Guide to Operation of the Bruker ESP 300E

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1 Introduction and Scope of Coverage

The Bruker ESP 300E EPR spectrometer and associated remote data workstation make up a modern system for the acquisition and analysis of EPR spectra. As with many technologically advanced instruments, the mastery of the skills necessary for the safe and efficient operation of this spectrometer may at first seem overwhelming. This guide is in no way intended to replace the information contained in the several manuals supplied by Bruker and other companies; it is instead meant to serve as an introduction to the basic operating procedure, so as to help ensure that a minimum of improper operating procedures occur. The diverse research interests of those using this instrument preclude the development of a condensed guide that meets very specific requirements; therefore, one should not hesitate to use all available resources to learn the necessary correct experimental techniques. Those resources include the set of manuals provided by the manufacturers, the MR Facility director or assistant director, and other experienced researchers.

2 Spectrometer Operations

2.1 Powering Up the System

1. Switch on the magnet power supply by pressing POWER ON button. The ELECTR ON button is normally left on at all times.

2. Switch on the frequency counter.

3. Enter the ESP 300E program. This program is started by default when the computer is powered on; however, one can "shell out" to the command line of the OS-9 operating system from the ESP 300E acquisition program with the F1 key. The operating system is characterized by a blue background and the $ prompt. Hit the Esc key to return to the ESP 300E program.

4. Verify that the N2 (g) line is attached to the waveguide via the hose barb located at the rear, and that there is a gas flow in the range of ~ 2-5 L · h⁻¹x100, as displayed on the flow gauge of the Variable Temperature unit located above the magnet. This gas flow ensures that the waveguide and cavity are kept free of moisture from the atmosphere.

2.2 Acquiring a Spectrum

It is assumed here that the appropriate microwave cavity is already properly installed and has an existing calibration file. For details on the proper installation of a microwave
cavity, see section 3.1.3 of the ESP 300 Technical Manual, or consult the assistant director of the MRF. **It is absolutely essential that the microwave cavity does not become contaminated with paramagnetic junk! Do not leave the cavity open to the outside world, and be certain that anything you put into it is clean.** There are solid collet plugs that should be installed whenever there is no sample in the cavity. Remember: accidents do happen; if you break a sample tube in the cavity, **PLEASE report it directly to CIC staff so that the necessary cleaning and recalibration can be done before others’ experiments are corrupted.**

1. Read in the signal channel calibration file for the microwave cavity in use by:
   
   a) Enter the Calibration menu; select D (use the F10 key to expand/reduce the one-letter commands on the left of the screen) for a directory of calibration files.
   
   b) Enter the pathname of the directory containing the desired calibration file (should be /h0/CAL).
   
   c) Highlight the name of the file that corresponds to the installed cavity, then press Enter. The first four digits of the filename correspond to the serial number on the rear of the cavity. Hit Enter again to confirm.
   
   d) The proper constants are automatically read into the program from the file.
   
   e) Hit the Esc key to return.

2. Activate the microwave bridge controller menu, located at the top of the screen, using the F3 key (and perhaps the F5 key as well). A white border indicates that it is activated; the absence of a white border indicates it is deactivated. The F3 key toggles between active and inactive. The F5 key toggles between the microwave bridge controller menu and the system information display.

3. Insert or change the sample:
   
   a) Verify that the microwave bridge is in the STAND BY mode. The ← and → keys toggle through the various modes.
   
   b) Verify that the attenuation ≥ 60 dB; use the ↓→ and ↑← keys (one set changes the value by unity, and the other set changes the value by tens) to set if necessary.
   
   c) Remove the sample or top collet plug and verify that the correct collet is installed for use with your sample.
d) Clean your sample tube with ethanol on a tissue, and then insert the tube carefully into the sample cavity. Adjust the lower pedestal to position the sample to the correct depth: the sample should be in the center of the cavity. Tighten the collet nuts snugly, but do not over tighten.

e) Set the attenuation to 25 dB. This is typically a good starting point for microwave power in the event that you are unsure of what value to use; it can be changed later if so desired.

4. Switch from STAND BY to the TUNE mode with the → key.

5. Initiate the auto-tune procedure by pressing the PgUp or PgDn key. The microwave bridge display will change as the tune procedure progresses; when it is finished, the OPERATE mode will be displayed and you should use the F3 key to deactivate the microwave bridge controller. Note: the tuning procedure can also be done manually; however, this is not normally necessary. Aqueous samples are a possible exception. See section 2.4 of this document and/or section 3.4 of the ESP 300 Technical Manual for the manual tuning procedure.

6. The →← key initiates the automatic fine-tune feature. You should monitor the LOCK OFFSET and DIODE CURR meters. The needles should be very nearly centered; if they are not, then the microwave cavity has become detuned. This can usually be corrected by fine-tuning: verify that an acquisition is not in progress and that the microwave bridge controller is activated, then press the →← key; the instrument will do an automatic fine tune procedure similar to the regular auto-tune procedure. It is often necessary to do a fine tune procedure when working at high powers (e.g., less than about 10 dB attenuation) due to changes in the temperature of the microwave cavity.

7. Enter the Parameters menu by pressing the P key. The F10 key toggles the verbose menu key on and off. Select the appropriate submenus and enter the desired parameters suitable for your sample.

Consider the following settings as starting points for parameters, but note the cautions below.

- **For organic radicals** (g-factor ≈ 2):
  (a) Center Field = 3400 G
  (b) Sweep Width = 50-100 G
  (c) Microwave Power = 1.00 mW
  (d) Modulation Frequency = 100 kHz
  (e) Modulation Amplitude = 0.105 G
  (f) Receiver Gain = 1.00e+04
  (g) Conversion Time = 40.96 ms
  (h) Time Constant = 0.32 ms
  (i) Resolution of Field Axis = 2-4 K points

- **For inorganic radicals** (g-factor ≈ 2 and ≈ 4):
  (a) Center Field = 3000 G
  (b) Sweep Width = 3000 G
  (c) Microwave Power = 1.00 mW
(d) Modulation Frequency = 100 kHz
(e) Modulation Amplitude = 10.45 G
(f) Receiver Gain = 1.00e+04
(g) Conversion Time = 163.8 ms
(h) Time Constant = 5.12 ms
(i) Resolution of Field Axis = 1-2 K points

• CAUTIONS

It is important to know how the various parameters affect the resulting spectrum. The following is a brief description of the most critical parameters. For more details, consult the ESP 300 Technical Manual or an advanced EPR text.

a) Microwave Power: An excessive power setting, P can cause a decrease in, or elimination of, the EPR signal due to sample saturation. This can especially be a problem for samples having relatively long relaxation times (those with sharp resonance lines). Signal intensity is proportional to √P in the region where saturation does not occur. It can be a good idea to perform an experiment in which the integrated intensity is measured as a function of power microwave power; the linear region of a plot of I vs. √P indicates suitable power levels.

b) Modulation Frequency: Sharp lines can be broadened by excessive values for modulation frequency (MF). This is usually not a problem except for very sharp lines (>> 50 mG), and 100 kHz MF is generally used. The ESP 300 Technical Manual recommends using MF [kHz] ≤ 3 x 10^-4 ∆Bpp [G], where ∆Bpp [G] is the line width in Gauss of the sharpest line.

c) Modulation Amplitude: Increasing the modulation amplitude (MA) will, in general, increase the EPR signal to a point. Excessive values, those greater than the line width will broaden and distort the resonance line. For a non-distorted line shape, use values of 1/10 ∆Bpp ≤ MA ≤ 1/2 ∆Bpp.

d) Receiver Gain: The best signal-to-noise ratio (S/N) is obtained with an optimal receiver gain setting. Too low a setting will result in poor S/N, and an excessive setting will result in a spectrum that is clipped off at the top and bottom.

e) Conversion Time: The noise, N, in an EPR spectrum is inversely proportional to the square root of the conversion time; therefore, noise can be filtered out to some extent via longer conversion time values.

f) Time Constant: The time constant (sometimes referred to as the response time) also filters out noise, but here the relationship is N ∝ τ^-1, where τ is the time constant. However, if the time constant is too large, the signal itself can be filtered out; a rule-of-thumb for undistorted line shapes is to use a scan time >10 τ, where “scan time” is the time required to scan through the narrowest resonance line. Try τ ≈ scan time for better S/N.
8. Under the Measurement Mode Parameters submenu, select the REPLACE mode when acquiring a spectrum using a single scan. The ADD mode is used for signal averaging.

9. In the Parameters main menu, press the > key to “Set page parameters to instrument,” then press Esc to exit the Parameter menu.

10. Enter the Acquisition menu, then initiate spectral acquisition by selecting C for Data Acquisition with ER023M.

2.3 Shutting the System Down

1. Activate the microwave bridge controller menu.

2. Increase the attenuation to 60 dB.

3. Switch to STAND BY mode.

4. Deactivate the microwave bridge controller menu.

5. Remove the sample from the cavity, and insert a clean collet plug into the top collet to keep dirt out of the cavity.

6. Set the modulation amplitude to zero by:
   a) Press the LOC button on the ER 023M Signal Channel to enter the local mode.
   b) Press the MA button, then
   c) Press the ## key on the signal channel keypad until the display indicates MA OFF.

7. Set Field Controller values by:
   a) Press the LOC button on the ER 032M Field Controller.
   b) Key in 0 (zero) from the keypad, then press SW to set the sweep width to zero.
   c) Key in 50, then press CF to set the magnetic field to its minimum value

8. Switch off the frequency counter.

9. Shut down the magnet power supply with the POWER OFF button.

10. Turn off the display screen on the spectrometer console.

11. Clean up your work area before leaving.
2.4 Manual Tuning

As previously noted, manual tuning is generally not necessary, as the automatic tuning procedure usually gives good results. However, manual tuning may be the only option for lossy samples (i.e., solvents having high dielectric constants, such as water and acetonitrile) or for better tuning at high microwave powers. An incidental benefit of manual tuning is that the operator gains a better understanding and appreciation for how the spectrometer operates, as it becomes less of a "black box." In addition to the arrow keys for setting the attenuation and initiating the auto-tune procedure, the operator will use the ↑ and ↓ keys and rotary encoder knobs 1, 2 and 3 in the manual tuning procedure.

1. Follow the directions of subsection 2.1 (Powering Up the System), and items 1-4 of subsection 2.2 (Acquiring a Spectrum).

2. Activate the microwave bridge using the F3 key and set the microwave attenuation to 25dB.

3. Use the → key to select the Tune mode.

4. Tune the klystron frequency using rotary encoder knob 1 to center the mode dip. It may be necessary to adjust the mode amplitude in the display by changing the attenuation.

5. Tune the signal (reference) phase using rotary encoder knob 2 to make the mode shoulders symmetric. Retune the frequency as needed to keep the dip centered.

6. Fine tune the klystron frequency:
   a) Press the → key to go to the OPERATE mode.
   b) Adjust the frequency (knob 1) to center the needle of the LOCK OFFSET meter.

7. Adjust the bias level:
   a) Set the attenuation to 50 dB.
   b) Adjust the bias using rotary encoder knob 3 until the needle of the DIODE CURRENT meter is centered. This corresponds to 200 µA of diode current.

8. Match (critically couple) the microwave cavity:
   a) Decrease the attenuation by 10 dB.
   b) Use the ↑ and ↓ keys to adjust the cavity iris in such a way as to keep the DIODE CURR meter needle centered.
   c) Repeat steps (a) and (b) until an attenuation of 10 dB is reached, adjusting the frequency (knob 1) as needed to maintain a centered needle on the LOCK OFFSET meter.
d) At 10 dB, adjust the signal phase (knob 2) until a local maximum is achieved in the diode current. Adjust the cavity iris to center the needle. At this stage, if the cavity is properly matched, changing the attenuation from 10 to 50 dB should not cause a change in the diode current. Use this as a test. If a change of diode current is observed, repeat the bias level adjustment and matching steps until virtually no change in diode current is observed when changing the attenuation from 10 to 50 dB.

9. If it is necessary to work at power levels less than about 10 dB, set the attenuation to 0 dB and fine tune the cavity iris again to center the needle on the DIODE CURRENT meter. Note that high power levels tend to heat the sample cavity, causing the tuning characteristics to change. Wait for thermal equilibrium to be achieved at the desired power setting, and then retune the cavity as needed.

3 Data Handling

Data handling integration, peak-picking, noise reduction, baseline subtraction, etc, can be performed at either the spectrometer console or the remote PC data station. The user should be aware that there are differences in the details of using the two different utilities: the spectrometer operation is under the control of the Bruker ESP 300E software, which also controls data handling, whereas the remote data station uses Bruker WIN-EPR data handling software; the commands are entirely different for these two programs. From either the spectrometer console or the remote data station, one can do both printing and plotting to the attached HP LaserJet 4L printer. A few words are in order about saving your data. There can be several stages to saving data; the most basic level is to save the data to the hard drive under your own /h0/GROUP/USER subdirectory. Users are responsible for properly saving their data; any "filename.spc" or "filename.par" files appearing in inappropriate directories will be deleted. The hard drive is good for temporary data storage before backup to more permanent storage. The hard disk has a capacity of 84.04 Mbytes. Given this relatively small capacity regular disk clearing is required. It is highly recommended that all users transfer their data via FTP to the workstation PC at the conclusion of their daily experiments. All user files should be saved under the C:Documents and Settings\UWCHEM\My Documents\EPRData\Group\User subdirectory, and/or can be saved to either 100 Mbytes Zip disk or CD-W for permanent storage. There are two file formats in effect: the ESP 300E program creates and uses the original file format; WIN-EPR permanently converts this format to the file format it uses; therefore, unless the data are saved in their original form, you will be restricted to using only WIN-EPR for data handling once the files have been converted.
3.1 Transferring Files via FTP

1) Double-click on the **WS_FTP95LE** icon at the PC data station to begin the file transfer program.

2) Connect to **mambo**, which is the Bruker ESP 300E spectrometer console computer. (It may be necessary to reboot the spectrometer console computer in the event that the transfer program is unable to connect to **mambo**.)

3) Set the local and remote directories to those which are applicable:
   a) Set the desired directory on the spectrometer console by entering `/h0/GROUP/USER`.
   b) Set the desired local directory on the PC by entering `C:\Documents and Settings\UWCHEM\My Documents\EPRData\Group\User`.

4) Transfer the files from the spectrometer console to the PC by highlighting the appropriate file names in the Remote Site box on the right side of the FTP window and pressing the **LEFT** pointing arrow in the center of the FTP window.

5) When finished with file transfer, close the FTP connection and then exit the **WS_FTP95LE** program.

3.2 Data Handling on the ESP 300E Console

The File Handling menu can be used to read and write spectral and parameter files on the console. See the ESP 300E Technical Manual for instruction and examples in using basic data handling methods. The ESP 300E/ECS Software Manual contains more detailed information.

3.3 Data Handling on the Remote Data Station

Consult the WIN-EPR manual for details pertaining to data analysis using WIN-EPR.