120f Effects of Reversible Association on Size Exclusion Chromatography of Proteins

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Reversible association reactions of proteins can cause peak splitting, merging, tailing, and fronting in size exclusion chromatography (SEC). The reactions can lead to loss of purity or yield. Therefore, it is important to understand and to control the effects of reversible association in SEC. Theoretical analysis and simulations based on a general rate model are used to understand the effects of dimer formation in SEC. Dimensionless group analysis shows that N_{k+}, which is the ratio of association rate and convection rate, and N_{k-}, which is the ratio of dissociation rate and convection rate, are the two key dimensionless groups in this system. A diagram based on N_{k+} and N_{k-} is built according to the analysis and the simulation results. When both N_{k+} and N_{k-} are small, the individual forms behave as separate species and show separated peaks. When N_{k+} is large but N_{k-} is small, dimers dominate and show one single dimer peak. When N_{k+} is small but N_{k-} is moderate, monomers dominate, but some dimers exist and they are partially separated from the monomers, resulting in one monomer peak with fronting. When both N_{k+} and N_{k-} are moderate, the monomer peak and the dimer peak merge, resulting in a broad peak. When N_{k+} is small but N_{k-} is large, monomers dominate, showing one single monomer peak. When both N_{k+} and N_{k-} are large, the individual forms behave as a single component with an average molecular weight, resulting in a merged peak with tailing. This diagram lumps the effects of association rate, dissociation rate, equilibrium constant, feed concentration, column length and linear velocity into the two key dimensionless groups. According to this diagram, peak shape and retention behavior can be well predicted by the two dimensionless groups, instead of the individual variables. This diagram is a convenient tool to predict the elution behavior in SEC with reversible dimerization. The results suggest potential strategies to control peak splitting or peak merging in such systems.