

## COMPUTER PROGRAMS FOR ESR SPECTRAL ANALYSIS\*

Jean C. KERTESZ, Michael B. WOLF, and Walter WOLF

*Department of Biomedical Chemistry, School of Pharmacy, University of Southern California,  
Los Angeles, California 90007*

A computerized on-line system utilizing time-sharing services for the signal analysis and display of the electron spin resonance (ESR) spectra of organic free radicals is described. The system provides a broad analysis capacity for arithmetic and transfer operations on spectra; curve smoothing and filtering by a moving average function; differentiation and integration functions; the addition, subtraction, and inversion of spectra; and correction factor routines required to match spectral parameters. Data collection is performed by a minicomputer housed in the laboratory which transmits this data to a large time-sharing facility for further analysis. The resultant data may be displayed in either a digital or graphic format. Applications of the programs to determine free radical spin concentrations, to analyze spectra consisting of overlapping peaks, and to enhance low intensity signals are illustrated.

Electron spin resonance spectra  
Computer-assisted spectrum analysis

Organic free radicals  
Time-sharing minicomputer interface

### 1. Introduction

The system to be described presents a computerized method for signal analysis of ESR spectra of organic free radicals, in particular of thiyl and related free radicals important in radiobiological processes. The ESR characteristics and properties of thiyl free radicals, generated by ceric ion oxidation utilizing ESR continuous and stopped-flow techniques, have been previously described [1-8]. Other workers have dealt with the development of computer programs for specific aspects of ESR spectral analysis, ions and free radicals in the solid state [9], or for study of microcrystalline symmetry at the site of paramagnetic ions [10], by facilitating calculations and evaluation of the parameters in generalized spin Hamiltonians; and for interpretation and simulation of the hyperfine structure of organic free radicals with nuclear spin of 1/2 and 1 [11]. Other studies [12, 13] have demonstrated a computerized method of resolution enhancement of spectra consisting of overlapping lines and with incompletely resolved

hyperfine structure.

In this paper, we report on the development of a program with broad analysis capacity of general applicability for ESR spectra, adapted from a study of a computer program called CONVOL which utilizes convolutional techniques and is in a batch environment exclusively, devised by Borg [14, 15] in Fortran for the CDC-6600. The present data transfer and computer analysis system is programmed in CPS/PL/1 [16] and is supported within the time-sharing environment at the University of Southern California IBM/370, Model 158 computer facility, which allows a user access to this facility via a standard telephone connection.

This program, named MAINCV, which is flow charted as Appendix I, is capable of accomplishing the following spectral manipulations:

- (1) Transfer operations permit the storage of spectral data, i.e., the read into file function, on a disk data set so that any spectrum can be recalled on demand.
- (2) Curve smoothing and filtering can be performed by means of a moving average operation.
- (3) Differentiation and integration functions are particularly useful in kinetic studies. Integration of peak areas allows the determination of free radical

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concentrations. The program yields a digital table of the spectrum, from which a graphic display of the spectrum can be produced from the paper tape which is compatible with the recorder's plotter. The area of the spectrum may be calculated directly from the digital table of the second integral. Correction factors are included, making the resultant spectra compatible with the recorder's plotter.

- (4) Addition and subtraction functions permit the simulation of complex spectra from simpler component peaks and the observation of underlying peaks otherwise hidden in a spectrum resulting from a mixture of several free radical species. The program outputs (a) a digital table, (b) the area of the resultant spectrum, and (c) a paper tape from which a graphic display may be obtained.
- (5) Routines for correction factors have been incorporated. These are required when spectra to be compared are recorded under different settings (e.g., field set, scan range, and receiver gain amplification). Spectra can be shifted horizontally or vertically; the base for field in gauss can be expanded or shortened; and receiver gain differences are compensated for. In addition, to secure a workable integration baseline of absorption curves, the spectra may be tilted.

ESR spectra are accumulated on a Varian E-3 ESR spectrometer and temporarily stored and digitized in a Hewlett Packard Multichannel Analyser 5400A (H/P). The data are then transferred to paper tape by means of a MATHATRONICS® 480 desk model computer PTP-TELETYPE® unit and read into the CPS computer file, from which the needed data can be recalled. A plot is obtained by inputting the paper tape containing the results of the computer analysis into the H/P, which in turn directs the recorder of the E-3 ESR spectrometer. All of these operations for frequently required ESR spectral analyses may thus be completed entirely within the laboratory, with the aid of a time-sharing computing system which provides for rapid turnaround.

In addition several programs were developed for the Mathatronics computer which duplicate some of the capabilities of the MAINCV program, such as integrations and double integrations, yet do not require the use of the time-sharing services. These permit some further computations, including various programs for analysis of kinetic decay curves. Programs

for this small computer may be used only when a plot of the results is not required, since the output format is incompatible with the input parameters of the H/P, and are thus not discussed in detail below.

## 2. General description of the programs

The MAINCV program consists of five overlaid procedures which occupy 24 K bytes of storage, including statements, file buffers, and array storage areas. All multispectra functions are performed on three matrices, each of which is capable of storing approximately 1/3 of 880 possible data points. This abbreviated number of data points is necessary due to the limitation on program memory space. A common block consisting of the matrices allowed for the successful overlay of all 5 CONVOL procedures. The maximum number of data points, 880, was set by the limitation of the 5 procedures.

MAINCV is an interface program which only serves to overlay the other procedures. The capability of each program is described below:

(a) CONV1 performs the file management function by creating new files with data from the H/P. It has the capability to list the file contents in a format compatible with the input requirements of the H/P.

(b) CONV15 permits the addition and subtraction of spectra, which are (multi-spectral) functions utilizing several spectra at one time, and spectral inversion. Computations follow the general equation:

$$Y_n = X_n + k(Z_n) \quad (1)$$

where  $Y_n$  is the data point for channel  $n$  in the resultant spectrum,  $Z_n$  is the data point for channel  $n$  in the spectrum which is to be added to or subtracted from  $X_n$ , the data point in channel  $n$  of spectrum  $X$ . When spectra  $X$  and  $Z$  are added together,  $k = 1$ . When  $Z$  is to be subtracted from  $X$ ,  $k = -1$ . For the inversion function,  $k = -1$  and  $X_n = 0$  for all values of  $n$ . For all three of these options the resultant spectrum may be shifted vertically to achieve a practical baseline, consistent with the input requirements of the H/P.

(c) CONV2 accomplishes the routines for correction factors, including:  $X$ -shift, a horizontal or channel shift which corrects for varying scan ranges;  $Y$ -shift, a vertical shift;  $Y$ -mult, which standardizes peak heights; and Ramp, for baseline corrections.

An  $X$ -shift occurs when the stored spectrum is read from the file into the matrices. For a (downfield) shift to the left, channels in the file are bypassed before transfer into the matrices begins. For an (upfield) shift to the right, the initial channels of the matrices are set to zero. Then readout from the file begins with the first file entry.

$Y$ -shift and  $Y$ -mult represent arithmetic functions to be performed on the data point entries, and may be represented by eq. (2):

$$Y'_n = M(X_n - B_0) + B_0 + S \quad (2)$$

Here  $Y'_n$  is the data point for channel  $n$  in the resultant spectrum,  $X_n$  is the data point for channel  $n$  in the spectrum to be altered,  $M$  is the  $Y$ -mult value by which  $X_n$  is multiplied,  $S$  is the  $Y$ -shift value which is added to  $X_n$ , and  $B_0$  is the zero baseline.

During the  $Y$ -mult operation, the original baseline of spectrum  $X$  would normally be altered. To prevent this, the original baseline of spectrum  $X$ , termed the zero baseline, is subtracted from  $X_n$  before multiplication by  $M$ , and is subsequently added to the result. This variable,  $B_0$ , also appears in the routines for integrations and differentiations described below, where inaccuracies in its assumed value must be corrected by using the Ramp function.

The Ramp function allows the error in integrals caused by improper choice of baseline to be corrected by adding or subtracting an increasing number from left to right at a linear rate. All numbers remain integers.

(d) CONV25 computes the moving average, integration, and derivative functions.

The moving average function is an adaptation of the curve smoothing capacity of the previous CONVOL program devised by Borg [14]. In this procedure two data points which are separated by ten channels are averaged to produce a new intermediate entry. This convolutional "window" then advances one channel at a time to compute new values continually from the given data points.

Integration of the ESR first derivative signal for the estimation of free radical concentrations was adapted for the computer from previous numerical methods [17] and is computed according to the equation:

$$Y''_n = \left[ \sum_{i=0}^n (X_i - B_0) \right] + B_0 \quad (3)$$

where  $Y''_n$  is the data point for channel  $n$  in the resultant spectrum,  $X_i$  represents the data point for channel  $i$  of the spectrum to be integrated, where  $i = 0, 10, 20, 30, \dots, n$ , and  $B_0$  is the zero baseline. In the program for integration written for use with the Mathatron,  $i$  may advance by any arbitrary step; in all other respects, the Mathatron program and MAINCV calculate integrals identically. The zero baseline,  $B_0$ , is added to the resultant summation to return spectrum  $Y$  to the original baseline in spectrum  $X$ .

For the derivative function, the following equation is used:

$$Y'''_n = X_n - X_{(n-10)} + B_0 \quad (4)$$

where  $Y'''_n$  is the data point for channel  $n$  in the resultant spectrum,  $X_n$  is the data point for channel  $n$  in the spectrum to be differentiated;  $X_{(n-10)}$  is the data point of the latter spectrum in the tenth channel preceding  $X_n$ , and  $B_0$  is the zero baseline. Again,  $B_0$  is added to return spectrum to the original baseline.

When integrating, the value for the zero baseline is estimated such that the area defined by the derivative of the absorption curve lying above the chosen zero baseline is equal to that area defined by the curve below the zero baseline. This should result in an absorption curve that returns to the same vertical level at which it begins. However, minor inaccuracies in the choice of a zero baseline can result in a large error in the integration. For example, a first derivative ESR peak is illustrated in fig. 1(a) and its integral in fig. 1(b). The estimated zero baseline in this case is seen to be too small, so the integrated curve does not return to its initial level. The triangular area indicated in fig. 1(b) represents the integration of the area which was not subtracted in the summation of eq. (3), due to an inadequately small selection of  $B_0$ . MAINCV subtracts an amount proportional to the appropriate triangular area from the absorption spectrum and integrates the result to obtain the corrected second integral (fig. 1(c)), thus markedly facilitating

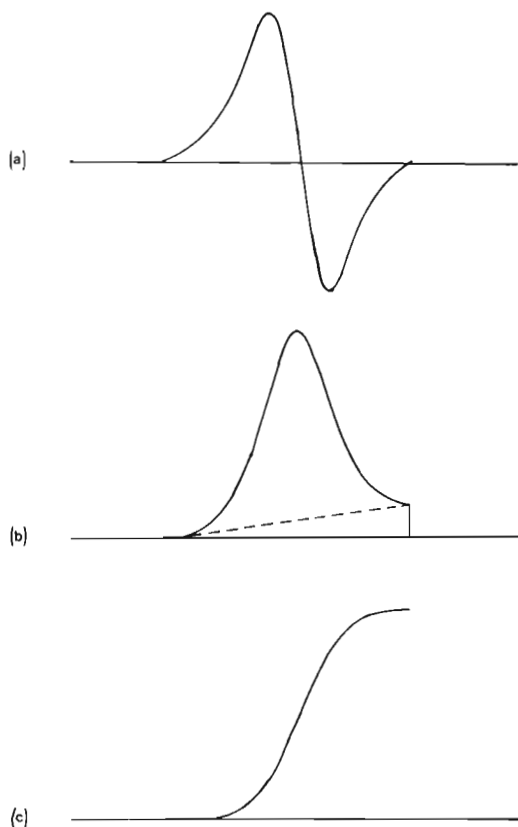


Fig. 1. First and second integrals of the central peak of the ESR first derivative triplet obtained by mixing solutions 0.02 M cysteine · HCl in 0.225 N HCl with 0.02 M  $\text{CeK}_2(\text{NO}_3)_6$  in 0.2 N HCl at room temperature. Flow rate, 5.4 ml/sec. Mod. amp. 2.5 gauss. (a) First derivative peak, (b) absorption curve, and (c) integral of corrected absorption curve.

the correction procedure [17, 18]. The input parameter for this zero baseline correction procedure is Ramp, mentioned above, which is thus equal to the deviation of the vertical level of the downfield end of the absorption (integrated) spectrum from the upfield end (tail).

In estimation of spin concentrations from ESR first derivative spectra, which depend on a variety of possible systematic and random signal errors, determination of the corrected second integral has been shown to be the most reliable method [18]. The magnitude of the effects of individual errors have been discussed in detail elsewhere [18]. The applica-

tion of the programs to the determination of spin concentration is discussed in section 6.1.

(e) CONV3 performs *X*-mult (a horizontal multiplication), and Markers functions, and stores the convoluted spectra in file. *X*-mult and Markers functions are both executed when the matrices are entered back into the file.

Only values of one-half or two are possible for the *X*-mult function. For a value of one-half, every other channel of the resultant spectrum is bypassed while storing it in file. For a value of two, the arithmetic average of each adjacent pair of channels is stored as a new channel, in between the original pair. Both functions are performed from left to right. Thus, when the *X*-axis is expanded by a factor of two, it is necessary to recenter the resultant spectrum. This may be accomplished by performing an *X*-shift of a selected number of channels to the left at the same time or before the *X*-mult of two is performed. If, for example, the spectrum is shifted 256 channels to the left, the resulting full-scale display will represent only the second and third quarters of the original spectrum.

When contracting the *X*-axis by a factor of two, it is simpler to center the result "manually" (see section 5.1), utilizing the E-3 ESR spectrometer, than to perform the *X*-shift function.

When the Markers function is employed, an arbitrary value is added to every hundredth channel of the spectrum in order to produce "spikes" in the graphic display. These "spikes" act as reference points to facilitate comparison of the graphic display with the digital data table.

### 3. Operating instructions

After LOGIN is established, loading and execution procedures are as follows:

```
LOAD (MAINCV)
XEQ
```

MAINCV will call the other programs as they are required. Thus, no additional direct LOAD command is needed. The following reminders are printed:

```
880 CHANNELS—MAXIMUM SPECTRUM LENGTH
"999" MARKS END OF SPECTRUM
```

CONV1 is loaded and executed. Computer response:

```
STORE OR END = 0  CONVOLVE = 1
SPECTRUM SUB/ADD OR INVERT = 2  LIST = 3
#JB
?
```

All possible options are presented here. At the completion of any option, the computer will return to this point. Execute the desired function by entering its corresponding number.

### 3.1. Storing a spectrum

Computer response:

```
WHICH FILE (0 = END)?
```

Two options are now presented:

a. A spectrum may be stored. Type a number or letter from 1 to 9 or A to Z. The selected number or letter becomes the file identifier and will be required whenever the particular file is to be retrieved. Computer response:

```
ENTER DATA, "STOP" = END OF ANNOTATION
```

The spectrum data, settings and comments may now be entered. To end loop, enter:

```
STOP
```

Computer response:

```
A(1)
```

"A" is an array of eleven entries. Eleven data points are now entered. The program will prompt the user for the matrix "A".

If an error occurs during the input process, an appropriate message will be printed and prompting will continue from the beginning. Should a terminal error occur a message such as:

```
370. WAS THE LAST LINE EXECUTED.
```

will appear indicating that the program should be restarted at the next line by typing:

```
XEQ 380. THRU ...
```

A spectrum can only be entered in its entirety, because the file lines do not have identifiers. The end of spectrum is indicated by inputting a "999".

b. This program may be terminated by typing in "0".

### 3.2. Multi-spectra functions

Should the following options be chosen, control returns to MAINCV which calls CONV15. If computer response is:

```
ADD = 1  SUBTRACT = 2  INVERT = 3
#JOB
?
```

Type 1, 2, or 3. These three options presented are treated as follows:

(1) or (2). Addition or Subtraction

Computer response:

```
SPEC(X) = SPEC(Y)(+, -) SPEC(Z)
```

```
Y =
```

```
?
```

This represents a request for the File Number of spectrum (Y) to be used in the above equation. Computer response:

```
BASELINE = ?
```

```
BL
```

```
?
```

During the addition or subtraction of spectra, the resultant baseline is either raised or lowered, respectively. Thus, the value of the desired resultant baseline must be entered. Computer response:

```
Z =
```

```
?
```

Enter File Number of spectrum (Z). Computer response:

```
X = ?
```

```
?
```

Enter File Number of spectrum (X), the file in which the resultant spectrum is stored. Before this new file is created, an opportunity is provided to enter data and comments as in section 3.1. The resultant spectrum will have as many channels as the shortest spectrum initially entered.

(3). Invert, Computer response:

```
SPEC(X) = INVERT SPEC(Z)
```

Then follow procedure in options (1) or (2), starting with the second computer response.

### 3.3. Convolution

If the option "CONVOLVE" is chosen, the program will return to MAINCV which will call CONV2. The computer will print:

```
1. X-SHIFT  2. Y-SHIFT  3. Y-MULT  4. RAMP
5. MOVING AVERAGE  6. INTEGRATION
7. DVT.    8. X-MULT  9. MARKERS
YES = 1   NO = 0;   FORMAT --
1,2,3,4,5,6,7,8,9
CV1
?
```

Thus all nine convolutional options are now presented. CONV2 performs computations only on the first four options selected. If options 5 through 9 are selected, then appropriate flags are set in the common blocks which will cause the other programs to be loaded and the required calculations performed. The chronological order of functions is:

```
X-shift and Y-mult
Y-shift and Ramp
Moving average
Integration or Derivative
X-mult
Markers
```

The format to be followed to call any of the nine functions involves the entry of a sequence of a total of nine ones or zeros, separated by commas, directly under the nine printed numerals, each of which corresponds to one of the numbered functions listed above, i.e., 1 corresponds to X-shift, 2 to Y-shift, etc. If a 1 is entered below a given numeral, the corresponding function will be performed. If a 0 is entered, it will not. For example, if X-shift, ramp, integration, and markers are desired, enter:

```
1,0,0,1,0,1,0,0,1
```

Computer response:

```
WHICH FILE?
```

Enter desired file (spectrum) to be convoluted. Instructions for individual functions follow:

```
1. X-SHIFT - Computer response:
CHANNEL SHIFT -- TO RIGHT = +,
TO LEFT = - CV1
?
```

```
2. Y-MULT -- Computer response:
Y-MULT -- VALUE?
```

```
CV3
```

```
?
```

Enter multiplication factor.

Computer response:

```
ZERO BASELINE = ?
```

```
BAS
```

```
?
```

```
3. Y-SHIFT -- Computer response:
```

```
Y-SHIFT -- VALUE?
```

```
CV2
```

```
?
```

```
4. RAMP -- Computer response:
```

```
RAMP -- VALUE?
```

```
CV4
```

```
?
```

Enter value to be added to the right end of the integrated spectrum, such that it equals the value of the initial points of the spectrum.

Computer response:

```
HOW MANY CHANNELS IN SPECTRUM?
```

```
M
```

```
?
```

Control returns to MAINCV and CONV25 is called if any of options 5 through 7 are to be performed.

```
5. MOVING AVERAGE -- No inputs.
```

```
6,7. INTEGRATION or DERIVATIVE -- Computer response:
```

```
INTEGRATION, DVT --- ZERO BASELINE?
```

```
ZB
```

```
?
```

For an INTEGRATION function, the computer response is: DO YOU WANT TO INTEGRATE AGAIN? -- YES = 1 NO = 0

```
PN
```

```
?
```

For a DERIVATIVE function, the computer response is: DO YOU WANT A SECOND DVT? -- YES = 1 NO = 0

```
PN
```

```
?
```

If a one is entered, the computer will respond: INTEGRATED SPECTRUM

Following this statement, every tenth term in the spectrum will be printed, so that a suitable RAMP value may be determined.

At this point, the terms are not yet “normalized” (see below). As a result, it is possible for negative terms to be included. The listing will end with:

```
RAMP, CHANNELS, BASELINE -- VALUES?
RAM
?
```

The numbers should all be entered together, but separated by commas.

During the course of the integration and derivative functions, results will be either too large to plot or too small to be useful. Thus, a “normalizing” function is applied. This function scales the vertical range of the spectrum, such that the smallest data point equals 10,000 and the largest 90,000. In this fashion, negative numbers are prevented from appearing in the resultant spectrum and the size of the graphic displays are standardized. When plotting the result, a normalized spectrum will cover the full scale on the E-3 ESR spectrometer recorder when the H/P display is set at 200 counts/division. Computer response is:

```
(INTEGRATION, DVT)
PLOTTING MULTIPLICATION FACTOR
```

This is followed by the number by which the spectrum was multiplied.

Once again, the computer will present the option for repeating this function. If a zero is entered, computer response is:

```
DO YOU WANT TO PLOT RESULT?
YES = 1 NO = 0
PL
?
```

If the NO option is chosen, the computer response is:

```
NUMERICAL RESULT --
```

Again, every tenth number of the spectrum will be listed, and control will return to MAINCV, bypassing CONV3.

If the spectrum is to be plotted, it is necessary to store the resultant spectrum in a new file. Thus, if a “1” is entered, control returns to MAINCV and CONV3 is called if either options

8 or 9 are to be performed. The computer response is:

```
ENTER RESULT INTO WHICH FILE?
```

The file number or letter is entered. Computer response is:

```
HEADING, “STOP” = END OF ANNOTATION
```

Then, enter data, settings of convolutions, and comments as described in storing a spectrum, Sec. 3.1.

```
8. X- MULT -- Computer response:
X-MULT -- *1/2 = 0, *2 = 1
XM
?
```

The options of contracting or expanding the spectrum by a factor of two are thus presented. For contraction, 0 is typed, and for expansion, 1 is typed.

```
9. MARKERS -- No inputs.
```

Markers cannot be removed. Thus, this function should be utilized only after all other functions have been performed.

### 3.4. Spectrum read-out (LIST)

Computer response:

```
FILE NUMBER --?
```

Enter File Number of spectrum to be read. The computer will now print out the contents of this file. If the data is then to be read back into the H/P Digitizer, the tape punch should be on. Upon completion, the program will return to CONV1. The programs may then be terminated, if desired. Although the programs are normally stopped at the beginning of the storing function, they can be terminated at any point by pressing CTRL-Q.

#### 4. Sample run

Input parameters are explained in detail in section 3.

?LOAD(MAINCV)

?XEQ

NOTE:

880 CHANNELS -- MAXIMUM SPECTRUM LENGTH

"999" MARKS END OF SPECTRUM

STORE OR END = 0 CONVOLVE = 1 SPECTRA SUB/ADD OR INVERT = 2 LIST = 3

#JB

?0

WHICH FILE (0 = END) ?

?1

ENTER DATA, "STOP" = END OF ANNOTATION

?SPECTRUM PEAK #3 FILE 1 870 CHANNELS

?STOP

A(1)

?0000 0 48973 49520 49585 50754 48696 48564 48447 48864 48779

A(1)

?0010 49417 48830 48452 49004 50047 50731 48915 47709 48426 49412

A(1)

?0020 49750 48377 48426 48174 47670 47932 48426 48527 47138 48267

≈

A(1)

?0840 50423 48404 48480 49701 48896 49238 49923 48821 48481 49409

A(1)

?0850 49142 48520 48321 48331 48322 48342 48516 48083 47357 48271

A(1)

?0860 48323 49043 49598 49159 50015 50620 50171 50565 50473 50622

A(1)

?0870 50279 50570 50062 48942 49680 48370 49793 48328 48321 999

STORE OR END = 0 CONVOLVE = 1 SPECTRA SUB/ADD OR INVERT = 2 LIST = 3

#JB

?1

1. X-SHIFT 2. Y-SHIFT 3. Y-MULT 4. RAMP 5. MOVING AVERAGE

6. INTEGRATION 7. DVT 8. X-MULT 9. MARKERS

YES = 1 NO = 0; FORMAT --

1,2,3,4,5,6,7,8,9

CV1

?1,0,0,0,0,1,0,0,0

WHICH FILE?

?1

CHANNEL SHIFT? -- TO RIGHT = +, TO LEFT = --

CV1

?-10

INTEGRATION, DVT --- ZERO BASELINE = ?

ZB

```

?48300
DO YOU WANT TO INTEGRATE AGAIN? -- YES = 1 NO = 0
PN
?1
INTEGRATED SPECTRUM
49417
50867
50993

17853
17876
-30424
0
RAMP, CHANNELS, BASELINE -- VALUES?
RAM
?35400, 860, 48300
(INTEGRATION, DVT) PLOTTING MULTIPLICATION FACTOR --
.053238958406398
DO YOU WANT TO PLOT RESULT? YES = 1 NO = 0
PL
?0
NUMERICAL RESULT --
90011
90042
90079

10076
-1410340
-30424
0
STORE OR END = 0 CONVOLVE = 1 SPECTRA SUB/ADD OR INVERT = 2 LIST = 3
#JB
?0
WHICH FILE (0 = END) ?
?0
END OF RUN
?

```

## 5. Manipulations external to the programs

### 5.1. Manual

An inversion of the ESR first derivative spectral presentation is sometimes required for the comparison of two or more spectra in order to match peak maxima and minima. Spectra may be either (1) read directly into the H/P for digitization while simultaneously plotting them on the E-3 ESR spectrometer

recorder during data collection, or (2) first plotted on the E-3 recorder and then later entered into the H/P digitizer by manually "tracing" (following) the recorded plot on the E-3 recorder with the recorder pen control, while the H/P is accumulating. In case (1) the CONV15 program Invert function may be used when spectral inversion is required, whereas in case (2) this manipulation may be accomplished by manually reversing the spectrum sheet before "tracing".

The CONV2 X-shift and Y-shift correction functions may similarly be approximated with the manual "tracing" procedure, by shifting the spectral sheet the number of magnetic field divisions equivalent to the selected number of channels to the right or left, on the recorder platen, before "tracing". Performing these functions by this procedure is of only limited accuracy.

Manipulations of the results of a computer analysis in terms of the display plotted out may also be accomplished in a "manual" fashion. As indicated in section 3.1., when an X-mult function of one-half is applied to a spectrum, the resultant contracted spectrum appears in the first two quarters of the oscilloscope display. In order to recenter the spectral plot on the recorder chart paper, instead of performing the computerized X-shift function, the recorder arm may be placed at the appropriate division of the chart paper before the direct readout from H/P storage.

## 5.2. Electronic

All manipulations of spectral data for entry from the E-3 spectrometer into the H/P digitizer or for readout from the H/P digitizer, which may be accomplished by a change in setting on either instrument, are defined as electronic. Changes in the signal peak amplitude, analogous to the computerized Y-mult function, may thus frequently be easily affected. The H/P display counts/division control may be varied for a proportional increase or decrease in peak amplitude ( $y$  value) of spectra read out onto the E-3 recorder. Variations in the E-3 recorder scan time produce a directly proportional change in the  $y$  value, when data are first entered into the H/P digitizer from the E-3 by either of the two methods described in section 5.1.

The H/P digitizer possesses a rollover capability which allows stored spectra to be shifted vertically, a manipulation analogous to the computerized Y-shift function, when the results of the other computer analyses are ready to be plotted out onto the E-3 recorder.

## 6. Applications

### 6.1. The determination of free radical spin concentrations

The accurate determination of free radical concen-

trations is of considerable importance in the analysis of ESR spectral data, particularly in kinetic studies [8]. The procedure for estimating spin concentrations, once a suitable primary standard has been selected [15, 16], requires a comparison of the integrated areas of the first derivative peaks recorded for standard and unknown samples. As it has been shown [16] that corrections may be made for differences in the ESR characteristics and instrument settings, optimum for the recording of sample and unknown, the absolute number of spins,  $N_s$ , of an unknown sample may be calculated, when the peak areas are determined by the computer analysis described in section 2, by means of the following expression:

$$N_s = \frac{C_s(A)(S_r)^2(V)(\Delta\text{Chan})^2}{g^2(P^{1/2})(H_{\text{mod}})(G)(\theta)(S_t)}$$

Here,  $C_s$  is a proportionality constant which is determined by the spectral parameters and known spin concentration of the standard;  $A$  is the area of an unknown spectral peak expressed in the arbitrary units of the MAINCV program;  $S_r$  is the spectrum scan range in gauss;  $V$  is the input range volts setting of the H/P digitizer;  $\Delta\text{Chan}$  is the number of channels between data points used in calculating the double integral and represents the size of the horizontal interval between data points in gauss;  $g$  is the  $g$ -value of the unknown;  $P$  is the microwave power;  $H_{\text{mod}}$  is the modulation amplitude;  $G$  is the spectrometer gain;  $\theta$  represents the area of the peak integrated divided by the total area of all peaks in the unknown spectrum; and  $S_t$  is the recorder scan time setting when the data is entered into the H/P digitizer from the E-3, either fed in directly, or at a later "tracing".

### 6.2. Subtraction of spectra consisting of overlapping lines

Subtraction of spectra by computer programs aids in the identification of component free radical species, either by revealing underlying peaks otherwise hidden or by separating peaks generated by different free radical species, which may be partially superimposed upon one another. An example of the latter case, which permits the individual ESR properties and characteristics of a partially masked peak to be more accurately measured, is illustrated in fig. 2. The ESR

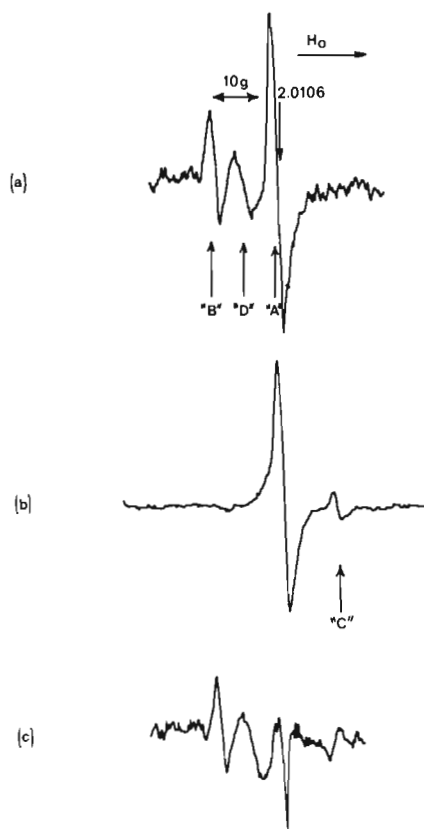


Fig. 2. Spectral subtraction of the first derivative ESR spectra of solutions obtained by mixing 0.02 M penicillamine in 0.1 N HCl with (a) 0.005 M  $\text{CeK}_2(\text{NO}_3)_6$  and (b) 0.04 M  $\text{CeK}_2(\text{NO}_3)_6$  at 0.2 ml/sec. Mod. amp. 2.0 gauss, (c) is the resultant spectrum when (b) is subtracted from (a).

spectrum recorded in fig. 2(a) consisted of three singlets (labeled "A", "B", and "D"), representing three individual free radical species, generated upon ceric ion oxidation of the thiol, penicillamine. At high ceric ion concentrations the "B" and "D" singlets were seen to disappear (fig. 2(b)) and a fourth singlet, the "C" signal, was recorded at high field along with the "A" singlet, which represents the thiyl ( $\text{RS}\cdot$ ) free radical. The nature and identity of these four free radicals have been discussed in detail elsewhere [1–8]. Due to the proximity of the intense "A" signal, the "D" peak cannot be seen in its entirety (fig. 2(a)). Thus, in order to clarify the nature of the "D" signal, the magnetic field masked by "A" needed to be un-

covered. To this end, fig. 2(b) was subtracted from fig. 2(a), after matching the maxima of the first derivative peaks of each. The resultant spectrum, illustrated in fig. 2(c), shows the more clearly defined broad "D" singlet, and a small spike peak minimum remnant of the "A" signal. The "A" singlet could not be eliminated entirely in this subtraction, since the  $X$ -mult function for matching peak widths can only be varied as a function of two.

### 6.3. Enhancement of low intensity signals

The thiyl free radical of cysteine is generated in greatest concentration upon ceric ion oxidation at an optimum pH of 1.5 [7]. Its ESR triplet signal weakens with increasing pH and is no longer observable above pH's higher than 7.5. Even at this pH, the signal is not

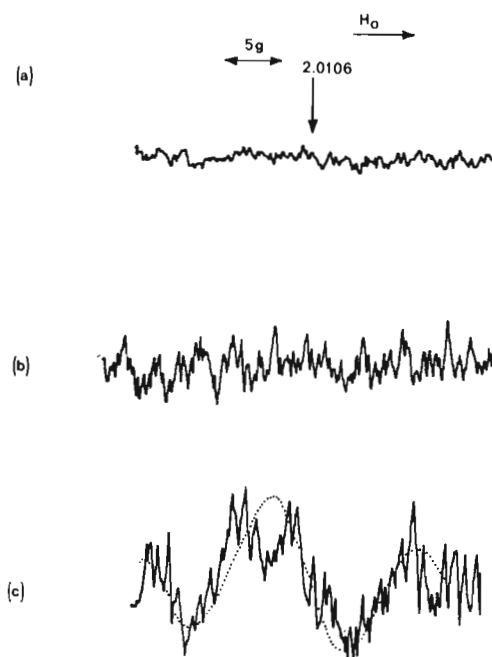


Fig. 3. Signal noise improvement obtained through use of moving average function for the first derivative ESR spectra of solutions obtained by mixing 0.02 M cysteine · HCl in 0.06 N NaOH with 0.005 M  $\text{CeK}_2(\text{NO}_3)_6$  at pH 7.5. Flow rate 1.0 ml/sec. Mod. Amp. 2.5. gauss. ESR receiver gain was (a)  $1.5 \times 10^6$ , (b)  $4.0 \times 10^6$ . (c) represents the improvement of spectrum (a), when moving average is followed by electronic amplification. The dotted line superimposed on this scan outlines the cysteinyl triplet signal.

distinguishable at high ESR spectrometer amplification alone (see figs. 3(a) and (b)). However, when the spectrum of fig. 3(a) was scanned three times with the moving average function and amplified by electronic means (see section 5.2.), it was possible to reproducibly verify the existence of the cysteine thiol radical triplet, as illustrated in fig. 3(c). The enhanced signal, although still noisy, coincided well with the characteristic cysteine triplet, and the spin concentration, determined after integration, fell within the expected range at this pH [7].

## 7. Hardware and software specifications

The program was written in CPS/PL/1 for the IBM/370 Model 158 computer system. The ESR spectra to be analyzed were recorded with a Varian E-3 ESR spectrometer. This was interfaced to a Hewlett Packard Multichannel Analyzer 5400A (H/P) by means of a Varian E-227 interface assembly. The H/P to E-3 interface for the control of the plotting range was affected by additional specially-built electronic cir-

cuitry (VIHP). Data was recorded on paper tape by means of the PTP-TELETYPE<sup>®</sup> unit associated with the Mathatronics 480 desk model computer. The teletype could run in either a 20 or 60 mil loop. The transition time for data was set at 100 baud for the teletype with the Mathatronics computer. The data rate between the Mathatron and the teletype was 83 msec. A standard dial-up telephone interface to the IBM computer locked out the teletype unit.

The interfacing for transfer of data from the E-3 spectrometer to the H/P digitizer is illustrated in fig. 4. Results of MAINCV computer calculations are punched onto paper tape by means of the teletype unit and read back into the H/P digitizer. Plotting of this data is achieved by utilizing the recorder of the E-3 spectrometer to display the output of the H/P digitizer by means of the interfacing illustrated in fig. 5.

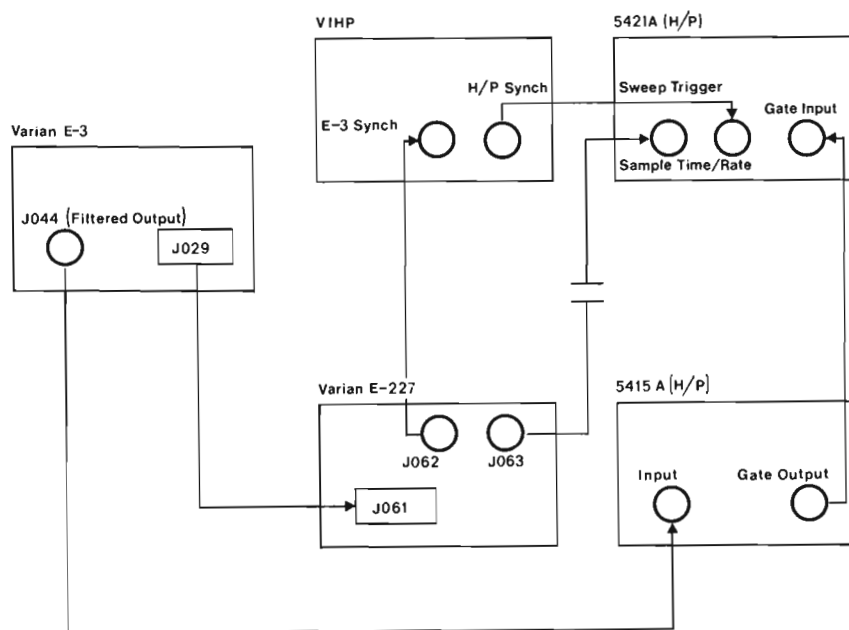


Fig. 4. Schematic representation of the interfacing devised to enter data into the Hewlett Packard Multi-channel Analyzer from the Varian E-3 Spectrometer.

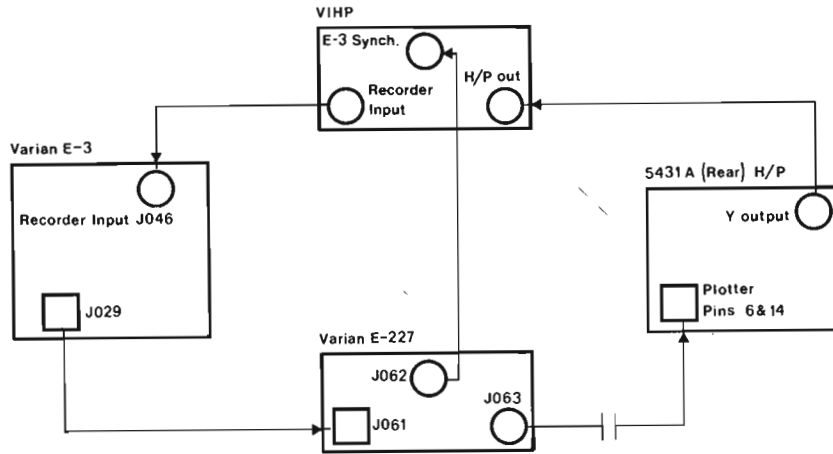
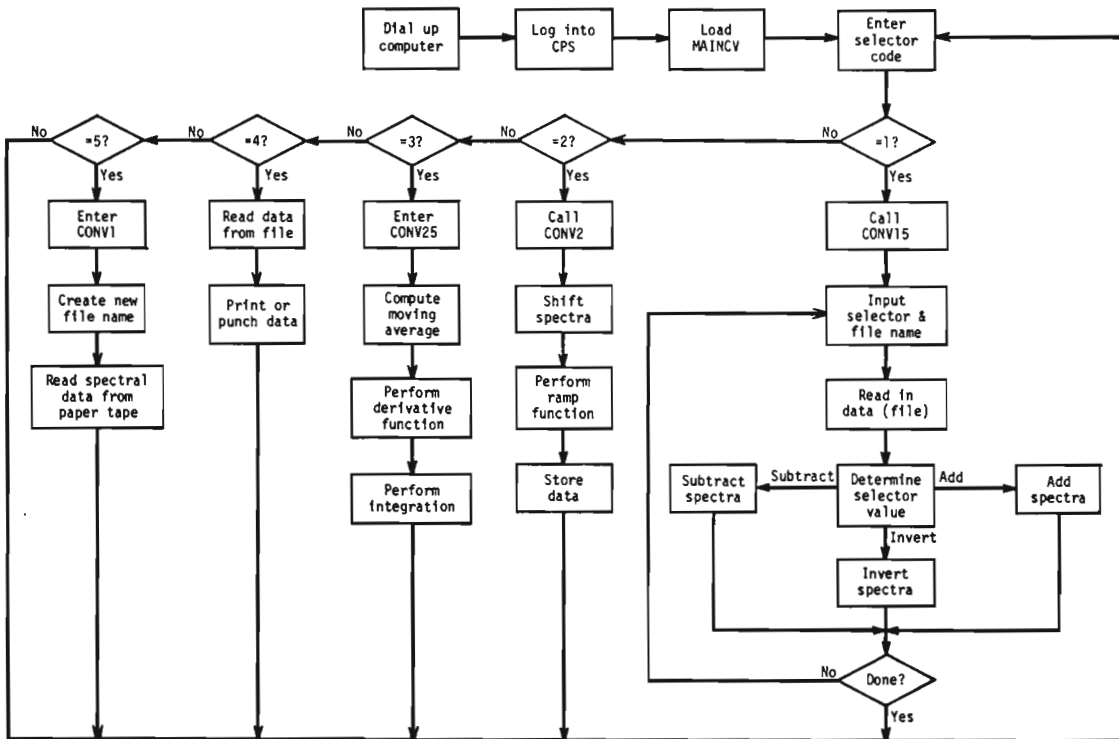


Fig. 5. Schematic representation of the interfacing devised to plot the results of the computer calculations, stored in the Hewlett Packard Multi-channel Analyzer, out on the Varian E-3 Spectrometer.

8. Appendix I

ESR SPECTRUM ANALYSIS PROGRAM FLOWCHART



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