子量多6994.6Da, 13987.8Da以及20847.4Da的峰，鉴定为IgG连接1个ODN(IgG-1ODN)，2个ODN(IgG-2ODN)和3个ODN(IgG-3ODN)的峰。增加的6994.6Da为IgG上连接了SMCC linker及HS-C6修饰的ODN分子产生的分子量。

HRMS的结果验证了CZE中峰2，峰3和峰4确实是IgG偶联了不同数量寡核苷酸，而且确认峰2,3,4分别为IgG-1ODN，IgG-2ODN和IgG-3ODN。

在游离IgG的峰簇中还看到分子量加221.8Da的异构体峰，为IgG连上了一个linker，与图2中的AOC偶联过程相符合，偶联物从图2虚线处断开即为IgG加221Da的分子量。

进一步将原始数据上传至SCIEX Biologics Explorer软件进行完整分子量分析，计算IgG，IgG-1ODN，IgG-2ODN及IgG-3ODN各峰的峰面积见图5(Volume)，通过峰面积计算各峰的比例分别为32.90%，44.54%，20.52%及2.03%。

CZE与HRMS两种结果计算的各组分比例比较如表2。从表2可以看出，CZE和HRMS两种技术计算的不同组分的比例比较一致。

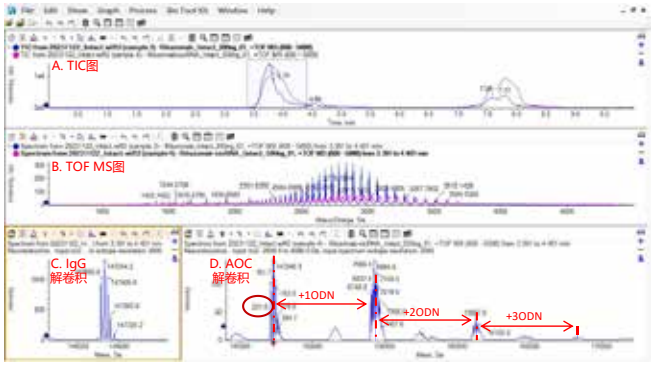


图4. IgG标准品和AOC样品的HRMS图谱。A.总离子流图(TIC图，蓝色为IgG标准品的，红色为AOC样品的)。B. TOF MS图(蓝色为IgG标准品的，红色为AOC样品的)。C. IgG标准品的解卷积结果。D. AOC样品的解卷积结果。

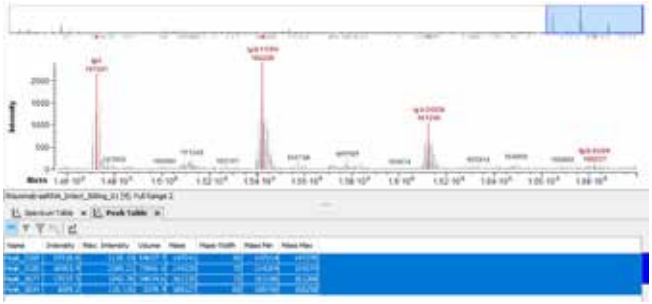


图5. SCIEX Biologics Explorer分析AOC完整分子量

表2. AOC药物中各组分比例

	IgG %	(IgG-1ODN) %	(IgG-2ODN) %	(IgG-3ODN) %
CZE*	39.22	41.33	16.99	2.46
HRMS	32.90	44.54	20.52	2.03

*注：CZE条件也可以分离检测到游离ODN及ODN与连接肽相连的峰，因为HRMS条件下无法检测到游离的ODN，因此，在与HRMS计算的组分对比时CZE也没有考虑ODN的比例

4. 结论

- 利用PA800 Plus，采用CZE方法有效分离了AOC药物中的游离IgG，不同ODN偶联数量的AOC以及游离ODN等不同形态的分子，实现AOC药物与游离杂质之间的分离。
- 利用X500B高分辨质谱，进一步验证了CZE分离得到3个IgG-ODN偶联物相关的峰分别是IgG-1ODN，IgG-2ODN和IgG-3ODN。
- CZE和LC-MS两种方法计算的各组分比例高度一致，说明两种方法在对AOC药物进行组分分离和结构确认时可以相互佐证，得到更为准确可信的结果。

5. 参考文献

1. Lin Shen, et al. Chemical Biology Approaches toward Precise Structure Control of IgG-Based Antibody-Oligonucleotide Conjugates. Chembiochem.2023 May 2;24(9)

高通量BioPhase™ 8800系统对抗体偶联药物的纯度和电荷异质性分析

Purity and charge heterogeneity analysis of antibody-drug conjugate by high throughput BioPhase™ 8800 system

唐红梅，王文涛，陈泓序，罗继，郭立海
Tang Hongmei, Wang Wentao, Chen Hongxu, Luo Ji, Guo Lihai
SCIEX, 中国;
SCIEX, China;

关键词： BioPhase™ 8800系统，十二烷基磺酸钠-毛细管凝胶电泳，毛细管等电聚焦电泳，抗体偶联药物

1、前言

抗体偶联药物（Antibody-drug conjugate, ADC）是通过连接子将小分子药物与单抗偶联在一起，利用单克隆抗体的特异性将强效小分子药物递送到特定组织，从而能够将有效剂量的治疗剂成功递送，起到治疗疾病的作用。近年来，毛细管电泳技术已成为蛋白药首选的分析方法，广泛应用于产品的研发和放行检测中。《抗体偶联药物药学研究与评价技术指导原则》中明确提出，ADC药物需要对其异质性进行深度分析，可以分别采用十二烷基磺酸钠-毛细管凝胶电泳（CE-SDS）和毛细管等电聚焦（cIEF）对ADC药物的原液及制剂的分子大小异质性和电荷异质性进行有效分析。随着生物制药市场竞争不断加大，如何有效加快生物药的研发进程是现在很多企业迫切需要解决的问题之一，高通量检测仪器的使用能够很好的应对这一挑战。BioPhase™ 8800多通道毛细管电泳系统创新性采用了八通道分离毛细管，大大缩短了方法开发和优化的时间，能够有效加速样品研发进程。

本文利用BioPhase™ 8800多通道毛细管电泳系统分别对ADC药物的纯度及电荷异质性进行了有效分析，并对方法的稳定性进行了详细考察。

2、实验部分

2.1 实验试剂和样品信息

CE-SDS实验试剂： 用于BioPhase™ 8800系统的CE-SDS蛋白分析试剂盒（SCIEX, C30085），包含凝胶缓冲液、样品缓冲液、10 kDa内标、碱性冲洗液、酸性冲洗液、CE级水。巯基乙醇（Sigma-Aldrich, M7154）、碘乙酰胺（Sigma-Aldrich, I-1149）。

cIEF实验试剂： 用于BioPhase™ 8800系统的毛细管等电聚焦（cIEF）试剂盒（SCIEX, C30101），包含cIEF胶、阳极液、阴极液、阳极稳定剂、阴极稳定剂、化学迁移剂、中性毛细管冲洗液、甲酰胺、尿素和CE级水。

样品为半胱氨酸偶联的ADC药物，通过液相色谱质谱联用（LC-MS）方法分析得到其药物抗体偶联比（Drug AntibodyRatio, DAR）为7.9，样品浓度10.0 mg/mL。

2.2 样品前处理及试剂制备

CE-SDS样品前处理： 取两支1.5 mL 离心管，分别依次加入85 µL样品缓冲液（pH 6.5）、10 µL ADC样品（盐浓度小于50 mM），然后分别向两支离心管中各加入5 µL 2-巯基乙醇（还原法，CE-SDS-R）和250 mM的碘乙酰胺溶液（非还原法，CE-SDS-NR），充分涡旋，置于70 ℃水浴10 min。冷却到室温后，短暂离心去除气泡，吸取100 µL置于96孔样品盘中，每个样品孔中各加入一滴矿物油，作为待测溶液。

cIEF试剂制备： 配置方法详细请参考BioPhase™ 8800系统的《cIEF等电聚焦试剂盒说明书》。

cIEF实验样品预混液制备： 建议按照实际样品数量加1的原则，计算各个试剂所需的体积进行预混液的配制。每个样品中各

个试剂的比例如下：200 µL 3.75 M 尿素-cIEF胶、12 µL 3-10两性电解质、20.0 µL 阴极稳定剂、2 µL阳极稳定剂和各2 µL的pI marker（pI 10.0, 9.5,7.0）。各取10 µL样品与240 µL样品预混液涡旋混匀，依次转移至96孔样品盘第一列A-H通道中（如图1b所示），等待分析。

2.3 仪器及方法

BioPhase™ 8800多通道毛细管电泳系统CE-SDS及cIEF实验样品盘摆放如图1所示：

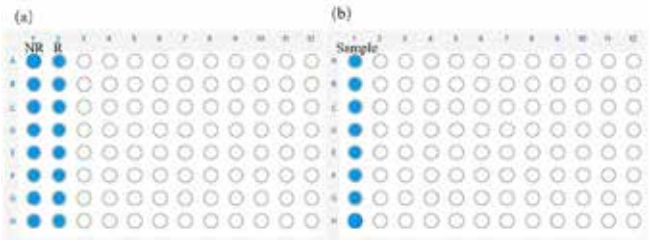


图1. BioPhase™ 8800系统进行ADC药物分析的样品摆放。（a）CE-SDS-NR和CE-SDS-R实验;（b）cIEF实验；

CE-SDS方法实验条件：采用BioPhase™ 8800多通道毛细管电泳分析系统，UV检测器，采集波长220 nm，采集频率：4 Hz；毛细管卡盒：50 µm × 30 cm BioPhase 8800 熔融石英毛细管卡盒（SCIEX，5080121）；毛细管温度：25 ℃；样品室温度：25 ℃；进样条件：-5 kV，20 s；分离条件：-15 kV，30 min。在CE-SDS实验中，采用快速冲洗的分离方法[1]，见下图2；毛细管活化和关机方法均参照《BioPhase™ 8800 CE-SDS蛋白分析试剂盒说明书》。

cIEF方法实验条件：采用BioPhase™ 8800多通道毛细管电泳分析系统，UV检测器，采集波长280 nm，采集频率：4 Hz；毛细管卡盒：50 µm × 30 cm BioPhase™ 8800中性涂层毛细管卡盒（PN 5080119）；毛细管温度：20 ℃；样品室温度：10 ℃；进样条件：25 psi，99 s；聚焦条件：25 kV，15 min；迁移条件：30 kV，30 min。cIEF实验中毛细管活化方法、分离方法和关机方法均参照《BioPhase™ 8800毛细管等电聚焦试剂盒说明书》。

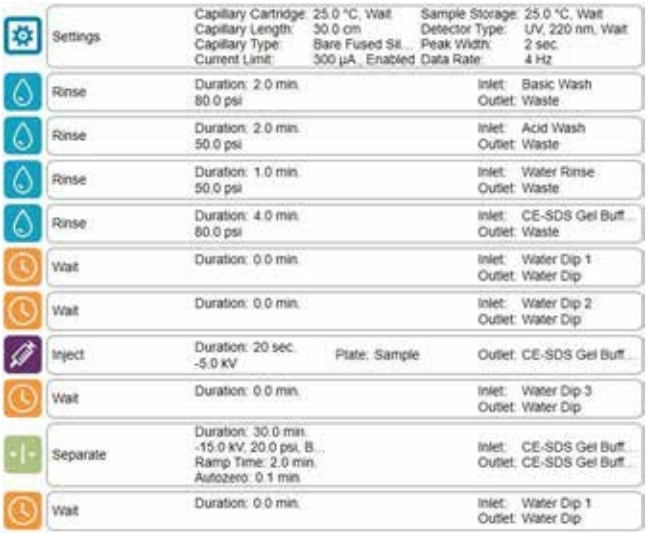


图2. Biophase™ 8800系统CE-SDS快速冲洗分离方法

4、结果与讨论

4.1 BioPhase™ 8800系统对ADC药物的纯度分析

对于半胱氨酸偶联的ADC药物，在生产过程中首先使用还原剂打开抗体中的链间二硫键，小分子药物再通过连接子与半胱氨酸上游离的巯基随机进行偶联，进一步通过疏水作用结合为一个整体。在进行ADC药物的CE-SDS纯度分析时，样品缓冲液中高浓度的SDS会破坏了不同蛋白亚基之间的疏水作用，导致ADC药物解离形成多种不同的碎片，包括结合或未结合小分子药物的轻链（LC）和重链（HC）。此外，在生产工艺的开发过程中，可能会存在单抗还原不彻底的情况，导致ADC药物中存在结合或未结合小分子药物的重轻链（HL）和重重链（HH），从而降低了ADC药物的DAR值。对于半胱氨酸偶联的ADC药物来说，如果所有链间二硫键都还原并与小分子药物成功实现偶联，理论的DAR值为8，本实验中采用的ADC样品通过LC-MS方法实测的DAR值为7.9，说明该ADC药物中存在少量的链间二硫键未完全还原。如图3上所示，在ADC药物的CE-SDS-NR分析谱图中共观察到4个峰，1~4号峰分别对应LC、HC、HL和HH，并且这些片段上均结合了小分子药物。如图3下所示，CE-SDS-R分析谱图中共观察到3个峰，通过与CE-SDS-NR谱图相比，可推断5号峰为未偶联小分子药物的LC，6号峰为偶联小分子药物的LC，7号峰为偶联小分子药物的HC。

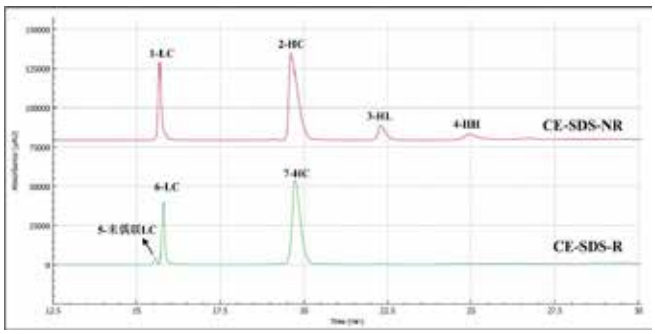


图3. Biophase™ 8800系统上进行ADC药物纯度分析的电泳谱图，CE-SDS-NR(上)和CE-SDS-R(下)

4.2 BioPhase™ 8800系统对ADC药物纯度分析的重复性考察

为了更好的验证BioPhase™ 8800系统对ADC药物纯度分析的重复性，分别对同一样品的8通道（A~H）同时连续进样6次测试，考察了单次进样中8通道间分析结果的一致性，以及单通道连续进样6针的重复性。如图4a所示，实验结果表明单次进样8通道间分析谱图表现出良好的一致性，LC和HC的迁移时间的RSD值均小于0.8%，并且校正峰面积百分比的RSD值也均小于0.5%（见表1）。如图4b所示，单通道连续6针分析结果的重复性也很好，LC和HC的迁移时间的RSD值< 0.5%，并且CAP的RSD值也< 0.4 %（见表2）。实验结果证明：BioPhase™ 8800系统进行ADC药物的纯度分析重复性好。

表1. BioPhase™ 8800系统进行ADC药物CE-SDS-R纯度分析时单次进样卡盒8通道间分析数据统计表（n=8）

通道编号	LC		HC	
	MT	CAP	MT	CAP
A	15.80	25.35	19.73	72.04
B	15.69	25.39	19.61	71.95
C	15.72	25.40	19.61	71.94
D	15.73	25.32	19.63	72.06
E	15.73	25.32	19.66	72.07
F	15.68	25.19	19.56	72.19
G	15.78	25.28	19.73	72.07
H	15.99	25.62	20.06	71.75
RSD%	0.63	0.49	0.80	0.18

备注：MT，迁移时间；CAP，校正峰面积百分比，下同。

表2. BioPhase™ 8800系统进行ADC药物CE-SDS-R纯度分析时A通道连续6针分析数据统计表（n=6）

运行编号	LC		HC	
	MT	CAP	MT	CAP
Rep- 1	15.80	25.35	19.73	72.04
Rep- 2	15.82	25.14	19.75	72.26
Rep- 3	15.84	25.22	19.79	72.21
Rep- 4	15.89	25.18	19.86	72.23
Rep- 5	15.93	25.12	19.92	72.26
Rep- 6	15.98	25.26	19.98	72.10
RSD%	0.44	0.34	0.50	0.13

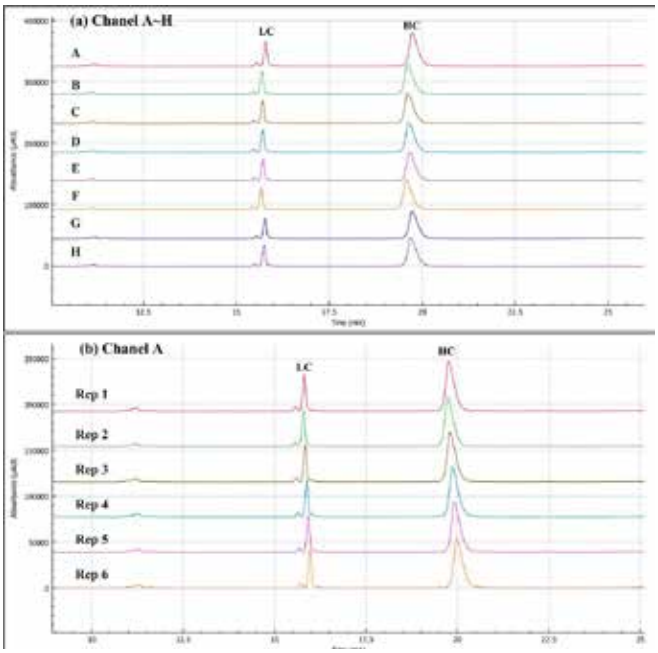


图4. BioPhase™ 8800系统上进行ADC药物的CE-SDS-R纯度分析时8个通道间及同一通道连续六针的电泳叠加图。（a）8通道A-H；（b）A通道重复6针；

4.3 BioPhase™ 8800系统对ADC药物等电点和电荷异质性分析

由于疏水性小分子药物的偶联，ADC药物的疏水性增加、表面电荷分布发生改变，从而引起ADC药物电荷异质性增强。本文采用cIEF方法对ADC药物的等电点和电荷异质性进行了有效分析，分析结果如图5所示。首先利用三个pI marker的迁移时间和理论pI值建立线性方程（R^2=0.99），通过各个电荷异构体的迁移时间进而可

以准确得到其对应的pI值。通过谱图可以看出：该ADC药物的电荷异质性较强，pI范围分布较宽，介于7.04~7.87之间，无明显主峰。

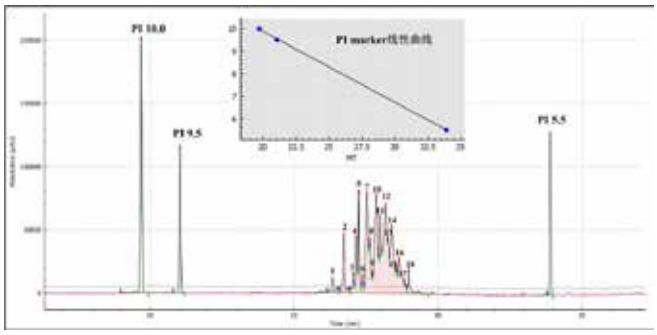


图5. BioPhase™ 8800系统ADC药物cIEF分析的电泳图（内插图为pI marker的理论pI值与迁移时间的线性曲线）

4.4 BioPhase™ 8800系统进行ADC等电点和电荷异质性分析的重复性考察

本文以峰10和峰12为例，重点考察了cIEF实验中同一样品在BioPhase™8800卡盒八通道间实验结果的一致性，以及在同一通道中连续进样的重复性。如图6a所示，单次进样8通道间cIEF分析结果表现出良好的一致性，峰10和峰12的pI值RSD均小于0.5%（如表3所示）。如图6b所示，单通道6针连续进样的重复性好，峰10和峰12的pI值RSD均小于0.3%（如表4所示）。实验结果证明：BioPhase™ 8800系统进行ADC药物的等电点和电荷异质性分析时重复性好。

表3. BioPhase™ 8800系统进行ADC药物cIEF分析时单次进样8通道间数据统计表（n=8）

通道编号	Peak 10	Peak 12
A	7.47	7.39
B	7.47	7.39
C	7.49	7.41
D	7.46	7.39
E	7.54	7.46
F	7.55	7.47
G	7.47	7.39
H	7.47	7.39
RSD%	0.49	0.48

表4. BioPhase™ 8800系统进行ADC药物cIEF分析时A通道连续6针分析数据统计表（n=6）

运行编号	Peak 10	Peak 12
Rep-1	7.47	7.39
Rep-2	7.47	7.39
Rep-3	7.48	7.41
Rep-4	7.47	7.39
Rep-5	7.43	7.35
Rep-6	7.46	7.38
RSD%	0.23	0.27

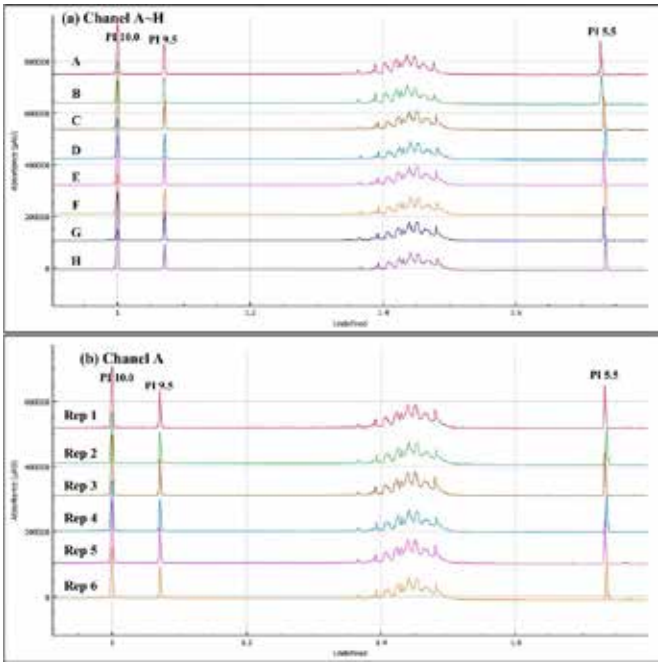


图6. BioPhase™ 8800系统上进行ADC药物cIEF等电点和电荷异质性分析时8个通道间及同一通道连续六针的电泳叠加图。（a）8通道A-H；（b）A通道重复6针；

5、结论

本文借助于BioPhase™ 8800多通道毛细管电泳分析系统对ADC药物的纯度以及电荷异质性进行了分析，主要优势如下：

1) 检测通量高：

a) CE-SDS纯度分析：平均每个样品的分析时间仅需约5 min；

b) cIEF等电点和电荷异质性分析：平均每个样品的分析时间仅需约7 min；

2) 重复性好：

c) CE-SDS纯度分析：同一样品的8个不同通道间以及单个通道连续6针进样的迁移时间RSD均小于0.8%，校准峰面积百分比RSD均小于0.5%。

d) cIEF等电点和电荷异质性分析：同一样品的8个不同通道间以及单个通道连续6针进样的pI值RSD均小于0.5%。

6、参考文献

[1]. SCIEX Tech Note：使用快速冲洗CE-SDS分离方法提高IgG纯度分析的检测通量（ RUO-MKT-02-15180-ZH-A ）

十六烷基磺酸钠-毛细管凝胶电泳法用于双抗的纯度分析

Purity analysis of bispecific antibody by capillary electrophoresis sodium hexadecyl sulfate (CE-SHS)

王文涛，罗继，陈泓序，郭立海
Wang Wentao, Luo Ji, Chen Hongxu, Guo Lihai

SCIEX，中国
SCIEX, China

Key words: 十六烷基磺酸钠-毛细管凝胶电泳法，双抗，纯度分析

1. 前言

十二烷基磺酸钠-毛细管凝胶电泳（CE-SDS）从2015年写入中国药典以来，被各大生物制药公司广泛用于检测治疗性蛋白制品的纯度分析，监控其生产过程中产生的可能影响药物疗效或患者安全的杂质，为产品放行提供纯度相关的检测数据。其中，SDS是在CE-SDS分析中常用的蛋白变性剂，主要有两方面作用：1）添加在前处理过程中使用的样品缓冲液中，用于蛋白的变性，并与蛋白进行有效结合（1g蛋白结合1.4g SDS）形成蛋白-SDS复合物，屏蔽了蛋白本身表面的电荷，保证不同分子量大小的蛋白具有一致的荷质比；2）添加到分离凝胶缓冲液中，用于保持蛋白-SDS复合物在分离过程中的稳定性。但随着蛋白药种类的增多，由于不同蛋白表面的带电性和糖基化存在差异，SDS并不能与所有蛋白都能实现较好的结合，常规CE-SDS方法的条件参数就不能有效适用于所有蛋白药物的分析。为此，需要根据样品特点，对CE-SDS方法中使用的样品缓冲液和分离凝胶缓冲液进行适当的优化。近年来，已有文献报道1，在分离缓冲液中添加更长烷基链的蛋白变性剂，如十六烷基磺酸钠（Sodium Hexadecyl Sulfate, SHS），能够有效增加变性剂与蛋白的结合，从而有效改善蛋白的分离。Jeff Beckman等人[1]研究发现，对于疏水性较强的单抗样品纯度分析来说，使用含有一定比例SHS的分离凝胶缓冲液相比于仅含有SDS的分离凝胶缓冲液来说，分离度提高了5倍，理论塔板数提高了8倍。本文将使用添加SHS的分离凝胶缓冲液进行的毛细管凝胶电泳纯度分析方法称为CE-SHS方法。

本文基于SCIEX成熟稳定的商品化分离凝胶（内含0.2%SDS，质量体积比，下同）和样品缓冲液（内含1%SDS），通过外加一定比例的SHS配置得到SHS分离凝胶缓冲液和SHS样品缓冲液，极大程度上改善了双抗样品在还原模式下分子量相差仅为1 kDa的两条重链的分离。此外，本文还详细探讨了在分离凝胶缓冲液和样品缓冲液中添加SHS对分离结果的影响，并对其可能的机理进行了阐述。

2. 仪器和试剂

2.1 仪器

SCIEX PA 800 Plus药物分析系统，配备二极管阵列检测器（PDA，波长220 nm），32 Karat™ 软件10.3（SCIEX）；离心机；分析天平；水浴锅。

2.2 试剂及耗材

IgG Purity/Heterogeneity Assaay Kit（SCIEX, PN：A10663），其中分离凝胶缓冲液含有0.2%SDS。2- 巯基乙醇（Sigma-Aldrich, PN M7154），Centricon YM-30超滤管（Millipore, PN 4208），十六烷基磺酸钠（Sigma-Aldrich, PN CDS000697）。Low pH Sample buffer (pH 6.5)（SCIEX, C57805），其中含有1%SDS。

2.3 SHS分离凝胶缓冲液和SHS样品缓冲液的配置

1）固定SDS浓度比例不变（0.2%），不同SHS浓度的分离凝胶缓冲液配置：取10 mL IgG Purity/Heterogeneity Assaay Kit中的商品化分离凝胶，分别加入0.02g、0.04g、0.06g SHS配置得到含有

不同SHS浓度的分离凝胶缓冲液，分别记为0.2%SHS、0.4%SHS、0.6%SHS分离凝胶缓冲液。由于SHS的烷基链更长，溶解性较SDS低，需要加热促进其溶解。

2）固定SHS浓度比例不变（0.2%），不同SDS浓度的分离凝胶缓冲液配置：取10 mL 0.2% SHS凝胶，其中已经含有0.2%SDS和0.2%SHS，分别加入0.03g、0.08g、0.18g SDS配置得到含有不同SDS浓度的分离凝胶缓冲液，最终三种凝胶中SDS的浓度一次为0.5%、1%、2%。SDS的溶解性好，无需加热即可很快溶解。

3）SHS样品缓冲液配置：取10 mL低pH样品缓冲液(pH 6.5)，加入0.02g SHS，配置得到本实验中使用的0.2% SHS样品缓冲液。

2.4 样品及前处理

本实验使用的双抗样品浓度为10 mg/mL，所含盐的终浓度小于50 mmol/L。准备两个1.5 mL离心管，其中一个离心管加入85 µL商品化样品缓冲液（40 mM 磷酸缓冲液体系，pH 6.5，1%SDS），另一个离心管加入85 µL自制0.2% SHS样品缓冲液（40 mM 磷酸缓冲液体系，pH 6.5，1%SDS+0.2%SHS）。然后分别依次加入10 µL双抗样品、5 µL 2-巯基乙醇。盖上瓶盖，充分混合后离心1 min。置于70℃水浴加热10 min，取出后冷却至室温即可上机实验。

2.5 运行方法

PA 800 Plus毛细管电泳仪，检测器：二极管阵列检测器，220 nm；采集频率：4 Hz；样品室温度：25 ℃；毛细管温度：25℃；窗口狭缝：2号（200 µm ×100 µm）；毛细管：熔融石英毛细管，内径50 µm，20/30.2 cm 有效/总长。

本实验使用的毛细管活化和关机方法请参考lgG Purity/Heterogeneity Assaay Kit试剂盒说明书，分离方法采用快速冲洗CE-SDS分离方法[2]，具体设置如下：

3. 结果与讨论

3.1 CE-SHS方法对双抗纯度的分析

本实验采用的双抗样品中，重链部分由于糖基化修饰的差异，两条重链分子量仅相差1 kDa左右（分别记为HC1和HC2），LC-MS分析结果如图2A所示。由于分子量差异较小，采用商品化试剂盒方法中的分离凝胶缓冲液和样品缓冲液，HC1和HC2之间无法实现有效分离，如图2B（下）所示。分别在凝胶缓冲液和样品缓冲液中加入0.2 %SHS后，双抗的两条重链HC1和HC2在分离度上发生明显的改善。实验结果可以看出：通过对分离凝胶缓冲液和样品缓冲液中变性剂的优化，极大程度上改善了蛋白的分离，这将有助于生物制药公司对其产品生产工艺的优化和完善。

3.2 SHS对双抗还原CE-SHS纯度分析的影响

为了更好的明确是在分离凝胶缓冲液中还是在样品缓冲液中添加SHS对双抗两条重链的分离度改善更大，本文通过设计单一变量实验进行了确认。如图3A所示，在保持分离凝胶缓冲液为0.2%SHS凝胶不变的情况下，不管是采用自配的0.2%SHS样品缓冲液（图3A上）还是采用商品化样品缓冲液（图3A中），双抗的两条重链的分离效果基本一致，分离度分别为0.53和0.55；该双抗的还原CE-SHS纯度分析结果也一致，纯度分别为96.07%和96.08%，如表1中实验1和2。相比而言，在保持样品缓冲液为自配的0.2 %SHS样品缓冲液不变的情况下，在分离凝胶缓冲液中添加0.2%SHS较商品化分离凝胶（图3A下）来说具有更好的分离效果，如表1中实验1和3。实验结果可以说明：在分离凝胶缓冲液中添加变性剂SHS更加有助于对该双抗在还原模式下两条重链的分离。

为了实现更佳的分离效果，本文还对分离凝胶缓冲液中的SHS浓度进行了考察，分别设置为0.2%、0.4%、0.6%。如图3B所示，由上往下，随着分离凝胶缓冲液中的SHS比例逐渐升高，该双抗的两条重链分离逐渐提高，分别为：0.53、0.86、0.93，如表1中对应的实验1、4、5所示。这可能是由于SHS的疏水性较SDS更强，SHS

图1. CE-SDS快速冲分离方法

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
1		Rinse - Pressure	70.0 psi	3.00 min	BI:B1	BO:B1	forward, In / Out vial inc 8	SDS Gel rinse to fill the capillary with SDS gel
2		Wait		0.00 min	BI:A1	BO:A1	In / Out vial inc 8	ddH2O, use for dipping to clean capillary tip
3		Wait		0.00 min	BI:A4	BO:A4	In / Out vial inc 8	ddH2O, use for dipping to clean capillary tip
4		Inject - Voltage	5.0 KV	20.0 sec	SI:A1	BO:C1	Override, reverse polarity	Sample injection
5		Wait		0.00 min	BI:B4	BO:B4	In / Out vial inc 8	ddH2O, use for dipping to avoid sample carry over
6	0.00	Separate - Voltage	15.0 KV	30.00 min	BI:C1	BO:C1	1.00 Min ramp, reverse polarity, both, In / Out vial inc 8	SDS Gel for separation
7	5.00	Autozero						
8								

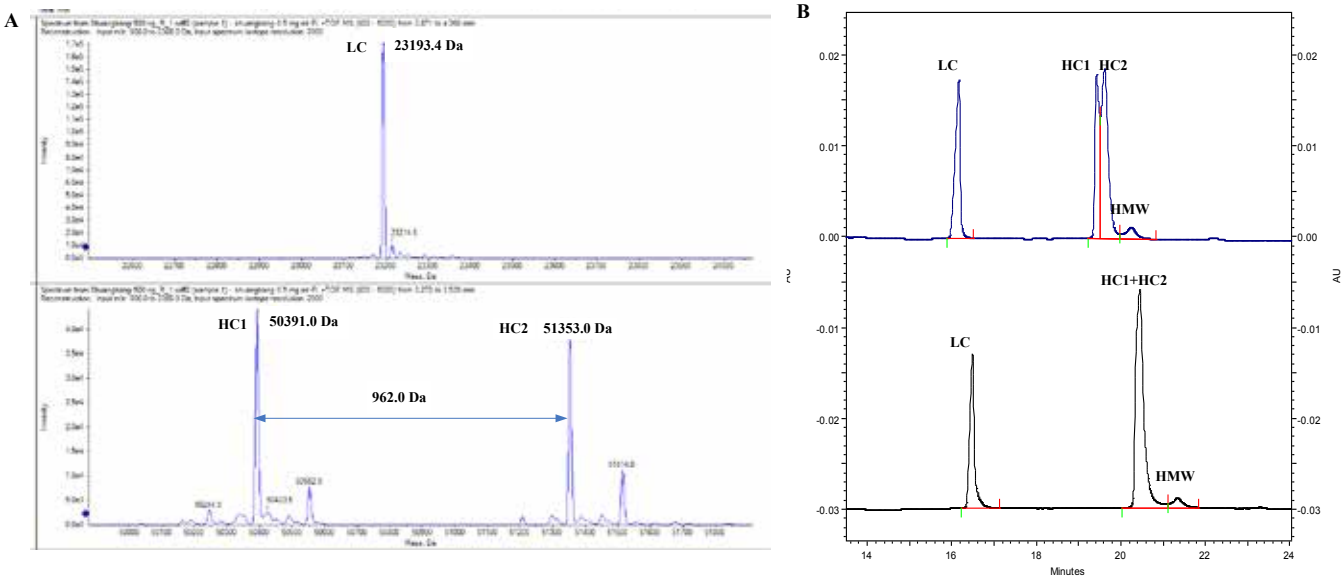


图2. 双抗样品的LC-MS还原分子量分析去卷积谱图（A）及还原CE-SHS纯度分析电泳图（B）。图2A（上）双抗还原后的轻链（LC）；图2A（下）为双抗还原后的两条重链（HC1、HC2）；图2B（上）和图2B（下）中使用的分离凝胶缓冲液和样品缓冲液类型存在差异，分别对应于表1中实验1和3；

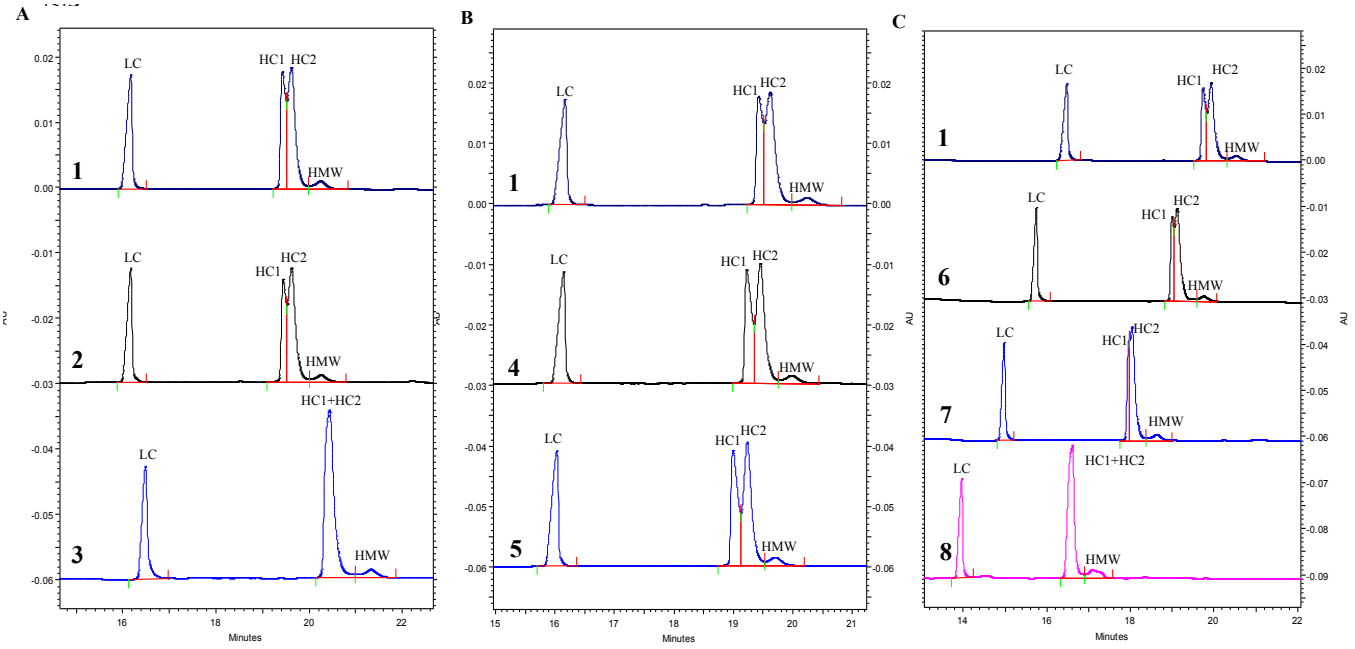


图3. 不同分离凝胶缓冲液和样品缓冲液对双抗还原模式下两条重链分离情况的比较。图中标注的编号为实验编号，不同实验对应的分离凝胶缓冲液和样品缓冲液存在差异，如表1所示。

表1. 变性剂SHS的添加对双抗的还原CE-SHS纯度及HC1-HC2间分离度数据统计表

实验编号	变性剂组成		双抗纯度/%	HC1-HC2间分离度
	分离缓冲液	样品缓冲液		
1	0.2%SDS+0.2%SHS	1%SDS+0.2%SHS	96.07	0.53
2	0.2%SDS+0.2%SHS	1%SDS	96.08	0.55
3	0.2%SDS	1%SDS+0.2%SHS	95.41	0
4	0.2%SDS+0.4%SHS	1%SDS+0.2%SHS	96.09	0.86
5	0.2%SDS+0.6%SHS	1%SDS+0.2%SHS	96.25	0.93
6	0.5%SDS+0.2%SHS	1%SDS+0.2%SHS	96.59	0.35
7	1%SDS+0.2%SHS	1%SDS+0.2%SHS	95.4	0.1
8	2%SDS+0.2%SHS	1%SDS+0.2%SHS	92.65	0

与蛋白的疏水性基团会更容易结合。随着分离凝胶缓冲液中的SHS浓度增多，SHS与SDS竞争与蛋白结合，导致两条重链之间的差异性增大，从而促进了两条重链的分离。但在实验过程中发现，当分离凝胶缓冲液中SHS浓度为0.4%和0.6%时，随着时间延长，由于SHS在常温下的溶解度较低，分离凝胶缓冲液容易出现浑浊，所以本文采用了0.2%作为SHS的最终添加浓度。此外，本文还在0.2%SHS分离凝胶缓冲液的基础上提高了SDS的浓度，分别为1%、2%、4%，探讨了分离凝胶缓冲液中SDS的浓度对该双抗的两条重链分离度的影响。如图3C所示，由上往下，随着0.2%SHS分离凝胶缓冲液中SDS浓度的逐渐提高，双抗的两条重链的出峰时间逐渐缩短，分离度降低，分别为：0.53、0.35、0.1、0，如表1中实验1、6、7和8所示。这可能是因为随着分离凝胶缓冲液中的SDS增多，一方面SDS会与分离凝胶缓冲液中的SHS竞争，降低了两条重链与变性剂（包括SDS和SHS）结合物的差异，从而导致两条重链的分离度降低；另一方面，分离凝胶缓冲液中更多的SDS与蛋白在分离过程中进一步结合，致使其荷质比升高，从而导致其出峰提前。

综合多个实验因素可以看出：在分离凝胶缓冲液中添加变性剂SHS更加有助于对该双抗在还原模式下两条重链的分离。为此，本文采用自配的0.2% SHS凝胶缓冲液作为对该双抗纯度分析的分离凝胶，用于监控其生产工艺的合理性。

3.3 CE-SHS双抗纯度分析重复性考察

为了更好的证明该方法的稳定性，本文采用0.2%SHS分离凝胶和0.2%SHS样品缓冲液对该双抗还原模式下的纯度分析进行了重复性考察，如图4和表2所示，详细展示了连续4针的分析结果。轻链和两条重链（HC1、HC2）的迁移时间RSD均小于0.34%，校正峰面积百分比均小于0.61%，不同蛋白片段之间的分离度RSD均小于1.62%。结果证明：优化后的CE-SHS方法具有很好的重复性，适合于该双抗的纯度分析。

表2. 双抗样品CE-SHS纯度分析的迁移时间、校正峰面积百分比及分离度数据统计表

编号	MT/min				CAP/%				Resolution (USP)		
	LC	HC1	HC2	HMW	LC	HC1	HC2	HMW	LC-HC1	HC1-HC2	HC2-HMW
Rep1	16.34	19.61	19.81	20.43	32.93	25.15	38.08	3.84	12.05	0.60	1.50
Rep2	16.30	19.56	19.76	20.38	32.96	24.85	38.34	3.85	12.06	0.59	1.50
Rep3	16.26	19.51	19.72	20.32	32.96	25.06	38.18	3.81	12.02	0.60	1.48
Rep4	16.22	19.46	19.66	20.27	32.55	25.15	38.37	3.92	12.04	0.58	1.47
RSD/%	0.32	0.33	0.32	0.34	0.61	0.56	0.36	1.02	0.14	1.62	1.01

备注：MT为迁移时间，CAP为校正峰面积百分比

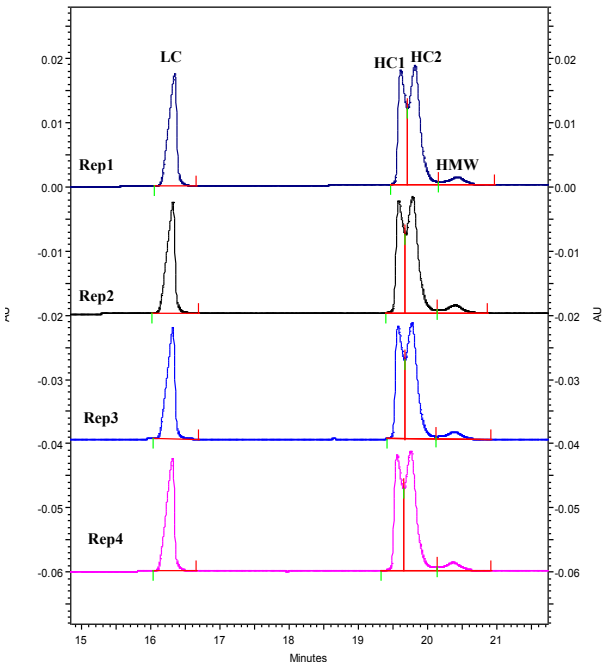


图4. 双抗样品CE-SHS纯度分析的电泳图

4. 结论

本文通过在商品化分离凝胶缓冲液和样品缓冲液中的变性剂进行了优化，开发了对双抗纯度分析的CE-SHS方法，并对其可能影响分离的一些因素进行了考察。该方案主要优势主要体现在：

1. 分离凝胶缓冲液中SHS的加入有效改善了分子量相近的蛋白片段的分离。
2. 该方法重复性好，轻链和两条重链的迁移时间RSD均小于0.34%，校正峰面积百分比均小于0.61%，不同蛋白片段之间的分离度RSD均小于1.62%。
3. 该方案为复杂生物制品的纯度分析提供了一种新的优化思路，如双抗、高糖基化融合蛋白、重组蛋白疫苗等；

参考文献

1. BECKMAN, Jeff, et al. Purity determination by capillary electrophoresis sodium hexadecyl sulfate (CE-SHS): a novel application for therapeutic protein characterization. Analytical chemistry, 2018, 90.4: 2542-2547.
2. 高铁, 刘冬科, 陈泓序. 使用快速冲洗CE-SDS分离方法提高IgG纯度分析的检测通量, RUO-MKT-02-15180-ZH-A.

CE-SDS方法对多肽药物的纯度分析

Purity analysis of polypeptide by CE-SDS method

刘冬科，王文涛，陈泓序，刘冰洁

Liu Dongke, Wang Wentao, Chen Hongxu, Liu Bingjie

SCIEX，中国

SCIEX, China

关键词： CE-SDS；多肽药物；司美格鲁肽；胰岛素

1. 前言

多肽药物通常由10-100个氨基酸组成，分子量介于小分子化药和抗体类生物药之间。作为现代药物研发的重要组成部分，多肽药物在代谢性疾病（如糖尿病、肥胖症）、肿瘤靶向治疗、心血管疾病、罕见病及抗感染等领域取得了显著成功，诞生了诸如胰岛素、司美格鲁肽、利拉鲁肽等一系列重磅药物。

十二烷基磺酸钠-毛细管凝胶电泳（CE-SDS）从2015年写入中国药典以来，被广泛用于分析治疗性蛋白制品的纯度。随着多肽药物快速发展，国家药监局药审中心（CDE）制定的《化学合成多肽药物药学研究技术指导原则（试行）》、中国检验检测学会（CITS）发布的《靶向治疗用多肽药物技术规范》（T/CITS 490-2025）都强调了采用多种不同原理的分析方法进行多肽药物的有关物质检查，以避免单一方法的分离局限性。本文在CE-SDS药典方法基础上进行优化，开发出适用于多肽药物的纯度分析方法，以用于评估多肽药物的产品质量。

2. 仪器及方法

毛细管电泳仪PA800 Plus，配备紫外检测器（UV，波长200 nm）。毛细管长度：20 cm/30 cm（有效/总长），进样条件：-5kV，20sec；分离电压：-20 kV，15 min；毛细管温度20℃，样品室温度20℃，毛细管的预处理、针与针间冲洗程序：使用碱性冲洗液在70 psi压力下冲洗3 min，酸性冲洗液70 psi冲洗1 min，去离子水70 psi冲洗1 min，背景电解质70 psi冲洗5 min。

2.1 试剂及样品

IgG 分子大小变异体分析试剂盒（SCIEX，A10663），包含50 µm内径熔融石英毛细管、SDS凝胶缓冲液、0.1 M氢氧化钠、0.1 M盐酸。pH 6.8 SDS样品缓冲液（100 mM Tris-HCl，含有1%SDS，SCIEX C44807）。β-巯基乙醇（Sigma, PN M7154）、碘乙酰胺（Sigma-Aldrich PN I1149）。

司美格鲁肽、胰岛素样品由某企业提供，初始浓度不等，用水将浓度调整至约1.5 mg/mL，各取上述浓度的样品40 µL，分别添加pH 6.8的SDS样品缓冲液60 µL、β-巯基乙醇/ 250 mM碘乙酰胺5 µL，涡旋混匀，70℃孵育10分钟。冷却至室温后2000 rpm离心2 min，再将处理好的样品转移至样品瓶中进行电泳分析。样品溶剂同样进行与样品一致的处理，作为空白对照。

3. 结果与讨论

3.1 多肽药物CE-SDS方法优化

在常规CE-SDS-UV方法上，多肽药物出峰受到系统峰（鱼翅峰）较大的干扰。经荧光标记后在CE-SDS-LIF上检测，目标峰也会受到过量游离荧光染料的干扰。探索发现使用pH 6.8 的SDS样品缓冲液变性样品，并在200 nm波长下检测，可显著降低鱼翅峰的响应，适当提升出峰速度（增大分离电压或反端进样）可进一步降低鱼翅峰附近的基线波动，避免对目标峰的干扰（未展示图谱）。

3.2 司美格鲁肽纯度分析结果

司美格鲁肽图由31个氨基酸组成，分子量约4.1 kDa，含一对链内二硫键。图1展示了非还原CE-SDS的纯度分析效果，参考空白对照对样品目标峰进行合理积分，可观察到司美格鲁肽2样品（光照14天处理）较司美格鲁肽1（4度冷藏避光存放）主峰占比明显下降，纯度由99.37 %降低至91.00 %，聚体峰占比升高，由0.63 %升高至9.00 %，该方案能够准确地监测到样品纯度变化。

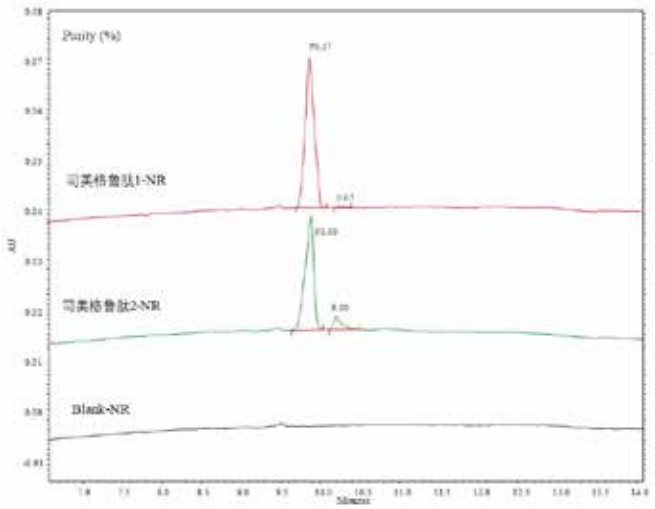


图1. 司美格鲁肽纯度分析电泳图（司美格鲁肽1：4度冷藏避光存放；司美格鲁肽2：光照14天处理）

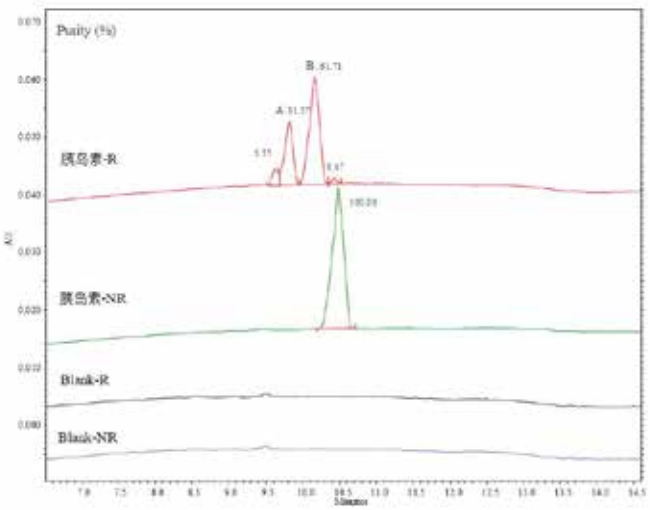


图2. 胰岛素纯度分析电泳图

3.3 胰岛素纯度分析结果

胰岛素由两条肽链通过两对链间二硫键组成，A链21个氨基酸（约2.4 kDa，含一对链内二硫键），B链30个氨基酸（约3.4 kDa），共51个氨基酸，分子量约5.8 kDa。图2展示了胰岛素在还原、非还原CE-SDS下的纯度分析效果，参照空白对照对目标峰进行合理积分，非还原条件下胰岛素样品只有一个主峰，纯度为100 %，还原条件下检测到A链、B链两个主峰及两个相关杂质峰，样品纯度为92.98 %（A%+B%）。该方法表现出较高的分辨率，可实现2.4 kDa的A链与3.4 kDa的B链的基线分离。

4. 结论

本文建立了CE-SDS方法分析多肽药物的纯度，方法对一条多肽链组成的司美格鲁肽，两条多肽链组成的胰岛素均具有良好的分离效果，并可准确反应不同条件处理的多肽药物纯度变化。该方法可作为平台化方法指导企业建立多肽药物的质量标准，以用于药物研发、生产各阶段的产品质量控制。

Subunit analysis of NISTmAb by capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) and capillary isoelectric focusing (cIEF)

Marcia Santos
SCIEX, USA

This technical note demonstrates a simple and effective workflow for characterizing the critical quality attributes of a protein therapeutic using CE-SDS and cIEF with UV detection. In this study, NISTmAb as a surrogate molecule employs intact and subunit strategies using the BioPhase 8800 system (Figure 1).

CE-SDS and cIEF with ultraviolet (UV) or laser-induced fluorescence detection (LIF) are well-known biochemical assays applied from discovery and screening through cell culture optimization, pre-clinical and clinical trials, to commercial release, as represented by the BioPhase 8800 and PA 800 plus systems.

In this workflow, we illustrate the importance of orthogonal assays and demonstrate how CE-SDS of subunits can assist in peak assignments of cIEF for subunit NISTmAb.

This experimental approach utilizes the Genovis Smart Enzymes FabULOUS and FabRICATOR for targeted digestion at and below the hinge region.

This procedure can be applied to other complex biotherapeutic proteins, particularly those requiring custom modifications for their clinical purpose.

Key features of the intact and sub-unit analysis using CE-SDS

- **Easy method development:** Fast and efficient experimental conditions screening (CE-SDS and cIEF) reduces method development time from months to weeks
- **Simple workflow:** Easy-to-follow workflow enables sample digestion and preparation for CE analysis on the same day
- **Comprehensive analysis:** The flexibility of cIEF chemistry allows the separation under both native and denatured conditions
- **Seamless method adoption:** Workflow can be applied to both the BioPhase 8800 system and the PA 800 plus system
- **Streamline development:** rapid subunit analysis of common PTMs better enhances the ability to interpret and attribute charge heterogeneity shifts



Figure 1: Subunit characterization approach of NIST using CE-SDS and cIEF.

Introduction

Protein therapeutics, including monoclonal antibodies (mAbs) and next-generation Ab molecules, are pivotal in enhancing health and quality of life for many. However, the analytical characterization of these molecules requires cutting-edge tools that provide reliable and robust methods to understand critical quality attributes. These attributes include common, but noteworthy, post-translational modifications such as oxidation, deamidation, glutamic acid cyclization, glycosylation, and C-terminal lysine clipping.[1] Expression systems, such as cell line type or cell culture conditions, can induce these modifications, consequently impacting product safety, quality and efficacy.

Liquid chromatography (LC) combined with high-resolution mass spectrometry (HRMS) of intact or digested molecules are widely employed in the industry. However, despite the resolving power of these technologies, fully characterizing these molecules' complex heterogeneity still requires orthogonal strategies. Techniques such as cIEF and CE-SDS are instrumental in understanding structural changes due to post-translational modifications (PTMs) or fragmentation resulting from stability issues or production processes.[2]

In thNISTmAb was used as the test compound for this study. It was analyzed by CE-SDS under non-reduced and reduced conditions before and after digestion with the Smart Enzymes from Genovis—FabRICATOR and FabULOUS. cIEF experiments were conducted to demonstrate this workflow with intact NISTmAb at near-native conditions (without urea) and NISTmAb subunits (figure 1).

The multi-capillary capabilities of the BioPhase 8800 system were instrumental in enabling the screening of reducing agents, CE-SDS sample buffer composition and pH, cIEF master mix conditions like ampholyte composition, urea concentration, focusing time for both intact and subunit analysis, significantly reducing overall development time.

In this work, the digested samples were split between CE-SDS and cIEF assays. Utilizing 2 BioPhase 8800 systems, the characterization process, following optimization, was completed within 2 weeks, encompassing the repeatability of sample preparation (results not shown).

Methods

Samples: NISTmAb, Humanized IgG1k Monoclonal Antibody (P/N: 8671) was from NIST (Gaithersburg, MD).

Reagents: The BioPhase CE-SDS Protein Analysis Kit (P/N: C30085) and cIEF Charge Heterogeneity analysis kit (P/NC30101) were from SCIEX. The Dithiothreitol (DTT) (P/N D5545), β-mercaptoethanol (β -ME) (P/N: M3148-25ML) and iodoacetamide (IAM) (P/N: I6125) were from Sigma-Aldrich (St. Louis, MO). The ampholyte mixtures 3-10 and (narrow range) were from Cytiva (Marlborough, MA).

Capillary cartridges: For the BioPhase 8800 system, the CE-SDS analysis was performed using a BFS capillary cartridge – 8 x 30 cm (P/N: 5080121) from SCIEX. The cIEF experiments were performed using a neutral coated capillary cartridge – 8x30 cm (P/N 5080119) also from SCIEX.

Capillary electrophoresis instrument and consumables: The BioPhase 8800 system (P/N: 5083590F) and a plate starter kit (P/N: 5080311) were from SCIEX. CE-SDS analysis was performed using UV detection at 220 nm, and cIEF at 280 nm.

Instrument methods:

The CE-SDS capillary conditioning, separation and shutdown methods were used as described in the CE-SDS application guide[3]. The cIEF capillary conditioning and shutdown methods were used as described in the cIEF applications guide [4], except for the separation method, which used 1hz and 8s for data rate and peak width at half height. All other parameters remain the same as described in the cIEF application guide[4].

Software: BioPhase software, version 1.2 e-license, was used to create methods and sequences for data acquisition and data analysis on the BioPhase 8800 system.

Sample preparation for CE-SDS analysis under reducing and non-reducing conditions: 2 µL of 10 kDa marker 83 µL of SDS sample buffer pH 9 were added to 10 µL of the NISTmAb (10mg/mL) sample. Then 5 µL of β -ME or 250mM IAM for reduced and non-reduced conditions were mixed well. The mixtures were heat-denatured at 70°C for 10 minutes (min) before CE-SDS analysis.

Sample preparation for cIEF analysis: In a 5 mL microcentrifuge tube, 200µL of cIEF gel (no urea added) was mixed with 25 µL of cathodic stabilizer, 12 µL of Ampholyte

mixture 3-10, 3µ od cathodic stabilizer and 2 µL of pI markers 10 and 4.1.

NISTmAb digestion for subunit analysis using FabRICATOR:

In a 0.5 mL microcentrifuge tube, 10 µl of 10 mg/mL NISTmAb was mixed with 10 µL of Fabricator (10 U/µL) and 2 uL of 220 mM of DTT. The mixture was incubated at 37 °C for 2 hours.

NISTmAb digestion for subunit analysis using FabULOUS:

10 µl of 10 mg/mL NISTmAb was mixed with 10 µL of FaBULOUS (10 U/µL) and 2 uL of 220 mM of DTT. The mixture was incubated at 37 °C for 2 hours.

Results and discussion

The digested samples were prepared and divided for the CE-SDS reduced and non-reduced conditions as well as cIEF analysis. These samples were analyzed using 2 BioPhase 8800 systems to ensure sample preparation consistency and repeatability.

CE-SDS OF NISTmAB digested with FabRICATOR

Figure 2 shows an overlay of the electropherograms obtained from NISTmAb under reduced and non-reduced conditions and digested with FabRICATOR. The electropherogram (e-gram) on the top shows the CE-SDS separation of NISTmAb under non-reducing conditions. As expected, the separation shows a major peak corresponding to the monomer of NISTmAb at approximately 2.2 minutes in relative migration time (RMT). The green trace shows a separation of NIST after digestion with FabRICATOR under non-reducing conditions. The separation indicates a complete digestion, as no monomer signal is detected. The 2 significant peaks correspond to the

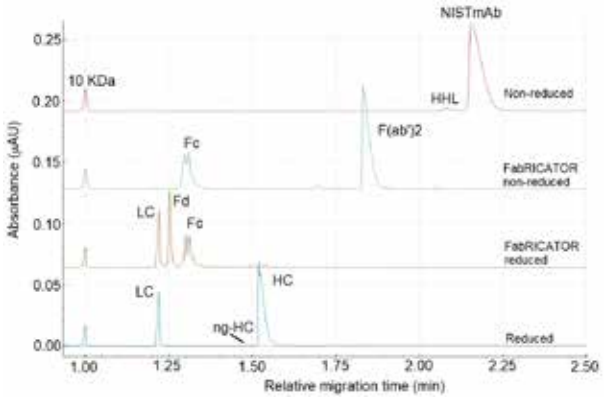


Figure 2: Overlay of electropherograms obtained of NISTmAb under different conditions.

crystallizable fragment (Fc), and antigen-binding fragment (F(ab')2) at around 1.31 min and 1.83 min, respectively. The double peak observed for Fc is likely a result of the presence of different glycan species.[5] The brown trace represents the NISTmAb that has been treated with FabRICATOR and subsequently reduced. As anticipated, the F(ab')2 fragment breaks into LC and Fd. The peak at 1.22 min is confidently assigned to the LC (light chain) because it overlays nicely with the LC of NISTmAB under reducing conditions (blue trace at the bottom). Therefore, the peak at 1.25 min is assigned to the Fd fragment by exclusion.

CE-SDS OF NISTmAB digested with FabULOUS

Figure 3 presents an overlay of the electropherograms of NISTmAb under reducing conditions treated with FabULOUS. The digestion site for FabULOUS is above the hinge region, and cleavage occurs under reducing conditions, producing Fc, LC, and Fd fragments (excluding the hinge region). Notably, the Fc* and Fd* fragments generated by FabULOUS exhibit slight size differences compared to those produced by FabRICATOR. Due to FabULOUS cleaving above the hinge region, the Fd* fragment is a few amino acids (aa) shorter, while the Fc* fragment is a few aa longer, compared to the Fc and Fd subunit FabRICATOR produces.

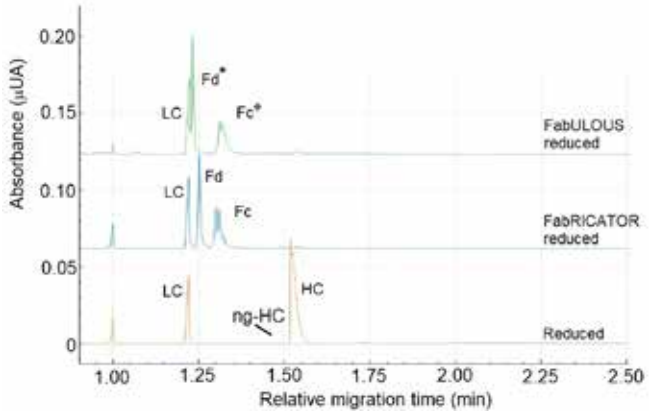


Figure 3: Overlay of e-gram obtained from NISTmAb under different conditions.

Peak assignment was conducted by comparing the e-gram of the sample digested with FabULOUS (green trace) to those of NISTmAb digested with FabRICATOR under reducing conditions (blue trace) and the reduced NISTmAb (brown trace). As expected, the e-gram of NISTmAb digested with FabULOUS and FabRICATOR display very similar profiles, as both reactions

yield comparable fragments. However, the Fc* fragment from FabULOUS digestion migrates slightly later than the Fc from FabRICATOR due to its longer length. Conversely, the shorter Fd* fragment migrates faster than the Fd from FabRICATOR. The final peak corresponds to the LC, assigned based on the comparison with reduced NISTmAb.

cIEF NISTmAB digested with FabRICATOR and FabULOUS

Monoclonal antibodies (mAbs) generally exhibit post-translational modifications (PTMs) such as deamidation, isomerization, oxidation, glycosylation, and C-terminal lysine clipping, etc.[1] These modifications alter the overall charge of the antibody. However, enzymatic digestion can significantly simplify the charge heterogeneity profile, enhancing mAb characterization.

cIEF is a high-resolution separation technique based on a protein's isoelectric point (pI). Interpreting cIEF separations can be challenging, as it is typically performed on intact molecules, resulting in an electropherogram that usually displays 1 main peak corresponding to the primary antibody variant. Acidic variants arise from modifications such as succinimide formation, C-terminal amidation, asialylation, and oxidation. Basic variants result from modifications like deamidation, sialylation, C-terminal lysine clipping, and glycation.

As discussed in the introduction, subunit analysis is essential for gaining insights into the structural changes that may occur in different domains of the antibody, which in turn induce changes in the charge heterogeneity of these molecules.

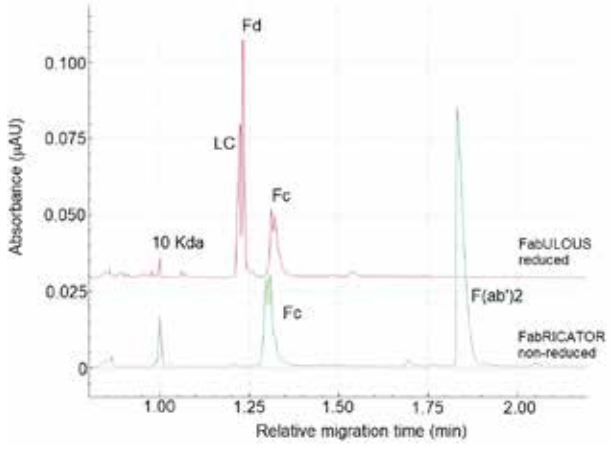


Figure 4: Comparison between CE-SDS runs of NISTmAb digested with FabULOUS and FabRICATOR under non-reduced conditions.

In this workflow, we illustrate the importance of orthogonal assays and demonstrate how CE-SDS of subunits can assist in the peak assignment of cIEF for subunit NISTmAb.

Figure 4 shows the e-gram of CE-SDS separation of NISTmAb after digestion with FabULOUS under reducing conditions and FabRICATOR under non-reducing conditions. These conditions were selected based on the optimal working conditions for the enzymes. Since the same samples were used for both CE-SDS and cIEF analysis, the species observed in CE-SDS e-gram should also appear in the cIEF separation, which was performed without urea.

Figure 5 shows 4 major subunit species in the e-grams: F(ab)2, Fc, Fd and LC. Fc and F(ab)2 are produced from the FabRICATOR digest, while Fc, Fd, and LC are generated from the FabULOUS digest. As the Fc subunit is expected to be present after digestion using both enzymes, the prominent peak observed in both cIEF separations at a pI of approximately 9.5 can be attributed to the Fc subunit. The second most prominent peak in the red trace at around pI 7.5 can be attributed to the other subunit F(ab') 2, from the FabRICATOR digest.

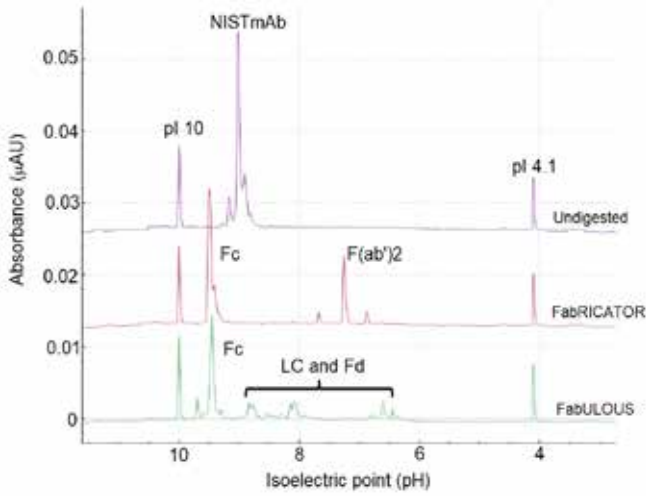


Figure 5: Overlay of cIEF runs of undigested NISTmAb, and digested with FabRICATOR (non-reduced) and FabULOUS (reduced). The focusing time for the undigested sample was 30 minutes and 25 minutes for the FabRICATOR and FabULOUS digested samples.

For the cIEF separation of the NISTmAb sample treated with FabULOUS, the peak detected at approximately pH 9.5 is attributed to the Fc region. This assignment is based on comparing the FabRICATOR and FabULOUS digests, as this

peak is consistently observed in the e-gram of both digests. The smaller species are attributed to the LC and Fd variants, whose identities will need to be confirmed through future icIEF-UV/MS analyses.

Conclusions

- A straightforward workflow for subunit analysis using CE-SDS and cIEF was developed for peak assignment.
- The BioPhase 8800 facilitated rapid screening of conditions to aid in the development of CE methods.
- Batch sample preparation for both cIEF and CE-SDS analysis ensures sample preparation consistency.
- Subunit analysis with CE-SDS gives insight into the structural changes that may occur to different domains of the antibody.
- Subunit information from CE-SDS can assist in attributing PTMs to changes in the charge heterogeneity of these molecules.

Reference

1. Benchaar, S. et al. (2015), Structural Elucidation of Chemical and Post-translational Modifications of Monoclonal Antibodies, ACS Publications, Washington, DC, [online].
2. Banerjee, S. BioProcess International 22(4)I, April, 2024.
3. CE-SDS Protein analysis kit for BioPhase 8800 system – Application guide.
4. Capillary isoelectric focusing (cIEF) for the BioPhase 8800 system – Application guide.
5. Krupke, A. et al. Intact NIST monoclonal antibody characterization —Proteoforms, glycoforms—Using CE-MS and CE-LIF, Cogent Chemistry (2018), 4: 1480455

Acknowledgments

The author would like to thank the Genovis team, which includes Laurent Rieux, John Turman, and Philip Widdowson, for their invaluable discussions.

毛细管区带电泳方法进行生长激素（GH）电荷异构体的分析

高铁，任挺钧，陈泓序
CE & Biopharma，SCIEX

1. 前言

生长激素（GH）为DNA重组人生长激素，具有人体生长激素同等作用。毛细管区带电泳的方法在2006年1月欧洲药典的增补中替代了等点聚焦进行对于生长激素电荷异构体的分析[1]。

本技术说明参考欧洲药典，重现了生长激素电荷异构体分析的方法[2]。

2. 试剂与方法

试剂

磷酸铵，磷酸，氢氧化钠，去离子水（Millipore），生长激素（由厂家提供）。

样品前处理

去离子水稀释样品至1 mg/mL。

缓冲液配置

将0.66 g磷酸铵溶于50 mL去离子水中，充分溶解，用磷酸调至pH为6.0，所得溶液过滤膜处理。

运行方法

PA800 Plus毛细管电泳仪；检测器：UV，200 nm，采集频率：2 Hz；样品室温度：4 ℃；毛细管温度：30 ℃；窗口狭缝：2号（100 × 200 μm）；毛细管：熔融石英毛细管，50 μm内径，80/70 cm有效/总长；毛细管的预平衡程序和电泳分离程序如图1，2。

3. 结果

生长激素的电泳图如图3，其中4号峰为生长激素主峰，迁移时间小于主峰的为碱性异构体，而大于主峰的为酸性异构体。按照峰面积百分比计算可得生长激素的比例为91.81%，碱性异构体的比例为1.42%，酸性异构体的比例为6.77%。

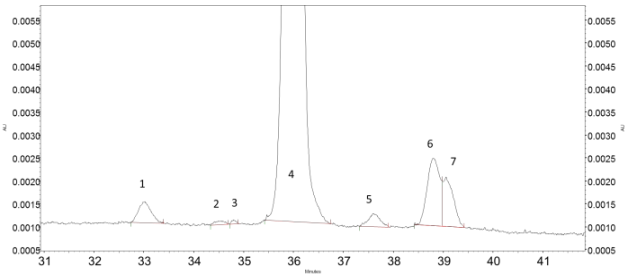


图 3. 生长激素（GH）电泳图谱（4 号峰为 GH；1、2、3 为碱性异构体；5、6、7 为酸性异构体）

Initial Conditions | PDA Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	20.00 min	BI:D1	BO:B1	forward	1M NaOH 1.4 mL - Water 0.8 mL
2		Rinse - Pressure	20.0 psi	10.00 min	BI:E1	BO:B1	forward	Water 1.4 mL - Water 0.8 mL
3		Rinse - Pressure	20.0 psi	20.00 min	BI:B1	BO:B1	forward	Buffer 1.4 mL - Water 0.8 mL
4								

图 1. 毛细管预平衡程序

Initial Conditions | PDA Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	20.00 min	BI:F1	BO:B1	forward	0.1M NaOH 1.4 mL - Water 0.8 mL
2		Rinse - Pressure	20.0 psi	20.00 min	BI:B1	BO:B1	forward	Buffer 1.4 mL - Water 0.8 mL
3		Inject - Pressure	0.7 psi	5.0 sec	SI:A1	BO:C1	Override, forward	Sample 200 uL - Buffer 1.4 mL
4		Inject - Pressure	0.7 psi	4.0 sec	BI:B1	BO:C1	No override, forward	Buffer 1.4 mL - Buffer 1.4 mL
5	0.00	Separate - Voltage	22.0 KV	80.00 min	BI:C1	BO:C1	1.00 Min ramp, normal polarity	
6	80.00	End						
7								

图 2. 分离程序

参考文献

1. S.M Storms, A. Feltus, A.R. Barker, M.A. Joly, M. Girard; Determination of Somatropin Charged Variants by Capillary Zone Electrophoresis; Pharmeuropa Scientific Note 2009-1,25-35.

2. Somatropin for injection; European Pharmacopoeia 6.0; 2935-2937.

毛细管电泳法用于重组人血清白蛋白分子大小变异体和等电点分析

Analysis of size variants and isoelectric point of recombinant human serum albumin by capillary electrophoresis

王文涛，唐红梅，罗继，陈泓序，郭立海
Wang Wentao, Tang Hongmei, Luo Ji, Chen Hongxu, Guo Lihai

SCIEX, 中国;
SCIEX, China;

Keywords: Recombinant human serum albumin, capillary electrophoresis, size variants, isoelectric point

1. 前言

人血清白蛋白是人血浆中最丰富的蛋白质，占血浆总蛋白的50%～60%，能够维持机体营养与渗透压，在机体中具有重要的生理功能，被称为血液里的“黄金救命药”。在生物医药领域，人血清白蛋白不仅可以作为药物直接经静脉注射使用，而且还可以作为优良的药物载体，延长药物的体内半衰期。此外，也可作为疫苗等生物制剂的稳定剂，或作为CHO等细胞的培养基使用。

目前临床上应用的人血清白蛋白主要来自于血液提取，然而人类血液中可能存在传染性疾病因子，且即使经过纯化也难以保证不会带入产品中。此外，人类血液供应有限，极大程度上限制了人血白蛋白的生产。利用基因重组技术生产重组人血白蛋白可以使白蛋白的生产摆脱原料的限制，同时也可以避免血浆原料引入的 病原微生物的污染。为了确保产品进入临床研究的安全性、有效性和稳定性，必须采用适当的方法对重组人血清白蛋白样品进行严格的质量控制。

毛细管电泳法（CE）以其自动化程度高、定量准确和分离效率高等特点，广泛应用于蛋白药物的质量监控。本文采用毛细管凝胶电泳（CE-SDS）方法和毛细管等电聚焦（cIEF）方法对重组人血清白蛋白样品的分子大小变异体和等电点进行了分析，并对其批次一致性进行了评估。

2. 仪器和试剂

2.1 实验仪器及方法设置

采用SCIEX公司PA 800 Plus药物分析系统，分子大小变异体分析采用二极管阵列（PDA）检测器，检测波长220 nm；等电点分析采用紫外（UV）检测器，配备280 nm滤光片。缓冲液托盘的放置及毛细管预冲洗、分离方法、关机方法请参考IgG和cIEF试剂盒说明书。

2.2 实验试剂及样品

分子大小变异体分析：IgG 分子大小变异体分析试剂盒（SCIEX，A10663）；等电点分析：cIEF等电点分析试剂盒（SCIEX，A80976）。其他试剂请参照试剂盒说明书推荐厂家购买并进行配制。

不同批次的重组人血清白蛋白样品（HSA-Lot 1、HSA-Lot 2），浓度均为1.0 mg/mL，样品基质为10.0 mM PBS溶液，理论等电点约为4~6之间。

2.2 样品制备及前处理

样品制备：取400 µL置于10 K超滤管中进行超滤浓缩至体积约为40 µL，配成初始浓度约为10.0 mg/mL的溶液，并且保证盐浓度低于50 mM。

CE-SDS分子大小变异体分析样品前处理：取初始浓度为10.0 mg/ml的重组人血清白蛋白溶液样品10 µL，加入85 µL的样

品缓冲溶液，再加入250 mM 碘乙酰胺5 μL，涡旋混匀； 70℃孵育 10 min；冷却至室温后6000 rpm离心1 min 。全部转移至样品瓶中，立即进行分析。

cIEF等电点分析样品前处理：先将下列试剂混合到0.5 mL离心管中得到样品预混液：200 μL 3.75 M 尿素-cIEF胶、12 μL两性电解质 (Pharmalyte 3~10)、20 μL 阴极稳定剂、2 μL阳极稳定剂和各2 μL的pI 标准品(pI 10.0, 9.5, 4.1)。将预混液完全涡旋混匀后，取240 μL预混液与10 μL 样品涡旋混匀，然后将200 μL液体转移至毛细管电泳仪的样品瓶中，立即进行分析。

3 结果与讨论

3.1 CE-SDS分子大小变异体分析

在生物制药生产领域，CE-SDS毛细管凝胶电泳方法已经广泛应用蛋白纯度分析，主要是按照蛋白的分子量大小进行分析。在还原型CE-SDS实验分析中，重组人血白蛋白样品的碎片峰较多，并且有部分杂质峰会与系统峰重合，影响分子大小变异体的计算，所以对于重组人血白蛋白样品分子大小变异体的计算选用非还原型CE-SDS实验。如图1所示，重组人血白蛋白样品在非还原型CE-SDS实验分析中共检测到5个峰，其中3号峰为主峰，主峰迁移时间为20.72 min，主峰分子大小变异体为89.62%。

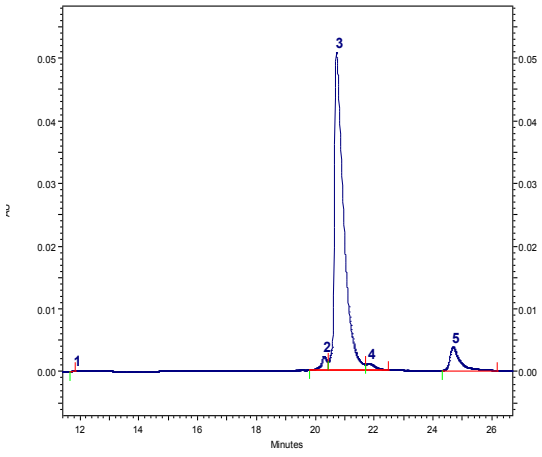


图1. 重组人血清白蛋白样品CE-SDS分子大小变异体分析谱图。1,2,4,5为杂质峰；3为主峰。

本文对重组人血白蛋白CE-SDS分子大小变异体分析的重复性进行了考察，同一个样品重复进样6针，重复性结果如表1所示。同一个样品连续6针进样，峰型一致，主峰的迁移时间RSD值以及校正峰面积百分比的RSD值均在0.1%以内。实验结果表明：CE-SDS方法重复性良好，可有效应用于重组人血白蛋白样品的分子大小变异体分析。

表1. 重组人血清白蛋白样品重复进样6针CE-SDS分析重复性结果

名称	主峰迁移时间（min）	主峰校正峰面积（%）
Run-1	20.76	91.54
Run-2	20.79	91.44
Run-3	20.79	91.48
Run-4	20.77	91.49
Run-5	20.80	91.35
Run-6	20.79	91.42
RSD%	0.07	0.07

本文利用非还原型CE-SDS方法对两个批次的重组人血清白蛋白样品（HSA-Lot 1、HSA-Lot 2）进行了分子大小变异体分析，结果如图2所示，HSA-Lot 1和HSA-Lot 2主峰的迁移时间分别为20.72 min、20.74 min；主峰校正峰面积百分比分别为89.62%、 91.72%。实验结果证明：CE-SDS方法可有效应用于重组人血清白蛋白样品的批次一致性分析。

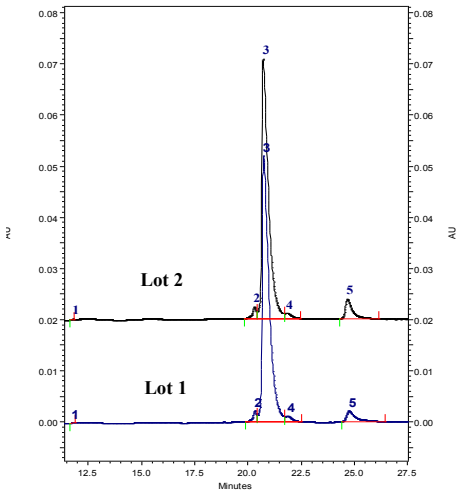


图2. 不同批次重组人血清白蛋白样品CE-SDS分子大小变异体分析谱图。1,2,4,5为杂质峰；3为主峰。

3.2 cIEF等电点分析

本文采用cIEF方法对重组人血清白蛋白样品进行了等电点分析。如图所示：重组人血清白蛋白样品异构体数目较少，可见明显主峰，2号峰为主峰，pI值为6.08（图2A）HSA-Lot 1的等电点范围为5.47~6.16。 cIEF方法基于蛋白等电点大小差异进行分析，分离度高可有效应用于重组人血清白蛋白样品的等电点分析和批次一致性分析。

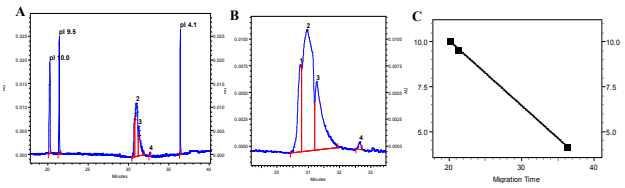


图3. 重组人血清白蛋白样品cIEF等电点分析结果。
图A为CIEF分析谱图；图B为样品峰放大图；图C为pI marker与迁移时间之间的线性曲线。其中，1号峰为碱性异构体，3和4号峰为酸性异构体

本文对cIEF等电点分析的重复性进行了考察，同一个样品重复进样5针，重复性结果如下表2所示。同一个样品重复进样5针主峰的迁移时间RSD值以及pI值的RSD值均在0.1%以内。实验结果表明：cIEF方法重复性良好，可有效应用于重组人血白蛋白样品的等电点分析。

此外，本文也可采用cIEF方法，对不同批次的重组人血清白蛋白样品进行了等电点分析。 HSA-Lot 1的等电点范围为5.47~6.16；重组人血清白蛋白样品异构体数目较少，可见明显主峰，2号峰为主峰，pI值为6.08；HSA-Lot 2的等电点范围为5.46~6.17；与HSA-Lot 1相似，2号峰为主峰，pI值为6.07。

表2. 重组人血清白蛋白样品重复进样6针cIEF分析重复性结果

编号	PI范围	不同峰的pI值重复性			
		1	2	3	4
1	5.46~6.15	6.15	6.08	5.98	5.46
2	5.47~6.16	6.16	6.08	5.98	5.47
3	5.46~6.15	6.15	6.07	5.97	5.46
4	5.47~6.16	6.16	6.08	5.97	5.47
5	5.47~6.16	6.16	6.08	5.97	5.47
RSD		0.09%	0.07%	0.09%	0.10%

备注：2号峰为主峰，1号峰为酸性异构体，3和4号峰为碱性异构体

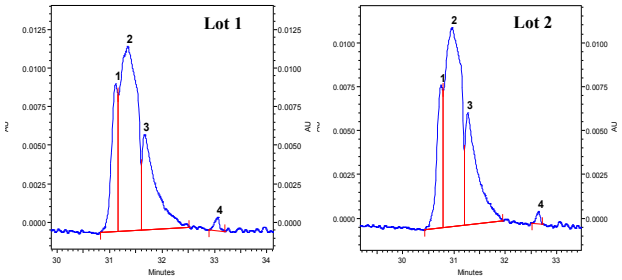


图4. 不同批次重组人血清白蛋白样品cIEF等电点分析结果。1号峰为酸性异构体，3和4号峰为碱性异构体

4、结论

本文基于SCIEX 成熟稳定的IgG分子大小变异体分析试剂盒和cIEF分析试剂盒方法，可以实现对重组人血清白蛋白样品分子大小变异体和等电点的质量控制，并可用于重组人血白蛋白样品的批次一致性分析。

毛细管等电聚焦电泳对胰岛素类产品的等电点分析

Isoelectric point analysis of insulin products by capillary isoelectric focusing electrophoresis

刘冬科，罗继，陈泓序，刘冰洁
Liu Dongke, Luo Ji, Chen Hongxu，Liu Bingjie

SCIEX，中国
SCIEX, China

Key words: Capillary isoelectric focusing electrophoresis, Glargine insulin, Aspartate insulin, protamine human insulin

引言

胰岛素类产品是指以重组技术表达的人胰岛素、人胰岛素序列突变体及其脂肪酸修饰物等，是治疗糖尿病的主要药物之一。2022年4月，国家药品监督管理局药品评审中心公布了《胰岛素类产品生物类似药药学研究与评价技术指导原则》[1]，其中明确指出：应采用多批次代表性样品，在确保研究方法准确、可靠的前提下，对样品的结构特征、理化性质和生物学活性展开研究，其中在结构表征部分明确提到需要对胰岛素类产品的等电点进行研究。

毛细管电泳（CE）以其自动化程度高、分离模式多样、定量准确和分离效率高等特点，广泛应用于生物制品的质量监控。其中，基于等电点进行分离的毛细管等电聚焦电泳（cIEF）方法已经广泛应用于单抗的等电点分析，成功被《中国药典》三部通则3129收录，为等电点分析的金标准。本文借助于cIEF方法对三种重组胰岛素类产品：甘精胰岛素、天冬胰岛素、精蛋白胰岛素，进行了等电点分析，详细考察了方法的重复性，并将该方法应用于胰岛素类产品的批次一致性分析。

2. 实验部分

2.1. 仪器及方法

采用SCIEX PA 800 Plus药物分析系统，等电点分析实验需用紫外检测器（UV），配备280 nm滤光片。缓冲液托盘的放置及毛细管预冲洗、分离方法、关机方法请参考SCIEX cIEF试剂盒说明书。

2.2. 试剂及样品

等电点分析：SCIEX cIEF等电点分析试剂盒（PN. A80976）。其他试剂请参照试剂盒说明书推荐厂家购买并进行配制。

本文使用了三种不同的胰岛素制剂：甘精胰岛素、天冬胰岛素、精蛋白胰岛素以及三个不同批次的精蛋白胰岛素，浓度均为3 mg/mL，样品无需超滤可直接使用。

2.3. 样品处理

分析多个样品时，SCIEX推荐按比例预先配置master mix，master mix可简化样品配置并且使误差最小化。按以下比例配置多个样品的master mix量：200 µL 6.25 M 尿素-cIEF胶、12.0 µL 3-10两性电解质、20.0 µL阴极稳定剂、2.0 µL阳极稳定剂和2.0 µL每个pI标准品，置于1.5 mL离心管中，涡旋混匀。取240 µL master mix与10 µL蛋白样品混合，充分涡旋后，将200 µL样品转移至内插管中，低速离心除去气泡后，将PCR样品管放入样品瓶中并用蓝色盖子盖好，放入样品托盘中，最后将样品托盘放入仪器。

3. 结果与讨论

3.1. 不同胰岛素产品等电点分析

本文利用cIEF方法对三种胰岛素类产品的等电点进行了分析。甘精胰岛素仅能够观察到1个主峰，pI值为7.10（图1A）。门冬胰岛素有2个电荷异构体的峰，对应的pI值分别为5.66和5.62（图

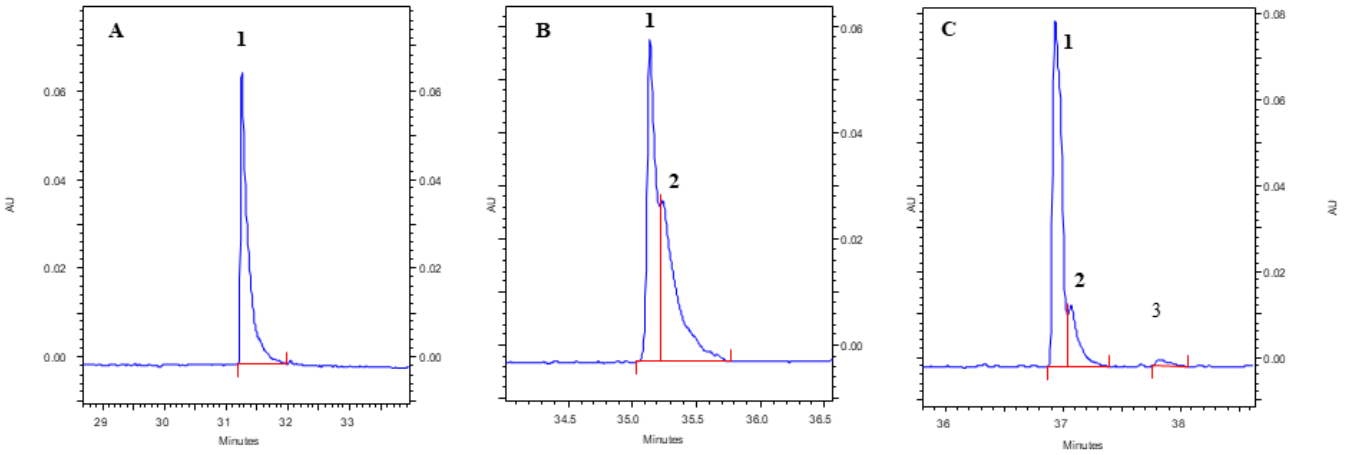


图1. 三种胰岛素产品的cIEF分析谱图 。图1A~1C依次为甘精胰岛素、门冬胰岛素和精蛋白胰岛素；1为每种胰岛素的主峰，2和3为对应的碱性异构体。

1B ）。精蛋白胰岛素的有3个电荷异构体的峰，对应的pI值分别为6.04，6.00，5.78（图1C ）。

为考察CIEF方法的重复性，本文以门冬胰岛素为例，连续测试6针，结果表现出了高度的谱图一致性（ 图2 ），峰1和峰2迁移时间和pI值的RSD均小于0.2 %（ 表1 ），实验结果证明cIEF方法对于胰岛素等电点的分析表现出极佳的重复性。

表1. 门冬胰岛素连续6针cIEF分析迁移时间和pI值汇总表格

名称	迁移时间/min		pI值	
	Peak 1	Peak 2	Peak 1	Peak 2
Run 1	35.14	35.23	5.66	5.62
Run 2	35.16	35.28	5.65	5.61
Run 3	35.2	35.3	5.66	5.62
Run 4	35.25	35.32	5.64	5.63
Run 5	35.28	35.38	5.65	5.61
Run 6	35.26	35.33	5.65	5.61
平均值	35.21	35.31	5.65	5.62
RSD	0.16 %	0.15 %	0.13 %	0.15 %

3.2. 胰岛素类产品批次一致性分析

在胰岛素类产品的生产过程中，批次一致性分析对于药物申报来说非常重要。本文以精蛋白胰岛素为例，采用cIEF方法对其三个批次的样品进行了分析。三个批次的精蛋白胰岛素样品cIEF分析谱图一致性较好（ 图3 ），主峰及其碱性异构体的迁移时间和pI值的RSD均小于1 %（ 表2 ），实验结果说明该方法适合胰岛素类产品的批次一致性分析。

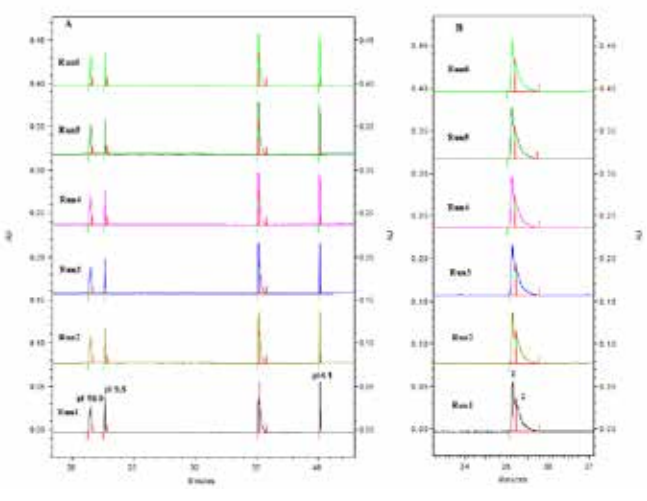


图2. A为门冬胰岛素制剂连续6针cIEF分析的叠加图 。B为图样品区域的放大图。

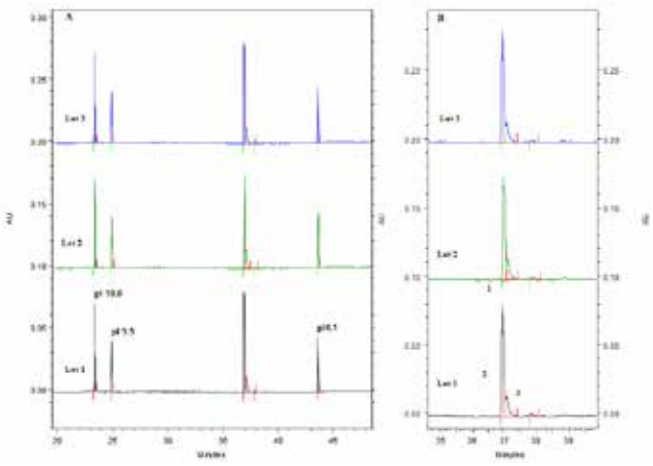


图3. A为三个不同批次的精蛋白胰岛素cIEF分析谱图的叠加图 。B为样品区域的放大图。

表2.不同批次精蛋白胰岛素cIEF测定结果汇总表

名称	迁移时间/min			pI值		
	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
Lot1	36.93	37.07	37.84	6.05	6	5.76
Lot2	36.96	37.1	37.85	6.04	5.98	5.78
Lot3	36.97	37.1	37.86	6.06	5.99	5.72
平均值	36.95	37.09	37.85	6.05	5.99	5.75
RSD	0.06	0.05	0.03	0.16	0.17	0.53

4. 结论

等电点作为胰岛素类产品的一项关键质量属性，在其生产过程中需要采用适当方法进行严格监控。cIEF方法作为《中国药典》认可的用于蛋白等电点分析的金标准，已经广泛应用于生物制品的等电点分析。基于SCIEX成熟稳定的PA 800 Plus药物分析系统及配套的cIEF等电点分析试剂盒，cIEF方法可以实现对胰岛素类产品的等电点准确分析，并可用于该类产品的稳定性研究、批次一致性分析，有效帮助生产企业对胰岛素类产品更好地进行质量控制。

参考文献

1. 胰岛素类产品生物类似药药学研究与评价技术指导原则，国家药品监督管理局药品评审中心，2022年4月

高灵敏度CE-SDS-LIF方法用于T7 RNA聚合酶纯度分析

Highly sensitive CE-SDS-LIF purity analysis of T7 RNA polymerase

张晓霞，唐红梅，陈泓序，罗继，郭立海
Xiaoxia Zhang, Hongmei Tang, Hongxu Chen, Ji Luo, Lihai Guo

SCIEX, 中国;
SCIEX, China;

Keywords: CE-SDS-LIF, mRNA vaccine, T7 RNA polymerase, Purity

1、前言

mRNA疫苗的研发与生产需要一系列酶的参与：mRNA T7 RNA聚合酶、无机焦磷酸酶、RnaseInhibitor、加帽酶以及2′ O-甲基转移酶、Poly(A)聚合酶和DNAase等7种酶。酶的纯度影响着体外转录的合成效率，从而影响终产品mRNA疫苗的纯度与活性。因此，需要一种分辨率高且检测灵敏度高的酶纯度检测方法，用于酶的质量控制，从而进一步获得高产量，高纯度的mRNA。

然而，RNA合成过程使用的酶的浓度往往都比较低，通常只有0.1-0.5 mg/ml左右，且盐浓度较高，利用传统的CE-SDS-UV蛋白纯度检测方法时灵敏度不足，本文以T7 RNA聚合酶为例，比较了UV和LIF两种检测器用于RNA 酶纯度检测，UV检测器只能勉强检测到一个主峰，无法检测到杂质峰，LIF检测器可获得更高的检测灵敏度，能清晰地分离和检测到T7 RNA 聚合酶中的杂质峰。同时，P503染料标记蛋白操作简单，无需换液操作，因此，非常适合用于酶纯度的检测。

2、试剂及方法

2.1 试剂及样品

试剂：

pH 9.0样品缓冲液（SCIEX IgG purity kit，A10663）；巯基乙醇（Sigma，PN 97622）；碘乙酰胺（Sigma，PN 11149）；0.1 mol/L氢氧化钠（SCIEX）、0.1 mol/L盐酸（SCIEX）；Gel buffer凝胶缓冲液（SCIEX，PN A30341）；P503染料（Sigma，30693）；去离子水（Millipore）。

样品：mRNA T7 RNA聚合酶，由某疫苗企业提供，浓度50U/μL。

2.2 样品前处理

CE-SDS-UV条件下样品制备：取蛋白样品10 μL，加入85 μL的样品缓冲溶液，再加入2-巯基乙醇5 μL，涡旋混匀； 70℃孵育10 min；冷却至室温后6000 rpm离心1 min 。取90 μL转移至PCR样品管中。

CE-SDS-LIF条件下样品制备：取蛋白样品10 μL，加入10 μL的样品缓冲溶液，再加入2-巯基乙醇2 μL，加入1 μL的P503染料，涡旋混匀，70℃孵育 10 min；冷却至室温，加入77 μL的去离子水，涡旋混匀后取90 μL转移至PCR样品管中。

2.3 仪器及方法设置

采用SCIEX PA 800 Plus药物分析系统，匹配UV和LIF检测器，UV检测器(214 nm)，窗口狭缝：2；LIF检测器（激发波长488 nm，发射波长600 nm）；采集频率：4 Hz；毛细管：熔融石英毛细管，50 μm内径，20/30.2 cm （有效/总长度）；毛细管温度：25 ℃；样品室温度：25 ℃；进样条件：电压进样，-10 kV，40 s；分离条件：-15 kV，40 min。

毛细管针间冲洗和胶填充：0.1 mol/L氢氧化钠溶液在70 psi压力下冲洗3 min，然后用0.1 mol/L盐酸溶液在70 psi压力下冲洗1 min，再用去离子水在70 psi压力下冲洗1 min，最后用SDS凝胶分离缓冲液在70 psi压力下冲洗10 min。

3、实验及结果

3.1 CE-SDS-UV与CE-SDS-LIF比较

比较了两种检测方法（CE-SDS-UV和CE-SDS-LIF）对T7 RNA聚合酶纯度检测结果，如图1。在相同的进样条件下，CE-SDS-UV方法仅能检测到一个主峰，计算纯度100%，无法对其纯度进行评估，而CE-SDS-LIF方法能清晰地检测到不同的杂质峰，计算酶纯度结果为97.53%。考虑到在mRNA疫苗合成过程中使用的几种酶浓度均比较低且盐浓度较高，UV检测器灵敏度无法满足杂质检测。因此，推荐使用CE-SDS-LIF方法进行酶纯度分析。

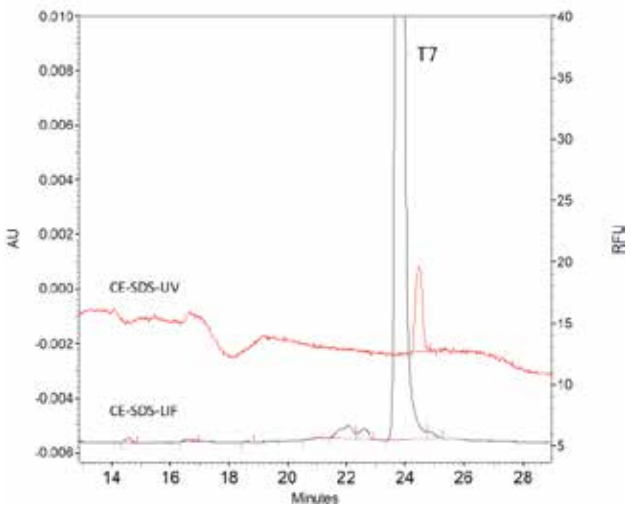


图1. T7 RNA聚合酶纯度电泳图。红色电泳图是CE-SDS-UV结果，响应值对应左侧Y轴； 黑色电泳图是CE-SDS-LIF结果，响应对应右侧Y轴。

3.2 CE-SDS-LIF方法的重复性

对T7 RNA聚合酶连续3针重复进样，CE-SDS-LIF结果如下图2示；主峰的迁移时间重复性RSD值为0.07%，校正峰面积百分比的RSD值为0.62%；

结果表明，该方法的重复性好，适用于mRNA疫苗合成过程中原料酶的纯度分析。

3.3 CE-SDS-LIF方法的线性范围及检测灵敏度

将T7 RNA聚合酶样品3倍逐级稀释后再按照CE-SDS-LIF的样品处理条件处理样品，线性和灵敏度结果如图3，从图3可看出T7 RNA聚合酶的主峰校准峰面积与浓度具有良好的线性关系（R^2>0.999），线性范围宽，覆盖3个数量级（0.023-50U/μL），检出限LOD为0.023U/μL，S/N为8.0。结果表明，该方法线性范围广，检测灵敏度高，可用于多种酶的纯度检测。

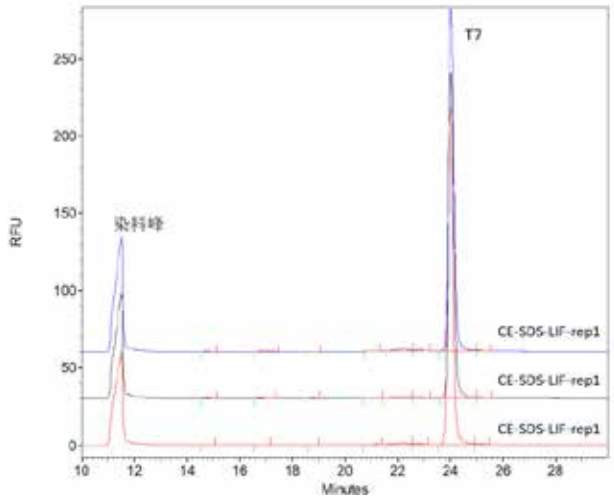


图2. mRNA T7 RNA聚合酶重复性结果（n=3）

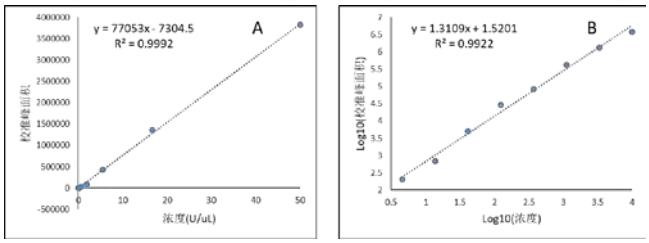


图3. T7 RNA聚合酶的线性图谱。图A为浓度和校准峰面积的线性关系，图B是对横纵坐标取对数log10的结果

4、结论

在mRNA疫苗合成过程中需要添加多种酶，但是这些酶的浓度较低且盐浓度较高，本文采用CE-SDS-LIF方式搭配P503染料，对T7 RNA聚合酶的纯度进行检测，同时考察了该方法的重复性，线性和检测灵敏度。

该方法重复性好，迁移时间重复性RSD值和校准峰面积重复性RSD值分别为0.07%和0.62%；线性范围广(0.023-50 U/μL)，跨3个数量级；检测灵敏度高，LOD为0.023 U/μL。P503染料标记简单，只需两步即可完成样品前处理，无需脱盐和换液等多种操作。可作为平台方法用于多种RNA 酶的纯度检测。

CE-SDS-LIF方法对单抗药物的纯度分析（5-TAMRA标记）

Purity Analysis of Monoclonal Antibodies (5-TAMRA Labeling) by CE-SDS-LIF

王文涛，高铁，张晓霞，陈泓序

Wang Wentao, Gao Tie, Zhang Xiaoxia, Chen Hongxu

SCIEX，中国

SCIEX, China

Keywords： Monoclonal antibodies; 5-TAMRA; CE-SDS-LIF;

前言

十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（CE-SDS）从2015年写入中国药典以来，被各大生物制药公司广泛用于治疗性单抗产品的分析表征、过程开发和质量控制[1]。在常规的CE-SDS实验中，依据蛋白本身的紫外吸收而分析的紫外/二极管阵列检测器（检测波长为214 nm或220 nm）被广泛应用于蛋白药的纯度分析。为了满足用户对低含量杂质的分析，通过对蛋白进行荧光标记，借助于激光诱导荧光检测器（LIF）可以有效提高检测灵敏度，改善基线的平整度，有利于积分，保证获得更加真实的纯度信息。本文详细介绍了使用荧光染料5-羧基四甲基罗丹明-琥珀酰亚胺酯（5-TAMRA）对单克隆抗体进行标记的过程，并结合LIF检测器对标记后的单抗样品进行了纯度分析。

2. 仪器及试剂

2.1. 仪器

SCIEX PA 800 Plus，配备LIF检测器（激发波长488 nm，发射波长560 nm），32 Karat™软件10.1（SCIEX）；离心机（Biofuge，Thermo）；分析天平、pH计（型号660，Beckman Coulter®）；

2.2. 试剂及耗材

SDS-MW Gel Buffer（SCIEX, PN A30341），荧光素校准溶液（SCIEX, PN726022），通用瓶（SCIEX, PN A62251），通用瓶盖（SCIEX, PN A62250），0.2 mL微量样品瓶（SCIEX, PN 144709）。其他试剂和耗材参考附录1。

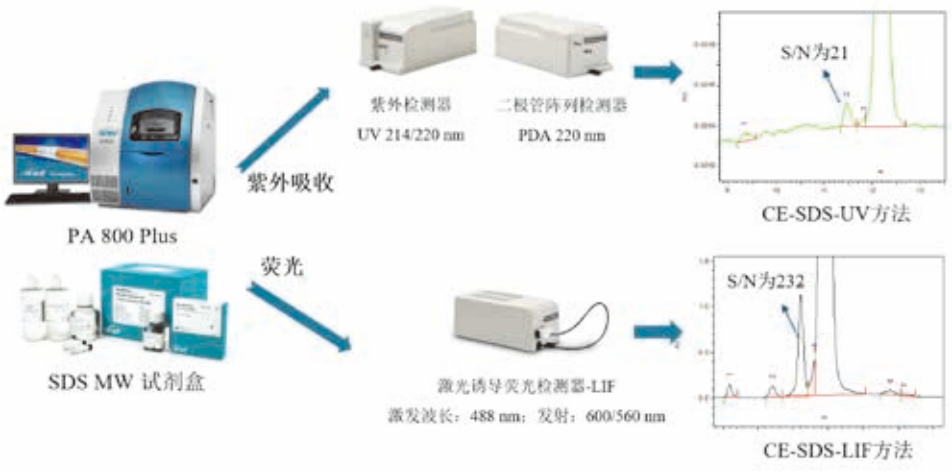


图1. 生物药纯度分析的CE-SDS方法。

3. 样品准备(~2.5小时)

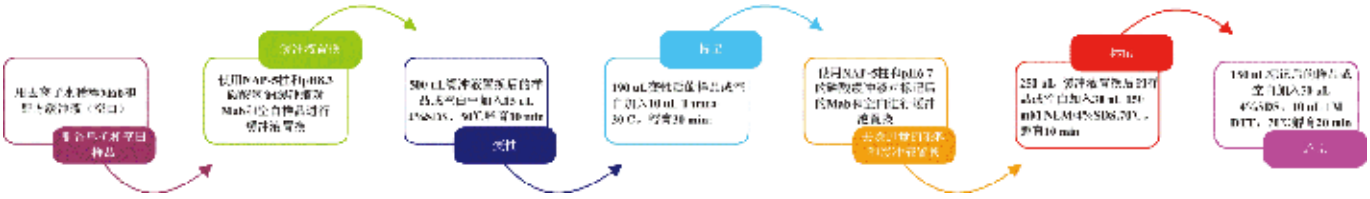


图2. 单抗样品CE-SDS-LIF纯度分析的前处理流程。

3.1. 试剂准备

(注：可根据实验样品数目调整以下步骤，但必须保持浓度不变。)

3.1.1. 标记反应缓冲液（0.1 M碳酸氢钠，pH 8.3）

a. 称取4.2 g碳酸氢钠转移到500 mL容量瓶中；

b. 加入250 mL 双蒸水（DDI）水并用磁力搅拌棒搅拌至完全溶解；

c. 取出搅拌棒，加水定容至500 mL，确认pH值为8.3 ± 0.2；

d. 使用0.2 µm尼龙过滤器过滤；

e. 2-8 ℃下保存，保质期1周；

注：如果pH超出范围，请勿调节pH值，请重新配置溶液；

3.1.2. 0.1 M磷酸缓冲液，pH 6.7

3.1.2.1 制备0.2 M磷酸二氢钠

a. 称取2.76 g磷酸二氢钠倒入含有50 mL DDI水的100 mL容量瓶中；

b. 搅拌至溶解；

c. 用DDI水定容至100 mL；

d. 用0.2 µm过滤器过滤；

e. 注明制备日期，2-8 ℃下保存；

3.1.2.2 准备0.2 M磷酸氢二钠

a. 称取2.84 g倒入含有50 mL DDI水的100 mL容量瓶中；

b. 搅拌至溶解；

c. 用DDI定容至100 mL；

d. 通过0.2 µm过滤器过滤；

e. 注明制备日期，2-8 ℃下保存；

3.1.2.3 在250 mL带刻度的玻璃烧杯中，将38.25 mL的0.2 M磷酸二氢钠溶液与36.75 mL的0.2 M磷酸氢二钠混合。使用1 mL枪头用DDI水定容至150 mL。

3.1.2.4 验证pH为6.7 +/- 0.1。

3.1.2.5 使用0.22 µm过滤器过滤，注明制备日期，2-8 ℃下保存，保质期2个月。

注意pH的测量，不要滴定调节pH值，如果pH值超出指定范围，请重新制备缓冲液各组分。

3.1.3. 4% SDS溶液

a. 将60 mL DDI水加入到250 mL烧杯中；

b. 放入磁力搅拌棒；

c. 称取4 g SDS，倒入装有水的烧杯中；

d. 搅拌直至完全溶解；

e. 用DDI水定容至100 mL；

f. 用0.22 µm过滤器过滤，注明制备日期，室温保存，保质期3个月。

3.1.4. TAMRA标记染料储备液

a. 在含有冻干5-TAMRA的小瓶中，加入3.6 mL二甲亚砜（DMSO）；

b. 涡旋溶解；

c. 每50 µL分装到棕色瓶中，盖上盖子；

d. 做好标记；

e. 保存在-15~-35 ℃范围的冷冻箱中；

f. 标注制备日期，保质期1年；

注意：使用前，立即制备并始终避光；

a. 取一瓶50 µL 5-TAMARA 标记染料储备液；

b. 加入366 µL DMSO；

c. 混匀后立即使用，丢弃未使用的溶液；

3.1.6. 1 M二硫苏糖醇（DTT）（需新鲜制备）

a. 将1.5 mg DTT加入到1.5 mL微量离心管中；

b. 用DDI 水定容至1 mL；

c. 涡旋溶解；

d. 立即使用，丢弃剩余溶液。

3.1.7. 150 mM N-乙基马来酰亚胺（NEM，溶在4% SDS溶液）

a. 称取0.185 g NEM；

b. 溶于约1.2 mL 4% SDS溶液中；

c. 室温储存，保质期1周。

3.2. 样品制备

1 mg/mL NIST样品制备：取50 µL 10 mg/mL 的NIST标准品，加入450 mL DDI水。对于其他抗体样品，需保证样品终浓度约为1.0 mg/mL，最终样品体积至少为500 µL。

空白样品制备：用DDI水稀释配方缓冲液，稀释倍数与抗体样品相同，现配现用。

3.3. 样品缓冲液置换

当配方缓冲液中含有胺时，如Tris、组氨酸，需对样品进行脱盐处理。

3.3.1. NAP-5柱平衡（~30分钟）：

a. 取2个NAP-5柱，分别对应NIST抗体和空白样品（用配方缓冲液代替）；

b. 将2个NAP-5柱放在支架上，并各对应一个烧杯用于收集洗脱液；

c. 将NAP-5柱的顶盖和底盖取下；

d. 丢弃NAP-5柱中的保护液；

e. 从NAP-5柱的顶部加入2 mL pH 8.3的碳酸氢盐缓冲液；

f. 让缓冲液透过NAP-5柱，丢弃洗脱液；

g. 重复步骤e~f 各5次。

注：并非所有NAP-5柱都有相同的流速，有时流速不同，是正常的。

3.3.2. NIST抗体和空白样品脱盐（10分钟）：

a. 标注每个NAP-5柱，分别为NIST抗体和空白样品；

b. 分别向2个NAP-5柱中各加入500 µL NIST抗体样品和500 µL空白样品；

c. 让溶液透过脱盐柱，丢弃洗脱液；

d. 在NAP-5柱下放置一个新的小瓶（1.5 mL离心管）；

e. 加入1.0 mL pH 8.3碳酸氢盐缓冲液，立即将洗脱液收集到一个样品瓶中；

f. 标注好每个小瓶，样品必须立即使用。

3.4. 样品变性

a. 取出脱盐后的抗体或空白样品各500 µL，分别加入15 µL的4% SDS，使最终的SDS浓度达到0.1%。

b. 轻轻混合并在50 ℃下加热10分钟；

重要提示：将水浴加热至50 ± 3 ℃，并在加热样品和空白前至少30分钟检查温度稳定性。

3.5. 样品标记

3.5.1. 样品标记

a. 将190 µL缓冲液置换后的样品转移到1.5 mL微量离心管中；

b. 加入10 µL 5-TAMRA荧光染料工作溶液；

c. 短暂涡旋；

d. 旋转下来；

e. 对于空白样品，重复步骤a~d；

f. 将样品和空白在30 ℃ +/- 3 ℃，孵育30分钟；

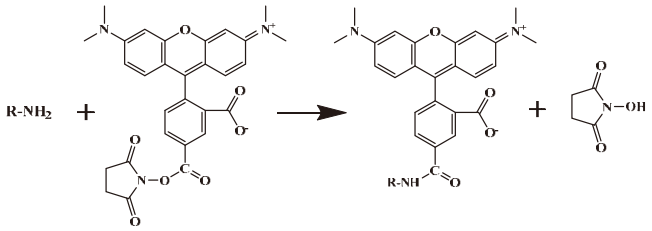


图3. 5-TAMRA标记反应原理。

3.5.2. 去除染料（样品在30 ℃处标记时执行此步骤）

3.5.2.1. NAP-5柱平衡（~30分钟）：

a. 取2个NAP-5柱，分别对应样品和空白样品（用配方缓冲液代替）；

b. 将每个NAP-5柱放在支架上，并各对应一个烧杯用于收集洗脱液；

c. 每个NAP-5柱取下顶盖和底盖；

d. 丢弃NAP-5柱中的保护液；

e. 在每个NAP-5柱的顶部加入2 mL pH 6.7的磷酸盐缓冲液；

f. 让缓冲液渗透NAP-5柱，丢弃洗脱液；

g. 重复e和f各 5次。

3.5.2.2. NIST抗体和空白样品染料去除以及缓冲液置换（~10分钟）：

a. 标注2个NAP-5柱分别为NIST抗体和空白样品；

b. 分别向2个NAP-5柱中各加入190 µL的NIST抗体样品和空白样品；

c. 让样品透过NAP-5柱，丢弃洗脱液；

d. 加入400 µL pH 6.7的磷酸盐缓冲液，丢弃洗脱液；

e. 在每个NAP-5柱下放置1.5 mL微量离心管；

f. 加入700 µL pH 6.7磷酸盐缓冲液，每一滴洗脱液收集一瓶；

g. 做好标记，样品需立即使用；

3.6. 样品制备

3.6.1. 非还原样品制备

非还原样品的制备步骤：

a. 将250 µL缓冲液置换后的样品和空白样品分别加入到1.5 mL Eppendorf管中。

b. 加入30 µL 4% SDS/150 mM NEM溶液并短暂涡旋。

c. 在70 ± 2 ℃水浴中孵育5 ± 1分钟。

d. 冷却至室温并将120 µL样品和空白转移至PA 800 Plus样品瓶中。

e. 准备好的样品可进行非还原CE-SDS-LIF分析。

3.6.2. 还原样品制备

样品/SDS络合物和二硫键还原步骤：

a. 转移150 µL标记后单抗样品和空白样品；

b. 加入50 µL的4% SDS溶液；

c. 添加10 µL的1 M DTT溶液；

d. 涡旋短暂混合并旋转；

e. 在70 ℃下孵育20分钟；

f. 冷却至室温并转移至PA 800 Plus样品瓶中；

g. 准备好的样品可进行还原CE-SDS-LIF分析；

4. 仪器准备、分离和分析方法

4.1. 仪器准备

a. LIF检测器和激光光源安装：按照PA 800 Plus和系统维护指南中的步骤进行操作；

b. LIF检测器校准：按照PA 800 Plus和系统维护指南中的步骤进行操作；

c. 创建项目：IgG-LIF项目，按照PA 800 Plus和系统管理指南中的步骤进行操作；

d. 创建IgG-LIF仪器：按照PA 800 Plus系统维护指南中的步骤进行操作；

e. 准备缓冲液托盘：按照IgG纯度和异质性分析指南的程序，了解运行的样品数；

4.2. PA 800 Plus的CE-SDS-LIF分离方法

毛细管电泳初始化条件设置：最大电压为30 kV，最大电流为300 µA，毛细管温度为40 ℃，样品保存温度为20 ℃。

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	10.00 min	BI:D1	BO:D1	reverse	0.1 N NaOH rinse to clean capillary surface
2		Rinse - Pressure	20.0 psi	5.00 min	BI:E1	BO:E1	reverse	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse - Pressure	20.0 psi	2.00 min	BI:F1	BO:F1	reverse	Water rinse to remove the acid residue
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	reverse	SDS-MW Gel Buffer rinse to fill the capillary
5	0.00	Separate - Voltage	15.0 KV	10.00 min	BI:C1	BO:C1	5.00 Min ramp, normal polarity, both	SDS-MW Gel buffer voltage equilibration
6								

图4. 毛细管平衡方法

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	3.00 min	BI:D1	BO:D1	reverse, In / Out vial inc 16	0.1 N NaOH rinse to clean capillary surface - Automatic incr
2		Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E1	reverse, In / Out vial inc 16	0.1 N HCl rinse to neutralize capillary surface silanol group -
3		Rinse - Pressure	70.0 psi	1.00 min	BI:F1	BO:F1	reverse, In / Out vial inc 16	Water rinse to remove the acid residue - Automatic increme
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	reverse, In / Out vial inc 16	SDS Gel rinse to fill the capillary with SDS gel - Automatic in
5		Wait		0.00 min	BI:A1	BO:A1	In / Out vial inc 16	ddH20, use for dipping to clean capillary tip - Automatic incr
6		Wait		0.00 min	BI:A4	BO:A4	In / Out vial inc 16	ddH20, use for dipping to clean capillary tip - Automatic incr
7		Inject - Voltage	5.0 KV	20.0 sec	BI:C1	SO:A1	Override, normal polarity	Sample injection.
8		Wait		0.00 min	BI:B4	BO:B4	In / Out vial inc 16	ddH20, use for dipping to avoid sample carry over - Automa
9	0.00	Separate - Voltage	15.0 KV	40.00 min	BI:C1	BO:C1	1.00 Min ramp, normal polarity, both, I	SDS Gel for separation - Automatic increment every 16 runs
10	5.00	Autozero						
11								

图5. 分离方法

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	10.00 min	BI:D1	BO:D1	reverse	0.1 N NaOH rinse to clean capillary surface
2		Rinse - Pressure	50.0 psi	5.00 min	BI:E1	BO:E1	reverse	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse - Pressure	50.0 psi	2.00 min	BI:F1	BO:F1	reverse	Water rinse to remove the acid residue
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	reverse	SDS Gel rinse to fill the capillary
5	0.00	Separate - Voltage	15.0 KV	10.00 min	BI:C1	BO:C1	5.00 Min ramp, normal polarity, both	SDS Gel for separation
6	10.00	Wait		0.00 min	BI:A1	BO:A1		Water used for capillary dip to prevent capillary from drying
7	10.10	Laser - Off						
8	10.20	End						
9								

图6. 关机方法

LIF检测器条件设置：LIF动态范围为100 RFU，激发波长为488 nm，发射波长为560 nm，采集频率为0.5 Hz。过滤器设置为“Normal”，峰宽采集点设置为16-25。使用直接吸收进行检测。

5. 实验结果

如图7上和下分别为5-TAMRA标记的NIST标准品在还原和非还原条件下的电泳谱图。在还原状态下，CE-SDS-LIF的方法非糖基化重链和糖基化重链分离度分别为1.86，还原状态下的纯度分析为96.90%。在非还原状态下，CE-SDS-LIF的方法纯度分别为96.76%。CE-SDS-LIF方法对于碎片、LC、HC、HL、HH以及HHL的响应更高；基线更加平整，便于更好的自动积分，有助于纯度的准确分析，便于得到更加真实的纯度信息。

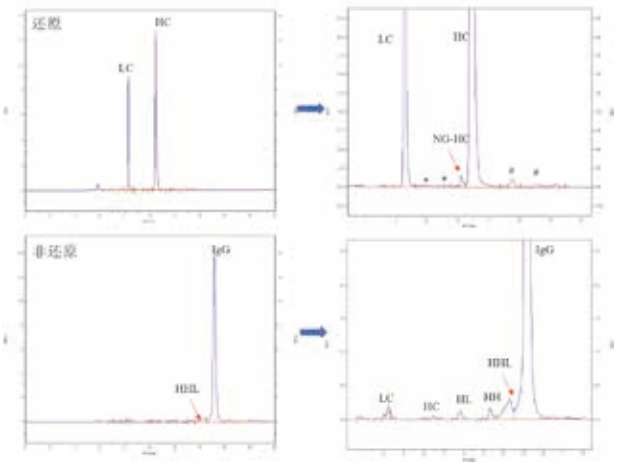


图7. NIST抗体的还原和非还原型CE-SDS-LIF分析电泳图；激发波长488 nm，发射波长560 nm，NIST终浓度为0.2 mg/mL。

6. 结论：

CE-SDS-LIF方法进行单抗的纯度分析，具有如下优点：

- 检测灵敏度高，有助于对低含量杂质的检测和纯度的准确分析；
- 基线更加平整，便于自动积分和定量；
- 有效帮助生物药企业提升检测标准和产品的质量控制；

参考文献

1. Optimization and Validation of a Quantitative Capillary electrophoresis Sodium dodecyl Sulfate Method for Quality Control and Stability monitoring of Monoclonal Antibodies; O. Salas-Solano, B. Tomlinson, S. Du, M. Parker, A. Strahan, S. Ma; Anal. Chem. 2006, 78, 6583-6594.

附录 1 CE-SDS-LIF（5-TAMRA标记）实验其他试剂和耗材。

试剂	制造商	货号
十二烷基硫酸钠（SDS）	JT Baker	PN 4095-04
磷酸二氢钠（NaH_2PO_4）	Sigma-Aldrich	PN S9638-25 g
磷酸氢二钠（Na_2HPO_4）	Sigma-Aldrich	PN S0876-500g
碳酸氢钠（$NaHCO_3$）ACS级别	Sigma-Aldrich	PN S-6014
NIST抗体参考物质	NIST	PN RM8671
二甲基亚砜（DMSO）	Sigma-Aldrich	PN 154938 或同等产品
5-TAMRA. SE, 单一异构体	Invitrogen	PN C2211
pH校准标准品	VWR	PN BK566575
D,L-二硫苏糖醇, ≥98％HPLC	Sigma-Aldrich	PN 43815-1G
N-乙基马来酰亚胺（NEM）	Sigma-Aldrich	PN E3876-25G或同等产品
Illustra NAP-5色谱柱, <0.5 mL	GE Life Sciences	PN 17085302
光敏低温储存盒	VWR	PN 80081-597或同等产品
精密级刻度量筒, 250 mL	VWR	S13746或同等产品
精密级刻度量筒, 250 mL	VWR	10545-960或同等产品
250 mL过滤器, 0.22 mm过滤器	VWR	PN#10040-464
1.25 mL棕色玻璃瓶, 带盖	VWR	PN 10798-846

注：以上列出的大多数项目均为建议的供应商，同等条件下，可选用其它替代产品。除非另有说明，请遵循储存条件和有效使用期。

SCIEX Now™支持网络

一站式满足您各种支持需求

产品和数据安全

合规性服务提升您的信心，帮助您保护数据安全，确保数据完整性，以及数据管理系统的溯源性。

新手上路

我们将帮助您在SCIEX Now 学习中心在线注册，邀请您在SCIEX Now 学习中心注册学习，并向您发送欢迎电子邮件。



实验室增强服务

SCIEX实验室增强服务计划，为您的实验室提供整体服务解决方案，以提高工作效率并减少系统停机时间。

SCIEX Now 学习中心

优质的内容，个性化的学习方式 - 使用全新的科学记忆方式设计的课程。

自我学习资源

我们的知识库和社区，将帮助您提升科学知识水平，并从SCIEX专家或者同行那里找到您所需要的答案。

全工作流程技术支持

只要您在实验过程中，遇到困难挑战，SCIEX 支持团队都会帮助您高效地解决问题，实现科学目标。

立即开始成功之路：sciex.com.cn/support

SCIEX临床诊断产品线仅用于体外诊断。仅凭处方销售。这些产品并非在所有国家地区都提供销售。获取有关具体可用信息，请联系当地销售代表或查阅https://sciex.com.cn/diagnostics 。所有其他产品仅用于研究。不用于临床诊断。本文提及的商标和/或注册商标，也包括相关的标识、标志的所有权，归属于AB Sciex Pte. Ltd. 或在美国和/或某些其他国家地区的各权利所有人。

© 2026 DH Tech. Dev. Pte. Ltd. MKT-39492-A

SCIEX
The power of precision

SCIEX 中国

北京分公司
北京市昌平区生命科学园科学园路18号院
A座一层
电话：010-5808-1388
传真：010-5808-1390
全国咨询电话：800-820-3488，400-821-3897

上海公司及中国区应用支持中心
上海市长宁区福泉北路518号
1座502层
电话：021-2419-7201
传真：021-2419-7333
官网：sciex.com.cn

广州办公室
广州国际生物岛星岛环北路1号
B2栋501、502单元
电话：020-8842-4017

官方微信：SCIEX-China

danaher