Rapid Analysis of Protein and Antibody Aggregation and Fragmentation using Nanoelectrospray Ion Mobility Spectrometry

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Summary
The analysis of protein aggregates and fragments is an important step in ensuring stable production of protein products. Size exclusion chromatography (SEC) is a widely accepted method for measuring the size and quantity of soluble aggregates. While SEC offers good sensitivity and precision with high throughput, at a relatively low cost, non-specific stationary phase adsorption may lead to erroneous estimates of aggregate and fragment levels, and larger aggregates may co-elute in the void volume. As such, a column-free technique with broader dynamic size separation range can bring added value to the analysis of protein aggregates and fragments. Here we describe the use of nanoelectrospray ion mobility spectrometry (ES-IMS) for the automated analysis of complex protein mixtures. The MacroIMS™ system relies on first-principle size analysis to determine the electrical mobility diameter and molecular weight of macromolecules and complexes between 8 kDa and > 100 MDa in a single run. A method has been developed to apply the ES-IMS to measure antibody aggregation levels. The data shows resolution of small Immunoglobulin (ca. 25 kDa) and heavy chain (ca. 50 kDa) fragments from the monomer, and dimer and higher order aggregates. Reference protein ladders were used to validate the method and the technique’s ability to accurately determine molecular masses of the native proteins. The described approach validates the use of ion mobility spectrometry of intact proteins. The described approach validates the use of ion mobility spectrometry of intact proteins.

Introduction
Protein-based therapeutics have been approved by Food and Drug Administration in clinical uses. Among these drugs, approximately 50% of sales are contributed by therapeutic monoclonal antibodies (Dimirov, 2012). Due to their biochemical properties, monoclonal antibodies have a propensity to aggregate (Lowe et al., 2011). Thus, aggregation becomes an important issue in the pharmaceutical and biotech industry. Aggregation can occur in the process of expression, purification, formulation, fill/finish, and storage (Cromwell et al., 2006). Antibody aggregation results in the loss of drug potency and may cause unnecessary immunogenicity (Rosenberg, 2006). Therefore, it is essential to monitor antibody aggregation in-process and final products. There are several bioanalytical methods to detect protein aggregation (Zhao et al., 2012).

Macron ion mobility spectrometry (MacroIMS) using charge-reduced electrospray ionization and differential mobility analysis is able to detect macromolecules such as protein complexes in the range of 8 kDa and > 100 MDa. Thus, MacroIMS is an excellent tool to address the issue of protein aggregation.

Working Principle of MacroIMS
In MacroIMS, macromolecules (e.g., proteins) are converted to gas-phase macromions using electrospray ionization (ESI). The multiply-charged ions that are generated in the nano ESI process are charge-reduced to a repeatable, known charge distribution using a soft X-ray source. Resulting +1 charge state macromions are transported to the ion mobility drift cell, where they are separated via their electrical mobility, a first principle function of size. The macron detector is used to quantify macromolecules of a given mobility. The detector works by condensing a vapor onto the macromolecules to form liquid droplets. These droplets are large enough to be counted individually when they pass through a laser and photodetector assembly. High performance liquid chromatography (HPLC) was applied to generate steady solvent flow and to deliver analytes to the Macro IMS System. Advanced chromatography-based system software is used for automated data acquisition and processing, as well as instrumental control. Audit trail and electronic signatures are also enabled by this 21 CFR Part 11-certified software package.

Separation of a Mixture of Multiple Proteins
Figure 1 shows a typical MacroIMS spectrum for a high molecular weight standard (GE Healthcare, HMW Electrophoresis Kit), prepared in 20 mM ammonium acetate. The upper left graph shows a contour plot of analyte size and molecular mass over run time, with concentration differences in colors. Integration of the 3-minute run time period leads to the upper right graph, displaying the size-specific molecular mass distribution of the protein. The lower left graph identifies the molecular masses present in the protein mixture.

Antibody Analysis
MacroIMS was used to analyze human IgG (Sigma, IgG from human serum) in 20 mM ammonium acetate pH 8.9. Figure 2 shows a size-specific molecular mass distribution of IgG. The light chain (25 kDa), heavy chain (50 kDa), monomer, dimer, trimer and tetramer were well identified.

Conclusion
Differences in the linearity of antibody concentrations between 2.5 - 200 μg/mL (17 - 1300 nM) as shown in Figure 3, linear antibody was found to be within the range tested here. We tested the linearity of antibody concentrations between 2.5 - 200 μg/mL (17 - 1300 nM) as shown in Figure 3, linear antibody was found to be within the range tested here.

KDa

Figure 2: The distribution of molecular mass of IgG. 200 μg/mL of IgG in 20 mM ammonium acetate pH 8.9 was analyzed using MacroIMS. (Inset) The distribution of molecular mass of IgG between 10 and 70 KDa.

References

Figure 3: Linearity of total antibody analyzed with MacroIMS using two droplet sizes. Figure 4 shows the proportion of dimer, trimer and tetramer in the total protein mixture as a function of concentration. Trimer and tetramer were observed at a concentration of 10 and 50 μg/mL, respectively. The results indicate that more aggregates are formed at higher concentrations. Our results corroborate the findings that proteins tend to aggregate at higher concentrations (Pease et al., 2008).

In addition, it is also possible that at higher concentrations two or more macromolecules may reside in a single drop, leading to multimer artifacts (i.e., coincidence). As shown in Figure 4, the results when using two different droplet sizes (98 nm and 135 nm) agree with each other, showing that this is not the case in this study.

Figure 4: The proportion of dimer, trimer and tetramer in the total IgG in two different drop sizes. Different concentrations of IgG were prepared in 20 mM ammonium acetate pH 8.9.

Linearity of Antibody and Antibody Aggregation
Different flow rates delivered by HPLC yield different droplet sizes. Sucrose was used to determine average droplet size. The values of droplet size were used to determine the cutoff values of concentration; that is, the concentration below which artificial aggregation (i.e., coincidence) will not occur (Pease, 2008). Two droplet sizes, 98 nm and 135 nm, were applied to the analysis of antibody samples. The cutoff concentrations are 505 μg/mL (3.37 mM) and 196 μg/mL (1.32 mM) to 98 nm and 135 nm, respectively.

Figure 5: The T.D. (a) and T.D. (b) have the same antibody concentrations and different droplet sizes (98 nm and 135 nm) as a function of run time. Two droplet sizes, 98 nm and 135 nm, were applied to the analysis of antibody samples. The cutoff concentrations are 505 μg/mL (3.37 mM) and 196 μg/mL (1.32 mM) to 98 nm and 135 nm, respectively.