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Effect of mass overloading on binding and elution of unstable proteins in Hydrophobic Interaction Chromatography

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Highlights

- Adsorption behavior of marginally stable proteins on a HIC medium was studied
- Influence of mass overloading on the retention pattern of proteins was determined
- A mechanistic model was developed and exploited to predict the column dynamics
- nanoDSF was used to determine protein stability versus mass overloading

2. Abstract

Adsorption behavior of unstable proteins, i.e., bovine serum albumin and α -lactalbumin, has been studied on a hydrophobic interaction chromatography medium under mass overloading conditions at different kosmotropic salt concentrations in the mobile phase. A mechanistic model has been formulated and used to describe kinetics and thermodynamics of protein interactions with the adsorbent surface. The model assumed two-site binding adsorption and reversible protein unfolding, which allowed predicting the inhibition of protein unfolding at high column loadings. A simplified procedure for the determination of model parameters has been developed, which was based on the inverse method. The model was successfully used to reproduce the pattern of chromatographic elution as well as the course of breakthrough curves. The model formulation was supported by Nano Differential Scanning

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