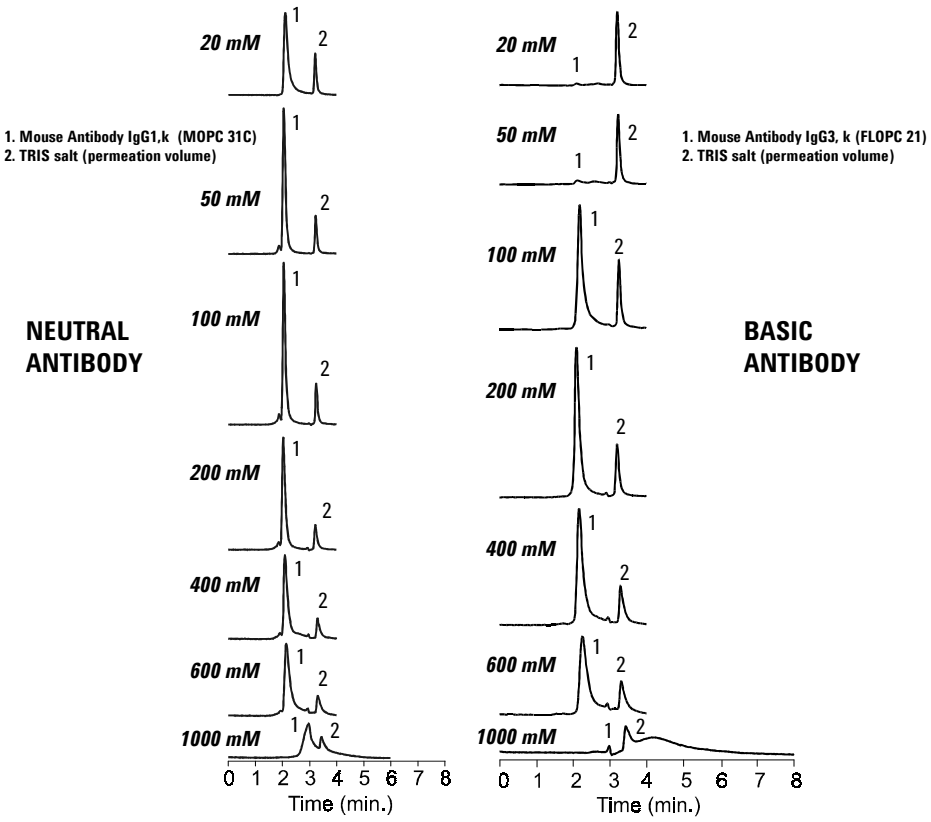
Effect of Mobile-Phase Ionic Strength on Neutral and Strongly Basic Antibodies at pH 7.0

Application

Biochemical

Robert Ricker

Separation of antibodies from antibody conjugants and reaction components is a job well suited to size-exclusion chromatography (SEC). However, mobile-phase ionic strength and pH have a strong influence over proper elution of antibodies and other biomolecules. The chromatograms below show the effect of phosphate buffer concentration on peak shape and retention.



Conditions:
ZORBAX GF-250 (4.6 x 250 mm) (Agilent P/N: 884973-701)
Mobile Phase: Sodium Phosphate, pH 7.0 (concentration as shown)
Injection volume 2µL, 0.96 mL/min, 35°C, Detect. UV (230 nm)

Highlights

- *All SEC columns have some electrostatic and hydrophobic characteristics. Therefore, take care to carry out experiments under conditions that promote ideal SEC behavior.*
- *Strongly basic molecules (right panel) interact with silica-based column packings to a much greater extent than neutral molecules (left panel). Note the complete adsorbance of IgG_3 at low ionic strength; and peak tailing of both antibodies at low and high ionic strength.*

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