# Data Acquisition and Control Program for Chromatographic and Spectroscopic Studies

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# A DATA ACQUISITION AND CONTROL PROGRAM FOR CHROMATOGRAPHIC AND SPECTROSCOPIC STUDIES

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## ABSTRACT

An IBM PC compatible computer was interfaced to an Ithaco 3962 lock-in-amplifier. The computer controlled the functions of the lock-in-amplifier, acquired and stored data, and allowed for real time or off-line processing of data. Computer/amplifier connection was made via RS-232-C serial interface. Programmed in Microsoft QuickBASIC, the computer assumed the role of a dedicated chromatographic integrator. This reduced the overall instrumentation expense by eliminating a dedicated chromatographic integrator. The computer program supplied much greater flexibility in control and data interpretation. To show its utility, the program was applied to a study of the infrared emission from a flame upon the introduction of hydrogen or carbon species, respectively, in the gaseous form and as contained in organic compounds. Emission was monitored at 2.7 and 4.3 $\mu$m by an infrared radiometer.

## INTRODUCTION

The objective of this project was to meet the need for a read-out device for spectroscopic and chromatographic data. These signals were being monitored by an infrared radiometer system. The radiometer system consisted of a lead selenide (PbSe) photoconductive cell, associated signal conditioning circuits, and an Ithaco 3962 lock-in-amplifier (LIA). The radiometer monitored a hydrogen/air flame into which the effluent from a gas chromatograph was routed. Organic compounds pass through a GC column at different rates, depending upon their interactions with the packing within the column. As the separated compounds eluted from the GC, they were combusted in the flame, resulting in predominantly carbon dioxide and water vapor formation. Each of these products are thermally excited in the flame and emit infrared radiation, at 4.3 $\mu$m for $CO_2$ and 2.7 $\mu$m for $H_2O$ (Hudson and Busch, 1987 and 1988). This radiation produced a signal in the Ithaco 3962 LIA proportional to the emission. While initial studies could rely on simply watching the numeric read-out of the LIA, more sophisticated work required data storage and further processing.

## EQUIPMENT AND METHODS

Computer equipment used included a 640K, floppy equipped IBM PC-Jr. and Zenith 12 MHz AT compatible 80286 PC. The program, written in QuickBASIC 4.5, was also run on an XT clone in the off-line mode to confirm graphics compatibility. The infrared radiometric system was set up as a flame infrared emission detector (FIRE) for gas chromatography and used the Ithaco 3962 LIA, a Varian Model 700 gas chromatograph, and PbSe FIRE detector. The PbSe device and electronics was initially described by Hudson and Busch (1988) and later modified by Hudson, Fau, Underhill, and Applequist (1990).

The Ithaco Model 3962 LIA is a phase and frequency locked, sensitive AC voltmeter (Ithaco, 1986). An LIA is used with a PbSe radiometer for several reasons. The nature of the PbSe photoconductor gives a greater sensitivity when operated in the alternating current, or chopped, mode. PbSe detection is enhanced by a factor of about 100 times in this case. Since the LIA is phase and frequency locked, many sources of noise can be discriminated against through its use. The Ithaco 3962 is also equipped with an 8088 microprocessor and IEEE-488 and RS-232-C ports for connection to external equipment. The internal programming of the 3962 supports several digital filtering and signal enhancement options. The addition of a computer system allowed a great deal of flexibility in the application of noise suppression and data

handling, both 3962 internal and on acquired data. QuickBASIC 4.5 (Microsoft Corporation, Redmond, WA) was chosen as the programming language because it offered the advantages of wide acceptance, ease of programming, speed, and significant enhancements over standard BASIC (Microsoft Corporation, 1987 and 1988). Initial versions of the described program were written using the authors knowledge of interpreted BASIC. As work proceeded, various features of QuickBASIC 4.5 were incorporated. The program version described will not run under interpreted BASIC, and may not under previous releases of QuickBASIC.

Samples of reagent grade hexane were repetitively injected on the GC column, a stainless steel ¼ in. o.d. 10% OV-101 on Chromasorb W-AW (Supelco, Bellefonte, PA), using a 10 ul liquid syringe (Hamilton, Reno, Nevada). Various sample amounts were used, up to the capacity of the syringe. Helium was used as the carrier gas, hydrogen and air as flame support gases. All gases were controlled using flow meters with integral flow controllers (Cole-Parmer Instruments, Chicago, IL). Flow rates for all data presented were: helium carrier, 40 ml/min; hydrogen, 100 ml/min; air, 330 ml/min. Column temperature was held at 75 °C.

## PROGRAMMING CONSTRAINTS

The overall goal of the program was to control and acquire data from the 3962, allow data storage and processing, and to present the data in a graphic display form representative of a GC chromatogram or spectroscopic display. For the infrared emission application, data would be presented with relative intensity as the y-axis in either case. The x-axis would represent time for GC studies and wavelength for spectroscopic studies. The ability to graph data in real time or data already stored on disk was required.

Control of the 3962 LIA was accomplished using the three letter commands recognized by its 8088 internal program. Each command may have an associated interrogative or passed parameter. The command 'BSS6' sent to the 3962 is interpreted as set the signal input sensitivity to the 1 mv range, while '?BSS' would return the current setting. All parameters had an associated initial level at 3962 start-up, however the program allows new settings to be selected from the keyboard, or input from a file.

Data was acquired at a rate of 1 Hz, ie, the 3962 sent a data block to the computer 1 time per second. The format consisted of a relative measurement number and the intensity data. Acquired data was plotted on the graphics screen and stored in an array until the end of an

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experiment, when the data could be saved to a disk file. The array was dimensioned large enough to allow data to be collected for about 100 minutes. Data acquisition rates could be changed to give longer monitoring time or shorter response time, as needed. Baud rate for the RS-232-C line was set at 4800. An examination of system bandwidth reveals that the data transfer rate of 1 Hz is sufficient for chromatographic peaks. Such peaks normally show a width at half height of at least 5 seconds, requiring a sampling rate greater than 0.4 Hz, which has been achieved. The LIA bandwidth is determined by the time constant of the low pass filter employed. The filter was set at 1 second for the data shown, giving a bandwidth of 0.16 Hz, again exceeded by the transfer rate.

Graphic display was achieved using the IBM CGA format. The incoming data needed to fit inside the PC screen, and therefore was scaled. The integration process required that an accurate average background, or baseline, level be determined. Since chromatographic data sometimes dips below this level, the baseline was displayed slightly above the screen bottom. Since the baseline is established under conditions of background signal (the flame itself emits infrared energy) and the analytical signal was often small in comparison, the baseline simply provided a reference point for the integration. The display was then actually a window viewing a portion of the overall infrared signal.

The useful interpretation of raw data during a chromatographic experiment requires that the time from sample injection to the time of a particular peak maximum be known and that the peak height or area be known. The first parameter is known as the retention time. The retention time is a qualitative parameter since individual compounds interact differently with the column packing and the compounds exit the column at different times. Peak height or area is the quantitative information in chromatography. The peak area is actually the best parameter, giving a more accurate determination of amount. It was therefore necessary that the program be able to integrate the area under the GC peaks. This required that routines be written to recognize the beginning and ending of an actual peak, and to then integrate the area contained under the peak down to the baseline level. Commercial integrator units usually print out integrations after the signal is acquired and plotted or graphed. Therefore, the program could make use of a nonreal-time integration.

### RESULTS AND DISCUSSION

In considering the goals of the program and the constraints presented by the Ithaco 3962, the program was menu driven. This menu presents a number of options, the five most important of which are the abilities to read current instrument setting, change settings, read experimental data in real time, read recorded data stored from previous runs, and to set some parameters in real time. Figure 1 shows the program main menu. The subroutines used to achieve the programs main functions are discussed below.

```
MODEL 3962 LOCK-IN AMPLIFIER INTERFACER VERSION 3.21

        1 - ENTER 3962 SETTINGS
        2 - SHOW ACTIVE 3962 SETTINGS
        3 - SET 3962 FROM FILED SETTINGS
        4 - SET SENSITIVITY
        5 - COLLECT SAMPLE
        6 - DISPLAY AND INTEGRATE SAMPLE
        7 - SAVE SAMPLE
        8 - LOAD SAMPLE
        9 - EXIT TO SYSTEM

    Enter selection:
```

Figure 1. Main menu.

In order to read current instrument settings, a series of print statements using the interrogative forms of the three letter commands followed immediately by an input were used. For example:

PRINT #1, "?BSS":INPUT #1,X,SS

was used to command the 3962 LIA to send the sensitivity setting to the computer, which ended up being stored in the variable 'S'. For the flame infrared emission detector chromatography system application, the parameters of interest were the header, sensitivity, time constant, reference or phase angle, and the error status. Often, during initial setup, one may want to set instrument parameters by hand and read them into the computer for future use. The ability to save these settings was incorporated.

The ability to enter new parameters from the computer provides remote control to the LIA. A subroutine was included to allow the operator to set many of the functions of the 3962 by selecting the functions and their parameters from a menu. Functions used less often, not included in the menu, could be entered by the operator as three letter commands. Figure 2 show the functions menu, while Figure 3 is an example for setting the sensitivity of the 3962 LIA.

```
The following can be set:

    1  - Analysis frequency range (Hz)
    2  - Reference mode
    3  - Sensitivity (V)
    4  - Time constant (s)
    5  - Automatic setting
    6  - Auto-ranging
    7  - Auto-tuning
    8  - Reference phase offset value
    9  - Meter sensitivity
    10 - Header
    11 - Sampling interval and output interval
    12 - OTHER

Press RETURN to end selection.
Enter selection:
```

Figure 2. Set functions menu (main menu item 1).

```
BTCx - Time constant (s)

POSSIBLE SETTINGS ARE:  0: 1m        5: 300m
                        1: 3m        6: 1
                        2: 10m       7: 3
                        3: 30m       8: 10
                        4: 100m      9: 30




Press RETURN to cancel.
Enter the setting:
```

Figure 3. Sensitivity Selection Menu (set functions menu item 3).

The most important single feature of the program is to plot or graph the data, both in real time and stored, to the computer screen. The Color Graphics Adapter (CGA) allows black and white images to be formed in a 200 (Y) by 640 (X) matrix (high resolution mode). X-axis data corresponds to run time, and run time may vary from as little as 2 minutes to as long as an hour in practice. Long GC runs were handled in two ways depending on whether real time or stored data were being plotted. In real time, X-axis data is directly plotted to the screen. At one Hz data collection rate, data points corresponding to from one to 640 seconds can be displayed at once. To display points above 640, the graphics screen is erased, and points are plotted beginning at the left side of the screen. In other words, after point 640 is plotted, the screen

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is erased and point 641 is plotted at the screens left edge. This is repeated each 640 points, until the end of the run is reached. Once data is stored, it may be recalled and redisplayed. Still using the 200 by 640 mode, the data may be plotted in a user specified window, in compressed or expanded form. Figure 4 shows data containing three obvious peaks,

F1--SHOW BASE LINE    F2--SHOW NOISE LINE    F5--CONTINUE
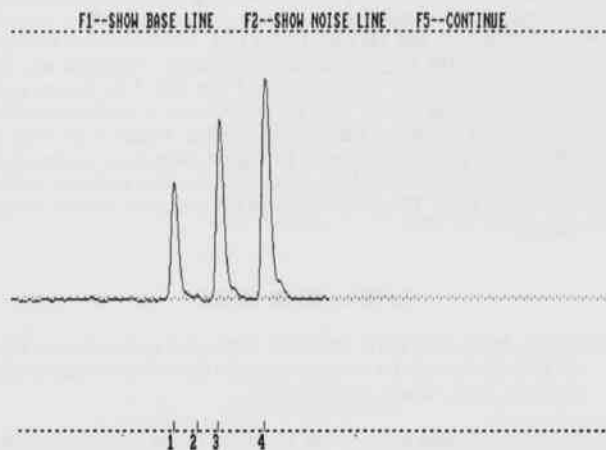


Figure 4. Chromatographic data, for multiple injections of n-hexane, redisplayed as recorded.

redisplayed as collected. Figure 5 shows the same data redisplayed, but scaled so that 225 data points are displayed. The screen "window" can be set to display any particular portion of the stored data, such as points 125 to 350, to allow various data features to be compressed or expanded for examination.

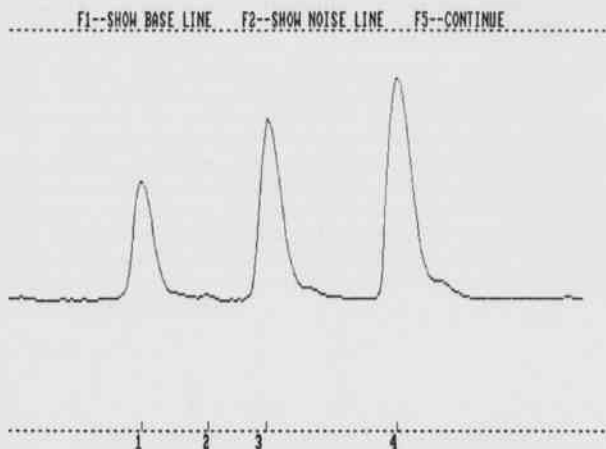F1--SHOW BASE LINE    F2--SHOW NOISE LINE    F5--CONTINUE



Figure 5. Chromatographic data, X scale expanded.

Y-axis, or intensity, data must be scaled to fit the screen for both modes of data display. In the real time mode, Y scaling is performed such that the bottom and top of the screen correspond to zero and full scale setting of the 3962 LIA. For data collected with a 10 mv LIA sensitivity setting, the graphics screen would represent approximately zero at the bottom and 10 mv at the top. Redisplayed data may employ a

user determined scaling factor. When the data file is read into the computer, an analysis of the data is performed which reveals several parameters including baseline, noise level, and an appropriate scale factor. The user may choose the option of setting the displayed "window" to any value. Fig. 6 shows data enlarged to reveal a small peak not clear in Fig. 4. The program "recognized" this as a peak, and so labeled it.

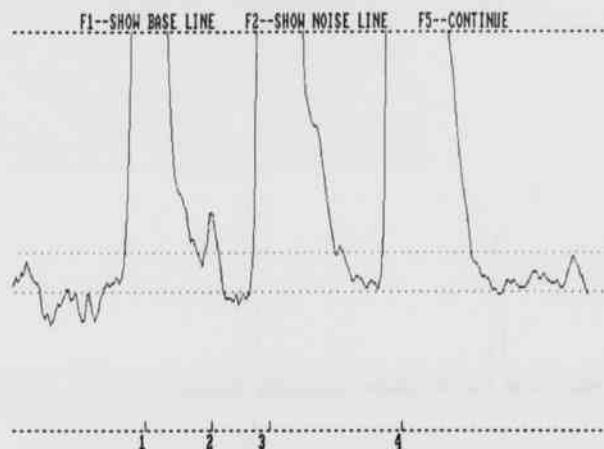F1--SHOW BASE LINE    F2--SHOW NOISE LINE    F5--CONTINUE



Figure 6. Chromatographic data, X and Y scales expanded.

Another feature found in redisplay of data is the raw/corrected data setting. Raw data is displayed within the window chosen, relative to actual zero. Keep in mind that the chromatographic baseline is actually the steady state hydrogen/air flame background. This background represents a large signal to the LIA (the DC offset value). Since the chromatographer is interested in the difference in the signal upon introduction of separated components from the GC column, the raw data display may not allow viewing of small peaks under high magnification "windowing". The corrected data setting displays the data centered on the average value, offset by a noise adjustment value, discussed in more detail below.

To correctly integrate peaks, a method of peak recognition must be incorporated into the program. The method must be fairly immune to noise, but still allow a proper level of sensitivity. This program has used two approaches to handling this problem. Both methods were used on the redisplayed data, as this was simpler to handle, and involved analyzing the first several data points recorded (defaults to 60). Method one averaged the data, which gives a baseline value and set a noise limit of the average + 10%. While this method worked in many cases, it would obviously fail for small signals superimposed on a large DC offset value, and for very noisy signals recorded with a small DC offset value. Method two evolved to correct these problems. This method averages the data and finds the standard deviation of the data (noise). The baseline is still the averaged value, but the noise limit is set at three times the standard deviation (99% confidence level). While this improved peak recognition compared to method one tremendously, it still detected erroneous peaks. An additional parameter of requiring a number of consecutive data points (default = 5) above the noise limit for peak recognition was added. This reduced falsing to a negligible level.

Integration was also handled two different ways. The first method simply summed individual data points minus the signal average. This technique was used with the first method of peak recognition, and, while functional, was changed to a more advanced technique, less prone to error. The second and currently used method involves the application of Simpson's Rule to individual points minus the baseline to more closely approximate the area under the peaks. Additionally, the points im-

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mediately before and after peak recognition are included in peak integration. Fig. 7 shows a simulated chromatographic run, in which peak two has twice the area of peak one, under noisy conditions. Table 1 shows the integrated areas for peaks one and two for conditions of noise and no-noise. A good correlation between these values is seen, indicative of good performance. The routine to generate noise and peaks for simulation is included as a test mode in the program.
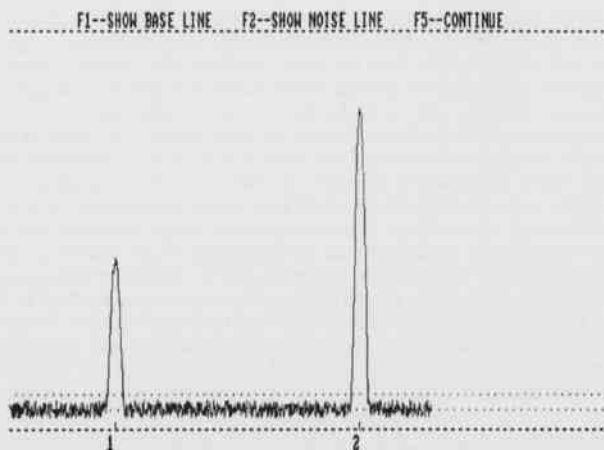


Figure 7. Simulated data showing the effect of noise on program integration routine.

Table 1. Noise/No Noise Simuleted Data Integration Results

| Peak No. | Location | Area |
|---|---|---|
| 1a | 220 | $2.359518 \times 10^{-4}$ |
| 2a | 732 | $4.754066 \times 10^{-4}$ |
| 1b | 220 | $2.400004 \times 10^{-4}$ |
| 2b | 732 | $4.800007 \times 10^{-4}$ |

"a" peaks taken under conditions of "noise"
"b" peaks, no noise conditions.

## CONCLUSION

The authors feel that the current program offers the user of an Ithaco 3962 a great deal of versatility in the collection and processing of data. While the application presented has involved gas chromatography, any application which involves an intensity versus time domain may be acquired, stored, and displayed. For example, the authors are currently constructing a spectrometer for infrared emission studies. The program will be used to collect intensity versus wavelength (time scanned) data. Other current work is underway to include commands to allow use with the Ithaco 3961 LIA and 3981 PC board LIA. Modifications will consist of changes in the file opening and three letter command set, and should allow higher data collection rates. The 3981 LIA system is internal to the computer chassis, with the exception of a small preamplifier unit, and further simplifies the instrumentation. Future work may include options to allow the program to acquire data from an analog to digital interface, which will allow application to other methods besides those using an LIA. Those interested in the program should contact the authors.

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