COMPARISON OF CONTINUOUS WAVE AND RAPID SCAN X-BAND ELECTRON PARAMAGNETIC RESONANCE OF IRRADIATED CLIPPED FINGERNAILS

Hanan Elajaili¹, Joseph McPeak¹, Alexander Romanyukha², Priyanka Aggarwal¹, Sandra S. Eaton¹ and Gareth R. Eaton^{1,*}

¹Department of Chemistry and Biochemistry, University of Denver, Denver, CO 80208 USA

²Naval Dosimetry Center, Bethesda, MD 20889-5614, USA

*Corresponding author: geaton@du.edu

X-band rapid scan electron paramagnetic resonance (EPR) measures the free radicals in irradiated clipped fingernails with higher signal-to-noise (S/N) and lower standard deviation of the signal amplitude for replicate measurements than does conventional continuous wave (CW) EPR in the same measurement time. For a clipped fingernail sample irradiated to 10 Gy and data acquisition time of 30 s with $B_1 = 8.5 \mu\text{T}$, the S/N for rapid scan is >2000 for the absorption spectrum and 1200 for the corresponding first derivative. The standard deviation for replicate measurements of signal amplitude is $\sim 1\%$. For CW spectra, the S/N depends on the modulation amplitude. The CW signal has a first derivative peak-to-peak linewidth of 0.95 mT. For 30 s of CW acquisition time, the S/N was 30 for a conservative modulation amplitude of 0.2 mT and B_1 of $2.3 \mu\text{T}$ or 90 for a modulation amplitude of 0.4 mT and B_1 of $3.2 \mu\text{T}$, which resulted in standard deviations of replicate measurements of 5% or 2%, respectively.

INTRODUCTION

It has been known for many years that multiple free radicals are generated in fingernails by mechanical processes and by radiation^(1, 2). There is renewed interest in quantitation of these electron paramagnetic resonance (EPR) signals because of their potential utility for measuring radiation dose, especially for medical triage in situations where there is a need to determine whether large numbers of people were exposed to medically significant doses of radiation^(3–13). Fingernail dosimetry has been applied to three cases of accidental radiation exposure⁽¹⁴⁾.

Extensive prior literature and presentations of unpublished results at scientific meetings reveal that the EPR signal in clipped fingernails depends on a wide range of variables, including how the nail was cut, storage humidity, time and temperature. The state of the art was reviewed recently^(10, 15). In addition, there are recent studies that seek to determine the optimum EPR frequency (e.g. X-band vs. Q-band^(16, 17)), microwave power, etc., for ascertaining the radiation dose). The purpose of this study is solely to compare two EPR data acquisition methods, conventional continuous wave (CW) and the newly developed rapid scan method⁽¹⁸⁾, for irradiated clipped fingernail samples. Comparisons in this study were performed at X-band in standard 4 mm tubes because the sample tube size was convenient. The sample tube size puts constraints on the size of clippings that can be analyzed.

Rapid scan EPR methodology was explained in references⁽¹⁸⁾ and⁽¹⁹⁾. The method has provided

substantially improved signal-to-noise (S/N) relative to CW EPR for a wide range of samples including paramagnetic defects in solids⁽²⁰⁾, the radiation-induced defects in teeth⁽²¹⁾, defects in gamma irradiated solids⁽²²⁾ and immobilized organic radicals⁽²³⁾. These papers demonstrate the improvement in S/N that can be achieved by rapid scan for samples with a wide range of relaxation times. A key feature of the rapid scan experiment is that the magnetic field is scanned through resonance thousands of times per second, which alleviates the need to select a modulation amplitude that is small relative to signal linewidth and permits detection of the full signal amplitude in every pass through the spectrum. Higher microwave B_1 can be used for rapid scan than for conventional CW, which also improves S/N ⁽¹⁸⁾.

MATERIALS AND METHODS

Fingernail clippings were collected using sharp scissors from both hands of one volunteer (60-year-old white male). Some of the collected clippings were cut in smaller pieces to make all pieces about the same size, e.g. 1–2 mm $\times$ 2–3 mm. As recommended in reference⁽⁶⁾, to eliminate (or significantly reduce) the mechanical stress caused by cutting, all collected fingernail clippings were soaked in distilled water for 10 min and dried on paper towels for 10 min. A pooled set of the treated fingernail clippings was split into three samples, one sample was kept unirradiated as a control and two other samples were irradiated using a calibrated ¹³⁷Cs source to 5 and 10 Gy. The

source dose rate was 0.55 Gy/min. More details on irradiation conditions and sources calibration can be found in reference⁽²¹⁾. After irradiation, samples were stored in a refrigerator at 4°C. When samples were outside of the refrigerator, tubes were protected from ultraviolet radiation by wrapping with aluminum foil. EPR measurements were made at times ranging from several weeks to months after irradiation, and the final comparisons reported in this article were obtained ~8 months after irradiation. No time dependence of signal amplitude or of the relative advantage of rapid scan to CW was noted.

Weighed amounts of clipped fingernail samples (20–21 mg) were placed in standard 4 mm outer diameter quartz tubes, filling the tubes to a height of about 1.0 cm. The relatively short sample lengths were selected to permit positioning the samples within the region of uniform B_1 in the resonators. Since the nail clippings do not pack uniformly in the resonator, changing the sample positioning changes the signal intensity. To evaluate the reproducibility of the spectroscopy, replicate measurements were performed by repositioning the sample in the resonator three times with retuning of the spectrometer and then recording three replicate spectra for each sample positioning.

Pending results of ongoing research in other laboratories in which attempts are being made to distinguish the mechanically induced signals from the radiation-induced signals (RIS), parameters were selected to maximize the overall S/N using each method of data acquisition with minimal lineshape broadening.

X-band CW spectra were measured at room temperature (nominally 20–21°C) on a Bruker EMX spectrometer, using an ER4122SHQE cavity resonator. For these non-lossy samples, the resonator Q is ~7000 and the resonator efficiency ($B_1/\sqrt{P}$) is about 0.2 mT W^{-1/2}⁽²⁰⁾. B_1 , the microwave magnetic field in the resonator, is calculated as the resonator efficiency times the square root of incident power. The CW signals were acquired using Bruker WinEPR software. X-band rapid scan spectra were measured on a Bruker E500T spectrometer using an ER4118-MD5 dielectric resonator. Following down-conversion of the microwave signal in a double-balanced mixer, the rapid scan EPR signals were digitized in a Bruker SpecJet II. The use of a dielectric resonator facilitates rapid magnetic field scans⁽¹⁸⁾. The dielectric resonator Q (~9000) is somewhat higher than of the SHQ resonator, but the EPR signal from the dielectric resonator is transmitted to the detection system through ~80 cm of lossy semirigid coaxial cable, whereas the EMX system is fully constructed of low-loss waveguide, so the potential increase in signal intensity due to the higher Q of the dielectric resonator is offset by the losses in the coax. The efficiency of the dielectric resonator is ~0.38 mT W^{-1/2}⁽²⁰⁾. The selection of

microwave power for each experiment was based on power saturation curves. A linear least squares fit through the point 0,0 and the signal amplitudes at the lowest 5 or 6 microwave powers were extrapolated to higher B_1 . The B_1 selected for rapid scan data acquisition (8.5 μ T) was the point at which the experimental signal amplitude was ~2% lower than the amplitude predicted by linear extrapolation of the non-saturated signal amplitude. For comparison with the rapid scan data, conventional CW data were obtained at both $B_1 = 2.3 \mu$ T, which is at the comparable position on the power saturation curve to the B_1 selected for rapid scan and also at $B_1 = 3.2 \mu$ T, which increases signal amplitude but causes some lineshape broadening. The CW spectra consisted of 1024 magnetic field points (102.4 pts/mT), and the rapid scan spectra after signal processing consisted of 3400 magnetic field points (400 pts/mT).

Processing of the rapid scan data was performed as previously reported⁽²¹⁾, using software written in MatLab. The residual background that remained after removal of the rapid scan background was fit to a fourth order polynomial and subtracted. Although the rates of scan through the signals are $<1/T_2$, and therefore the formal definition of rapid scan is not satisfied, the coherent signal averaging, direct detection and the ability to use higher B_1 results in improved S/N . The deconvolution procedure does not change the lineshape if there are no passage effects.

Digital filtering that was performed to decrease noise was limited to 5% broadening of the signal for both CW and rapid scan. To calculate the amplitudes of the noisy CW spectra, the signal intensity at 21 points (a range of 0.2 mT) centered on the apparent maxima and minima were averaged. The standard deviations of peak amplitudes were based on the absorption spectra for rapid scans and the first derivative for conventional CW. Three to six replicates were measured to define the standard deviations. The S/N is calculated as the ratio of the amplitude of the signal to the RMS (root mean square) error in a baseline region of the spectrum. The RMS error was calculated for 50 point segments of the baseline. This corresponds to 5% of the scan (0.5 mT) for CW and 1.5% (0.13 mT) for rapid scan. A series of values was calculated by stepping in one-point increment to check for consistency. Increasing the number of points that was used in the calculation did not significantly impact the calculated noise. The lowest value of the RMS noise within the baseline region was used in the calculation of S/N .

Electron spin relaxation time (T_1 and T_2) measurements were made by spin echo techniques on a Bruker E580 spectrometer using an ER4118X-MS-5 split-ring resonator. Variation of the 90° pulse lengths from 20 to 80 ns caused no significant change in the 2-pulse spin echo dephasing time (T_m), which

indicated that the decay was not impacted by spectral diffusion or instantaneous diffusion. It was assumed that $T_2 = T_m$.

RESULTS

Power saturation curves for the EPR signal in the clipped fingernail sample irradiated to 10 Gy obtained by CW and rapid scan spectroscopy are shown in Figure 1. Incident microwave power is on resonance for shorter time in the rapid magnetic field scan experiment than in the CW experiment, so one can apply higher microwave power, and therefore higher B_1 , without saturating the spin system in the rapid scan experiment than in the CW experiment. For the same extent of power saturation, the ability to apply higher microwave power without saturation of the signal results in a stronger EPR signal in the rapid scan experiment, as shown in Figure 1. The region of the power saturation curve in which signal increases linearly with B_1 extends to higher B_1 as the scan rate increases, with some limitations that depend on T_1 and T_2 ⁽¹⁸⁾. The shape of the rapid scan power saturation curve (Fig. 1) suggests that the S/N of the rapid scan spectrum could in principle be improved with a faster scan rate than was used, but limitations of the prototype spectrometer produced the best S/N at the scan frequency of 36 kHz, which is the frequency for which data are shown in the figures. The scan rate is the product of scan width times scan frequency. The maximum available

scan frequency is determined by the characteristics of the scan coils and the design of the scan driver.

CW spectra for the samples with 0 and 10 Gy doses, acquired with 0.2 mT modulation amplitude and $B_1 = 2.3 \mu\text{T}$, with 60 scans and a total data acquisition time of 20 min are shown in Figure 2. The CW results are in full agreement with previously published ones (Figure 2 in reference⁽⁶⁾) and indicate that only background and radiation-induced components are present in the spectra. The line-shapes are similar for the three dose levels. The EPR spectrum in fingernails has a very complex structure. According to reference⁽¹⁰⁾, it consists of several radiation-induced spectral components, along with several of non-radiation origin. These signals have very similar paramagnetic parameters and are difficult to distinguish when the EPR signal is measured at X-band. Two RIS in fingernails are of particular interest. RIS2 is most intense but unstable at room temperature; RIS5 is significantly less intense but very stable. In order to determine the radiation dose, which is estimated from the peak-to-peak amplitude of the radiation-induced components, it is necessary to separate the non-radiation component from the radiation-induced signals in the spectrum. A notable, non-radiation signal is the so-called mechanically induced signal, MIS, which is caused by the stress imposed on the fingernail during clipping. A fast and simple procedure of sample preparation that drastically reduces the impact of mechanical stress on dose measurements in fingernails has been developed⁽⁶⁾. It consists of a brief (10–15 min) soaking of fingernails in water followed by drying. Unfortunately, the most intense radiation-induced component in fingernails, RIS2 is also drastically reduced by soaking in water, whereas RIS5 is stable against

Figure 1. X-band power saturation curves for clipped fingernail samples irradiated to 10 Gy. For rapid scan ($\diamond$) the amplitude of the absorption spectrum was recorded with a scan frequency of 35.7 kHz and scan width of 8.5 mT. For CW ($\blacklozenge$) the amplitude of the first derivative peak-to-peak signal obtained with 0.20 mT modulation amplitude at 100 kHz was recorded. The y -axis is in arbitrary units that are selected to overlay the two data sets at low B_1 . The B_1 selected for acquisition of the data in Figures 2 and 3 are marked.

Figure 2. X-band CW EPR spectra of clipped fingernail samples irradiated with doses of 0 Gy (gray), and 10 Gy (black). The y -axis scales are the same for the two spectra. Spectra were recorded with 0.2 mT modulation amplitude, $B_1 = 2.3 \mu\text{T}$ and signal averaging 60 scans for a total data acquisition time of 20 min for each spectrum.

such treatment. In order to maximize radiation-induced components (RIS2+RIS5), the water soaking was applied only prior to irradiation to eliminate mechanically induced signals. For the same reason, prepared fingernail samples were stored in a refrigerator between EPR measurements. The spectra in Figures 2 and 3 were recorded 8 months after the samples had been prepared and irradiated. It is important to emphasize that these spectra demonstrate that the peak-to-peak amplitude in irradiated

samples still depends on the radiation dose, e.g. the radiation-induced component has not completely faded out. This result is in agreement with the previous study of the RIS stability⁽⁸⁾. One can also see from Figure 2 that the sample with dose of 10 Gy has ~35% of its intensity due to radiation-induced components. That is why it is interesting to study the 10 Gy sample using the rapid scan technique to see if it is possible to improve the S/N with this technique.

For the most accurate absolute spin quantitation results, B_1 is selected to be in the linear response region of the power saturation curve⁽²⁴⁾. The signal amplitude can be increased with little signal broadening by selecting a B_1 in the region slightly above the linear response region. For the rapid scan data shown in Figure 3 and for the CW data shown in Figures 2 and 3, B_1 was selected to cause <5% broadening of the signal. Since some improvement in S/N can be achieved with somewhat higher B_1 , at the expense of some lineshape broadening, a second set of CW data were recorded with B_1 increased by a factor of 1.5.

To be useful for dosimetry with large numbers of samples, rapid throughput and short data acquisition times are essential. Accurate signal quantitation requires high enough S/N to achieve a small standard deviation for replicate measurements. CW and rapid scan spectra for the 10-Gy irradiated fingernail sample, recorded with 30 s acquisition time are compared in Figure 3. For this sample, the S/N for the rapid scan absorption signal (Fig. 3A) is too high to measure accurately but is >2000. When the first derivative of the rapid scan signal was calculated numerically, the S/N decreased to 1200 (Fig. 3B). The first derivative of the rapid scan spectrum is overlaid in Figure 3B on the CW spectra obtained with a modulation amplitude of 0.2 mT, which is ~20% of ΔB_{pp} ~0.95 mT. The lineshapes obtained by CW and rapid scan are in excellent agreement. The S/N for the CW spectrum is ~30. When the modulation amplitude was increased to 0.40 mT and B_1 was increased to 3.2 μ T, the S/N improved to ~90, which is still much poorer than for rapid scan and the linewidth was broadened by 7%.

For dosimetry, a key figure of merit is the standard deviation of replicate measurements of signal amplitude. For the data acquisition conditions used to acquire the spectra shown in Figure 3, the standard deviation of the signal amplitude for the rapid scan was 1% and for the CW spectra was ~5%. Although the use of higher modulation amplitude (0.40 mT) and B_1 (3.2 μ T) that result in some signal broadening would be undesirable for absolute spin quantitation or to distinguish superimposed contributions to the spectrum, the more aggressive parameters could be used to obtain relative intensities of composite signals. Those settings resulted in ~2%

Figure 3. X-band spectra of clipped fingernail sample irradiated to 10 Gy obtained with 30 s data acquisition time. (A) Rapid scan absorption spectrum calculated by deconvolution of data recorded with 35.7 kHz scan frequency, 8.5 mT scan width and 0.5 mW microwave power ($B_1 = 8.5 \mu$ T). (B) First derivative of rapid scan signal (—, less noisy) superimposed on a CW spectrum (—) recorded with 0.13 mW microwave power ($B_1 = 2.3 \mu$ T), 0.20 mT modulation amplitude at 100 kHz and three scans.

standard deviation, which is still substantially poorer than for rapid scan data for the same acquisition time. To distinguish contributions from different radiation-induced signals, minimally broadened spectra are required.

For some samples with long electron spin relaxation times, pulsed EPR can produce data with higher S/N than is obtained by CW and may be competitive with rapid scan⁽¹⁸⁾. Spin echo signals were weak for the 10-Gy irradiation-clipped fingernail sample. The S/N , even after extensive signal averaging, was not adequate to definitively distinguish between single exponential and two exponential fits to the experimental data, so the reported values are single exponential fits. T_2 , measured by two-pulse echo decay, was ~ 0.40 μ s, and within experimental uncertainty the value measured was independent of magnetic field position within the broad line. The lack of dependence of T_2 on position in the line or on pulse length indicated that three-pulse inversion recovery should give a good approximation to the true T_1 . The value of T_1 measured at the position of maximum echo intensity was 20 μ s. The uncertainties in T_1 and T_2 are $\sim 10\%$. Note also that T_1 is long enough that 100 kHz magnetic field modulation could introduce distortions in CW spectra. Because of the relatively short T_2 , S/N obtained by pulse methods is poorer than by either CW or rapid scan.

CONCLUSIONS

The X-band rapid scan EPR data acquisition method provided significantly improved S/N and lower standard deviation in signal amplitude for the EPR signal in the irradiated clipped fingernail sample, relative to CW EPR and holds substantial promise for improving fingernail dosimetry.

ACKNOWLEDGEMENTS

Zhelin Yu (University of Denver) helped to set up initial rapid scan experiments. Richard W. Quine assisted with implementation of the rapid scan coil driver. David Robinson (University of Denver) assisted with replicate electron spin relaxation time measurements.

FUNDING

This work was supported in part by National Institutes of Health, United States (grant CA177744 to G.R.E. and S.S.E.).

DISCLAIMER

The views expressed in this paper are those of the authors and do not necessarily reflect the official

policy or position of the Department of the Navy, Department of Defense, nor the US Government.

REFERENCES

- Chandra, H. and Symons, M. C. R. *Sulphur radicals formed by cutting a-keratin*. *Nature* **328**, 833–834 (1987).
- Symons, M. C. R. *Whole body electron spin resonance imaging spectrometer*. In: *Bioradicals Detected by ESR Spectroscopy*. Ohya-Nishiguchi, H. and Paker, L. Eds. (Basel: Birkhauser Verlag) pp. 93–102 (1995).
- Black, P. and Swarts, S. G. *Ex vivo analysis of irradiated fingernails: chemical yields and properties of radiation-induced and mechanically-induced radicals*. *Health. Phys.* **98**, 301–308 (2010).
- Dalgarno, B. G. and McClymont, J. D. *Evaluation of ESR as a radiation accident dosimetry technique*. *Appl. Rad. Isot.* **40**, 1013–1020 (1989).
- He, X., Gui, J., Matthews, T. P., Williams, B. B., Swarts, S. G., Grinberg, O., Sidabras, J. W., Wilcox, D. E. and Swartz, H. M. *Advances towards using finger/toenail dosimetry to triage a large population after potential exposure to ionizing radiation*. *Radiat. Measur.* **46**, 882–887 (2011).
- Reyes, R. A., Romanyukha, A., Trompier, F., Mitchell, C. A., Clairand, I., De, T., Benevides, L. A. and Swartz, H. M. *Electron paramagnetic resonance in human fingernails: the sponge model implication*. *Radiat. Environ. Biophys.* **47**, 515–526 (2008).
- Reyes, R. A., Romanyukha, A., Olsen, C., Trompier, F. and Benevides, L. A. *Electron paramagnetic resonance in irradiated fingernails: variability of dose dependence and possibilities of initial dose assessment*. *Radiat. Environ. Biophys.* **48**, 295–310 (2009).
- Reyes, R. A., Trompier, F. and Romanyukha, A. *Study of the stability of EPR signals after Irradiation of fingernail samples*. *Health Phys.* **103**, 175–180 (2012).
- Romanyukha, A., Reyes, R. A., Trompier, F. and Benevides, L. A. *Fingernail dosimetry: current status and perspectives*. *Health Phys.* **98**, 269–399 (2010).
- Trompier, F., Romanyukha, A., Reyes, R., Vezin, H., Queinnec, F. and Gourier, D. *State of the art in nail dosimetry: free radicals identification and reaction mechanisms*. *Radiat. Environ. Biophys.* **53**, 291–303 (2014).
- Trompier, F., Kornak, L., Calas, C., Romanyukha, A., LeBlanc, B., Mitchell, C. A., Swartz, H. M. and Clairand, I. *Protocol for emergency EPR dosimetry in fingernails*. *Radiat. Meas.* **42**, 1085–1088 (2007).
- Trompier, F., Romanyukha, A., Kornak, L., Calas, C., LeBlanc, B., Mitchell, C. A., Swartz, H. M. and Clairand, I. *Electron paramagnetic resonance radiation dosimetry in fingernails*. *Radiat. Meas.* **44**, 6–10 (2009).
- Wilcox, D. E., He, X., Gui, J., Ruuge, A. E., Li, H., Williams, B. B. and Swartz, H. M. *Dosimetry based on EPR spectral analysis of fingernail clippings*. *Health. Phys.* **98**, 309–317 (2010).
- Trompier, F., Queinnec, F., Bey, E., DeRevel, T., Lataillade, J. J., Clairand, I., Benderitter, M. and Bottollier-Depois, J.-F. *EPR retrospective dosimetry with fingernails: report on first application cases*. *Health. Phys.* **106**, 798–805 (2014).

15. Marciniak, A. and Ciesielski, B. *EPR dosimetry in nails—a review*. Appl. Spectros. Rev. **51**, 73–92 (2016).
16. Romanyukha, A., Trompier, F., Reyes, R. A. and Melanson, M. A. *EPR measurements of fingernails in Q-band*. Radiat. Meas. **46**, 888–892 (2011).
17. Trompier, F., Romanyukha, A., Swarts, S., Reyes, R. and Gourier, D. *Influence of nails polish in EPR dosimetry with human nails*. Radiat. Meas. **75**, 6–8 (2015).
18. Eaton, S. S., Quine, R. W., Tseitlin, M., Mitchell, D. G., Rinard, G. A. and Eaton, G. R. *Rapid scan electron paramagnetic resonance*. In: Multifrequency Electron Paramagnetic Resonance: Data and Techniques. Misra, S. K. Ed. (Weinheim, Germany: Wiley), pp. 3–67 (2014).
19. Eaton, S. S. and Eaton, G. R. *Rapid-scan EPR of nitroxide spin labels and semiquinones*. In: Methods in Enzymology: Electron Paramagnetic Resonance Investigations of Biological Systems by Using Spin Labels, Spin Probes, and Intrinsic Metal Ions, Part A Vol. **563**, Qin, P. and Warncke, K. Eds. (U.K.: Academic Press), pp. 1–21 (2015).
20. Mitchell, D. G., Tseitlin, M., Quine, R. W., Meyer, V., Newton, M. E., Schnegg, A., George, B., Eaton, S. S. and Eaton, G. R. *X-Band rapid-scan EPR of samples with long electron relaxation times: a comparison of continuous wave, pulse, and rapid-scan EPR*. Mol. Phys. **111**, 2664–2673 (2013).
21. Yu, Z., Romanyukha, A., Eaton, S. S. and Eaton, G. R. *X-band rapid scan electron paramagnetic resonance of radiation induced defects in tooth enamel*. Radiat. Res. **184**, 175–179 (2015).
22. Shi, Y., Rinard, G. A., Quine, R. W., Eaton, S. S. and Eaton, G. R. *Rapid scan electron paramagnetic resonance at 1.0 GHz of defect centers in gamma-irradiated organic solids*. Radiat. Meas. **85**, 57–63 (2016).
23. Yu, Z., Quine, R. W., Rinard, G. A., Tseitlin, M., Elajaili, H., Kathirvelu, V., Clouston, L. J., Boratyński, P. J., Rajca, A., Stein, R., Mchaourab, H., Eaton, S. S. and Eaton, G. R. *Rapid-scan EPR of immobilized nitroxides*. J. Magn. Reson. **247**, 67–71 (2014).
24. Eaton, G. R., Eaton, S. S., Barr, D. P. and Weber, R. T. *Quantitative EPR* (New York: Springer-Verlag/Wein) (2010).