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have included several routines for calibration of the instrument reading to the digital value recorded by the computer and for calibration of the timing loops of the software on a specific computer to time measured by stopwatch. Timing accuracy is as good as manual stopwatch reading. Reading accuracy is very good, but falls off in the upper and lower 15% of the range. Reading precision is limited by the intrinsic 1 part in 256 precision of the ADC chip. This is probably about half the precision possible with careful manual reading of the Spectronic 20 meter but is better than the precision possible in reading the rapidly changing display commonly experienced in kinetics experiments.

## Modification

Although the program is set up to interface with the Spectronic 20 and to convert the percent transmittance data to absorbance, it has provisions for adding other instruments by specifying the calibration points and data transformations they require. Other default values may be altered temporarily from a menu. The constants have been grouped within one section of the program to allow easy permanent modification.

## Data Format

Data can be saved as time versus reading pairs in Data Interchange Format (DIF), comma-separated-value text files, or files compatible with the data analysis programs Scientific Plotter and Curve Fitter.<sup>2</sup> One of these formats can be read by most spreadsheet or other data manipulation programs.

Our software and instructions for building the ADC Interface Box will be available through Project SERAPHIM.

## Simulation of a Gas Chromatography-Mass Spectrometry Experiment with a Commercial Spreadsheet Program

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Commercial spreadsheet programs are becoming increasingly valuable in laboratory calculations and simulations (1). In our recent work on data reduction for gas chromatographic mass spectrometry (GC-MS), it became necessary to simulate a GC-MS experiment as a means of investigating, in a controlled way, the effects of chromatographic resolution, column bleed, noise, and background interference on the data manipulation. We developed the simulation using a commercially available spreadsheet

<sup>2</sup> Published by Interactive Microware, Inc., State College, PA.

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program, LOTUS 1-2-3. In addition to its value as a simulator, the method would seem to have value as a teaching tool for instruction of many aspects of multidimensional chromatographic/spectroscopic combinations. For example, in GC-MS the concepts of spectral clean-up and mass chromatograms are easily demonstrated. Other combination methods (LC-UV, GC-IR, LC-MS) would work equally well, with almost identical spreadsheet instructions. As examples, the concepts of absorbance ratioing (11), wavelength chromatography (12), and derivative spectroscopy (13) in ultraviolet spectroscopy or chemigrams (plots of integrated absorbance versus spectrum number) (14) in GC-IR data can be readily illustrated with spreadsheet simulation.

## Spreadsheet Organization

An IBM PC-XT with 640-K core memory was used for all work described. LOTUS 1-2-3 (version 2.0) was purchased from Lotus Development Corporation and was used without modification. The spreadsheet is organized as a large matrix, with the rows identified by numbers and columns identified by letters. The contents of each cell, or address, can be numbers such as data, alphanumeric labels, or calculations. In our particular application, the organization is as diagrammed in Figure 1. The first several columns are simulated chromatographic profiles of individual components as a function of time (Figure 1, columns B-E). Each row represents some arbitrary time increment. Although any number or shape for these profiles may be employed, we frequently used a simple Gaussian:

$$Y_{i,t} = \frac{A_i e^{-(t-\mu_i)^2/2\sigma_i^2}}{\sigma_i \sqrt{2\pi}} \quad (1)$$

where  $y_{i,t}$  is the value of the  $i$ th component in the  $t$ th time interval,  $A_i$  is a relative maximum peak height value,  $\sigma_i$  is related to peak width, and  $\mu_i$  gives peak position. Once the equations are entered, the relative intensity of peaks can be increased or decreased, or the peaks can be broadened or sharpened or moved about relative to one another by adjusting only  $A_i$ ,  $\sigma_i$ , and  $\mu_i$ , respectively. The sum of all these curves gives a "total ion profile" and represents the resulting chromatogram (Fig. 1, column G).

The mass spectrum of each pure component is listed in a row across the bottom of the spreadsheet (Fig. 1, rows 80-83). Masses are listed sequentially across the top (row 1); each cell entry corresponds to a relative abundance (normalized to 100) for the ion at that mass in the pure compound. For example, the mass spectrum of 1-chloro-nonane consists of eight ions with masses (and normalized relative abundances):  $m/z$  41 (40%), 43 (60%), 55 (45%), etc. This information is coded into Figure 1, Row 81.

The balance of the spreadsheet is the data array that would result if four compounds were eluted, with the profiles indicated in columns B-E, from a GC and were directed into a repetitively scanning MS. Each row (Fig. 1, rows 2-77) represents a mass spectrum from one scan of the mass range. The relative abundance of each mass is obtained by summing the contributions to that mass from each compound. These contributions are computed by multiplying the abundance of the ion of interest in each of the pure

spectra (Fig. 1, rows 80–83) by an intensity term defined by the corresponding chromatographic profile (columns B–E). The calculation is shown at the bottom of Figure 1. The calculation is repeated for every  $m/z$  value at each time point. This array is central to understanding the nature of GC–MS data, since it illustrates the three-dimen-

sionality of a chromatographic spectroscopic experiment: the spectral resolution elements span each row ( $m/z$ , in this case), the time axis is represented as a descent down a column, and the spectral intensity is communicated by the numbers in the cells. Slicing the data array horizontally corresponds to sampling the mass spectrum at some

point in time. If the chromatographic profiles of components overlap, the spectrum will represent a mixture of the two (or more) pure compounds, exactly as it would in a repetitively scanned spectrometer with scan time equal to the time increment of one row. Slicing the data array vertically corresponds to observing a given  $m/z$  as a function of time. In GC-MS, we would call this display a mass chromatogram (15).

## Illustration

Several features of the GC-MS simulation can be illustrated with a simple example. Four components are involved, with chromatographic profiles indicated in Figure 1. These might correspond to three analytes and an increasing column bleed. The first three are variations of the Gaussian equation (eq 1) to provide different peak heights, widths, and positions by adjustment of  $A_i$ ,  $\sigma_i$ , and  $\mu_i$  as described above. The column bleed is a simple exponential of the form:

$$y = It^n \quad (2)$$

where  $I$  and  $n$  adjust the rate of rise of the curve. When these four profiles are summed, the total ion profile in Figure 1, column G results. To examine the mass spectrum at any point in this chromatogram, one can simply graph the appropriate row at the desired time interval.

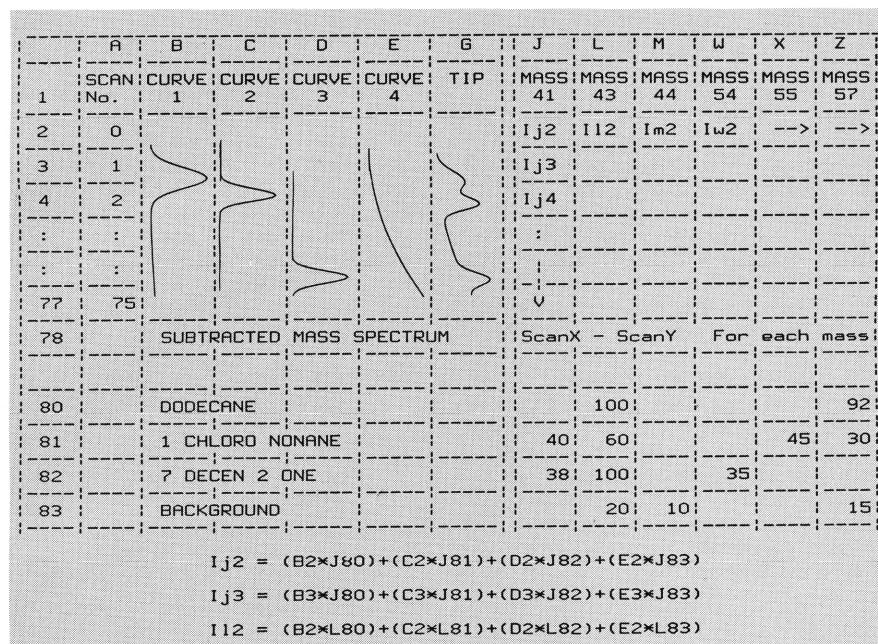


Figure 1. Spreadsheet organization. The numbers appearing in the array J2 to Z77 represent relative ion abundances.

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One exercise that can be readily demonstrated using the spreadsheet is the clean-up of mass spectra by spectral or background subtraction. To accomplish this with the spreadsheet, one simply subtracts the cell entries of one row from those of another. In our spreadsheet, we set aside one row (Fig. 1, row 78) to receive this difference. We only need to specify which two spectra we would like to subtract (by row numbers) and row 78 is updated, and the corresponding graph can be viewed. In the example of Figure 1, the two components eluting around scans 17–28 overlap badly. A spectrum from scan 22 results from a mixture of the two components (Fig. 2a). However, if we take a spectrum early in the peak (scan 17), which is predominantly from the first-eluting component, and subtract the spectrum from late in the peak (scan 28), predominantly from the later-eluting material, we can get a relatively clean spectrum of compound 1 (Fig. 2b) that closely matches the spectrum of the pure material. Similarly, reversing the order of subtraction (scan 28 – scan 17) provides a clean spectrum of compound 2 (Fig. 2c). In exactly the same way, the spectrum of the analyte that elutes on the rising column bleed can be obtained by subtracting, for example, scan 74 and scan 67. The only entries that need to be changed are the two scan numbers; the spreadsheet automatically performs the new subtraction and updates row 78.

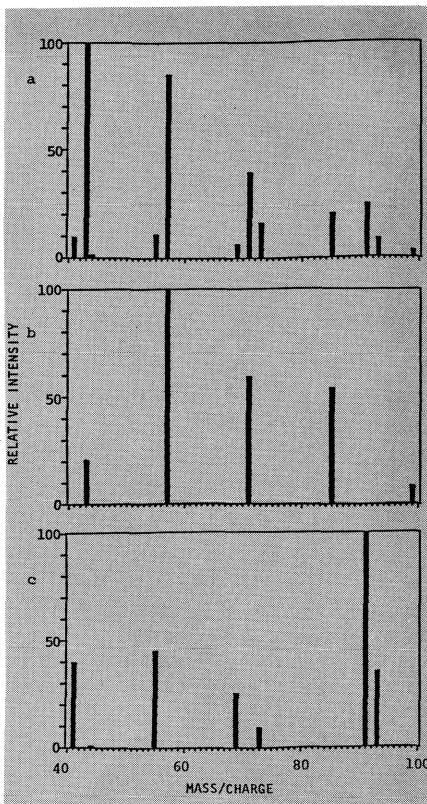


Figure 2. Mass spectra of (a) scan 22, (b) scan 17 – scan 28, and (c) scan 28 – scan 17 from simulated data set of Figure 1.

Students often have difficulty understanding the idea and utility of a mass chromatogram. Normally, this is a data display in which the computer plots the intensity of a requested  $m/z$  value as a function of time. The idea is readily grasped with the aid of the spreadsheet, however. The mass chromatogram is simply a slice vertically through the data array; a graph of any one column. Figure 3 shows the mass chromatogram of  $m/z$  41 (Fig. 3a) and the total ion profile (Fig. 3b). It is easy to see that analytes 2 and 3 have  $m/z$  41, while analyte 1 and the "column bleed" do not. One can see how this form of selective data reduction helps to resolve overlapping peaks, decrease background, and improve the signal-to-noise ratio by reducing chemical noise. Just as important, however, is noting the difference between a mass chromatogram and the result of a "single-ion monitoring" experiment. In the experiment we have been simulating, the entire mass range is recorded at set time intervals. A mass chromatogram is a selective display of a fraction of the collected data. In a single-ion monitoring experiment, only one  $m/z$  is recorded throughout the entire experiment, that is, the data are collected selectively. A graph of that data set would look like Figure 3a but would represent the entire collected data set. Each type of experiment has its own advantages and disadvantages (16).

To simulate more complicated (and more realistic) chromatograms, one could increase the complexity of the profiles in columns B–E (Fig. 1) to provide peaks that front or tail, small analyte peaks on the tail of a large solvent peak, a relatively constant, but high background, or random spectral noise. One would rarely need more than three or four profiles to illustrate any given situation; and the rapid spreadsheet, updating allows one to change profiles and peak positions with ease.

In conclusion, commercial spreadsheet software can provide a versatile and powerful means of simulating a data set for GC–MS or other chromatography–spectroscopy combinations. With relatively few commands, a variety of realistic chromatographic situations can be approximated. The spreadsheet helps to conceptualize the multidimensionality of the data set and aids in the illustration of spectral clean-up and mass chromatograms.

## Literature Cited

- Levkov, J. S. *J. Chem. Educ.* 1987, 64, 31.
- Coe, D. A. *J. Chem. Educ.* 1987, 64, 137.
- Mason, T. J.; Turrell, J. A.; West, C. W. *J. Chem. Educ.* 1985, 62, 344.
- Hurlbut, J. A.; Kavianian, G. R.; Lee, S. Y.; Nuttall, K. L.; Gentry, S. R.; Hassman, T. L. *J. Chem. Educ.* 1977, 54, 442.
- Hurlbut, J. A.; Bishop, C. V.; Brittain, P. C.; Preheim, C. W. *J. Chem. Educ.* 1975, 52, 100.
- Hurlbut, J. A.; Ball, T. N.; Pound, H. C.; Graves, J. L. *J. Chem. Educ.* 1973, 50, 149.
- Waddington, M. D.; Meany, J. E. *J. Chem. Educ.* 1978, 55, 60.
- Henderson, J. *J. Chem. Educ.*, in press.
- Jackson, D. F.; Henderson, J.; Berger, C., Jr.; Berger, C.; Estell, J. K. *J. Chem. Educ.*, the following paper.
- Henderson, J. *J. Chem. Educ.* preceding paper.
- Yost, R.; Stoveken, J.; MacLean, W. *J. Chromatogr.* 1977, 134, 73–82.
- Denton, M. S.; DeAngelis, T. P.; Yacynych, A. M.; Heineman, W. R.; Gilbert, T. W. *Anal. Chem.* 1976, 48, 20–24.
- Meal, L. *Anal. Chem.* 1986, 58, 834–836.
- Mattson, D. R.; Julian, R. L. *J. Chromatogr. Sci.* 1979, 17, 416–422.
- Hites, R. A.; Biemann, K. *Anal. Chem.* 1970, 42, 855–860.
- Anderegg, R. *J. Am. Lab.* 1985, 17(9), 20–30.

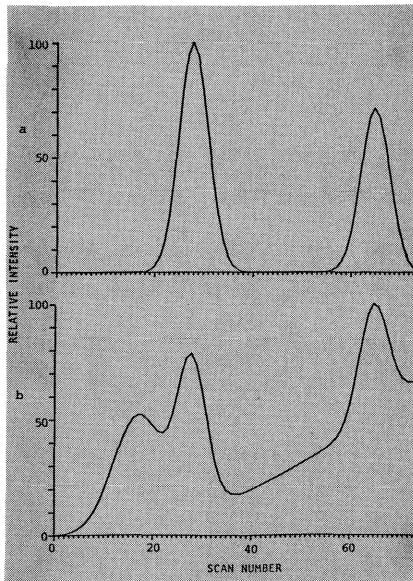


Figure 3. (a) Mass chromatogram of  $m/z$  41 and (b) total ion profile of simulated data set of Figure 1.

The simplest, but also most tedious, method of entering the data for the pure spectra into the spreadsheet is by manually typing the abundance values for each cell. Information can also be entered using the IMPORT command from a data file elsewhere. In our work, we have interfaced the PC directly to the data system of a Hewlett-Packard 5985 B GC–MS system and can dump spectra directly from data files or spectral libraries to the PC and from there into the spreadsheet.