SEC-CDMS ENABLES ONLINE BUFFER EXCHANGE AND CHARACTERIZATION OF PROTEIN-BASED THERAPEUTICS

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INTRODUCTION

Size exclusion chromatography (SEC) provides a gentle, size-based separation of large biomolecules and their complexes under native conditions. When coupled with mass spectrometry (MS), SEC can also function as an online buffer exchange step, enabling rapid transfer into MS-compatible solutions while minimizing variability associated with off-line sample preparation. Charge Detection MS (CDMS) is uniquely suited for the analysis of megadalton-sized species; however, integration with SEC has been limited by the low throughput of traditional single-ion CDMS approaches.

Here, we present preliminary results demonstrating a simple and practical coupling of SEC with high-throughput CDMS that is compatible with chromatographic timescales and requires sample volumes comparable to standard manual injections. The acquisition were supported by multiple ion charge extraction algorithm (MICE) to enable recovery of overlapping ion signals. This early work highlights the feasibility of extending SEC-CDMS into the megadalton regime and is illustrated using protein-based therapeutic samples.

METHODS

Samples: Standard empty and full CMV-GFP AAV8 capsids were sourced from Virovek Inc. at 2 × 10^{13} viral particles (vp)/mL. Volumetric mixtures of empty and full capsids ranging from 0% to 100% empty capsid in 25% increments were prepared. Dengue virus serotype 1 VLP at a concentration of 0.32 mg/mL was purchased from The Native Antigen Company. Keyhole limpet (Megathura crenulata) hemocyanin (KLH) in PBS solution at a concentration of 5 mg/ml was purchased from Sigma-Aldrich. Adalimumab at a concentration of 1 mg/ml was sourced from Humira.

SEC-CDMS: Low-flow, ultra-wide-pore (1000 Å) size exclusion chromatography (SEC) was performed on a Waters ACQUITY™ UPLC Premier System using narrow-bore columns to improve coupling with CDMS and minimize sample consumption. The column was maintained at 30 °C and samples were held at 5 °C prior to injection. Injection volumes were 10 µL for AAV and Dengue VLP samples and 1 µL for the Adalimumab and KLH samples. Separations were carried out at a flow rate of 50 µL/min using an isocratic mobile phase of 50 mM ammonium acetate solution. The SEC effluent was split post-column, delivering approximately 5 µL/min to a silica-fused tip for continuous electrospray ionization. Electrospray conditions included a nebulizer gas flow of 1.8 L/min (nitrogen), a source temperature of 150–250 °C, and a spray voltage of 3.65–3.9 kV to promote efficient desolvation. Reduced temperature and spray voltage settings were applied for KLH and adalimumab to minimize ion activation. Measurements were acquired on a Xevo™ CDMS System operated in continuous trapping mode with a trapping time of 100 ms. High-throughput data acquisition was enabled using the multiple ion charge extraction (MICE) algorithm. Data processing and visualization were performed using waters_connect™ Software.

SEC-CDMS EXPERIMENTAL SETUP

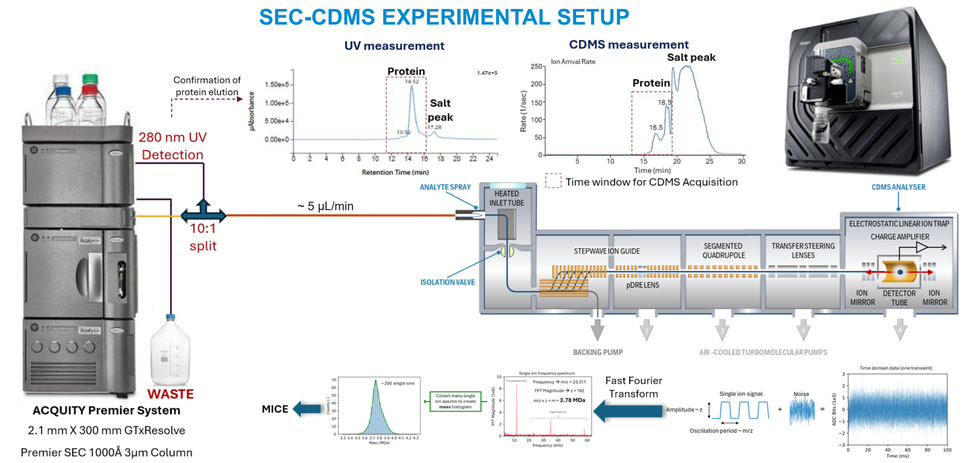

Figure 1: A schematic representation of the SEC-CDMS setup. The Waters Xevo CDMS Instrument is coupled to a Waters ACQUITY UPLC Premier System. Using a 10:1 flowrate split, ~ 5 µL/min of the eluate is introduced to the nano-ESI source which was adapted for continuous flow.

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RESULTS

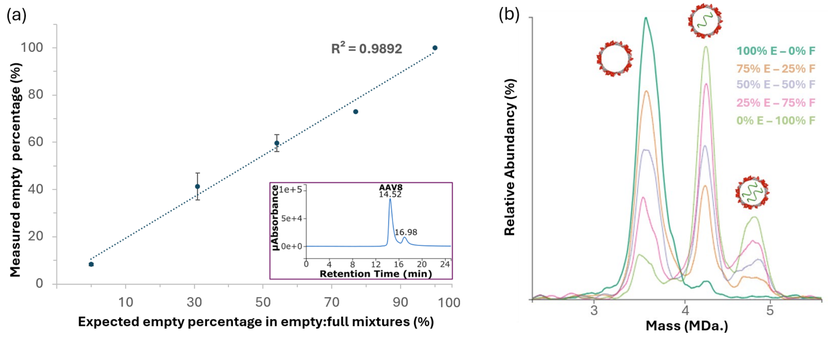

*Figure 2: **(a)** Relative quantification of empty capsid content in AAV8 empty and full capsid mixtures of varying ratios, eluting at approximately 15 mins. The measured percentage of empty capsids, is plotted against the expected percentage in defined empty:full volumetric mixtures. Linear regression demonstrates strong agreement (R^2 = 0.9892). Error bars represent experimental variability from duplicate measurements. **(b)** Representative CDMS mass spectra of AAV8 empty and full capsid mixtures. Overlaid spectra illustrate the relative abundance distributions corresponding to increasing empty capsid content, with distinct mass populations for empty and full capsids.*

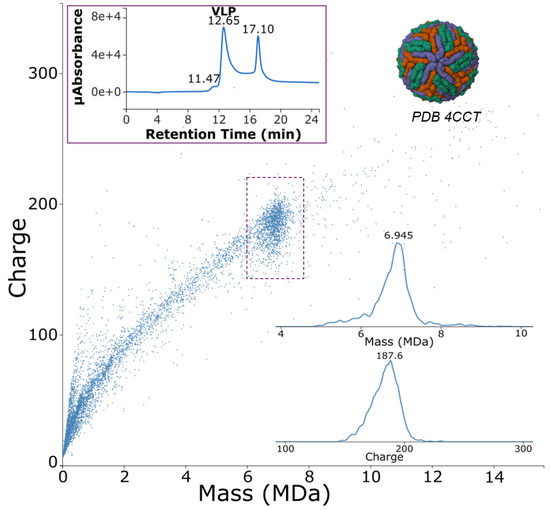

Figure 4: Charge versus mass scatter plot of dengue virus-like particles (VLPs), eluting from the SEC column around 13 minutes into the gradient (inset). Histogram insets show the extracted mass (~6.95 MDa) and charge (~188 e) distributions, consistent with intact VLP assembly.

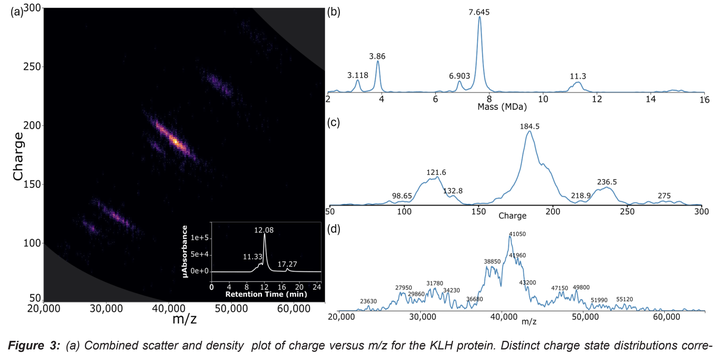

Figure 3: (a) Combined scatter and density plot of charge versus m/z for the KLH protein. Distinct charge state distributions corresponding to multiple oligomeric species are observed. (b) Mass distribution revealing several species at approximately 3.13, 3.88, 6.93, 7.67, and 11.24 MDa, eluting from the column between 10 –13 minutes into the gradient (inset). (c) Charge distribution extracted from the dataset, showing a dominant population at ~185 charges. (d) Representative m/z spectrum corresponding to the measured ions.

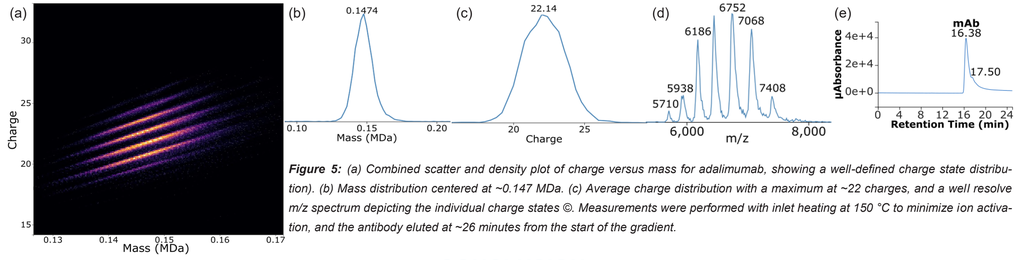

Figure 5: (a) Combined scatter and density plot of charge versus mass for adalimumab, showing a well-defined charge state distribution). (b) Mass distribution centered at ~0.147 MDa. (c) Average charge distribution with a maximum at ~22 charges, and a well resolve m/z spectrum depicting the individual charge states ©. Measurements were performed with inlet heating at 150 °C to minimize ion activation, and the antibody eluted at ~26 minutes from the start of the gradient.

CONCLUSION

- SEC-CDMS provides a simple and robust workflow for online buffer exchange and direct analysis of large, heterogeneous biomolecules, eliminating the need for offline preparation.
- The approach is compatible with chromatographic timescales and low sample volumes (1–10 µL), enabling efficient and practical analysis of valuable samples.
- High-throughput acquisition enabled by MICE supports ion fluxes compatible with SEC, addressing traditional limitations in CDMS throughput.
- The platform demonstrates accurate relative quantification (R^2 = 0.989) and clear resolution of complex assemblies, including antibodies, viral capsids, VLPs, and megadalton oligomers.
- These preliminary results highlight the feasibility of SEC-CDMS as a scalable approach and support its potential as a powerful tool for characterization of protein-based therapeutics and viral systems in the megadalton mass range.

REFERENCES

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2. Parikh, R. A.; Draper, B. E.; Jarrold, M. F. Multiple Ion Charge Extraction (MICE) for High-Throughput Charge Detection Mass Spectrometry. Analytical chemistry 2024, 96. https://doi.org/10.1021/acs.analchem.3c05087.

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