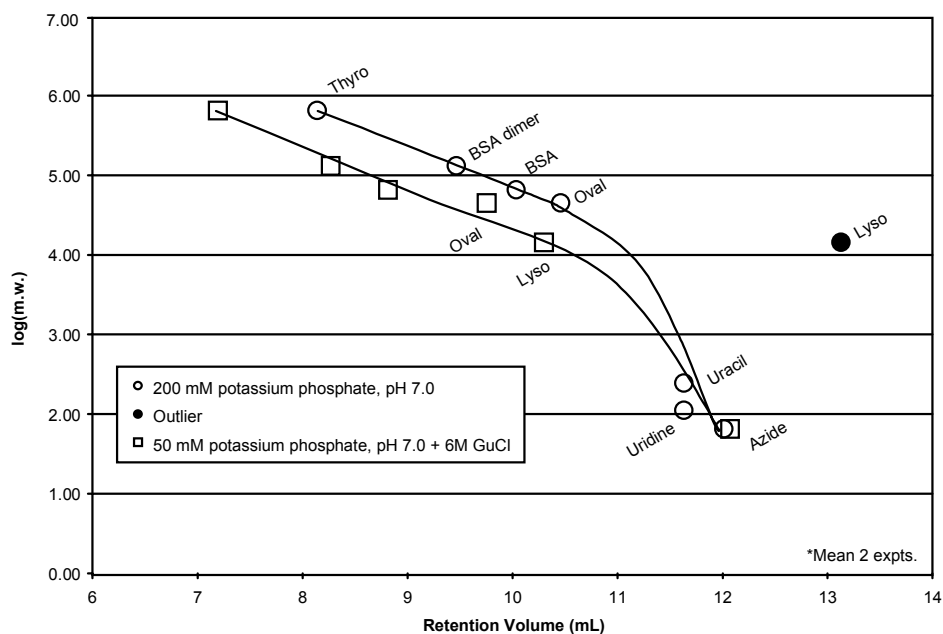# Comparison of Denaturing and Nondenaturing Mobile Phases in SEC: ZORBAX GF-450, +/- GuCl

Application

Biochemical

Robert Ricker

Size-exclusion chromatography is a powerful technique for the size-dependent separation of biomolecules. While standard, non-denaturing mobile phases are often desirable, denaturing mobile phases containing guanidine hydrochloride (or SDS), may be used to insure un-aggregated, monomers for proper size determination. As shown below, denaturation shifts molecules toward larger apparent size and smaller retention volume.

Chart: log(m.w.) versus Retention Volume (mL)

Legend:
- ○ 200 mM potassium phosphate, pH 7.0
- ● Outlier
- □ 50 mM potassium phosphate, pH 7.0 + 6M GuCl

Labeled points: Thyro, BSA dimer, BSA, Oval, Lyso, Oval, Lyso, Uracil, Uridine, Azide, Lyso

*Mean 2 expts.

Conditions:
LC: Hewlett Packard HP1050
Column: ZORBAX GF-450 (9.4 x 250), Agilent Part No. 884973-902
UV: 254 nm
Flow: 2.0 mL / min.; ambient
Inj. Vol.: 20 µL (1 µg / µL)

Highlights

- Agilent ZORBAX GF-450 columns separate biomolecules in a size-dependent manner within a molecular-weight range of 10,000-1,000,000 daltons. Note, some molecules (depending upon their characteristics) are retained or excluded by non-SEC mechanisms and are shifted from linearity.

- ZORBAX GF-450 columns are manufactured using extremely hard particles, allowing high flow rates and fast separations.

- When smaller molecules are used in combination with denaturing mobile phases, ZORBAX GF-250 may be used to obtain more-linear separation in the lower molecular-weight range.

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