WHAT'S INSIDE A VACCINE?

A CLOSER LOOK USING CHARGE DETECTION MASS SPECTROMETRY

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INTRODUCTION

- Vaccines are increasingly targeting complex diseases, driving demand for advanced analytical tools to characterize large, heterogeneous modalities such as virus-like particles (VLPs) and nanoparticle platforms.
- Charge detection mass spectrometry (CDMS) using an electrostatic linear ion trap (ELIT) enables direct mass measurement of single particles by independently determining *m/z* and charge, eliminating the need for charge state deconvolution.
- Building on prior success with viral vectors (AAV, adenovirus)[1,2], this work demonstrates application of the Xevo™ CDMS system for VLP characterization, supporting next-generation vaccine development.

METHODS

Samples: Commercial vaccine preparations including the inactivated poliomyelitis vaccine (IPOL), RotaTeq, and Engerix-B were sourced from various suppliers. Dengue, Norovirus, and Chikungunya VLPs were purchased from The Native Antigen Company. Samples were buffer exchanged into 200 mM ammonium acetate solution using either Micro Bio-Spin® P-6 size-exclusion columns (Bio-Rad Laboratories) or Amicon® 100 kDa molecular weight cut-off filters (Merck Millipore).

CDMS: Ions were generated in positive ion mode via static nanoelectrospray ionization (nanoESI) and analyzed using a Waters Xevo™ CDMS instrument. Signal processing and data visualization were performed using waters_connect™ CDMS Toolkit software. Ions were trapped for 100 ms, with a charge RMSD of ~0.9 *e*. Time-domain signals were Fourier transformed, where the measured frequency and magnitude corresponded to an individual ion's *m/z* and z, respectively, enabling direct mass calculation. Individual ion data were binned into histograms of *m/z*, charge, and mass spectra, along with 2-dimensional heat maps.

EXPERIMENTAL SETUP

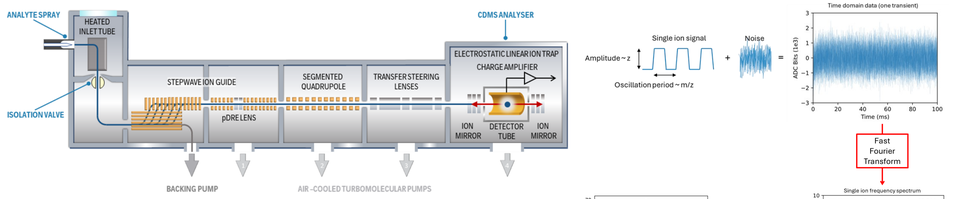

***Figure 1**: A schematic representation of the Waters ELIT-based CDMS instrument with diagrams showing how m/z and z information is obtained, ultimately providing mass information.*

- The CDMS mass analyser houses a conductive cylinder with two ion mirrors, which reflect the ion back and forth.
- When an ion enters and progresses through the detection cylinder, the charge on the ion is induced on to the cylinder.
- The induced charge is then detected by a low-noise charge sensitive amplifier, which results in a periodic signal that can be analysed using fast Fourier transform (FFT).
- The *m/z* of an ion is determined from the oscillation frequency and the charge from the signal amplitude.
- $m/z \times z \rightarrow \mathbf{m}$ for each ion

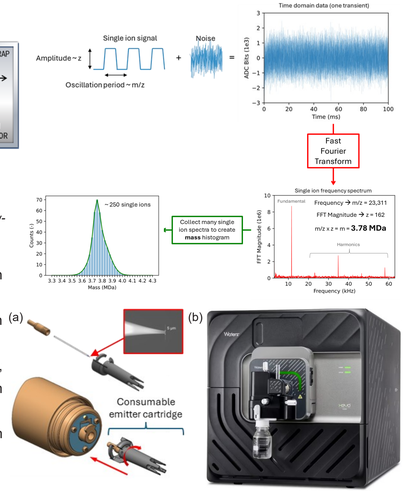

***Figure 2:** (a) emitters for nanoESI source and (b) Xevo CDMS instrument*

RESULTS

Engerix-B Vaccine

Engerix-B, composed of hepatitis B surface antigen (HBsAg) VLPs, is a recombinant vaccine used to prevent hepatitis B virus infection.

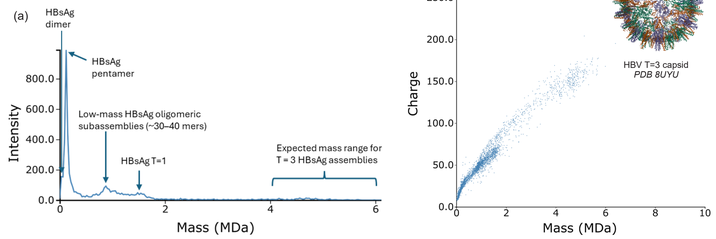

***Figure 3:** (a) CDMS mass spectrum of the Engerix-B vaccine (5100 ions acquired across the displayed mass range in a 12-minute acquisition). Signals consistent with HBsAg oligomeric subassemblies and assembled T=1 particles are observed; however, well-defined mass distributions corresponding to T=3 particles were not detected, although a small population of ions were detected in the expected mass range for these particles, potentially due to formulation effects of the vaccine. (b) Two-dimensional charge versus mass scatter plot of the Engerix-B vaccine, where each point represents an individual ion measurement.*

Rotateq Vaccine

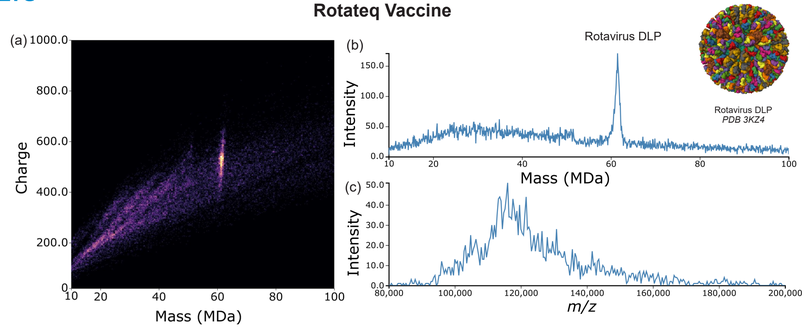

***Figure 5:** (a) 2D charge vs. mass combined scatter and density plot for RotaTeq, a live, oral rotavirus vaccine used to prevent rotavirus gastroenteritis. (b) mass spectrum for the Rotateq vaccine (6700 ions acquired across the displayed mass range within a 30-minute acquisition time) with the rotavirus double layered particle (DLP) detected at 61.07 MDa (-1.75%; FWHM 0.95 MDa). (c) Extracted m/z spectrum of the rotavirus DLP, displaying a broad distribution of ions across the m/z range of 80,000–200,000.*

IPOL Vaccine

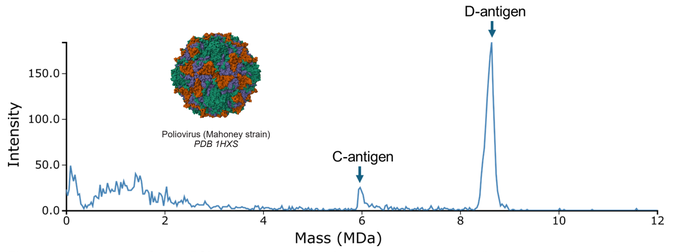

***Figure 4:** Mass spectrum for the IPOL vaccine, which is used to prevent poliomyelitis (polio) caused by poliovirus Types 1 (Mahoney strain), 2 (MEF-1 strain), and 3 (Saukett strain). 3300 ions were acquired across the displayed mass range within a 15-minute acquisition time. CDMS provided mass distributions for both empty (C-antigen) and full (D-antigen) particles present in the vaccine, with measured masses of 5.98 MDa (+1.53%; FWHM 0.12 MDa) and 8.62 MDa (+2.25%; FWHM 0.18 MDa), respectively. The observed mass excess likely arises from contributions such as counterions, residual non-volatile salts, trapped solvent, and formaldehyde-induced crosslinking[3].*

Expanding VLP Vaccine Characterisation

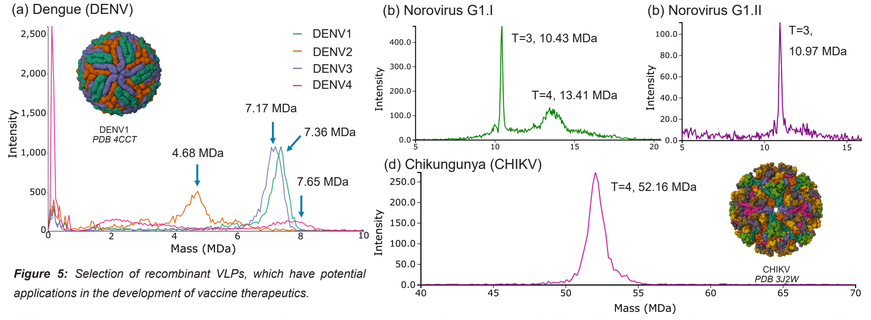

***Figure 5:** Selection of recombinant VLPs, which have potential applications in the development of vaccine therapeutics.*

(a) Dengue VLP serotypes 1-4 overlay mass plot (normalized so total intensity of each plot is equal). (b) and (c) mass spectra of norovirus VLP genotypes G1.I and G1.II, respectively. (d) CHIKV VLP mass spectrum. Ion counts ranged from 1500-15000 with 10-15 minute acquisition times for all VLPs shown. Classical capsid structures were detected for the norovirus and chikungunya VLP, whilst mainly sub viral assemblies were detected for the DENV serotypes.

CONCLUSIONS

Direct measurements of charge and m/z enable accurate, single-particle mass determination of viral antigens across the vaccine formulations investigated in this study. For the vaccine formulations, where the viral antigens were detected, the resulting mass distributions were in good agreement with the expected masses, whilst also accounting for counterions and adducts. Additionally, impurities, including defective particles (e.g., C-antigen in IPOL vaccine) were detected, along with incomplete particles and sub-assemblies for different VLPs.

REFERENCES

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