

Improving Resolution for Size Exclusion Chromatography Methods by Optimization of Linear Velocity

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

Overview

Size Exclusion Chromatography (SEC) is a technique for the separation of biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight (HMW) aggregates.

Flow rate is a common method parameter for most adsorptive LC methods. However, SEC is a unique separation modality in that lower flow rates often yield improvements in separation and resolution can improve quite considerably simply by running at a lower linear velocity.

Figure 1 demonstrates the utility of method improvements by running at a lower flow rate for the aggregate analysis of NIST mAb, a common standard protein. 0.4 mL/min gives a resolution (R_s) of 2.97 for monomer and HMW, with 0.35 mL/min giving R_s of 3.17 and 0.2 mL/min giving an R_s of 3.42. Results are summarized in **Table 1**.

LC Conditions

Column: Biozen™ 1.8 μm dSEC-2, 200 \AA
Dimensions: 300 x 4.6 mm
Catalog No: [00H-4787-EQ](#)
Mobile Phase: 0.2M Potassium Phosphate + 250 mM KCl, pH 6.2
Flow Rate: As Indicated
Injection Volume: 3 μL
Temperature: 25 $^{\circ}\text{C}$
Detection: UV @ 280 nm
Sample: NIST mAb, 10 mg/mL

Although the method will effectively take twice as long, since resolution is often a requirement to meet system suitability, the superior result with lower flow rate value may give a wider margin of error prior to method failure. As such, if one is prioritizing performance and robustness over throughput, lower flow rate is one of the easiest and practical method parameters to implement.

Figure 1. SEC Chromatogram Overlays for NIST mAb Run at Varying Flow Rates

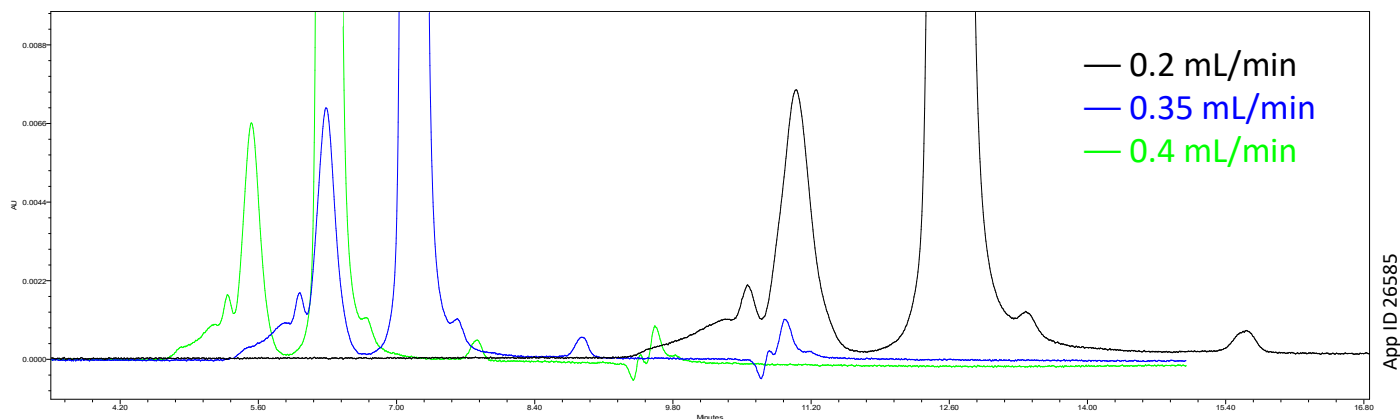


Table 1. Summary of Chromatographic Results with Modulating Flow Rate

Flow Rate (mL/min)	R_s 1,2 (HMW, Monomer)	R_s 2,3 (Monomer, Fragment 2)
0.2	3.42	9.04
0.35	3.17	8.66
0.4	2.97	8.09



Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/LiveChat.

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
info@phenomenex.com

🌐 **All other countries/regions**
Corporate Office USA

t: +1 (310) 212-0555
info@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com

BE-HAPPY™
GUARANTEE

Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.

www.phenomenex.com/behappy

Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions, which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks

Biozen and BE-HAPPY are trademarks of Phenomenex.

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.

© 2021 Phenomenex, Inc. All rights reserved.

