

Effect of Mobile Phase pH in Aggregate Analysis of Monoclonal Antibodies by Size Exclusion Chromatography

James Song, Brian Rivera, Dr. Bryan Tackett
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

Size Exclusion Chromatography (SEC) is a technique to separate biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. Silica-based SEC columns are modified with a hydrophilic stationary phase, typically to minimize electrostatic interactions of positive moieties on proteins and other analytes. However, depending on the physicochemical properties of the analyte, sample recovery may be inconsistent, as non-specific interactions of sample to stationary phase might occur.

One possible mechanism for adsorption is hydrophobic interaction, which may be pronounced especially with moderately high concentrations of phosphate. This might be further exacerbated when mobile phase pH is closer to the isoelectric point (pI) of the protein. In this application note, the effect of mobile phase pH is explored for two IgG1 monoclonal antibodies (mAbs).

Figure 1 shows an overlay of NIST mAb, with mobile phase pH at 6.2, 6.8, and 7.4. It is important to note that the concentration of phosphate buffer and co-solvent (potassium chloride) remained consistent for all assessments. The inset shows chromatograms overlay almost identically; additionally, there are nominal changes in aggregate recovery and resolution of monomer and high molecular weight peaks.

The NIST mAb does have a relatively high isoelectric point (measured pI is typically 9-9.5). As such, the mobile phase is more than 1.5 pH units below the isoelectric point, thus theoretically in a state where hydrophobic interactions are minimized. As such, a less basic mAb (Adalimumab, pI ~8.25) was also investigated, with **Figure 2** showing a similar overlay of SEC chromatograms at three different pHs. Again, nominal differences in chromatography are observed, with the inset showing overlays are virtually identical.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å

Dimensions: 300 x 4.6 mm

Part No.: [00H-4787-E0](#)

Mobile Phase: 200 mM Potassium Phosphate + 250 mM Potassium Chloride, pH as indicated

Flow Rate: 0.35 mL/min

Temperature: 25 °C

Detection: UV @ 280 nm

Sample: NIST mAb (30 µg)
Adalimumab (30 µg)

In summary, protein adsorption may occur in SEC methods. As such, investigating the pH of the mobile phase is critical in understanding experimental design for method optimization and robustness studies. The two monoclonal antibodies investigated here show little difference in chromatographic performance when running moderately high phosphate buffers and varying pH levels- 6.2, 6.8 and 7.4.



Figure 1. SEC chromatographic overlays of NIST mAb run with mobile phase at 3 different pH levels. There are negligible differences in aggregate by % peak area (~2.2%) as well as monomer and aggregate resolution (~2.8-2.9).

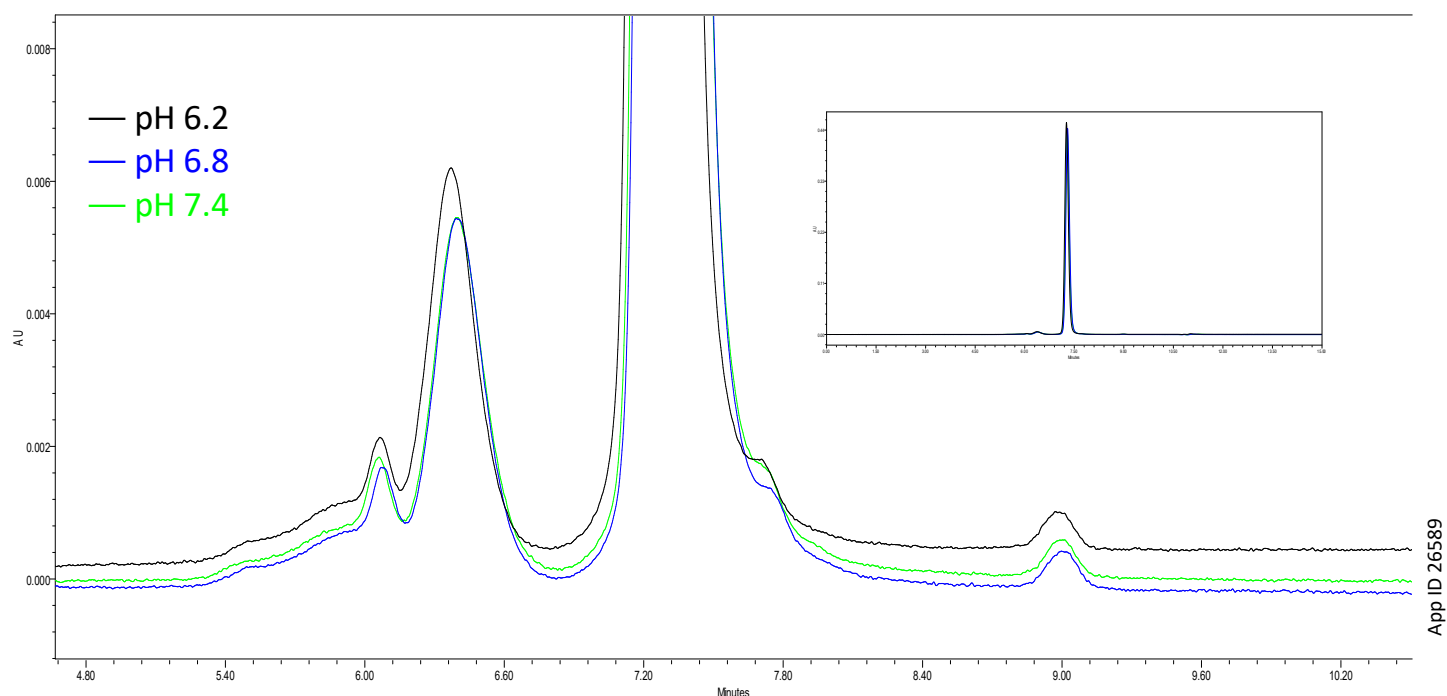
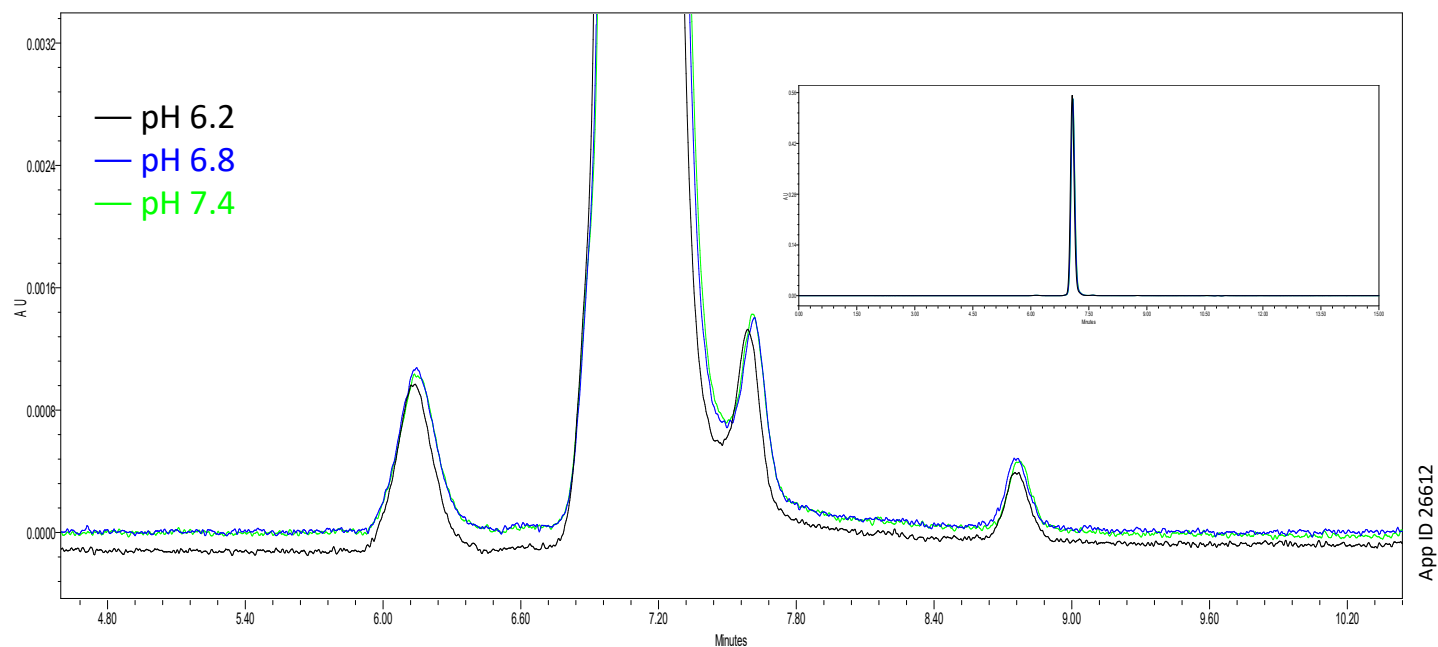


Figure 2. SEC chromatographic overlays of Adalimumab run with mobile phase at 3 different pH levels. Virtually no difference in peak area recoveries, resolution of monomer and dimer, and resolution of post peak fragment.



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Australia

t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria

t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium

t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada

t: +1 (800) 543-3681
info@phenomenex.com

China

t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic

t: +420 272 017 077
cz-info@phenomenex.com

Denmark

t: +45 4824 8048
nordicinfo@phenomenex.com

Finland

t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France

t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany

t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong

t: +852 6012 8162
hkinfo@phenomenex.com

India

t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia

t: +62 21 5010 9707
indoinfo@phenomenex.com

Ireland

t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy

t: +39 051 6327511
italiainfo@phenomenex.com

Japan

t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg

t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico

t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands

t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand

t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway

t: +47 810 02 005
nordicinfo@phenomenex.com

Poland

t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal

t: +351 221 450 488
ptinfo@phenomenex.com

Singapore

t: +65 800-852-3944
sginfo@phenomenex.com

Slovakia

t: +420 272 017 077
sk-info@phenomenex.com

Spain

t: +34 91-413-8613
espinfo@phenomenex.com

Sweden

t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland

t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan

t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand

t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom

t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA

t: +1 (310) 212-0555
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🌐 **All other countries/regions**
Corporate Office USA
t: +1 (310) 212-0555
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