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studied this reaction as a function of temperature and obtained for the third-order rate constant

$$k = 10^{7.6 \pm 0.2} \exp(-4500 \pm 200/RT) M^{-2} \text{ sec}^{-1}$$

At 30 °C, this yields a value of $2.26 \times 10^4 M^{-2} \text{ sec}^{-1}$ with error limits of 1.5×10^4 and $3.8 \times 10^4 M^{-2} \text{ sec}^{-1}$. We obtained the data in Table I by fitting the observed data to either a growth or a decay pseudo first-order expression by means of a nonlinear least-squares program (17). At concentrations of HCrO_4^- greater than 0.5mM, we observed the same initial deviation from pseudo first-order decay as had been previously reported (16).

We used the reaction of aqueous NaOH with 2,4-dinitrophenyl acetate (DNPA) to test the effect of various averaging schemes on the rate constant. This was a pseudo-first order, fixed wavelength experiment at 400 nm. From the results, we conclude that the rate constant is remarkably insensitive to the averaging scheme used. Only for a grouping factor of four is there any indication that excessive early averaging might cause problems.

A remaining problem with this system, caused by the need to use four valves between the syringes and the mixing chamber and to employ strain relief spirals, is the relatively large hold-up volume. The total hold-up volume of ~2 ml is detrimental when working with limited amounts of reactants. In addition, some back diffusion from the mixing chamber can occur, depending upon the solution densities and the time between successive pushes. This problem has been overcome by making two pushes in quick succession and discarding the first one.

Good quantitative performance under actual operating conditions at three different temperatures has also been demonstrated in the study of several enzyme reactions. These studies showed the ability of the stopped-flow system to give reproducible rate constants, enhanced S/N and to detect subtle changes in the absorption spectrum during reaction.

Computer-Interactive System for Stopped-Flow Kinetics with Rapid Scanning Molecular Absorption Spectrometry

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A rapid scan stopped-flow apparatus was interfaced to a remote PDP-8/I computer. The data transmission system permits high frequency (up to 10 MHz) parallel digital data transfer over several hundred feet. A phase-locked loop frequency multiplication system allows sampling to occur at a constant computer-limited rate which is independent of scan speed and is synchronized with the wavelength drive of the monochromator. The data acquisition software establishes a digital averaging procedure which produces real-time signal-to-noise ratio (S/N) enhancement. This averaging varies with time in such a way that adequate spectral and temporal resolution are maintained while storage requirements are minimal.

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The use of computers for real time data acquisition has become commonplace (1-3). Computer-coupled stopped-flow instruments have been reported (4-6), and a system with rapid scanning absorption spectrophotometric capabilities has been described (7,8). The present work describes a computer-interactive system for acquiring and processing data from a rapid scanning stopped-flow apparatus. A description of the stopped-flow system is presented in a companion paper (9). The interface system makes use of typical components which are not described in detail here; however, several unique features of the interface are discussed at length in the hope that they might find general applicability.

Briefly, the purpose of the interface system is not only to optimize the accuracy and efficiency of data collection and processing, but also to permit maximum interaction between the experimenter and the experiment. Principal features of the system include: processing of large quantities of rapidly changing three-dimensional data by a remote minicomputer; a phase-locked loop frequency multiplica-

tion system which allows the computer-limited sampling rate to be synchronized with the scanning monochromator; signal enhancement in real time with concomitant reduction in data storage without significant loss of information; and an interactive, versatile computation and display system to permit decision making during the experiment.

SYSTEM DESIGN CONSIDERATIONS AND CONSTRAINTS

The stopped-flow technique involves the rapid mixing of solutions from two syringes in a specially designed mixing and observation cell followed by rapid stopping of the mixed reactants in a third syringe located downstream. In our instrument, the extent of reaction is monitored spectrophotometrically either at a single wavelength or by rapidly scanning the absorption spectrum. (See Figures 6 and 7 respectively for example experimental results for the two modes.)

Rapid scanning stopped-flow studies present special problems to the experimenter which are not encountered in fixed wavelength work. Since the duration of a single experiment (or "push") ranges from a few seconds to several minutes, and the monochromator can scan at speeds up to 150 complete spectra per second, an enormous amount of three-dimensional data must be rapidly processed and stored. The previous system utilized analog data storage (10) with later digitization (11). This procedure introduced tape recorder noise which was particularly troublesome for small absorbance changes. Another major disadvantage was the inability to evaluate the data after each push.

Actual stopped-flow runs require only a small fraction of the total experimental time, so that a computer dedicated only to this system is not necessary. Therefore, although the stopped-flow apparatus was located in the basement of our building, it was interfaced to an existing computer system located on the fourth floor. Thus, needless duplication of expensive equipment, particularly peripheral devices, was avoided. (The computer has now been relocated in the sub-basement. To move the computer and transmission lines required only about two days.) A remote parallel digital data transmission system of the type used should be generally applicable where the availability of computers is limited and high frequency data transmission over relatively long distances is required.

SIGNAL ENHANCEMENT IN REAL TIME

In typical rate studies, the signal varies rapidly at first and then progressively more slowly as the reaction approaches completion (or equilibrium). It is therefore desirable to use a wide noise equivalent bandwidth at the beginning of a reaction, but this necessity decreases as time increases. Most systems are designed for a fixed bandwidth; that is, electrical filters are selected prior to the experiment and then not changed during the data acquisition. Even in these cases, one must be careful in selection of the filters since analog filtering can distort the signal shape. In contrast to this, digital filtering (appropriate averaging or smoothing) can provide a bandwidth which can be varied with time as needed.

Narrow slitwidths, resulting in decreased light intensities, coupled with the mechanical vibrations of the monochromator are the primary causes for the inherently poorer signal-to-noise ratios (S/N) associated with scanning experiments. This is particularly troublesome in the case of enzyme reactions where absorbance changes are generally small and spectral changes are often subtle. Optimum on-line use of the minicomputer is achieved by sampling the data signal at a maximum rate which is limited by the computer software and not by the experiment (12). Data stor-

age requirements are reduced and real time S/N enhancement in both the wavelength and time domains is achieved by using software to perform time-dependent digital averaging.

DETERMINATION OF THE MAXIMUM SAMPLING RATE

In the rapid scanning stopped-flow experiment, full-scale signal deflections occur on the millisecond time scale as a result of changing wavelength and extent of reaction. With traditional fixed wavelength systems, a stable clock oscillator provides a convenient sampling frequency which remains constant during the entire reaction. More sophisticated instruments have utilized programmable clocks to decrease the sampling rate as the reaction proceeds, which reduces the quantity of stored data. These approaches were rejected for several reasons: sampling, not precisely synchronized with the monochromator wavelength drive, would preclude effective spectral averaging; decreasing the sampling rate during the course of the reaction would lead to poorly defined spectra since the absorbance continues to change with wavelength on the millisecond time scale even at equilibrium; some of the most vital data for testing complex rate expressions occur near the end of the reaction where the S/N is poor in the absence of averaging. In the present work, the guiding principle was to use a constant sampling frequency and to perform appropriate digital filtering. This results in real S/N enhancement and simultaneously reduces absolute storage requirements.

It was estimated that an average of 40 μ sec would be required by the computer to complete the data acquisition, averaging, and storage cycle. Thus, the maximum sampling frequency was ~ 25 kHz. This sampling frequency is well within the capabilities of a moderately priced medium speed analog-to-digital converter (ADC). We also wanted to synchronize sampling with the wavelength drive of the Model 108 monochromator (Perkin-Elmer Corp., Norwalk, CT). Wavelength is scanned by the wobble of a rotating mirror. A gear attached to the mirror shaft has 136 teeth which interrupt a light beam and produce a signal, called the gear-tooth signal (GT), whose frequency varies with the scan speed up to a maximum of 10.2 kHz at a scan rate of 150 spectra per second. Once per revolution the light beam is reflected from a polished gear tooth to produce the beginning of scan (BS) pulse. The GT signal is the input to a frequency multiplication system which ultimately generates the trigger pulse used for sampling. In this way, a sampling frequency of 20.4 kHz is readily obtainable at any scan speed and is compatible with the time requirements of both the computer and the ADC. The commercial monochromator was modified in this laboratory to produce the GT and BS pulses.

AVERAGING SCHEME

Stopped-flow data must be stored initially in core memory because of the time scale of the experiment and the relative slowness of the available peripheral computer storage (magnetic tape). For a given push, data storage is limited to whatever space remains after resident system software has been loaded. This fact was of primary importance in the development of the data acquisition software for the scanning system. At a sampling frequency of 20.4 kHz, one would rapidly exhaust the storage capacity of the 8K computer. While a bandwidth which simply decreases with time in a pre-determined way would be satisfactory for data obtained at fixed-wavelength, it is not applicable when scanning, since the signal changes with time in a way which is determined by the spectrum and the scan rate and not merely by the rate of reaction. We have devised a

scheme which operates equally well in either mode. For simplicity, its operation with fixed-wavelength data will be considered first.

Consider a point on an absorption band which decays as a function of time (see Figure 1 for example). Initially the signal is changing very rapidly with time and excessive averaging would distort the decay curve. At long times, when the signal is slowly changing with time, we would like to average a number of adjacent samples into a single point (not simply decrease the sampling rate) in order to increase the S/N in the very important "tail" of the reaction. Thus, what is needed is a variable frequency bandpass as the reaction proceeds. The averaging process chosen is best represented by an example.

Consider a reaction in which the absorbance of species A decays in a second order fashion with a half-life of 50 msec. Suppose we have an absorbance with an initial S/N of 100 (based upon four samples per point). As the reaction proceeds through six half-lives, the absorbance of A decreases to 1.56% of its initial value with a concomitant decrease in S/N expected if the number of samples per point were not changed. Of course, the actual change in S/N with absorbance will be a complex function of the absorbance since there are several sources of noise in the experiment. For convenience, a constant noise level is used in this example. In the averaging scheme selected for this example, the total time is arbitrarily divided into 10 groups of 16 data points each (see Figure 1). In an actual experiment, the number of groups and the number of points would be pre-set by the experimenter. These parameters are chosen according to the rate of reaction and the number of half-lives to be measured. A grouping factor is selected which determines the modulus by which the averaging bandwidth is increased as the reaction proceeds. Each of the stored *points* in group 1 is actually the average of four 49- μ sec *samples* (20.4 kHz) and the first group extends in time to 3.14 msec of reaction. The second group also contains 16 points, each of which is the average of eight 49- μ sec samples. Group 2 extends in time to 9.4 msec. In this way each group contains twice as many samples per point as the preceding group and requires twice as long a time. For an arbitrary grouping factor, g , group number, n , elapsed time at the end of the group, t_n , and samples per point s_n , we have

$$s_n = g^{n-1} \cdot s_1 \quad (1a)$$

$$t_n = \frac{(g^n - 1)t_1}{g - 1} \quad (1b)$$

After six half-lives, only 160 points are stored; however, 65,472 samples are actually measured. One obvious advantage of the technique is that the total number of storage locations is reduced without ignoring useful information. Second, we can expect significant S/N enhancement. For a random constant noise level with a signal level S_n in group n , the S/N is given by

$$(S/N)_n = g^{(n-1)/2} \cdot (S/N)_1 \cdot \frac{S_n}{S_1} \quad (2)$$

For example, at the ends of the first (Group 5) and sixth (Group 10) half-lives, we can expect S/N values of 200 and 35, respectively. Because of the S/N enhancement, small changes in the signal at long times can still be detected. Obviously, for very fast processes little can be done to improve the S/N in real time since excessive averaging would lead to signal distortion.

The ability to scan wavelength during reaction adds another dimension to the averaging problem. In this case, the number of points per spectrum (P/S) is selected by averaging an appropriate number of adjacent *samples* as the spectrum is scanned. Once this selection is done the aver-

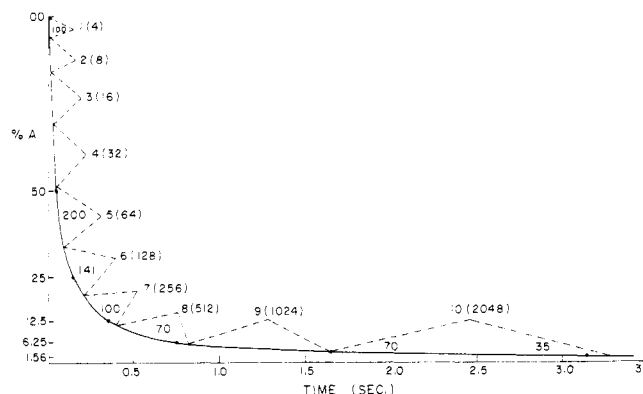


Figure 1. Example of the averaging scheme for a fixed wavelength case

The numbers in parentheses following the group number are the number of samples averaged into each point. Approximate S/N values are given within the triangles

aging bandwidth for the wavelength variable remains fixed (for that reaction) while averaging in *time* proceeds according to the scheme outlined above for fixed wavelength. For moderately fast reactions involving broad featureless spectra, the S/N is enhanced by averaging more adjacent *samples*. As wider wavelength regions are scanned or as the complexity of the spectrum increases, the number of points per spectrum required for adequate resolution also increases. As the number of points per spectrum increases, however, the total number of spectra which can be stored for a given push decreases. The averaging bandwidth for the wavelength variable is therefore determined by the reaction conditions, wavelength resolution desired, and the amount of storage available.

Averaging in *time* is accomplished by averaging appropriate numbers of consecutive spectra. Analogous to the fixed wavelength case, once the number of samples per point and points per spectrum are determined, the time course of the reaction is divided into groups. Each group contains the same pre-determined number of spectra. Each of the "stored" spectra within a particular group actually represents the average of C consecutive spectra where C is determined by the group number n and grouping factor g according to Equation 3

$$C_n = g^{n-1} \quad (3)$$

The total number of samples averaged into each point of the stored spectrum is given as before by Equation 1a. The grouping factor has a marked effect upon the total time span. For example, choosing a grouping factor of 2 with 10 spectra per group and 5 groups yields a total of only 50 stored spectra out of 310 measured spectra. For a grouping factor of 3, the 50 stored spectra would result from 1210 collected spectra. Obviously when the *time* development at a particular wavelength is extracted from the scanning record, appropriately averaged times must be used.

A software generated variable bandwidth averaging scheme is extremely versatile, limited essentially by the experimental conditions and/or data storage capacity. There is a trade off between resolution and S/N enhancement on the one hand and storage limitations on the other. The relationship among the sampling parameters for the available storage in our system is given by

$$(P/S)(S/G)(NG) \leq 3456_{10} \quad (4)$$

for P/S points per spectrum, S/G spectra per group, and NG groups.

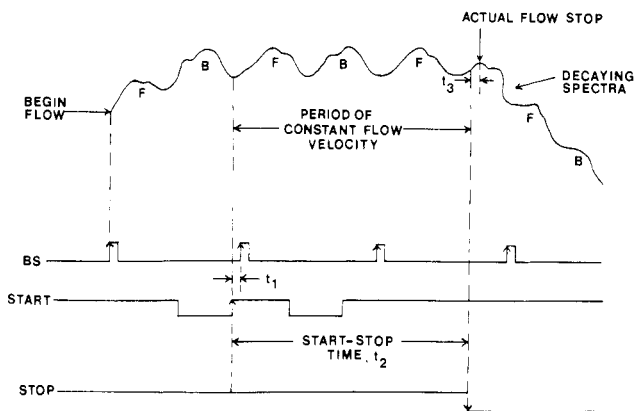


Figure 2. Timing diagram for a scanning experiment

F and B refer to forward and backward scans respectively. The other symbols are defined in the text

One of the primary advantages of the variable bandwidth technique is that both rapid and slow processes may be recorded in the same experiment. Early groups contain the rapidly changing information while later groups continue to follow slow processes. Even reactions with induction periods can be effectively studied since any number of spectra or samples can be skipped before data collection begins.

SYSTEM CONTROL AND TIMING

On line experiments require certain instrument-generated triggers or timing pulses so that the exact zero of time can be determined. The timing problem had been particularly troublesome in our previous scanning experiments because the time at which flow stops had no relation to the wavelength of the spectral scan. One must have an accurate knowledge of the zero of time in order to establish initial absorbance values at all wavelengths and to avoid using data collected during the period of deceleration. In order to directly compare replicate pushes in the scanning mode, therefore, one must determine precisely when flow stops with respect to any particular wavelength. This is accomplished by using trigger signals from the stopped-flow apparatus.

Two metal flags, attached to the stopper plunger, break light beams and cause light-sensitive transistors to give the trigger signals shown in Figure 2. Two light beams, fixed with respect to the stopping plate are used. Initially, both metal flags are below both beams. As the plunger moves towards the stopping plate, the lower beam is interrupted by both flags in succession to give the start signal shown in Figure 2. The rising edge, produced as the first flag clears the light beam, is used to start both the time-shift clock [computer real-time clock (13)] and the flow velocity clock (Heath Universal Digital Instrument). The time-shift clock measures the time (t_1) between the start pulse and the next beginning of scan (BS) pulse.

When the stopping plunger is at a known small distance (d) from the stopping plate, the first flag interrupts the upper light beam and gives a stop pulse which stops the Flow Velocity Clock and yields the time t_2 . Since the separation of the two phototransistors is known, the average flow velocity can be calculated from t_2 . Measurement of the flow velocity profile showed that the velocity is constant after the start pulse. From Figure 2, it can be seen that knowledge of the flow velocity, the timing information, and the distance, d , permits computation of the time, t_3 between the stop pulse and the actual flow stop. Also, the wavelength at the time of flow stop can be determined.

Data acquisition begins with the first sample pulse after the time shift clock is stopped, always at the beginning of a

new spectral scan. For fixed wavelength pushes, a Wavetek model 116 Signal Generator is used to provide the 20.4-kHz sampling trigger and the stop flag is used to initialize data acquisition. Once within the sampling mode, the software takes control until sufficient data are collected as determined by the variable bandwidth averaging scheme described above.

SYSTEM HARDWARE DESCRIPTION

A block diagram of the overall system is shown in Figure 3. The interface system was constructed from Heath modules (Heath Co., Benton Harbor, MI) and printed circuit blanks. Most of the individual circuits such as data latches, octal decoders, etc., were constructed on Heath printed circuit boards and used generally available IC components. The sampling system utilizes an Analogic MP250 fast acquisition Sample and Hold Amplifier (SHA) and an MP2212 12-bit ADC (Analogic Corp., Wakefield, MA). The display and plotting system uses Analogic MP1810 high-speed 10-bit DAC's.

A DEC Digital PDP-8/I computer (Digital Equipment Corp., Maynard, MA) with 8K of core was used. Computer peripherals include ASR-33 and -35 Teletypes, a high-speed paper tape punch/reader, dual DEC-tape drives, an IBM cardpunch, a line printer, a Tektronix model 611 storage display scope and a Houston Instruments Model 6550 incremental plotter. Data collected initially in core memory are subsequently stored on DEC-tape. After preliminary examination of the data on the display scope, selected "time cuts" at various wavelengths are punched onto IBM cards for more sophisticated analysis with the MSU CDC 6500 computer.

REMOTE DIGITAL TRANSMISSION SYSTEM

Since the stopped-flow instrument is located in the basement and the PDP-8/I computer is on the fourth floor, a special system for transmitting data between the two locations was required. Because of the distance involved (~150 feet), direct analog transmission cannot be used without signal degradation and noise pickup. It was therefore necessary to transmit data in digital form. The Input/Output bus drivers provided with the computer are incapable of operating over this distance. Remote digital data transmission kits for small computers have recently become available (1). However, data transmission is serial and too low in speed for our needs.

In the present system, Texas Instruments line drivers and receivers (Texas Instruments Corp., Dallas, TX) are utilized in a "party-line" configuration to transmit information in parallel form between the two locations. High speed (up to 10 MHz) data transmission without signal reflections is possible along a terminated transmission line. In such a system, noise is primarily common mode and is rejected by a set of differential-input line receivers and a balanced (two-wire) transmission system. The transmission system is capable of driving and receiving noise-free data at frequencies up to 10 MHz and distances of several thousand feet (14).

The transmission system consists of 10 dual TI SN75109 Line Driver and 10 dual SN75107 Line Receiver integrated circuits (~\$3.00 per dual circuit) powered by Analog Devices (Norwood, MA) Micro Logic 5 VDC power supplies at each end of the transmission line. This line is a Belden 8769 cable (Newark Electronics) which contains 19 twisted pairs of No. 22 conductors (~\$1.00/foot). All drivers and receivers at each end were mounted on a single 6-in. \times 9-in. circuit board which is connected to the interface with 3M type flat connection cables. The estimated material costs for the complete data transmission system were \$600.

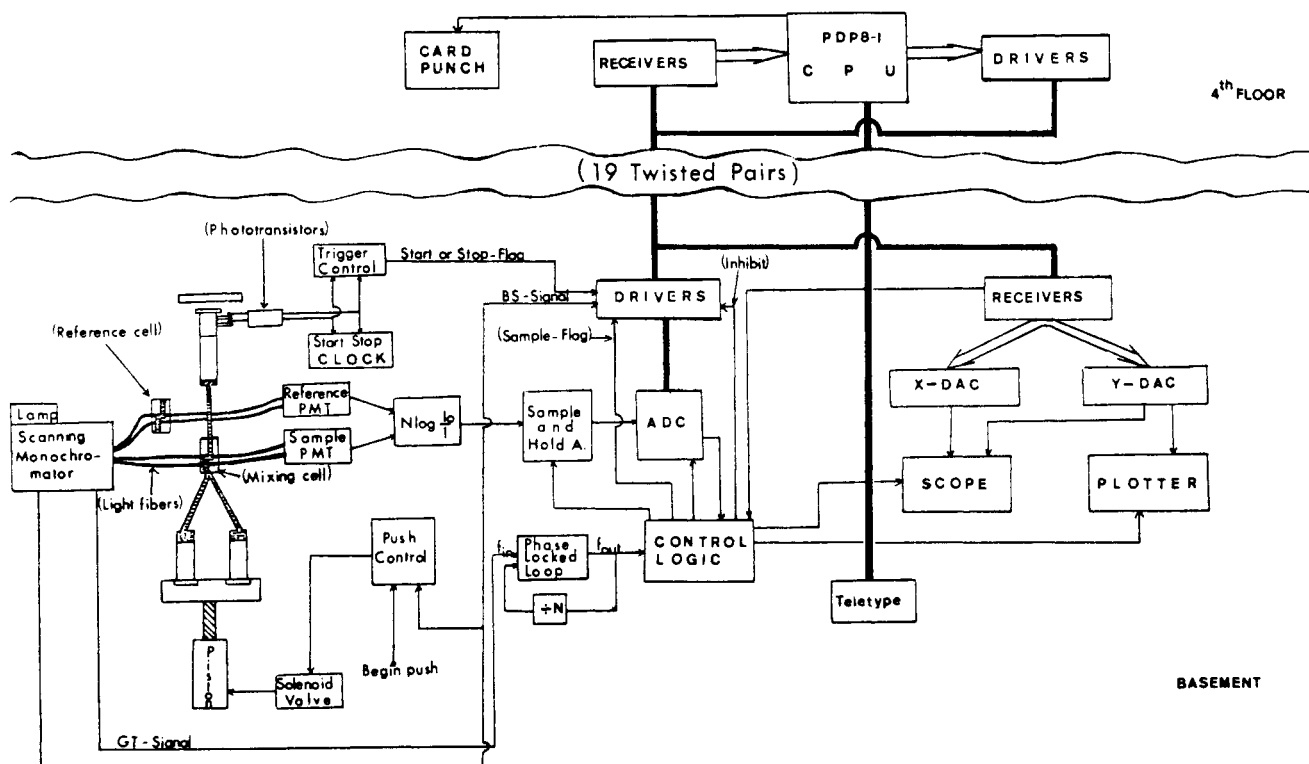


Figure 3. Block diagram of the computer-interfaced stopped-flow system

This kind of transmission system, in a "party-line" configuration, may be extended to include a number of stations which share a common transmission line. In this way, several laboratories can use a single transmission line in conjunction with the receiver strobe and/or driver inhibit facilities to place several instruments on-line with a remote computer system.

GENERATION OF SAMPLING FREQUENCY

To sample data at a constant rate which is synchronized with the monochromator even when the scan speed is changed requires multiplication of the frequency of the GT pulses. A programmable clock or voltage controlled oscillator could be used to achieve virtually any sampling rate desired. However, the system described here permits synchronization of data sampling with the wavelength drive.

Since operation at the maximum sampling rate demands extensive time averaging of spectra, all sampling pulses must be synchronized with the scanning monochromator. The Phase-Locked Loop and Divide by N circuit (PLL/N) (Figure 3) was constructed to multiply the frequency of the fundamental timing trigger (the GT frequency) which varies from 0.2 to 10.2 kHz by an appropriate integer (N) to produce the 20.4-kHz sampling frequency. The phase-locked loop (PLL) continually corrects its output frequency to keep the two input signals f_{in} and f_{out}/N in phase and of equal frequency. As the monochromator scanning rate is changed from one speed to another, the value of N must also be changed in order to maintain the 20.4-kHz sampling rate.

The GT pulses arise from the main gear on the rotating mirror shaft. Variations in speed of rotation caused by "play" in the gear train would lead to non-reproducibility of the wavelength of a particular sample were it not for the frequency-locked circuit. The PLL/N circuit is capable of following the "wander", thereby allowing digital averaging of consecutive spectra to be an even more effective technique for enhancing S/N without degrading the resolution of the spectra. The frequency multiplication process per-

mits interpolation between GT pulses and, since both the GT and BS pulses originate from the same rotating gear, synchronization of the beginning of scan with individual spectral features is automatic. The PLL/N circuit was constructed on a Heath PC board from commercially available components (Signetics NE 565A PLL and Motorola MC 4018P programmable counter).

SYSTEM SOFTWARE

This system requires a long and sophisticated set of computer programs. Only 8K of core was available and about half was needed for data storage. To utilize the available core efficiently we divided our software into two main parts: 1) approximately 2.5K of core resident routines which include the PS/8 resident monitor, an I/O package, and a supervisor routine; 2) non-core resident routines called segments which are loaded into core from DEC-tape one at a time as needed under control of the supervisor. Control over the system software is performed via Teletype and scope-displayed instructions. This has proved to be a very efficient way to utilize 4K of core memory for ~18K of software. The number of segments can be easily expanded if necessary. All programs were written in the PAL-8 programming language.

The overall system is so complex that flow charts cannot be included here. The operation of the TAKE mode in the SCANNING option is outlined below. Additional options in this segment provide routines for absorbance calibration with neutral density filters, wavelength calibration with absorbing glasses, and determination of solvent background as well as background at the end of reaction. The experimenter merely sets or changes the data collection parameters, selects the appropriate option, and initiates the push. The digital sample collection then takes place according to the following sequence of events:

- 1) The start flag occurs, signalling that the push has begun. The clocks are started.
- 2) Data collection begins after the falling edge of the next BS pulse, the time-shift clock is read and the value is

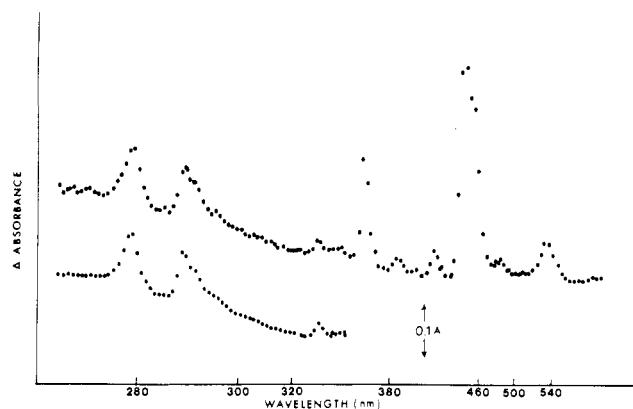


Figure 4. Holmium oxide glass spectrum collected while scanning at 75 spectra per second

The top spectrum is from a single scan while the bottom one is the average of 100 scans

stored with the other experimental parameters.

3) The next sample trigger (from PLL/N) activates the HOLD mode of the SHA.

4) The same sample trigger is delayed $\sim 5 \mu\text{sec}$ in order to allow the SHA to "settle", and then triggers the ADC. The conversion time of the ADC is variable but is presently set at $44 \mu\text{sec}$.

5) 100 nsec before the 12 parallel digital bits are ready, the ADC generates an End of Conversion pulse which after a suitable delay opens the data latch and sends a SAMPLE READY signal to the sample flag.

6) The computer recognizes the SAMPLE READY FLAG, removes the inhibit on the data line drivers, and loads the sample into its accumulator.

7) The computer clears the sample flag and proceeds to average and store the data.

The next sample trigger begins the process again. Since the computer keeps track of BS pulses, the samples are averaged and stored in the correct sequence with respect to both time and wavelength.

Our scanning system produces both a forward and a back-scan on each revolution (one is the mirror image of the other). According to the data acquisition routines, averaging of adjacent spectra does not occur in Group 1. In this way, it is possible to collect both the forward and back-scans to obtain better time-resolution in the early stages of the reaction. In the remaining groups, back-scans cannot be collected because the time is needed for spectral averaging.

When the fixed wavelength option is selected, the sampling and digitization process begins with the stop flag and goes through steps 3-7.

Each data file (one per push) is stored permanently on DEC-tape. Selected data during flow can be averaged to improve initial absorbance readings which can then be subtracted from the raw data if desired. Solvent backgrounds and/or the absorbance at infinite times can also be subtracted. Subsequently, the desired wavelength or time displays can be examined to indicate possible modifications in the experiment. Since the data are routinely transferred to the CDC 6500 computer for kinetics analysis, we have not included data-fitting routines as part of the on-line software package.

Displays of spectral growth and decay with arbitrary scale expansion and selection of particular spectral scans individually or collectively can be obtained on the storage display scope. Time developments at any particular wavelength can be examined also on the scope with arbitrary starting and ending times as well as appropriate scale ex-

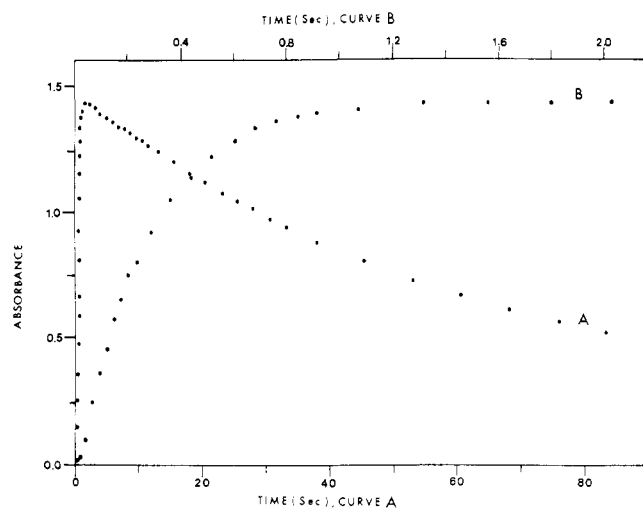


Figure 5. Time profile of the formation and subsequent decomposition of peroxychromic acid

These data were obtained from a time-cut at 600 nm of scanning data collected at 75 scans per second

pansions. In this way, the absorbance during any time period at any wavelength can be examined as soon as the push is over. Hardware and software are also available to plot on the incremental plotter whatever data are displayed on the scope. Finally, absorbance values displayed on the scope can be punched onto computer cards for more detailed data analysis.

SYSTEM PERFORMANCE

In order to evaluate the overall accuracy and precision of the data Input/Output system, known voltages from a Fluke voltage reference source were measured by the computer system and the results were compared by outputting the data to punched cards. The overall accuracy of one part in 4096 showed that the accuracy and precision are limited by the word-size used.

Sampling frequency measurements were also performed with the aid of the computer. For scanning pushes, resolution in time (and wavelength) is dependent essentially on the proper operation of the frequency multiplication system. With proper tracking and multiplication, the PLL system was reproducible to within one sample per scan. Thus, the basic digital interface system has proved both accurate and reliable. Elimination of transmission and read-out errors resulted in a substantial improvement in S/N over the previous FM tape system.

RESULTS

Wavelength calibration is carried out during each run with either holmium oxide or didymium glass filters. Typically, scan ranges of from 100 to 600 nm are used depending upon the system under study and response limitations of the photomultiplier tubes. We have used RCA 6903 tubes for the 250- to 550-nm range and cooled EMI 9684B tubes for the 350- to 950-nm range.

Scan speeds from 3 to 150 spectra per second are available with the Perkin-Elmer Model 108 Scanning Monochromator. We have found a speed of 75 spectra per second suitable for most applications. Static (time independent) calibration spectra are collected at the same scan rates as those for the stopped-flow experiments. Examples of the wide scan-range available and the enhancement obtained by averaging are given in Figure 4. For work requiring higher resolution, slower scan speeds with more points per spectrum can be used in conjunction with narrower slits and more extensive averaging. The system display software per-

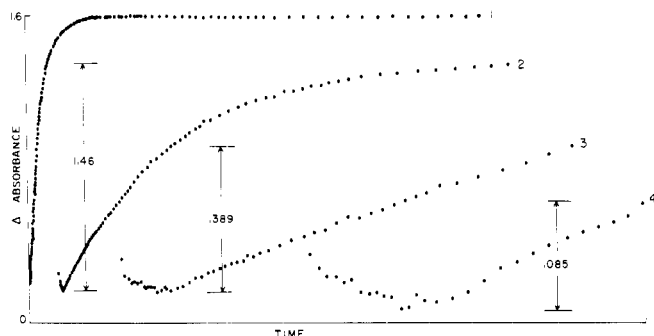


Figure 6. Time-development of the FeSCN^{2+} complex at 460 nm obtained in a fixed wavelength experiment

Total times for those portions displayed are: (1) 8 sec; (2) 400 msec; (3) 40 msec; (4) 11 msec. Note the expansion of the absorbance scale between traces 2, 3, and 4

mits full scale expansion both vertically and horizontally of any portion of the spectrum as an additional means of detailed wavelength examination.

The ability to examine both short-time and long-time data by using time dependent spectral averaging is illustrated in Figure 5 by two time-cuts at one wavelength. This shows the absorbance changes which accompany the reaction of chromic acid with hydrogen peroxide in acidic solution to form peroxychromic acid which subsequently decomposes to give non-absorbing products (15). A time-expansion of the growth portion of the overall growth and decay is shown. The reader is reminded that a single scanning push provides the kinetics at all wavelengths of interest. The display software can display any number of "time-cuts" from a scanning push simultaneously for direct comparison.

Of course, the scanning system can also be used at fixed wavelength for the study of faster reactions. Figure 6, curve 1 illustrates the kinetics of growth of the complex formed by the reaction of $0.01M \text{ Fe}^{3+}$ with $0.005M \text{ SCN}^-$. Curve 2 is an expansion of the first 400 msec of curve 1 (this is a closer look at the same data, not a new push); similarly, curve 3 is the first 40 msec and curve 4 is the first 11 msec of the reaction shown in curve 1. Note the expansion of both the time and absorbance scales. This scale expansion procedure is useful when examining flow velocity and stopping characteristics of the system, fast and slow processes, large and small absorbance changes, etc. Similar flexible display capabilities are utilized for both wavelength and time displays of data obtained in the scanning mode.

The entire system has been used to study the kinetics of a number of complex reactions involving such diverse fields as transient-state enzyme kinetics and solvated electron reaction rates. The performance characteristics have fully lived up to the expectations developed during the calibration runs. As an example, sample spectra obtained during the equine liver alcohol dehydrogenase (LADH) catalyzed reduction of the substrate analog N,N -dimethylnitrosoaniline with NADH, (16), are shown in Figure 7. Three wavelength-dependent time developments have been extracted

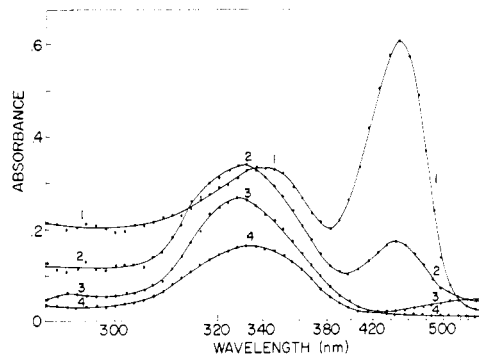


Figure 7. Overall spectral changes for the LADH catalyzed reaction

Spectrum 1 is the initial spectrum showing absorption bands of substrate (340 and 440 nm). Spectrum 2 shows the shift of the 340 absorption band during decay of substrate. Spectrum 3 shows the two transient absorption bands (325 and 540 nm) and spectrum 4 shows the residual absorption (free and enzyme-bound) of NADH. Note the improved S/N in spectrum 4, especially at the shorter wavelengths

and analyzed from these data which were obtained in the scanning mode.

Details about the construction and performance characteristics of the stopped-flow system are given in a companion paper (9). Copies of the software are available upon request.

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