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., $dK/dX \neq 0$ (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: $dC_1^*/\{[k_{ln} + k_{nl}]C_1^* - k_{nl}C_T^*\} = -dt$,

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

$$dC_1^*/\{[k_{ln}(C_1^*)^2 + k_{nl}C_1^*] - k_{nl}C_T^*\} = -dt,$$

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

$$dC_1^*/\{[k_{ln}(C_1^*)^3 + k_{nl}C_1^*] - k_{ln}C_T^*\} = -dt,$$

(IV) $n = 4$, Monomer-tetramer system: $dC_1^*/\{k_{ln}(C_1^*)^4 + k_{nl}C_T^*\} = -dt.$ (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

ROBERT CLEGG, *Max Planck Institute for Biophysical Chemistry, Department of Molecular Biology, 3400 Göttingen, West Germany, and*
MACK J. FULWYLER, *Becton Dickinson Electronics Laboratory, Mountain View, California 94043 U. S. A.*

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

ability to reduce the mixing dimensions as much as possible. Optical detection of the progress of the reaction is accomplished by measuring the fluorescence of the liquid jet at different positions along the emerging stream.

KINETIC TRANSIENTS

A Wedding of Empiricism and Theory

Alan H. Colen, *Veterans Administration Hospital, Kansas City, Missouri 64128, and The University of Kansas School of Medicine, Lawrence, Kansas 66044 U. S. A.*

The purpose of the study of transient-state kinetics is to separate events in time and thus contribute to a detailed understanding of underlying mechanism. We have recently developed some simple but novel methods to achieve this, including the rapid-flow calorimetric determination of the kinetics of oxime formation (1) as a model for imine formation in enzymatic reactions, the stopped-flow ultraviolet spectrophotometric measurement of hydrogen exchange in nucleotides and of the effect of binding by dehydrogenases on that process (2), and the observation of transient features of enzymatic reactions over a period of minutes by using the cryoenzymological approach of Douzou and Fink (3). Thus, either by mixing and observing rapidly or by slowing the reaction sufficiently we can observe and characterize complex behavior ordinarily inaccessible to steady-state or relaxation kinetics techniques.

Many qualitative empirical conclusions can be obtained from studies of kinetic transients: "lags" give information about the kinetics of formation of precursor complexes; the existence of a "burst" suggests that there is a slow step late in the mechanism, possibly involving the formation of tight product complexes; time-dependent spectral shifts often help identify reaction intermediates; and preincubation effects (or lack thereof) give clues about slow steps and obligatory pathways in biochemical reactions. All of these phenomena can yield detailed information about the relative rates of formation and breakdown of complexes with effectors.

Quantitative mathematical and theoretical treatment of biochemical transients and related fast reactions is required, however, to give flow methods their full power as tools for the determination of mechanism. Although detailed treatments are available and many transient kinetics studies have been performed, some powerful but simple empirical methods for obtaining and handling data to yield a quantitative understanding of the processes underlying observed transients far from equilibrium have not been fully exploited. Two such approaches are developed and illustrated here with applications to the study of the glutamate dehydrogenase-catalyzed reaction, relating empirical observation to mechanistic description.

The first method is the study of the initial velocities of transients. These may be measured either directly for initially linear time-courses or by extrapolation of nonlinear experimental time-course curves to an empirically determined experimental time