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TECHNICAL NOTE

APPARENT MOLECULAR WEIGHT ON CE-SDS AND SDS-PAGE

THE ADVANTAGES OF MAURICE CE-SDS OVER SDS-PAGE

CE-SDS has increasingly replaced traditional SDS-PAGE, particularly in the biopharmaceutical industry, for use in the characterization, stability, and purity studies of proteins.[1] CE-SDS on the Maurice and Maurice S. systems from ProteinSimple provides clear advantages over SDS-PAGE. Automation, accurate quantitation, better reproducibility, and increased throughput result in higher data quality with significant time savings. To learn more about the advantages of CE-SDS over SDS-PAGE, and a direct comparison of data obtained by the two methods, refer to our Application Note on Comparing SDS-PAGE with Maurice CE-SDS for Protein Purity Analysis.

DETERMINING THE APPARENT MOLECULAR WEIGHT OF A PROTEIN

While CE-SDS has significant advantages over SDS-PAGE, it has been observed that there can be discrepancies in apparent molecular weight (MW) between the two methods.[2,3] Although CE-SDS and SDS-PAGE are typically not intended for high-resolution MW determination, many of their applications require a reasonable estimate of apparent MW.[1] Differences in apparent MW between the two methods can arise from several sources, including the choice standards used for reference, secondary interactions with the separation matrix, and post-translational modifications of the proteins analyzed. This Technical Note highlights relevant peer-reviewed publications that address these factors for comparing MW between CE-SDS and SDS-PAGE.

CHOOSING THE RIGHT MARKER

In a research article published recently in *Electrophoresis*, Wiesner and her team at the University of Braunschweig sought to figure out the disparity in the performance of MW determination of proteins between SDS-PAGE and CE-SDS.[4] To do so, they tested several different sample preparation conditions, including sample buffer, denaturation temperatures, and different reducing agents, but found that none had a pronounced impact on the MW determination. In contrast, Wiesner and colleagues found that the selection of the MW marker plays a decisive role in determining the accurate apparent MW of a protein. For example, when directly comparing five different MW markers on SDS-PAGE, the deviation in MW determination can exceed 10% (**FIGURE 1**). Therefore, a comparison of MWs obtained by SDS-PAGE and CE-SDS should be based on the same markers. Wiesner and colleagues also noted that when the same MW marker was analyzed by 10% SDS-PAGE and CE-SDS on Maurice, the linear range was larger on Maurice, between 20 and 150 kDa, while on SDS-PAGE is was between 20 and 100 kDa (**FIGURE 2**).[4] Thus, the linear range of a marker depends on the separation matrix that is used.

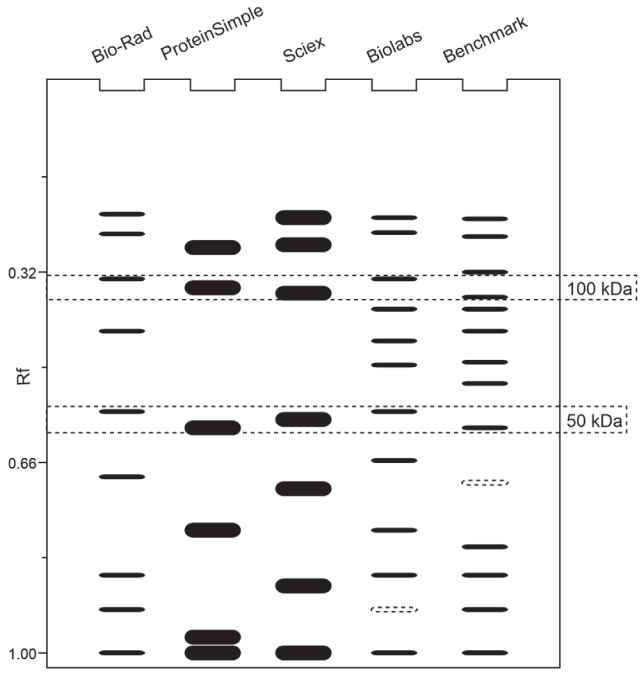

FIGURE 1. Comparison of MW markers of five different manufacturers by 10% SDS-PAGE. The relevant lanes of the gel were reconstructed for better illustration using ChemDraw 19.0.1.28 (PerkinElmer Informatics, Inc.); Bio-Rad = Precision Plus Protein™ Standard unstained by Bio-Rad; ProteinSimple = Molecular Weight Marker Maurice CE-SDS by ProteinSimple; Sciex = ProteomeLab™ MW Sizing Standard by Sciex; Biolabs = Unstained Protein Standard by New England Biolabs; Benchmark = Benchmark™ Unstained Protein Ladder by Novex by life technologies. (Adapted from Wiesner et al. 2020 CC BY 4.0)

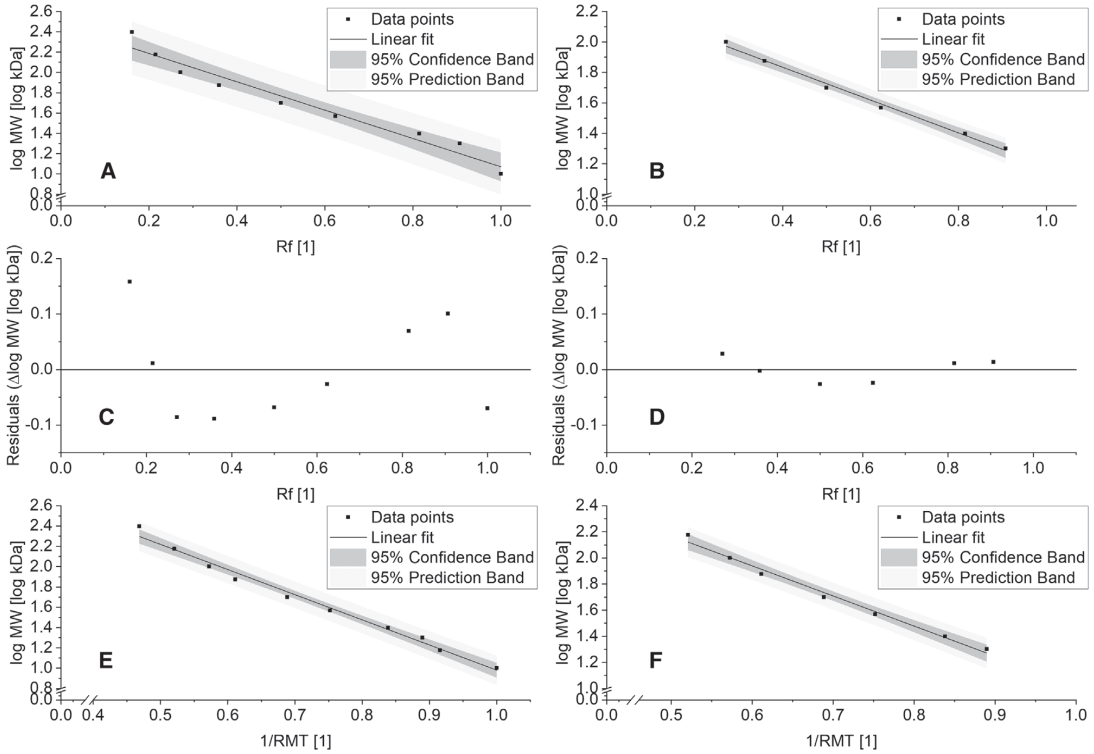

FIGURE 2. Linear regression plots based on the logarithm of the MW versus the relative migration distance (Rf) respectively the reciprocal relative migration time (RMT) of MW marker proteins (Precision Plus ProteinTM Standard from Bio-Rad). (**A**) Linear regression of the data points while all points included on SDS-PAGE; (**B**) Linear regression of the data points, deviating points excluded on SDS-PAGE; (**C**) Residual plot of the data points out of (**A**); (**D**) Residual plot of the data points out of (**B**); (**E**) Linear regression of the data points while all points included on CE-SDS; (**F**) Linear regression of the data points, deviating points excluded on CE-SDS. (Adapted from Wiesner et al. 2020 CC BY 4.0)

THE IMPACT OF POST-TRANSLATIONAL MODIFICATIONS

Post-translational modifications can have a significant impact on the electrophoretic mobility of proteins. This is well-known to occur for glycosylated proteins, which migrate more slowly than their aglycosylated counterparts.[5] Glycans do not bind SDS, causing the proteins to move more slowly than typical during electrophoresis, resulting in a higher apparent molecular weight.

To explore the impact of glycosylation on electrophoretic mobility, a recent study compared the MWs of several different glycoproteins between Maurice CE-SDS and traditional SDS-PAGE.[6] While the MWs of glycosylated proteins determined from SDS-PAGE were close to or slightly exceeded the theoretical values, the values of the same proteins appeared to be much greater on Maurice, with an average of >10 kDa increase per glycan site over the theoretical values. A likely explanation for this dramatic increase is that the various glycan chains can interact differently with the gel matrix of SDS-PAGE and the non-covalent sieving matrix of Maurice CE-SDS, resulting in higher MW determination on Maurice compared to SDS-PAGE. It should be noted that these attenuations were not restricted to Maurice, as they applied to other CE-SDS platforms as well. Nevertheless, proteins that were treated with the glycosidase PNGase F resulted in migration rates close to the theoretical values, which supports the notion that glycosylation contributes to this increase in MW. These results demonstrate that glycosylation can be a major factor in MW discrepancies, and treatment with a glycosidase may be necessary for accurate comparison of MW between the two methods. Deglycosylation also improves peak efficiencies and reduces peak broadening, as a major source of protein heterogeneity is eliminated. In addition to glycosylation, the study also noted that extensive disulfide bonds may also contribute to MW discrepancies under non-reducing conditions. For example, IgG1 showed a significantly decreased electromobility under non-reducing conditions on Maurice compared to SDS-PAGE, despite having only two *N*-glycans.[6]

CONCLUSION

Between CE-SDS and SDS-PAGE methods, sample preparation conditions like temperature, reducing reagent, and sample buffer do not have a pronounced influence on MW determination. By contrast, the choice of MW standard, linear range, secondary interactions conferred by the separation matrix, and the presence of post-translational modifications like glycosylation and disulfide bond formation have a significant impact on MW determination. These factors should be considered when comparing the apparent MW of proteins between these two methods.

REFERENCES

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