SCANNING MOLECULAR SIEVE CHROMATOGRAPHY OF INTERACTING PROTEIN SYSTEMS

EFFECT OF KINETIC PARAMETERS ON THE LARGE ZONE BOUNDARY PROFILES FOR LOCAL EQUILIBRATION BETWEEN MOBILE AND STATIONARY PHASES

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Large zone reaction boundary profiles for molecular sieve chromatography as affected by kinetic parameters have been simulated for local equilibration between the mobile and stationary phases. Our studies of monomer-dimer and monomer-tetramer systems indicate that in a slowly equilibrating system, the kinetic controls operating between the mobile and stationary phases contribute most significantly to the overall boundary profile. In a rapidly equilibrating system, however, the kinetic parameters k_{ij} and k_{ji} operating in the mobile phase are the principal determinants of the reaction boundary, while the kinetic effects of k_{ii} and k_{jj} between the mobile and stationary phase are minimal.

The solute partition cross-section and axial dispersion operator (1) of the boundary profiles of such an interacting protein system under kinetic control have been simulated by (*a*) time discretization, (*b*) the finite difference approximation of Fick's second law (2) and (*c*) the kinetic expression for the concentration of monomer, $C_{1(t)}^*$, as a function of equilibration time (3). Here, the expression for idealized chromatographic continuity is (4):

$$(\partial C_i^*/\partial t) = L_i(\partial^2 C^*/\partial X^2) - (F/\xi_i)(\partial C/\partial X) - \sum_{j\neq i} k_{ij}(C_i^*)^j + \sum_{j\neq i} k_{ji}(C_j^*)^i - \sum_{i} k_{ii}C_i^* + \sum_{j} k_{jj}C_i^{**}, \qquad (1)$$

where k_{ij} is the chemical reaction rate from species i to j. C_i^* is the concentration of species i. The $-\sum_{j\neq i} k_{ij}(C_i^*)^j + \sum_{j\neq i} k_{ji}(C_j^*)^i$ term represents a local equilibration in the mobile phase and $-\sum_i k_{ii}C_i^* + \sum_j k_{jj}C_i^{**}$ represents an equilibration between the mobile and stationary phases. F is the elution flow rate, ξ_i is the solute partial cross-section, and L_i the axial dispersion coefficient.

In considering a monomer-n-mer system, the kinetic effect varies with the distribution of the concentration of monomer along the column. Due to kinetic effects, the column equilibrium coefficient for a given self-associating species in such a system is not always constant. Hence, the apparent equilibrium constant will vary as a function of the distance coordinate, i.e., $\mathrm{d}K/\mathrm{d}X \neq 0$ (5).

The general kinetic expressions for the concentration of monomer as a function of equilibration time, $C^*_{1(t)}$, in the mobile phase for various types of self-associating systems become:

(I) $n = 1$, Isomerization: $\mathrm{d}C^*_1/\{[k_{ln} + k_{nl}]C^*_1 - k_{nl}C^*_T\} = -\mathrm{d}t,$

(II) $n = 2$, Monomer-dimer system:

$$\mathrm{d}C^*_1/\{[k_{ln}(C^*_1)^2 + k_{nl}C^*_1] - k_{nl}C^*_T\} = -\mathrm{d}t,$$

(III) $n = 3$, Monomer-trimer system:

$$\mathrm{d}C^*_1/\{[k_{ln}(C^*_1)^3 + k_{nl}C^*_1] - k_{ln}C^*_T\} = -\mathrm{d}t,$$

(IV) $n = 4$, Monomer-tetramer system: $\mathrm{d}C^*_1/\{k_{ln}(C^*_1)^4 + k_{nl}C^*_T\} = -\mathrm{d}t.$ (2)

Our simulation studies in monomer-dimer and monomer-tetramer systems have indicated that in a slowly equilibrating system, the kinetic rate constants k_{ii} and k_{jj} between the mobile and stationary phase contribute significantly to the overall gradient boundary profile. In contrast, in the rapidly equilibrating system, the kinetic parameters k_{ij} and k_{ji} in the mobile phase are the principal determinants of the reaction boundary. Although the kinetic effects of k_{ii} and k_{jj} may be noted, their contribution to the overall boundary profile is minimal.

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A SOLUTION MIXER WITH 10-μs RESOLUTION

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An apparatus has been developed which mixes two solutions within a time limit of 10–100 μs. The technique is based upon the mixing properties of a fluid flowing around a sphere. The two solutions are juxtaposed and constrained to a very thin layer as they flow around the sphere. The time resolution of the method depends upon the