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## AN INEXPENSIVE MICROCOMPUTER-BASED STOPPED-FLOW DATA ACQUISITION SYSTEM

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**ABSTRACT** A low-cost (<\$2,500) microcomputer-controlled data acquisition system for use with a stopped-flow instrument is described. Data acquisition, reduction, signal averaging, kinetic modeling, and plotting are performed under software control. Applications to biological and inorganic systems are presented.

### INTRODUCTION

Kinetics studies typically generate enormous amounts of raw data that traditionally have been laboriously hand-digitized and reduced. The described microcomputer-controlled data acquisition system improves accuracy, eliminates the labor-intensive steps, and permits data acquisition, reduction, and plotting of final results in seconds without the operator ever having to handle raw data.

### METHODS

#### *Instrumentation*

The stopped flow instrument has been described previously (1). In all experiments reported concentrations are before mixing.

The computer system consists of an MITS Altair 8800 microcomputer (Altair Corp., Chicago, Ill.) using an Intel 8080A microprocessor (Intel Corp., Santa Clara, Calif.), 9,216 bytes of semiconductor random access memory, 2,048 bytes of permanent read-only memory (PROM), and an ASR33 Teletype interface (Processor Technology 3P+S serial card). The data acquisition control and clock circuitry plugs directly into the Altair bus; it uses ~50 7400 type small and medium-scale integration integrated circuits and was wire-wrap constructed on a Vector 8800V prototyping card (Vector General, Inc., Woodland Hills, Calif.). The separate analog card contains a Hybrid Systems ADC-550-10E-G 10 bit, 27- $\mu$ s analog-to-digital converter (ADC) (Hybrid Systems Corp., Burlington, Mass.) and two 371I-10 10-bit digital-to-analog converters (DAC's), which communicate with the control card through multiconductor cables. The real-time clock controls the conversion rate of the ADC and is selectable in a 1, 2, 4, 5, 8, 10, 16 sequence from 40  $\mu$ s to 160 s per data point.

Analog signals for the ADC and the DAC's are run through coaxial cables to an external control center. The analog input voltage signal is buffered with operational amplifiers with selectable normal or inverted levels (1 or 2 gain), and variable DC offset ranges ( $\pm 10$ , 1, and 0.1 V). A Datel IC-SHM-1 sample-and-hold amplifier (Datel, Inc., Holly Hill, Fla.) between the buffer amplifiers and ADC eliminates digitization errors for rapidly changing signals. An LM311 comparator trigger circuit provides normal oscilloscope triggering functions ( $\pm 10$  V level control, rising or falling edge, internal or external triggering, and an light-emitting diode status indicator). The DAC outputs are converted to 0–10.23 V to drive a display oscilloscope and *x-y* plotter.

## SOFTWARE

The PROM stores machine language utility (768 bytes) and data acquisition (256 bytes) routines. PROM permits immediate loading of paper tape programs including the MITS 8 K BASIC Interpreter upon power up. The acquisition software, accessed through a BASIC subroutine call, permits selection of the real-time clock interval, execution of the experiment, and examination and plotting of stored data or calculated results.

The acquisition software instructs the interface control card from commands entered on the Teletype and the computer's switch register. After a keyboard-issued arming command and manual operation of the stopped-flow instrument, flow stoppage triggers collection of 101 "decay" points and 101 "infinity" points separated by a 200 time interval delay. Other collection schemes are readily implemented. After acquisition, stored data may be visually evaluated for data quality, and the best region to fit selected from the computer generated oscilloscope display or an *x-y* plot. Upon return to BASIC, on-line data reduction is performed by least-squares programs in BASIC. As configured, approximately 100–120 lines of

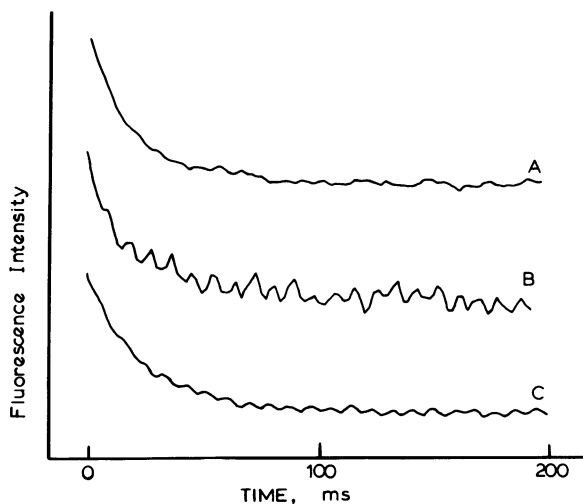


FIGURE 1 A. Tryptophan fluorescence monitored on the mixing of equal volumes of IA-BSA (ca.  $1.5 \times 10^{-5}$  M) in 0.04 M  $\text{NaClO}_4$  at pH 3.5 and 0.05 M phosphate (pH 8.0). Increasing fluorescence intensity is down. On-line computer-calculated refolding rate constant is  $28.8 \pm 0.4 \text{ s}^{-1}$  ( $r = 0.97$ ). B. The same as A with the initial protein concentration reduced to  $5 \times 10^{-6}$  M to increase the noise level. Refolding rate constant is  $24.8 \pm 0.6 \text{ s}^{-1}$  ( $r = 0.85$ ). C. Results of recording and on-line ensemble averaging 16 transients of B. Refolding rate constant is  $32.3 \pm 0.5 \text{ s}^{-1}$  ( $r = 0.98$ ).

BASIC programming are accommodated. A first-order rate constant error estimates are typically computed in <5 s for 101 data points.

## RESULTS

The kinetics of iodoacetamide-blocked bovine serum albumin (IA-BSA) refolding have recently been characterized by stopped-flow monitoring of the protein's tryptophan fluorescence after a rapid pH jump (1). We reexamined this system using our micro-computer-interfaced stopped-flow instrument.

Fig. 1 shows computer-acquired and -plotted data taken with good signal-to-noise ratio (S/N) (1A), poor S/N (1B), and with computer-controlled ensemble averaging of the noisy data (1C). The computed constants are in excellent agreement with the previously reported value of  $32 \text{ s}^{-1}$  (1).

We also examined the reactions of 0.02 M  $\text{NaHCO}_3$  with 0.01 N HCl ( $3.4 \times 10^{-5}$  M bromophenol blue indicator) and of  $10^{-3}$  M  $\text{Fe}(\text{NO}_3)_3$  in 0.1 M  $\text{H}_2\text{SO}_4$  with 1.0 M KSCN. The  $\text{NaHCO}_3$  reaction yielded a rate constant of  $21.1 \text{ s}^{-1}$  ( $24^\circ\text{C}$ ), which compares well with the literature value of  $19.0 \pm 2.0 \text{ s}^{-1}$  ( $24.1^\circ\text{C}$ ) (2).

For the  $\text{Fe}^{3+}$  system, four replicate runs ( $24^\circ\text{C}$ ) yielded a lifetime of  $57.5 \pm 0.5 \text{ ms}$ , which compares well with an estimated value of 45 ms calculated for a slightly different ionic strength (3). The total time to acquire all four transients, display and verify their quality on the oscilloscope, compute the rate constants, and plot the observed and calculated best-fit curves on an x-y recorder was under 20 min.

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## ON THE INTEGRATION OF COUPLED FIRST-ORDER RATE EQUATIONS

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Differential rate equations for many chemical reaction mechanisms are inherently linear or can be linearized through judicious choice of experimental conditions.

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The author of this paper did not attend the actual Discussion. The present text was submitted and circulated to participants before the meeting.