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NATIONAL BUREAU OF STANDARDS

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Real-Time Acquisition and Processing of Fluorimetry Data

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[2] Part of the Center for Radiation Research.
[3] Located at Boulder, Colorado 80302.
[4] Part of the Center for Building Technology.

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Peter S. Shoenfeld

The National Bureau of Standards Analytical Chemistry Division uses a centralized computer system to automate a number of experiments. Computer software for a fluorimetry application is described in this report. The file and program structure used is quite general and can be applied to other experiments as well.

Key Words: Analytical chemistry; computers; data acquisition; data processing; fluorimetry; laboratory automation; real time.

1. INTRODUCTION

The National Bureau of Standards Analytical Chemistry Division operates a generalized laboratory automation facility which has been used to computerize a number of experiments. The fluorimetry experiment described herein is typical of a general class of these where minimal control is desired, but acquisition and special processing of data are extremely important.

Considerable effort was expended in devising a file structure which would accommodate a variety of experiments. The data acquisition program can be used for many experiments with little or no modification.

The major participants in this project were Peter Shoenfeld, Lawrence Kaetzel, and Fillmer Ruegg. Mr. Shoenfeld designed and programmed the software and wrote this report. Mr. Kaetzel tested the software, and Mr. Ruegg designed and implemented the hardware interfacing. The entire project was under the administrative supervision of James R. DeVoe.

2. THE ANALYTICAL CHEMISTRY DIVISION COMPUTER SYSTEM

The Analytical Chemistry Division Computer System is built around a Univac Series 60[1] (formerly known as EMR 6135) general purpose computer. This computer has 32,000 words of 16-bit main memory, two million words of high speed disk storage, a magnetic tape drive, and other peripheral devices. A generalized interface has been developed which allows the computer to communicate simultaneously with numerous laboratory interfaces via a bidirectional data bus. Information sent from the laboratory to the computer includes data from the experiment and parameters dialed in thumbwheels on the laboratory interface control panel. Information sent from the computer to the laboratory includes control signals for the experiment and output to optional Teletypes, CRT displays, and drum plotters. Communication of experimental parameters is generally done through the control panel thumbwheels.

Data transmissions from the laboratory to the computer are in the form of standard records of several different types. All records begin with an address word indicating the type and origin of the records. Up to fourteen additional words follow, depending on the record type. The individual record types are described briefly below and in more detail in Appendix A.

Setup records are sent at the beginning of each experiment to notify the computer of the experiment's onset. A setup record is triggered by pushing the "Setup" and "Send" buttons on the laboratory panel when the panel is in its inactive state. This record consists essentially of the information dialed in on the panel thumbwheels.

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[1] In no case does the identification of trade names imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the equipment identified is necessarily the best available for the purpose.

Re-setup records are identical in content to setup records, but are triggered when the "Setup" and "Send" buttons are pushed while the experiment is running. These records are generally used to transmit new parametric information and to denote major breaking points in the experiment.

Data records contain up to 32 bits of experimental data. The triggering logic is generally hardwired for the individual experiment.

Precursor data records (PCD's) contain the time of year, in milliseconds, and the contents of the input parameter thumbwheels on the panel. They may also contain several words of additional information hardwired for the individual experiment. PCD's are most frequently used to denote the beginning of a series of data records, but they may be used for other purposes as well.

Terminate records consist essentially of a single word and are used to signal the end of an experiment. They are triggered by pushing the "Terminate" and "Send" buttons on the panel.

Experiments which have their own data buffer, such as multichannel analyzers, may also send data to the computer in a high-speed, buffered mode. This is done by first sending the computer a PCD with a special bit set to indicate the presence of buffered data. It is then the computer's responsibility to "read-out" the buffer in the laboratory.

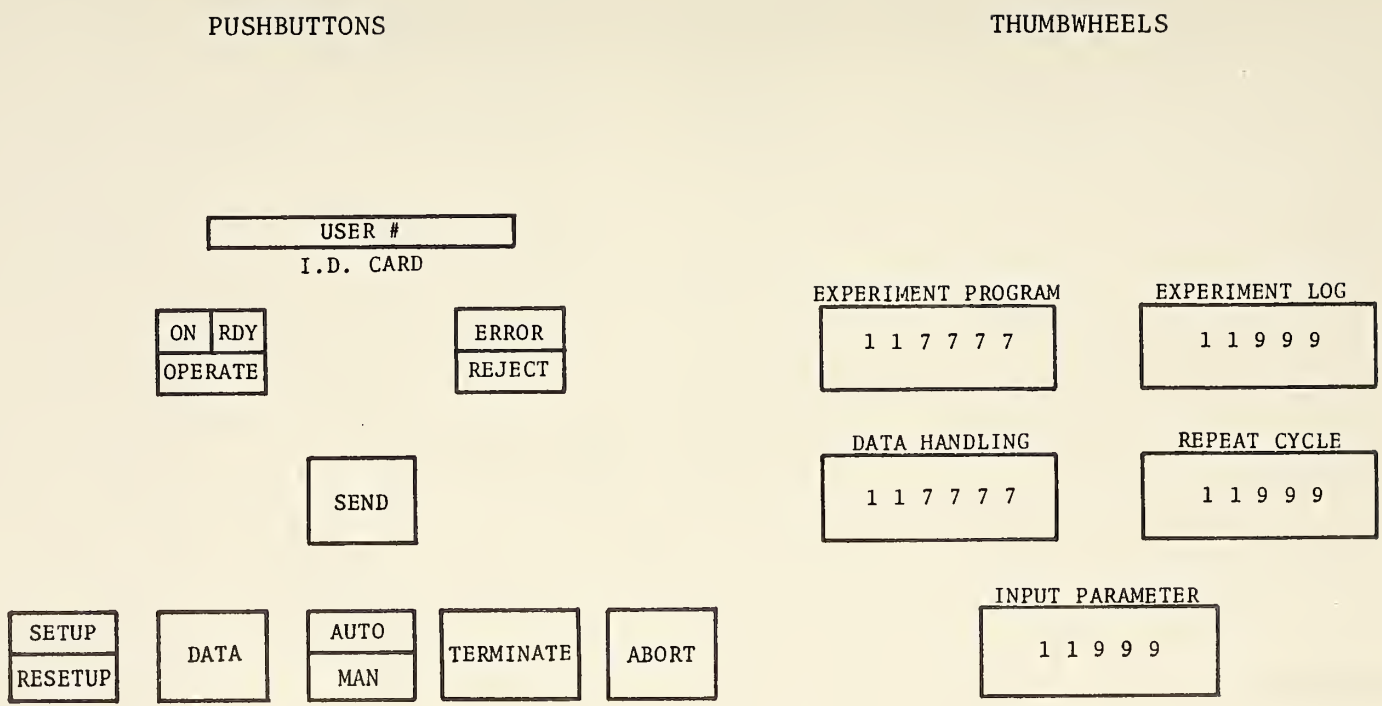
Four major software elements are involved in automating an experiment; the real-time operating system (ASSET), the data acquisition system (DACQ), the file management system (FMGR), and the experiment control program. ASSET provides the multiprogramming, scheduling, and input-output facilities which make all else possible. The experiment control program is the ultimate recipient of all records transmitted from the laboratory to the computer and is the source of control signals and output messages sent back to the laboratory. It is written in FORTRAN and is tailored to the particular experiment. DACQ provides the software interface between each experiment and its associated experiment control program. FMGR facilitates the dynamic creation of sequential disk files for the storage of experimental data. Generally, such a file is created each time an experiment is run. The experiment control program transfers data to disk via calls to FMGR.

3. THE FLUORIMETRY EXPERIMENT

An NADH (reduced nicotinamide adenine dinucleotide) reference standard is being developed for use in clinical laboratories. This experiment is used in determining the theoretical maximum reaction velocity of the NADH sample and the Michaelis Constant associated with the NADH-enzyme combination.

The fluorimeter is used to measure the fluorescent intensity of a fluorescing sample, since dimunition in fluorescent intensity as a function of time serves as a measure of reaction rate of NADH with the alcohol dehyrogenase and the acetaldehyde substrate. A photomultiplier output signal is monitored continuously by a strip chart recorder and a digital voltmeter (DVM). The DVM is sampled at discrete time intervals, with its output going to the computer. The sampling rate is set manually and may vary from 0.1 to 20 readings per second.

The experiment, for automation purposes, can be considered to consist of a number of "runs" each containing three "phases." In a given phase a record of fluorescent intensity vs. time is obtained, with the light source intensity remaining constant. Typically, a run might be five minutes in duration with the entire procedure taking several hours.



1. Diagram of Laboratory Instrument Console

In phase one of each run, the fluorescent intensity of a standard quinine sulfate solution is measured. This is used to correct for instrumental fluctuations between runs. In phase two, a mixture containing NADH, a buffer solution, and an enzyme is placed in the machine and its fluorescent intensity is measured. In phase three, acetaldehyde is added to the solution, causing a reaction. The resulting fluorescent decay curve is measured. Values of initial reaction rate (V_0) and initial NADH concentration (N_0) are calculated from the data obtained for each run. Between runs, the NADH concentration is varied. A plot of $1/V_0$ vs. $1/N_0$ is obtained with each run contributing a single point.

4. FLUORIMETRY DATA ACQUISITION PROGRAM

FLACQ, the fluorimetry data acquisition program, is the experiment control program associated with the fluorimetry experiment. Its generalized structure makes it applicable to other experiments as well.

A. Experiment and File Structure

An "experiment" consists of that quantity of data collected between the time the control panel is "Set Up" and the time it is "Terminated." Experiments are identified by the log number which is dialed into the control panel thumbwheels and is transmitted in the setup record. An experiment includes a variable number of "runs," each of which includes a variable number of "phases." A "file" is associated with each experiment. This file is created when the setup record is received and is filled with data during the course of the experiment. After the experiment, the file may be processed to obtain numeric output, or it may be transferred to an archive tape for later processing or processing on another computer.

The control panel "Data Handling" and "Repeat Cycle" thumbwheels are used to communicate control information when the experiment is set up. The data handling thumbwheels should be zero unless we are restarting an earlier experiment (see 4.B.). The right two repeat cycle thumbwheels should contain the maximum number of phases expected per run and the left two should contain the maximum number of DVM values (in hundreds) expected per phase. An absolute maximum of 500 values per phase is currently assumed.

The information transmitted to the computer for each phase consists of a PCD followed by a number of data records, one for each time sampled DVM reading. In addition to the customary information, the PCD is hardwired to contain the sampling rate and a scale factor corresponding to instrumental sensitivity (see Appendices A and B). The last data record of a phase is marked by a special stop bit. Buttons marked "Start" and "Stop" are mounted on the DVM panel. Pushing the Start button causes a PCD to be sent and sampling to start. Pushing the Stop button causes sampling to cease with the last reading being marked by a stop bit. If the maximum expected number of points per phase is reached before the Stop button is pushed, the computer sends back a signal which has the same effect as pushing the Stop button.

Phases are numbered sequentially within each run by the computer. Runs are numbered sequentially within each experiment. A new run starts whenever either the maximum expected number of phases per run is reached or a re-setup record is sent to the computer.

Each file consists of a label record, also called Record 0, followed by a number of subfiles, also called logical files, one for each phase. The label record contains certain standard identifying information, a copy of the setup record, and the run number and phase number of the last phase currently in the file. Each subfile contains a header entry followed by the DVM readings for the associated phase in floating point format. The header entry contains a copy of the PCD record which initiated the phase as well as the run number and phase number of that phase. See Appendix C for further details.

B. Program Features

1. Checkpoints and Restarts

At the end of each phase, certain measures are taken by FLACQ to facilitate restarts. The fields in the label record denoting last run number and phase number are updated with the run number and phase number from the phase just completed. This information is needed to resume the numbering sequence in the event of a restart. The file is also checkpointed. If a computer failure occurs, the only information lost from the file is that which was written since the last checkpoint.

Restarts are of two types. One type is used after a deliberate interruption and the other after a computer failure.

The experiment may be terminated at any time. If a phase is in progress when the terminate button is pushed, it is simply truncated at the terminate point. The experiment may be later restarted by sending a "Set Up" with the same log number dialed in the control panel thumbwheels and the data handling thumbwheels set to 0001. Phases recorded subsequently will be added to the old file and the numbering sequence will be resumed at the breaking point.

The second type of restart is used after the system has failed and has been brought back into service. The run number and phase number of the last completed phase are dialed into the input parameter thumbwheels and the data handling thumbwheels are set to 0002. Sending a setup then restarts the experiment, using the dialed in information to resume the numbering sequence.

2. Teletype Output

Although disk is the primary medium for recording data, messages are sent to a laboratory Teletype monitoring the progress of the experiment. These are explained individually below.

Event	Message
Start of experiment (Setup)	PREFATORIAL DATA Message followed by identifying information from setup record.
Start of experiment	DUPLICATE LOG NO. Message appears only if log no. dialed in duplicates that of a file already on disk.
Restart	RESTART RUN NO. XX PHASE NO. XX These are the run and phase nos. of the last phase acquired before restart.
Restart	RESTART--FILE MISSING Message appears only if restart is attempted with a log no. for which there is no corresponding file on disk.
Start of phase	RUN XX PHASE XX
End of Phase	VALUE = XXXXXXXXX. DVM reading in last data record of phase. Message may not appear until start of next phase.
End of phase	END OF PHASE Message may not appear until start of next phase.
End of Run	END OF RUN Message may not appear until start of next run.
End of experiment (Terminate)	EXPERIMENT TERMINATED

C. Program Description

1. Major Variables

Major program variables are identified below. Minor variables, not involved in controlling program flow, are omitted.

NPHASE -- Maximum number of phases per run; obtained from setup record.

NPTS -- Maximum number of points per phase; obtained from setup record.

IPHASE -- Current phase number.

IRUN -- Current run number.

IPTS -- Number of points collected so far in current phase.

ACTIV -- Logical switch indicating phase in progress.

FLUSH -- Logical switch indicating that a "Stop" has been sent to the DVM and that further data should be ignored.

VALUE -- Floating point value extracted from data record.

2. Flowcharts

The basic functions of the fluorimetry acquisition program are shown in the flowcharts which follow. Input-Output status checks and other minor details are omitted for clarity. The program is organized around the different possible input record types and the flowcharts reflect this.

The "End-of-Phase Subroutines" is a separately compiled external FORTRAN subroutine, called SUBROUTINE EPHASE. The "Terminate Routine" is a bit different from the other routines in that DACQ enters it directly on receipt of a terminate record instead of passing the record to the experiment control program as is done with the other record types.

3. Error Codes

The acquisition program uses Subroutine ERROR to report input-output failures and other difficulties on the console Teletype in the computer laboratory. The message typed is always of the form ERROR 6XX or ERROR 7XX. When ERROR 7XX is typed, the experiment is automatically terminated. Error codes typed and their meanings are listed below:

ERROR 600 -- Data record received at time when this is illegal.

ERROR 610 -- Unrecognizable record received or PCD record received at time when it's illegal.

ERROR 720 -- I/O failure.

ERROR 723 -- File manager error, or duplicate log no. on Setup, or file missing on Restart.

D. Operating Instructions

Computer related operating procedures are described individually below:

To begin experiment -- Insert User I.D. Card. Set control panel dials as follows:

Experiment Program -- 67 (Identifies FLACQ).

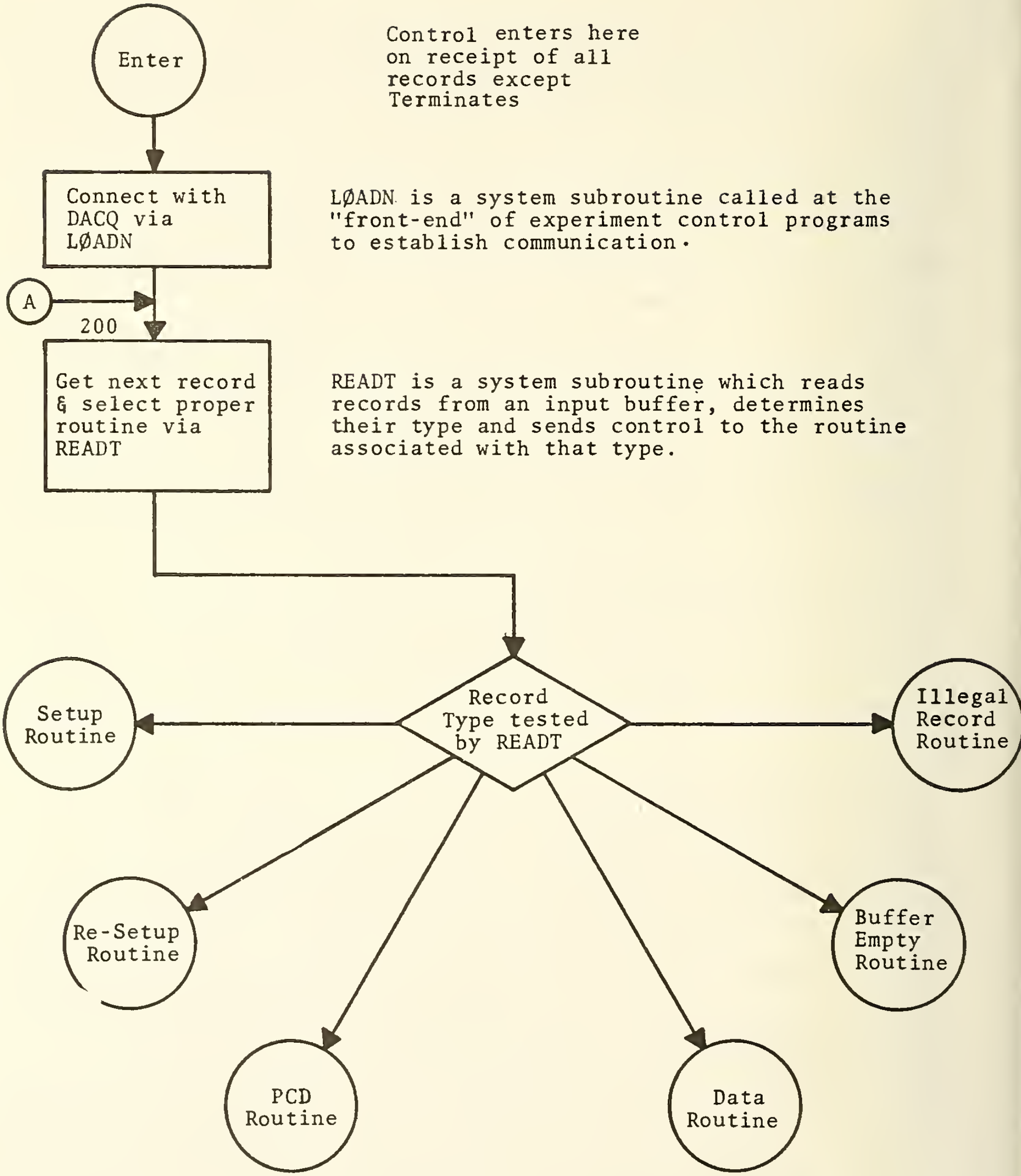
Experiment Log No. -- new value (Identifies file).

Data Handling -- 0000

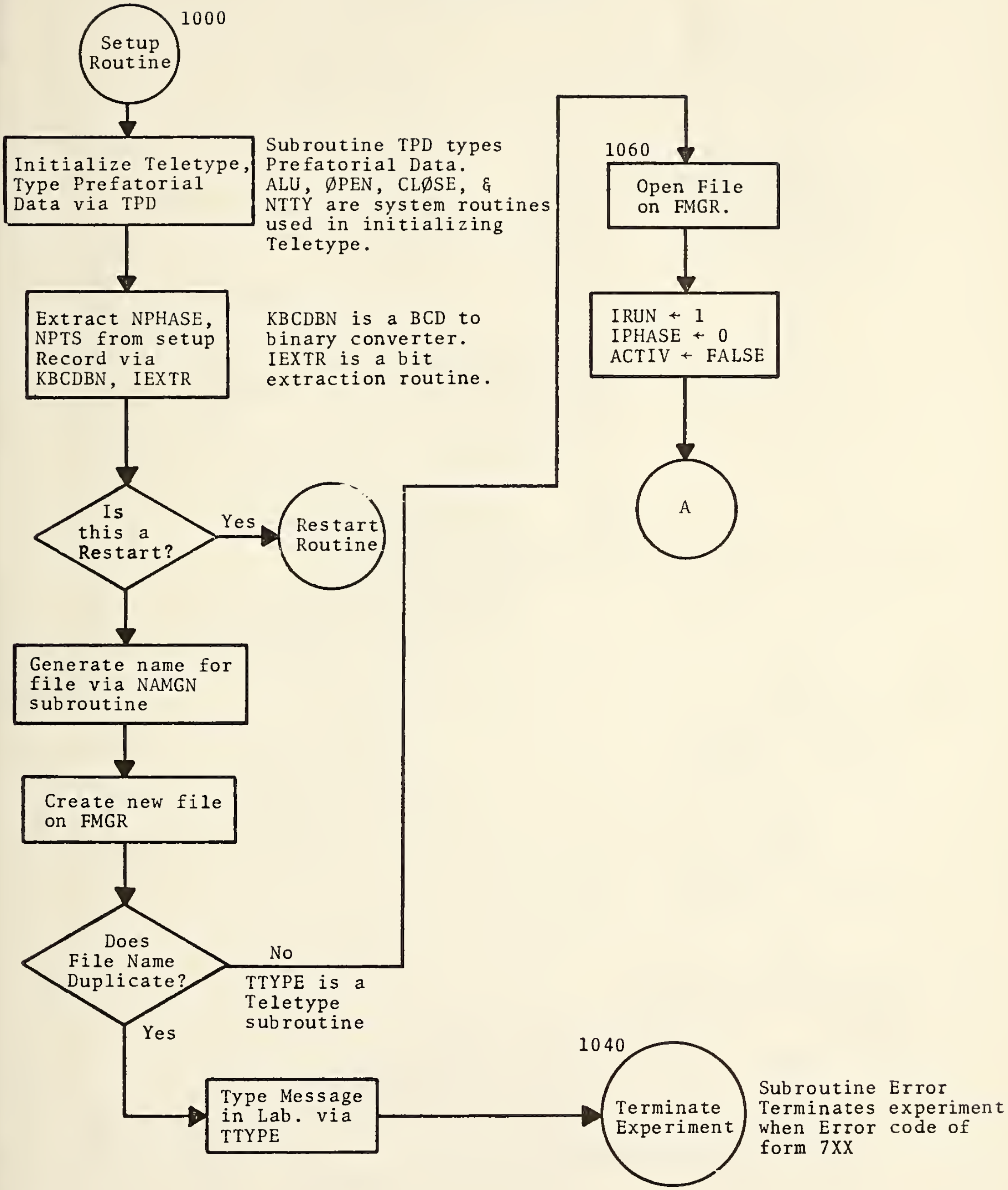
Repeat Cycle -- Maximum number of phases per run in right two dials, maximum number of points (hundreds) per phase in left two dials.

The DVM rate dial should be set. Control Panel should be in ON RDY state. Turn on Teletype. Push "Setup," then "Send." Control panel should switch to "Operate" state and the Teletype should type "Prefatorial Data." If "Duplicate Log No." is typed, try again with a new log number.

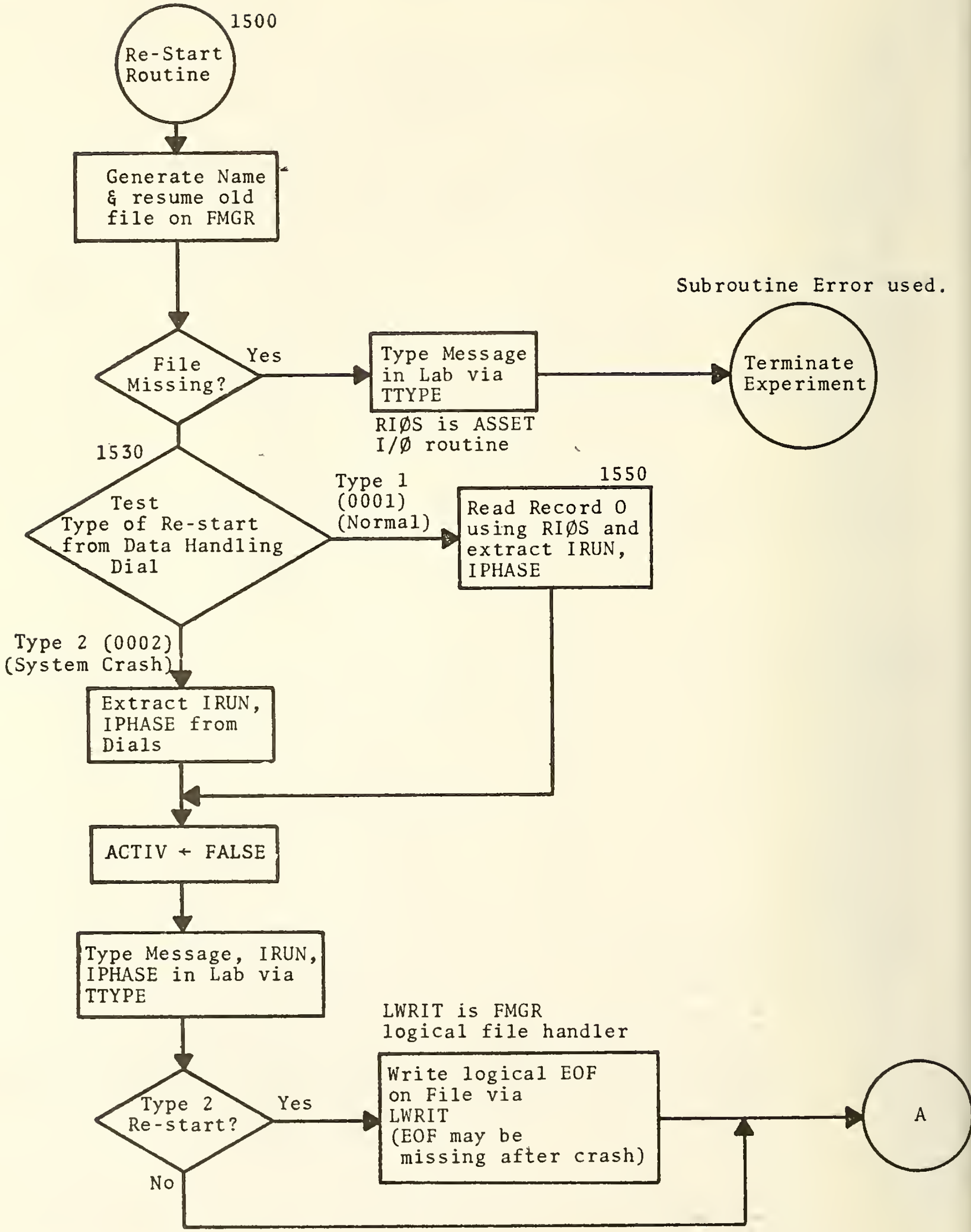
MAIN ROUTINE



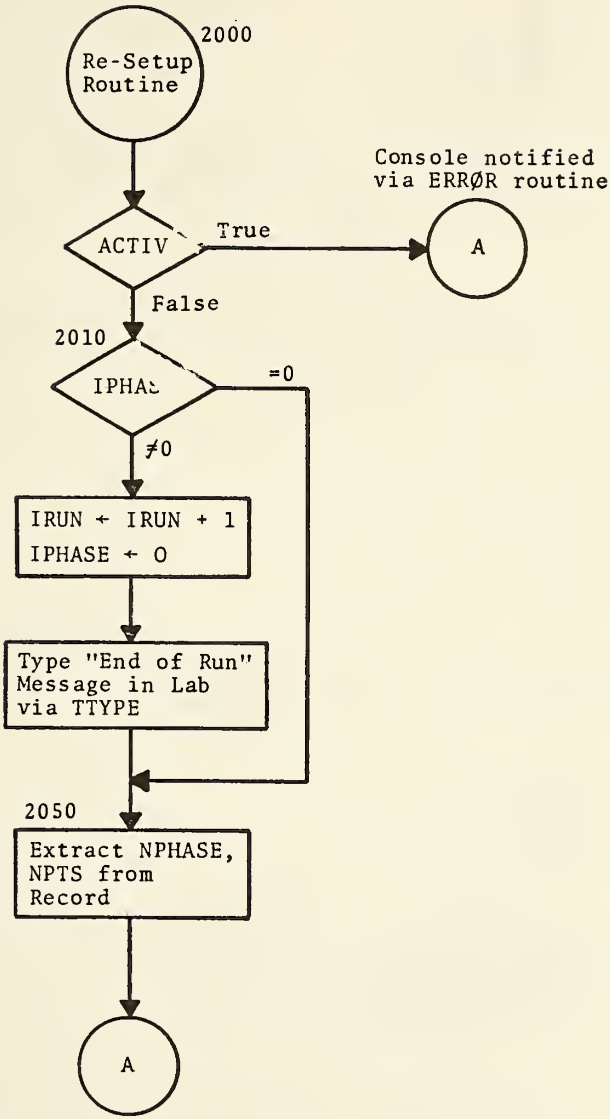
SETUP ROUTINE



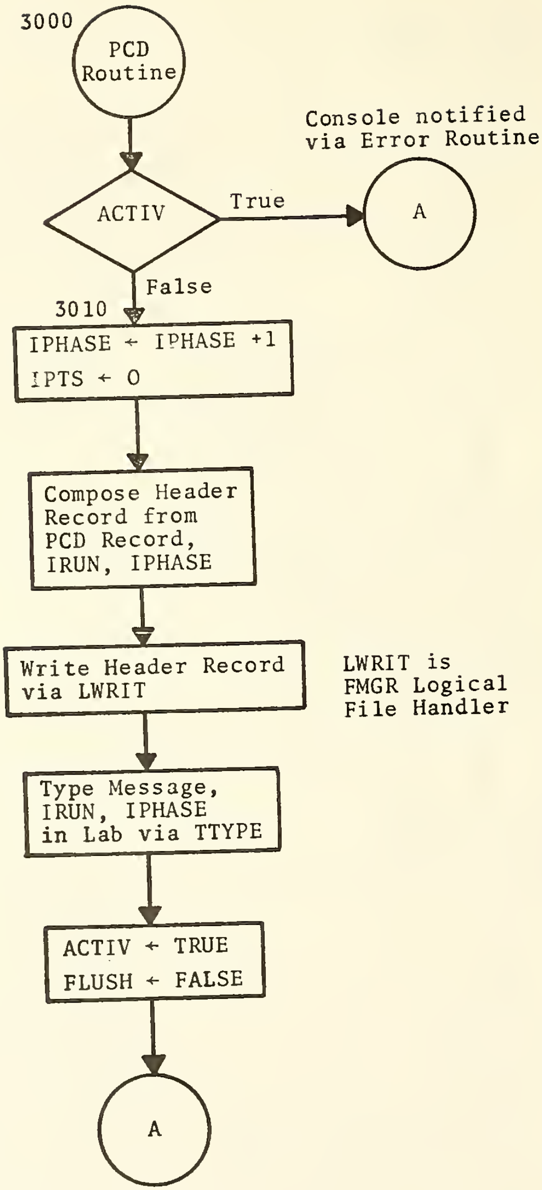
RE-START ROUTINE



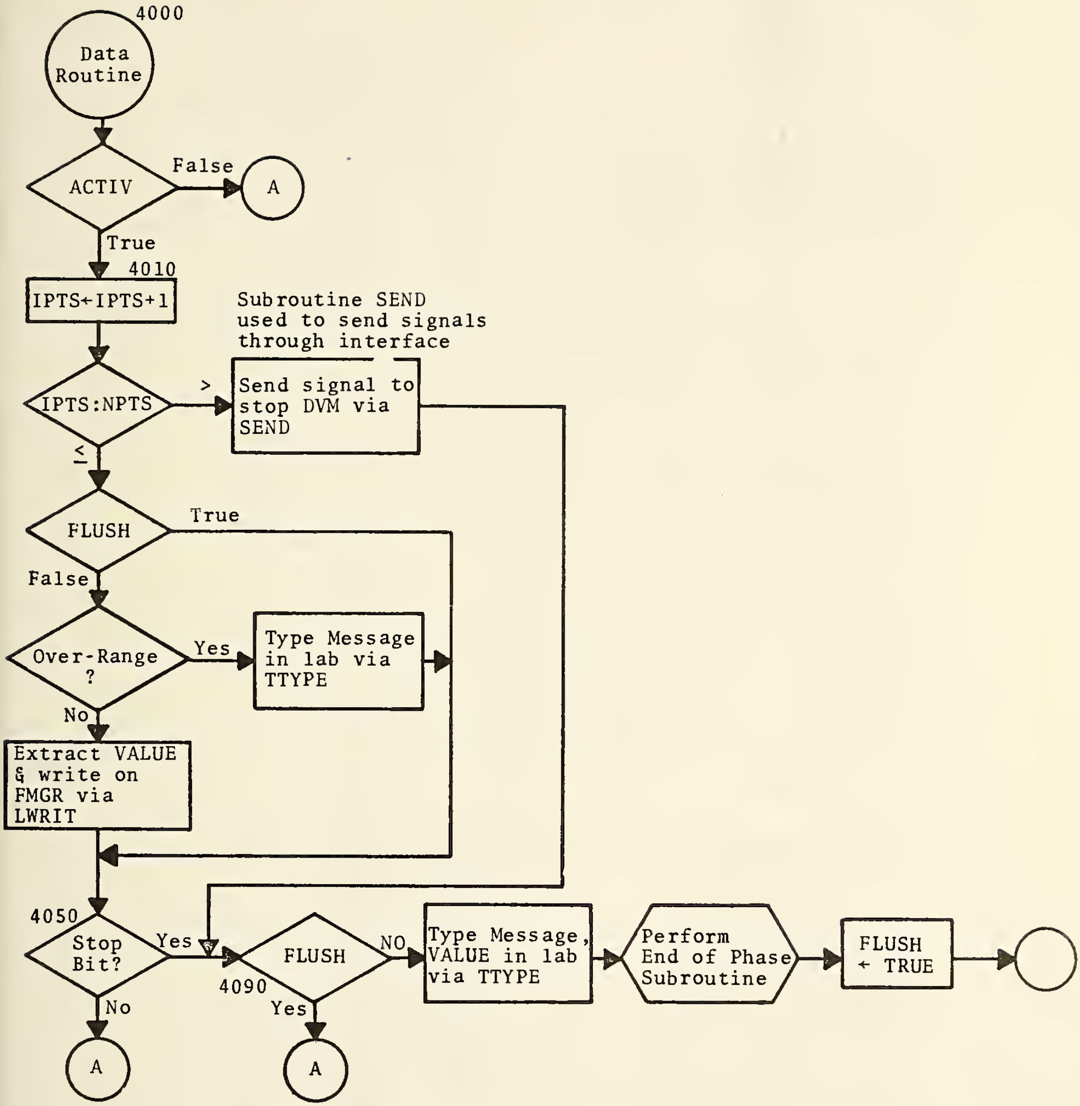
RE-SETUP ROUTINE



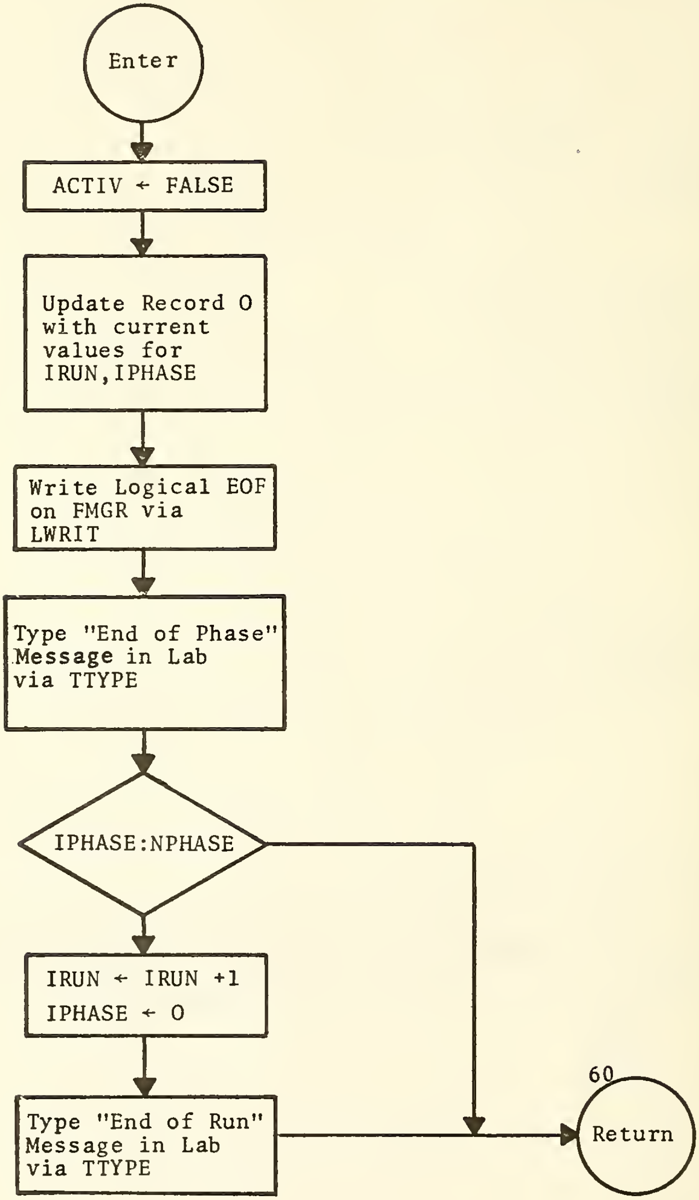
PCD ROUTINE


3000
PCD Routine
Console notified via Error Routine
ACTIV
True
A
False
3010
IPHASE ← IPHASE +1
IPTS ← 0
Compose Header Record from PCD Record, IRUN, IPHASE
Write Header Record via LWRIT
LWRIT is FMGR Logical File Handler
Type Message, IRUN, IPHASE in Lab via TTYPE
ACTIV ← TRUE
FLUSH ← FALSE
A

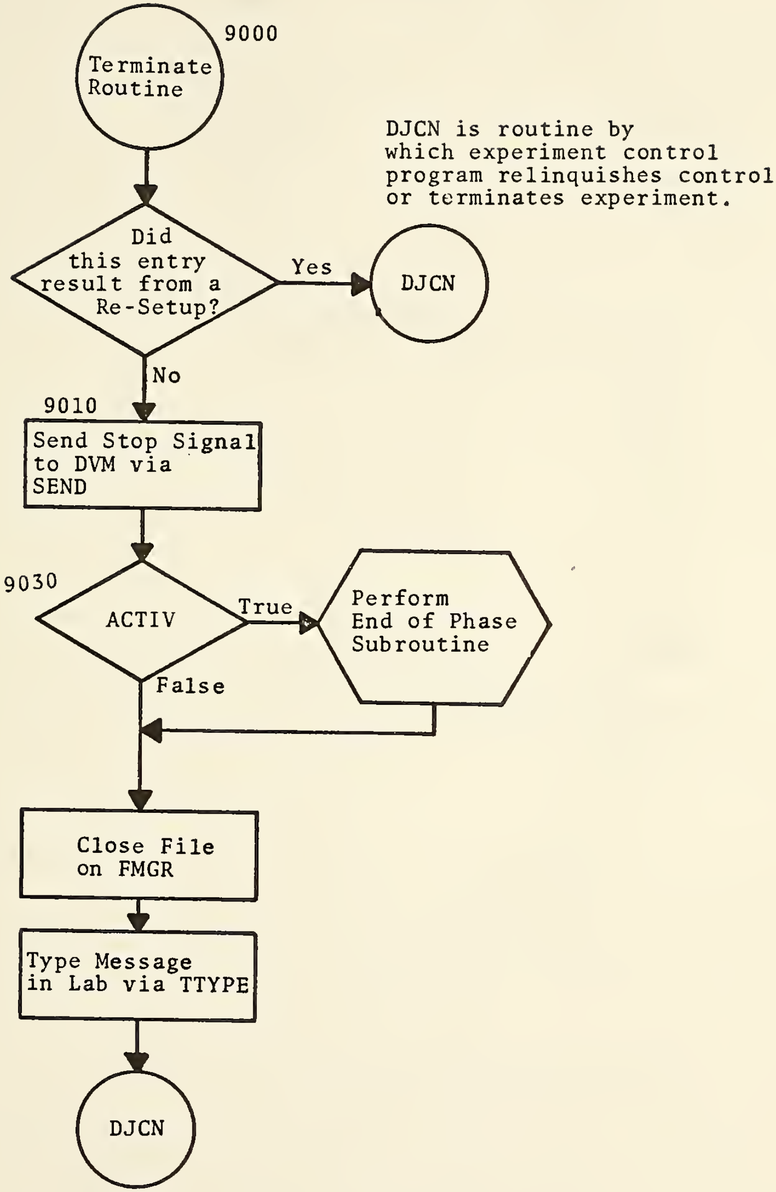
DATA ROUTINE



END OF PHASE SUBROUTINE

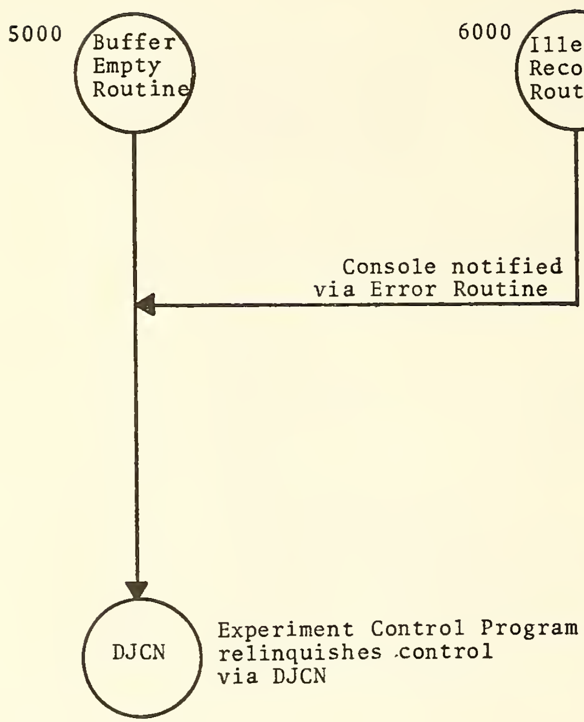


TERMINATE ROUTINE



BUFFER EMPTY AND

ILLEGAL RECORD ROUTINES



To begin phase -- Push start button on DVM. Teletype should type out run and phase numbers.

To begin new run before reaching maximum phase number -- Set control dials as at beginning of experiment. Push "Re-Setup," then "Send." If "Error" lights on panel, try again. Teletype should respond with "End of Run."

To Terminate Experiment -- Push "Terminate," then "Send." Teletype should respond with "End of Experiment."

To Restart after normal Terminate -- Follow same procedure as at beginning of experiment, but with 0001 in the Data Handling dials. Teletype should respond with "Prefatorial Data" followed by the run and phase numbers at which the interruption occurred. If no file can be found on disk which matches the Log Number dialed, the Teletype will say so.

To Restart after computer failure -- The Data Handling dials should contain 0002. The Input Parameter dials are used to resume the run and phase number sequence with the run number in the left two dials and the phase number in the right two. The run and phase number of the last completed phase should be used unless we are at the beginning of a run, in which case we use the run number of that run and a phase number of 0. Procedure is like that immediately above otherwise.

Spooling of Teletype messages sometimes results in a delay before their receipt in the laboratory. The user should wait for a message about twenty seconds before concluding that the system has failed.

5. FLUORIMETRY DATA PROCESSING PROGRAM

A. Program Description

This program performs computations on the file produced by the acquisition program (see Appendix C). It is run as a non-resident foreground program on the Univac Series 60

(EMR 6135) computer. It reads the file from disk and produces processed output on the line printer.

1. General Description

The program begins execution by reading the I.D. No., Code, and Log No. needed to identify the file from the console Teletype. A heading reflecting this information is printed. Successive phases are then read and processed. After the last phase has been processed, a summary table is printed giving the reciprocals of initial reaction rate and NADH concentration for each run completely processed. The program expects phases numbered 1, 2, and 3, in that order, in each run and does not care about the sequence of run numbers. Any sequence errors are detected and handled.

Phase 1 Processing

The average and standard deviation of the intensity values on the file are computed and printed out using subroutine STAT2. The average intensity is saved for phase 3, where it is used as the normalizing quinine sulfate value.

Phase 2 Processing

The average and standard deviation of the intensity values are computed and printed out as in phase 1.

Phase 3 Processing

A least squares line is fitted to the values received, and the slope and y-intercept are printed out. The y-intercept is in millivolts and the slope is computed using a time unit equal to the constant time separation between points. Residuals and their standard deviation are computed and printed out in units of volts. A sensitivity correction is computed using the sensitivity from the header record. The time unit is converted to minutes using the rate from the header. The period between readings, the number of readings. the sensitivity, and the quinine sulfate intensity saved from phase 1 are all printed out. Initial intensity, reaction rate, and NADH concentration are computed and printed out using the formulas discussed in (2). The run number and the reciprocals of the initial reaction rate and NADH intensity are saved for printout in the summary at the end of processing.

The usefulness of the processing program is presently severely limited by the presence of certain excursions, or "blips", in the phase 3 data. These occur approximately 40% of the time. This problem should be corrected by either modifying the instrument or by the computational filtering off of the "blips" in the phase 3 processing routine before further applying the program to the present apparatus. The program presently assumes that it is operating on a nearly linear initial portion of the reaction rate curve. Some modification of this assumption might also be desirable.

Tables and Constants

A table in the program (CTAB) is used to obtain initial NADH concentration as a function of initial intensity via table lookup and linear interpolation. Short tables are also used to interpret the sensitivity and data rate bits in the header record. The program presently assumes a constant background intensity of .0006.

Error Conditions

Error indications appear on the line printer in the computer laboratory as listed below:

FILE MISSING -- No file on disk matching identifiers entered on console Teletype, no recovery.

FMGR ERROR -- I/O error, no recovery.

FILE ERROR -- Error in logical file structure, no recovery.

PHASE NO. BAD -- Phase encountered with phase number other than 1, 2, or 3. Phase is identified on printout but not processed.

SEQUENCE ERROR -- Phase encountered out of the expected 1, 2, 3 phase number sequence within each run. Phase is identified on printout but not processed.

If a run contains less than three phases on the file, and they are sequenced correctly, the phases present will be processed, but no entry for that run will appear in the summary table at the end of the printout.

If a phase contains more than 300 points, the excess points are ignored.

2. Flowcharts

The general flow of the processing program is shown. The "Phase 1, 2, and 3 routines" are internal branches. STAT2 is an external subroutine used to compute the average and standard deviation of a linear array.

3. Derivation of Formulas

The formulas derived here are used during phase 3 processing for computing initial intensity (I_0), initial NADH concentration (N_0), and initial reaction rate (V_0).

Notation

Q = Average fluorescent intensity (volts) obtained with quinine sulfate in Phase 1; used to normalize between runs

S = Fluorimeter sensitivity in Phase 3

C = Normalization factor to be applied to Phase 3 intensity data

F = y intercept of observed fluorescence curve (volts)

V = -Slope of observed fluorescence curve (volts/minute)

$N(t)$ = NADH concentration as function of time

N_0 = Initial ($t=0$) NADH concentration = $N_{(0)}$

$I(t)$ = Fluorescent intensity as function of time

I_0 = Initial ($t=0$) fluorescent intensity = $I_{(0)}$

I_{BG} = Background fluorescence intensity

I_r = Portion of fluorescent intensity resulting from reaction = $I-I_{BG}$

V_0 = Initial reaction rate = $(dN/dt)_{t=0}$

From the observed Phase 3 fluorescence curve we wish to calculate V_0 and N_0. We use an experimentally obtained table of NADH concentration vs. fluorescent intensity to obtain N_0 after calculating I_0.

By first principles we have

(i) $dN/dt = -\lambda N$ for some constant λ.

We assume fluorescent intensity to be proportional to NADH concentration. Thus, $N(t)/I_r(t)$ = constant. Differentiating, $(dN/dt)/N = (dI_r/dt)/I_r$. Thus, $dI_r/dt = -\lambda\ I_r$ by (i).

Integrating and substituting $I-I_{BG}$ for I_r we get

$I(t) - I_{BG} = (I_0-I_{BG})\ e^{-\lambda t}$. Linearizing,

$I(t) - I_{BG} = (I_0-I_{BG})\ (1-\lambda t)$ or

(ii) $I(t) = I_0 - \lambda\ (I_0-I_{BG})\ t$.

Now F and $-V$ are the y-intercept and slope of the observed fluorescence curve. We apply a factor $c = S/(Q \times 3.0)$ to account for sensitivity and to normalize between runs, obtaining corrected values $F' = cF$ and $V' = cV$. The factor

3.0 arises from an assumed sensitivity during the Phase 1 quinine sulfate observations. We then have $I_0 = F'$ by (ii) and we get N_0 from I_0 by table lookup and linear interpolation.

We have $V' = \lambda(I_0 - I_{BG})$ from (ii). Solving for λ, we get

(iii) $\lambda = V' \left(\frac{1}{I_0 - I_{BG}}\right)$.

Evaluating (i) at t=0 we get

(iv) $V_0 = -(dN/dt)_{t=0} = \lambda N_0$

Combining (iii) and (iv) we get

(v) $V_0 = V' \left(\frac{N_0}{I_0 - I_{BG}}\right)$. This is the equation used in calculating V_0.

B. Operating Instructions

1. Enter SNR,45,12 on the console Teletype to begin execution (schedules non-resident program number 45 at priority 12).

2. Console Teletype will request a word on input three times. Request and proper operator response are given below.

ID

I user's I.D. No., in this case 575

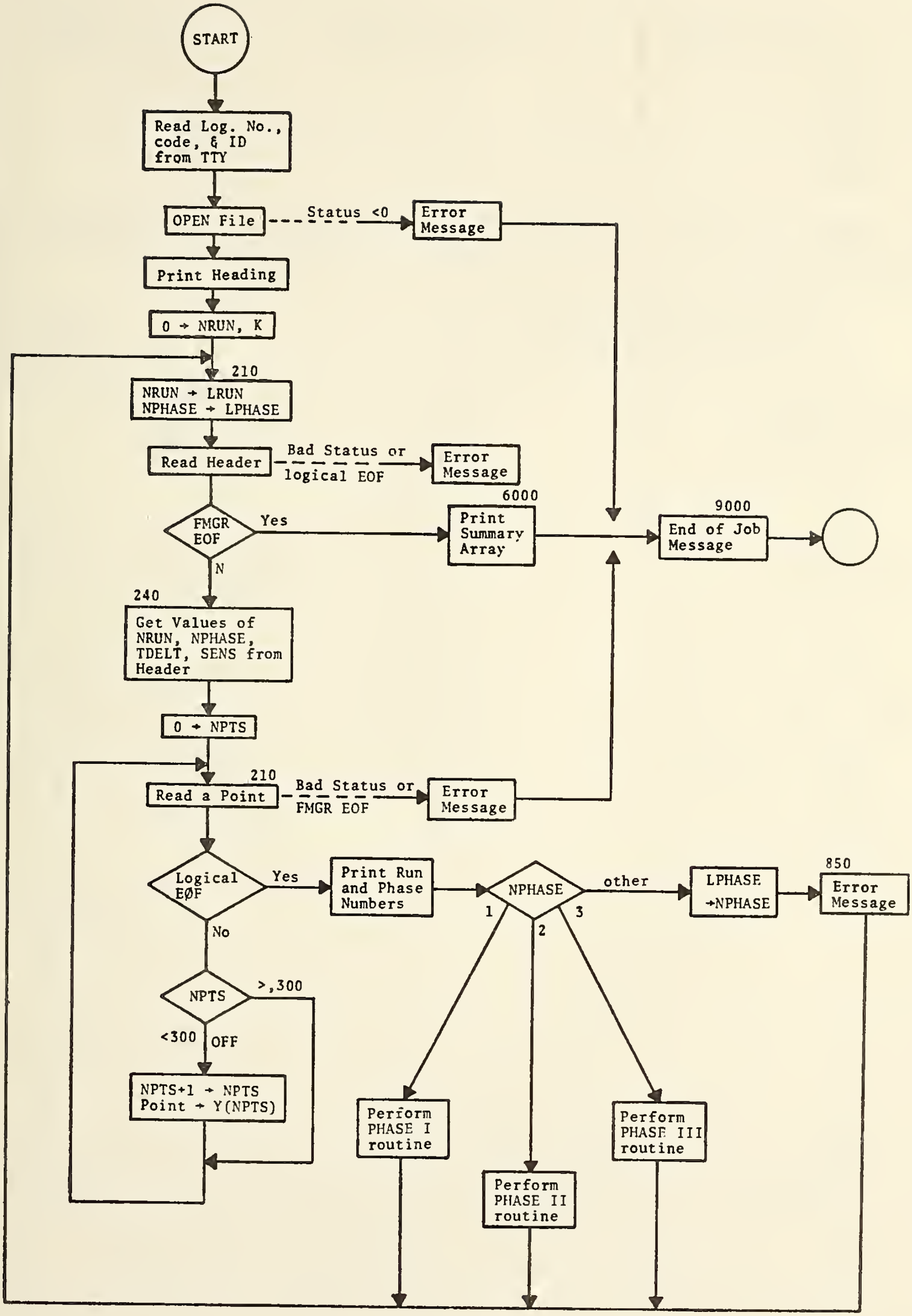
CODE

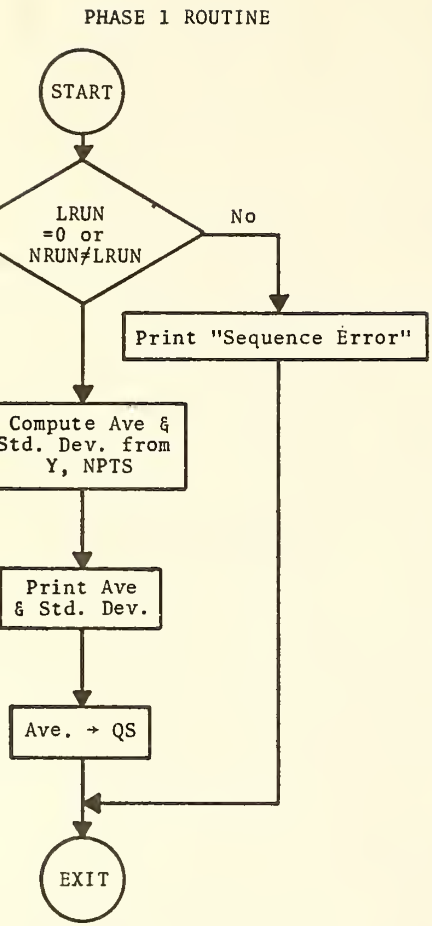
I identifying code word in file name, in this case 00000

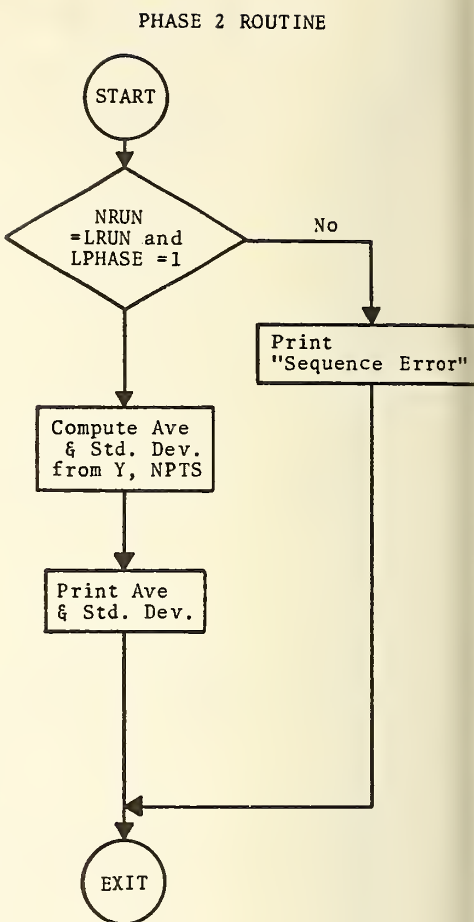
LOG NO.

I five digit log number dialed into control panel when file was created

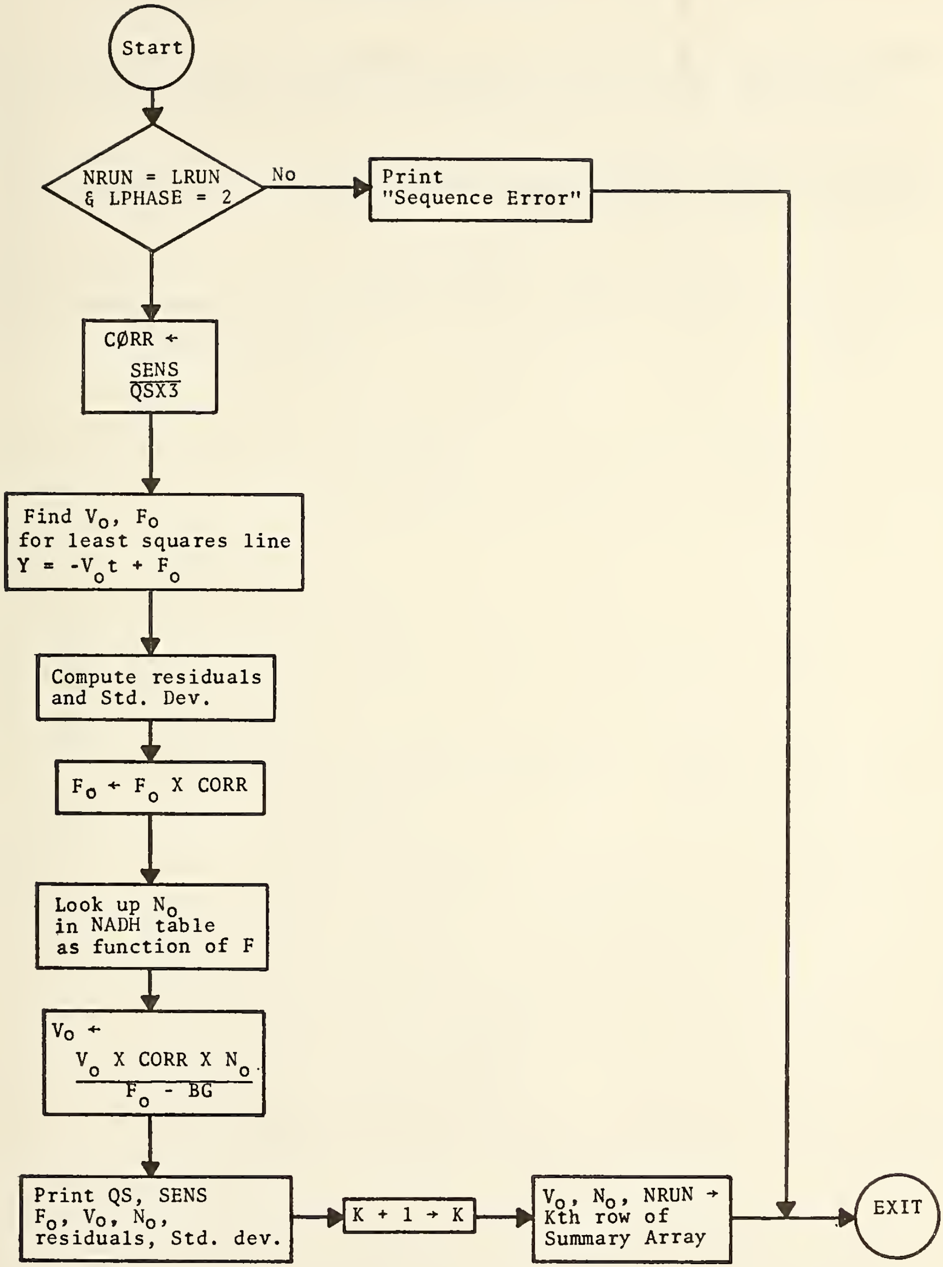
3. Program runs to completion.


START
Read Log. No., code, & ID from TTY
OPEN File
Status <0
Error Message
Print Heading
0 → NRUN, K
210
NRUN → LRUN
NPHASE → LPHASE
Read Header
Bad Status or logical EOF
Error Message
FMGR EOF
Yes
N
6000
Print Summary Array
9000
End of Job Message
240
Get Values of NRUN, NPHASE, TDELT, SENS from Header
0 → NPTS
210
Read a Point
Bad Status or FMGR EOF
Error Message
Logical EØF
Yes
No
Print Run and Phase Numbers
NPHASE
other
1
2
3
LPHASE →NPHASE
850
Error Message
NPTS
>,300
<300
OFF
NPTS+1 → NPTS
Point → Y(NPTS)
Perform PHASE I routine
Perform PHASE II routine
Perform PHASE III routine


PHASE 1 ROUTINE
START
LRUN
=0 or
NRUN≠LRUN
No
Print "Sequence Error"
Compute Ave &
Std. Dev. from
Y, NPTS
Print Ave
& Std. Dev.
Ave. → QS
EXIT


PHASE 2 ROUTINE
START
NRUN
=LRUN and
LPHASE =1
No
Print
"Sequence Error"
Compute Ave
& Std. Dev.
from Y, NPTS
Print Ave
& Std. Dev.
EXIT

PHASE 3 ROUTINE



6. LITERATURE

DeVoe, J. R., Shideler, R. W., Ruegg, F. C., Aronson, J. P., Shoenfeld, P. S., Anal. Chem. 46, 509 (1974).

ASSET IV Real-Time Monitor User's Manual, EMR Computer, Bloomington, Minn., 1970.

FORTRAN IV User's Manual, EMR Computer, Bloomington, Minn., 1970.

APPENDIX A. GENERAL RECORD DESCRIPTIONS

The records transmitted from the teleprocessor (low-speed) to the computer all consist of an address word plus up to fourteen additional words. Bits 1-8 of the address word contain the laboratory address of the device sending the record. Bits 9-10 are unused. Bits 11-16 are control bits identifying the record type.

1. Setup Records

A Setup record is sent when the experimenter pushes his "SETUP" and "SEND" buttons. It contains fourteen words as well as the address word with contents as follows:

Word	Contents
* Identifier Octal 100000	
address	Bits 16 and 15 on (140xxx, x=address), other control bits off
1	Bits 1-11, User No. (from I.D. Card); Bits 12-16 No. words in PCD in addition to address word (hard-wired), see below
2-4	Time of year to nearest millisecond, see below
5	Experiment log No. (from thumbwheels)
6	Input Parameter (from thumbwheels)
7	No. of high-speed words (Mantissa bits 1-13, Exponent -1 bits 14-16)
8	Nonresident program number of applications program (from thumbwheels)

* The Identifier is placed in front of the address word of the Setup Record by DACQ when it is passed to an application program It is not transmitted from the teleprocessor.

9	Repeat cycle; No. of PCD Records to be processed before forced termination (from thumbwheels) 4 BCD digits
10	Data Handling (from thumbwheels)
11-14	Special purpose; not generally used

Time of Year Format

Word	Bits	Contents
2	15-16	Day; 1st BCD digit
	11-14	Day; 2d BCD digit
	7-10	Day; 3d BCD digit
	5-6	Hour; 1st BCD digit
	1-4	Hour; 2d BCD digit
3	12-14	Minute; 1st BCD digit
	8-11	Minute; 2d BCD digit
	5-7	Second; 1st BCD digit
	1-4	Second; 2d BCD digit
4	9-12	Millisecond; 1st BCD digit
	5-8	Millisecond; 2d BCD digit
	1-4	Millisecond; 3d BCD digit

No. of PCD Words Format (Bits 12-16, Word 1)

Contents	No. of Words
10000	14
01000	12
00100	10
00010	8
00001	6

2. Re-Setup Records

A Re-Setup Record is sent when the experimenter pushes his "SETUP" and "SEND" buttons. It contains fourteen words as well as the address word with contents as follows:

Word	Contents
* Identifier	Octal 100000
address	Bits 16 and 14 on (octal 120xxx, x=address), other control bits off
1-14	Same as Setup Record

* The Identifier is treated the same way as in Setup Records.

3. Data Records

A Data Record is sent when the experimenter pushes his "DATA" and "SEND" buttons when in manual mode and automatically when in automatic mode. It contains two words as well as the address word with contents as follows:

Word	Contents
* Identifier	Octal 2000
address	All control bits off
1-2	Data

* PCD and Data Record Identifiers are generated by DACQ and replace the address word when the record is passed to an application program.

4. PCD (Precursor Data) Records

PCD Records are generally used to transmit control information. They are usually sent periodically according to a hard-wired scheme which is application dependent. The number of words sent in addition to the address word may be six, eight, ten, twelve, or fourteen. This number is application dependent, but fixed for a particular application. Contents are as follows:

Word	Contents
* Identifier	Octal 40000
address	Bits 16 and 13 on (010xxx, x=address), bit 11 may also be on to signal a high-speed transmission; other control bits off
1-6	Same as Setup Record
7-14	Application dependent when present

* PCD and Data Record Identifiers are generated by DACQ and replace the address word when the record is passed to an application program.

5. Terminate Records

A Terminate Record is sent when the experimenter pushes his "TERMINATE" and "SEND" buttons. It contains fourteen words as well as the address word with contents as follows:

Word	Contents
address	Bits 16 and 12 on (004xx, x=address), other control bits off
1-14	Same as setup record (currently ignored).

APPENDIX B. RECORD FORMAT DETAILS FOR FLUORIMETRY EXPERIMENT

Setup Record

Repeat Cycle Dial -- Max. No. Phases/Run in bits 1-8, Max. No. Hundred Points/Phase in bits 9-16

Log No. Dial -- used for naming file as is customary

Data Handling Dial -- 0000 means normal setup

0001 means restart after normal terminate

0002 means restart after computer failure

Input Parameter -- meaningful only when restarting after computer failure

Bits 1-8 give phase no. to resume with

Bits 9-16 give run no. to resume with

PCD Record

PCD's are nine words long. Words 1-7 are standard and word 9 is not used. Bits 4-8 of word 8 indicate sensitivity and bits 9-16 indicate data rate as shown below.

Bits 4-8	Full Scale DVM Value in Volts
00001	1.0
00010	0.3
00011	0.1
01000	0.03
10000	0.01

Bits 9-16	Time Interval between Readings in Seconds
00000001	0.05
00000010	0.1
00000011	0.2
00001000	0.5
00010000	1.0
00011000	2.0
01000000	5.0
10000000	10.0

Data Record

Word 1 -- Address word

Word 2 --

- Bit 5 -- Stop Bit, 1 means stop
- Bit 4 -- Over-range Bit, 1 means over-range
- Bit 3 -- Sign Bit, 1 means minus
- Bits 1-2 -- Most significant BCD digit (3 is max.)

Word 3 -- Least significant four BCD digits

Appendix C. Fluorimetry File Description

	1st Phase of 1st Run				Last Phase of Last Run			
Rec. 0	Header	Data	Logical E.O.F.	...	Header	Data	Logical E.O.F.	FMGR E.O.F.

Record 0

0	20 34	35	36	49
FMGR Standard Information	Setup Record Info. inserted by NAMGN	IRUN	IPHASE	

IRUN & IPHASE are the Run No. & Phase No. of the last phase on the file

Header

1 9	10	11	12
PCD as received	Run No.	Phase No.	

Data

Each data block contains an indeterminate number of floating point (2 word) entries.

Logical E.O.F.'s

These are generated by LWRIT and detected by LREAD. More than one logical E.O.F. may appear between phases; since an extra logical E.O.F. is sometimes inserted by the Re-start procedure.

APPENDIX D. SAMPLE DATA PROCESSING OUTPUT

```
                                FLUORIMETRY PROCESSING
ID= 575      CODE=      0     LOG NO.=      3

  RUN NO      1 PHASE NO.      1
INTENSITY    .3759            STD. DEV.    .0020

  RUN NO      1 PHASE NO.      2
INTENSITY    .3069            STD. DEV.    .1069

  RUN NO      1 PHASE NO.      3
SLOPE     -.3004            YINT  315.7137

                                  RESIDUALS
 -.0014   .0019   .0032   .0025   .0018  -.0009  -.0016  -.0013

 -.0000  -.0007  -.0014  -.0021  -.0018  -.0005  -.0022   .0001

 -.0006  -.0013   .0020   .0003  -.0004   .0009   .0032   .0005

STD. DEV.    .0017      PERIOD  .01667      NO. OBS.    24
QUININE SULFATE    .3759         SENSITIVITY  1.0000
INTENSITY    .2799     INIT.RATE 12.0098      CONCEN.209.8913
```

RUN NO 2 PHASE NO. 1
INTENSITY .3086 STD. DEV. .0017

RUN NO 2 PHASE NO. 2
INTENSITY .3020 STD. DEV. .0005

RUN NO 2 PHASE NO. 3
SLOPE -.9689 YINT 308.9615

RESIDUALS

-.0100	-.0090	-.0101	-.0101	-.0081	-.0091	-.0082	-.0092
-.0092	-.0073	-.0093	-.0093	-.0064	-.0104	-.0074	-.0065
-.0065	-.0055	-.0036	-.0076	-.0016	-.0046	-.0017	.0003
.0003	.0002	.0012	.0002	.0021	.0031	.0071	.0040
.0040	.0060	.0060	.0099	.0079	.0069	.0108	.0108
.0078	.0097	.0097	.0127	.0136	.0116	.0136	.0135
.0145	.0165	.0155	.0174	.0164	.0184	.0183	.0183
.0223	.0222	.0192	.0222	.0221	.0211	.0241	.0270
.0230	.0240	.0260	.0249	.0279	.0269	-.2352	-.2352

STD. DEV. .0421 PERIOD .01667 NO. OBS. 72
QUININE SULFATE .3086 SENSITIVITY 1.0000
INTENSITY .3337 INIT.RATE 59.8335 CONCEN.317.4258

```
  RUN NO      3 PHASE NO.     1
INTENSITY   .6724          STD. DEV.   .0037

  RUN NO      3 PHASE NO.     2
INTENSITY   .3054          STD. DEV.   .0017

  RUN NO      3 PHASE NO.     3
SLOPE     -.3723        YINT  290.7699

                                RESIDUALS
 -.0014   .0010  -.0007  -.0003   .0011  -.0005  -.0002  -.0028

 -.0014  -.0010  -.0017   .0017   .0001   .0004   .0008   .0012

 -.0004  -.0001  -.0007  -.0013   .0030  -.0016  -.0002   .0032

  .0005  -.0021   .0003   .0007   .0010  -.0006  -.0002  -.0009

  .0005   .0029  -.0017   .0016   .0010  -.0016   .0008  -.0009

 -.0025   .0009   .0012   .0026   .0020  -.0006   .0017  -.0009

  .0025  -.0002   .0012   .0006  -.0000   .0023  -.0003   .0001

  .0005  -.0012   .0012  -.0024  -.0001  -.0017  -.0003  -.0009

 -.0016  -.0002  -.0018  -.0005  -.0001  -.0027  -.0013  -.0010

 -.0006  -.0002   .0002   .0005   .0009   .0023  -.0024   .0000

  .0004   .0018   .0011

STD. DEV.   .0014     PERIOD  .01667     NO. OBS.   83
QUININE SULFATE   .6724        SENSITIVITY  1.0000
INTENSITY   .1441     INIT.RATE  4.2765     CONCEN. 55.4318
```

(RUNS 4-22 OMITTED FROM APPENDIX)

SUMMARY

RUN	1/INIT.RATE	1/CONCENTRATION
1	.0833	2.3378
2	.0167	1.4668
3	.2338	1.3698
4	.2581	1.6481
5	.2611	1.5998
6	.2389	2.0528
7	.3069	2.5986
8	.2927	.0567
9	-580.1123	.0454
10	3.8618	.0546
11	3.4137	.0516
12	1.2163	.0538
13	-50.3861	.0545
14	12.5003	.0544
15	.9596	.0518
16	2.3378	408.0000
17	1.4668	407.0000
18	1.3698	412.0000
19	1.6481	415.0000
20	1.5998	416.0000
21	2.0528	416.0000
22	2.5986	.0360

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The National Bureau of Standards Analytical Chemistry Division uses a centralized computer system to automate a number of experiments. Computer software for a fluorimetry application is described in this report. The file and program structure used is quite general and can be applied to other experiments as well.

17. KEY WORDS *(six to twelve entries; alphabetical order; capitalize only the first letter of the first key word unless a proper name; separated by semicolons)*

Analytical chemistry; computers; data acquisition; data processing; fluorimetry; laboratory automation; real-time.

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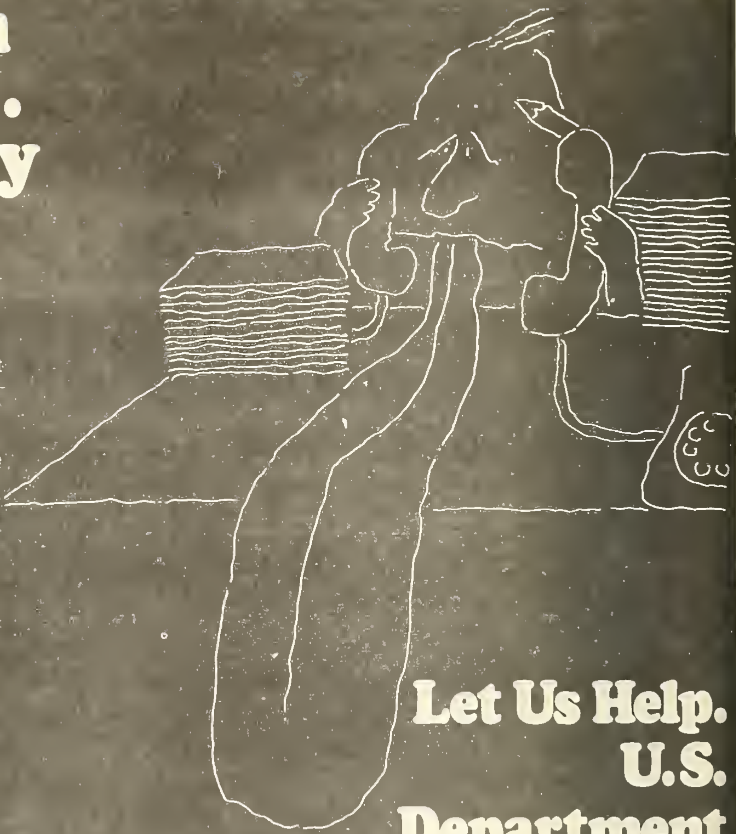

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