Enhancing Characterization of Antibody-based Biologics using Differential Mobility and Mass Spectrometry

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Introduction

An important class of emerging biologics currently being developed are antibody drug conjugates (ADC). Due the large size of these molecules, the process used to manufacture them and the associated heterogeneity, detailed characterization of the material is required. During this characterization, multiple analytical approaches will be used; intact MW determination, heavy- and light-chain MW, amino acid sequencing, etc. For the analysis of the some of the high molecular weight species (intact or reduced), LC frequently leads co-elution of the various forms, which can lead to overlapping deconvolution. Differential mobility separation (DMS) is a technique that can separate ions in the gas phase, prior to MS analysis and can complement the LC separation. Since parameters such as size, conformation and dipole moment have been show to play a role in the separation of species in a DMS cell, this technique could be used to provide additional selectivity during the characterization of ADC and simplify the data processing.

Experimental

- **Sample Preparation:** IgG1was cleaved at the hinge region to generate F(ab') and Fc fragments enzymatically.

  DTT reduction is achieved by incubation at 37ºC for 30min with 10mM DTT at pH 7.5 with Ammonium Bicarbonate.

  IgG1 was also enzymatically digested with trypsin to provide signature peptides based on standard protocols.

- **HPLC Conditions:** A Shimadzu Prominence-XR LC system with Agilent Poroshell SB 300 C8 (1x75mm) at 70°C with a gradient of eluent A water/acetonitrile (98/2) + 0.1% formic acid and eluent B water/acetonitrile (5/95) + 0.1% formic acid was used at a flow rate of 400µL/min. For peptide analysis, a Phenomenex Luna C18 (2x50mm) was used at 45°C with a 5 min gradient (0-45% organic) at 450µL/min

- **MS/MS Conditions:** A SCIEX Turbo V™ source and Electrospray Ionization (ESI) probe was used. A TripleTOF® 5600 LC-MS system and a QTRAP 5500 LC-MS System, both equipped with a SelexION® DMS Technology Device.

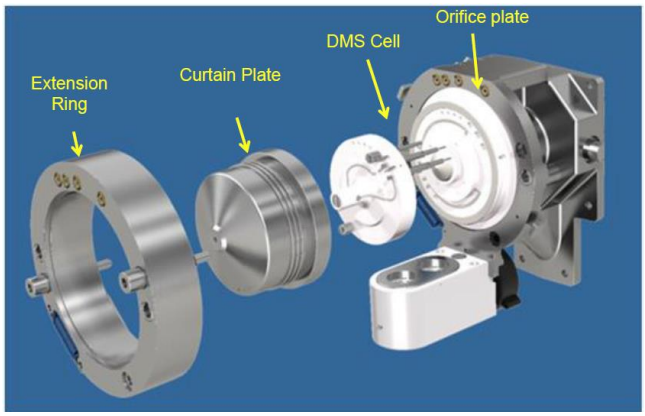

Figure 1 Components associated with SelexION® Technology, Cell dimension are 1 x 10 x 30 mm (gap height x width x length)

Results

The DMS cell provides a means of separating ions at atmospheric pressure, ahead of any mass analysis step, thus in many cases, improving ion selection prior to MS/MS analysis. The separation capability offered by differential mobility are affected by several parameters associated with the ions such as size, conformation, dipole moment and interaction with chemical modifier (gas vapor of organic solvent) added to the transport region. For any ion, as the separation voltage (SV) is increased it is possible to adjust the compensation voltage (CoV) to ensure proper transmission of the analyte for mass detection. Figure 2 shows the typical behavior observed for various analyte type under different conditions. Of particular interest in the analysis of ADC, it is possible to ensure discrimination of ions that could cause interference in the detection of analyte and lead to more complex data deconvolution scheme required. When performing analysis on a DMS enabled TripleTOF® 5600 LC-MS System, one could acquire data in one of 2 modes; 1) CoV mapping and 2) target CoV values. In CoV mapping mode, the instrument is set to acquire data over a wide range of CoV values, all at the same SV value. This can be performed on LC time scale, thus providing a complete view of all species and their behavior at the selected SV value. Figure 3 shows a CoV map for an intact mAb sample (A) and that for a mAb sample that was enzymatically cleaved at the hinge region to generate the Fc and Fab chain (B). As can be seen, the DMS can easily separate each of these species, which leads to simplified mass spectral data.

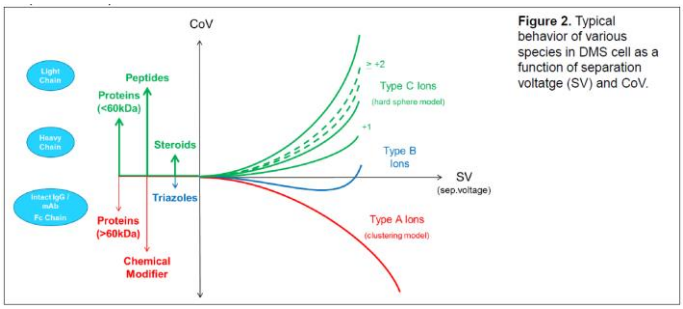

Figure 2. Typical behavior of various species in DMS cell as a function of separation voltatge (SV) and CoV.

Figure 2 Typical behavior of various species in DMS cell as a function of separation voltage (SV) and CoV

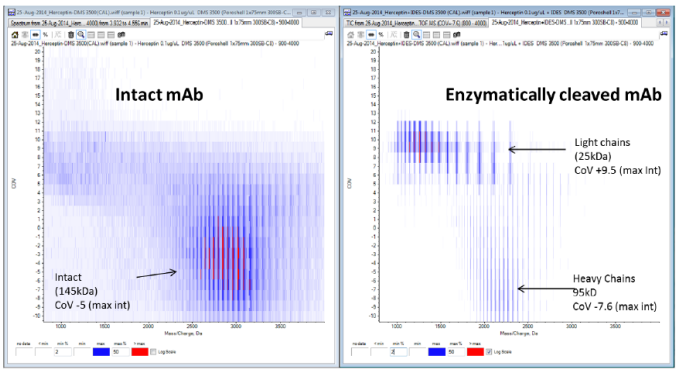

Figure 3 Separation of mAb and enzymatically cleaved mAb. SV was set to 3500V and all data is average over the elution profile of the analyte (LC gradient 3 min). As can be seen, the light chain can easily separated from the heavy chain.

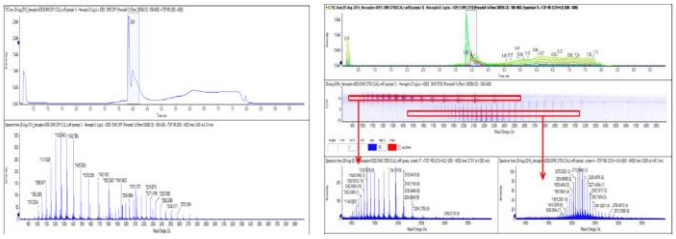

Figure 4 Analysis of enzymatically cleaved mAb with (A) all DMS cell parameter turned off (i.e. SV=CoV=0) and (B) in CoV mapping mode with SV set to 3500V. When DMS cell is activated, it is possible to get simplified mass spectrum for both the Fc as well as the Fab region even under co-eluting conditions (bottom).

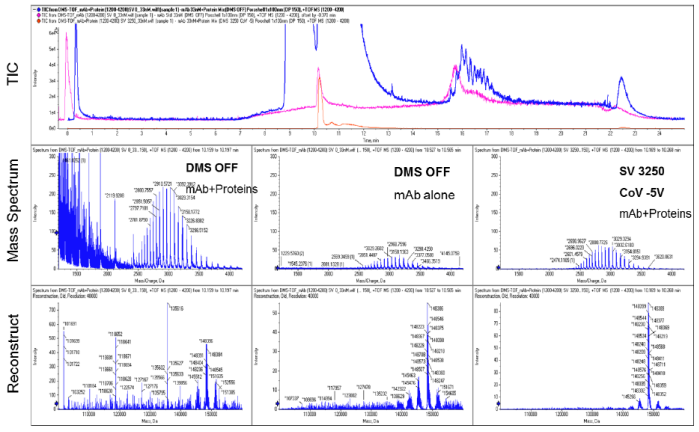

Figure 5 Effect of DMS on mass spectra deconvolution of mAb data in the presence of other protein species in solution. When the DMS is OFF, lower m/z ions contribute to additional chemical noise. When the DMS is activated, only mAb signal is detected, thus reducing nois contribution in the deconvoluton and simplifying data processing.

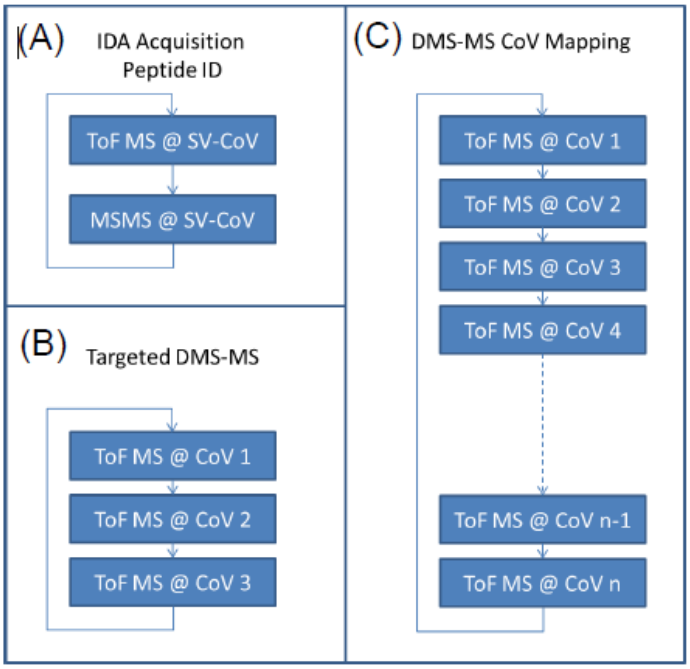

Figure 6 LC Acquisition workflow typically used with DMS to support characterization of ADC. (A) The IDA acquisition workflow is used to improve peptide analysis by reducing chemical noise associated with peptide detection while automatically selecting precursor ions for MSMS analysis. Both the MS and MSMS data are collected at same SV/CoV values. (B) Targeted DMS-MS analysis to improve spectral quality of associated with subunit of mAB (heavy and light) and intact mAb. Typical target CoV value would be CoV of +10, +5 and -5 all at the same SV value. (C) DMS-MS CoV Mapping on LC time scale. This provides a wide range of CoV values to generate 3D map of the ions behaviors at a specific SV value. The data displayed in Figure 3 and 4 were generated using this acquisition mode.

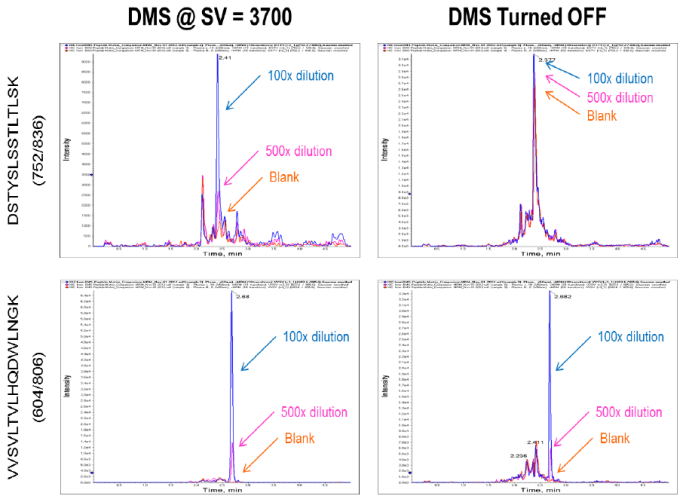

Figure 7 Improving detection and quantitation of digested mAb signature peptide with DMS under rapid LC analysis (5 min gradient). When DMS is active, the matrix blank and low level sample are readily distinguished. When the DMS is OFF (or removed) the signature peptide exhibit significant interferences at the same R.T. or near the elution time of the peptide. Using the DMS ensures more selective detection and simplifies reliable peak integration for quantitation.

Conclusions

- The implementation of differential mobility (DMS) as a 'front end' separation prior to mass spectrometric detection provides a generally deployable tool for pharmaceutical organizations that need to be conscious of method deployment through all departments or with external collaborators.
- Implementation of DMS in the source region also reduces the dependence on complex software for data processing because data can be processed with existing software packages. This facet allows novice users to access the technology immediately.
- The general utility of Differential Ion Mobility Separation is evident from the wide variety of biomolecules studied and separated.
- The ability to interface all types of mass spectrometry platforms makes this tool applicable to all stages of production, development, and clinical stages of biotherapeutic development from characterization to bioanalysis.

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