Selecting Fit-for-Purpose SEC Columns for Biotherapeutic Aggregate Analysis

Pore size is a crucial characteristic of SEC columns because it dictates separation efficiencies within a suitable range.

Considerations for Method Development

Stationary Phase

Size exclusion chromatography is a non-adsorptive separation technique. To achieve efficient separation, reducing hydrophobic interactions is critical. Biosep™ and Biozen™ SEC columns mitigate hydrophobicity with hydrophilic stationary particle chemistries.

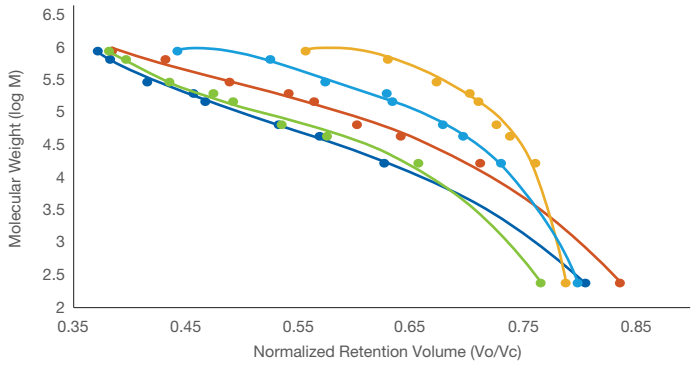
Pore Size

As a rule of thumb, the pores should be approximately two to three times larger than the largest analyte. If a column is supposed to separate an antibody sample with aggregates (≥ 10 nm), monomer (5 nm) and fragments (≤ 5 nm), a pore size of 25 nm (250 Å) is appropriate.

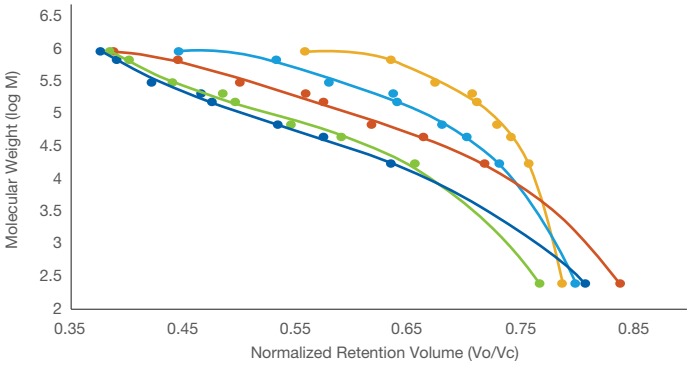
Other Parameters

Flow rates and additive selections (salts, Arginine, organic solvents) can impact separation efficiencies. Reducing the hydrophobicity of the stationary phase enables the use of friendlier additive concentrations and mitigates potential secondary interactions, ensuring optimal separation, column efficiencies, and high-quality data.

100 mM Sodium Phosphate, pH 6.8



200 mM Potassium Phosphate + Potasssium Chloride, pH 6.2



SEC-2, 150 Å
SEC-3, 300 Å
dSEC-2, 200 Å
dSEC-7, 700 Å
BioSep SEC 4000s, 500 Å

Protein Mix (MW): Uridine (244), Myoglobin (1700), Ovalbumin (44000), BSA (66463), IgG (150000), B-Amylase (200000), IgA (300000), Thyroglobulin (670000), IgM (900000)

A Continuing Success of Biozen SEC Columns for Biotheraputic Aggregate Analysis

BioSep™ Aqueous GFC/SEC
1992- 2015
First generation SEC columns for biomolecules

Biozen™ by Phenomenex
2018
SEC-2, 150 Å & SEC-3, 300 Å

Biozen™ by Phenomenex
2021
dSEC-2, 200 Å

Biozen™ by Phenomenex
2024
dSEC-7, 700 Å

Need help selecting your fit-for-purpose SEC column? Chat with us at

Phenomenex.com/chat

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