Determination of molecular weight of Keratin using CE-SDS MW protein kit (Beckman Coulter)

1) Preparation of protein standard/sample

1.1 Protein Standard; the protein size standard (10-225 kDa) was prepared following the PA800 plus application guide.

- Remove protein size standard from fridge, leave it at room temperature for 15 min.
- Mix standard and pipet 5 uL of size standard into a micro-centrifuge tube.
- Add 42.5 uL of sample buffer (containing SDS) into the micro-centrifuge tube.
- Add 1 uL of internal standard protein (10 kDa) into the micro-centrifuge tube.
- Add 2.5 uL of mercaptoethanol, cap tube tightly and mix thoroughly.
- Heat the mixture in heating block at 95-100^{o}C for three minutes.
- Cool the tube before injection. The protein size standard is good for 24 hours.

The protein size standard was mixed with SDS sample buffer at an elevated temperature. The reaction should produce SDS-protein complexes having similar charge densities. When standard was electrophoretically separated, their mobilities would depend on their sizes. Smaller proteins have greater mobilities.

1.2 Protein sample

- Dilute protein sample with the SDS-MW sample buffer for a total 95 uL volume to give a final protein concentration range of 0.2 – 2.0 mg/mL.
- Add 2 uL of internal standard (10 kDa) into the micro-centrifuge tube.
- Add 5 uL of mercaptoethanol, cap the tube tightly and mix thoroughly.
- Heat the mixture in a heating block at 95-100oC for three minutes.
- Cool the tube before injection. The protein sample is good for 24 hours.

2) CE instrumentation setup

- Installing capillary column

  A 50 um ID bare fused silica column was used for separation, Total length 34 cm, effective length 24 cm (the shortest length that can put in a cartridge). Flush the column with 1M NaOH, HCl, DI water for 30 min (each of those solutions) for new capillary column.

- Method setup for HP3D CE

  Column: Bare-silica column 50 um ID, 360 um OD, L 34/24 cm

Preconditioning

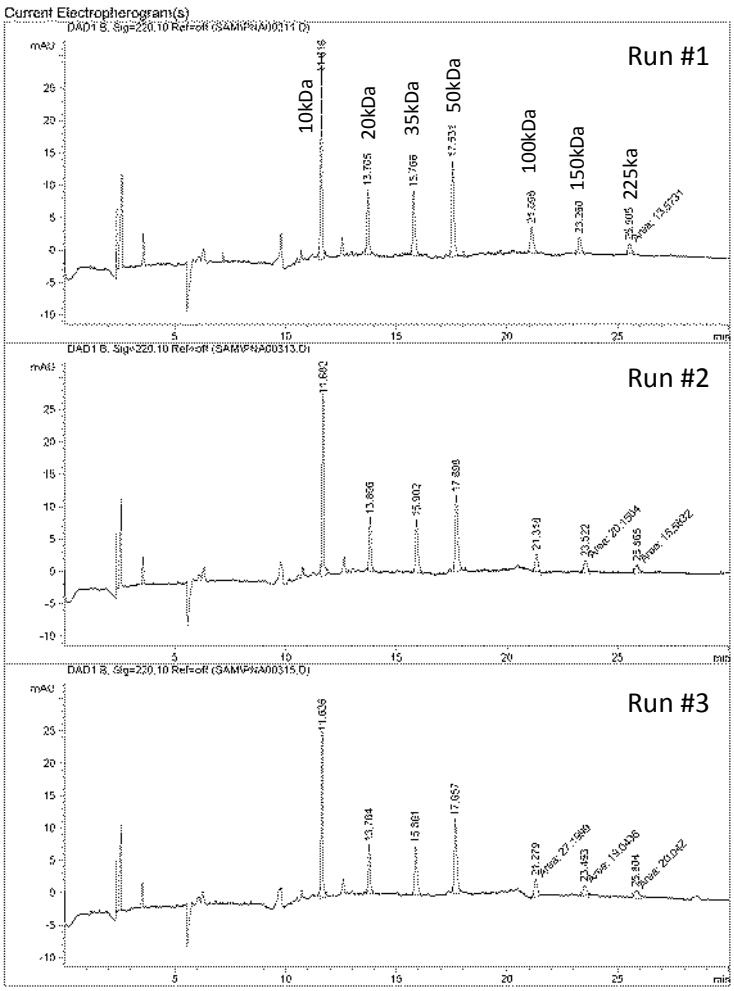
Event	Duration	Comments
Flush - 0.1 M NaOH	3 min	0.1 M NaOH rinse to clean capillary surface
Flush - 0.1 M HCl	3 min	0.1 M HCl rinse to neatralize capillary surface
Flush - DIW	3 min	Water rinse to remove the acid residue
Flush - SDS buffer gel	15 min	SDS gel rinse to fill the capillary with SDS gel

Post conditioning

Flush - DIW	10 min	Remove the used gel inside the capillary tube

Injection: Negative polarity -5kV, 30 second
Voltage: Negative polarity -18 kV
Column temperature: 25^{o}C
Detection: 220 nm or 220 nm with reference at 350 nm.
Note: SDS gel buffer can be used to multiple runs.

- Electropherogram



Integration Results

Signal 1: DAD1 B, Sig=220,10 Ref=off (SAM\PNA00311.D)
Integrated with enhanced integrator!

Peak #	Time [min]	Type	Area [mAU*s]	Height [mAU]	Width [min]	Start [min]	End [min]
1	11.616	BB	148.04034	31.60137	0.0747	11.513	11.753
2	13.705	BB	55.85342	10.05051	0.0848	13.605	13.846
3	15.766	BB	70.95741	9.93377	0.1073	15.647	15.953
4	17.531	BB	109.62124	14.76437	0.1146	17.413	17.740
5	21.095	BB	37.23710	4.02707	0.1399	20.945	21.340
6	23.260	BB	23.66271	2.59814	0.1383	23.100	23.486
7	25.505	MM	13.57309	1.50944	0.1499	25.410	25.677

Signal 2: DAD1 B, Sig=220,10 Ref=off (SAM\PNA00313.D)
Integrated with enhanced integrator!

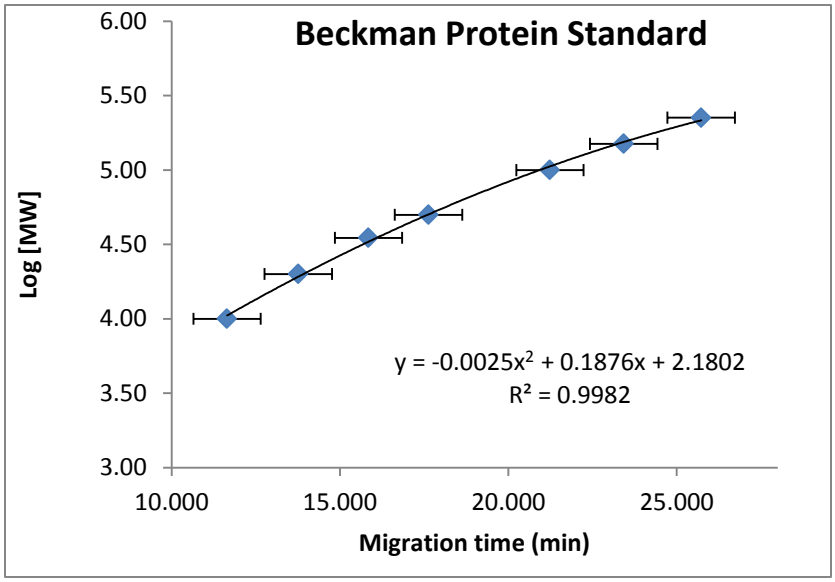
Peak #	Time [min]	Type	Area [mAU*s]	Height [mAU]	Width [min]	Start [min]	End [min]
1	11.682	BB	132.22824	28.21833	0.0747	11.581	11.820
2	13.806	BB	48.94861	8.24074	0.0893	13.700	13.966
3	15.902	BB	60.76453	8.12632	0.1152	15.774	16.100
4	17.698	BB	98.20544	11.80102	0.1251	17.567	17.913
5	21.318	PP	23.59450	2.73652	0.1326	21.158	21.540
6	23.522	MM	20.15043	1.87796	0.1788	23.374	23.722
7	25.865	MM	15.58316	1.31674	0.1972	25.692	26.029

Signal 3: DAD1 B, Sig=220,10 Ref=off (SAM\PNA00315.D)
Integrated with enhanced integrator!

Peak #	Time [min]	Type	Area [mAU*s]	Height [mAU]	Width [min]	Start [min]	End [min]
1	11.636	BB	126.04147	25.18464	0.0784	11.531	11.780
2	13.764	BB	45.07071	7.56658	0.0935	13.651	13.926
3	15.861	PB	56.48566	7.49915	0.1159	15.736	16.073
4	17.657	BB	92.42345	11.46198	0.1181	17.530	17.860
5	21.279	MM	27.19990	2.81616	0.1610	21.107	21.458
6	23.493	MM	19.04355	1.68168	0.1887	23.307	23.690
7	25.804	MM	20.04203	1.32340	0.2524	25.667	26.070

- Estimation of protein molecular weights (plot of electrophoretic mobility and log MW)

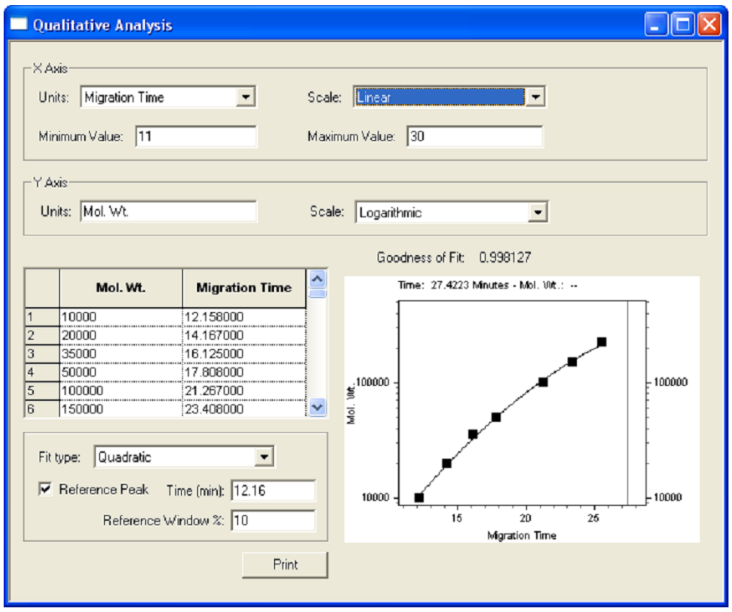
Molecular weight	Log [MW]	Migration time			Avg	SD
		Run 1	Run 2	Run 3		
10000	4.00	11.616	11.682	11.636	11.645	0.034
20000	4.30	13.705	13.806	13.764	13.758	0.051
35000	4.54	15.766	15.902	15.861	15.843	0.070
50000	4.70	17.531	17.698	17.657	17.629	0.087
100000	5.00	21.095	21.318	21.279	21.231	0.119
150000	5.18	23.260	23.522	23.493	23.425	0.144
225000	5.35	25.505	25.865	25.804	25.725	0.193



- Quadratic equation is applied to the plot according to PA800 plus application guide.

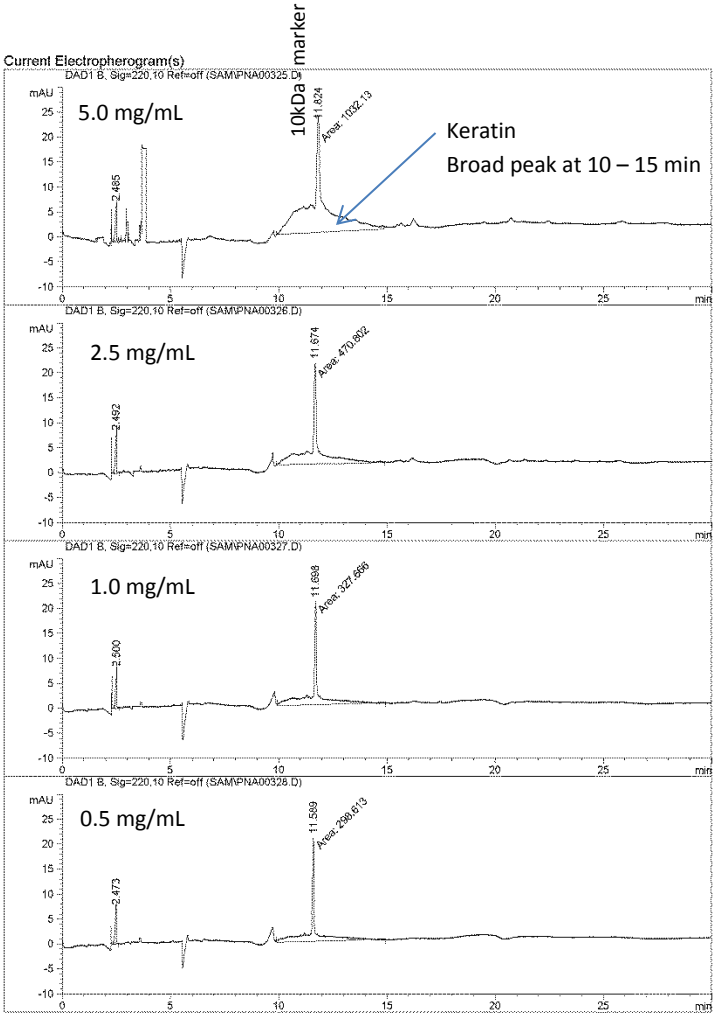
- N = 3

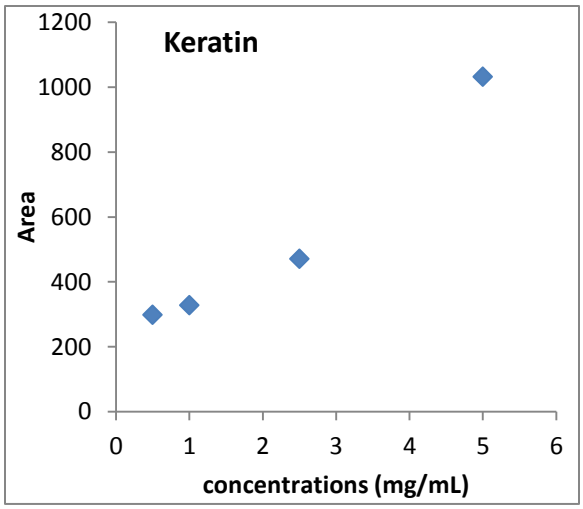
Figure 1.7 Qualitative Analysis Tab for Updating the Size Calibration Curve



3) Keratin

Keratin samples at different concentrations (5, 2.5, 1, 0.5 mg/mL) were prepared following 1.2 and injected in CE system.





- Keratin showing broad peak at 10 – 15 minute.
- From the migration times, molecular weight of keratin can be calculated in a range of **6.4 – 27 kDa**.