Assessment of Column Priming for sub-2 µm Size Exclusion Columns

Dr. Ivan Lebedev, Brian Rivera, and Dr. Bryan Tackett
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

Size Exclusion Chromatography (SEC) is a technique for the separation of large molecules such as proteins and polymers based on their size in solution. SEC methods are commonly used for the quantitation of aggregates in a biotherapeutic sample. Consequently, any non-specific adsorption to the column media or hardware could compromise data, as peak areas may be underreported.

As a failsafe for aggregate and sample adsorption to the column, column "priming" is often included in an analytical method sequence. This includes several injections of a protein; commonly an inert protein like bovine serum albumin (BSA) or reference standard. This helps minimize the chance for sample adsorption to occur.

In this application, we demonstrate a priming protocol, wherein 10 subsequent injections of BSA are injected onto a freshly packed 150 x 4.6 mm ID column with sub-2 µm particles. **Table 1** shows a summary of total peak areas for monomer and dimer. **Figure 1** shows a chromatographic overlay.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Part No.: 00F-4787-E0
Dimensions: 150 x 4.6 mm
Mobile Phase: 200 mM Potassium Phosphate + 250 mM KCl, pH 6.2
Flow Rate: 0.35 mL/min
Injection Volume: 10 µL
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: Bovine serum albumin (10 mg/mL)

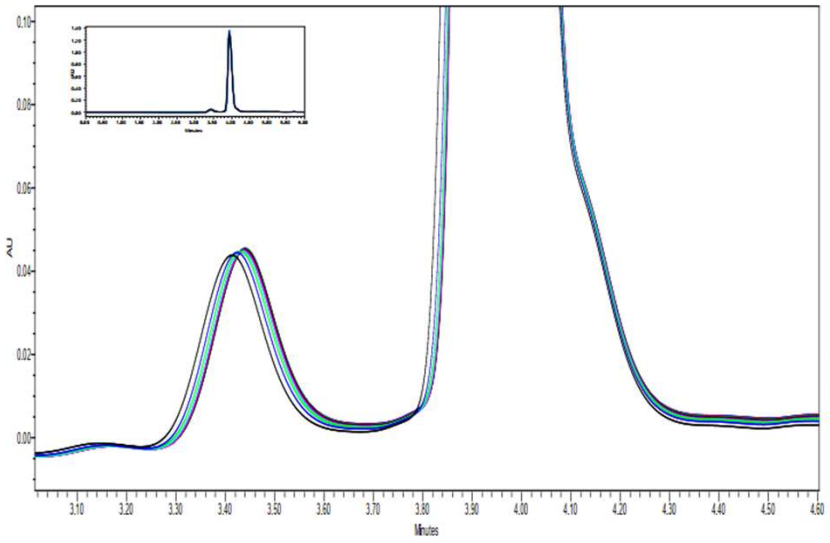
Upon initial injection, peak areas for both monomer and dimer show almost complete recovery. Additionally, as shown in the inset for **Figure 1**, only slight shifts in retention time for monomer and dimer are observed. This indicates that the extent of priming necessary for the Biozen dSEC-2 chemistry is nominal.

In summary, although the practice of column priming is typically performed, column selection may minimize the need for extensive priming.

Table 1. Retention Times for Protein Standards

Injection	Peak Area, Monomer	Peak Area, Dimer
1	10757707	462531
2	10774224	474567
3	10780383	475693
4	10775497	478940
5	10773430	480767
6	10779073	480131
7	10785349	481109
8	10785780	480578
9	10782926	481759
10	10771993	482644
average	10776636.2	477871.9
%CV	0.1%	1.3%

Figure 1. Chromatographic Overlays for BSA



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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
info@phenomenex.com

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