Simplifying CE-SDS Data Processing

Approach for Mitigating Product Peak Migration Time Drift

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Introduction

Capillary electrophoresis SDS (CE-SDS) is an industry standard for fragment analysis of monoclonal antibodies and other novel large biologics. The Sciex PA 800 Plus is one such system that demonstrates high sensitivity and resolution in the support of this analysis.

An industry known issue with CE-SDS is drift over time of the product peaks, giving rise to complications in data analysis, peak identification, and comparability. During each experiment it was observed that the inlet gel buffer vials are subject to evaporation (Figure 2), and this was believed to be correlated to the occurrence of profile drift (Figure 3). A collaboration between Sciex and MedImmune undertook an investigation into the use of mineral oil layered on top of gel buffer solutions during system preparation.

PA 800 Plus Pharmaceutical Analysis System

It was hypothesised that evaporation of water from the gel buffer during the course of the sequence leads to concentration of the gel buffer components. This would result in changes to the migration of the product through the capillary during electrophoresis, giving rise to the observed drift. The addition of mineral oil was proposed as a mechanism to reduce gel buffer evaporation. This would result in consistent migration times of the various product peaks and minimised profile drift.

Experimental work showed that the addition of mineral oil to the Gel-R buffer vials stabilised the migration time in both reduced and non-reduced separations (Figure 1).

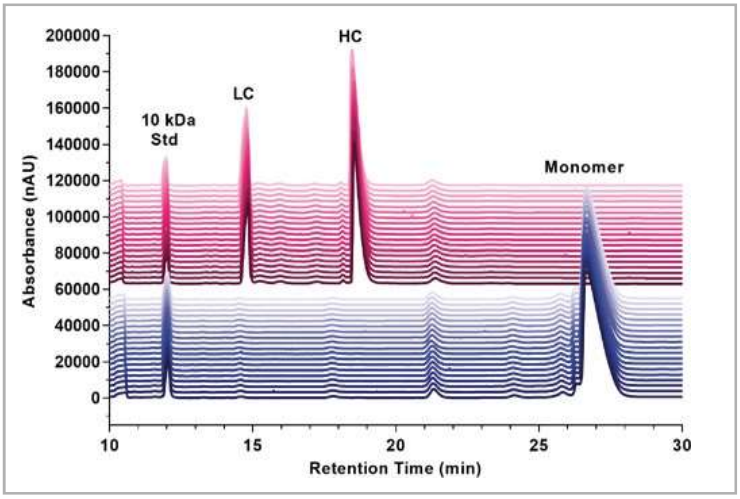

Figure 1. Stable migration times with modified method. Stressed reduced (red) and non-reduced (blue) NIST mAb.

Key Feature of CE-SDS Method Update

- Stabilises migration time of all samples in CE-SDS sequences
- Simplifies data analysis in chromatography data software
- Maximises the data which may be analysed automatically

Figure 2. Gel-R without mineral oil at the start of sequence (left) and the end of the sequence (right).

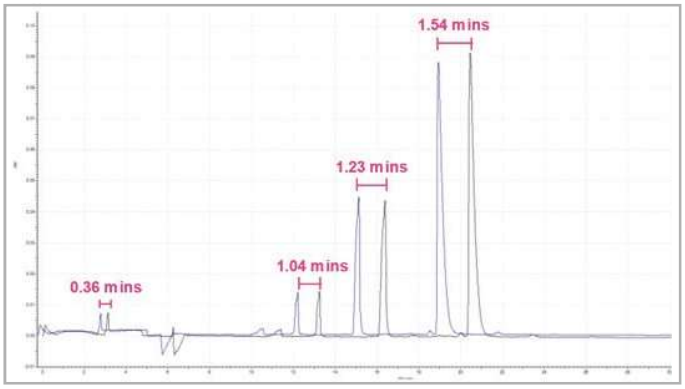

Figure 3. Product profiles demonstrating peak migration time drift, with HMWs experiencing an increased rate of drift.

Study Design - Introduction

For this investigation we proposed that the use of mineral oil layered over gel buffer loading vials would result in reduction of product peak migration time drift and improvements in consistency and comparability of CE-SDS product profiles. We evaluated the following criteria for any potential deleterious effects from the use of mineral oil during assay preparation;

- Accuracy
- Precision
- Linearity
- Autosampler Stability
- LOD / LOQ
- Product profiles

These were tested using the NIST mAb, both thermally stressed and unstressed, under both reducing and non- reducing conditions.

Materials and Equipment

Material / Equipment	Supplier	Catalogue No.
PA 800 Plus	SCIEX	A66528
NIST mAb	NIST	RM 8671
IgG Purity / Heterogeneity Assay Kit	SCIEX	A10663
Mineral Oil	SCIEX	608114
EZ-CE Capillary Cartridge	SCIEX	A55625
Iodoacetamide (IAM)	Thermo Scientific	90034
β-mercaptoethanol (BME)	Sigma Aldrich	M6250
NANOpure Diamond™ Milli-Q H_2O	Barnstead	N/A
Dri-Block® DB-2D	Techne	N/A

Table 1. Materials and equipment.

Method

Thermally stressed material was generated by incubating NIST mAb at 67°C for 24 hours to produce low molecular weight impurities.

The Sciex IgG purity assay kit was used for sample preparation with either IAM for non-reducing or with BME for reducing conditions. Samples were prepared to 1.0 mg/mL, spiked with a 10 kDa internal standard and heated at 65°C for 10 minutes. All capillary electrophoresis separations were performed on a Sciex PA 800 Plus Pharmaceutical Analysis System.

H_2O (Cycle 17-24)	H_2O (Cycle 17-24)				
H_2O (Cycle 9-16)	H_2O (Cycle 9-16)				
H_2O (Cycle 1-8)	H_2O (Cycle 1-8)				
H_2O (Cycle 17-24)	Gel-R (Cycle 17-24)	Gel-S (Cycle 17-24)	NaOH (Cycle 17-24)	HCl (Cycle 17-24)	H_2O (Cycle 17-24)
H_2O (Cycle 9-16)	Gel-R (Cycle 9-16)	Gel-S (Cycle 9-16)	NaOH (Cycle 9-16)	HCl (Cycle 9-16)	H_2O (Cycle 9-16)
H_2O (Cycle 1-8)	Gel-R (Cycle 1-8)	Gel-S (Cycle 1-8)	NaOH (Cycle 1-8)	HCl (Cycle 1-8)	H_2O (Cycle 1-8)

Figure 4. Inlet buffer tray layout with Gel-R vials highlighted

Capillary Electrophoresis

- Column: 20 cm effective length (30 cm total, 50 µm ID bare fused silica capillary (EZ-CE Capillary Cartridge))
- Separation gel buffer: HR
- Sample storage temp: 25°C
- Separation temp: 25°C
- Separation voltage: 15 kV (0.17 min ramp time), E=1000 V/cm
- Polarity: Reversed (cathode at injection side)
- Sample Injection: 1kV for 40 seconds
- Detection: PDA detector at 214 nm
- Method run time: 54 mins / sample

Data acquisition was performed by 32 Karat (version 10.1 SCIEX) and data analysis performed on Empower™ version 2 chromatography data software.

Results

We observe that over the course of a run the level of evaporation from Gel-R buffer vials is significantly reduced after the application of mineral oil during assay preparation (Figure 5), when compared against vials prepared without mineral oil (Figure 2).

Precision: Precision was assessed by analysing 20 replicates of NIST mAb within a single run. Migration times of the product peaks under non-reducing (monomer peak) and reducing conditions (Light chain (LC) and Heavy chain (HC) peaks) were evaluated, and results compared between runs that used Gel-R vials either with or without the addition of mineral oil.

Figure 6 is an overlay of NIST mAb product profiles under reducing conditions (red) and non-reducing conditions (blue) without the addition of mineral oil to Gel-R buffer vials. It shows that the migration times of the product peaks increased slightly with each subsequent injection in the sequence, highlighting the issue outlined in the introduction. This drift was not proportional for the entire product profile, with higher molecular weight species (HMWs) experiencing a greater degree of shift in retention time (Figure 3). Therefore, the data processing difficulties could not be resolved through the application of a simple correction factor.

For NIST mAb on a 20 cm separation method, the difference in migration times of the same product peak can drift by over 1 minute within a single run (Figure 6, Figure 8 and Figure 9). The precision data where mineral oil was added to Gel-R buffer vials demonstrated a drastic reduction in migration time drift, with product peaks showing no more than 0.2 minutes difference throughout the run (Figure 7 and Table 3).

The addition of mineral oil did not alter the signal (absorbance) or the peak shape, no atypical peaks were produced, and the reported results by % time corrected area (% TCA) were comparable to runs using Gel-R buffer vials without mineral oil (NMT 1% difference).

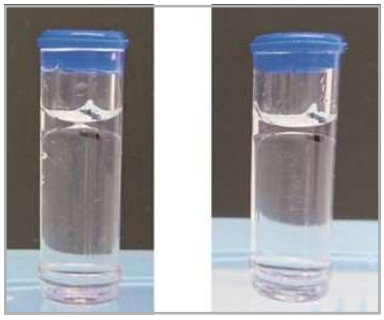

Figure 5. Gel-R with mineral oil at the start of sequence (left) and the end of the sequence (right).

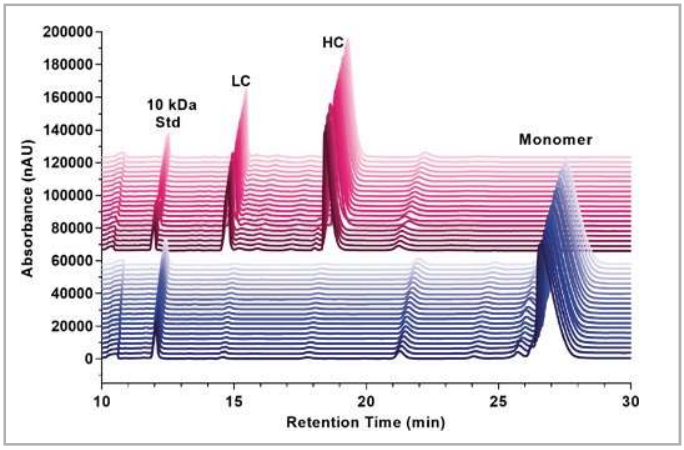

Figure 6. Stressed reduced (red) and non-reduced (blue) NIST mAb without mineral oil.

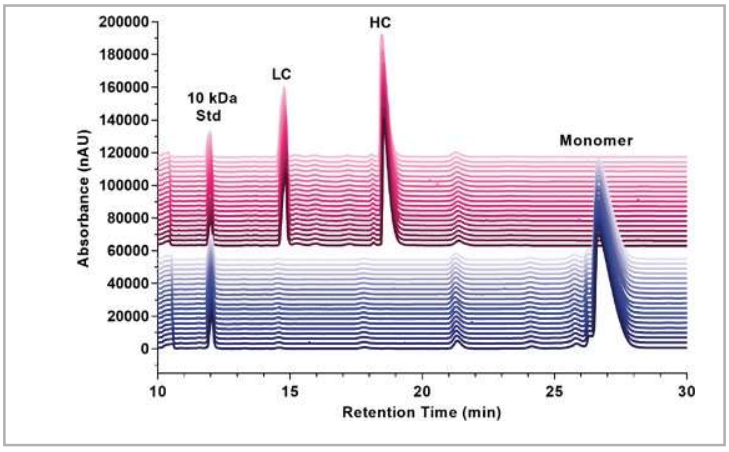

Figure 7. Stressed reduced (red) and non-reduced (blue) NIST mAb with mineral oil.

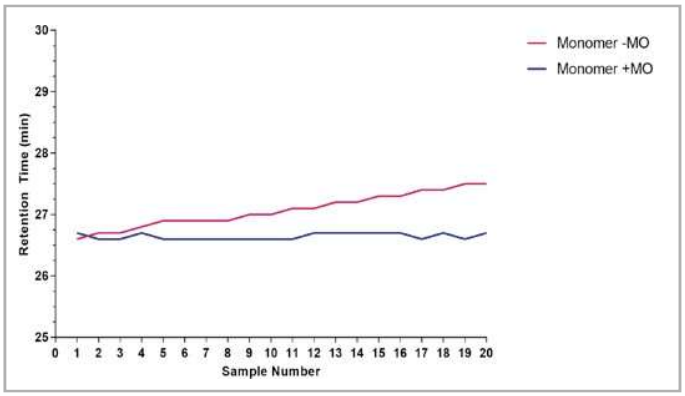

Figure 8. Retention times of NIST mAb product peaks under non-reducing conditions with (+MO) and without (-MO) mineral oil.

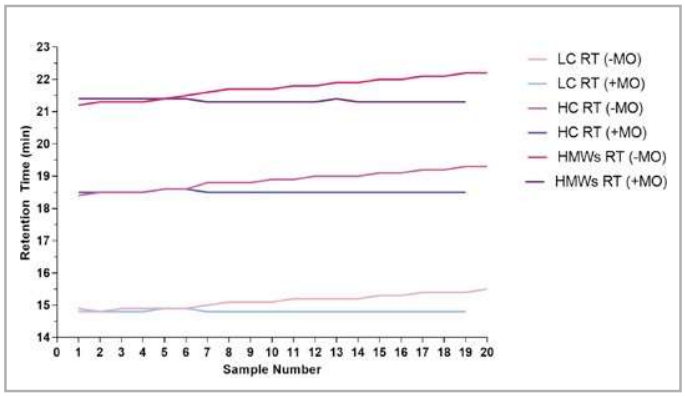

Figure 9. Retention times of NIST mAb product peaks under reducing conditions with (+MO) and without (-MO) mineral oil.

Linearity: NIST mAb was prepared in triplicate at 0.8, 0.9, 1.0, 1.1 and 1.2 mg/ml and analysed for linear regression (R^2) and accuracy of purity levels in the presence and absence of mineral oil, under both reducing and non-reducing conditions. Figure 10 and Figure 11 show that the R^2 value was greater than or equal to 0.98 for both conditions, independent of whether or not mineral oil was added to Gel-R vials. These results demonstrate that the use of mineral oil had no impact on linear regression for the NIST mAb under reduced or non-reduced conditions.

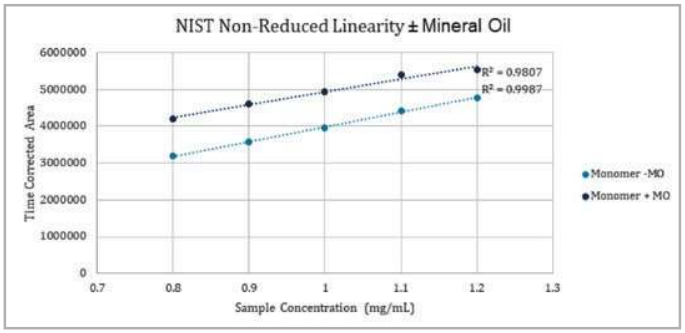

Figure 10. Linearity results for NIST mAb under non-reducing conditions with (+MO) and without (-MO) mineral oil.

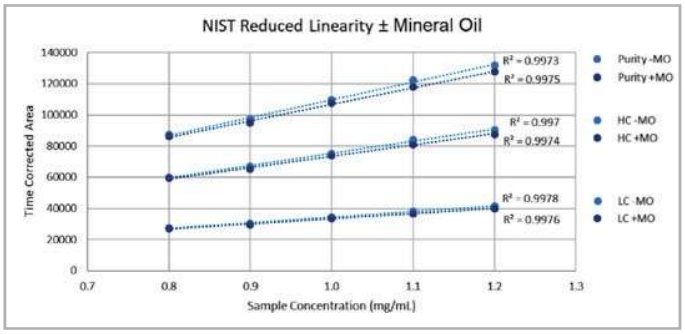

Figure 11. Linearity results for NIST mAb under reducing condition with (+MO) and without (-MO) mineral oil.

Accuracy: Data from the linearity study were used in the assessment of the impact upon accuracy. Results for % purity, % leading peak (LP), and % heavy chain leading peak (HCLP) differed across all n=15 replicates by not more than (NMT) 1%. Results at each concentration (n=3) differed from the average of the whole data set (n=15) by NMT 1%.

Test	Linearity (non-reducing)		Linearity (reducing)	
	LOQ	LOD	LOQ	LOD
With Mineral Oil	0.15	0.05	0.14	0.04
Without Mineral Oil	0.21	0.07	0.13	0.04

Table 2. Calculated LOQ / LOD as % TCA for NIST mAb under reducing and non-reducing conditions, with and without mineral oil.

LOD and LOQ: The limits of detection / quantitation were determined using data from the linearity results (Table 2). A single peak was selected and results were calculated as follows.

$$LOQ\ (mg/mL) = \left(\frac{10x\ Std\ Error}{Slope}\right)$$

$$LOQ\ (\%\ CPA) = \left(\frac{LOQ\ (mg/mL)}{peak\ conc\ (mg/ml)}\right)$$

$$LOD = \frac{LOQ}{3}$$

These results demonstrate that the LOD / LOQ values were not impacted by the application of mineral oil to Gel-R vials.

Autosampler stability: Autosampler stability was assessed by running triplicate injections of NIST mAb prepared under both reducing and non-reducing conditions at 0, 15, 30, 45 and 60 hours, both with and without the application of mineral oil.

Figure 12 and Figure 13 demonstrate that the use of mineral oil eliminated the migration time drift over a 60-hour sequence, and did not negatively impact upon the resolution of the product profile.

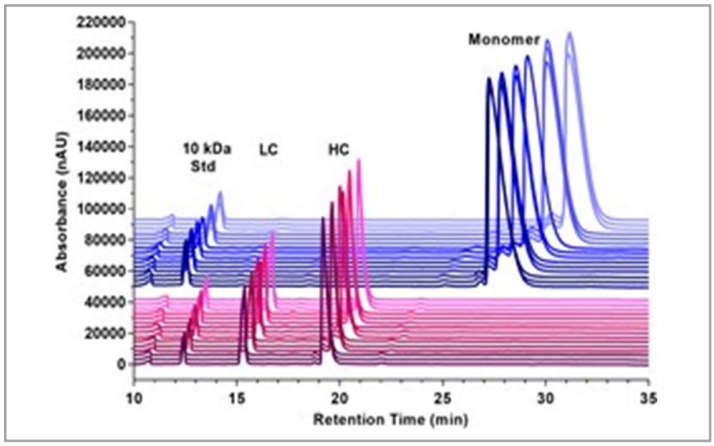

Figure 12. Autosampler stability of NIST mAb without mineral oil under reducing (red) and non-reducing (blue) conditions.

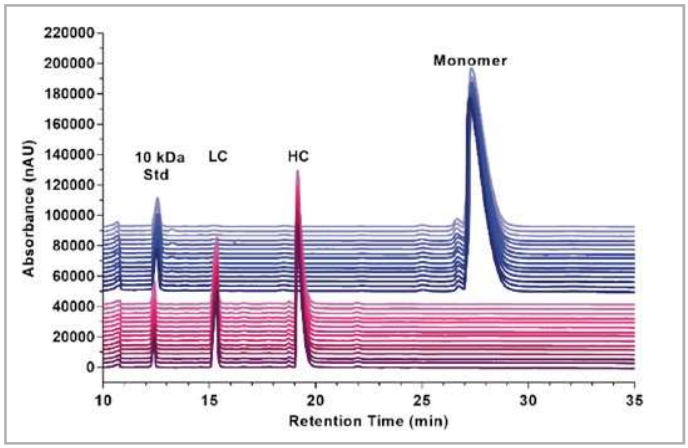

Figure 13. Autosampler stability of NIST mAb with mineral oil under reducing (red) and non-reducing (blue) conditions.

Table 3: Summary of results from impact assessment of mineral oil upon assay quality for both reducing and non-reducing CE-SDS

Assessment	Non-reducing		Reducing	
	- Mineral Oil	+ Mineral Oil	- Mineral Oil	+ Mineral Oil
Avg Retention Time (n=20, mins)	Monomer = 27.1	Monomer = 26.6	LC = 15.6 HC = 19.4	LC = 15.4 HC = 19.2
Max Retention Time Drift (n=20, mins)	Monomer = 0.9	Monomer = 0.1	LC = 0.7 HC = 0.9	LC = 0.1 HC = 0.1
Avg Peak Response (nAU)	Monomer = 66130	Monomer = 64830	LC = 45086 HC = 90707	LC = 44367 HC = 86932
Accuracy	0.4 %	0.2 %	0.2 %	0.2 %
Precision	Monomer = 0.2 % CV	Monomer = 0.1 % CV	LC = 0.2 % CV HC = 0.1 % CV	LC = 0.2 % CV HC = 0.1 % CV
Linearity	Monomer = 0.999	Monomer = 0.981	LC = 0.998 HC = 0.997	LC = 0.998 HC = 0.997
LOQ	0.21 %	0.15 %	0.13 %	0.14 %
Autosampler Stability	Purity = 0.6 % difference over 60 hrs	Purity = 0.9 % difference over 60 hrs	Purity = 0.1 % difference over 60 hrs	Purity = 0.0 % difference over 60 hrs

Conclusions

We have demonstrated that the addition of mineral oil to the Gel-R buffer vials during CE-SDS assay preparation significantly reduced evaporation of the gel buffer. This resulted in a significant improvement in the consistency of product profiles by almost completely mitigating product peak drift.

Throughout this study there were no observable changes to the product profiles as a result of using mineral oil, and no new or spurious peaks were detected.

Using NIST mAb as a test molecule, we have also shown through a series of experiments that there were no deleterious effects upon the assays accuracy, precision, linearity, LOD/LOQ, or autosampler stability. There were no observable changes in signal response, and all reportable results in the form of % TCA were comparable between runs using mineral oil and runs that did not.

The analysis of samples that displayed greater consistency in product profiles with more reproduceable migration times for product peaks, simplified the data processing aspect of CE-SDS testing significantly. Correct assignment of peak IDs was more reliable as integration windows could be tightened, without risk of peak migration time shift leading to mislabeling or errors in purity calculations.

Together, this eases the demand on analysts as the need for manual adjustment of integration windows on a sample by sample basis was reduced.

It is our recommendation that the application of mineral oil to Gel-R buffer vials should be included as a regular part of assay setup. Since mineral oil is added to buffer vials that are used for capillary reconditioning and does not directly come into contact with prepared sample, it is not expected that there would be any product specific issues with its use.

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