

macroIMS™ Macroion Mobility Spectrometer Analysis of Antibody Aggregates and Fragments

Application Note CHEMC-008

Background

Antibodies have traditionally been thought of as molecules which aid in the immune system. Today they are being applied for even more diverse uses, including as novel vaccine carriers for drug therapeutic delivery to target and find specific proteins. To ensure the quality of antibody products and refine the production process for antibodies, a highly sensitive assay for high mass aggregates and low abundance fragments is required.

The *macroIMS* Macroion Mobility Spectrometry method allows rapid, highly sensitive analysis of antibody aggregates and antibody fragments. To demonstrate the capabilities of *macroIMS* for antibody analysis applications, an experimental protocol to generate antibody fragments is detailed below. Immunoglobulin aggregates analysis is also demonstrated on a stored human IgG sample, which naturally forms multimers.

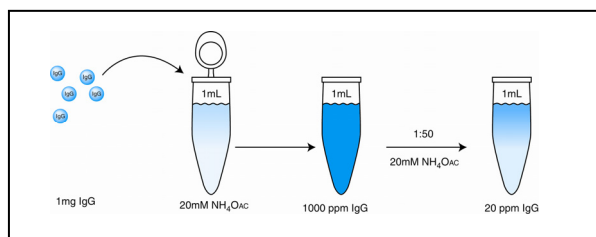
Objective

To demonstrate the ability of the *macroIMS* system to measure the quality of antibody solutions, by detecting antibody aggregates and fragments.

Methods

Antibody Preparation Procedure

1. Prepare 1000 ppm IgG solution by dissolving 1 mg salt free lyophilized IgG powder in 1 ml 20 mM ammonium acetate buffer.
2. Dilute sample of 1000 ppm stock IgG with 20 mM ammonium acetate buffer to obtain ~20 ppm solution to run in *macroIMS*.



Antibody samples are prepared in electroprayable buffer, in this case 20 mM NH₄OAc

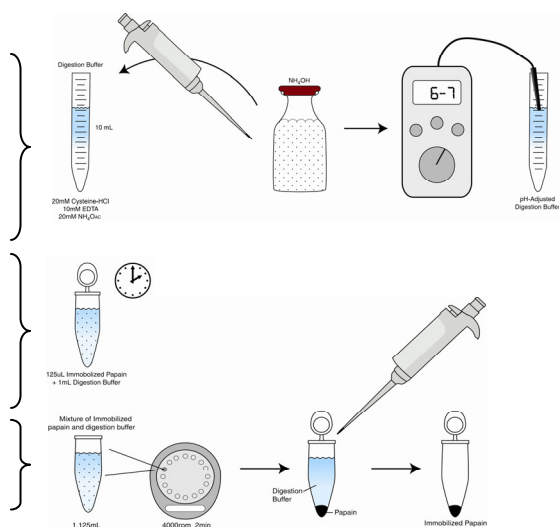
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Antibody Fragmentation Procedure

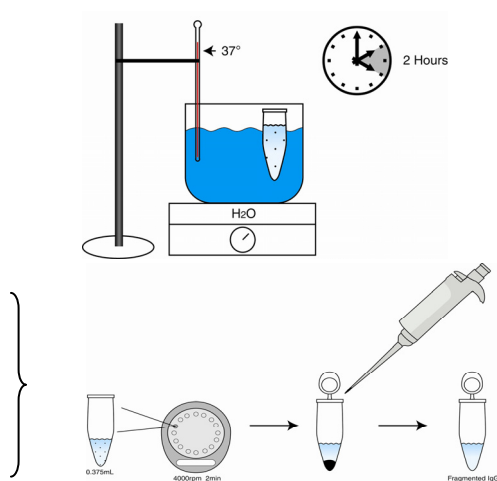
Immobilized Papain*/Digestion Buffer Preparation

1. Just before use, prepare 10mL digestion buffer to a final concentration of 20 mM Cysteine-HCl, 10 mM EDTA, and 20 mM ammonium acetate.
2. Adjust digestion buffer to a pH of 6–7 by addition of ammonium hydroxide.
3. Place 125 μ L of 50% Immobilized papain slurry in a 1.5ml vial.
4. Add 1 ml of digestion buffer to the slurry and allow to equilibrate (~15 min).
5. Remove digestion buffer by centrifuging at 4000 rpm for 2 min and pipette off the supernatant.
6. Repeat steps 4 and 5 for an additional washing of the papain slurry.
7. Resuspend the gel in 125 μ L of digestion buffer.



Digestion Procedure

1. Combine 125 μ L digestion buffer with 125 μ L 20ppm IgG solution and add to the suspension from step-7. Heat in a water bath at 37 degrees Celsius for 2 hours. Remove sample vial and briefly vortex approximately every 10 minutes to re-suspend the papain protease for increased antibody digestion. (Only necessary for full digestion, not necessary for fragmentation demonstration where partial digestion is acceptable)
2. After 2 hour incubation of sample, centrifuge the sample vial at 4000 rpm for 2 minutes to separate the immobilized papain from the fragmentation product.
3. Pipette off the supernatant and place in a new 1.5 ml vial.
4. Desalt 50 μ L of the supernatant by nanosep centrifugation.



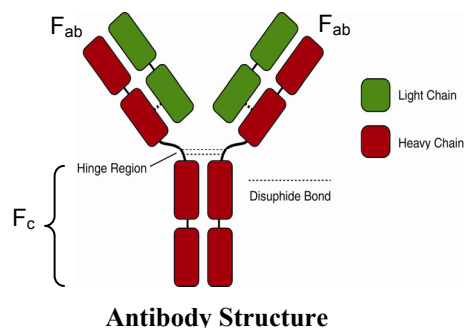
macroIMS Protocol

1. Run 20 mM ammonium acetate buffer in *macroIMS* system to ensure clean, low background.
2. Prepare and run stock 1000 ppm IgG solution for 5 min to coat capillary surface.
3. Prepare and run 20 ppm IgG sample in *macroIMS* and obtain spectra at 60 second scan times until the spectra appear stable.
4. Once stable narrow the scan range to 10–300 kDa and increase scan time to 180 seconds.
5. Repeat steps 1–4 for the fragmented IgG sample.

* Papain is an enzyme that breaks down antibodies, and has an optimal pH range of 6-7 and requires the presence of cysteine residues for activation

Expected Results

Papain is a cysteine-endopeptidase that causes cleavage of the IgG antibody, typically at the hinge region. Papain cleavage produces F_{ab} and F_c fragments of relatively similar masses around 50 kDa. Upon comparison of the native sample to the degraded sample, the antibody peak around 150 kDa should be diminished upon degradation along with a corresponding peak formation around the fragmentation location of 50 kDa.



Results

Figure 1 displays the *macroIMS* spectrum for the IgG antibody in solution with 20 mM ammonium acetate. Antibody monomer is detected at 160 kDa while lower quantities of IgG dimer and trimer are found at 305 and 457 kDa, respectively. Antibody aggregates can result from extended storage of sample at room or elevated temperatures, and are thought to be precursors to particulates.

Figure 2 displays the *macroIMS* spectrum for the partially-digested IgG antibody sample. The papain that was used to digest the IgG is a biological protein that cleaves peptide bonds and tends to cleave the antibody within its hinge region to yield the F_{ab} and F_c fragments, all of ~50 kDa. The Figure 2 *macroIMS* spectrum shows that some antibody remains at 160 kDa (incomplete digestion), with the peak at 51 kDa representing the F_{ab} and F_c fragments. There was also incomplete fragmentation due to the partial digestion conditions applied (no mixing over 2 hour incubation), which resulted in the $F(ab')_2$ peak at 107 kDa. Since the intent of the digestion procedure described was to demonstrate *macroIMS* detection of antibody fragmentation, not necessarily complete digestion, the 107 kDa peak was expected. Light Chain at 25 kDa was also evident from the spectrum, detectable in the presence of the dominant F_{ab}/F_c peak. The sensitive detection of antibody fragments, concurrently with the intact antibody and its multimers, is useful to determine antibody sample stability and purity.

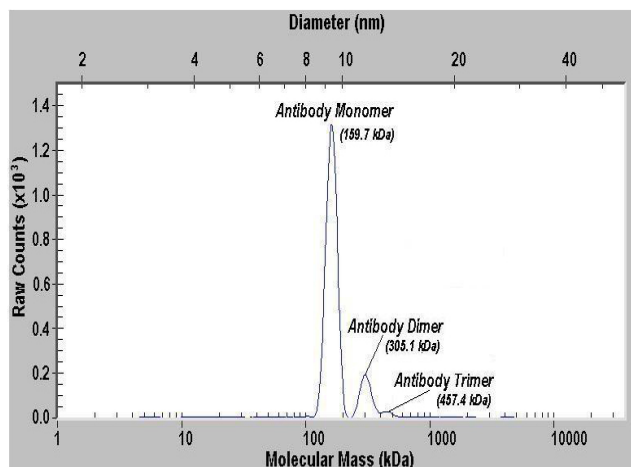


Figure 1: *MacroIMS* spectrum of Stored IgG Antibody at 20 ppm in 20 mM Ammonium Acetate Buffer

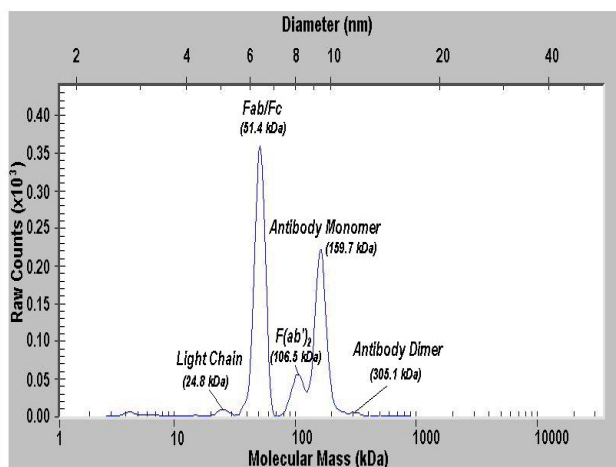


Figure 2: *MacroIMS* spectrum of IgG sample at 22 ppm in 20 mM Ammonium Acetate buffer after 2 hrs immobilized papain digestion

Conclusions

The *macroIMS* system clearly displays the ability to differentiate between IgG, monomer, dimer and trimer. It also shows the ability to display the fragmentation components of IgG; F_{ab} , F_c , $F(ab')_2$, as well as light chain fragments. *MacroIMS* provides a highly sensitive assay for high mass aggregates and low abundance fragments of human IgG, and is envisioned as an important tool in biotechnology for the analysis of antibody purity, stability and quality.

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