

Micro Computerized EPR

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We introduce an inexpensive commercial microcomputer-based data acquisition, processing and simulation system configured with an Apple II Plus and a Varian E-Line EPR spectrometer. The interfacing and applications are described. The high level programming languages PASCAL and BASIC are used together with ASSEMBLER subroutines. All the problems normally encountered in liquid EPR data acquisition and data handling can be solved, and the necessary programs are available. The capacity of the system in data acquisition and manipulating is 10 000 points. The simulation program uses 1 000 channels and the time needed is normally about 1–2 min.

The applicability of electron paramagnetic resonance spectrometry (EPR) is greatly extended when the spectral data can be acquired and analysed in digital form. The digital record can be used to remove baseline drift, enhance resolution by means of multiple scans, add and subtract spectra, derive and integrate spectra, perform digital filtering, and many other operations as well. With a gaussmeter and microwave counter it is possible to measure g values and hyperfine coupling constants with a very high degree of accuracy.

A number of applications have been published, in which an EPR spectrometer is connected to a microcomputer and EPR spectra are collected for further processing.^{1–13} The rapid development of microcomputers programmable in high level languages offers the possibility of versatile on-line functions implying economical and easy solutions to EPR problems.

The computer system we used was an Apple II Europlus microcomputer, one of the most widely-used personal computers, together with two floppy disc drives, video monitor (ASA), Anadex DP-8000 dot-matrix printer, 12-bit analog-to-digital (A/D, Mariacchi) and 12-bit digital-to-analog (D/A), Tecmar DA101) converter cards, IEEE-488 (=GP-IB) card (Model 7490 GPIB interface, California Computer Systems), Apple II communications interface card (A2BOO3X, RS-232) and Apple II language card. The EPR spectrometer was a Varian E-line spectrometer equipped with frequency lock (Varian), gaussmeter (Varian E-500) and microwave-counter (Takeda Riken TR52110). The gaussmeter and the microwave counter have the IEEE-488 interface. In addition to the on-line solution, dataprocessing and simulation programs were developed.

INTERFACE

The interface between the computer system and the measuring instrument, an EPR spectrometer, consists of two parts: a connection between the microcomputer and the

Table 1. Read/write connections.

Name	Pin	Address		Voltage on/off	Description
		dec	hex		
Connections write					
AN3	12	-16289	C05F	on	Computer Control on
AN2	13	-16291	C05D	on	Computer lifts voltage for stepping motor
		-16292	C05C	off	Computer drops voltage to 0 V for stepping motor
AN1	14	-16293	C05B	on	Direction to left
		-16294	C05A	off	Direction to right
AN0	15	-16295	C059	on	Pen up
		-16296	C058	off	Pen down
AN3	12	-16290	C05E	off	Computer Control off
AN2	13	-16291	C05D	on	Activated (5 V)
AN1	14	-16293	C05B	on	Computer gives down-going pulse=
		-16294	C05A	off	manual start
AN0	15	-16295	C059	on	Activated (5 V)
Connections read					
PB0	2			on	Value of stepping motor value < 128
				off	value >= 128
PB1	3			on	Left limit value < 128
				off	value >= 128
PB2	4			on	Right limit value < 128
				off	value >= 128

Varian EPR spectrometer and a connection between the gaussmeter and the microwave counter.

As a starting point for interface planning, we decided that the information in the input from the Varian recorder should be the exact result of the EPR measurement. This required only a simple transformation in voltage, from the of ± 7.5 V of the Varian instrument to the ± 5 V of the microcomputer. The voltage information is communicated to and from the microcomputer by means of A/D and D/A converters. The A/D and D/A converters have a resolution of 12 bits, which means 4096 points in *y*-direction.

The Varian E-line series EPR recorder is of the stepped sweep type; a square-wave generator is used to advance the pen along the *x*-axis in discrete steps, approximately 10 000 steps being required to complete each traversal of the spectrum. The stepwise traversal of the recorder acts as a calculator of channels in the *x*-direction. The stepping motor is connected to one of the three single-bit inputs of the Apple game I/O connector (PB0, pin

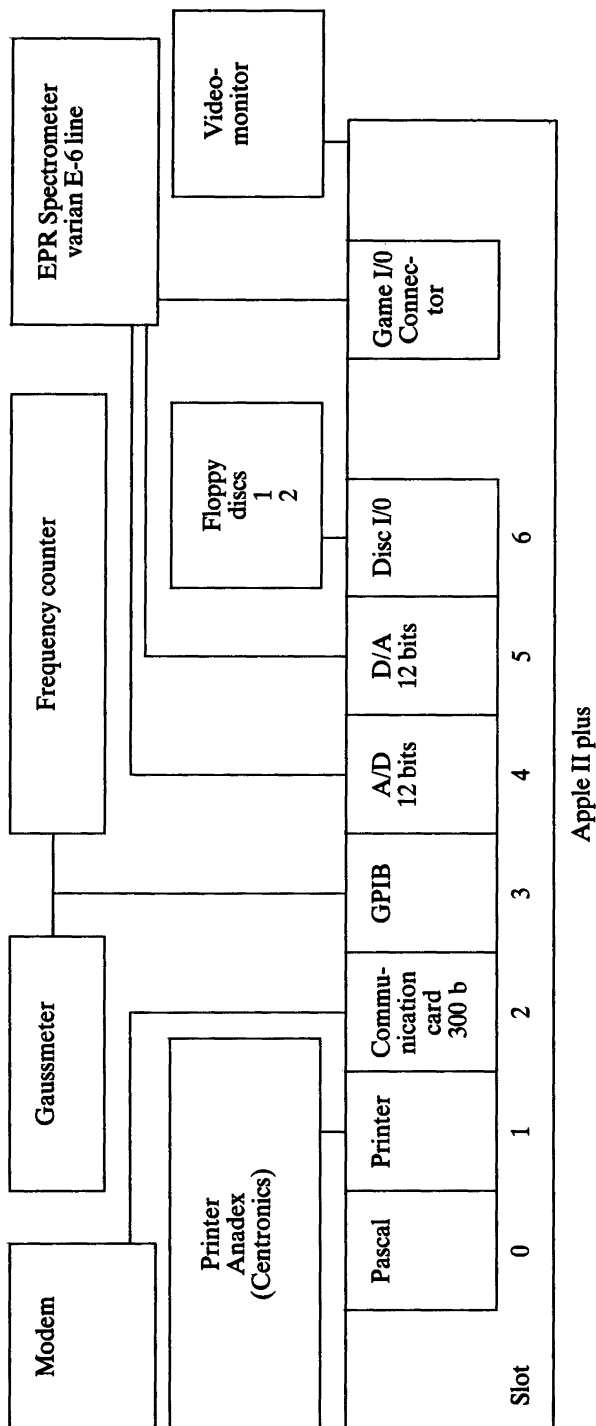


Fig. 1. Block diagram of the system.

2). The other two single bit inputs are used to specify the state of the right carriage limit (PB2, pin 4) and the left carriage limit (PB1, pin 3).

One of the annunciator outputs of the game I/O connector (AN3, pin 12) is used for turning the computer control on and off. The limited number of output ports in Apple II makes it necessary to use one of the annunciator pins for two purposes: when AN3 is on, the AN1 (pin 14) defines the direction of the recorder (to right or to left: 0 or 1); and when AN3 is off, AN1 starts the recorder with a downgoing pulse 5 V–0 V–5 V (manual start). AN2 (pin 13) is connected to the input of the *x*-direction stepping motor in the recorder, and it acts only when the computer control is on. The downgoing voltage from the computer (5 V to 0 V) advances the pen one step in the *x*-direction. The fourth annunciator, AN0 (pin 15), is used for pen up-down action. A summary of the game I/O connections is provided in Table 1.

The second part of the interfacing is easy. The IEEE-488 card is located in the Apple slot 3. The only problems left are those of software.

A block diagram of the system is shown in Fig. 1.

PROGRAMMING LANGUAGES

The two main tasks in an EPR study are data collection and spectrum simulation. To meet the different requirements of a rapid information flow and easy programmability, the following division of programming languages is suggested. The slow, but easily programmable BASIC language can be used in data collection and the fast, but complicated PASCAL language in simulation. The PASCAL programs can be linked to ASSEMBLER subroutines, which are needed for I/O actions: A/D, D/A converters and single bit inputs. These ASSEMBLER subroutines are shown in Fig. 2.

DATA ACQUISITION AND PROCESSING

The routines that do not necessarily need high speed and that are programmed in BASIC in this system are the following: data acquisition, *g* value measurements, the digitalizing of graphical information, statistical determination of coupling constants, plotting of data and both integration and derivation.

Data acquisition can also be carried out with exact magnetic field values, calculated with the aid of a gaussmeter and microwave frequency counter. We have two modes of data acquisition: one (16 min, 10 000 points) without field connection and a slower one with field correction (120 min, 10 000 points). Multiple collection is also possible. The field frequency lock is needed in multiple collection without field correction. SMOOTHING is possible and is performed in the usual way.

The g value measurement program reads the values of the gaussmeter and microwave counter, using the IEEE-488 line, when the pen after the first derivation signal, crosses 0-level in a downward motion. The 0-level is adjusted by setting the Receiver Gain of the spectrometer to 'off' or by setting the pen in the middle of the EPR first derivative peak and read the value of the *y*-coordinate into the microcomputer as the 0-level. The microcomputer calculates the *g* value from the resonance condition. Standard samples must be used to calibrate the system. The accuracy of this type of measurement is within ± 0.0000005 for consecutive runs.

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(a)
;PROCEDURE TAKE(VAR KE,KF:INTEGER);
  .PROC ARVO,2
RET      .EQU 0
ADDR     .EQU 2
CHNSET   .EQU 0C080
SAMPLE   .EQU 0C081
HOLD     .EQU 0C082
HIGH     .EQU 0C083
LOWE     .EQU 0C084
  PLA
  STA RET
  PLA
  STA RET+1
  PLA
  STA ADDR
  PLA
  STA ADDR+1
  LDY #040
  LDA #0
  STA CHNSET, Y
  LDA SAMPLE, Y
  LDX #3
LOOP     DEX
         BNE LOOP
WAIT     LDA HOLD, Y
         LDA LOWE
         BMI WAIT
         AND #00F
         LDY #0
         ROL A
         ROL A
         ROL A
         ROL A
         LSR A
         LSR A
         LSR A
         LSR A
         STA (ADDR),Y
         INY
         LDA #0
         STA (ADDR),Y
         PLA
         STA ADDR
         PLA
         STA ADDR+1
         LDY #0
         LDA HIGH
         STA (ADDR),Y
         INY
         LDA #0
         STA (ADDR), Y
         LDA RET+1
         PHA
         LDA RET
         PHA
         RTS
         .END

(b)
;PROCEDURE PUT(VAR KA,KB:INTEGER);
  .PROC PANE,2
RET      .EQU 0
KALOC    .EQU 0C0D0
KBLOC    .EQU 0C0D1
  PLA
  STA RET
  PLA
  STA RET+1
  PLA
  STA KALOC
  PLA
  PLA
  STA KBLOC
  PLA
  LDA RET+1
  PHA
  LDA RET
  PHA
  RTS
  .END

(c)
;PROCEDURE LIMITS (VAR KA,KB:INTEGER);
  .PROC OTA,2
RET      .EQU 0
ADDR     .EQU 2
KALOC    .EQU 0C062
KBLOC    .EQU 0C063
  PLA
  STA RET
  PLA
  STA RET+1
  PLA
  STA ADDR
  PLA
  STA ADDR+1
  LDY #0
  LDA KBLOC
  STA (ADDR),Y
  INY
  LDA #0
  STA (ADDR),Y
  PLA
  STA ADDR
  PLA
  STA ADDR+1
  LDY #0
  LDA KALOC
  STA (ADDR),Y
  INY
  LDA #0
  STA (ADDR),Y
  LDA RET+1
  PHA
  LDA RET
  PHA
  RTS
  .END

```

Fig. 2. Listings of the ASSEMBLER subroutines; (a) for A/D converter, (b) for D/A converter and (c) for recorder limits (left and right).

Statistical determination of hyperfine coupling constants is based on relative frequency distributions of EPR line differences. This empirical probability gives the order of hypothetical coupling constants. The hypothetical hyperfine coupling constants can be used in simulation, as we know from known EPR spectra that the most probable hypothetical

couplings are the real ones. This method does have limitations, of course – for example, if only a few wellresolved line-differences can be obtained. The size of a class interval can be changed in the program. In the beginning it is better to calculate crude values with a broader class width, and the best accuracy is finally obtained by putting the class width to 0.01 G. The microcomputer program reads the values of the magnetic and microwave fields from the gaussmeter and microwave counter for every resolved EPR line of the spectrum, in the same way as in the *g* value program, and then it calculates the coupling constants from a selected value area. This method easily gives the solution of the EPR spectrum. The hyperfine coupling constants can be calculated very exactly by measuring the differences between “clean” lines and then calculating the standard deviation of the group. Accuracy was within 0.01 G when we used the value 2 x standard deviation.

The digitalizing program is written such that the EPR spectrometer acts free of control by the computer except for the A/D converter, the value of which is read once for every step of the recorder stepping motor. The sweep of the recorder must be set slow enough for the operator to be able to follow the plotted curve at all times. In this way it is possible to digitalize any graphical information.

Integration can be carried out as on-line or off-line calculation from the collected spectra. The first five and the last six points determine the base-line and the integral function and/or the second integral can be obtained.

Derivation can also be performed from the collected spectra. If the signal to noise ratio is good and the line width not too small, any desired derivative can be obtained. The program is structured to calculate the derivatives one by one.

The plotting routine is the usual program transmitting the spectrum y-value through the D/A converter and other recorder functions through the game I/O connectors, flip flops and annunciators.

SPECTRUM SIMULATION

The normal simulation program was designed such that the *number of coupling constants* is *not limited* by the structure of the program. The vector model meets this criterion.¹⁴ The RAM memory available in the Apple II plus microcomputer (64 kbytes) sets the limit, which is 16 different coupling constants with 1 K points in the calculation. This number is sufficient for all problems to be solved. Another requirement is that it must be possible to carry out the simulation for the spin quantum number of any nucleus.

The structure of the simulation program is as follows. First, the stick spectrum is calculated with the aid of vectors that include all quantum number combinations. All combinations are calculated by changing the first vector through all values, separately for each value of the second vector *etc.*

The intensities are effectively calculated recursively in the proton or spin 0.5 case, but for all other nuclei the intensity is calculated by the relation¹⁵

$$\text{INT}(N,J) = \sum_{K=0}^{(J-1)/R} (-1)^K \frac{N! (N+J+R \times K-2)!}{K! (N-K)! (N-1)! (N+J-R \times K-2-N+1)!}$$

where $\text{INT}(N,J)$ is the value of *J*:th number of the *N*:th row of a Pascal-like triangle having from a top row of $R=2 \times I+1$ elements of one. *I* is the nuclear spin. In subsequent rows every

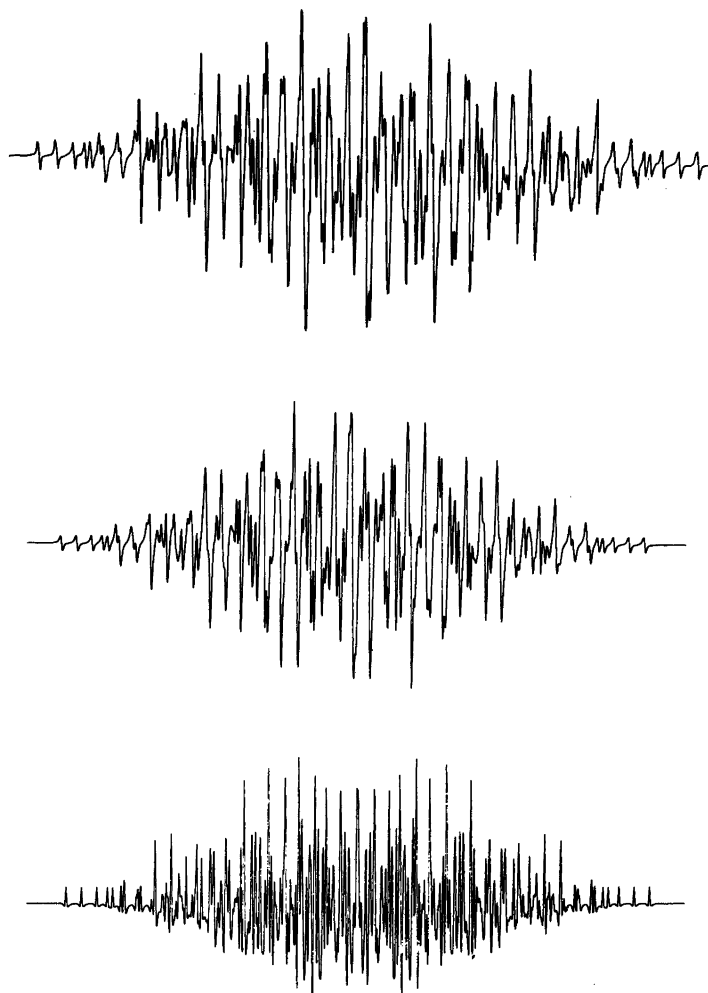


Fig. 3. The EPR spectrum of the radical cation of 2-methylnaphthoquinone in trifluoromethanesulfonic acid at room temperature a, simulated spectrum as first and second derivatives b and c. Eight different coupling constants are used in the simulation; one quartet $a(3\text{H})=3.34$ G, and seven doublets $a(1\text{H})=2.05$ G, $a(1\text{H})=1.56$ G, $a(1\text{H})=1.16$ G, $a(1\text{H})=0.59$ G, $a(1\text{H})=2.09$ G, $a(1\text{H})=2.21$ G, and $a(1\text{H})=2.19$ G with Lorentzian linewidth of 0.07 G.

element is the sum of the R nearest elements above it.

The calculated absorption of the stick spectrum is located to one of 1000 possible channels determined by the scaling of the spectrum and translation of the middle point of the spectrum. In every channel the located absorption is increased in value by the intensity value. In this way only one 1000 dimensioned vector is needed for the stick spectrum. Then the *lineshape* is calculated with either a Lorentzian first or second derivative or a Gaussian first derivative or as a mixed lineshape, as desired. Lineshape is calculated from the

peak-to-peak value as a convolution of stick and lineshape for every stick separately. Computation time is saved by starting the convolution a distance 6–8 times the peak-to-peak linewidth before absorption and continuing as much after. This procedure gives a spectrum where the cut-off cannot be seen.

The calculated spectrum can be displayed on the video monitor if desired, but only roughly owing to the limited resolution of the graphics (279×192). The spectrum is then *plotted* with the aid of the ASSEMBLER subprograms linked to PASCAL routines. The A/D converter program needs two bytes, the most significant and the least significant, if 12 bits are involved. The annunciator outputs are easily programmed in PASCAL because the TTLOUT functions are in the APPLE PASCAL library. The simulated spectrum can be stored on floppy disc if desired.

To estimate the effectiveness of the algorithm and of the Apple microcomputer, we performed a simulation of the earlier published spectrum of the radical cation of 2-methyl-naphtho-quinone (Fig. 3).¹⁶ The spectrum was simulated with eight different hyperfine coupling constants. The time needed for calculation of the first or second order spectrum was about 6 min, and that for plotting about 4 min.

Carbon-13 simulation is done with a simulation program structured on the basis of the chaining capability. The Apple CHAINSTUFF subroutine makes it possible to chain different programs together without observing the memory limitations very strictly. The printing subroutine can also be added.

Second order simulation for any nucleus can be done with the chaining version.

Superimposed spectra are easily calculated by temporarily saving the stick spectra of both components on the floppy disc and then calculating the lineshape for the superimposed spectrum.

CONCLUSION

The configuration is cheap and effective enough for normal EPR research. No extra parallel ports are needed for interfacing when using the Apple I/O connector. All problems arising with normal, liquid phase EPR work can be handled. The major advantage of our system over commercial systems is that programs can be enlarged or changed according to laboratory requirements. The project described above was carried out between 1980 and 1982.

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