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Stability of X-band EPR signals from fingernails under vacuum storage

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Abstract

EPR signals of different origin have been tested in human finger- and toe-nails with an X-band EPR technique for different conditions of nail storage. Three different signals were identified, namely a singlet at $g=2.005$, a doublet at $g=2.004$ with a splitting constant $A=1.8$ mT, and an anisotropic signal at $g_1=2.057$, $g_2=2.029$ and $g_3=2.003$ (positions of local extrema). All EPR spectra from nails, whether irradiated or mechanically stressed, can be described as a superposition of these three signals. The singlet is responsible for the background signal (BG), is the main component of radiation-induced signals (RIS) for low doses (100 Gy or lower) and also contributes to mechanically-induced signals (MIS). This signal is quite stable under vacuum storage, but can be reduced almost to zero by soaking in water. The behavior of this signal under ambient conditions depends on many factors, such as absorbed dose, air humidity, and ambient illumination intensity at the place of storage. The doublet arises after exposure of nails to high (few hundreds Gy and higher) doses or after mechanical stress of samples. Depending on how this signal was obtained, it may have bulk or surface locations with quite different stability properties. The surface-located doublet (generated on the nail edges during cutting or clipping) is quite unstable and decays over about two hours for samples stored at ambient conditions and within several seconds for samples immersed in water. The volume-distributed doublet decays within a few minutes in water, several hours at ambient conditions and several days in vacuum. The anisotropic signal may also be generated by both ionizing radiation and mechanical stress; this signal is quite stable in vacuum and decays over several days at ambient conditions or a few tens of minutes in water. The reference lines for the above-described three EPR signals were obtained and a procedure of spectra deconvolution was developed and tested on samples exposed to both ionizing radiation and mechanical stress.

Keywords

nails; emergency dosimetry; EPR signals; vacuum storage

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1. Introduction

Recently Sholom and McKeever (2016) have proposed and tested a so-called vacuum-stored, dry-nail, dose reconstruction technique that allows the reconstruction of doses as low as 2 Gy from nails up to at least 7 days after exposure. This technique exploits a feature of a radiation-induced EPR signal at $g=2.005$ to remain stable if the nail samples are stored in vacuum after exposure. A similar stability was also observed by Trompier et al. (2009) for samples stored in 0 % humidity, which is the case for vacuum storage as used by Sholom and McKeever (2016). The dose reconstruction protocol proposed by Sholom and McKeever (2016) used an approach that the signal observed at $g=2.005$ is a superposition of just two reference signals: a singlet at $g=2.005$ and a doublet at $g=2.005$ with a splitting constant of 1.8 mT; see also Black and Swarts (2010). This approach, in combination with individual calibration of the sample background, mechanically-induced and radiation-induced signals (BG, MIS and RIS, respectively), resulted in quite large uncertainty in the reconstructed doses (the average standard deviation was about 0.45 Gy for the dose range 0–10 Gy). One of the possible reasons for this large uncertainty may be related to the much more complex structure of the signal at $g=2.005$, which was not taken into account by Sholom and McKeever (2016). It is known that several EPR signals may contribute to the cumulative signal around $g=2.005$. For example, Trompier et al. (2014) claimed to identify five radiation-induced signals (RIS1-RIS5, using the nomenclature of these authors), four mechanically-induced signals (MIS1-MIS4) and one background signal in the $g=2.005$ region, i.e. 10 signals in all. In the present work, the stability of the different EPR signals from nails was studied for the different storage conditions, including storage in vacuum. Special attention was paid to the problem of isolation of the different EPR signals. To do this, we increased the width of the permanent magnetic field sweep up to 250 G (25 mT) in order to cover all EPR signals contributing to the $g=2.005$ region. (A 100 G sweep, which was used by Sholom and McKeever (2016), is not enough for full observation of some of the EPR signals.) Also, we used a different reference sample to calibrate the spectra as a function of g -factor, as well as to normalize the EPR signal intensities with respect to microwave cavity Q -factor variation. Sholom and McKeever (2016) used a $\text{Mn}^{2+}:\text{MgO}$ reference sample for such calibration and normalization. However, its 3rd line overlaps with some of the target EPR signals; so, for some experiments we replaced the $\text{Mn}^{2+}:\text{MgO}$ reference with a Bruker ER 4119HS-2100 reference sample that only has a single EPR line at $g=1.9800$, which is very favorable for EPR studies of nails.

In this paper we demonstrate that the overall EPR spectrum can be described by the superposition of just three EPR signal and use this to describe the shape of the overall EPR spectrum as a function of either irradiation and mechanical stress, as well as the changes in the spectra as a result of storage under different environmental conditions.

2. Materials and Methods

Ten volunteers provided toe- and finger-nails for this study. Nails were collected during routine hygiene procedures and were stored at ambient conditions between clipping and submitting to the research laboratory. Before any measurement, the nail clippings were further cut (if necessary) to pieces of 4–5 mm length (in order to fit the size of the EPR

sample tube) and soaked in water for one hour followed by drying in a vacuum oven also for one hour. This procedure removed all possible pre-experiment, radiation-induced and mechanically induced signals and minimized the background signal intensity.

Mechanically induced signals (MIS) were produced in two different ways: using scissors (or clippers) to cut the nail clips into smaller parts, and using a hydraulic press (with no extra cuts) to squeeze the nails. In the former case, it is presumed that the MIS centers are primarily formed on the cutting edges (surface location) while in the latter case the MIS centers may be generated within the volume of a sample (bulk location). To distinguish between different components of the MIS, we also measured the samples at different values of microwave power.

Because at least one MIS is known to demonstrate a complex anisotropic structure (from sulphur radicals, as described by Chandra and Symons, 1987), we tested the possible angle dependences of these sulphur-related signals using plates of size approximately $4 \times 3 \times 0.7$ mm³ cut from toenails. In this experiment, a single plate squeezed with a hydraulic press was rotated inside the EPR sample tube relative to the direction of the permanent magnetic field, and the corresponding EPR spectra were recorded.

Radiation-induced signals (RIS) were obtained after irradiation of the samples to different doses in the range 2 Gy–20 kGy. Again, the sulphur-related centers were tested with a nail plate at different angles relative to the direction of the permanent magnetic field.

Background signals (BG) were obtained in two different ways: after storage of the original nail clippings at ambient conditions for several days and also after soaking the samples in water for one hour. We also examined how environmental illumination intensity (from incandescent lamps) may affect this signal.

The stability of the signals was tested under two extreme conditions: storage in a vacuum (zero humidity) and storage under water (100% humidity). Some experiments were also conducted in ambient laboratory conditions, which are characterized by quite stable temperatures in the range 20–22°C, but also by significant day-to-day variations of ambient humidity in the range 20–68 %.

A Bruker EMX spectrometer equipped with a Bruker 4119 cavity was used for EPR spectra acquisition. The spectrometer was operated with the following parameters: microwave frequency 9.98 GHz, incident microwave power 200 mW – 6.2 μ W (attenuation 0–45 dB), sweep width 10 or 25 mT, modulation frequency 100 kHz, modulation amplitude 0.4 mT, receiver gain 1000, spectral resolution 1024 or 2048 points, sweep time 21 or 42 s, number of scans 1–8. A Bruker ER 4119HS-2100 reference sample, which has a single line at $g=1.9800$, was used to adjust spectra of the nail samples to the same g -factor values and to normalize EPR signal intensities with respect to the microwave cavity Q -factor variation. Some measurements were conducted using a $\text{Mn}^{2+}:\text{MgO}$ reference sample.

A Carver model-C laboratory press and standard nail clippers and/or scissors were used to generate the MIS. The press is equipped with a gauge that allows the control of the press

force starting from about 200 lbs (890 newtons). The force was converted to units of the pressure (bars) using division of force by the sample surface area.

A Bel-Art Scienceware F42010 compact vacuum desiccator was used to store the samples in vacuum. By using a Robinair 15115 vacuum pump, a partial vacuum of 7.59 mm Hg can be obtained in this desiccator within a few minutes. The vacuum could be held for at least 48 hours.

A 250 mCi $^{90}\text{Sr}/^{90}\text{Y}$ beta source was used for irradiation of all samples. The source was calibrated against a NIST secondary standard ^{60}Co source in terms of absorbed doses to water using Luxel Al₂O₃:C OSL dosimeters.

GE crystal clear incandescent bulbs with brightness of 1490 and 690 lumens were used to test the possible influence of environmental illumination light on the BG signal.

EPR spectra deconvolution was conducted with a Matlab code according to the method of Sholom and Chumak (2003). The corresponding nail reference signals were obtained from the nail experimental spectra and adapted for use with this code.

3. Results and Discussion

3.1 MIS signals and their properties

Examples of MIS are shown in Fig. 1 for scissor-generated (fig. 1(a)) and press-formed (fig. 1(b)) signals. The signal in Fig. 1(a) was recorded at 1 min after cutting while those in Fig. 1(b) were collected at 20 min after pressing. Positions of three main signals are shown by arrows in Fig. 1(a); details of these signals are presented in Fig. 2.

To deconvolute the spectra shown in Fig. 1 and similar ones, we used the matrix method developed and described by Sholom and Chumak (2003). This method requires some number of reference spectra, which could be obtained using combinations of experimental spectra recorded at different times after clipping/squeezing or after storage at different conditions. The example MIS reference spectra are shown in Fig. 2. A MIS singlet, at $g=2.005$, was obtained from a sample stored at ambient conditions for 3 days after cutting. A MIS doublet, at $g=2.004$ and a splitting constant $A=1.8$ mT, is a result of subtraction of two spectra, recorded at 1 min and 2 h after cutting with a scissor. The difference between these two spectra gave the signal that had decayed during this time, namely, the doublet. A MIS anisotropic signal (MIS-AS), at $g_1=2.057$, $g_2=2.029$ and $g_3=2.003$ (positions of local extrema), is a result of subtraction of spectra of two squeezed samples (7.3 kbar and 1.6 kbar) recorded after storage at ambient conditions for 3 days after stress. The MIS singlet, MIS doublet and MIS-AS signals are equivalent to the MIS₂, MIS₃ and MIS₁ signals described by Trompier et al. (2014). We did not observe an equivalent of the MIS₄ signal reported by Trompier et al. (2014).

A similar approach was used by He et al. (2011). They also used only three reference spectra (a MIS singlet which had the same a spectral shape as RIS; a MIS “broad” and a MIS doublet) to deconvolute the MIS+RIS spectra. The only difference between the sets of reference spectra presented by He et al. (2011) and those developed in the present work is

for the MIS-AS (MIS-broad in He et al., 2011): our reference spectrum for MIS-AS includes also the low-field part of this signal, which is omitted by He et al. (2011). It should be noted that the MIS-broad signal presented in the follow-up work by the same team (He et al., 2014) has a clearly different shape around the MIS singlet: specifically, there is no shoulder and the location of the minimum is shifted towards higher magnetic fields. The method used by He et al. (2011) for spectra decomposition is essentially the same as was proposed by Sholom and Chumak (2003); the only difference is that the former method applies the matrix deconvolution to the raw sample spectrum while the spectrum of the empty sample tube is subtracted from the sample spectrum before deconvolution in the latter method.

The properties of the MIS singlet and doublet signals have been studied in many publications (e.g. Wilcox et al., 2010; Black and Swarts, 2010; Trompier et al., 2014), but very little information is available for the MIS-AS, including, in particular, how this signal may affect the shape and interpretation of the main RIS at $g=2.005$.

Fig. 3 (a) shows the spectra of a sample stressed to a pressure of 12.1 kbar and recorded 2 days after stressing for different values of microwave power (storage under ambient conditions). For comparison, the spectra of a sample exposed to 100 Gy dose are shown in Fig. 3 (b) for the same values of microwave power. Fig. 4 demonstrates the corresponding microwave power dependences for signal intensities measured at three characteristic points of the MIS-AS ($g_1=2.057$, $g_2=2.029$ and $g_3=2.003$ for (fig.4(a)), or for the 100 Gy RIS signal (fig. 4(b)). Similar dependences were observed by Wilcox et al. (2010), but the latter were obtained just for one characteristic point of the MIS-AS signal in the narrower range of microwave power. (The MIS-AS signal in the latter work is called MIS2.). It is seen from Figs. 3–4 that the spectra following 12.1 kbar of stress and measured after 2 days of storage under ambient conditions is dominated by the anisotropic signal: the doublet has already decayed during the 2 days of storage. (We provide more data about the stability of this signal in Section 3.2, below.) The singlet contribution is negligible, which is confirmed by both the spectra in Fig. 3 (a) and the corresponding microwave power saturation curves in Fig. 4 (a). Such observations allow one to determine how the MIS-AS may affect the main dosimetric signal (i.e. the RIS at $g=2.005$). It is seen in Fig. 2 that the MIS-AS high-field negative peak (between $g=2.003$ and $g=2.008$) overlaps entirely with the $g=2.005$ signal; so, if two signals are added, one can see some visual reduction of the $g=2.005$ signal maximum and an increase of its minimum. The shape of the composite signal will therefore depend on the ratio of the MIS-AS and $g=2.005$ signal intensities. This is simulated in Fig. 5 for two different cases: for fixed MIS-AS and variable RIS (fig. 5(a)) and for fixed RIS and variable MIS-AS intensities (fig. 5(b)). One important observation from Fig. 5 is that the visual peak-to-peak intensity of the $g=2.005$ signal is changed if the MIS-AS is present, which may result in an increase of overall uncertainty of dose reconstruction. We demonstrate in Section 4 below how to account for this possible issue. Another observation from Fig. 5 is that some feature is formed around $g=2.008$ (the location of MIS4 signal in Trompier et al., 2014) due to overlapping of MIS-AS with the singlet. Depending on the ratio of MIS-AS and singlet intensities, this feature might be improperly identified as a separate signal, which could be the case for the MIS4 signal.

Fig. 6 (a) shows the spectra of a nail plate stressed with a pressure of 3.2 kbar and recorded at different angles between the direction of the permanent magnetic field and the plate surface. An angle of 90 degrees means that the magnetic field is perpendicular to the plate surface while 0 degree corresponds to the parallel orientation. Corresponding angle dependences for intensities measured for three signal extrema are shown in Fig. 6 (b). A recurrence period of 180 degrees is seen, which means that there is a spatial alignment of the corresponding paramagnetic radicals in the nails. Although anisotropic properties of whole nail clips were already mentioned by Wilcox et al. (2010), no data were provided, and we believe that data in Fig. 6 is the first experimental confirmation of anisotropy of the MIS-AS. Additional experiments are required to specify in more detail the spatial ordering of MIS-AS signals.

3.2 Stability of MIS signals

Fig. 7 shows the MIS evolution in two samples: stressed with scissors (a) and squeezed with 12.1 kbar pressure (b). Both samples were stored at ambient conditions after stressing. Quite different behavior of the doublet in the two tested samples is seen in fig. 7(a): this signal almost disappears 2 h after exposure in the case of generation by scissors (in accordance with the previous observation by Wilcox et al. (2010)), but is clearly seen even 1 day after squeezing with a press. We measured the time dependence of the signal intensity at $g=2.012$, which corresponds to the low-field maximum of the doublet. The results are presented in Fig. 8 for scissor- and press-generated MIS. One can see in this figure again the better stability of the press-generated MIS doublet.

It is speculated that the difference in the stabilities of the MIS doublets is related to the different locations of the corresponding EPR radicals: in the case of scissor (or clipper) use, the radicals may be formed primarily on the edges of the cut (surface location) while the press-generated radicals may be distributed throughout the volume of the sample (bulk location). This notion is supported by the data shown in Fig. 9, in which the scissor- and press-generated MIS are shown after soaking for different times. It is seen in Fig. 9 (a) that the MIS doublet completely disappears from the scissor-stressed sample after soaking for just 1 minute, which is in agreement with the previous studies (see e.g. Tromprier et al., 2007). At the same time, this signal is still resolved in a press-stressed sample (Fig. 9 (b)) after soaking for 3 minutes and was only reduced to almost zero after soaking for 5 minutes.

Another observation from Fig. 9 is that the MIS-singlet and MIS-AS also have different stabilities (which may also be related to the surface/bulk location of the radicals) depending on how they were formed. This observation leads to a suggestion that the ratio of MIS singlet and MIS-AS intensities for any specific sample may change with fading time, and that correlation of the two signals, which was observed by He et al. (2014) at some specific fading time, may depend on the fading time chosen.

Fig. 10 demonstrates how different MIS signals in the press-stressed sample decay with soaking time. (For comparison, in a scissor-stressed sample, as in Fig. 9 (a), all MIS signals disappear after soaking in water for 1 minute.) It is seen in Fig. 10 that the decay curves for all press-generated MIS follow the same pattern, which suggests that different MIS radicals interact with water in similar ways.

Fig. 11 shows the spectra of the MIS in scissor- and press-stressed samples stored in a vacuum. It is seen that the MIS-AS is stable for both the tested samples while the stability of the MIS-doublet is better for the press-stressed sample compared to the scissor-stressed specimen. The MIS-singlet is also stable in vacuum, as was reported by Sholom and McKeever (2016).

The observation that the decay rate of the MIS-doublet for the scissor-generated centers is slower than for vacuum storage compared with storage under ambient conditions means that molecules of oxygen or water, which are absent in vacuum, may play a catalyst role in the process of MIS-doublet decay. The enhanced stability of the MIS-doublet was also observed by He et al. (2014) for samples stored in carbon dioxide. The authors of the latter work speculate that oxygen is important in the decay of the MIS-doublet, but the role of air humidity remains unclear from their reported data.

3.3 Stability of RIS signals

The RIS signals produced by ionizing radiation are very similar to those observed after mechanical stress and described in Section 3.1 above. Some representative spectra of samples exposed to the doses in the range 0–20 kGy are shown in Fig. 12. All spectra in this figure were recorded about 1 h after exposure. The 20 kGy spectrum was multiplied by a coefficient 0.3 for clarity. It is seen that the spectra for doses below 100 Gy consist mainly of a singlet at $g=2.005$. At higher doses, both the doublet and the anisotropic signal become apparent (see 1 kGy spectrum in Fig. 12) and become dominant in the spectrum of a 20 kGy irradiated sample. The radiation-induced spectrum can be described by superposition of the same 3 EPR components as the mechanically induced spectrum, as observed in Fig. 13. This figure shows the corresponding reference spectra obtained from the experimental spectra of samples exposed to different doses and recorded at different times after irradiation. The RIS-singlet at $g=2.005$ was recorded from a 200 Gy irradiated sample after storage in vacuum for 7 days; a RIS-doublet at $g=2.004$ with a splitting constant $A=1.8$ mT is a result of subtraction of two 20 kGy spectra, recorded at 1 h after exposure and after storage in vacuum for 2 days; and the RIS-anisotropic signal (RIS-AS) is a result of subtraction of a 20 kGy spectrum (recorded 1h after exposure) and the RIS-doublet. RIS-AS has $g_1=2.058$, $g_2=2.031$ and $g_3=2.012$ (positions of local extrema). Its shape is slightly different from that of MIS-AS around $g=2.005$ due to a relatively small contribution from the RIS-singlet (which is saturated at much lower doses). The RIS singlet, RIS doublet and RIS-AS signals are equivalent to the RIS2, RIS3 and RIS1 signals described by Trompier et al. (2014). We did not observe an equivalent of the RIS4 signal reported by Trompier et al. (2014), possibly because this signal is not resolved in the X-band or because RIS4 might be an artificial signal caused by overlapping of RIS-AS and singlet signals, as was supposed in case of the MIS4 signal above.

Fig. 14 shows spectra for the sample exposed to 20 kGy and recorded at different values of microwave power after storage for 2 days under ambient conditions. It is clear that in this dose region RIS-AS is dominant and that this signal is the same as MIS-AS in Fig. 3 (a). RIS-AS also demonstrates similar anisotropic properties. In Fig. 15 are shown the RIS-AS spectra from a nail plate after a dose of 20 kGy and recorded at three different orientations

with respect to the permanent magnetic field direction. (Only parts of the spectra around $g=2.025$ are plotted for clarity.) Some anisotropy is seen, but to a lesser extent when compared with that of MIS-AS (Fig. 6 (a)). The RIS-AS (and by inference the MIS-AS) is proposed to be related to the breaking of sulphur-sulphur bonds in cystine (Trompier et al., 2014). The observed differences in anisotropy suggest that the alignment of the radicals after formation via irradiation is different from that after formation by mechanical stress. In particular, the formation of the signal by pressing may increase the probability of alignment of the radicals along the direction of the applied force, which in the present case is perpendicular to the plate surface. However, some variability in the anisotropy properties of the signal due to different nail plates being used in the two experiments may also play some role.

Fig. 16 demonstrates how different RIS signals in a sample exposed to 500 Gy decay with soaking time. For this specific dose, only two RIS signals were detected, namely, the singlet and the doublet. It is seen that the decay curves for these two signals have approximately the same pattern, but the observed rates are much slower than those for the corresponding MIS radicals (compare with Fig. 10). One can suggest that RIS radicals are generated at even deeper locations than the corresponding bulk MIS centers.

Data shown in Fig. 16 clearly demonstrates that there is no stable component of the RIS singlet; this signal continues to reduce with increase of soaking time reaching the background level at the total soaking time of about 1 h. This observation is in agreement with some previous observations (see e.g. Fig. 5 in Black and Swarts, 2010; Fig. 3 in Marciniak et al., 2014 (compare data at 2.3 and 7.7 days, which were recorded after first and second soaking, respectively), Fig. 3 in Sholom and McKeever, 2016), but contradicts data reported in Trompier et al. (2014), who observed the stable RIS signal after sample soaking for 10 min. Trompier et al. (2014) called this signal RIS5 and concluded, from measurements in Q-band EPR, that RIS2 and RIS5 have different values of g -factor (2.004 and 2.005, respectively). These data has been recently revised by Tipikin et al. (2016), who tested 30 samples from 15 different donors in W-band EPR. These authors came to a conclusion that both RIS2 and RIS5 are the same and arise from the same radical species. In our opinion, RIS5 is just a remainder of RIS2 after sample soaking for 10–15 min.

Fig. 17 illustrates how the RIS-singlet from samples exposed to different doses in the range 0–100 Gy changes with time for storage under different conditions. Five aliquots from the same sample were exposed to doses of 0, 3, 10, 30 and 100 Gy and measured at (a) 1 h, (b) 7 days and (c) 37 days after exposure. (Note: Between the first and second measurements the samples were stored in a vacuum for 7 days while between second and third measurements they were stored under ambient conditions for an extra 30 days.) It is seen that the RIS-singlet is quite stable under vacuum storage, but decays strongly under ambient conditions, in accordance with data previously reported by Sholom and McKeever (2016) and by Marciniak et al. (2014). Trompier et al. (2009) also observed the different stability of the RIS-singlet in samples with different nail humidity content (i.e. percentage of water in nail samples). The stable RIS-singlet in their work corresponded to 0 % humidity, which is the case if the samples are dried and then stored in vacuum.

An interesting observation from the data in Fig. 17 is that all signals, independent of the initial dose, become approximately the same after prolonged (approximately one month) storage in ambient conditions. It is clear that the samples should be stored under conditions of controlled humidity (preferably, in a vacuum) for all times between harvesting and EPR measurement in order to provide reliable results.

3.4 Stability of BG signals

The properties of the background signal (BG) are quite similar to those of the RIS-singlet. The BG decreased with increase in soaking time in the same way as described in Reyes et al. (2008); it becomes approximately constant after a total soaking time of approximately 1 hour.

If a sample was soaked for long time and then left for storage in vacuum or under ambient conditions, the BG intensity was seen to grow, which was already described by Sholom and McKeever (2016) and others (Reyes et al., 2012; Wang et al., 2015; Marciniak and Ciesielski, 2016). It is speculated that exposure to environmental light may contribute to this observed growth. Fig. 18 supports this idea. The figure shows three spectra obtained using different aliquots of the same sample. All aliquots were soaked for 1 h at the beginning in order to minimize BG intensity (spectrum labeled BG in Fig. 18). The two other spectra in Fig. 18 were recorded after exposure for 3 h to clear, incandescent lamps of different power (50 W and 100 W, respectively). A clear increase in the BG signal is observed due to exposure to the lamps.

These observations suggest an equilibrium state for the EPR radicals responsible for the BG signal. This equilibrium state depends, among other factors, on environmental humidity and ambient illumination. For example, after recording the spectra shown in Fig. 17 (c), we relocated the samples to another room with brighter illumination and measured the EPR spectra again after storage in this room for 5 days. The signals from all aliquots were again the same, but with an average value approximately 50% higher than those seen in Fig. 17 (c). This suggests that the radicals responsible for the BG signal came to a new equilibrium level. He et al. (2014) showed that the BG signal is stable for at least four hours if the nails are stored in nitrogen but increased during the same storage time in oxygen. More experiments, including different temperature, humidity and illumination conditions, are required to clarify the complex behavior of the BG signals.

4. Spectral deconvolution for vacuum-stored samples

It is clear from the above data that the cumulative EPR signal at $g=2.005$ in nail samples subjected to both or either irradiation and mechanical stress can be described by superposition of the same three signals. These are a singlet (which contributes to the RIS, MIS and BG signals), a doublet (observed in the low dose range and which mainly contributes to MIS) and an anisotropic signal (also contributing to MIS for doses below 100 Gy, but seen as a contributor to RIS at very high doses). The reference spectra shown in Figs. 2 and 13 were used to fit the experimental spectra according to the procedure described by Sholom and Chumak (2003). The RIS-singlet and RIS-doublet are the same as the corresponding MIS-singlet and MIS-doublet; therefore, it doesn't matter which are used as

the references for the singlet and doublet. For the anisotropic signal, however, the MIS-AS was used because, as was already mentioned, the RIS-AS includes some small contribution from the singlet and because, for emergency-exposed samples, the main contribution to the anisotropic signal is expected from mechanical stress during sample harvesting, not from exposure. The results are shown in Fig. 19 for an unirradiated sample and for a sample exposed to 10 Gy. Both samples were given an extra cut to simulate harvesting. In the case of the 10 Gy sample this extra cut was applied immediately after irradiation. Samples were recorded for 1 h and 1 day after the corresponding treatments; for all times (except during the EPR measurements themselves) the samples were stored in a vacuum.

Satisfactory fits of the experimental spectra with three selected reference spectra are observed. The individual contributions of each reference spectrum are shown in Table 1 together with the corresponding peak-to-peak amplitudes. (The latter are determined for the cumulative signal around $g=2.005$ using the same units as for the intensity of the singlet line.) As expected, the main change between the spectra recorded 2 h and 1 d after exposure/cutting was observed for the doublet; the singlet and the anisotropic signal intensities remained quite stable. (The singlet intensity from the 10 Gy sample dropped by about 7% in accordance with previous observations (Sholom and McKeever, 2016).) It is also seen from Table 1 that the peak-to-peak method gives overestimates for the $g=2.005$ singlet in the case of unexposed samples (especially measured soon after cutting) and underestimates in the case of samples irradiated to 10 Gy dose. This is because the doublet reduces the singlet minimum. On the other hand the anisotropic signal increases the singlet minimum, but also slightly reduces its maximum. The resultant visual change of the singlet intensity, with overlapping with doublet and anisotropic signals, will depend on the ratio of the three signals.

As was already mentioned in Section 3.1 above, the matrix decomposition method used by He et al. (2011) to fit the spectra of nails exposed to both mechanical stress and ionizing radiation is similar to that described by Sholom and Chumak (2003). He et al. also used three reference spectra (singlet, doublet and “broad”), which are quite similar to the current reference spectra (singlet, doublet and AS shown in Figs. 2 and 13). The main difference between two sets of reference spectra is in the range of the permanent magnetic field in which the spectra were recorded. In the present study, it is 25 mT, which covers the entire AS (viz. “broad”) signal while in the He et al. (2011) the reference spectra were recorded only within a 15 mT range, which includes only the central part of the AS (“broad”) signal. Such extension of the deconvolution range to 25 mT should result in better accuracy during spectra decomposition. Also there is some difference in shape between the MIS-AS from this paper and MIS-broad reference spectra from He et al. (2011) at the location of the MIS-singlet signal, which might be caused by a contribution of the residual MIS-singlet to the MIS-broad signal.

5. Summary and Conclusion

The stability of three main EPR signals (namely a singlet at $g=2.005$, a doublet at $g=2.004$ with a splitting constant $A=1.8$ m, and an anisotropic signal at $g_1=2.057$, $g_2=2.029$ and $g_3=2.003$) has been studied in nail samples exposed to different doses or stressed/squeezed

with different forces and stored under different conditions. Both the singlet and the anisotropic signal were quite stable in a vacuum and, together with the unstable doublet signal, form a cumulative EPR signal at about $g=2.005$.

Two types of a MIS doublet signal were observed depending on how they were generated. It is speculated that the doublet observed after nail stress with scissors/clippers and characterized by a very short life time has a surface location while that generated after squeezing with a press and which shows a much longer life time is a bulk signal.

Anisotropic properties of MIS-AS and RIS-AS have been described. While both signals demonstrated similar anisotropic behavior, the observed effect is more pronounced for the press-generated MIS-AS.

It has been found that the BG signal is the singlet signal and is sensitive to environmental light. It is suggested that there is an equilibrium state for the EPR radicals responsible for the BG signal, which depends, among other factors, on environmental humidity and ambient illumination.

A procedure of spectral deconvolution has been tested on irradiated and stressed vacuum-stored samples. The data show that the intensity of the $g=2.005$ singlet signal may be overestimated or underestimated, depending on the ratio of the three signals, if the peak-to-peak method is used to quantify the signal around $g=2.005$ without spectra deconvolution.

Finally, the correspondence between the three signals used in this work and the previous complex nomenclature adopted in earlier publications to describe the EPR spectra of nails (RIS1-RIS5; MIS1-MIS4; BKG) is shown in Table 2. We recommend that the earlier nomenclature be dropped in favor of just three EPR components, namely the singlet, doublet and anisotropic signals.

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HIGHLIGHTS

- EPR spectra from irradiated or stressed nails are described by just 3 signals.
- The signals are a singlet, a doublet and an anisotropic signal.
- All three contribute to the observed net signal around g-factor 2.005.
- The singlet and anisotropic signals are stable in vacuum.
- Spectra deconvolution was tested on irradiated and stressed samples.

Figure 1.

EPR signals of nails generated by cutting with scissors (a) or by squeezing with a hydraulic press (b); shown are the spectra corresponding to different pressure values). Spectrum (a) was recorded at 1 minute after cutting while the spectra in (b) were acquired 20 minutes after squeezing. The positions of the three main signals are shown by arrows in (a); details of these signals are given in Fig. 2 below.

Figure 2.
Reference MIS spectra.

Figure 3.

Spectra of a sample squeezed with 12.1 kbar and recorded at 2 days after squeezing at different values of microwave power (plot a). For comparison, the similar microwave power spectra are shown in plot b for a sample exposed to 100 Gy. Singlet lines at $g=1.980$ at the right sides of plots are from the Bruker reference sample. 0 dB attenuation corresponds to the maximum power of the spectrometer (200 mW) while 42 dB corresponds to about $12.5\text{ }\mu\text{W}$.

Figure 4.

Microwave power saturation curves for (a) a 12.1 kbar sample (intensities were measured for signals at $g_1=2.057$, $g_2=2.029$ and $g_3=2.003$) and (b) for a 100 Gy irradiated sample (intensity was determined for a $g=2.005$ signal).

Figure 5. Simulation of the composite signal for different combinations of the MIS-AS and $g=2.005$ RIS. Fig. 5(a) shows the signals with fixed MIS-AS and variable RIS intensities while 5(b) demonstrates the spectra with variable MIS-AS and fixed RIS intensities.

Figure 6. Angle dependences for the MIS-AS measured in the nail plate squeezed with 3.2 kbar pressure. (a) Spectra for three orientations of the nail plate with respect to the permanent magnetic field direction; (b) The intensities measured at $g_1=2.057$, $g_2=2.029$ and $g_3=2.003$.

Figure 7.

MIS signals evolution in samples stressed with scissors (a) and a hydraulic press (b). The samples were stored under the ambient conditions after stressing. “Fd1min” means fading for 1 min, etc.

Figure 8.
Time dependences of the MIS doublets generated by a scissor and a press. Signals were measured at $g=2.012$. Storage under the same ambient conditions.

Figure 9.

(a) MIS in the samples stressed with scissors, or (b) a press, after soaking for different times. “Fd1min” means fading for 1 min while “soak 1min” means soaking in water for 1 min, etc.

Figure 10.
Decay of the different MIS in a press-stressed sample as a function of soaking time.

Figure 11. MIS in the samples stressed with scissors (a) or a press (b) and stored in vacuum. “Fd15min” means fading for 15 min, etc.

Figure 12.

RIS in the samples exposed to different doses in the range 0-20 kGy. All spectra were recorded at about 1 h after exposure. The 20 kGy spectrum was multiplied by a coefficient 0.3 for clarity.

Figure 13.
Reference RIS spectra.

Figure 14.

Spectra from a sample exposed to 20 kGy and recorded at different values of microwave power after storage for 2 days under ambient conditions. Power is changed from 18 dB attenuation to 45 dB attenuation, in 3 dB steps. (45 dB corresponds to about 6.2 μ W.)

Figure 15.

Angle dependences for the RIS-AS measured in a nail plate exposed to a dose of 20 kGy. Spectra around $g=2.025$ are shown for three orientations of the nail plate with respect to the permanent magnetic field direction.

Figure 16.

Decay of the RIS in a sample exposed to 500 Gy, as a function of soaking time.

Figure 17.

(a) RIS in samples exposed to different doses in the range 0–100 Gy and measured at 1 h after exposure, (b) 1 week after exposure (vacuum storage), and (c) 37 days after exposure (storage in vacuum for 1 week followed by storage under ambient conditions for an extra 30 days). “D0” means dose D=0 Gy, etc.

Figure 18.

BG signals in samples from the same individual after exposure for 3 h to the clear incandescent lamps of different power (50 W and 100 W). Also shown is the signal (BG) from an unexposed aliquot.

Figure 19.

Examples of spectra fitting using RIS-singlet, MIS-doublet and MIS-AS reference signals (see Fig. 2 and Fig. 13). Figures 19(a) and (b) correspond to spectra from an unexposed sample recorded at 2 h and 1 day after extra cutting while (c) and (d) show the spectra from a sample exposed to 10 Gy and also recorded at 2 h and 1 day after irradiation/extra cutting (both treatments were applied to this sample approximately at the same time). All times except EPR measurements samples were stored in vacuum. “Fd2h” means fading for 2 h, etc.

Table 1

Results of spectra deconvolution for unirradiated and 10-Gy exposed and stressed samples using three reference signals

Sample dose/Fading time	Singlet intensity, arb. units	Doublet intensity, arb. units	Anisotropic signal intensity, arb. units	Peak-to-peak amplitude *
0 Gy/2 h	0.84	0.39	0.95	0.98
0 Gy/1 d	0.84	0.27	0.92	0.86
10 Gy/2 h	1.5	0.4	1.08	1.41
10 Gy/1 d	1.4	0.28	1.15	1.34

* The peak-to-peak amplitude is expressed in the same units as the singlet intensity to allow direct comparison.

Table 2

The correspondence between the three signals used in this work and the nomenclature from Trompier et al., 2014.

EPR signal used in this work [*]	Equivalent EPR signals from Trompier et al., 2014 [*]
Singlet	BKG, RIS2, RIS5 ^{**} , MIS2
Doublet	RIS3, MIS3
Anisotropy signal	RIS1, MIS1

^{*} We didn't observe RIS4 and MIS4 signals;

^{**} According to Tipikin et al., 2016, RIS2 and RIS5 are the same signals.