Application Note

Overcoming the Challenges of Large Biomolecule Analysis with Maurice™

Rapid Analysis of IgM Proteins with the Turbo CE-SDS™ Cartridge

Introduction

Immunoglobulin M (IgM) molecules are increasingly being explored for their therapeutic potential, particularly because of their pentameric or hexameric structure that confers high avidity, in turn enabling them to bind with antigens that are present at low concentrations[1]. Currently, there are over 100 IgM-based therapeutics in clinical trials for the treatment of several diseases. Examples include Imvotamab (non-Hodgkin's lymphoma), IgM-8444 (colorectal cancer), and IgM-7354 (solid tumors)[2]. More recently, the intranasal administration of an IgM-based therapeutic against SARS-CoV-2 was reported, showing better potency than an IgG-based version and demonstrating an ability to target a wider range of viral variants[3]. As more reports unfurl on the therapeutic applicability of IgM proteins, the greater the demand will be for suitable characterization tools.

Key Takeaways

1. The Maurice Turbo CE-SDS™ cartridge offers high throughput CE-SDS analysis.
2. Large proteins with MW of 900 kDa can be rapidly analyzed using Turbo CE-SDS.
3. The study illustrated in this application note demonstrates IgM molecular weight determination, protein identification, stability assessment, and method reproducibility.

The large size of IgM molecules (~950 kDa), however, can cause challenges in their characterization. In this application note, we showcase how capillary electrophoresis dodecyl sulfate (CE-SDS) with the Turbo CE-SDS™ cartridge on the Maurice platform can be used for analysis of IgM proteins. With its ability to perform both CE-SDS and imaged capillary isoelectric focusing (icIEF) characterization of a wide range of proteins including mAbs, fusion proteins, ADCs, etc., the use of one Maurice system can result in significant savings in capital, time, and labor.

About Maurice

Maurice is a multi-functional capillary electrophoresis (CE) platform that enables both CE-SDS and icIEF analysis. Whether you are analyzing protein size and purity or pI charge heterogeneity, the pre-assembled cartridges and easy workflow let you develop your methods in a day. Set up your method, prepare your samples and load them onto the Maurice instrument, plug in the relevant cartridge (CE-SDS PLUS, Turbo CE-SDS, or cIEF), and hit Start. You will get high-quality, 21 CFR Part 11 compliant data in minutes. Cleanup on the Maurice system is simple and automated, letting you easily switch between your CE-SDS and icIEF methods without risk of cross-contamination.

The study in this application note utilized the Maurice Turbo CE-SDS™ cartridge to rapidly analyze IgM molecules. The Turbo CE-SDS cartridge enables rapid and high throughput CE-SDS analysis, providing results 5X faster than traditional CE-SDS methods and thus ideal for upstream applications. Shown in this application note are data on IgM purity (non-reduced and reduced), stability, identity, and reproducibility analysis.

Materials and Methods

The materials used in this study are listed in **Table 1**. IgM samples were diluted with the Maurice CE-SDS 1X Sample Buffer to a final concentration of 1 mg/mL. Different concentrations of dithiothreitol (DTT) were added to samples for stability studies. All samples were heated at 70°C for 10 minutes, cooled on ice for 5 minutes, and then subjected to centrifugation. The samples were then loaded onto the Maurice instrument and injected for 8 seconds at 3500 V and separated for 14 minutes at 4200 V. All data were analyzed using Compass for iCE software.

Material	Vendor	Catalog #
Maurice Turbo CE-SDS Size Application Kit	ProteinSimple, a Bio-Techne brand	PS-MAK01-TS
Maurice Turbo CE-SDS Cartridge		PS-MC02-TS, PS-MC01-TS
Maurice CE-SDS Molecular Weight Markers		046-432
Maurice CE-SDS IgG Standard		046-039
Maurice CE-SDS 25X Internal Standard		046-144
Dithiothreitol (DTT)		042-251
Iodoacetamide (IAM)	Millipore Sigma	16125
β-mercaptoethanol (β-ME)		M-3148

TABLE 1. List of materials and reagents used in this study.

Results

Analysis of Large Molecular Weight Proteins

FIGURE 1 demonstrates how the Turbo CE-SDS method was able to baseline separate the IgM molecule despite being of such a large molecular weight, in just 14 minutes. A typical IgG molecule, as shown, weighs 150 kDa. In contrast, IgM is known to have a MW of 950 kDa. The heavier well-resolved IgM peak demonstrates that the Turbo CE-SDS method allows for the identification and characterization of proteins of wide molecular weight range without needing additional method development or modification. The broadness of the IgM peak could be attributed to the bulky pentameric structure of the molecule. Profiles of IgM under non-reduced and reduced conditions are shown in **FIGURES 2A** and **2B** respectively.

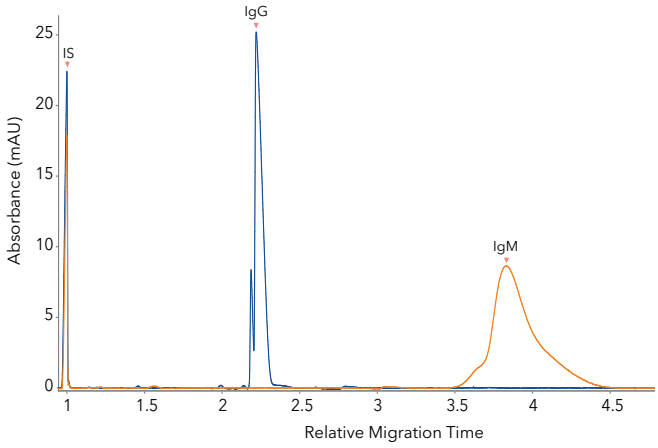

FIGURE 1. Turbo CE-SDS baseline separates IgG and IgM molecules. An overlay of IgG and the IgM profiles demonstrates the advantage of Turbo CE-SDS in analyzing large proteins with molecular weight approaching 950 kDa.

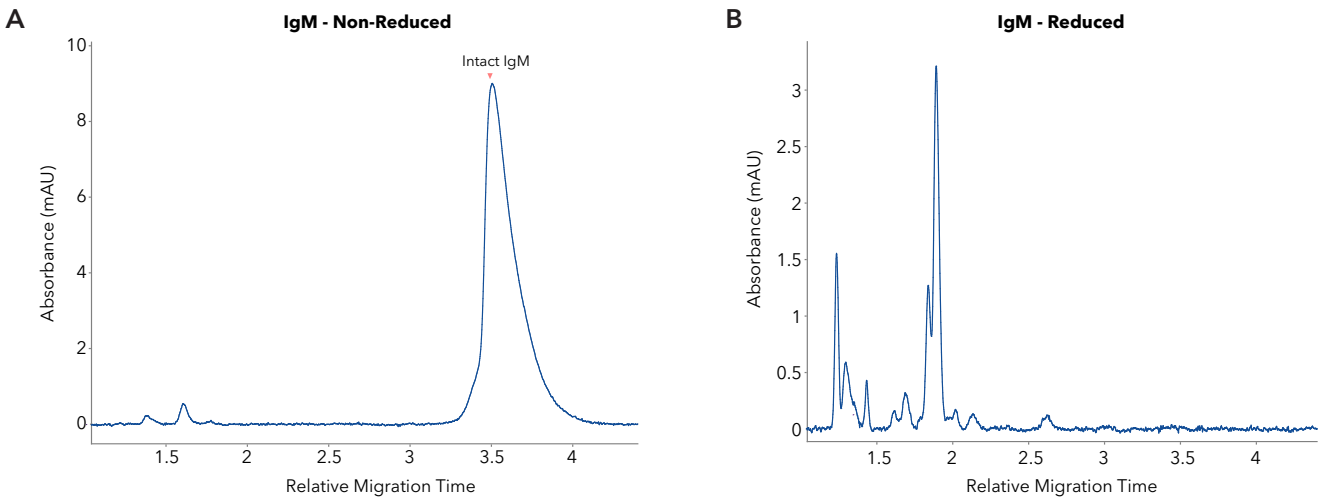

FIGURE 2. Representative electropherograms of IgM samples under A. non-reduced conditions and B. reduced conditions. The IgM subunits are held together by disulfide bonds, resulting in one big peak as shown under non-reduced conditions. Conversely, reduction of the disulfide bonds causes the IgM subunits to separate into their individual heavy and light chains, thus altering their electrophoretic mobility.

Stability Assessment

DTT is a commonly used reducing agent in CE-SDS experiments. It can be used in smaller quantities than β-mercaptoethanol because of its lower oxidation-reduction potential and comparative resistance to oxidation[4].
To effectively determine the stability-indicating property of the Turbo CE-SDS method, IgM samples were treated with varying concentrations of DTT to induce partial and complete reduction and analyzed on the Maurice system. The results are shown in **FIGURE 3**, where denaturation is apparent at a DTT concentration as low as 0.5 mM.
A steady increase in IgM subunits that were held together by disulfide bonds, is seen with an increase in DTT concentration. The marked changes in different IgM sample profiles confirm that the Turbo CE-SDS method is stability-indicating.

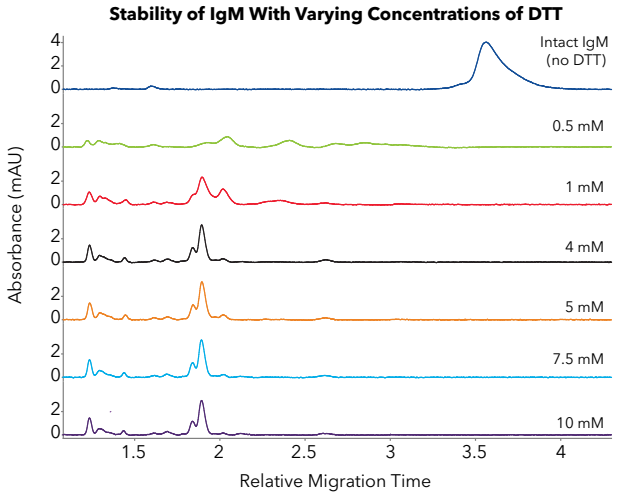

FIGURE 3. Turbo CE-SDS is a stability-indicating method for IgM analysis. Six different concentrations of DTT were added to IgM samples and their profiles were compared with a control sample (no DTT, top profile). Complete reduction of the sample was observed for samples with 0.5-4 mM DTT.

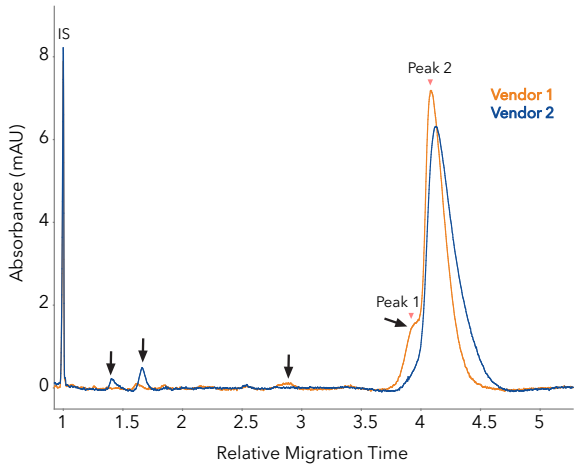

FIGURE 4. Turbo CE-SDS is a suitable identity assay for IgM molecules. IgM samples from two different vendors were analyzed, and the Turbo CE-SDS method was sensitive enough to detect minor differences between the two samples, as indicated by the arrows.

Sample Identification

The Turbo CE-SDS method was also used to distinguish between IgM samples from two different vendors. **FIGURE 4** shows the representative electropherograms of both samples, where key differences between the two are easily detected by this method, making it a suitable identity assay.

Reproducibility

Finally, the reproducibility of the Turbo CE-SDS method was evaluated, as shown in **FIGURE 5A**. Representative electropherograms from a 96-injection batch are shown. Compass for iCE software has a "Lane View" feature that shows a gel-like representation of the results (**FIGURE 5B**).

The method demonstrated excellent reproducibility; relative standard deviation (%RSD) values for the percent peak area of Peak 1 and Peak 2 were **1.74** and **0.11**, respectively for 96 injections.

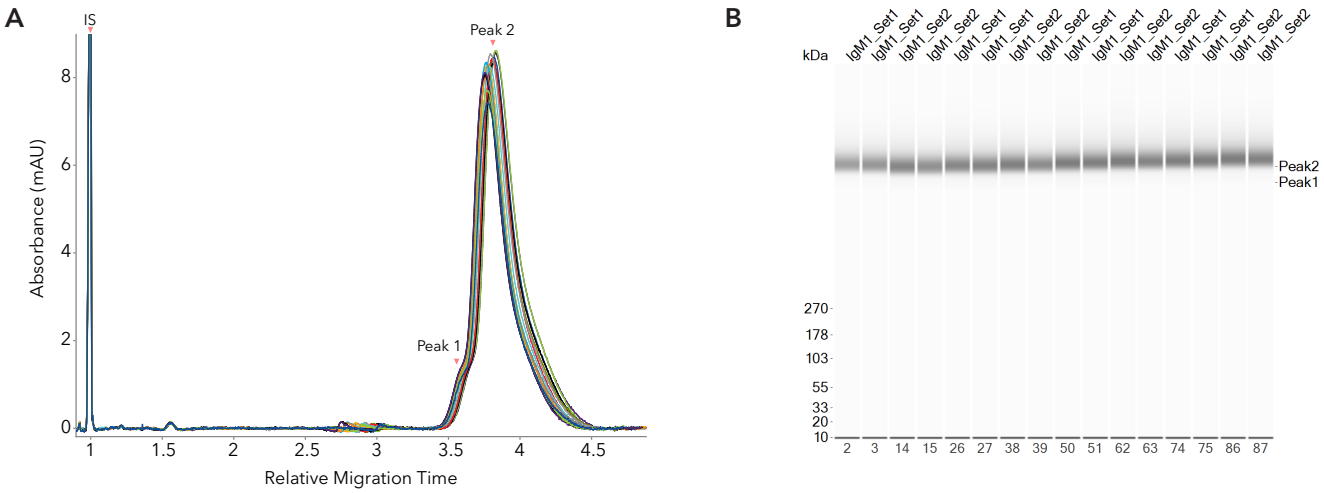

FIGURE 5. The Turbo CE-SDS method is highly reproducible. **A.** 16 representative electropherograms from a batch of 96 IgM sample injections, with %RSD values of 1.74 and 0.11 for Peak 1 and Peak 2 respectively, showing outstanding reproducibility. **B.** Gel-like view of the same results, obtained from the "Lane View" feature in Compass for iCE software.

Conclusion

Large molecules like IgM proteins are challenging to characterize using traditional CE & HPLC methods, yet it is critical to have the right analytical tools to do so, especially with a rising interest in IgM-based therapeutics. This application note demonstrated the suitability of the Maurice Turbo CE-SDS method for fast and high-throughput analysis of IgM molecules. The results illustrate that the method is stability-indicating, is an identity assay, and is highly reproducible, rendering it a critical tool in the development and manufacturing of IgM-based therapeutics. The Maurice system is already widely used in the characterization of a host of biotherapeutics including monoclonal antibodies, viral vectors, fusion proteins, ADCs and more. With IgM molecules added to its analytical portfolio, the Maurice system truly serves as a one-stop solution for the characterization of protein purity, charge, and identity, enabling you to make better analytical decisions in developing lifesaving biotherapeutics for your patients faster. Visit our website to learn more.

References

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3. Ku, Z., Xie, X., Hinton, P. R., Liu, X., Ye, X., Muruato, A. E., Ng, D. C., Biswas, S., Zou, J., Liu, Y., Pandya, D., Menachery, V. D., Rahman, S., Cao, Y. A., Deng, H., Xiong, W., Carlin, K. B., Liu, J., Su, H., Haanes, E. J., … An, Z. (2021). Nasal delivery of an IgM offers broad protection from SARS-CoV-2 variants. Nature, 595(7869), 718–723. https://doi.org/10.1038/s41586-021-03673-2

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