Charge Characterization of Viruses and Virus Like Particles (VLPs) Using icIEF with UV Fluorescence Detection

Jiaqi Wu, Will McElroy, and Chris Heger

ProteinSimple, a Bio-Techne Brand, 3001 Orchard Parkway, San Jose, CA 95134, USA

INTRODUCTION

icIEF is well-established as the gold standard tool in the biopharmaceutical industry for protein charge characterization. Newer therapeutic modalities are coming to market, including viruses and virus like particles. A challenge associated with icIEF analysis of virus and virus like particle (VLP) samples is sample aggregation during IEF. For most samples (mAbs, proteins), adding solubilizers into the sample solution, such as urea and non-ionic surfactants, can prevent aggregation. However, intact viruses and VLPs may disassociate when these additives are used. While reducing the final sample concentration can help minimize aggregation, UV light absorbance-based detection may not possess sufficient sensitivity to analyze these samples at lower concentrations. In the presentation, we will illustrate the results of using an icIEF-UV fluorescence instrument for charge characterization of viruses and VLP samples. The UV fluorescence method requires no dye labeling and shows significantly higher sensitivity than UV absorption detection.

MATERIALS & METHODS

Maurice™

icIEF Cartridge

Maurice is a benchtop system that runs both cIEF and CE-SDS methods. In cIEF mode, molecules are detected with both absorbance and Native Fluorescence (NF) modes using whole column detection for each run.

icIEF METHODS

Norovirus

Norvirus GII.4 VLP was obtained Cedarlane (REC31620). The sample was directly diluted into final IEF buffer containing 4% Biolyte 3-10, 0.35% methylcellulose, 25% formamide. Focusing at 1.5 kV for 1 min, then at kV for 7 min. Fluorescence exposure time: 80 seconds

Polio Virus

Inactive poliomyelitis vaccine BRP was obtained from EDQM/DEQM (P2160000). Sample directly diluted into final IEF buffer containing 4% Biolyte 3-10, 0.35% methylcellulose, 40% SimpleSol®. Focusing at 1.5 kV for 1 min, then at 3 kV for 8 min.

RESULTS

Norovirus VLPs

Aggregation in IEF process and the impact of sample concentration

At the end of IEF process, protein molecules are focused to their pI points where their net surface charge is zero. At the protein pI, chances for aggregation significantly increase. **Figure 1** shows an example of Norovirus VLP particles, where the spike peaks are generated by aggregation. As shown in the figure, in the two consecutive sample injections, the spikes are not reproducible. The spikes disappear when lowering the sample concentration (**Figure 2**). When the final sample concentration is at or below 17 µg/mL, the spikes disappear. However, at such a low sample concentrations, no sample peaks can be observed by UV absorbance.

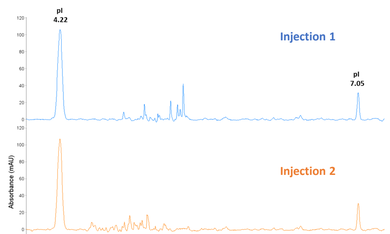

Figure 1. UV absorption electropherograms of two injections of the Norovirus particles. Using absorbance, with at 29ug/mL, the sample does not produce a repeatable profile.

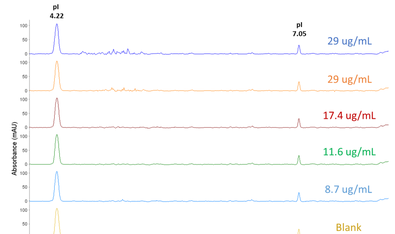

Figure 2. UV absorption electropherograms of different Norovirus particles at different final sample concentrations. Diluting the VLP sample does appear to remove aggregation, but no absorbance signal is observed for the VLP.

UV fluorescence detection

For these lower sample concentrations, the UV-induced native fluorescence on Maurice can detect the sample. **Figure 3** shows the comparison between the absorbance and native fluorescence detection for the same sample injection. A clear signal is seen using native fluorescence.

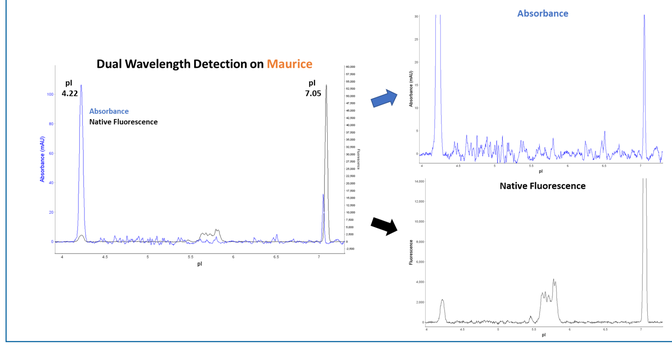

Figure 3. Dual wavelength detection on Maurice affords signal for VLP. UV absorption signal at 280 nm (blue) and native fluorescence signal (black) from the same sample injection

Method Reproducibility and Linearity

The sample peak pattern detected by the native fluorescence detection is reproducible. **Figure 4** shows three injections of the sample.

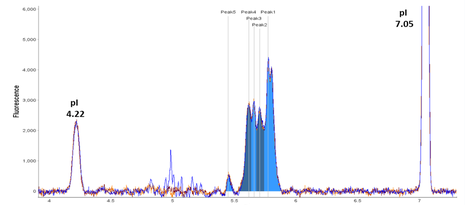

Figure 4. VLP method is reproducible. Triplicate injections of Norovirus particles at final concentration of 17.4 µg/mL are shown and overlay well. 5 peak groups are readily quantifiable.

The VLP sample was measured with NF at lower concentrations as shown in **Figure 5**. The fluorescence detection has good linearity (**Figure 6**) with a limit of quantitation of ~1.5 µg/mL.

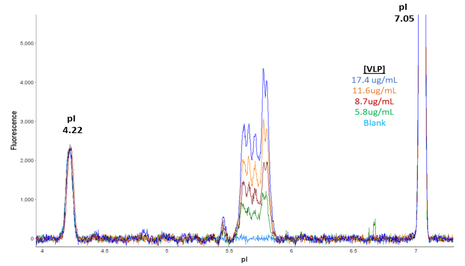

Figure 5. VLP titration. VLP was titrated from 17.4ug/mL to 5.8ug/mL and compared to a blank.

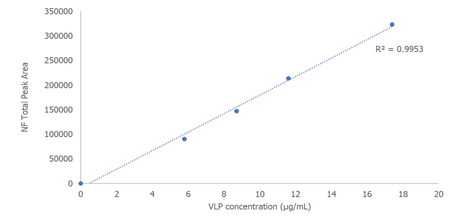

Figure 6. Linearity assessment for VLP titration data. The VLP sample was titrated from 17.4ug/mL down to 5.8ug/mL and compared to blank.

Inactivated Polio Virus (Polio Vaccine)

Leveraging native fluorescence for vaccines

Another product that benefits from the approach is inactive polio virus, as it aggregates during IEF process. To avoid the aggregation, the sample needs to be run at lower concentrations. **Figure 7** shows the result of the inactive virus after diluted into the final IEF buffer ~13-fold. At this concentration, sample aggregation is eliminated. As expected, the signal intensity in UV absorption detection is at the noise level. The sample peaks can be detected by the UV fluorescence detection as shown in Figure 7 (black). The peak pattern is reproducible as shown in **Figure 8**.

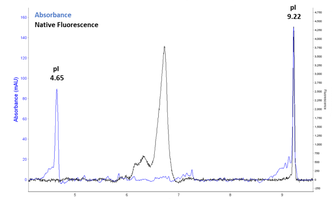

Figure 7. Comparison of UV and NF for polio vaccine sample diluted 13X.

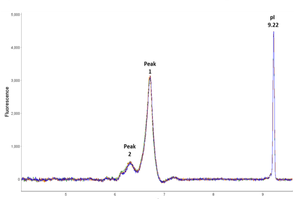

Figure 8. Triplicate injections in native fluorescence for polio vaccine sample.

The vaccine sample was measured with NF at lower concentrations as shown in **Figure 9**. The fluorescence detection has good linearity for both peaks, with a limit of quantitation around 1:100 for both peaks.

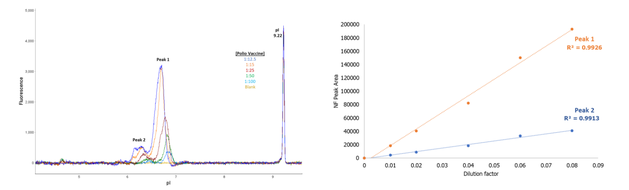

Figure 9. Polio vaccine titration. Polio vaccine was diluted 1:12.5-1:100 and compared to a blank (left). Peak area vs sample concentration for the two peaks was graphed to assess linearity for each peak (right).

CONCLUSIONS

- Sample aggregation during isoelectric focusing may be avoided for virus and VLP samples by reducing sample concentration.
- At such low sample concentrations, UV absorption detection usually is not sensitive enough to detect the samples.
- Maurice's native fluorescence provides enhanced detection for samples at lower concentrations and expands the applicability of icIEF methods to newer, more complex therapeutic modalities.

CONTACT: Jiaqi.wu@bio-techne.com

bio-techne.com