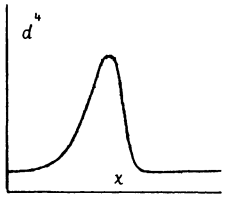

FIGURE 5

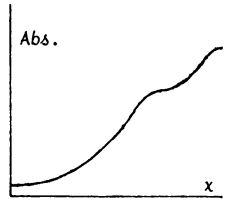

FIGURE 6

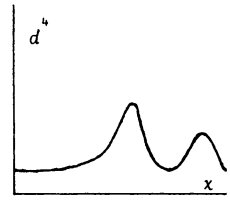

FIGURE 7

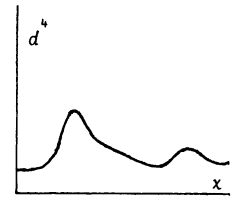

FIGURE 8

times involved, provided they are sufficiently separated in time (if not, a shifting of the peak positions will occur; however, this can be corrected by a simple iterative computer program).

Our instrumentation consisted of a Durrum stopped-flow apparatus (Durrum Instrument Corp., Sunnyvale, Calif.) and a Tektronix digital processing oscilloscope (DPO) coupled to a programmable desk calculator (TEK 31 Tektronix, Inc., Beaverton, Ore.). The logarithmic time base was generated by feeding an adjustable ramp function into a four-decade logarithmic amplifier (Solid State Electronics Corp., Sepulveda, Calif., 3076) and connecting its output to the external-volts output of the DPO's time base (7B70). The direct record of the reaction captured by the DPO had to be smoothed first in the calculator to eliminate the excessive noise which hindered the subsequent differentiation of the curve. Fig. 4 is the record of the pseudo-first-order reaction between Fe^{+++} and SCN^{-} before curve smoothing. Fig. 5 is the derivate of the smoothed version of the same curve raised to the fourth power. Figs. 6 and 7 are corresponding curves for the reaction between chymotrypsin and *p*-nitrophenyl acetate. Fig. 8 represents the amylose/iodine reaction. The latter figures indicate the presence of two distinct processes in these complex reactions, respectively.

Our work suggests that the logarithmic recording of kinetic results combined with the processing of the data in a DPO can be a very useful tool in the fast-reaction kinetic studies of biochemical reactions.

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FAR-ULTRAVIOLET STOPPED-FLOW CIRCULAR DICHROISM

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To follow directly the secondary structure changes involved in rapid protein-folding processes, we have designed a stopped-flow circular dichroism (CD) instrument capable of millisecond-range time resolution in the far-ultraviolet region. A stabilized Xe light source, piezo-optical birefringence modulator and phase-sensitive, heterodyning

lock-in amplification techniques are utilized in conjunction with a special stopped-flow observation chamber to produce and detect the small, rapidly varying signals. We present initial results of the application of the instrument to a complicated protein subunit folding and assembly reaction system involving reorganization on the secondary, tertiary, and quaternary structural levels. These results demonstrate the instrument's utility for separating out the kinetics and, thus, for elucidating the interplay of the structural changes at those various levels.

To illustrate instrumental capabilities, the acid denaturation of ferrihemoglobin at three pH values and a pH-jump renaturation of denatured heme-free α globin are presented, as monitored by CD at 222-nm. The fastest of these reactions, exhibiting a 42-ms reaction half-time for a total CD change of 88 millidegrees (17.2×10^3-deg-cm^{2}dmol^{-1} mean residue ellipticity), was detected with a signal-to-noise ratio of 5 to 1.

At 222 nm, with an effective OD between 0.5 and 1.5 and a smoothing time constant of 4 ms, the noise level does not exceed approximately 17 millidegrees with current components and design. The noise is sharply reduced by increasing wavelength by even a few nanometers. Modifications to reduce the noise at 222 nm by an order of magnitude are outlined.

With a total accessible wavelength range from ~200 nm to ~800 nm, the instrument should prove a useful tool in kinetic investigation of a wide array of reactions involving altered optical activity and modification of chiral centers.

A SIMPLE SYSTEM FOR MIXING MISCIBLE ORGANIC SOLVENTS WITH WATER IN 10–20 ms FOR THE STUDY OF SUPEROXIDE CHEMISTRY BY STOPPED-FLOW METHODS

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We describe a simple device capable of mixing dimethyl sulfoxide (DMSO) and aqueous solutions for spectrophotometric observation of superoxide (O_2^-) chemistry with common stopped-flow methodology (1). Studies on superoxide and particularly on its dismutation catalyzed by so-called superoxide dismutases have been forced to rely on the expensive pulse radiolysis technique or poorly defined chemical or biochemical techniques to produce superoxide (2). Pulse radiolysis can generate superoxide concentrations up to 300 μM, while chemical methods produce steady-state concentrations 1,000 times lower. A method for dissolving high concentrations (>100 mM) of commercially available potassium superoxide in DMSO has been