

Generating a Calibration Curve with UHPLC Size Exclusion Column

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. To determine the hydrodynamic radius (R_h) of an unknown, a calibration curve can be generated with polymer standards. The relative retention times are plotted against the log MW for each respective standard, and a polynomial regression analysis is performed. Here we demonstrate the use of protein standards, with well-characterized hydrodynamic radii and molar masses, to generate calibration curves.

Figure 1 highlights the separation of common protein standards using a gel filtration standard for analytical and large-scale size exclusion analysis. **Table 1** shows a summary of relative elution times for these standards analyzed by SEC. Relative retention times plotted are then plotted against the log R_h for each respective standard, and a polynomial regression analysis is performed.

Figure 2 shows the calibration curve using a third order polynomial regression generated from standards and log R_h . Based upon the elution volume or retention time for NIST mAb (**Figure 3**), the measured R_h is 50.2 Å. This is within 97.2 % of the reported value of 5.2 Å for NIST mAb.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Part No.: [00H-4787-E0](#)
Dimensions: 300 x 4.6 mm
Mobile Phase: 200 mM Potassium Phosphate, 250 mM Potassium Chloride, pH 6.2
Flow Rate: 0.35 mL/min
Injection Volume: 10 µL
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: Protein Standards

In summary, using a calibration curve generated by well-characterized standards can be used to determine the hydrodynamic radius of an unknown. Further, it may potentially be used for a stability indicating or biosimilarity method, as changes in post-translational modification such as deamidation and glycosylation can be detected with a well-developed analytical SEC method.

Table 1. Retention Times for Protein Standards

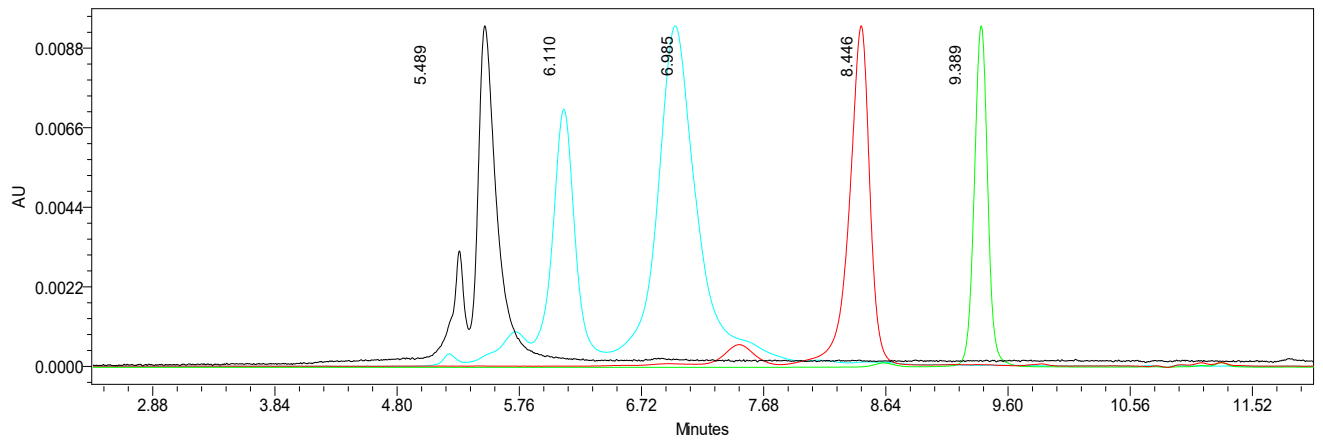
Analyte	Retention Time (min)	Relative Elution Volume (mL)	Theoretical R_h (Å)
Bovine Thyroglobulin	5.489	3.85	86
IgA	6.122	4.3	76
IgG	6.99	4.91	51
Ovalbumin	8.447	5.93	28
Myoglobin, horse heart	9.389	6.59	18.4
NIST mAb	7.078	4.97	52



Figure 1. SEC Chromatograms for Protein Standards

Chromatographic overlays of protein standards, y-axis normalized.

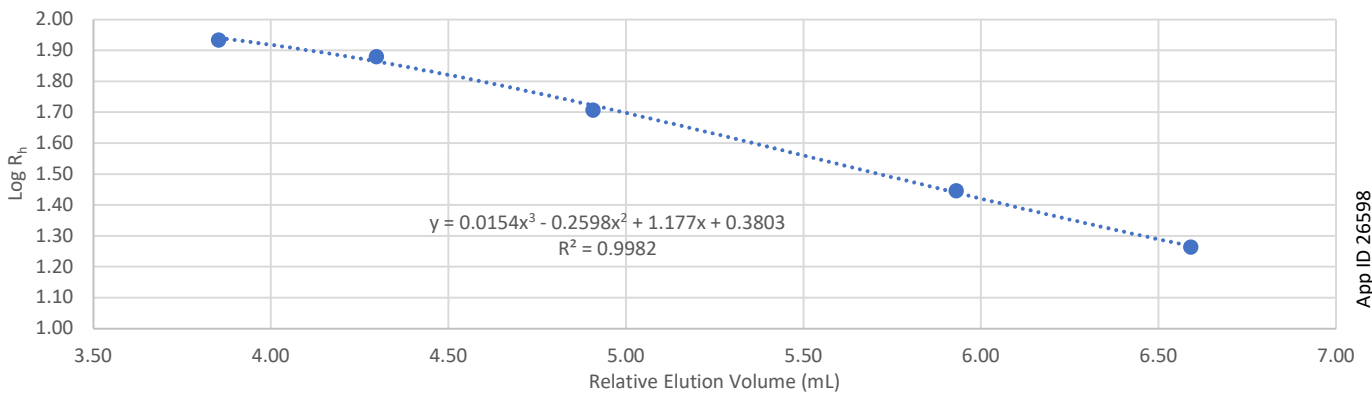
Black- Thyroglobulin, Teal- Gamma Globulin (IgA/IgG), Red- Ovalbumin, Green- Myoglobin



App ID 26592

Figure 2. Calibration Curve, Hydrodynamic Radius (R_h)

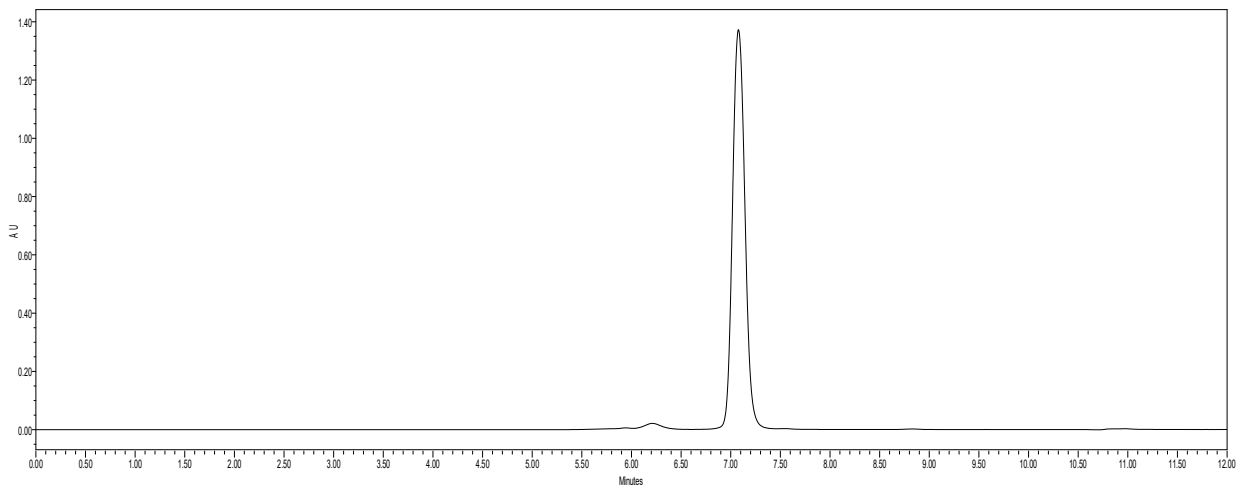
Calibration curve generating using relative elution volume of protein standards, plotted against $\log R_h$. A third order polynomial regression was used to calculate R_h of various analytical samples.



App ID 26598

Figure 3. Representative SEC Chromatogram for NIST mAb

Retention time for NIST mAb is 7.078 min. Extrapolated hydrodynamic radius is 50.2 Å



App ID 26593



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.

