

Universal mobile phase for size-exclusion chromatography (SEC)

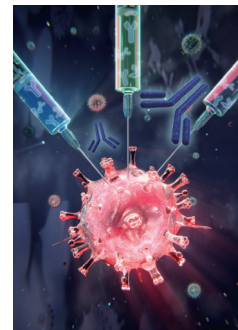
Arg-SEC Mobile Phase

Size-Exclusion Chromatography (SEC) is an essential technology for developing biopharmaceuticals. SEC is normally carried out in aqueous solution so that the mobile phase has a minimal impact on the target biopharmaceutical proteins, e.g., not disrupting the protein structure. However, such mobile phase is not often optimal for the performance of SEC. Proteins often bind to the SEC column surface, leading to a compromised protein elution and peak resolution. Such binding can cause incorrect determination of the size of the eluted proteins and the amount of the aggregated species. To overcome these problems, arginine-based mobile phase^{*1} has been developed. This mobile phase reduces non-specific protein binding to many SEC columns, while not affecting the protein structure. Depending on the strength of non-specific binding, there are 3 mobile phases: strong, standard and mild.

This product is manufactured with permission from Ajinomoto Co., Inc. based on the patent JP 4941882.^{*2}

^{*1} Arginine is effective in suppressing non-specific molecular interactions.

^{*2} JP: 4941882, US: 7501495, EP: 1698637



Features:

- **Increased recovery of proteins and peptides that are more hydrophobic and have stronger tendency to stick to the columns.**
- **Correct determination of protein aggregates that have stronger tendency to stick to the columns than the monomeric species.**
- **Optimal for quality control.**
- **Effective separation of protein conjugates, e.g., ADC and sticky cytokines.**

Advantages of using this product:

1. Quantitative analysis of protein (in particular sticky protein) samples.
2. Applicable to many commercial SEC columns.
3. Reduce lot-to-lot variability due to non-specific protein binding.
4. Less non-specific protein binding increases the life time of the columns.
5. Reduce the time required to condition the columns.
6. Increase the purification yield as a result of less binding.

Arg-SEC Mobile Phase Series

Product ID	Application	Example
Standard	First choice, all proteins	IgGs
Strong	More hydrophobic, sticky proteins	TGF- β 3, Aggregates
Mild	Oligomeric proteins with weak subunit-subunit interaction	Hemoglobin*

Filtered through 0.22 μ M filter, ready to use, pH 6.8, contains arginine.

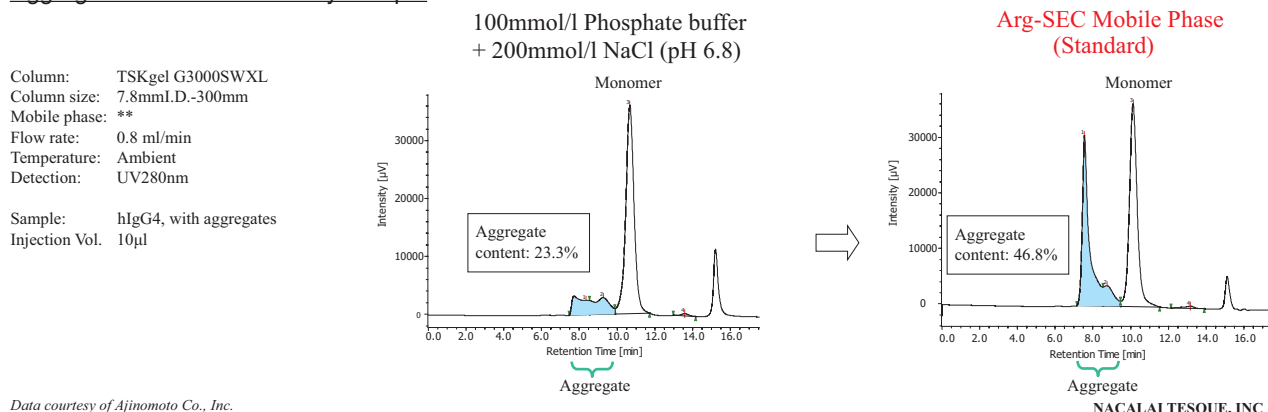
*Such a weakly associating oligomeric protein as hemoglobin may dissociate to monomers in the presence of arginine.

Comparison 1

Data 1: Aggregate content of hlgG4 mAb determined by the standard strength Arg-SEC mobile phase in comparison with NaCl-based SEC mobile phase.

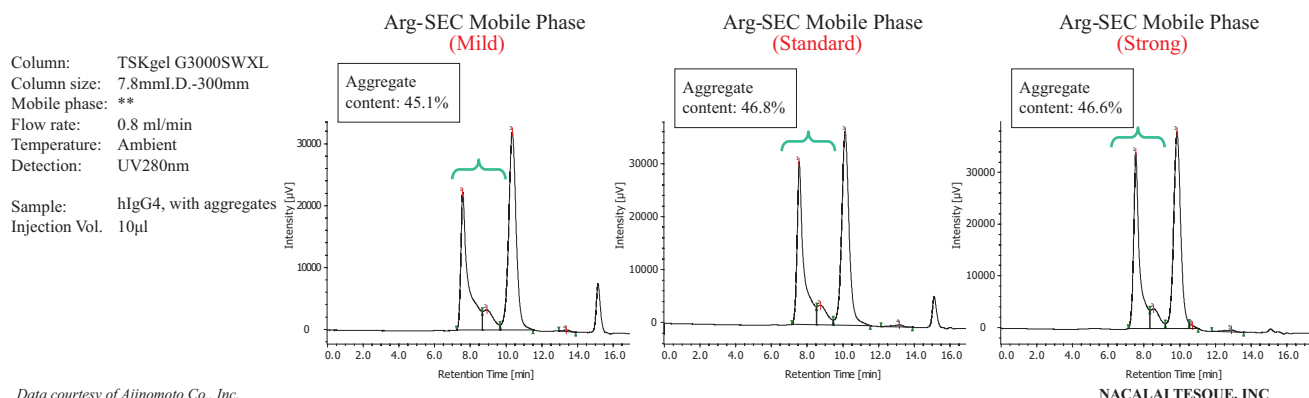
A mAb, hlgG4, containing aggregated species, was analyzed by SEC using our standard Arg-SEC mobile phase and NaCl-based 100 mM phosphate. Although the monomer content was identical between these 2 mobile phases, the aggregate content was much higher with our Arg-SEC mobile phase. This is due to suppression of non-specific adsorption of the aggregates to the SEC column by Arg-SEC mobile phase, but not the NaCl-based mobile phase.

Aggregate Content of Antibody Sample



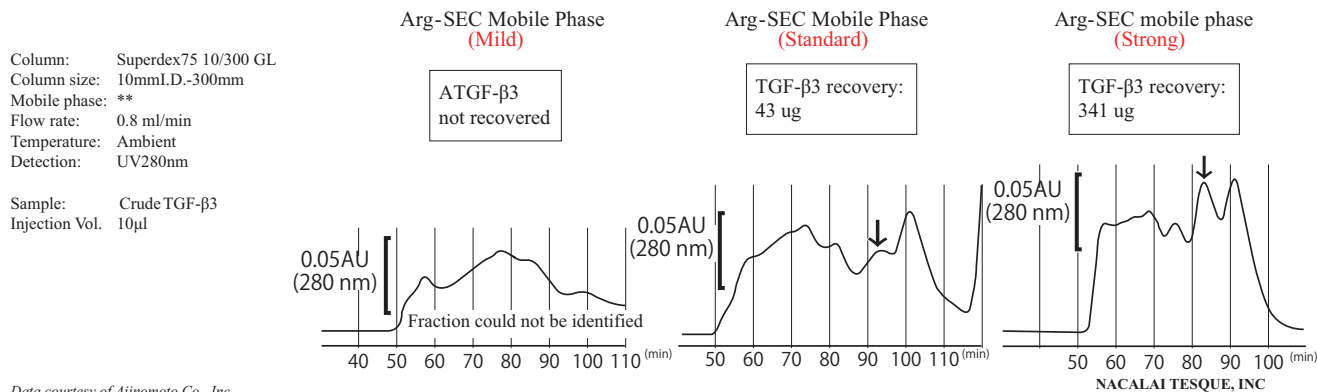
Data 2: Comparison of Mild, Standard and Strong all showed comparable aggregate recovery and monomer peak size. For this particular analysis, any Arg-SEC mobile phase can be used.

Aggregate Content of Antibody Sample



Data 3: Comparison of Mild, Standard and Strong showed significant differences for the recovery of TGF-β3. Such a stocky protein as TGF-β3 cannot be recovered with the NaCl-based mobile phase nor with Arg-SEC Mild, but can be recovered by Standard and Strong. A much greater recovery was obtained with the Strong.

Purification of Crude TGF-β3



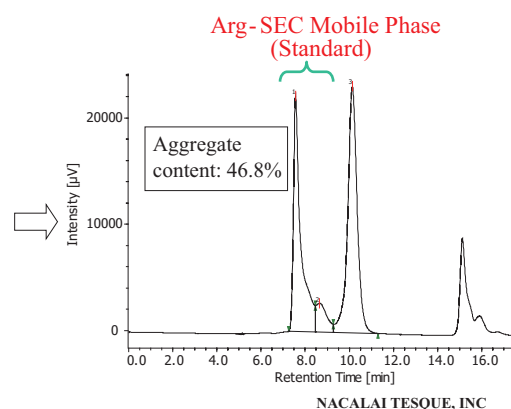
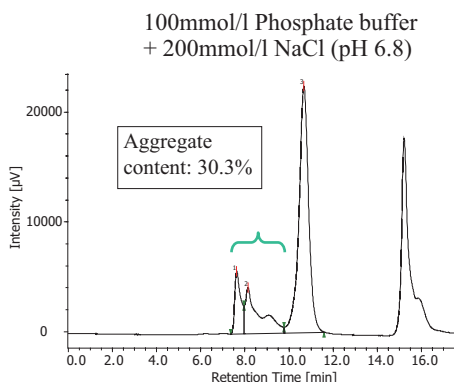
Comparison 2

Arg-SEC Standard mobile phase works on any column and gives a consistently higher aggregate recovery than NaCl-based mobile phases:

Arg-SEC can overcome differences in non-specific characteristics of three commercial columns. With the NaCl-based mobile phase, 3 columns gave a low and variable aggregate content due to different surface properties of the columns and non-specific binding. On the contrary, Arg-SEC mobile phase suppressed non-specific binding on all 3 columns and gave a consistent and expected aggregate content. Such effects should help minimize the lot-to-lot or column-to-column variation of non-specific protein binding.

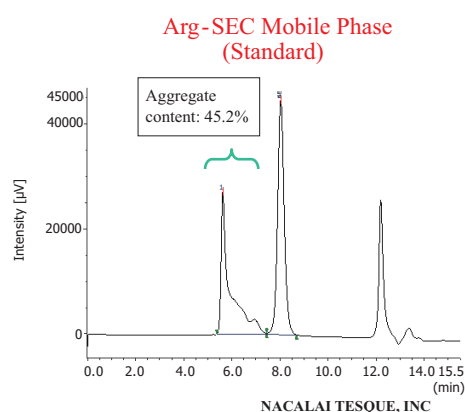
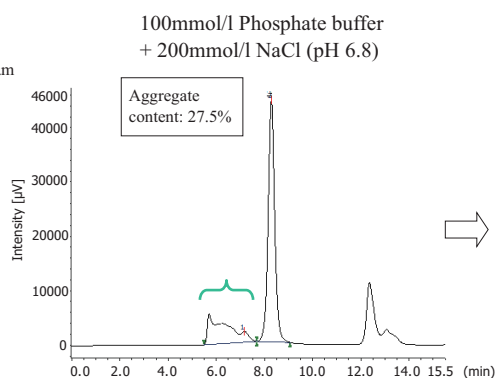
Column A

Column: TSKgel G3000SWXL
Column size: 7.8mm I.D.-300mm
Mobile phase: **
Flow rate: 0.8 ml/min
Temperature: Ambient
Detection: UV280nm
Sample: mIgG1, with aggregates
Injection Vol. 10µl



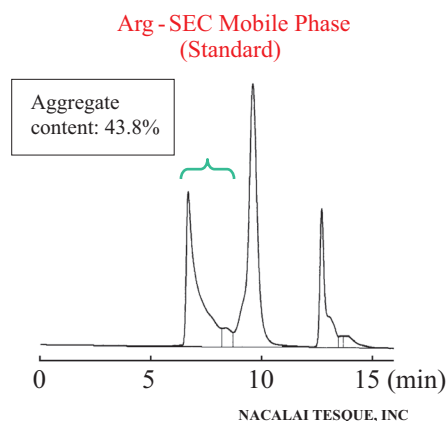
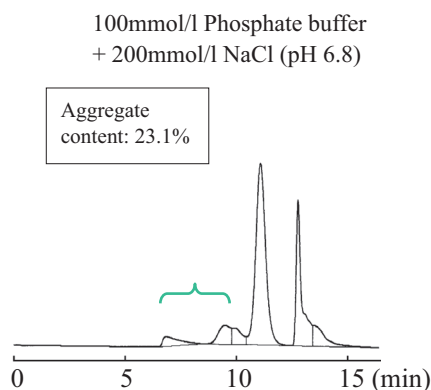
Column B

Column: AdvanceBIO SEC 300 Å, 2.7µm
Column size: 7.8mm I.D.-300mm
Mobile phase: **
Flow rate: 0.8 ml/min
Temperature: Ambient
Detection: UV280nm
Sample: mIgG1, with aggregates
Injection Vol. 10µl



Column C

Column: COSMOSIL 5Diol-300-II
Column size: 7.5mm I.D.-300mm
Mobile phase: **
Flow rate: 0.8 ml/min
Temperature: 30°C
Detection: UV280nm
Sample: mIgG1, with aggregates
Injection Vol. 10µl



Applications

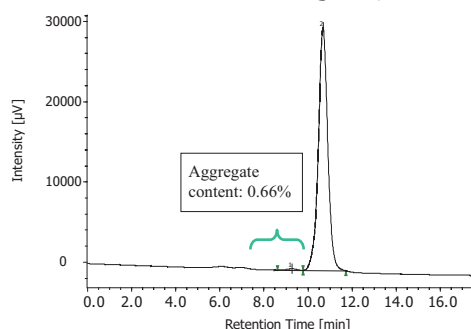
Arg-SEC can give correct estimate of minute amount of aggregate content. Arg-SEC mobile phase gave a slightly, but significantly, higher aggregate content (both panels) and a higher protein recovery (bottom panel) than NaCl-based mobile phase.

Aggregate Content of Antibody Sample

100mmol/l Phosphate buffer
+ 200mmol/l NaCl (pH 6.8)

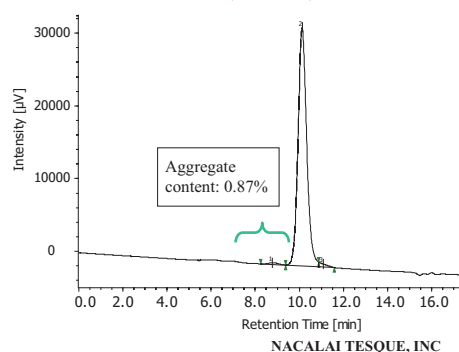
Column: TSKgel G3000SWXL
Column size: 7.8mm I.D.-300mm
Mobile phase: **
Flow rate: 0.8 ml/min
Temperature: Ambient
Detection: UV280nm

Sample: hIgG4 monomer
Injection Vol. 10µl



Data courtesy of Ajinomoto Co., Inc.

Arg-SEC Mobile Phase
(Standard)



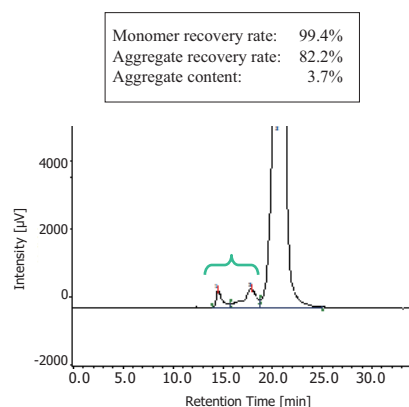
NACALAI TESQUE, INC

Aggregate Content of Antibody Sample

100mmol/l Phosphate buffer
+ 200mmol/l NaCl (pH 6.8)

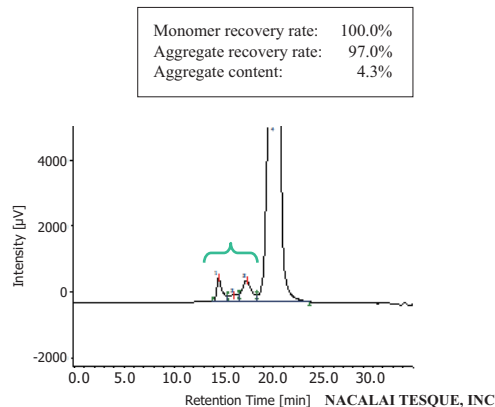
Column: Superdex200 Increase
Column size: 10mm I.D.-300mm
Mobile phase: **
Flow rate: 0.8 ml/min
Temperature: Ambient
Detection: UV280nm

Sample: mIgG1 (15µg)



Data courtesy of Ajinomoto Co., Inc.

Arg-SEC Mobile Phase
(Mild)



NACALAI TESQUE, INC

Caution:

Arginine has moderate absorbance in far UV region and thus care should be exercised when using 220 nm. Using UV 280 nm is recommended.

Ordering Information

Product Name	Grade	Storage	Product No.	PKG Size
Arg-SEC Mobile Phase(Standard)	SP	Room Temperature (Refrigerate after opening)	16999-31	1 L
Arg-SEC Mobile Phase(Strong)			17000-51	1 L
Arg-SEC Mobile Phase(Mild)			16998-41	1 L

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