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COMPUTER PROGRAMS IN BASIC LANGUAGE FOR GRAPHITE FURNACE ATOMIC ABSORPTION USING THE METHOD OF ADDITIONS PART 1. OPERATING INSTRUCTIONS

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**COMPUTER PROGRAMS IN BASIC LANGUAGE
FOR GRAPHITE FURNACE ATOMIC ABSORPTION
USING THE METHOD OF ADDITIONS
PART 1. OPERATING INSTRUCTIONS**

DISCLAIMER

1. The following information was obtained from the records of the Department of the Interior, Bureau of Land Management, regarding the status of the land owned by the United States in the State of California, as of January 1, 1960:

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FOREWORD

This report covers work performed by the Lawrence Livermore Laboratory for the United States Environmental Protection Agency under Interagency Agreement 78-D-X0085.

Part 2 ("Documentation") of this report describes in detail the various aspects of each of the programs discussed here in Part 1, and contains flow charts, definitions of variables, and symbol tables for these programs.

CONTENTS

Foreword	iii
Abstract	1
Introduction	1
General Description of Programs	2
Operating Instructions	11
Introduction	11
Timing Algorithm	11
Automatic Operation	14
Manual Operation	17
Final Report	22
Helpful Hints	26
Appendix A: Concise Operating Instructions	27
Appendix B: Timing Algorithm	31
Appendix C: Typing In Data for Familiarization	33

LIST OF ILLUSTRATIONS

1.	Method of additions	2
2.	Data file for spiked samples	4
3.	Data file for completed samples	5
4.	Sample ID file	6
5.	Flowchart for the main program (MADSTART.GF), operating in the manual mode	7
6.	Flowchart for the main program (MADSTART.GF), operating in the automatic sampler mode	8
7.	Flowchart for the regression and statistics subprogram (MADFIT.GF)	9
8.	Flowchart for the report subprogram (MADREP.GF)	10
9.	Terminal output, illustrating user interaction with the timing algorithm	12
10.	Terminal output, illustrating typical user responses for an automatic run	15
11.	Terminal output, showing a sample wheel pattern	16
12.	Sample output during an automatic run	18
13.	Sample responses during a manual run	19
14.	Example of an interim report	21
15.	Typical output in response to the SX/P and UX/P commands	23
16.	Terminal output, illustrating responses for a final report	24
B1.	Timing for data collection	31

LIST OF TABLES

1.	Responses to CSI double asterisk prompt	20
C1.	Suggested values for hand entry of data	34

COMPUTER PROGRAMS IN BASIC LANGUAGE FOR GRAPHITE FURNACE
ATOMIC ABSORPTION USING THE METHOD OF ADDITIONS
PART 1. OPERATING INSTRUCTIONS

ABSTRACT

These instructions describe how to use BASIC language programs to process data from atomic absorption spectrophotometers using the graphite furnace and the method of additions calibration technique. The instructions cover loading the programs, responding to computer prompts, choosing among various options for processing the data, performing operations with an automatic sampler, and producing reports. How the programs interact with each other is also explained. Examples of computer/operator dialogue are presented for typical cases. In addition, a concise set of operating instructions is included as an appendix.

INTRODUCTION

The method of additions or standard additions technique¹ is used to develop a calibration curve when there is a reason to suspect that the physical and chemical properties of the sample matrix itself might interfere significantly with the signal attributable to the analyte (the substance being measured). The method consists of adding known amounts of a standard solution of the analyte to aliquots of the sample solution. The usual practice is to do this in such a way that each sample solution, plus added standard solution, is diluted to a constant volume. The known amounts of standard solution added to the sample solutions are called spikes.

The idea of the method is that the unknown solutions, spiked with standards, will be affected by the sample matrix in the same ways as the unspiked sample. If this is so and if the response is linear, a plot of signal vs concentration may be extrapolated to give the analyte concentration in the sample solution (Fig. 1).

¹J. Ramirez-Munoz, *Atomic Absorption Spectroscopy* (Elsevier Publishing Co., New York, 1968), pp. 328-338.

FIG. 1. Method of additions. The instrument response to the unspiked sample is plotted on the ordinate, and the sample concentration is read directly from the negative x-axis.

The method of additions is often used with the graphite furnace method of atomic absorption spectrophotometry, since it is known to have a high probability of matrix interference.

GENERAL DESCRIPTION OF PROGRAMS

The main program is linked to three subprograms. The names assigned to the BASIC language program and subprograms are as follows:

1. MADSTART.GF--the main program.
2. MADDAT.GF for data accumulations and interpretation.
3. MADFIT.GF for linear regression and statistics.
4. MADREP.GF for report generation.

The main program (MADSTART.GF) does the initialization, allows the operator to investigate the timing of his data logging, sets parameters for data logging, logs in samples in either an automatic or manual mode of

operation, and interprets commands to the program with the command string interpreter (CSI). The main program also contains sections that allow some editing of the data files.

The data-gathering program (MADDAT.GF) takes the raw data, converts them to optical absorbance, and calculates a smoothed peak height and integral. A plot of the data can be produced on the line printer or terminal.

The calculational program (MADFIT.GF) does the first-degree linear fit and calculates the concentration of the unknown by mathematically extrapolating the equation for this fit. This computation is somewhat different from the regression routines used in the previous calibration programs in that it uses each individual reading of the spiked sample, rather than an average, to develop the necessary mathematical matrices. In this way the statistical standard deviation for the extrapolation routine may be determined more conveniently. The standard deviation of the extrapolation is only valid if equal numbers of replicates (if any) are run on each spiked sample.

Finally, the report program (MADREP.GF) reports results during the analysis and formulates a final report when the analysis is complete.

A run will be described as being one complete set of data on a set of spiked unknowns, resulting in an answer for an unknown sample. A set of spiked samples will include the unspiked sample. The spiked sample data will be stored as shown in Fig. 2. (This figure is a reasonable representation of how the data are organized in the computer-generated file.) When a run is completed, the operator should respond (manual mode) to the CSI request by typing MS (more samples). The CSI then queries the operator whether he wants to run this sample again or another sample. In either case, essential information obtained from the spiked sample data file is transferred to the sample data file, and the spiked sample data file is cleared. If the same sample is run again, the spiked sample data file is again used to compute sample information, and this data file is again stored in the sample data file. If a sample is run more than once, we can determine another statistic--the usual standard deviation of the sample. We thus have two sets of statistics to carry along with each determination: the standard deviation within a sample, that is, the standard deviation of the extrapolated calibration curve, and when two or more runs are made, the standard deviation among samples.

First spiked sample	Second spiked sample	
Av signal	Av signal	
Spike conc	Spike conc	
Number of reps	Number of reps	
--	--	
Signal, 1st rep	Signal, 1st rep	Up to six spiked samples →
2nd	2nd	
3rd	3rd	
4th	4th	
5th	5th	
6th	6th	

FIG. 2. Data file for spiked samples. These files are cleared for each new sample.

The information about unknowns is stored in the same file as spiked samples, starting at what might be called record 7. Two records are kept for each unknown (see Fig. 3). As many as six runs can be made on a single sample. The data contained in these files may be viewed by giving certain commands (as explained below) to the CSI.

There is one other file the operator should be familiar with. This is the sample ID file, which contains information on the sample, as shown in Fig. 4. If one of the samples is a reagent blank, it must have the letters RB as the first two characters in its title. Only the first RB found is considered the reagent blank. Figures 5, 6, 7, and 8 are flowcharts that show the general organization and flow of the programs. These flowcharts will be helpful to the operator by allowing him to determine which program is currently the operating program, as well as giving him an insight into the state of that program.

Unknown #1

Av conc	Av variance within sample
Dilution factor	Variance among samples
Number of reps	Number of reps
Number of spikes	— —
Conc, 1st run	Variance, 1st sample
2nd	2nd
3rd	3rd
4th	4th
5th	5th
6th	6th
Slope, 1st run	1st coeff of determination
2nd	2nd
3rd	3rd
4th	4th
5th	5th
6th	6th

Additional samples →

FIG. 3. Data file for completed samples. The variance among samples is for $N1$ degrees of freedom, where $N1$ is the number of samples. It is recalculated as $N1-1$ degrees of freedom when output. (See Part 2.)

Sample title	
Number of spiked unknowns	
Number of runs	
Conc of 1st spike	
2nd	Additional samples →
3rd	
4th	
5th	
6th	
Dilution factor	

FIG. 4. Sample ID file. The sample title may comprise 25 characters.

FIG. 5. Flowchart for the main program (MADSTART.GF), operating in the manual mode. The circled letters indicate links either within the main flowchart or with the flowcharts in Figs. 6, 7, and 8.

FIG. 6. Flowchart for the main program (MADSTART.GF), operating in the automatic sampler mode. The circled letters indicate links with the flowchart in Fig. 5.

FIG. 7. Flowchart for the regression and statistics subprogram (MADFIT.GF). Circled letters indicate links with the flowchart in Fig. 5.

FIG. 8. Flowchart for the report subprogram (MADREP.GF). Circled letters indicate links with the flowchart in Fig. 5.

OPERATING INSTRUCTIONS

INTRODUCTION

These instructions are intended for the operator who is to run the BASIC programs that are used with atomic absorption (AA) graphite furnace spectrophotometers using the method of additions. (The BASIC interactive programming language used in this document is Data General's extended BASIC, operating under their Real Time Disk Operating System, RDOS.) More complete descriptions of these programs are found in Part 2. A set of concise operating instructions is included in Appendix A.

The computer will request an identification code after the operator presses the ESC key. If the proper code letters are typed, followed by a carriage return (all responses are entered into the computer by typing a carriage return), the computer will display on the terminal some message indicating that the BASIC system is up. The operator may then load the appropriate program by typing LOAD "MADSTART.GF". To start the program he types RUN. Alternatively, he may type RUN "MADSTART.GF", which loads and starts the program.

The program requires the operator to reply to a number of questions. In general, the proper responses are indicated in the question itself. If this is not so, the correct response will be Y or N, for yes or no. Only the first one or two letters of a response are significant; other letters are ignored. If the response is not accepted by the program, the question is usually repeated.

Illustrations are provided in this report for your assistance in understanding the instructions. They are reproductions of the output of a Texas Instruments "Silent 700" terminal, taken from demonstration runs of the program. You may find it helpful to follow them along with the descriptions.

TIMING ALGORITHM

The first section of the program begins with the algorithm that determines the proper timing for the computer to capture the signal from the graphite furnace. (See Appendix B for a description of the algorithm.) The program asks TIME TO DEFINE PEAK IN SECONDS? (Fig. 9). The operator should


```

***** MAINTAKT.64 *****
14:54:14
14:54
TIME TO DEFINE PEAK IN SECONDS ? 8
TIME BETWEEN POINTS IS 5.00 SECONDS
TOTAL TIME IS 12.5 SECONDS
DELAY TIME IS 15.0167 SECONDS
INJECT SAMPLE BY HAND FOR TIMING ?
Y
BASELINE: LEADING OR COMBINED (LEAD) ? L
PK. HEIGHT: OR REG. INTEGRAL (PKPK) ? P
ENTERING DATA TAKING CALL 7
SIG= .195721
TIMING O.K. ? M
TIME TO DEFINE PEAK IN SECONDS ? 10
TIME BETWEEN POINTS IS 7.00 SECONDS
TOTAL TIME IS 17.5 SECONDS
DELAY TIME IS 14.5833 SECONDS
INJECT SAMPLE BY HAND FOR TIMING ?
Y
BASELINE: LEADING OR COMBINED (LEAD) ? L
PK. HEIGHT: OR REG. INTEGRAL (PKPK) ? P
ENTERING DATA TAKING CALL 7
SIG= .229097
TIMING O.K. ? Y
REQUIRE PLOT ? Y/N? IF Y THEN A PLOT IS MADE FOR EVERY INJECTION ? M
SET OR CHANGE PARAMETERS Y/N ? N
AUTOMATIC OR MANUAL ARM ? M
SAMPLE NO. 1 SAMPLE I.D. ?

```

FIG. 9. Terminal output, illustrating user interaction with the timing algorithm.

respond with his best estimate of the time between the first rising data point of the peak and the last falling data point of the peak; that is, the time for only the peak to appear, not the baseline. The program then calculates everything else, using the 150 points available.

After calculating the time between points and rounding to the nearest 1/60 of a second (the maximum data-taking frequency), the program prints TIME BETWEEN POINTS IS x/60 SECONDS, where x is the number of 1/60-second "ticks" between points. If the interval is less than 1/60 of a second, the program prints INTERVAL TOO SHORT and requests the user to input a new time. If the interval is 1/60 of a second or a multiple thereof, the program calculates the total time interval for taking data and prints TOTAL TIME IS x SECONDS, where x is the total time for taking data, including the baseline.

The program must then calculate a delay time. This is the time the computer waits after a data-taking routine is called by BASIC. The delay begins when the strip-chart recorder is automatically turned on, which occurs when the furnace is nearing its maximum temperature. This delay time is based on the time interval between the recorder signal and the onset of the maximum temperature cycle of the furnace. This time interval is a constant for each instrument, but it probably varies somewhat from instrument to instrument. It should be determined with a stopwatch. For the instrument used here, the time was 16.8 seconds. This time is stored in a variable called K(35) and is marked as TIME TO FURNACE ON in the program. The program calculates the delay time on the basis of the number of points required to define the leading baseline (set at 20 at line 545). The message DELAY TIME is x SECONDS is printed. If the delay time is less than 1/60 of a second, the computer prints DELAY TIME IS TOO SHORT. The program goes back to the beginning of the timing section and requests the operator to enter a new time for defining the peak.

If the delay time is okay, the operator must then decide if he wants to check it with an actual run by answering the query INJECT SAMPLE BY HAND FOR TIMING? If this step is necessary, the parameters for taking preliminary data are selected by the operator. (A plot of the curve is automatically produced on the line printer during the trial reading.) The parameters are selected by answering the appropriate program prompts: BASELINE: LEADING OR COMBINED (L/C)? and PK HEIGHT, OR REG. INTEGRAL (P/RI)?

The program then links to MADDAT.GF, and the operator injects the sample. Data are taken and a plot produced. If the data and the plot of the data indicate that the timing was able to capture a well-defined curve, or if the operator had decided previously not to do a preliminary timing run, the operator answers Y to the question TIMING O.K.? Otherwise, the program returns to the beginning of the timing algorithm. The operator then has an opportunity to change his parameters for the actual sample analysis runs.

The question REQUIRE PLOT Y/N? must be answered next. The usual answer here is N (no); otherwise the program will produce a plot on the line printer for every sample injected. The question SET OR CHANGE PARAMETER Y/N? is then asked. If the answer to this question is Y, the program returns to the parameter section. If the answer is N, the previously selected parameters or the default parameters are selected. It is possible to go through the program without injecting a sample and without setting the parameters. The default

parameters produce no plot, do not use a trailing baseline, and take the peak height of the signal for the signal value.

AUTOMATIC OPERATION

After the timing and control parameters have been set, the program asks AUTOMATIC OR MANUAL A/M (see Fig. 10). We will first take the case of entering the sample data when using an automatic sampler. First, in response to NO. OF SAMPLES?, enter the total number of samples to be run. Next, enter the number of replicated readings in response to GOING TO TAKE MORE THAN ONE READING PER SPIKED SAMPLE Y/N? and HOW MANY FOR EACH SPIKED SAMPLE? The number of replicates is also set on the dial of the automatic sampler, which causes the sampler arm to inject the same solution the proper number of times before advancing to the next solution.

Next the program asks REGULAR OR IRREGULAR RUN R/I? If the operator responds R, he need not enter spike concentrations more than once, since they will be taken as identical for each unknown. For an irregular run, the program prints the instruction INCLUDE ZERO IN CONCENTRATION OF SPIKES, then SAMPLE NO. 1 and SAMPLE I.D.? After receiving an answer, the program inquires SAMPLE TO BE RUN HOW MANY TIMES?, then CONC OF SPIKE x? for each spiked sample, in order of increasing spike concentration. The x stands for an integer (1, 2, 3,...,6) that identifies the spiked unknown. These questions are repeated for unknown numbers 2 to N1, where N1 is the total number of samples. For a regular run, the spike concentration questions are given only once--before the sample IDs are entered.

Next comes the opportunity to print out a sample wheel pattern (Fig. 11); that is, the way the automatic sampler should be loaded to correspond with the information given the computer program earlier. This pattern can be printed out on the terminal, the line printer, or both. If a sample is to be run more than once, extra sets of spiked samples must be placed in the automatic sampler in the proper sequence, as indicated by the sample wheel pattern. The program prompts are: DO YOU WANT SAMPLE WHEEL PATTERN PRINTED OUT (Y/N)? and WHERE? TYPE 0 FOR LINE PRINTER, 1 FOR TERMINAL, 2 FOR BOTH. These prompts appear at the top of Fig. 11.

```

TIMING O.K. ? Y
REQUIRE PLOT Y/N ? N
SET OR CHANGE PARAMETERS Y/N ? N
AUTOMATIC OR MANUAL R/M ? A
NO. OF SAMPLES ? 4
GOING TO TAKE MORE THAN ONE READING PER SPIKED SAMPLE Y/N ? Y
HOW MANY FOR EACH SPIKED SAMPLE ? 2
REGULAR OR IRREGULAR RUN R/I ? I
SAMPLE NO. 1 SAMPLE I.D. ? FIRST #1
SAMPLE TO BE RUN HOW MANY TIMES ? 2
NO. OF SPIKES ? 3
INCLUDE ZERO IN CONCENTRATION OF SPIKES
CONC OF SPIKE 1 ? 0
CONC OF SPIKE 2 ? 50
CONC OF SPIKE 3 ? 100
SPIKES O.K. Y/N ? Y
SAMPLE NO. 2 SAMPLE I.D. ? SECOND #2
SAMPLE TO BE RUN HOW MANY TIMES ? 2
NO. OF SPIKES ? 2
INCLUDE ZERO IN CONCENTRATION OF SPIKES
CONC OF SPIKE 1 ? 0
CONC OF SPIKE 2 ? 25
SPIKES O.K. Y/N ? Y
SAMPLE NO. 3 SAMPLE I.D. ? ROTHIRD
SAMPLE TO BE RUN HOW MANY TIMES ? 2
NO. OF SPIKES ? 2
INCLUDE ZERO IN CONCENTRATION OF SPIKES
CONC OF SPIKE 1 ? 0
CONC OF SPIKE 2 ? 20
SPIKES O.K. Y/N ? N
HOW MANY SPIKE CORRECTIONS ? 1
NUMBER AND CONC. ? 2 ? 10
SAMPLE NO. 4 SAMPLE I.D. ? FOUR #4
SAMPLE TO BE RUN HOW MANY TIMES ? 2
NO. OF SPIKES ? 4
INCLUDE ZERO IN CONCENTRATION OF SPIKES
CONC OF SPIKE 1 ? 0
CONC OF SPIKE 2 ? 1
CONC OF SPIKE 3 ? 2
CONC OF SPIKE 4 ? 3
SPIKES O.K. Y/N ? Y
SAMPLE I.D.'S O.K. Y/N ? N
HOW MANY I.D. CORRECTIONS ? 1
SAMPLE NO. ? 3
CORRECT I.D. ? RBTHIRD

```

FIG. 10. Terminal output, illustrating typical user responses for an automatic run.

DO YOU WANT SAMPLE WHEEL PATTERN PRINTED OUT (Y/N) ? Y
 WHERE ? TYPE 0 FOR LINE PRINTER, 1 FOR TERMINAL, 2 FOR BOTH 2

***** SAMPLE WHEEL PATTERN *****

POSITION		I.D.	SPIKE
1	B	BLANK	
2	1 1 <1	FIRST #1	0
3	1 2 <1	FIRST #1	50
4	1 3 <1	FIRST #1	100
5	B	BLANK	
6	1 1 <1	FIRST #1	0
7	1 2 <1	FIRST #1	50
8	1 3 <1	FIRST #1	100
9	B	BLANK	
10	1 1 <1	SECOND #2	0
11	1 2 <1	SECOND #2	25
12	B	BLANK	
13	1 1 <1	SECOND #2	0
14	1 2 <1	SECOND #2	25
15	B	BLANK	
16	1 1 <1	PTHIRD	0
17	1 2 <1	PTHIRD	10
18	B	BLANK	
19	1 1 <1	PTHIRD	0
20	1 2 <1	PTHIRD	10
21	B	BLANK	
22	1 1 <1	FOUR #4	0
23	1 2 <1	FOUR #4	1
24	1 3 <1	FOUR #4	2
25	1 4 <1	FOUR #4	3
26	B	BLANK	
27	1 1 <1	FOUR #4	0
28	1 2 <1	FOUR #4	1
29	1 3 <1	FOUR #4	2
30	1 4 <1	FOUR #4	3

FIG. 11. Terminal output, showing a sample wheel pattern.

The program stops here to allow the operator to check the correct loading of the sampler. Note that blanks are run before every new sample. When the operator is ready, he types a carriage return in response to the message TYPE CARRIAGE RETURN WHEN READY TO BEGIN. The program then takes over automatic operation of the sampler and graphite furnace until all of the samples have been run. Because of the mechanical setting of the autosampler, the automatic mode requires that, when replicate runs are made, blanks be run in replicate also. The values of the blanks are averaged for the calculations only in the automatic mode.

Part of the output during an automatic run, using data entered via the terminal, is shown in Fig. 12. After the automatic run is over, the program reverts to the manual mode as indicated by an asterisk(*). In the manual mode, the operator may run more samples, ask for reports, or terminate the runs as explained in the next section.

MANUAL OPERATION

When the operator chooses the manual mode of operation by responding with an M to the question AUTOMATIC OR MANUAL A/M?, he must instruct the program for each action by responding with the appropriate command to the double asterisk prompt(**). The appropriate commands are summarized in Table 1. If a command that is not in the program's repertoire is given, a SYNTAX ERROR message appears, and the double asterisk is repeated.

The first question after selecting the manual mode is SAMPLE NO. 1 SAMPLE I.D.? After being given the sample identification code, the program asks NO. OF SPIKES?, then queries for the spike concentrations for this one sample, as explained under automatic mode. A double asterisk is then given, and the operator enters appropriate responses, as listed in Table 1 (see Fig. 13).

The usual procedure is to enter the spikes and read them until all have been read the selected number of times (S1/1, S2/1, S1/2, etc.), then type a C to calculate the regression curve. However, the manual mode allows considerable flexibility as to how the operator proceeds. After gaining sufficient expertise, he can use the responses in Table 1 in many different ways. Responses to the C command are the same as those shown in Fig. 12 for the automatic run. Blanks (B) may be read at any time and are subtracted from

```

TYPE CARRIAGE RETURN WHEN READY TO BEGIN ?
1      B      BLANK
B
PEAK HEIGHT? 0
B
PEAK HEIGHT? 0
NEW AVERAGED BLANK= 0
  2      3 1      FIRST #1      0
11/1
PEAK HEIGHT? .1
1 1 < 1      SIG.= .1      AVE.SIG.= .1

11/2
PEAK HEIGHT? .11
1 1 < 2      SIG.= .11      AVE.SIG.= .105

  3      1 2      FIRST #1      50
12/1
PEAK HEIGHT? .2
1 2 < 1      SIG.= .2      AVE.SIG.= .2

12/2
PEAK HEIGHT? .21
1 2 < 2      SIG.= .21      AVE.SIG.= .205

  4      1 3      FIRST #1      100
13/1
PEAK HEIGHT? .3
1 3 < 1      SIG.= .3      AVE.SIG.= .3

13/2
PEAK HEIGHT? .31
1 3 < 2      SIG.= .31      AVE.SIG.= .30%

  5      STDs IN CALIB SET

STANDARD DEV OF SPIKE MEASUREMENTS= 3.36641
COEFF OF DETERM= .996264
CONC= 52.1172 + 498.131 *ABS

1 DIFF : .186569 - 0 = .186569
2 DIFF : 49.9997 - 50 = -2.74658E-04
3 DIFF : 99.8128 - 100 = -.187164

CONC= 52.1172      AVG CONC= 52.1172
  5      B      BLANK
B
PEAK HEIGHT? 0
B
PEAK HEIGHT? 0
NEW AVERAGED BLANK= 0

```

FIG. 12. Sample output during an automatic run.

```

♦ ♦ MD
REPEAT THIS SAMPLE? Y
♦ ♦ S1/1
PEAK HEIGHT? .1
S 1 / 1      SIG.= .1      AVE.SIG.= .1

♦ ♦ S1/2
PEAK HEIGHT? .91.09% ? .09
S 1 / 2      SIG.= 9E-02    AVE.SIG.= 9.5E-02

♦ ♦ S2/1
PEAK HEIGHT? .2
S 2 / 1      SIG.= .2      AVE.SIG.= .2

♦ ♦ S2/2
PEAK HEIGHT? .19
S 2 / 2      SIG.= .19     AVE.SIG.= .195

♦ ♦ S3/1
PEAK HEIGHT? .3
S 3 / 1      SIG.= .3      AVE.SIG.= .3

♦ ♦ S3/2
PEAK HEIGHT? .29
S 3 / 2      SIG.= .29     AVE.SIG.= .295

♦ ♦ C
3 STDs IN CALIB SET

STANDARD DEV OF SPIKE MEASUREMENTS= 3.22524
COEFF OF DETERM= .996264

CONC= -47.1353 + 498.129 *ABS

1 DIFF : .187012 - 0 = .187012
2 DIFF : 50 - 50 = -3.05176E-05
3 DIFF : 99.9129 - 100 = -.187119

CONC= 47.1353      AVG CONC= 49.6262
♦ ♦ R
INDIVIDUAL SAMPLE STATISTICS Y/N ? Y
TYPE 0 FOR REPORT ON LINE PRINTER, 1 FOR TERMINAL, 2 FOR BOTH
? 2

```

FIG. 13. Sample responses during a manual run.

TABLE 1. Responses to CSI double asterisk prompt.

Response	Function
SX/Y	Get data for spiked sample X, replicate Y.
UX/Y	Display average concentration for unknown X and concentration for replicate Y.
B	Get data for blank solution.
R	Print interim report. If no more samples, print final report.
C	Calculate first-degree linear regression curve and print curve parameters, unknown concentration, and statistics.
MS	Perform another complete run on the same sample or begin run on a new sample.
UX/P	Display raw data files for unknown X, and concentration and statistics.
SX/P	Display raw data file for spiked standard X.
SX/E or UX/E	Erase results (zero raw data files) for spiked sample X or final results on unknown X.
SX/YD or UX/YD	Delete results on spiked sample X, replicate Y, or current run Y on unknown X file. Unknown file deletions are not allowed following MS.

the subsequent sample readings. Only the most recent blank is used; any previous blank will have been overwritten by the current one. Each spiked unknown must be repeated the same number of times within a run for the spike statistics to be valid.

After all of the spiked samples have been read the correct number of times, the operator can type R, which will link to the report program. Certain questions to be answered for producing an interim report are shown in Fig. 13. An example of an interim report is shown in Fig. 14. The individual statistics section is simply bypassed when N is given instead of Y, in response to the question INDIVIDUAL SAMPLE STATISTICS Y/N?

If, after the report section has been scrutinized, the data for the run seem correct, the operator may signal for another run or a new sample by typing MS in response to the double asterisk. After receiving the response

***** GRAPHITE FURNACE ANALYSIS REPORT *****
 ** METHOD OF ADDITION **

** SAMPLE RESULTS **

SAMPLE I D	NO. OF REPS	AVERAGE CONC.	STD. DEV. AMONG SAMPLES	AV. STD. DEV. WITHIN SAMPLE
FIRST #1	2	49.6262	3.52276	3.29658
SECOND #2	2	24.6287	1.75024	2.77017
RBTHIRD	2	9.9962	1.05533	.564155
FOUR #4	2	2.01328	.119832	8.78401E-02

SAMPLE I D NO. AND CONCENTRATION OF SPIKES

FIRST #1	3	0	50	100
SECOND #2	2	0	25	
RBTHIRD	2	0	10	
FOUR #4	4	0	1	2
		3		

INDIVIDUAL STATISTICS

NO.	SAMPLE I D	CONC.	STD. DEV. IN IMPL	COEFF. OF DM.	SLOPE
1	FIRST #1	52.1172	3.36641	.996264	498.131
1	FIRST #1	47.1353	3.22524	.996264	498.129
2	SECOND #2	25.8663	2.85191	.990099	247.524
2	SECOND #2	23.3911	2.68595	.990099	247.524
3	RBTHIRD	10.7458	.614754	.997238	52.4861
3	RBTHIRD	9.25339	.508547	.997738	47.5113
4	FOUR #4	1.92915	8.49954E-02	.996673	
98.6809					
4	FOUR #4	2.09862	9.05954E-02	.996543	100.66

MORE MEASUREMENTS Y/N ? N
 DO YOU WANT A FINAL REPORT Y/N ? N

END AT 6010

◆

FIG. 14. Example of an interim report.

MS, the program responds with REPEAT THIS SAMPLE? If the response is yes (Y), the counters and files are adjusted for another run on the same sample. If the response is no (N), the computer prints SAMPLE NO. 2 SAMPLE I.D.?, and the operator proceeds as explained for sample No. 1, entering the concentration of spikes and running the spiked samples until satisfied with the data.

The raw data in either the spiked sample file or the unknown file may be examined at any time by typing a SX/P or a UX/P, where X is the number of the spiked sample or unknown, respectively (see Fig. 15). The data are displayed as stored in the file. (Reference to Fig. 3 will help clarify the meaning of the numbers.) In addition, the average concentration, the average standard deviation of the spike, and the sample standard deviation are displayed for the unknown file.

An entire set of spiked sample data or unknown data may be erased by typing SX/E or UX/E, where X is the spike concentration number or sample number. After erasing a complete set of sample data, an MS must be entered. An individual spiked sample value may be deleted by typing SX/YD, where X is the spiked sample number and Y is the replicate number. (If the same spiked sample and replicate number is run twice, the second value overwrites the first; there is no need to delete the first value before the second run.) Results of an individual sample run may be deleted by typing UX/YD. In this case the files are adjusted so that there are no empty spaces between values. Individual sample run deletions can only be made during the actual run of that sample.

FINAL REPORT

The operator ends the cycle of running unknowns following an interim report. After typing R and getting the interim report, the query MORE MEASUREMENTS Y/N? appears. When the operator responds N, the program asks DO YOU WANT A FINAL REPORT Y/N? If the operator responds N, the program ends. A Y response begins a final report section, which allows one to correct for sample dilutions, search for a reagent blank and subtract it if desired, and enter experimental information as a heading for the final report (see Fig. 16). There is also an option to change the file names used to write this report. The report may be then called up independently using RUN "MADREP.GF" and these file names, entered in reply to the computer prompts. This will generate

```

♦ ♦ SX/P
.295 100 2 0 .3 .29 0 0 0 0
♦ ♦ SX/P
.195 50 2 0 .2 .19 0 0 0 0
♦ ♦ SX/P
9.5E-02 0 2 0 .1 4E-02 0 0 0 0
♦ ♦ C
3 STDS IN CALIB SET

STANDARD DEV OF SPIKE MEASUREMENTS= 3.22524
COEFF OF DETERM= .996264
CONC= -47.1353 + 498.129 *ABS

1 DIFF : .187012 - 0 = .187012
2 DIFF : 50 - 50 = -3.05176E-05
3 DIFF : 99.8129 - 100 = -.187119
CONC= 47.1353 AVG CONC= 49.6262
♦ ♦ UX/P
AVE CONC= 49.6262

49.6262 1 2 3 52.1172 47.1353 0 0 0 0 498.131 498.129 0 0
0 0

SAMPLE STD DEV= 3.52276
AVERAGE SPIKE STD DEV= 3.29658

10.8675 6.20492 2 0 11.3327 10.4022 0 0 0 0 .996264 .996264
0 0 0 0

♦ ♦ R
INDIVIDUAL SAMPLE STATISTICS Y/N ?

```

FIG. 15. Typical output in response to the SX/P and UX/P commands.

INDIVIDUAL STATISTICS

NO.	SAMPLE ID	NO.	STD. DEV. IN. SAMP.	COEFF. OF VAR.	LOPE
1	FIRST #1	52.1172	3.52276	6.76264	498.131
1	FIRST #1	47.1254	3.22524	6.82264	498.129
2	SECOND #2	29.658	3.72731	12.57805	274.389
2	SECOND #2	27.3411	3.66595	13.40099	247.524
3	PTHIRD	10.2454	1.614754	15.7313	52.4861
3	PTHIRD	4.42675	1.33437	29.94036	46.4919
4	FOUR #4	1.42915	1.44954E-02	1.496278	
4	FOUR #4	21.0362	2.05454E-02	1.996543	100.66

MORE MEASUREMENTS IN THIS

DO YOU WANT A FINAL REPORT (Y/N) :

NO. OF DILUTION : 2

SAMPLE NUMBER, VOL. 1, DILUTED TO VOL. 2 = 2 + 1 = 3

SAMPLE NUMBER, VOL. 1, DILUTED TO VOL. 2 = 4 + 2 = 6

CORRECTION : 0.0000

REAGENT BLANK : SAMPLE NO. 3 = 10.0861

SUBTRACT REAGENT BLANK : 0.00

INDIVIDUAL SAMPLE STATISTICS (Y/N) :

DATE OF ANALYSIS : 5.1.78

OPERATOR : WALTER BOYLE

INSTRUMENT CONDITION : NA-TYPED DATA

STANDARD SOURCE : SIMULATED STANDARD

ELEMENT NAME AND ELEMENT

METHOD : AUTOAMPLER, MANUAL, OF BOTH : MANUAL

COMMENT : DEMO PROGRAM

UNIT : PPM

TYPE 0 FOR REPORT ON LINE PRINTER, 1 FOR TERMINAL, 2 FOR BOTH

1 2

***** GRAPHITE FURNACE ANALYSIS REPORT *****

** METHOD OF ADDITION : **

DATE: 5.1.78
 OPERATOR: WALTER BOYLE
 INSTRUMENT CONDITION: NA-TYPED DATA
 STANDARD SOURCE: SIMULATED STANDARD
 ELEMENT: NO ELEMENT
 METHOD: MANUAL
 COMMENT: DEMO PROGRAM
 UNIT: PPM

** SAMPLE RESULTS **

SAMPLE ID	NO. OF REPS	AVERAGE CONC.	STD. DEV. AMONG SAMPLES	AV. STD. DEV. WITHIN SAMPLE
FIRST #1	2	39.5401	3.52276	3.29658
SECOND #2	2	94.0126	3.72448	3.05155
PTHIRD	2	0	1.93303	1.731735
FOUR #4	2	70.4692	1.119832	8.78401E-02

REAGENT BLANK (SAMPLE NO. 3) = 10.0861 HAS BEEN SUBTRACTED

FIG. 16. Terminal output, illustrating responses for a final report.

another report for that particular run. The program understands when it is being called up independently rather than being linked to another program, since it starts at slightly different locations in each case. Thus, old reports may be kept on file on the disk storage device if an accurate record of file names is kept.

At the beginning of the final report section, the program asks NO. OF DILUTIONS? If the operator has not diluted the samples or wants to ignore this section, he types the number 0, otherwise he types in the number of dilutions he wishes to enter. Then, for each dilution, the computer prompts SAMPLE NUMBER, VOL 1, (DILUTED TO) VOL 2, and the operator must enter three numbers in that order. For instance, if sample number 3 (sample numbers are established by the order in which they were entered into the program) was diluted 5 ml to 250 ml, the operator would respond 3 in response to the first question mark, 5 to the second question mark, and 250 to the third. The line would appear as ? 3 ? 5 ? 250. Of course, a carriage return must be given with each response. Since a dilution factor is calculated from a ratio of the last two numbers, the response ? 3 ? 1 ? 50 would be equivalent to the first response. The question is asked for each diluted sample, then an opportunity for corrections is presented. The correction cycle sends the operator back to the beginning of the routine, but only those solutions that were entered wrong the first time need be reentered.

If the operator responds with an N to the query CORRECTIONS Y/N?, the report-writer program looks for a reagent blank. If it doesn't find a sample ID with RB as its first two letters, it continues. If it does find such a sample, it prints out the sample number and concentration on the terminal and inquires SUBTRACT REAGENT BLANK Y/N? If the reply is Y, the concentration of the reagent blank will be subtracted from each unknown before the final report is written. This subtraction will be noted on the final report.

After the operator sets the individual statistics flag with a Y or N response, the program asks for information for the heading of the report, as shown in Fig. 16. This information is in the form of string literals, and the operator may respond with any desired input. Then the report is printed on the output device selected by the operator, as shown in Fig. 13 for the interim report. Another report is printed each time a positive response is given to the question ANOTHER REPORT Y/N? Then, when an N is received, the program moves on to the section that allows data file names to be changed for

future reports on the same data. Finally, the program ends. It should be noted that when the program ends the subprogram MADREP.GF is in core. To start a new methods of addition program, the operator must reload the main program by typing LOAD "MADSTART.GF" then RUN, or RUN "MADSTART.GF", as discussed earlier.

When the report-writer program is called up as an independent program (RUN "MADREP.GF"), the program asks for the three file names it needs to build a report. It then goes to the reagent blank routine, and after the operator decides about including individual statistics, the program prints a report and goes into the extra report cycle. The dilution factors and headings have already been stored in the appropriate data file and are used in producing this report.

HELPFUL HINTS

One can gain familiarity with these programs simply by typing in data corresponding to the averaged signal from the sample beam. To allow this hand entry of data, the program must be modified so that it skips the real data acquisition section and instead asks for typed data. The necessary modifications are presented in Appendix C.

The programs usually allow corrections to be made when entering long lists of data, i.e., sample IDs, spike concentrations, and dilution factors. Most errors or mistakes can be fixed, and the program resumed at the line on which it stopped, by entering RUN XXXX, where XXXX is the line number. Values can be set using an equals sign, e.g., X = 2. Files can be closed by the command CLOSE. The computer can also be used in a calculator mode, e.g., PRINT (X + Y)/Z will print the answer directly on the terminal.

Finally, a word of warning. Because the program links back and forth from one subprogram to another, it is important for the operator to know what program he actually has in core before doing a SAVE. A SAVE stores the program on the disk and will overwrite another program of the same name. Thus, if the operator performs a SAVE, incorrectly assuming that one of the main programs is in core, the program that is in core will overwrite the main program, which will be lost. He can recover it by reading it back from a magnetic tape record. Because disk records are volatile, magnetic tape records should always be kept as a backup.

GDV/ew

APPENDIX A
CONCISE OPERATING INSTRUCTIONS

I. Initial conditions for computer-assisted analysis

A. Normal instrument setup

1. Set wavelength.
2. Start cooling.
3. Set lamp current.
4. Set timing and temperature for graphite furnace.
5. Optimize optics.
6. Turn on inert gas.

B. Autosampler setup

1. Power to AS-1 ON.
2. Start to AS-1 OFF.
3. Injection counter to 1.

C. NOVA Interface setup

1. interface switch to COMPUTER.
2. Press RESET on Interface to initialize status (unnecessary but good habit).

II. Starting the master control program MADSTART.GF

A. LOGON to computer

1. Turn on computer terminal.
2. Press the escape key until the phrase ACCOUNT-ID: appears.
3. Type in the Graphite Furnace/Method of Additions account identifier (e.g., GFMA1) and press RETURN.
4. The log-on time and system message will appear, followed by a star(*) .

B. Starting the computer program MADSTART.GF

1. After the star, type RUN "MADSTART.GF" and press RETURN.
2. The program will control computer prompts, responses, and activities through the course of the analysis.
3. The user must answer each prompt and press RETURN when the answer has been typed.
4. The program will usually allow the user to correct certain types of run information during the course of the run, but it is wise to be careful when answering computer prompts.

III. Setting parameters for peak data acquisition (MADSTART.GF)

A. Timing for peak detection

1. Input time for peak definition (atomization time)--5 to 15 seconds usually.
2. Check timing (optional). (If this option is chosen, it will constitute the first injection of the run. The first injection in any computer-controlled analysis requires that the user start the AS-1 changer at the appropriate time. The computer will prompt the user to start the AS-1 changer and will wait until the user signifies completion of the task.)

- a. Put a detectable test sample in the current sampling position.
 - b. The computer will display the command *****START SAMPLER NOW*****, and the user must push the START button on the AS-1 sampler. The user must not tamper with the AS-1 until the analysis is complete, unless more than 30 samples are being run. This restriction applies to both manual and automatic sequencing. Special instructions for running more than 30 samples are described under IV-C below.
 - c. The sample will then be injected, dried, charred, and atomized, and a plot of the burn will be output to the line printer, showing the peak data.
 - d. Adjust data-taking time, if necessary.
- B. Defining data reduction and plot parameters
1. Respond to prompt **REQUIRE PLOT? Y/N** (normally N, unless plot is needed for each injection).
 2. Respond to prompt **BASELINE: LEADING OR COMBINED (L/C)?** (normally L for peak height measurements and C for peak integral measurements).
 3. Respond to prompt **PK. HEIGHT, OR REG. INTEGRAL (P/RI)?** (normally P).

IV. Procedures for automated sequencing

- A. Setting up sample wheel pattern
1. Input number of unknowns.
 2. Input number and value of spikes for each unknown.
 3. Input sample identifier for each unknown.
 4. Input number of repeat injections at each location (advance AS-1 counter to desired position).
 5. Get output of sample wheel pattern.
 6. Load the first 30 samples in the wheel in the same order they appear on the wheel pattern report. (Blanks are required at the beginning of each sample set.)
 7. Check instrument adjustments.
 8. Position sample wheel to location #1.
- B. Starting automated run
1. To begin automatic sequencing, press RETURN in answer to the **READY** prompt.
 2. If no timing peak was taken, the first blank in the automated sequence will constitute the first sample injection of the run. The user will be instructed to start the sampler, as described in III-A-2 above.
 3. The computer will take control of the sequencing until it has completed all the samples in the wheel pattern or until it has reached position #30 of the sample wheel.
- C. Running more than 30 samples in an automated sequence
1. The AS-1 sampler will stop after sampling the 30th position.
 2. The computer will complete data reduction on the last burn and will suspend operations until the AS-1 is restarted.

3. The analyst may then load another wheel of samples or reload the same wheel with up to 30 more samples. (The samples must be in the order defined in the sample wheel pattern.)
4. Position the sampler to the #1 position and press the AS-1 START button.
5. The computer will then take over control of the sampling until it reaches the end of the pattern or until it has sampled the last chamber in the wheel.

D. Completing an automated run

1. The computer will complete processing of data and output two stars(**), indicating that it has completed the automatic sequence and is ready for operator commands.
2. Data for each sample set may be reviewed using the appropriate commands.
3. Other samples may be run in this phase using manual sequencing, as described under V below.
4. Reports may be generated any time control is returned to the user. The user is given the opportunity either to terminate the run after a report has been generated or to continue manual operations.

- V. Procedures for manual sequencing (MADSTART.GF) using the AS-1. (The manual mode requires more knowledge and effort on the part of the analyst. It is a highly flexible command-structured system, which allows for improved accuracy and precision. It facilitates the rigorous analysis of one unknown, and may be highly useful in determining the concentration range and method requirements for measuring an analyte in a set of similar samples.)

A. Preparing to run one sample set

1. After following the setup procedures in I, II, and III, above, enter sample identifier.
2. Enter spike values for sample.
3. Measure blank value using B command when the two stars(**) appear. If this is the first measurement in the sequence, the user will be instructed to start the sampler as was described in III-A-2 above.
4. Each measurement requires positioning the sample wheel to the desired location and typing in the appropriate command to the terminal (e.g., B, S1/1, S4/2, etc.). The computer will start the injection cycle, process the peak data, and return control to the user after each measurement.
5. The user must not stop the AS-1 sampler during manual sequencing.

B. Completing a manually sequenced sample run

1. Once the spikes for the sample have been measured (at least one time), the user may develop the method of additions line through the points using the C command.
2. Divergent points may be eliminated and files updated with the appropriate commands (e.g., S1/2D, S3E, etc.).
3. When all the samples for the unknown have been run and a good curve has been established, a report may be issued on the results using the R command.
4. Another sample may then be run using the MS command and following the procedure outlined in this section.

APPENDIX B
TIMING ALGORITHM

The timing algorithm depends on two pieces of information: (1) that 150 points are always taken to define the curve and (2) that the interval between the time the graphite furnace call is enabled by the autoswitch and the startup of the ignition cycle of the furnace is a constant. This constant is called the hardware delay (see Fig. B1). If we decide on a constant number of points to define the leading baseline (20 points are used here) and the trailing baseline (40 points), then 90 points are left to define the curve. All that need be entered is the time required to define the peak; the timing algorithm will then calculate everything necessary for the BASIC call to take data.

FIG. B1. Timing for data collection.

The following algorithm is implemented in lines 500 to 700 of MADSTART.GP.

1. Determine the numbers of points needed in each section of the curve: the leading baseline, the peak, and the trailing baseline. We have found that 20, 90, and 40 points, respectively, are reasonable values. Also, determine the hardware delay for the instrument.

2. Determine the time for defining the peak, using preliminary runs and prior information. The peak definition time begins when the signal first rises above the baseline and ends when it first returns to the baseline.

3. Determine the total time for the run by calculating the rate needed to define the peak (seconds/point), then multiplying by the total number of points (150).

4. Determine the time for the leading baseline by taking proportional parts of the total time. For example, if the total time to define the curve were 15 seconds, the leading baseline would take $20/150$ times 15 seconds, or 2 seconds.

5. Subtract the result in step 4 from the experimentally determined hardware delay. This gives the delay time; that is, the time between the call being enabled and the call actually beginning to take data.

Values for the graphite furnace call must be integers. Times are recalculated as the number of $1/60$ -second intervals, and these are rounded to integer values. If the interval is less than $1/60$ of a second, an error message is generated, and the user is directed to try a new value.

APPENDIX C
TYPING IN DATA FOR FAMILIARIZATION

Since hand-entered data are treated as final absorbances, the data-taking subprogram (MADDAT.GF) is not used. The routine to take data starts at line 6000 in MADSTART.GF.

At line 6006, replace the REM *** FOR HAND ENTRY OF DATA GOTO 6050 with a GOTO 6050. (Also, if a beam correction routine is used, the REM *** FOR HAND ENTRY OF DATA GOTO 3085 at line 3022 must be replaced with GOTO 3085.) The program responds with PEAK HEIGHT? The operator may then type in appropriate absorbance values. Blanks may be entered as zero. They are ignored in this procedure. Some suggested values are given in Table C1.

TABLE C1. Suggested values for hand entry of data.

ID	No. of spikes		Concentration of spikes			Dilution factor	Nominal concentration	
FIRST	3		0	50	100	1		50
SECOND	2		0	25		4		25
RB THIRD	2		0	10		1		10
FOUR	4		0	1	2	3	40	2

Peak height values								
FIRST								
RUN #1	S1/1	0.1	S2/1	0.2	S3/1	0.3		
	S1/2	0.11	S2/2	0.21	S3/2	0.31		
RUN #2	S1/1	0.1	S2/1	0.2	S3/1	0.3		
	S1/2	0.09	S2/2	0.19	S3/2	0.29		
SECOND								
RUN #1	S1/1	0.1	S2/1	0.2				
	S1/2	0.11	S2/2	0.21				
RUN #2	S1/1	0.1	S2/1	0.2				
	S1/2	0.09	S2/2	0.19				
RB THIRD								
RUN #1	S1/1	0.2	S2/1	0.4				
	S1/2	0.21	S2/2	0.39				
RUN #2	S1/1	0.19	S2/1	0.41				
	S1/2	0.2	S2/2	0.4				
FOUR								
RUN #1	S1/1	0.02	S2/1	0.029	S3/1	0.04	S4/1	0.05
	S1/2	0.019	S2/2	0.03	S3/2	0.041	S4/2	0.049
RUN #2	S1/1	0.021	S2/1	0.031	S3/1	0.04	S4/1	0.051
	S1/2	0.020	S2/2	0.032	S3/2	0.041	S4/2	0.052