Extending the Molecular Weight Range for Maurice CE-SDS

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Introduction

The CE-SDS mode of the Maurice™ platform has several critical advantages over traditional CE-SDS instrumentation including ease-of-use, faster analysis, and a flatter baseline. While most researchers utilize Maurice CE-SDS to examine the purity of monoclonal antibodies, in some cases the test samples can be outside the range of our current Molecular Weight Standards. While a separation system can be optimized for proteins with various sizes, it is often more desirable if a single separation system can be applied to a diverse group of molecules.

To address these points, we will show how to create an extended molecular weight range by minor modification for Maurice CE-SDS using the current Maurice CE-SDS separation system and commercially available materials.

Methods and Materials

Instrument: Maurice CE instrument and CE-SDS cartridge.

Maurice

CE-SDS cartridge

Size Application Kit

Samples and Reagents:

- Maurice CE-SDS Application Kit (ProteinSimple)
- Maurice CE-SDS Molecular Weight Markers (ProteinSimple)
- CE-SDS Internal standard (IS, ProteinSimple)
- Maurice pI 3.38 IEF Marker (ProteinSimple)
- Thyroglobulin (Sigma, T1001-100)
- IGSF3 (R&D Systems, Custom product)
- Iodoacetamide (IAM), 250 mM solution prepared in deionized water
- b-mercaptoethanol (b-ME)
- Insulin analytical reference standards (Human, Lispro and Glargine) were purchased from the United States Pharmacopeia (USP).

Sample Preparation and Run Conditions:

Sample Preparation: All samples, both reduced and non-reduced, were run at 0.5 mg/mL prepared in ProteinSimple CE-SDS Sample buffer. The Maurice CE-SDS Molecular Weight Markers sample was prepared as follows for data presented in Figures 2-7.

Low Molecular Weight (LMW) Ladder preparation: The Maurice CE-SDS Molecular Weight markers sample was denatured at 70C for 10min with 11.5mM IAM. The pI 3.38 marker, reconstituted in 50uL 1X Sample Buffer, was then added at a ratio of 1:20 (pI 3.38 marker:MW ladder).

High Molecular Weight (HMW) ladder preparation: Thyroglobulin (4 mg/mL) was heated to 70C for 10min in the presence of 11.5mM IAM before being diluted 5-fold in heat-denatured (95C, 10min, non-reduced) MW ladder.

Run conditions: All samples were injected for 20 seconds at 4600 V, followed by separation at 5750 V for 20min for the analysis of LMW proteins and 60 minutes for the analysis of HMW proteins.

Results and Discussion

Figure 1 shows the commercially available MW marker ladder from ProteinSimple. This ladder spans 10-270 KDa.

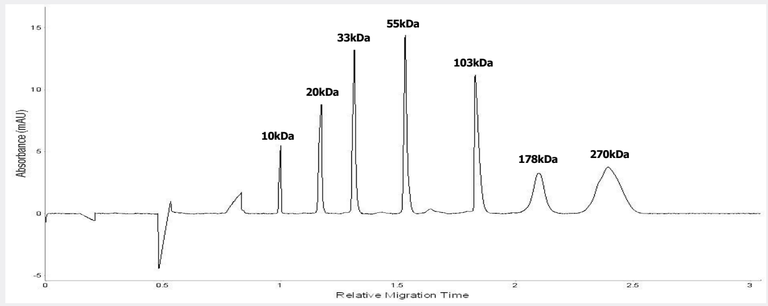

Figure 1. ProteinSimple Maurice Molecular Weight marker Ladder, run under reducing conditions, as recommended in the product insert.

Extending the Molecular Weight Ladder for Analyzing Small Proteins

For low molecular weight samples, the MW ladder can be extended at the low molecular weight region by the addition of a small peptide. In this example, the Maurice pI 3.38 peptide marker was added to the non-reduced, heat-denatured MW ladder (prepared as described in Materials and Methods), shown in **Figure 2**. The resulting calibration curve of the molecular weight between the 0.5-55kDa range is shown in **Figure 3**.

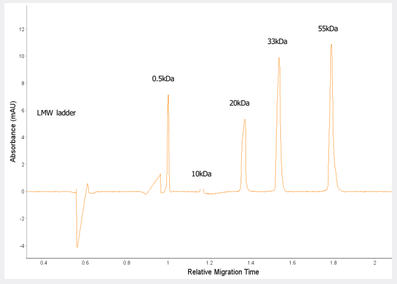

Figure 2. Addition of Maurice IEF peptide to MW ladder. The pI 3.38 marker was spiked into the MW ladder, and assigned a MW of 0.5kDa. This peak can then be set as the internal standard (IS).

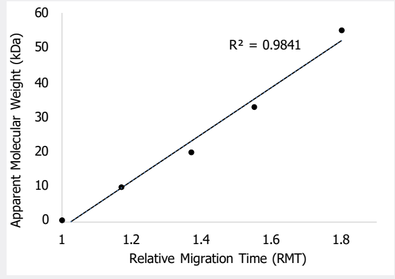

Figure 3. Molecular weight calibration curve in the low molecular range with the added pI 3.38 marker. A good linear relationship between RMT and apparent molecular weight is achieved from 0.5-55kDa.

We obtained three insulin standards (Human, Lispro and Glargine) from the USP and analyzed them using the low molecular range ladder (**Figure 4**). The apparent molecular weights of the insulin samples were well-correlated to calculated molecular weight derived from the amino acid sequence (7.04kDa measured vs. 5.7kDa theoretical).

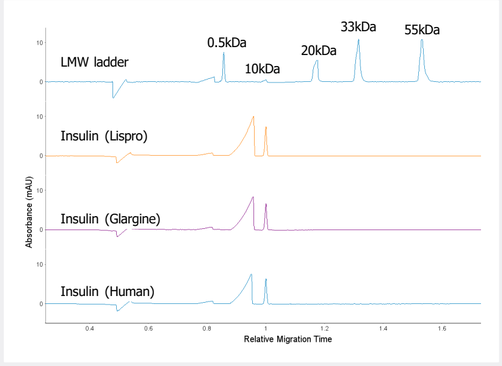

Figure 4. Analysis of USP Insulin proteins using the LMW ladder. Three insulin proteins were obtained from the USP and analyzed by Maurice CE-SDS.

Extending the Molecular Weight Ladder for Analyzing Very Large Proteins

For high molecular weight samples, the range of the molecular weight marker can be extended by adding a single protein (Thyroglobulin) that yields two additional HMW peaks: 330 kDa and 660 kDa. To retain the integrity of the 660kDa peak (dimer), Thyroglobulin needs to be gently denatured at 70C while the commercial ladder needs to be denatured for 10min at 95C to result in a clean profile. The ladder shown is a mix of both. The electropherogram of the ladder for high molecular weight protein analysis is shown in **Figure 5**. The calibration curve of the molecular weight between 10-660kDa range is shown in **Figure 6**.

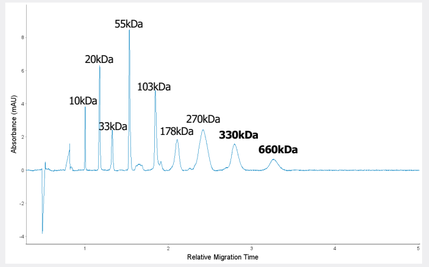

Figure 5. Adding a HMW protein to MW ladder. Thyroglobulin was prepared independently, then then combined with heat-denatured MW ladder. The separation time was extended to 60min to allow larger proteins sufficient time to pass through the detection window.

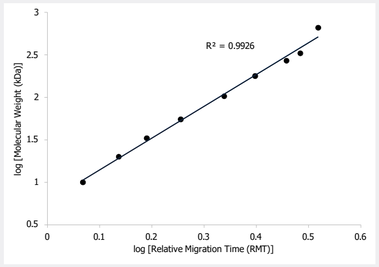

Figure 6. Fitting for HMW ladder. A log scale was adapted to generate best-fit for the additional HMW proteins that were added to the ladder.

We analyzed IGSF3 using the extended high molecular range ladder. The results are shown in **Figure 7**. The measured molecular weights (apparent MW) of the samples are overlaid on the electropherograms.

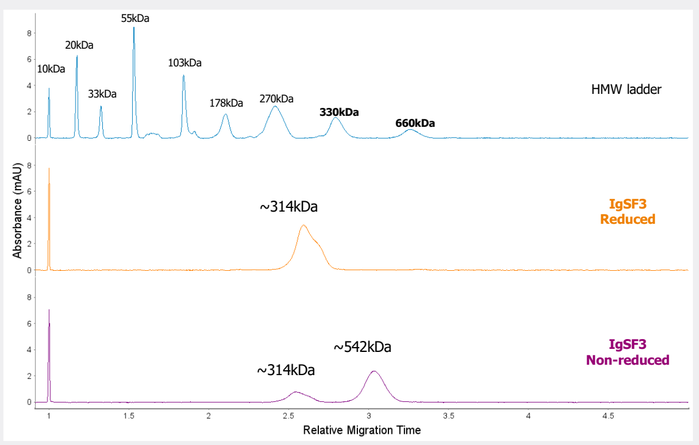

Figure 7. Analysis of two high molecular weight proteins. To verify the apparent molecular weight determination, IGSF3 (reduced and non-reduced) was run alongside the HMW ladder.

Conclusions

- The detectable apparent molecular weight range of Maurice can be extended at both the low and high molecular weight ends by adding off-the-shelf reagents.
- The MW ladder is run non-reduced, but denatured at 95C for 10min in the presence of alkylating agent to avoid HMW protein reduction.
- For LMW protein analysis, the pI 3.38 marker can be added to the MW ladder. Non-reduced insulin analytical standards from the USP were analyzed with the LMW ladder.
- For HMW protein analysis, non-reduced, alkylated thyroglobulin can be added to the MW ladder to extend the apparent molecular weight range to ~700kDa.