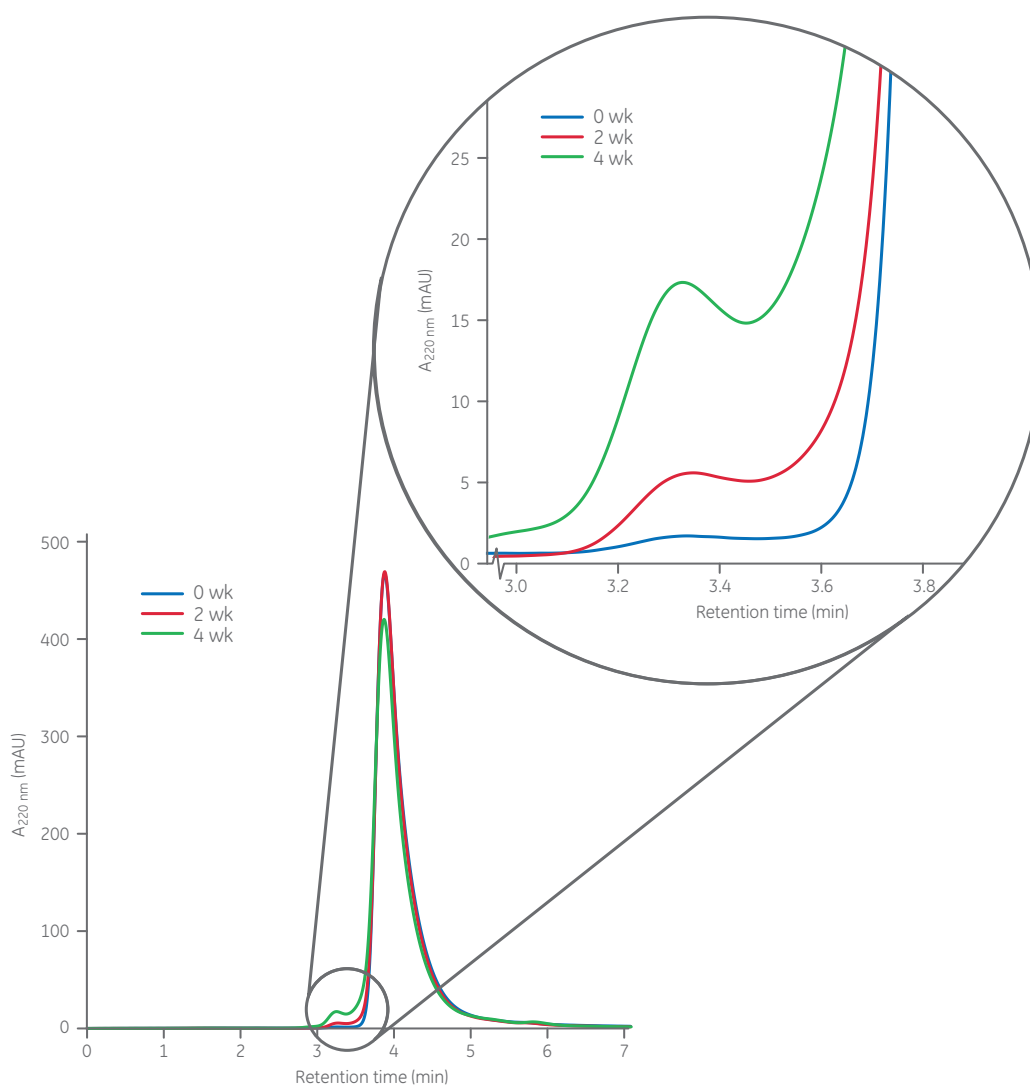# Protein analysis with size exclusion chromatography (SEC)

Fundamentals. New-generation agarose resins. Applications.

Protein analysis with size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) is currently the most powerful chromatography technique for obtaining reliable information about the size of biomolecules under native conditions. As such, it is widely used in several different analytical applications from basic research to quality control of biotherapeutics.

This white paper presents fundamentals about analytical SEC and describes how our new-generation agarose-based SEC columns are used in analysis of biomolecules.

Introduction to protein analysis with SEC

Since the introduction of biologic-based therapeutics, the need for checking the presence of protein aggregates has been of particular concern given their potential effect on efficacy and immunogenicity. SEC is a very effective method for protein analysis and it allows true size profiling of protein samples due to the mild separation conditions that can be used to obtain high-resolution separations. This is a great advantage compared to other size-separation techniques, such as ultrafiltration or dialysis. A protein can occur in different "size forms" (monomeric, aggregated, degraded, complexed, Fig 1) and those different forms exhibit different functions. As many protein "size forms" are held together by noncovalent means, they are often weak and could be easily broken if using nonnative (i.e., denaturing) conditions.
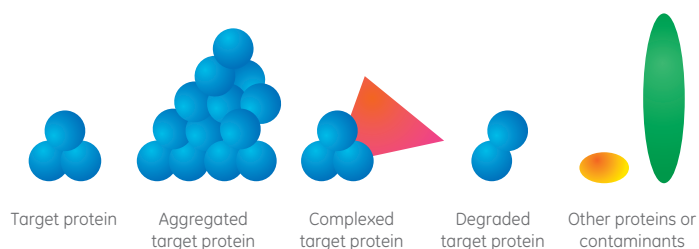

Fig 1. Schematic illustration of different "size forms" of a protein.

The use of SEC makes it possible to size profiling the sample, for example when you need to:

- Monitor protein prep quality
- Evaluate protein stability
- Study complex formation
- Evaluate tendency to aggregate and quantity of aggregates
- Evaluate tendency to degrade and quantity of degraded forms
- Identify protein interaction partners and interaction conditions

Factors to consider when selecting an SEC column for protein analysis

When choosing SEC columns for protein analysis many parameters need to be considered with resolution being one of the most important. Resolution is influenced by several factors including:

- **Resin properties**
  - Resin chemical composition
  - Particle (bead) size and size distribution
  - Pore size and selectivity
  - Fractionation range

- **Column related factors**

- **Running conditions**
  - Sample volume and injection technique
  - Buffer composition
  - Flow rate

- **LC system configuration**
  - Tubing diameter and flow path volume

After the selection of SEC resin, column dimension and sample volume are the two factors that affect the resolution of the separation the most.

Resin properties

Resin chemical composition

Different resin types are available today, such as agarose, silica, and metacrylate. Silica-based and agarose-based resins are the two most used types of SEC resins for analytical purposes. For a comparison of both resin types, see *Silica vs agarose resins*.

Particle size and size distribution

Particle size is important for the resolution in SEC. A rule-of-thumb is that smaller particle and narrow size distribution usually give the highest resolution. Particle size ranges used for analytical SEC currently available on the market including benefits and challenges are listed in Table 1.

The size range of 4 to 12 µm is traditionally the standard for analytical SEC resins. However, the trend is towards smaller particles of < 2 µm, with the use of ultra high-performance liquid chromatography (UHPLC) systems for even faster separations in high-throughput mode.

Columns packed with particles < 4 µm in diameter typically provide rapid runs and high resolution. However, very high back pressures are generated when these columns are operated at high flow rates, often exceeding the pressure specifications of standard high-performance liquid chromatography (HPLC) systems. Also, the LC system needs to have very small internal (dead) volumes so that the high resolution obtained over the column is not lost elsewhere in the flow.

Keep in mind that the high pressure obtained under UHPLC conditions generates high temperatures and shear stress, which might affect sensitive proteins in particular (1–4).

Table 1. Particle size ranges currently available for analytical SEC

4 to 12 µm particles (standard for analytical SEC)	< 4 µm particles (includes particles for UHPLC)
Benefits	*Benefits*
• Usually provides adequate resolution and run times	• Rapid runs
• Standard HPLC equipment can be used	• High resolution
• Lower price for equipment compared with UHPLC	*Challenges*
• Milder conditions can be used than with UHPLC	• Very high back pressure; demands specific equipment
	• Loss of resolution due to LC system dead volumes
	• Heat generation and shear stress at high flow rates could affect proteins

Pore size and selectivity

Pore size is a complex function of the pore diameter, the pore shape, the number of pores, and pore connectivity. All pores are not equal even within a single chromatography particle. Describing the pore size of a chromatography resin with a single number such as average pore size is therefore an oversimplification.

The relationship between resolution and selectivity for a SEC resin is described in the selectivity curve. The steeper the selectivity curve, the higher the resolution that can be achieved. Resolution is also affected by band-broadening, which is dependent on the particle size of the SEC resin.

Fractionation range

The fractionation range defines the range of molecular weights of molecules that have access to the pores in the resin; molecules within this range can be separated by high-resolution fractionation. The exclusion limit for a SEC resin indicates the size of the molecules that are excluded from the pores of the resin and which therefore elute in the void volume.

In Figure 2, the fraction ranges of globular proteins and peptides with various molecular weights on the new-generation agarose-based resins can be seen.
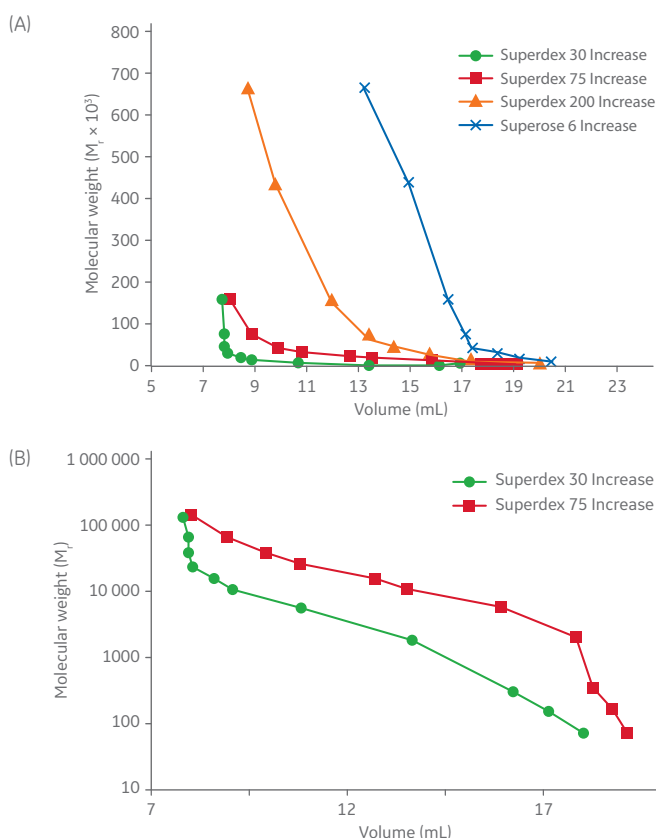

Fig 2. (A). Fractionation range of globular proteins and peptides of various molecular weights on Superdex™ 30 Increase 10/300 GL, Superdex 75 Increase 10/300 GL, Superdex 200 Increase 10/300 GL, and Superose™ 6 Increase 10/300 GL prepacked chromatography columns. Note that the whole fractionation range of Superose 6 Increase is not covered in this diagram. (B). Zoomed image clearly shows the differences in selectivity between Superdex 30 Increase and Superdex 75 Increase columns.

Column-related factors

The height of the packed bed affects both resolution and the time taken for elution. The efficiency in SEC increases with the square root of bed height (see formula below). Doubling the bed height gives an increase in resolution equivalent to $\sqrt{2} = 1.4$ (40%).

$$\text{Column efficiency (N/m)} = 5.54 \frac{(V_e/W)^2}{L}$$

where

V_e = peak elution (retention) volume
W = peak width at half peak height
L = bed height (m)
Note that V_e and W are in same units

However, longer columns also mean longer run times. There is a tradeoff between resolution and runtime that needs to be defined by the needs for the analysis at hand.

As the separation takes place in only one column volume, it is very important to have a well-packed bed.

Running conditions
Sample volume and injection technique

In analytical SEC, the sample volume should be approximately 0.3% of the bed volume to achieve optimal results.

It is also relevant to consider how the sample loop (capillary) is filled and emptied, a factor that is often is overlooked. The sample assumes the shape of an arrow with a broad head and long shaft in its passage through the capillary, rather than the shape of a compact plug. The sample therefore occupies a longer section of the capillary than expected, which can lead to a peak broadening effect.

To ensure reproducible analysis, always ensure that the syringe is completely filled for manual injection. For autosampler injection, it is important to check the settings on how the injections are performed to avoid inconsistent results.

Buffer composition

Buffers and sample are important parameters to achieve high resolution. The buffer composition and how it affects resolution will be discussed later in the document.

Flow rate

One of the most important parameters that affects resolution is the flow rate (especially for large proteins). If the analysis shows low resolution between protein peaks, the first action should be to set a lower flow rate for the run.

Liquid chromatography system configuration
Tubing diameter and flow path volume

The configuration of the liquid chromatography (LC) system needs to be optimized to achieve the highest resolution. For maximum resolution, system volumes should be kept at a minimum. Short, narrow capillaries should be used and unnecessary system components should be bypassed. Resolution is affected by different inner diameters (i.d.) and length of the tubing in the system (from sample load to column inlet and from column outlet to the UV cell). The narrower the tubing used, the higher the resolution achieved.

Figure 3 shows the effect that a five-fold difference in tubing diameter has on resolution. Improved resolution was observed on the 0.15 mm capillary compared with the 0.75 mm capillary.

Tubing length is equally important, and longer tubing than necessary should never be used. This is even more important for sub-2 µm particles.
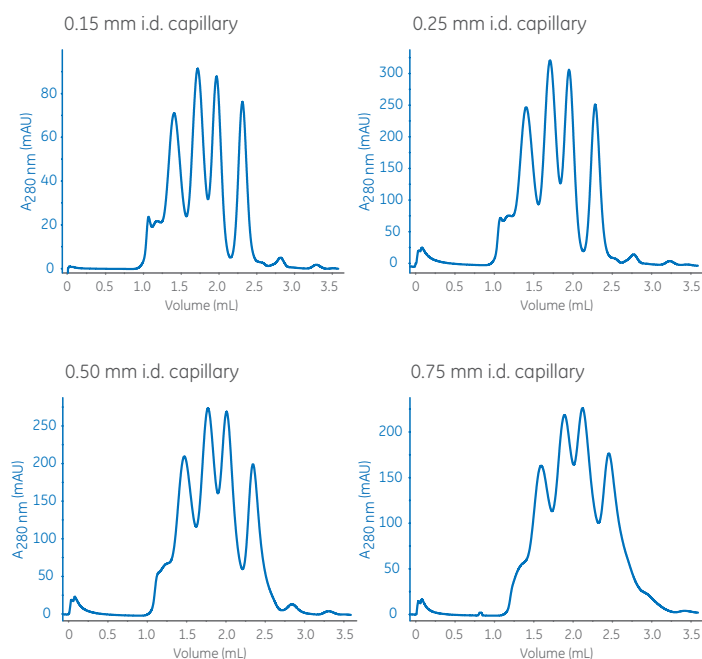

Fig 3. Effect of increasing capillary inner diameter on resolution in protein purification.

Silica vs agarose resins

Silica- and agarose-based resins are the two most used types of SEC resins available for analytical purposes. Agarose is obtained from natural sources and contains a very low amount of ionic and hydrophobic groups that could interact with the compound of interest. In contrast, silica-based resins have silanol groups that require coating before use by SEC.

Properties of silica-based and agarose-based resins are listed in Table 2.

Table 2. Properties of silica and agarose particles[1]

Property	Silica particles	Agarose particles
Chemical composition	SiO_2	Polysaccharide (D-galactose-3,6-Anhydro-L-galactose)
Surface coating	Silanols are blocked to minimize adsorption	Not required
pH stability	Typically, pH 2–8 (operational)[2]	Typically, pH 3–11, operational[2] pH 1–12, cleaning-in-place (CIP)[3]
Mechanical stability	Rigid, high pressure stability	Rigidity achieved by chemical cross-linking
Porosity	Can be controlled to achieve the desired separation range	Can be controlled to achieve the desired separation range

[1] This information is taken from product information on vendor's web pages.
[2] pH range where resin can be operated without significant change in function.
[3] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

Similar resolution for both silica and agarose resins

As seen in Figure 4, the agarose-based SEC resin of Superdex 200 Increase 10/300 GL column gives similar resolution compared with the silica-based resin of TSKgel column, even though agarose beads are larger. Thus, one cannot rely solely on bead size to compare the ability of a resin to give good separation.
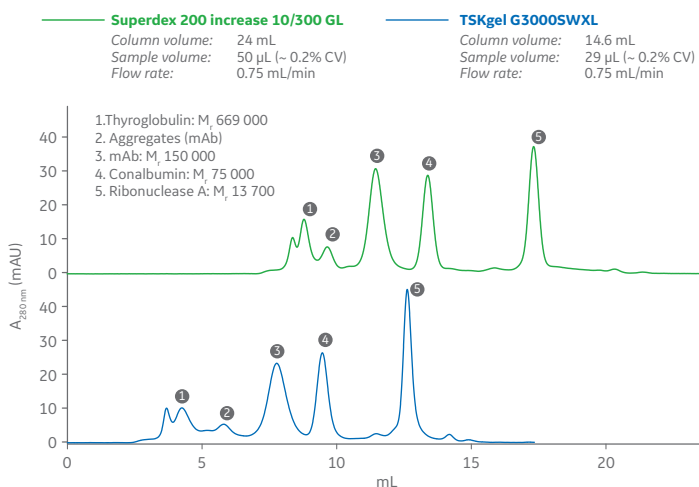

Fig 4. Separation of the agarose-based Superdex 200 Increase resin with particle size of 8.6 µm (green) and silica-based TSKgel with 5 µm particles (blue). The information presented is based on benchmarking studies performed at GE Healthcare Bio-Sciences AB, Uppsala from June to July 2014. The purifications were performed in triplicate, using the recommended protocols from the suppliers. The data used to support this study can be made available upon request at protein.purification@ge.com

Higher alkali stability of agarose resins enables efficient cleaning

Superdex Increase and Superose Increase are agarose-based resins, which tolerate high pH and can therefore withstand NaOH cleaning. This means that they can be efficiently cleaned and there is little if any need to use a guard column.

The recommended process for cleaning the column is to inject NaOH after 10 to 20 separation cycles. As shown in Figure 5, sample injections 1 and 200 on a Superdex 75 Increase column gave consistent results for efficiency and resolution when the NaOH cleaning regime had been applied.
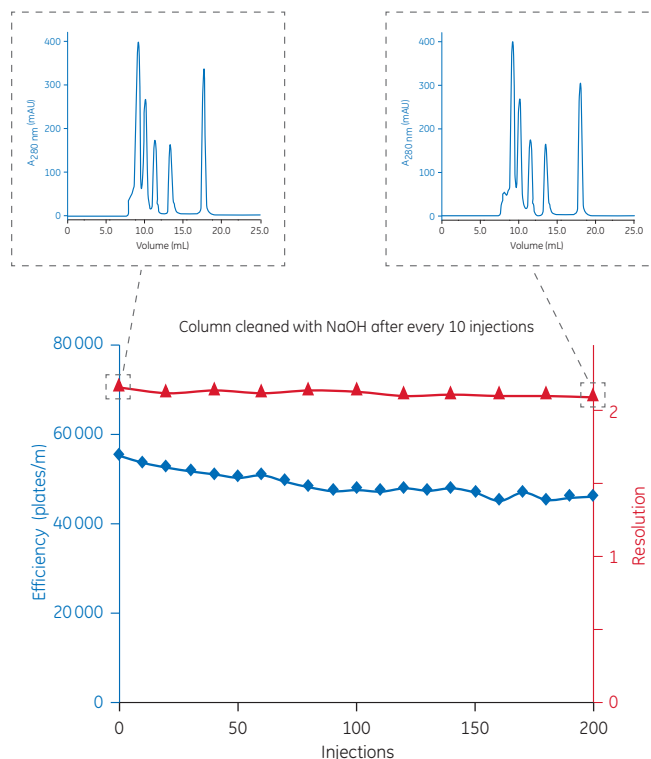

Fig 5. Recommendation for agarose-based resins: perform regular cleaning with NaOH after 10 to 20 separation cycles. Red curve shows resolution and blue curve efficiency over 200 runs.

Silica-based resins are mechanically very stable below a pH of 7.0 but do not tolerate pH > 8.0. Silica columns typically do not tolerate the common cleaning regimes for protein chromatography columns since those regimes involve NaOH, which gives high pH (5, 6). Between pH 7.0 and 8.0, performance of the columns might be affected with a change in resolution as the outcome.

An advantage with agarose-based resins is that the same SEC column can be used for different samples. This is not the case with silica-based resins as they do not tolerate cleaning in NaOH.

SEC columns packed with new-generation agarose-based resins

The new generation of agarose-based resins—Superdex 30 Increase, Superdex 75 Increase, Superdex 200 Increase, and Superose 6 Increase—are well-suited to biomolecular analysis. The columns together cover separation of a broad molecular weight range (M_r 100 to 5 000 000) from small peptides up to large protein complexes (Fig 6).

The resins are produced with a novel manufacturing method resulting in more rigid, smaller beads with a narrower particle size distribution, compared with the original Superose/Superdex resins. The consequence of this improvement is shorter runtime and increased resolution compared to the original Superose/Superdex columns[1].
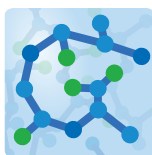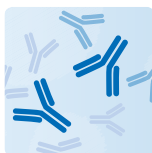

Superdex 30 Increase

Peptides and other small biomolecules
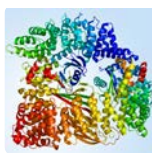Fractionation range M_r ~ 100 to 7000

Superdex 75 Increase

Recombinant tagged proteins
Fractionation range M_r ~ 3000 to 70 000

Superdex 200 Increase

mAb and other antibodies
Fractionation range M_r ~ 10 000 to 600 000

Superose 6 Increase

Larger proteins and protein complexes[1]
Fractionation range M_r ~ 5000 to 5 000 000

[1] Superdex 30 Increase replaces Superdex Peptide, Superdex 75 Increase replaces Superdex 75, Superdex 200 Increase replaces Superdex 200, and Superose 6 Increase replaces Superose 6.

Fig 6. Typical applications and fractionation ranges of Superdex 30 Increase, Superdex 75 Increase, Superdex 200 Increase, and Superose 6 Increase.

Which format for which application?

New-generation SEC resins from GE are packed in three different column sizes to fit different needs in biomolecular analysis (Fig 7).

10 mm × 300 mm

- High-resolution analysis (25 to 500 µL sample volume)
- Best choice if sample amount is not limited

3.2 mm × 300 mm

- High-resolution analysis (4 to 50 µL sample volume)
- Low sample and buffer consumption

5 mm × 150 mm

- Purity check
- Rapid screening
- Low sample (4 to 50 µL sample volume) and buffer consumption

Fig 7. Three different column sizes of for various application needs.

Compelling reasons to use new-generation SEC columns for protein analysis

As described previously, there is no single resin that offers the optimal fit for all proteins and conditions. However, agarose-based resins have a number of features highlighted here that ensure good analytical SEC data.

High reproducibility

As protein drugs are increasing in complexity at the same time as legislation on drug safety becomes ever stricter, the requirements on SEC columns' ability to detect impurities has also increased.

Superdex Increase and Superose Increase resins and prepacked columns have high reproducibility, buffer stability, and lifetime stability including stability towards high pH. Low nonspecific interactions are also important.

The narrow specification for particle size range and selectivity of the new generation of Superdex Increase and Superose Increase resins result in low batch-to-batch variation and thus high consistency and reproducibility.

Column-to-column reproducibility

In Figure 8, results from tests of column packing robustness are shown on Superdex 200 Increase 10/300 GL columns. Three columns from different lots, packed with the same resin lot, were tested and the results show very high reproducibility.

Columns: Superdex 200 Increase 10/300 GL
Sample: 1. mAb5 (monomer) – M_r 150 000
                     2. Fab (mAb5) – M_r 50 000
                     3. dAb (mAb 5) – M_r 13 000
Sample volume: 50 µL (0.2% CV)
Buffer: 20 mM NaH_2PO_4, 300 mM NaCl, pH 7.4
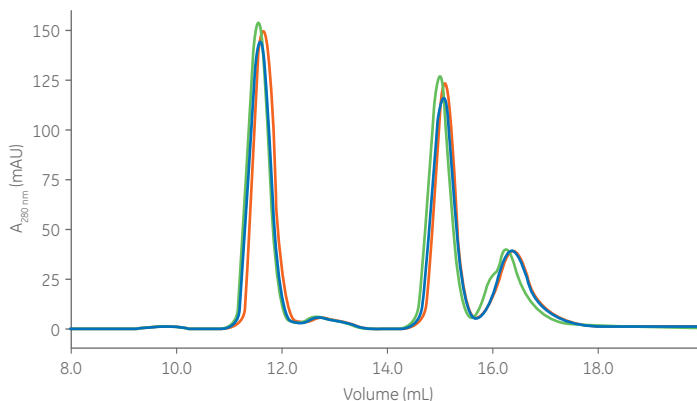Flow rate: 0.75 mL/min

Fig 8. Three column lots of Superdex 200 Increase 10/300 GL show high lot-to-lot reproducibility of column packing.

Resin batch-to-batch reproducibility

Results from batch-to-batch reproducibility of different resin lots of Superdex 200 Increase are shown in Figure 9. Six different resin lots were compared for resolution and retention volume and show minor differences in relative standard deviation (RSD) of < 6% for resolution and < 10% for retention volume.
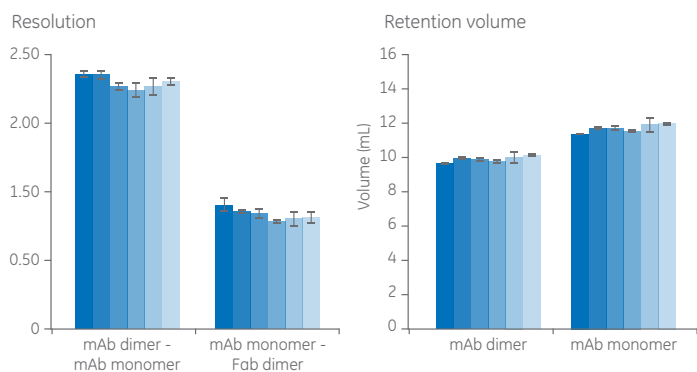

Fig 9. Comparison of six different Superdex 200 Increase batches of resin on resolution and retention volume in the purification of mAb and mAb aggregates. Same sample and running conditions as for Figure 8.

Lifetime stability

Reproducible results are essential in all research. The long working life and high reproducibility of Superdex Increase and Superose Increase prepacked columns are the result of optimized design, stable properties of the resin, and controlled production procedures. In Figure 10, a total of 350 injections of a sample mix consisting of proteins and peptides with low concentration was performed on Superdex 30 Increase 10/300 GL column. Peak areas and resolution were essentially unchanged during the study.

Column: Superdex 30 Increase 10/300 GL
Sample: 1. Cytochrome C (M_r 12 300), 0.16 mg/mL
                     2. Aprotinin (M_r 6500), 0.16 mg/mL
                     3. [Ile^7]-Angiotensin III (M_r 897), 0.08 mg/mL
                     4. Triglycine (M_r 189), 0.16 mg/mL
                     5. Glycine (M_r 75), 5.6 mg/mL
Sample volume: 50 µL
Buffer: 20 mM phosphate buffer, 280 mM NaCl, pH 7.4
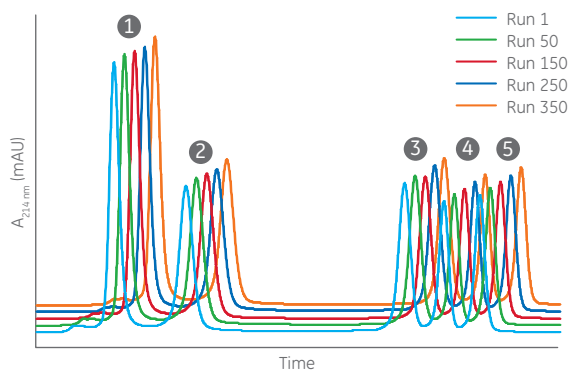Flow rate: 0.8 mL/min
System: HPLC

Fig 10. Repeated injections of a sample mix consisting of proteins and peptides on Superdex 30 Increase. Results from run 1, 50, 150, 250, and 350 are shown. Peaks of the proteins and peptides are labeled 1 to 5.

Easy inspection of the packed resin bed

Superdex Increase, and Superose Increase are prepacked glass columns. The transparency of the glass makes it easy to inspect the packed bed ensuring user confidence of the integrity of the resin before using the column. This is typically not possible with steel columns.

For protection, the glass is covered with a thin protective film Tricorn (10/300 GL and Tricorn 5/150 GL columns) or the glass column is placed in a protective shell (3.2/300 columns).

Stability with a range of buffers

Properties of proteins such as number of charge groups, hydrophobicity, and size influence separation by SEC. Resins must therefore be chemically stable in a wide range of buffers to minimize interaction from the resin and changes in retention time, peak area, and resolution. Buffers with pH between 6.0 and 8.0 are used in SEC applications since many proteins are stable in that pH range.

Further, it is important to select an ionic strength that is beneficial for the protein of interest. Higher ionic strength decreases the retention time for positively charged proteins and increases it for negatively charged proteins. An ionic strength that is too high might also increase hydrophobic interactions between resin and proteins, which affects retention time as well as peak area and resolution.

Superdex Increase and Superose Increase resins are stable over a wide pH range. Retention time and resolution are consistent with commonly used buffers. This is confirmed in Figure 11, where a mix of six biomolecules was consistently separated on Superdex 75 Increase 10/300 GL column using buffers (pH 6.7 and 7.4) commonly used in SEC applications.

In conclusion, the buffer stability of agarose resins such as Superdex 75 Increase gives flexibility in the choice of the buffer to allow easier selection of the optimal buffer for the target biomolecule.
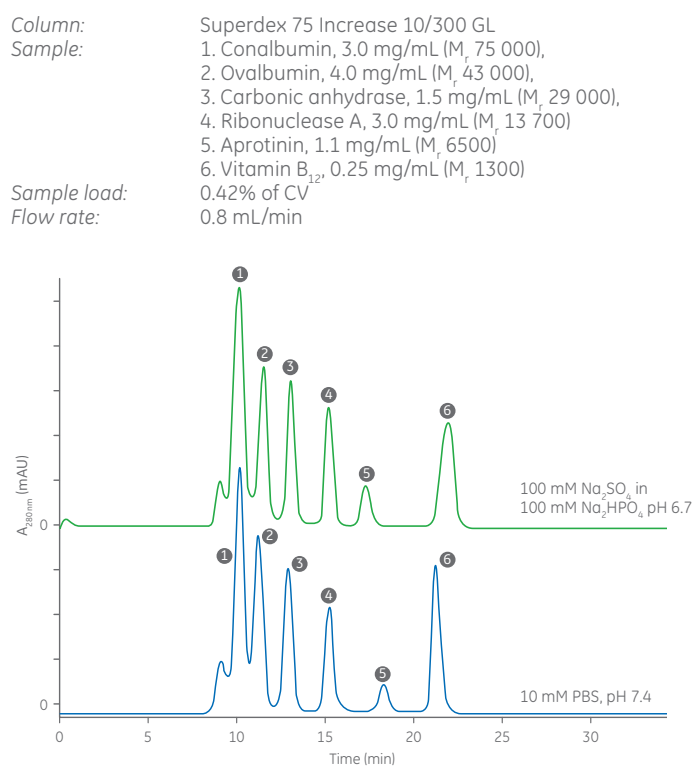
Column: Superdex 75 Increase 10/300 GL
Sample: 1. Conalbumin, 3.0 mg/mL (M_r 75 000),
2. Ovalbumin, 4.0 mg/mL (M_r 43 000),
3. Carbonic anhydrase, 1.5 mg/mL (M_r 29 000),
4. Ribonuclease A, 3.0 mg/mL (M_r 13 700)
5. Aprotinin, 1.1 mg/mL (M_r 6500)
6. Vitamin B_{12}, 0.25 mg/mL (M_r 1300)
Sample load: 0.42% of CV
Flow rate: 0.8 mL/min

Fig 11. Separation of a mixture of six biomolecules on Superdex 75 Increase 10/300 GL using 100 mM Na_2SO_4 in 100 mM Na_2HPO_4 (above) and the same column using 10 mM PBS (140 mM NaCl, 2.7 mM KCl, 10 mM sodium phosphate, pH 7.4, below). Six peaks were detected after separation.

Successful use of new-generation SEC columns: application examples

SEC Increase columns from GE have been used in several published studies for analytical SEC applications (7–9) such as molecular mass determination (10, 11), analysis of oligomeric state (11–14), for SEC-multiangle light scattering (MALS) experiments (15–17), complex formation studies (7), and to prepare samples for protein structure determination using cryo-EM (18), or X-ray crystallization (19, 20).

SEC analysis using these columns covers a wide variety of research areas such as drug resistance (15, 21), developing antiviral therapies (14), and studies of HIV (11).

Other examples of how these columns have been used for analysis are described here.

Screening of mAb aggregates

Screening for mAb aggregation is important in the production of mAb. When performing aggregate analysis on mAbs there is often no issue when the aggregate level is above 1%. At lower levels, however, issues can arise in integration of the aggregate peak.

Figure 12 shows that it is possible to determine ≥ 0.1% aggregate with 4-min run time on Superdex 200 Increase 5/150 GL. Thus, this column is a good screening tool to evaluate conditions for mAbs.

Column: Superdex 200 Increase 5/150 GL
Sample: mAb
Sample volume: 5 µL
Flow rate: 0.75 mL/min
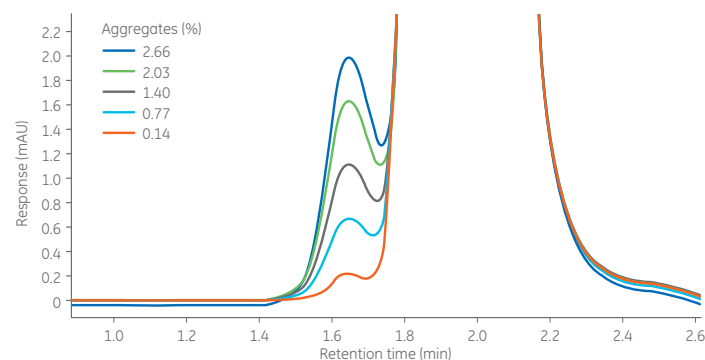System: Agilent™ 1260 Infinity II Bio-Inert LC System (UHPLC)

Fig 12. Screening of mAb aggregates on Superdex 200 Increase 5/150 GL at runtimes down to 4 min.

Studying aggregation and degradation over time

In structural and functional protein studies, it is essential that the prepared protein does not aggregate, oligomerize, or degrade. With analytical SEC, tendency to aggregate and other size changes can be studied under different conditions, such as in storage stability analyses of biopharmaceuticals.

In the examples in Figures 13 and 14, Superdex 75 Increase 5/150 GL was used to monitor small changes in size homogeneity of two proteins stored under different conditions and over different time periods (weeks). The HPLC system used was equipped with an autosampler to allow analysis of many samples during long, unattended, overnight SEC runs.

Column: Superdex 75 Increase 5/150 GL
Sample volume: 10 µL
Flow rate: 0.5 mL/min
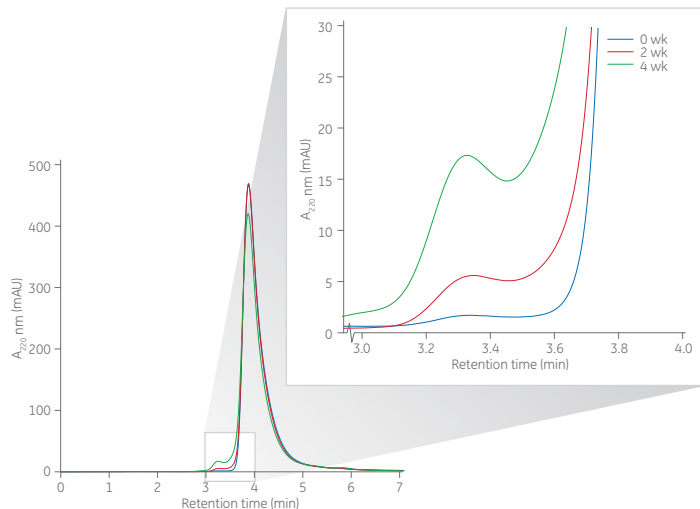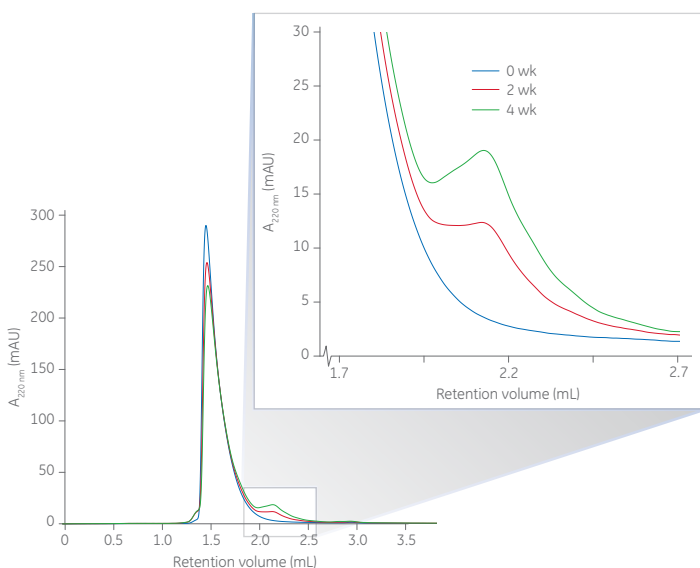*System:*   Agilent 1100

Fig 13. Monitoring of size-homogeneity changes of proteins during storage (0, 2, and 4 wk) using Superdex 75 Increase 5/150 GL for rapid analyses of higher molecular weight variants. The area under the curves for the much larger main peaks decreased over time and corresponded to the area increase seen for the small fronting peaks.

Column: Superdex 75 Increase 5/150 GL
Sample volume: 10 µL
Flow rate: 0.5 mL/min
System: Agilent 1100

Fig 14. Monitoring of size-homogeneity changes of proteins during storage (0, 2, and 4 wk) using Superdex 75 Increase 5/150 GL for rapid analyses of degraded products. The area under the curves for the much larger main peaks decreased over time and corresponded to the area increase seen for the small tailing peaks.

Studying the initiation of α-synuclein aggregation by an aldehyde

Lewy bodies, abnormalities found inside nerve cells in patients with Parkinson's disease and related neurodegenerative disorders, constitute mainly aggregated forms of the protein α-synuclein. Aldehydes formed during oxidative stress are believed to be involved in the formation of Lewy bodies. Superose 6 Increase 3.2/300 column was used for analysis of oligomerized α-synuclein for further *in vitro* and *in vivo* studies (Fig 15).

Column: Superose 6 Increase 3.2/300
Sample volume: 10 µL
Flow rate: 0.05 mL/min
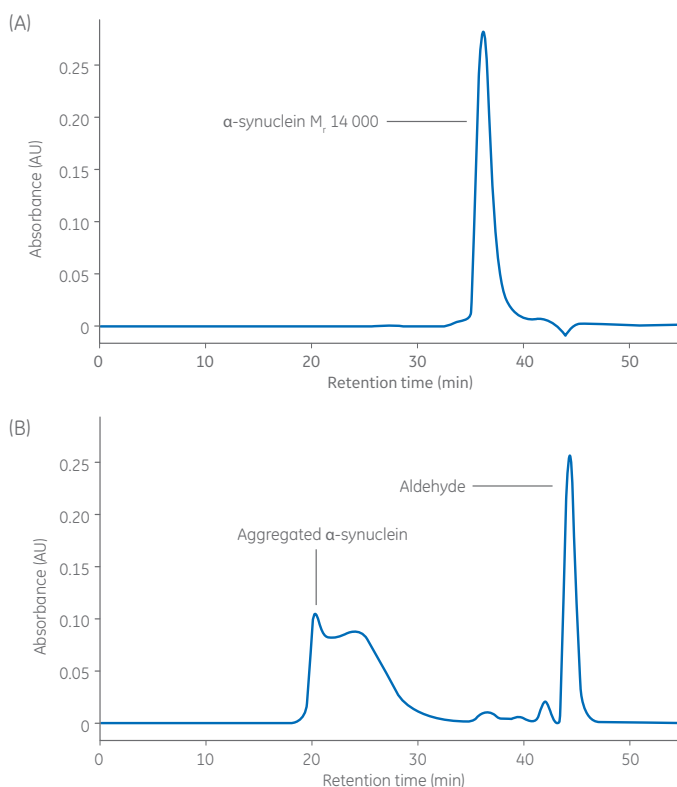*System:*   Merck Hitachi D-7000 LaChrom

Fig 15. Superose 6 Increase 3.2/300 used for preparation of oligomerized α-synuclein for further *in vitro* and *in vivo* studies. In chromatogram (A) a monomeric form of **α**-synuclein is seen. In chromatogram (B), no monomers of the protein remain after incubation with aldehydes; only oligomerized α-synuclein is present. Data courtesy of Dr. Joakim Bergström, Rudbeck Laboratory, Uppsala University.

Conclusions

GE's range of new-generation, agarose-based SEC Increase columns are should be the first choice for high-resolution analysis of proteins and other biomolecules. The narrow particle size distribution of Superose/Superdex Increase resins ensure reproducible results.

The high pH and buffer stability and excellent resolution combine to make the new-generation SEC prepacked columns the go-to columns for analytical SEC.

References

1. Fekete, S. *et al.* Critical evaluation of fast size exclusion chromatographic separations of protein aggregates applying sub-2 μm particles. *J. Pharm. Biomed. Anal.* **5 (78–79)** 141–149 (2013). https://doi.org/10.1016/j.jpba.2013.02.013

2. Vajda, J. and Romling, R. New approaches to HPLC analysis of antibody aggregates and fragments, *Chromatogr. Today* **5**, 44–47 (2012).

3. Liu, Y. *et al.* Coil-stretch transition of high molar mass polymers in packed-column hydrodynamic chromatography. *Macromolecules* **38**, 7476–7484 (2005). https://doi.org/10.1021/ma050964g

4. Goyon, A. *et al.* Evaluation of size exclusion chromatography columns packed with sub-3 μm particles for the analysis of biopharmaceutical proteins. *J. Chromatogr. A* **1498**, 80–89 (2017). https://doi.org/10.1016/j.chroma.2016.11.056

5. Jungbauer, A. Chromatographic media for bioseparation, *J. Chromatogr. A* **1065**, 3–12 (2005). http://dx.doi.org/10.1016/j.chroma.2004.08.162

6. Hahn, R. Methods for characterization of biochromatography media, *J. Sep. Sci.* **35**, 3001–32 (2012). https://doi.org/doi:10.1002/jssc.201200770

7. Schuch, B. *et al.* The exosome-binding factors Rrp6 and Rrp47 form a composite surface for recruiting the Mtr4 helicase. *EMBO J.* **33(23)**, 2829–2846 (published online Oct. 15. 2014). https://doi.org/10.15252/embj.201488757

8. Petrovic A. *et al.* Structure of the MIS12 Complex and molecular basis of its Interaction with CENP-C at human kinetochores. *Cell* **167(4)**, 1028–1040 (2016). http://dx.doi.org/10.1016/j.cell.2016.10.005

9. Mazzorana, M. *et al.* Ca-dependent folding of human calumenin. *PLoS One.* **11(3)**, e0151547 (published online Mar. 18.2016). https://doi.org/10.1371/journal.pone.0151547

10. Akita, H. *et al.* Characterization of the *Kluyveromyces marxianus* strain DMB1 YGL157w gene product as a broad specificity NADPH-dependent aldehyde reductase. *AMB Express* **5**, 17 (published online Mar 3. 2015). https://doi.org/10.1186/s13568-015-0104-9

11. Baig, T. T. *et al.* Determinants of Efficient Degradation of APOBEC3 Restriction Factors by HIV-1 Vif. *J. Virol.* **88(24)**, 14380–14395 (2014). https://doi.org/10.1128/JVI.02484-14

12. Behr, S. *et al.* Insights into the DNA-binding mechanism of a LytTR-type transcription regulator. *Biosci. Rep.* **36(2)**, e00326 (published online Apr. 27 2016). https://doi.org/10.1042/BSR20160069

13. Kaundal, S. *et al.* Dual role of a biosynthetic enzyme, CysK, in contact dependent growth inhibition in bacteria. *PLoS One* **11(7)**, e0159844 (published online Jul. 26 2016). https://doi.org/10.1371/journal.pone.0159844

14. Michinori Kakisaka, M. *et al.* A novel antiviral target structure involved in the RNA binding, dimerization, and nuclear export functions of the influenza A virus nucleoprotein. *PLoS Pathog.* **11(7)**, e1005062 (published online Jul. 29 2015). https://10.1371/journal.ppat.1005062

15. Shuhua Thong, S. *et al.* Defining key roles for auxiliary proteins in an ABC transporter that maintains bacterial outer membrane lipid asymmetry. *eLife.* **5**, e19042 (published online Aug. 16 2016). https://doi.org/10.7554/eLife.19042

16. Gaik, M. *et al.* Structural basis for assembly and function of the Nup82 complex in the nuclear pore scaffold. *J. Cell Biol.* **208(3)**, 283–297 (2015). https://doi.org/10.1083/jcb.201411003

17. Yan, R. *et al.* The eukaryotic-specific ISD11 is a complex-orphan protein with ability to bind the prokaryotic IscS. *PLoS One* **11(7)**, e0157895 (published online Jul. 18. 2016). https://doi.org/10.1371/journal.pone.0157895

18. Fernandez-Leiro, R. *et al.* cryo-EM structures of the *E. coli* replicative DNA polymerase reveal its dynamic interactions with the DNA sliding clamp, exonuclease and τ. *eLife* **4**, e11134 (published online Oct. 24 2015). https://doi.org/10.7554/eLife.11134

19. Heintz, U. and Schlichting, I. Blue light-induced LOV domain dimerization enhances the affinity of Aureochrome 1a for its target DNA sequence. *eLife.* **5**, e11860 (published online Jan. 12 2016). https://doi.org/10.7554/eLife.11860

20. Malaby, A. W. *et al.* Methods for analysis of size-exclusion chromatography–small-angle X-ray scattering and reconstruction of protein scattering. *J. Appl. Crystallogr.* **48(4)**, 1102–1113 (2015). https://doi.org/10.1107/S1600576715010420

21. Hürlimann, L. M. *et al.* The Heterodimeric ABC Transporter EfrCD mediates multidrug efflux in *Enterococcus faecalis*. *Antimicrob. Agents Chemother.* **60(9)**, 5400–5411 (2016). https://doi.org/10.1128/AAC.00661-16

Ordering information

Size exclusion chromatography columns

Product	Code number
Superdex 30 Increase 10/300 GL	29219757
Superdex 30 Increase 5/150 GL[1]	Custom product
Superdex 30 Increase 3.2/300	29219758
Superdex 75 Increase 10/300 GL	29148721
Superdex 75 Increase 5/150 GL	29148722
Superdex 75 Increase 3.2/300	29148723
Superdex 200 Increase 10/300 GL	28990944
Superdex 200 Increase 5/150 GL	28990945
Superdex 200 Increase 3.2/300	28990946
Superose 6 Increase 10/300 GL	29091596
Superose 6 Increase 5/150 GL	29091597
Superose 6 Increase 3.2/300	29091598

Whatman™ syringe filters for sample clarification (prior to injection to the HPLC system)

SPARTAN™ HPLC Syringe Filter, 13 mm, regenerated cellulose, 0.2 µm, w. minitip outlet[2]	10463042
Mini-UniPrep™ Syringeless Filter, translucent housing, standard cap, 0.2 µm, regenerated cellulose[2]	UN203NPERC

[1] Not available as standard product. Can be ordered as custom product.

[2] Other syringe filter diameters, pore sizes, and membrane materials are available.
Visit gelifesciences.com/LabFiltration.

GE Healthcare Bio-Sciences AB
Björkgatan 30
SE-751 84 Uppsala
Sweden