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# Modification of Particle and Hardware Surfaces for Efficient Biotherapeutic Method Development

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## PURPOSE

Undesired analyte-to-surface interactions create challenges for analyzing critical quality attributes for mAb based biotherapeutics. This phenomenon is often cited as an obstacle that has hindered sensitivity and reproducibility in separation sciences.

To overcome these challenges, modifications were incorporated into hardware and particle surfaces to minimize metal-based adsorption and evaluated for two critical quality attributes, size and charge variant analysis. By deploying this novel surface technology, challenges associated with metal-sensitive analytes can be mitigated and consequently drive products to market faster.

## OBJECTIVE(S)

By reducing metal-based adsorption of mAb based biotherapeutics, analytical methods will benefit from an increase in sensitivity and resolution while utilizing simpler mobile phases and reduce the need for passivation of the chromatographical flow path.

## METHOD(S)

**SEC Analysis**

| | |
|---|---|
| LC system: | ACQUITY™ UPLC™ H-Class Bio System (QSM) |
| Column: | XBridge™ Premier Protein SEC 250 Å, 2.5 µm, 4.6 x 150 mm Column<br>BioResolve™ SEC mAb 200 Å, 2.5 µm, 4.6 x 150 mm Column |
| Mobile Phase A: | 200 mM Sodium Phosphate Buffer pH 6.8 |
| Mobile Phase B: | 1.0 M NaCl |
| Mobile Phase C: | Milli-Q™ Water (18.2 MΩ) |
| Column temp.: | 30 °C |
| Injection volume: | 1 µL of 2 mg/mL NISTmAb RM 8671 |
| Flow Rate: | 0.350 mL/min |
| Wavelength: | 280 nm |
| Isocratic Gradient: | 0 mM NaCl – 100 mM Phosphate pH 6.8 (50% A/50% C)<br>50 mM NaCl – 100 mM Phosphate pH 6.8 (50% A/5% B/45% C)<br>100 mM NaCl – 100 mM Phosphate pH 6.8 (50% A/10% B/40% C)<br>200 mM NaCl – 100 mM Phosphate pH 6.8 (50% A/20% B/30% C) |

**IEX Analysis**

**SCX-MS Conditions**

| | |
|---|---|
| LC system: | ACQUITY Premier System (BSM) |
| Column: | BioResolve SCX mAb Column, 3 µm, 2.1 mm X 100 mm |
| Mobile Phase A: | IonHance™ CX-MS pH Concentrate A |
| Mobile Phase B: | IonHance CX-MS pH Concentrate B |
| Column temp.: | 40 °C |
| Injection volume: | 2 µL of 10 mg/mL Infliximab innovator and biosimilar |
| Wavelength: | 280 nm |
| MS system: | RDa™ detector |
| Scan mode: | Positive |
| Scan range: | 400 – 7000 *m/z* |
| Scan rate: | 1 Hz |
| Capillary voltage: | 1.5 kV |
| Cone voltage: | 125 V |
| Probe temp: | 350 °C |

Gradient:

| Time (min) | Flow (mL/min) | %A | %B | Curve |
|---|---|---|---|---|
| Initial | 0.100 | 54.3 | 45.7 | Initial |
| 45.00 | 0.100 | 40.0 | 60.0 | 6 |
| 46.00 | 0.100 | 2.0 | 98.0 | 6 |
| 49.00 | 0.100 | 2.0 | 98.0 | 6 |
| 50.00 | 0.100 | 54.3 | 45.7 | 6 |
| 70.00 | 0.100 | 54.3 | 45.7 | 6 |

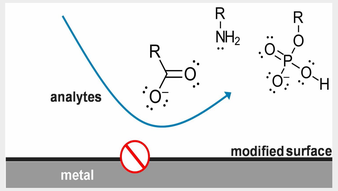
**SCX-UV Conditions** (Unlisted parameters are identical to SCX-MS conditions)

| | |
|---|---|
| Injection volume: | 1 µL |
| Mobile Phase A: | BioResolve CX pH Concentrate A |
| Mobile Phase B: | BioResolve CX pH Concentrate B |

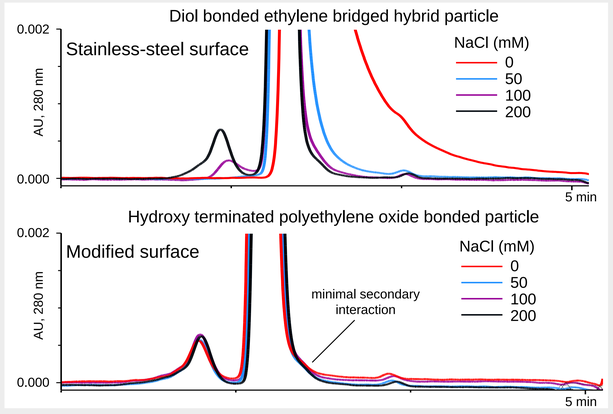
Gradient:

| Time (min) | Flow (mL/min) | %A | %B | Curve |
|---|---|---|---|---|
| Initial | 0.100 | 81.5 | 18.5 | Initial |
| 45.00 | 0.100 | 61.0 | 39.0 | 6 |
| 46.00 | 0.100 | 10.0 | 90.0 | 6 |
| 49.00 | 0.100 | 10.0 | 90.0 | 6 |
| 50.00 | 0.100 | 81.5 | 18.5 | 6 |
| 70.00 | 0.100 | 81.5 | 18.5 | 6 |

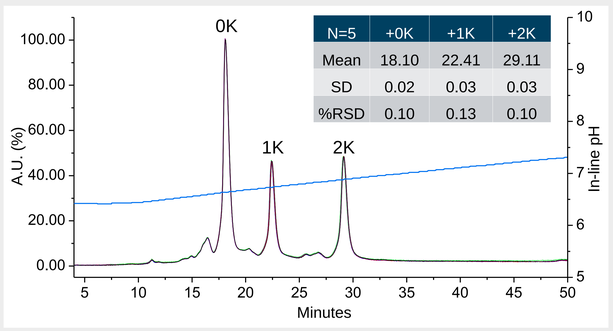
## RESULT(S)



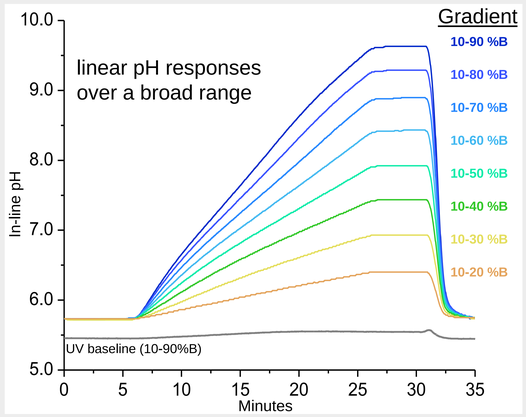

*Modifications were incorporated onto particle and hardware surfaces to offer maximum performance when evaluating metal-sensitive analytes.*



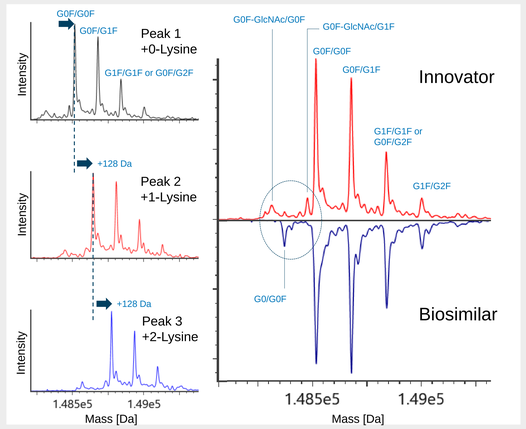

*The column with hydroxy terminated polyethylene oxide bonded particles (bottom) shows excellent monomer peak shape across the entire range of NaCl concentrations and no change in aggregate recovery between 50-200 mM NaCl. The stainless-steel column with diol bonded ethylene bridged hybrid particles (top) needed an addition of 200 mM of NaCl to match the same performance.[1]*




*The ACQUITY Premier System with modified hardware surfaces reproducibility separated three dominant charge variants in infliximab with retention time %RSDs below 1%.[2]*




*In-line pH measurements of the IEX pH buffers were observed to have a linear and proportional response over a broad range. This broad coverage enables charge variant analysis of a wide variety of proteins and coupled with optical and MS compatibility, empowers faster development and manufacturing of drug products.[2]*




*The dominant charge variants were assessed by MS and a mass shift of ~128 Da was observed in the MS spectral data indicating the addition of a lysine residue. Further investigations into an infliximab innovator and biosimilar revealed differences in glycosylation samples between the drug products (dashed circle).[2]*

## CONCLUSION(S)

- Modification of particle and hardware surfaces yielded reproducible and robust performance for size and charge variant analysis of mAbs.
- The modified SEC column maintained performance across the entire range of NaCl concentrations. Conversely, the column without modifications showed significant reliance on salt additives to match performance.
- The IEX pH buffers deliver a linear pH response over a broad range and are compatible with optical and MS detection systems to enable complete insight into charge variant analysis.
- Three dominant charge variants were resolved optically using the ACQUITY Premier System with modified hardware surfaces and showed reproducible retention times of %RSD well below 1%.
- By utilizing MS compatible buffers, IEX analysis can directly identify truncated species by mass and indicate varying glycosylates patterns between drug products to facilitate rapid assessment of charge variants as part of process development.

## REFERENCES

1. Kizekai L, Shiner SJ, Lauber MA. Waters ACQUITY and XBridge Premier Protein SEC 250 Å Columns: A New Benchmark in Inert SEC Column Design. Waters Application Note. 2022. 720007493.
2. Birdsall RE, Koshel B, Yu YQ. Accelerating Charge Variant Analysis of Biotherapeutics with the BioAccord™ System. Waters Application Note. 2022. 720007706.



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