

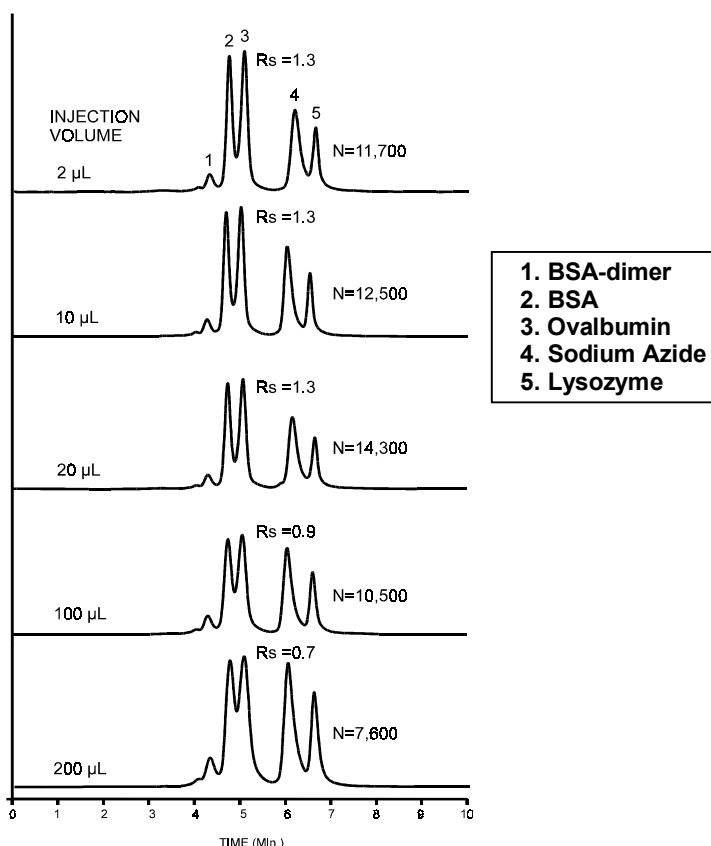
Effect of Injection Volume on Resolution in Size-Exclusion Chromatography

Application

Biochemical

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Injection volume is one of the critical factors to consider when optimizing size-exclusion chromatography (SEC). While sample concentration can be very high in SEC with little effect on resolution, large sample volumes can dramatically increase peak width. Protein samples of 2 to 200 μL were injected onto a 9.4 x 250 mm GF-250 column to obtain a guideline for effective injection volumes. Values are shown for resolution (R_s) of peaks 2 and 3, and for plates (N) of peak 5.



Conditions:
ZORBAX GF-250 (9.4 x 250 mm) (Agilent P/N: 884973-901)
Mobile Phase: 200mM Sodium Phosphate, pH 7.0
Injection 2-200 μL , 1 mL/min, Ambient, Detect. UV (225 nm)

Highlights

- Keep sample injection-volumes small (e.g., 2-20 μL) when maximum resolution is desired. Resolution and plate values decrease dramatically for the 100 and 200 μL injections on a 9.4 x 250 mm SEC column.
- In SEC, limits on load volumes can be compensated for by increasing sample concentration.



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