SDS-MW calibration and molecular weight estimation using the SCIEX PA 800 Plus Empower Driver version 1.3

Firdous Farooqui[1], Peter Holper[1], John D. Walsh[2], Handy Yowanto[1]
1 SCIEX, Brea, CA
2 Waters Corporation, Milford, MA

Introduction

CE-SDS, a specific CGE technique, involves the separation of protein-SDS complexes using a replaceable gel matrix. The gel is formulated to provide a sieving range of approximately 10 kDa to 225 kDa. Within this size range, the logarithm of protein molecular weight is linear with its migration time. Therefore, the molecular weight of an unknown protein can be estimated from a standard curve of known protein sizes[1]. Currently, Empower does not have pre-configured standard curve and MW estimation capability. This technical note demonstrates the molecular weight estimation of unknown protein peaks using Empower software CDS from data generated with the SCIEX PA 800 Plus Empower™ Driver v1.3.

Key features

- Baseline resolution of SDS-MW Size Standard peaks
- Internal software calibration of SDS-MW Size Standard peaks
- Seamless integration of the PA 800 Plus System into an existing centralized chromatography data system (CDS)
- Validated CE-SDS method on an automated instrument for robust and reliable analysis
- Comparable results between Empower Driver v1.3 calibration curve (R^2 = 0.999120) and 32 Karat software (R^2 =0.998241).

Methods

Instrument

All experiments were performed on a PA 800 Plus System (SCIEX, Brea, CA) equipped with a PDA detector. Data was collected using the PA 800 Plus Empower Driver v1.3. A 50 µm i.d., 30.2 cm long bare-fused silica capillary (SCIEX, P/N 338451) was installed on the PA 800 Plus System (20 cm effective length). The capillary temperature was maintained at 25°C in all separations. Voltage was applied using reverse polarity.

The following methods were used with the SCIEX PA 800 Plus Empower Driver v1.3:

- SDS MW Conditioning: conditions the capillary.

Figure 1: The PA 800 Plus Pharmaceutical Analysis System

- SDS MW Separation: performs the SDS-MW separation.
- SDS MW Shutdown: cleans the capillary at the end of the sequence for storage and turns off the UV lamp.

Chemicals and materials

SDS-MW Size Standard

The SDS-MW Size Standard contains 10 kDa, 20 kDa, 35 kDa, 50 kDa, 100 kDa, 150 kDa, and 225 kDa standards and is used to establish a calibration curve in order to estimate the molecular weight of a sample peak.

Internal Standard

A 10 kDa protein internal standard is used as a mobility marker. The relative migration times of all protein samples are calculated against the migration time of the 10 kDa standard, allowing for more accurate size estimation.

Reagents and buffers

SDS-MW Gel Buffer, SDS-MW Sample Buffer, 10 kDa protein internal standard, SDS-MW Size Standard, acidic and basic wash solutions were all manufactured by SCIEX (Carlsbad, CA) as part of the SDS-MW Analysis Kit (P/N 390953). The IgG control standard was manufactured by SCIEX (P/N 391743). 2-mercaptoethanol was obtained from Sigma (St. Louis, MO).

Sample preparation

Prepare the SDS-MW Size Standard

1. Allow the SDS-MW Size Standard to thaw at room temperature for 15 minutes.
2. Mix the SDS-MW Size Standard thoroughly and centrifuge briefly in a standard microcentrifuge.
3. Pipette 10 µL of the SDS-MW Size Standard into a 0.5 mL microcentrifuge tube.
4. Add 85 µL of the SDS-MW sample buffer to the microcentrifuge tube.
5. Add 2 µL of the 10 kDa internal standard to the microcentrifuge tube.
6. Inside a fume hood, add 5 µL of 2-mercaptoethanol to the microcentrifuge tube.
7. Seal the vial cap with Parafilm, mix thoroughly, and heat in a water bath at 100°C for 3 minutes.
8. Put the tube in a room-temperature water bath to cool for five minutes. The sample will remain stable for approximately 24 hours.
9. Transfer 100 µL of the prepared sample to a micro vial, put the micro vial into a universal vial, and cap the universal vial.

Prepare the IgG control standard (reduced)

1. The IgG control standard (~ 1 mL) was aliquoted into ~10 vials of 95 µL each and stored at -20°C to avoid multiple freeze-thaw cycles.
2. Thaw one of the 95 µL aliquots of the IgG control standard at room temperature.
3. Add 2 µL of the 10 kDa internal standard to the IgG tube.
4. Inside a fume hood, add 5 µL of 2-mercaptoethanol to the IgG tube.
5. Cap the tube and mix thoroughly.
6. Using a centrifuge, spin the tube for 1 minute at 300 × g.
7. Seal the vial cap with Parafilm and heat the vial at 70°C for 10 minutes.
8. Allow the vial to cool for at least 3 minutes.
9. Transfer 100 µL of the prepared sample to a PCR vial, put the PCR vial in a universal vial and cap the universal vial.

Results and discussion

All proteins were completely separated within 30 minutes using our recommended method. Figure 2 shows a typical separation of the SDS-MW Size Standard.

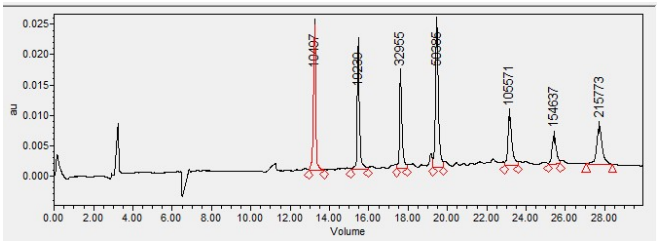


Figure 2: Separation of the SDS-MW Size Standard

Establishing the standard curve

A 10 kDa internal standard is used as a mobility marker and all protein mobility is calculated relative to this internal standard. See Figure 3 for a typical calibration curve obtained by plotting the Log Molecular Weight versus Volume (Migration Time) of each protein peak in the SDS-MW Size Standard. The molecular weight of an unknown protein peak can be estimated using this calibration curve. The calculated molecular weights are displayed in the electropherogram by displaying the MW (Daltons) column in Table properties. It is recommended that this curve be re-calibrated every 24 cycles. This is done by running the SDS-MW Size Standard and updating the calibration curve values for each standard to reflect the new run, as shown on Figure 3.

Figure 3 shows the SDS-MW analysis result of the SDS-MW Size Standard, demonstrating the analysis method separates the analytes by their molecular weights. The calibration curve correlation coefficient (R^2) generated using this analysis method in the SCIEX PA 800 Plus Empower Driver v1.3 was > 0.99.

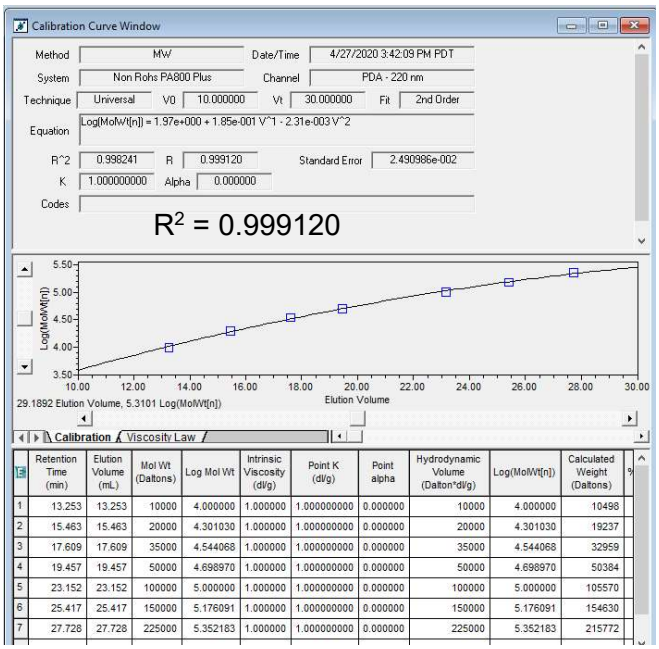


Figure 3: Calibration curve generated by the Empower software.

The calculated molecular weight of the SDS-MW Size Standard is shown in Table 1.

Table 4: ***Calculated molecular weights of the SDS-MW Size Standard using Empower software***

Estimated MW (Daltons)	Calculated MW (Daltons)
10,000	10,498
20,000	19,237
35,000	32,959
50,000	50,384
100,000	105,570
150,000	154,630
225,000	215,772

The Empower software data file of the SDS-MW Standard used to generate the standard curve in Figure 3 was converted and imported into 32 Karat™ software. The file was analyzed in 32 Karat™ software to generate a calibration curve (Figure 4).

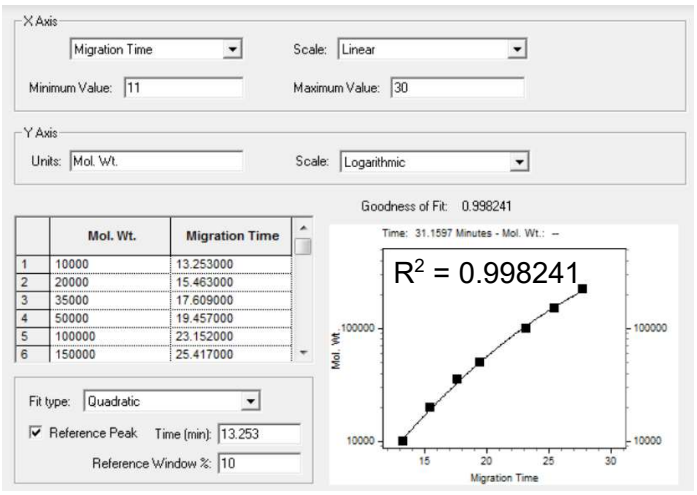


Figure 4: Linear curve of log of MW vs. Migration Time from Table 2 analyzed using 32 Karat™ software.

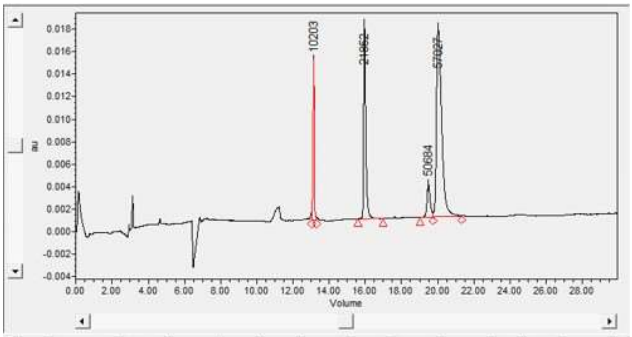
A comparison of the R^2 values generated by 32 Karat™ software and Empower software shows both calibration curves are identical. Thus, the calibration curve generated using data collected using the SCIEX PA 800 Plus Empower Driver v1.3 can be successfully used to estimate the molecular weight of an unknown protein peak.

Generating estimated protein molecular weights from the standard curve

Note: High levels of glycosylation and other attributes can affect peak migration when analyzed by CE-SDS. The molecular weight determination is an estimation and might not be representative of the theoretical molecular weight of each analyte analyzed.

A glycosylated IgG standard was analyzed under reducing conditions using the same separation conditions described above. The molecular weight of each resulting peak was estimated using the calibration curve generated with the SDS-MW Size Standard (Figure 5).

The following table and the annotation on each peak show the estimated molecular weight of the 10 kDa Internal Standard and each peak of the reduced IgG standard.



	Name	Migration Time (min)	MP (Daltons)	Area (µV*sec)	% Area	Height (µV)	% Height	Mw(LS) (Daltons)	Mz(LS) (Daltons)	Mz+1(LS) (Daltons)	Rgz (nm)	K(LS) (nm)	alpha(LS)	Line
1		13.154	10203	72793	12.40	14394	28.06							
2		15.954	21852	140553	23.93	17264	33.66							
3		19.484	50684	36227	6.17	2924	5.70							
4		20.028	57027	337695	57.50	16712	32.58							

Figure 5: Molecular weight estimation of reduced IgG standard

Conclusions

This technical note demonstrates that data collected using the SCIEX PA 800 Plus Empower Driver v1.3 can be used to generate a molecular weight calibration curve by plotting Log Molecular Weight versus Elution volume (migration time) of each protein peak in the SDS-MW Size Standard. The molecular weight of an unknown protein can be estimated by using this calibration curve.

References

1. *SDS-MW Analysis Kit Application Guide* (RUO-IDV-05-6934-B)

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices