**DIFFERENTIAL MOBILITY SPECTROMETRY ANALYSIS OF GLYCANS AND GLYCOPEPTIDES**

**INTRODUCTION**

Glycans and glycoprotein biomolecules perform numerous roles through their interactions in cellular environments, with roles ranging from immune recognition to cell signaling. For this reason, gaining greater insight into natural carbohydrates is critical in understanding the overall behavior of these biological systems. Over the past two decades, numerous methods have been developed to study these molecules. One such approach is differential mobility spectrometry (DMS), a powerful technique for the analysis of charged molecules. The technique relies on the use of two orthogonal electric and magnetic forces to sort charged molecules based on their size and charge. The fundamentals of the DMS device have been described elsewhere. In this study, we employed DMS-MS for the analysis of glycosylated species.

The separation of theionic and glycoprotein species studied here was accomplished because of differences in the DMS behavior between such ion species. In our previous studies, a clear size-dependent difference that allows the DMS to separate them based on their differing mobilities during the high- and low-field portions of the electric modulation applied across the DMS cell. In addition, our experiments allowed us to test the separation of glycosylated species. We showed that with the addition of water vapor in the DMS cell, this size-dependent difference was amplified, leading to the separation of isomeric carbohydrates. (Figure 1 and Figure 2). In addition, DMS can use a variety of chemical modifiers to achieve effective separation.

**MATERIALS AND METHODS**

**Sample Preparation**

Glycans were purchased from Sigma-Aldrich (Oakville ON, Canada), and diluted to 1 mg/mL with the ESI solvent containing methanol and water (50/50, v/v) with 0.1% formic acid. Dextran standards were purchased from Dextra Laboratories (Reading, UK), and diluted with acetonitrile and water (50/50, v/v) and purified through a C18 semi-preparative HPLC column. The DMS analysis was carried out in the Denver laboratory of Schlumberger (Denver, CO) and diluted to 1 mg/mL with the ESI solvent containing methanol and water (50/50, v/v) containing 0.1% formic acid.

**DMS-MS Conditions**

A commercially available (SelexION™ device, SCIEX) system was mounted in the atmospheric region between the SCIEX QTRAP® 5500 system's sampling orifice and a Turbo V™ ion trap mass analyzer. The instrument setup of the SelexION® system's three orthogonal electric fields were applied in the DMS cell. As such, each isomer required different DC compensation voltage (CV) and separation voltage (SV) to be transmitted through the DMS cell. As such, each isomer required different DC compensation voltage (CV) and separation voltage (SV) to be transmitted through the DMS cell.

**RESULTS**

**Glycans**

In this study, both separation voltage and compensation voltage were scanned synchronously. As the SV was stepped from 0 to 4000 V, the CV was controlled in 0.5 V steps, and compensation voltage (CV) were scanned synchronously. As the SV was stepped from 0 to 4000 V, the CV was controlled in 0.5 V steps. As shown in Figure 1, a more negative CV is necessary to transmit a smaller glycan through the DMS cell. The separation of carbohydrates based on size and/or charge was achieved using DMS. The separation of isomeric glycan and glycopeptide species studied here was accomplished because of differences in the DMS behavior between such ion species. In our previous studies, a clear size-dependent difference that allows the DMS to separate them based on their differing mobilities during the high- and low-field portions of the electric modulation applied across the DMS cell. In addition, our experiments allowed us to test the separation of glycosylated species. We showed that with the addition of water vapor in the DMS cell, this size-dependent difference was amplified, leading to the separation of isomeric carbohydrates. (Figure 2 and Figure 3). In addition, DMS can use a variety of chemical modifiers to achieve effective separation.

**Isomeric separation of sodiated glycans with DMS**

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**Glycopeptides**

**Sensory and Chemical Analysis of Glycopeptides**

In this study, the differential mobility spectrometry was used to analyze glycopeptide and glycoprotein isomer. With the addition of gas phase chemical modifier to the DMS, various isomeric species were successfully separated leading to isolation of isomeric species that could be used for the discrimination of isomeric carbohydrates. In addition, the separation of these isomers using DMS was successfully achieved. In addition, the separation of these isomers using DMS was successfully achieved. In addition, the separation of these isomers using DMS was successfully achieved. In addition, the separation of these isomers using DMS was successfully achieved. In addition, the separation of these isomers using DMS was successfully achieved.

**REFERENCES**


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