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Gradient Elution Behavior of Proteins in Hydrophobic Interaction Chromatography with U-Shaped Retention Factor Curves

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HIGHLIGHTS

- Proteins exhibit U-shaped retention factor vs ammonium sulfate molarity in HIC
- Gradient slope determines protein retention and recovery in LGE
- Local equilibrium model predicts critical gradient slope
- Detailed numerical model predicts recovery as a function of gradient slope

Abstract

Protein retention in hydrophobic interaction chromatography is described by the solvophobic theory as a function of the kosmostropic salt concentration. In general, an increase in salt concentration drives protein partitioning to the hydrophobic surface while a decrease reduces it. In some cases, however, protein retention also increases at low salt concentrations resulting in a U-shaped retention factor curve. During gradient elution the salt concentration is gradually decreased from a high value thereby reducing the retention factor and increasing the protein chromatographic velocity. For these conditions, a steep gradient can overtake the protein in the column, causing it to rebind. Two dynamic models, one based on the local equilibrium theory and the other based on the linear driving force approximation, are presented. We show that the normalized gradient slope determines whether the protein elutes in the gradient, partially elutes, or is trapped in the column. Experimental results are presented for two different monoclonal antibodies and for lysozyme on Capto Phenyl (High Sub) resin. One of the mAbs and lysozyme exhibit U-shaped retention factor curves and for each, we determine the critical gradient slope beyond where 100% recovery is no longer possible. Elution with a reverse gradient is also