Advanced CE system for IgG Purity and Heterogeneity Analysis

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Introduction

Monoclonal antibodies (mAbs) are routinely used as therapeutic and diagnostic products when treating a wide-variety of diseases. Due to the importance of these molecules, regulatory agencies have guidelines describing methods to assess product purity necessary for lot release and characterization. CE-SDS is one of the most common techniques used to monitor purity and heterogeneity.

iCE3 has been the go-to method for analyzing charge heterogeneity for mAbs. In this poster, we introduce Maurice, the latest member of the iCE platform family, who now gives you high resolution CE-SDS IgG data on top of the exceptional cIEF data you've come to expect.

Maurice's CE-SDS application gives you baseline resolution of reduced non-glycosylated and glycosylated IgG heavy chain in just 25 minutes with % Area RSDs less than 4%. A simplified workflow provides unparalleled ease-of-use. Once samples and reagents are prepared, it takes less than 10 minutes to install the cartridge and start your batch. And at the end of your batch, easy clean-up and automatic data analysis by Compass for iCE software lets you quickly start your next batch.

Method Principles

Maurice is a benchtop system who runs both cIEF and CE-SDS applications while Maurice S. is dedicated for CE-SDS applications. For CE-SDS applications, Maurice and Maurice S. use a cartridge capable of completing up to 100 sample injections (max 48/batch). The conditioning steps before separation equilibrates the system and ensures the formation of a uniform charge surface layer on the capillary surface. Samples are loaded into the capillary with electrokinetic injection and voltage is then applied to separate molecules by size. A point-detector monitors separation time in terms of Relative Migration Time (RMT) compared to a 10 kDA internal standard. An optional panel of MW markers can also be used if MW information is desired.

Figure 1. Maurice

Figure 2. CE-SDS cartridge

Method Workflow

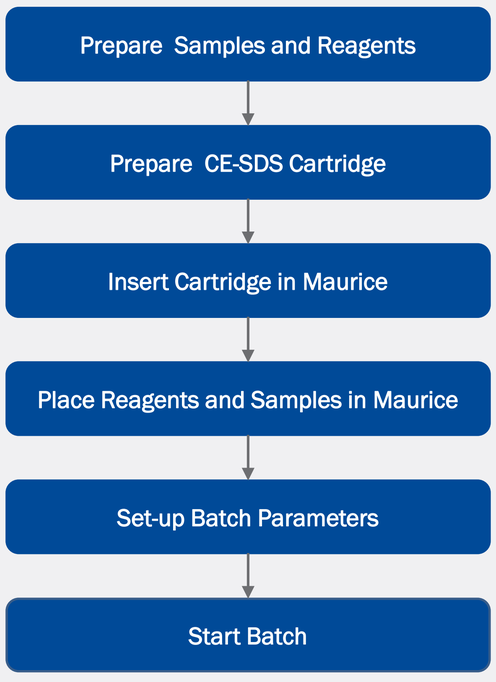

Figure 3. Maurice workflow

Resolving the IgG standard

Reduced and non-reduced IgG standard was run on Maurice. Light chain, non-glycosylated heavy chain, and glycosylated heavy chain were all baseline resolved in the reduced sample. Maurice easily detected IgG fragments the non-reduced IgG standard (>94% intact IgG).

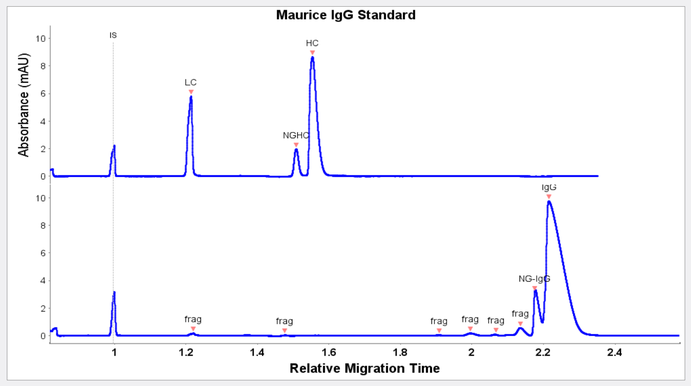

Figure 4. CE-SDS separations of (top) reduced and (bottom) non-reduced preparations of IgG Standard. IgG standard was reconstituted to 1 mg/mL with 1x Sample Buffer containing SDS. Reduced samples were treated with β-ME and non-reduced samples were alkylated with iodoacetamide before denaturing at 70 °C for 10 minutes. Reduced and non-reduced samples were separated on Maurice for 25 and 35 minutes, respectively.

Linearity

BSA was serially titrated and the peak area was used to demonstrate method linearity. Dynamic range was at least 2 logs with a $R^2 = 0.9998$

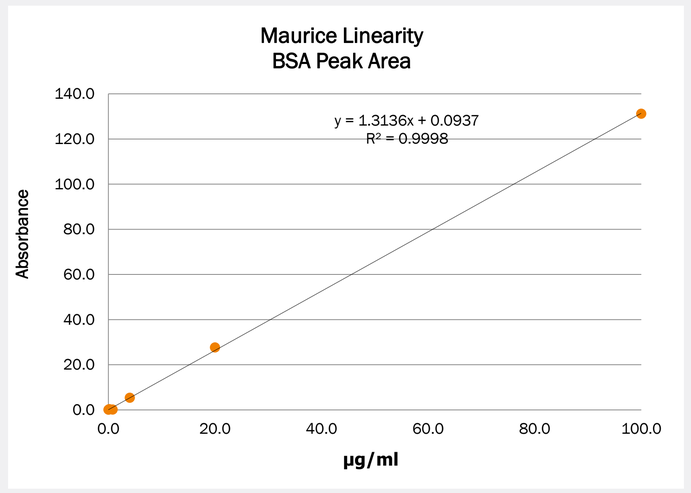

Figure 5. BSA was diluted in 1x Sample Buffer from 100 µg/mL down to 0 µg/mL. Samples were then reduced with β-ME and heated at 70 °C for 10 minutes

Sensitivity

The Internal Standard was serially diluted to determine method LOD and LOQ.

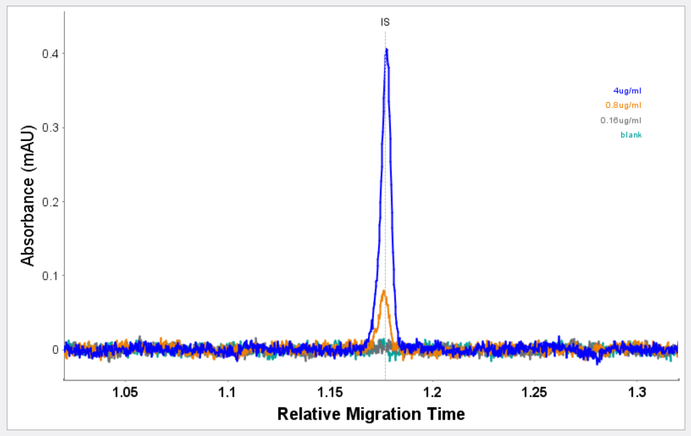

Figure 6. The Internal IgG Standard was diluted in 1x Sample Buffer from 4 µg/mL down to 0.16 µg/mL. Samples were then reduced with β-ME and heated at 70 °C for 10 minutes

Sample	LOD	LOQ
Internal Standard	0.21 µg/mL	0.71 µg/mL

Table 1. LOD and LOQ calculated using a serial dilutions of Internal Standard from 0 - 20 µg/mL. LOD and LOQ were calculated using peak height.

Consistent Relative Migration Time (RMT)

The RMT for the glycosylated heavy chain, non-glycosylated heavy chain, and light chain was tracked across 42 injections for migration consistency.

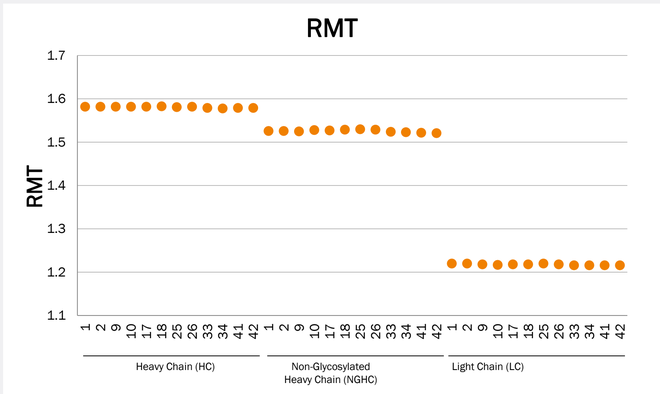

Figure 7. The relative migration time (RMT) across 42 injections. Data for 12 injections shown.

Peak	Average RMT	RSD (%)
Glycosylated heavy chain	1.581	0.106
Non-glycosylated heavy chain	1.526	0.191
Light chain	1.218	0.191

Table 2. Summary statistics for RMT prove consistent peak migration

Reproducible Quantitation

The % peak area and HC/LC ratio was monitored across 42 injections to demonstrate quantitation reproducibility.

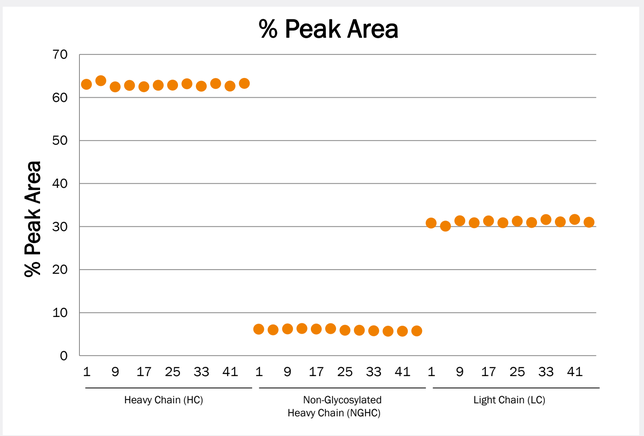

Figure 8. The % peak area across 42 injections. Data for 12 injections shown.

Peak	Average % Peak Area	RSD (%)
Glycosylated heavy chain	62.9%	0.652
Non-glycosylated heavy chain	6.0%	3.975
Light chain	31.1%	1.33

Table 3. Summary statistics for % peak area are prove consistent data quantitation

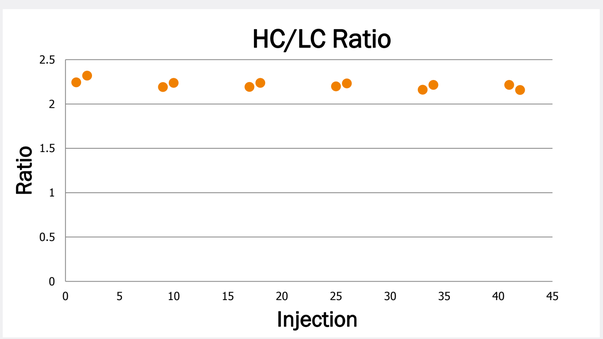

Figure 9. The HC/LC ratio across 42 injections. Data for 12 injections shown.

Peak	Average Ratio	RSD (%)
Heavy chain/Light chain	2.21	0.03

Table 4. Summary statistics of the HC/LC ratio prove consistent data quantitation

Conclusion

- High resolution CE-SDS data with baseline resolution of the non-glycosylated and glycosylated heavy chain.
- CE-SDS application is sensitive with a LOQ of 0.71 µg/mL for the internal standard and linear to R^2 of 0.9998 for BSA with at least 2 log dynamic range.
- Reproducible data
  - RMT RSD < 0.2%
  - % Composition RSD < 4%
  - HC/LC ratio RSD < 0.03%
- Maurice delivers results faster with a simple workflow.