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Original Article

An ESR study on fingernail as a biological dosimeter

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Abstract
Recent studies indicate that ESR based dosimetry in fingernails can be an effective method for estimating the absorbed dose and thus fingernails could be used as biological or personel dosimeters. In the present work, characteristic features of the radicals were investigated in detail for pre-irradiated, irradiated (gamma and UV), and mechanically induced fingernails by Electron Spin Resonance (ESR) spectroscopy. Heights of the resonance lines measured with respect to the spectrum base line were used to monitor microwave power saturation, washing treatment, absorbed dose, storage time and temperature dependent kinetic features of the radical species contributing to the formation of recorded experimental ESR spectra. From the recorded ESR spectra of fingernail samples collected from 20 donors showed that spectroscopic splitting factors g determined from recorded background spectra were 2.0042 and peak to peak width was found to be $\Delta H_{pp} \cong 0.45$ mT. In the interested low dose range (≤ 200Gy) irradiated fingernails showed a broad, unresolved ESR spectrum which was similar to the background signal except its high peak intensity. A linear dose-response curve was recorded for UV-irradiated fingernails. Washed fingernails with different solutions effected the decay rate of the samples. The radicals produced upon irradiation were fairly stable at room storage conditions. The act of cutting the fingernail samples generates sulphur centered radicals of α-keratin content of fingernail. Three radical species of were found for the mechanically damaged fingernail samples. The variations of the peak heights of the samples with temperature were related with the water content found in the keratin of fingernails. Decay activation energy for mechanically induced radicals in fingernail was calculated to be 23.5 kcal/mol. Basing on these results, it was concluded that human fingernail samples could be used as biological dosimeters and that ESR spectroscopy could be successfully used as a potential technique for monitoring its dosimetric behaviours.
Key Words: ESR, fingernail, irradiation, dosimeter, biological dosimeter

Introductıon

Nowadays a non-invasive, rapid and reliable biological/personel dosimeter is urgently needed to estimate the absorbed dose immediately after the radiation accidents which can be used for a large number of individuals rapidly and with sufficient accuracy. The potential for using Electron Spin Resonance (ESR) to measure absorbed doses was first recognized and reported by Brady et al. (1968). The use of ESR for biodosimetry is based on the capability of the technique with a very sensitive measurement for the unpaired electron species which are created in the samples upon irradiation/mechanical damage. ESR can be used for long lived paramagnetic species that are stabilized in the matrix of the samples such as bones, teeth, keratin of fingernails or human hair (Brady et al., 1968; Chandra and Symons, 1987; Dalgarno and McClymont, 1989; Symons, et. al., 1995; Desrosiers and Schauer, 2001). ESR dosimetry on fingernail was considered to be a method with great potential in the recent studies (Romanyukha, 2007; Reyes, et. al., 2008; Alexander, et. al., 2007; Trompier et al., 2007; 2009). Fingernails can sample easily and painless from the donors, the time required for dose evaluation is rather short (days, weeks) so ESR responses can be taken in very short time intervals.

Fingernails are composed of a fibrous proteins called α-keratin which contain large amounts of the sulfur bearing amino acid cysteine (Fig. 1), required for the disulfide bridges that give additional strength and rigidity to the structure. When the cysteine molecule attacks the free radicals, it loses the hydrogen atom in the thiol group and the remaining sulphur atom forms a compound with another sulphur atom on another cysteine molecule. Together, the two molecules form one cysteine molecule that contains two mutually connected sulphur atoms, and is therefore a disulphide (Fig 1b). This particular compound is very strong and it is responsible for the structure in many resistant peptides and proteins such as in hair and fingernails.
Applying mechanical stress (cutting) on the fingernail samples induces free radicals that can be demonstrated by ESR which are the precursors of this destruction. Some publications (Chandra and Symons, 1987; Dalgarno

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and Mcclymont, 1989; Wu et al., 1998; Romanyukha et al., 2007; Reyes, et. al., 2008) discussed the development of the ways to reduce the background and mechanically induced radicals (named as MIS) which is overlapped by the radiation induced radicals (named as RIS) so that dosimetric features of the fingernails could be shown efficiently.

There have been not so much studies dedicated to radiation or other physical properties for fingernails but a very important protocol for fingernail is published containing the collection, storage and the measurement by ESR spectroscopy (Alexander, et. al., 2007). But there is currently no sufficiently detail data about some of the topics such as UV-irradiation, long term stability features, temperature and kinetic dependences (annealing) of the radicals induced and proposals of radical species for mechanically damaged fingernails. These results could provide a general characterization for the radicals found in fingernails samples and will be very helpful in dose assesments. This study presents the interested answers, some of which will be first time exist in the literature. In the recent study, the ESR responses of fingernail samples upon gamma and UV-irradiation, microwave power saturation, mechanical stress effects, washing treatments on fingernails, stability features, temperature dependences and annealing studies were presented to characterize the radicals of fingernail samples as a whole (Pembegül, 1996).

Materıals and methods

The fingernail samples were collected from 20 donors by clipping them as long as possible with minimum of cut. Sharp surgical scissors were used to cut the fingernails. The pieces of fingernails were all about 1–2 mm wide and about 5 mm long. The typical sample mass was about 15 mg. The samples were kept in paper envelopes during the measurements and was stored in dark at room temperature. No further purification was performed and they were used as they were received without any washing treatment.

For the irradiation processes, UV lamp (Hg lamp, 300 W) and ^{60}Co gamma cell were used. In UV irradiation processes, the samples were put always with the same distance to the UV source and ESR signal variations with irradiation time were examined. The temperature rise of the UV lamp during irradiation is very small so the thermal effects on the samples were ignored. Gamma irradiations were performed at room temperature (293 K) in dark using a ^{60}Co gamma cell supplying a dose rate of 0.5079 kGy/h in Hacettepe University, Chemistry Department, Beytepe, Ankara. The dose rate was measured by a Fricke dosimeter and ESR investigations were performed on samples irradiated at 3 - 200 Gy dose ranges. ESR measurements were carried out using Varian 9''-E line-X band ESR spectrometer operating at 9.5 GHz and equipped with a TE_{104} rectangular double cavity containing a standard sample (strong pitch, g = 2.0028) in the rear resonator which remained untouched throughout the experiment. Signal intensities were calculated from first derivative spectra and compared with that obtained for the standard sample under the same spectrometer operating conditions. The recorded ESR signals are normalized to the intensities of standard material and mass of the samples. The evolutions of the ESR signal intensities with the applied microwave power; received radiation dose, storage time, temperature and annealing time monitored by measuring the heights of the characteristic resonance peaks from recorded experimental spectra. The spectrometer operating conditions adopted during the experiment are given in Table 1.

Sample temperature inside the microwave cavity was monitored with a digital temperature control system (Bruker ER 4111-VT). The latter provided the opportunity of measuring the temperature with an accuracy of ±0.5 K at the site of the sample. A cooling, heating and subsequent cooling cycle was adopted to monitor the evolution of the ESR line shape with temperature for fingernails. Sample temperatures were first decreased to 100 K starting from room temperature with an increment of 10 K, then increased to 470 K and finally was decreased again to room temperature.

A set of three different samples which were cut into small pieces (mechanically damaged) were annealed at 323, 340, 360 K for predetermined times, and the signal intensity data obtained were used to calculate the high temperature decay characteristics of the radical species responsible from the ESR spectra of fingernail. Digitized signal intensity data derived from room temperature ESR spectrum of a sample mechanically damaged and stored at liquid nitrogen temperature was used as input for spectrum simulation calculations basing on a model predicting the presence of three radical species of sulfure centered with different spectroscopic features.

Experimental results and discussion

Background (pre-irradiated and mechanically undamaged) spectra of human fingernails at room temperature

In background studies, the samples were clipped from the donors as long as possible with the minimum cut so as to ignore the effect of mechanical damage on the fingernail. Pre-irradiated nail samples were observed to exhibit an intrinsic background signal with an unresolved and complex ESR signals consisting of one

antisymmetric intense central resonance line (Fig. 2a) which is extended over a magnetic field range about 2.5 mT. The peak to peak width for the central line was $\Delta H_{pp} \cong 0.45$ mT and g values was calculated to be 2.0042.
The ESR spectra features of the pre-irradiated signal of the fingernail is similar with the gamma/UV irradiated fingernail samples. But the background signal of fingernail does not decay at room temperature in contrast with the irradiated/mechanically damaged samples. The background signal only decays at high temperatures such as upper than 320 K (Symons, et. al., 1995). The origin of the ESR signals recorded for the pre-irradiated nail samples is currently unknown (Trompier, et. al., 2009).

In addition to free radicals induced by irradiation (part 3.3), cutting the samples into small pieces (~1-3 mm) also demonstrate mechanically induced radicals (Part 3.4) with seven resonance lines (Symons, et., al., 1995). The spectra recorded for pre-irradiated, irradiated and mechanically damaged fingernail samples overlapped with each other (Fig. 2 b, c, d, e). So there is some studies in the literature (Chandra and Symons, 1987; Romanyukha, et. al., 2007; Reyes, et. al., 2008) to fade the backgroung signal of the fingernail so that more efficient results could be reached on dosimetric studies of fingernails.

Variations of the peak heights with microwave power

Variations of the mechanically induced radicals with the applied microwave power at room temperature (293 K) were studied first (Fig 3). This study gives an idea about the number of radical species induced during radiation/mechanical stress. The mechanical induced radicals (Fig. 2) are better seen at higher microwave power (>10 mW) values. The microwave saturation data related with the resonance lines were fitted to the the exponential growth equation given by Eq. 1 where b gives the saturation constant of the untreated fingernail samples.

$$I = a*[1 - \exp(-b*(P)^{1/2}] \qquad \text{(Eq. 1)}$$

Parameter values found are given in Table 2. These parameters given in this table were used in Eq. 1 to get theoretical decay data. They are represented as dotted lines in Fig. 3 with their experimental counterparts. As seen from Table 2, the saturation behaviours of mechanically treated fingernail samples were different indicating that more than one radical species is present in the sample. As seen from Fig. 3, the mechanically induced radicals did not saturate at high microwave power except resonance line 4 which is similar with a study which was also indicated that the background signal of the fingernail sample was saturated at about 2 mW (Reyes, et al., 2008).

Dosimetric features of fingernail

Dosimetric features of the fingernails were the main interested topic in the present study. UV and gamma irradiations were applied to the fingernail samples which were used as long as possible without taking into account the mechanical stress effect on the samples. There is no study in the literature on the UV irradiation of fingernail samples as far as we know. The variations of the peak-to-peak height of UV- irradiated fingernail samples with irradiation time is shown in Fig 4. A large and unresolved complex singlet (fingernails were not cut into small pieces) spectrum was recorded for UV irradiated fingernail samples. It was very similar to the background signal of fingernail with only a significant increase in the peak heights upon irradiation time. The increase in the irradiation time did not create any pattern change in the spectra of the samples irradiated, so it was concluded that irradiation dose was not an very important parameter in the shape of the ESR spectrum of fingernail. The origin of the increased signal intensities upon irradiation is related with the deformation of sulfur bonds, thus because of the sulfur containing radicals (Dalgarno and McClymont, 1989; Kudynski et al., 1994; Symons, et. al., 1995).

As seen from Fig. 3, the slope of the dose-response curve is correlated with a linear fit and gives an important idea about the dosimetric behaviour of the fingernail sample. The increase in the ESR peak intensity with irradiation time was biggest at 40 minutes in the study of 0-75 min irradiation time range. Fingernail samples collected from different donors were also irradiated with gamma rays in the dose range of 3-200 Gy by using ^{60}Co gamma cell. The samples which mechanically untreated were used in this part of the study.
A large, unresolved and complex similar singlet spectra were also recorded for gamma irradiated fingernail samples as the ones of UV-irradiated. The spectrum is also similar to the background spectrum of the fingernail except the high intensity of the peak heights. By the experimental results it was concluded that, the discrimination of fingernail sample irradiated at a dose as low as 3 Gy, from unirradiated one was possible even after one week due to the relatively high stabilities of the produced radical species. This value is 2 Gy in the literature (Dalgarno and McClymont, 1989).

Unfortunately a good relationship between the intensity of the peak heights of gamma irradiated samples with the absorbed dose could not be reached in this study because of the high intensity of the background signal of the irradiated sample. But it has been reported that fingernails have a linear dose dependence on the ESR radiation induced radicals in a broad dose range (Symons, et. al., 1995; Trompier, et. al., 2009).

The signal intensity of one of the fingernail samples which was 200 Gy gamma irradiated increased 1.3 times of its background signal where this value was 4.1 for a sample collected from a different donor. Thus radiation induced radicals can be also related with the invidual specifications of the donors. For a study held at 200 Gy gamma irradiation, fingernail sample was stored at liquid nitrogen temperature 77 K and a resolved spectrum with an increased intensity was recorded. The gamma irradiated samples decayed in a few weeks in contrast of the background signal of the sample which did not decay at room temperature so this parameter must be take into account in the dose assesments.

Variations of the peak heights with mechanical stress

Fingernail samples collected from 20 different donors and cutted (mechanical stress) in variable lenghts (1cm→1mm) and their ESR spectra were recorded. By sequential cutting a mechanically induced type of spectrum which has seven resolved resonance lines was observed for fingernail samples (Fig. 2b, c, d, e). The ESR spectrum patterns of the recorded mechanically damaged fingernails were not same in short time intervals after cutting procedure. The variations of the spectrum patterns of the mechanically damaged fingernails after the act of cutting 5 min, 20 min and 2 hours were given in Fig. 2 b, c, d respectively. Addition to this findings, just after the mechanical stress to fingernail one more resonance line appeared with very short life time at low magnetic field (g=~2.0997 G) but disappeared 2 minutes after the cutting. The spectrum pattern was unchanged 2 hours after the mechanical stress and after one day background signal becomes dominant which was also reported (Reyes, et al., 2008). These radicals are best studied at liquid nitrogen temperature (77 K) because of the good resolution in the spectra (Fig. 2e).

The signal intensity of the fingernails which were cut in 2 mm pieces was increased ~24% when they were compared with the ones which were cut in 3 mm pieces. The same ratio was 45% for the fingernails which were cut in 1 mm pieces. When the fingernails were pounded, the increase in the signal intensity was 64%. Thus the increase in the act of cutting the samples into smaller pieces increases the mechanically induced radicals in the samples as expected. Chandra and Symons (1987) noticed that there is a direct correlation between the number of cuts in a fingernail sample and the mechanical induced intensity. In the present study we did not reach a saturation in the ESR signal intensity in the sequential cutting in the range of 0.5-0.5 mm.

Addition to these findings, the ESR signal intensity of the fingernail is lower when the experiments were taken just after the treatment where the intensity higher (with the same mass) when the fingernails were cut and stored for a few days before the experiments. The reason for this is the variation of the water content in fingernail samples during storage time. When the fingernail is stored for a few days, water bounded to its structure leaves the sample (Milczarek, et. al., 1992; Wortmann, 1993) and more intense ESR peak heights can be recorded (see part 3.6). The similar results was also recorded for human hair sample (Çolak and Özbey, 2011).

The long term variation of the I_1, I_2+I_4 and I_5+I_7 peak heights with storage time is given also in Fig. 5. As seen from the figure, the peak heights of the selected resonance lines had sharp decreases after 4 hours the cut, after this value rate of decreasing was small. The half lifetime of the I_5+I_7 peak heights was observed to be 8 days. The decay constants for this study were calculated and given in Table 3. The decay data related with I_5+I_7 were fitted to the the first order decay kinetics given by Eq. 2 (Symons, et al., 1995) where I_o is related with the background signal of the fingernail, I indicates the intensity of the washed samples with different solutions and k indicated the decay constant.

$$I(t) = I_o + A*\exp(-k*t) \qquad \text{(Eq. 2)}$$

Parameter values given in this table were used in Eq. 2 to get theoretical decay data. They are represented as dotted lines in Fig. 5 with their experimental counterparts. As seen from Table 3, the decay constant of the central peak (I_5+I_7) is the biggest one when compared with the other resonance lines. The different decay rate of the resonance lines is another indication of more than one type of radical species formation during mechanical stress.

In the literature several studies were also done to fade the mechanically induced radicals so that to end the overestimate behaviour of mechanically stress to the absorbed dose. Mechanically damaged could give a dose offset up to 10 Gy (Romanyukha, et. al., 2007). The treatment of washing the fingernail samples was one part of our study (Part 3.5) for the same purpose.

The treatment of washing to the fingernail samples

Symons et al. (1995) have pointed out that the radiation-induced signals (RIS) in fingernails overlap with two non-radiation signals: a mechanically induced signal (MIS) originating from radicals induced by mechanical stress during the fingernail cutting, and a background signal whose origin is currently unknown. In recent studies (Chandra and Symons, 1987; Romanyukha, et. al., 2007; Reyes, et. al., 2008) different treatments

on fingernails with chemical reagents were applied to reduce the MIS and background signal so as to get a clear result on absorbed dose estimation of fingernails.

In the present study, the untreated fingernail samples (which are not mechanically damaged or irradiated) were put into the distilled water, acetone and ethyl alcohol solutions. After this procedure, the samples were dried for 4.5 hours at 323 K and for 17 hours at 313 K temperature storage conditions. The dried nail samples were cut in 1.5 mm pieces and the variations of the I_5+I_7 peak height with storage time at room temperature were investigated (Fig. 6). The washing treatment on the samples did not changed the peak intensities of the samples as seen in Fig. 6, the only aim of the different initial signal intensities was a clear image, it is better to normalize them in the actual case.

The decay constants were calculated for each procedure and given in Table 4. Parameter values given in this table were used in Eq. 1 to get theoretical decay data. They are represented as dotted lines in Fig. 6 with their experimental counterparts. The decay constant of the washed fingernail with distilled water is found to be greater than the washed samples with ethyl alcohol and acetone. So it will be better to use water in the washing procedure of fingernail so as to accelerate the fading of mechanically induced signals. It is seen that in a few hours, the effect of mechanical degradation can be ignored by this procedure (Fig. 6). Also it is seen from Table 4 that the decay rate is faster in washed and dried samples than the dried samples at the beginning (Table 3). It is similar with the documented report that, the decay rate of the freshly cut nails is higher than in drier nails (Trompier, 2009) which indicates that decay rate depends on the water content in fingernails

The aims of this study were to examine the fading feature of the mechanically induced radicals of the fingernail and to decide how to use the fingernail samples in the experiments. By the results concluded, the fingernails were preferred to use as they received, without washing treatment. Because the decay behaviour of the samples changed upon treatment of washing (Chandra and Symons, 1987; Trompier, et. al., 2007). Fingernails are also known to be an effective water absorber (Reyes, et al., 2008).

Addition to these findings, no ESR signal was obtained for wet samples until they are completely dried. It has already been documented that water treatment of irradiated fingernails causes a strong reduction of the radiation induced species (Trompier, 2007; Romanyukha, 2007). When the nail is wet, α-keratin absorbs water and β-keratin feature is indicated. β-keratins do not contain cysteine and cystine aminoacids so as there is no free radicals to give response to ESR spectroscopy, no signals could be recorded. Thus the moisure of the atmosphere during the experiments is very important (Brady et al., 1968; Milczarek et al., 1992).

Variable temperature study

Temperature variable studies were made at liquid nitrogen temperature (77 K) and in 100–470 K temperature range for mechanically damaged fingernail samples. At 77 K, a resolved ESR spectrum with high intensity were recorded. At low temperature study (290-100 K) the pattern of the spectra of fingernail remained unchanged while peak heights increased at lower temperatures and when the temperature again increased to room temperature (100-290 K) the intensities of the peak heights were observed to decrease reversibly, obeying Curie' Law as expected.

The results of high temperature study (290 - 470 K) of the I_5+I_7 resonance lines of the fingernail sample is given in Fig. 7. In this temperature dependence study, spectroscopic splitting factor (g-value) and peak-to-peak height ΔH_{pp} of the fingernails were found to be unchanged. In the heating process (290 – 470 K) the peak heights decreased in intensity on annealing above room temperature 290 K. The structure of fingernail begin change above 370 K temperatures as an indication of changes in ESR spectra. In the cooling process (470 - 290 K) the signal intensity nearly remained constant which shows that the mechanically induced radicals decayed unreversibly at high temperatures. So the decrease of the peak height at high temperatures can also be used to fade the mechanically induced radicals at high temperatures if fingernail samples are aimed to be used as biological dosimeters.

The decrease on high temperatures can be best explained by the water content found in α-keratin structure which depends on atmospheric humudity. By heating, weakly and strongly bounded water to the α-keratin begins to leave the sample and more intense ESR peak heights can be recorded at high temperatures as reported (Milczarek, et. al., 1992; Wortmann, 1993). The same behaviour was observed for human hair samples (Çolak and Özbey, 2011).

In the temperature studies the burnt fingernail samples were also investigated. The singlet spectrum of the burnt fingernail sample had ~10^2 times higher intensity than the untreated fingernail with the spectral parameters ΔH_{pp} = 0.53 mT and g = 2.0030. The stability of the resonance peak height the burnt fingernail was found to be very high at room temperature and atmospheric humidity so this sample can also be a good candidate for ESR standard material.

Radical decays in annealed samples

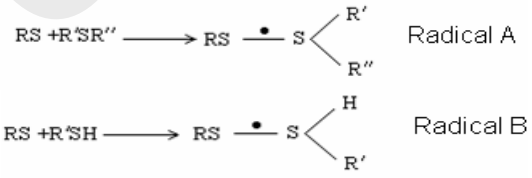
The mechanically induced radical species of fingernail samples are expected to have exhibit temperature dependent decay characteristics. Increase in the sample temperature is predicted to accelerate the decay rates of the involved radicals. It was found that it is the case. Variations of the I_5+I_7 peak height decay data for a sample mechanically damaged and annealed at 323, 340 and 360 K temperatures for predetermined times are summarized in Fig. 8. As the structure of fingernail changed above 370 K (part 3.6), annealing studies were held up to 360 K temperature. The decay constants (k) were determined at each annealing temperature. That is, the decay data related with I_5+I_7 peak height data were fitted to the Eq. 1 and I_o, k parameters were calculated at each annealing temperature. The results are presented in Table 5. Parameter values given in this table were used in Eq. 1 to get theoretical decay data. They are represented as dotted lines in Fig. 8 with their experimental counterparts. It is seen that the experimental and theoretical decay data correlate very well in the 290 - 470 K temperature range. The activation energies of the involved radical species were also calculated by Arrhenius equation with using the slopes of the straight lines best describing ln(k)-1/T graphs (Fig. 9) constructed by using decay constants given in Table 5. The activation energy, which is the energy needed for decaying the mechanically induced radicals in this case, is calculated to be 23.5 kcal/mol. The decay activation energy calculated for X-ray irradiated keratin is 14.5 kcal/mol (Rajewsky and Redhart, 1962).

Proposed radical species for mechanically induced radicals of fingernail samples

The structure of the mechanically induced radicals is rather complex and at least two components could be identified by varying the microwave power (Part 3.2), storage time dependence (Part 3.4) and temperature dependences (Part 3.6). It is known that cutting fingernails generates mechanically induced radicals and induced species is a sulfur centered radical (Rajewsky and Redhart, 1962; Chandra and Symons, 1987; and Symons, et al., 1995). It is expected that mechanical stress break the weak S-S bonds in favour of the stronger C-S bonds. The radical type of nail samples are expected to be very broad, but they have high affinity for sulphur. But Millington reports that (1997) the radicals proposed by Symons, et. al., (1995) would be highly sensitive to their environment and can not be observed experimentally.

Sulfure radicals have g tensors of orthorombic symmetry with an average value varying between 2.0037-2.0059 (Samoilovich and Tsinober, 1970; Bershov et al., 1975). The g values of the central resonance line of mechanically damaged fingernail (Fig. 2) were calculated to be 2.0042 stay in this range. However, all species produced after irradiation are expected to undergo immediate germination termination reactions (Tilquin, 1985) of different rates due to cage effect. Therefore, the amounts of the species responsible from the observed experimental ESR spectra would be different depending on the capacity of these species participating to the germination reaction.

We believe, accordingly, sulfure ionic radical (hereafter radical A), sulfure ionic radical with proton hyperfine splitting (hereafter radical B) and a molecular sulfur fragment with axially symmetric g tensor (hereafter radical C) properties are responsible from the mechanically induced fingernail samples (Fig. 1). Radical A and B are randomly oriented in fingernail structure and give intense ESR resonance line in the center of the spectrum and have the mechanisms of proposals of Chandra and Symons (1987).



Radical C is restricted in large extent due to the big group attached to it, so that it give rise to powder ESR spectra with principal g values varing between g_{xx}=2.0022-2.0031, g_{yy}=2.0015-2.0098 and g_{zz}=2.0058-2.0066 (Bershov et al., 1975). As for radical A, it experiences a high motional freedom, it give rise to a single resonance line of average spectroscopic g factor varying between 2.0037-2.0059 (Samoilovich and Tsinober, 1970).

The singlet which is related with radical A has a similar shape, linewidth with background spectrum and radiation induced radicals (Trompier, 2009). While the doublet component can completely decay (radical C), the singlet component does not completely disappear. This residual signal, whose origin remains unknown, is called a background signal (Fig. 2a).

Spectrum simulation calculations were performed to support the idea put forward and to determine correct spectroscopic parameters of the contributing radical species assuming the presence of three radical

species proposed above, exhibiting isotropic (species A and B) and axially symmetric (species C) g tensors. Room temperature experimental signal intensity data obtained for a sample mechanically damaged was used as input to carry out the simulation calculations. The results of these calculations are summarized in Table 6 and theoretical spectrum derived using the parameter values given in this table are presented in Fig 10 with its experimental counterpart. It is seen from this figure that while radicals A and B dominate the central part of the experimental spectrum, the radical C, with its relatively small weight, gives rise to the appearance of the resonance lines with low magnetic field (Fig. 10). A good agreement between experimental and spectrum calculated using parameters given in Table 6 was considered supporting the proposed the model based on the presence of three radicals with different spectroscopic features.

Conclusion

In the present work, the dosimetric features of fingernail samples were investigated in detail by some helping experimental parts such as microwave power saturations, effects of mechanical damages, washing procedures on the samples, temperature and annealing studies and finally proposals for mechanically induced radical species. All the results will give important features of fingernail samples to be used as biological or personnel dosimeters. The conclusions are summarized below:

- Radical species responsible for the pre-irradiated nail samples is probably because of the S-S bonds found in keratin protein which is named as radical A and B in the present study.
- Microwave power studies indicates that more than one type of radical is induced upon mechanical stres.
- Fingernail samples irradiated at a dose as low as 3 Gy can be distinguished from unirradiated one by ESR spectroscopy.
- A linear dose-response curve can be recorded in UV-irradiated fingernail samples with irradiation time.
- The origin of radiation induced radicals are radical A and B which has a very similar spectrum with pre-irradiated fingernail samples.
- The radiation induced signals in fingernails are stable for at least several days and much longer if the samples are collected within a few hours after the event and stored at lower temperature. It is seen from part 3.6 that the variations of signal intensities with temperature is reversible at low temperature range (100 - 290 K).
- Cutting of the fingernail creates a mechanically induced signal that overlaps with the radiation-induced signal. However, the decay rate of the mechanically induced radicals are fairly fast (Table 3) and in this decay can be accelerated by both simple chemical treatments (Table 4) and annealing studies (Table 5).
- Stability studies of the mechanically induced radicals indicated that radicals are quite stable. Stability of a sample makes a good candidate this sample to be a good dosimeter.
- At high temperatures the signal intensity decreases irreversibly and wet samples gave no ESR signal related with the water content of fingernail samples.
- Washing procedure with different solutions accelarates the fading of mechanically induced radicals and water is recommended to use for this purpose. And the decay rate is faster in washed samples (Table 4) than the dried samples (Table 3) related with the water content of keratin structure.
- The measurements can be made using conventional X-band ESR spectrometers, which are widely available.
- This technique may applicable in adults as the ESR signals also depends on the individual specifications of the donors.

Recent results indicate that ESR based dosimetry in fingernails can be an effective method for estimating absorbed dose and fingernail samples are good candidates for biological or personnel dosimeters.

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TABLE LEGENDS

Table 1 . The spectrometer operating conditions adopted during the experiment.
Table 2. Saturation parameters for microwave power variaitons of mechanically damaged fingernail samples.
Table 3. Decay constants for time variations of mechanically damaged fingernail samples stored at room conditions.
Table 4. Decay constants for washed fingernail samples with different solutions.
Table 5. Decay constants for I_5+I_7 resonance line at three different temperatures for a mechanically damaged fingernail sample.
Table 6. ESR parameters calculated for proposed contributing radical species

FIGURE LEGENDS

Figure 1. Molecular structure of two aminoacids found in human nail which are rich of sulfur a) cysteine, b) cystine.
Figure 2. ESR spectra of fingernail samples.a) Pre-irradiated (background) signal, b) mechanically damaged, after 5 min, c) mechanically damaged, after 25 min, d) mechanically damaged after 2 hours, e) mechanically damaged after 2 hours and stored at liquid nitrogen (77 K) temperature.
Figure 3. Variations of the assigned peak heights with the square root of microwave power at room temperature (293) for a mechanically damaged fingernail. I_2 (■), I_3 (o), I_4 (▲), I_5 (▷), I_6 (✱), I_7 (◆). Lines are obtained simply by joining data.
Figure 4. Variations of the peak-to-peak height of UV- irradiated fingernail samples with irradiation time.
Figure 5. Variations of the peak heights with storage time. I_1 (□); I_2+I_4 (o); I_5+I_7 (▲); dotted lines: theoretical

Figure 6. Variations of the I_5 +I_7 peak height of washed nail samples with storage time. Distilled water (□); acetone (o); ethyl alcohol (▲); dotted lines: theoretical.
Figure 7. Variations of the I_5+I_7 height with temperature in the range of 290–470 K. Bolds: heating; open: cooling.
Figure 8. Variations of the I_5+I_7 peak height with annealing time at three different temperatures. 323 K (□); 340 K (o); 360 K (Δ).
Figure 9. Arrhenius plot constructed for the I_5+I_7 peak height of the annealed at different temperatures. Symbol: experimental, dashed lines: theoretical.
Figure 10. Experimental (dotted line) and theoretical (solid line) ESR spectra calculated using parameter values given in Table 5. **a)** sum spectra; **b)** species A; **c)** species B; **d)** species C.

Table 1 . The spectrometer operating conditions adopted during the experiment. Central field 327.5 mTSweep width 10 mTMicrowave frequency 9.25 GHzMicrowave power 2 mWModulation frequency 100 kHzModulation amplitude 0.1 mTReceiver gain $1.25x10^3$ - $8x10^3$Sweep time 240 sTime constant 1 sTemperature 100 - 470 K

Table 2. Saturation parameters for microwave power variaitons of mechanically damaged fingernail samples.

Number of resonance line	**b**	**R^2**
2	0.119±0.029	0.97912
3	0.028±0.038	0.96491
4	0.011±0.330	0.41342
5	0.159±0.032	0.97429
6	0.303±0.039	0.96226
7	0.314±0.042	0.95513

Table 3. Decay constants for time variations of mechanically damaged fingernail samples stored at room conditions.

Resonance Lines	**I_o**	**Decay Constant ($k\ (min)^{-1} \times 10^3$)**	**Correlation Coeffiencient (r^2)**
I_1	0.115 (±0.002)	3.349 (±0.953)	0.96125
I_2+I_4	0.201 (±0.005)	2.043 (±0.725)	0.94733
I_5+I_7	0.896 (±0.006)	8.979 (±1.296)	0.95343

Table 4. Decay constants for washed fingernail samples with different solutions.

Washing Solution	**I_o**	**Decay Constant ($k\ (min)^{-1} \times 10^3$)**	**Correlation Coeffiencient (r^2)**
Distilled Water	0.682 (±0.015)	3.769 (±0.656)	0.94219
Ethyl Alcohol	0.586 (±0.792)	2.674 (±0.422)	0.97486
Acetone	0.66941 (±0.025)	2.376 (±0.291)	0.98373

Table 5. Decay constants for I_5+I_7 resonance line at three different temperatures for a mechanically damaged fingernail sample.

Annealing Temperature (K)	I_o	Decay Constant ($k\ (min)^{-1} \times 10^3$)	Correlation Coeffiencient (r^2)
323	0.853 (±0.027)	8.152 (±2.151)	0.97147
340	0.491 (±0.033)	57.010 (±10.125)	0.96753
360	0.572 (±0.026)	287.810 (±45.374)	0.96961

Table 6. ESR parameters calculated for proposed contributing radical species

	Spectroscopic Parameters			
Radical Species	g factor	Line Width (mT)	Hyperfine Splitting A_H(mT)	Relative Weight
A	**2.0004 (±0.0002)**	**1.60 (±0.02)**	**-**	**0.22 (±0.20)**
B	**2.0244 (±0.0002)**	**0.65 (±0.02)**	**0.60 (±0.02)**	**0.03 (±0.02)**
C	**2.0207 (±0.0002)**	**1.20 (±0.02)**	**-**	**0.73 (±0.20)**
	2.0982 (±0.0002)			

Figure 1. Molecular structure of two aminoacids found in human nail which are rich of sulfur a) cysteine, b) cystine.

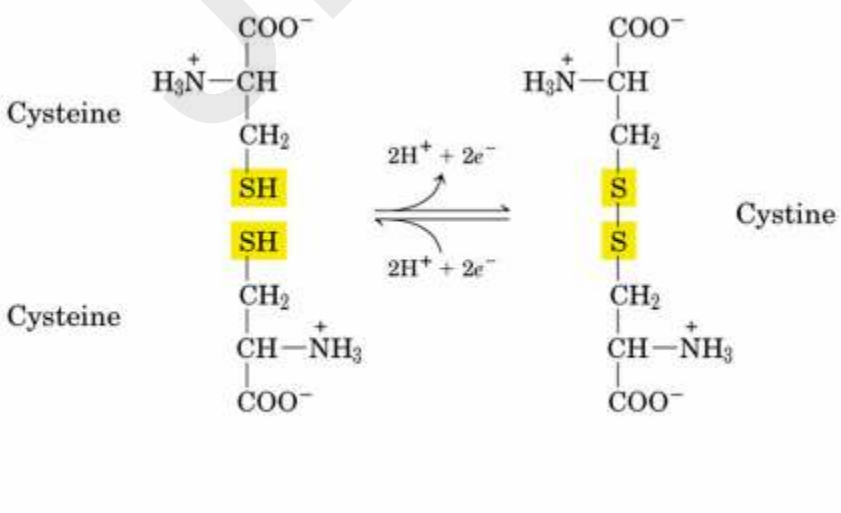
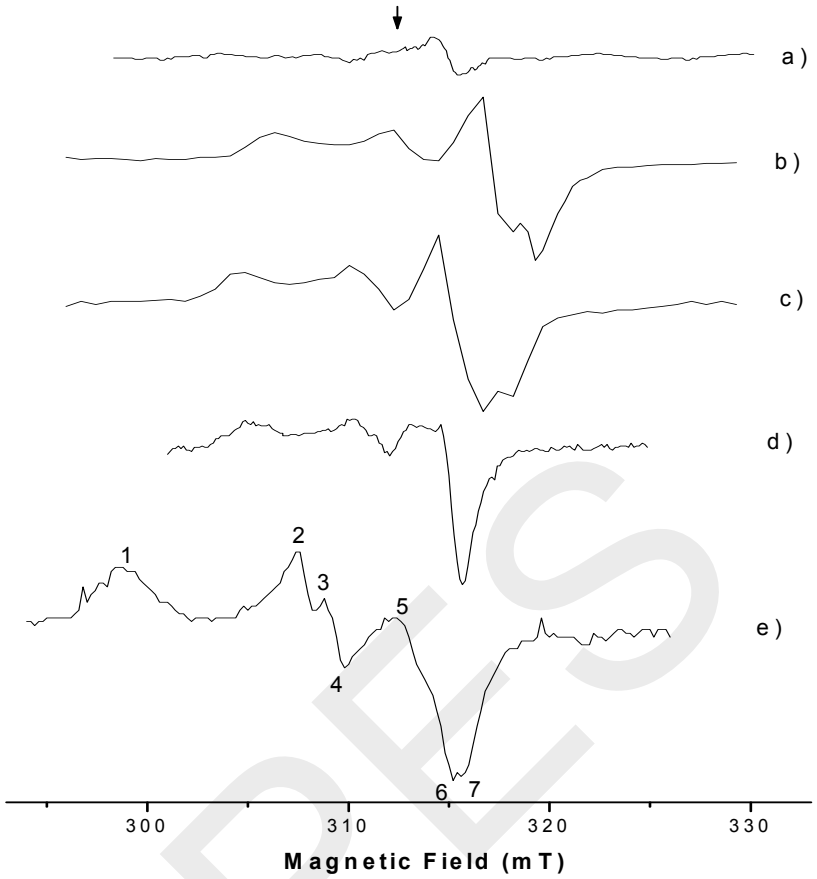


Figure 2. ESR spectra of fingernail samples.a) Pre-irradiated (background) signal, b) mechanically damaged, after 5 min, c) mechanically damaged, after 25 min, d) mechanically damaged after 2 hours, e) mechanically damaged after 2 hours and stored at liquid nitrogen (77 K) temperature.



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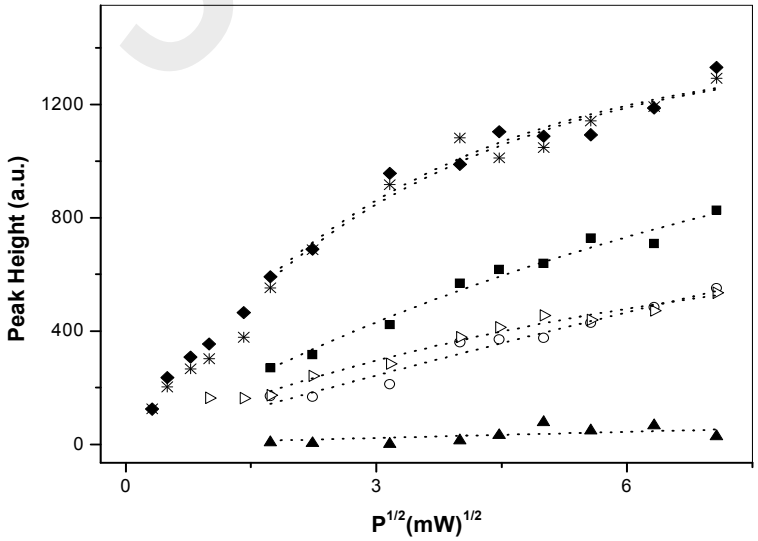

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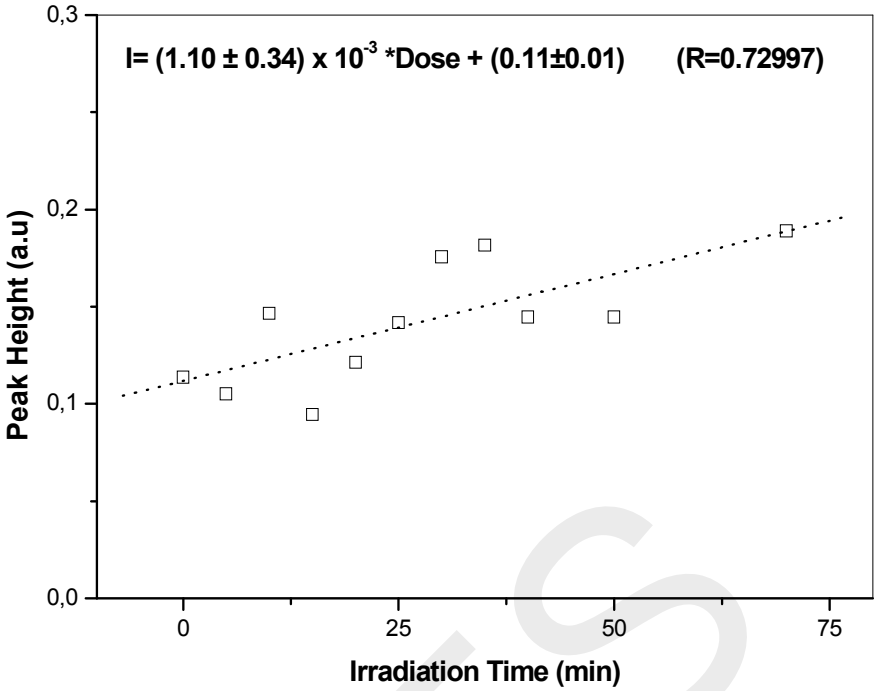


Figure 5. Variations of the peak heights with storage time. I_1 (□); I_2+I_4 (o); I_5+I_7 (▲); dotted lines: theoretical

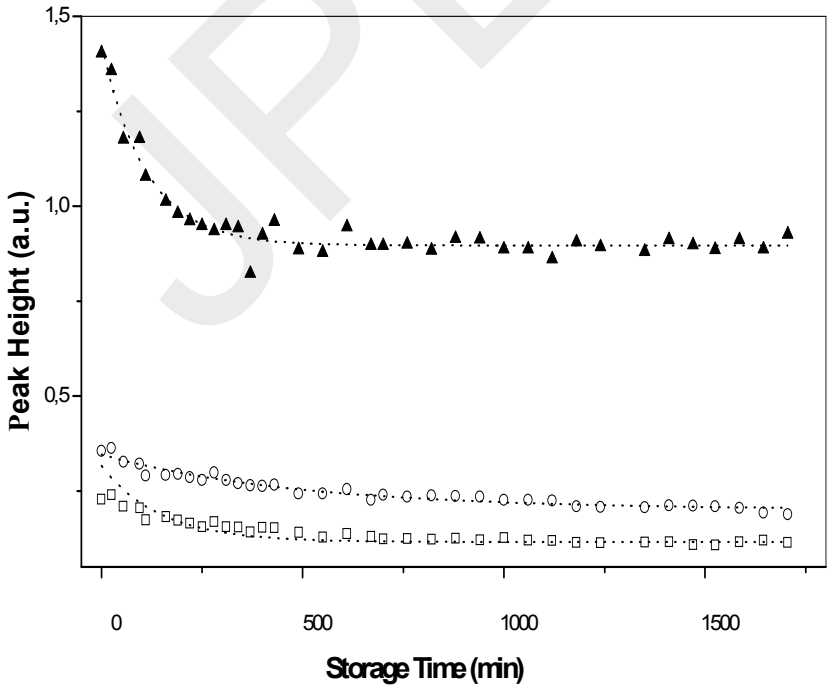


Figure 6. Variations of the I_5 +I_7 peak height of washed nail samples with storage time. Distilled water (□); acetone (o); ethyl alcohol (▲); dotted lines: theoretical.

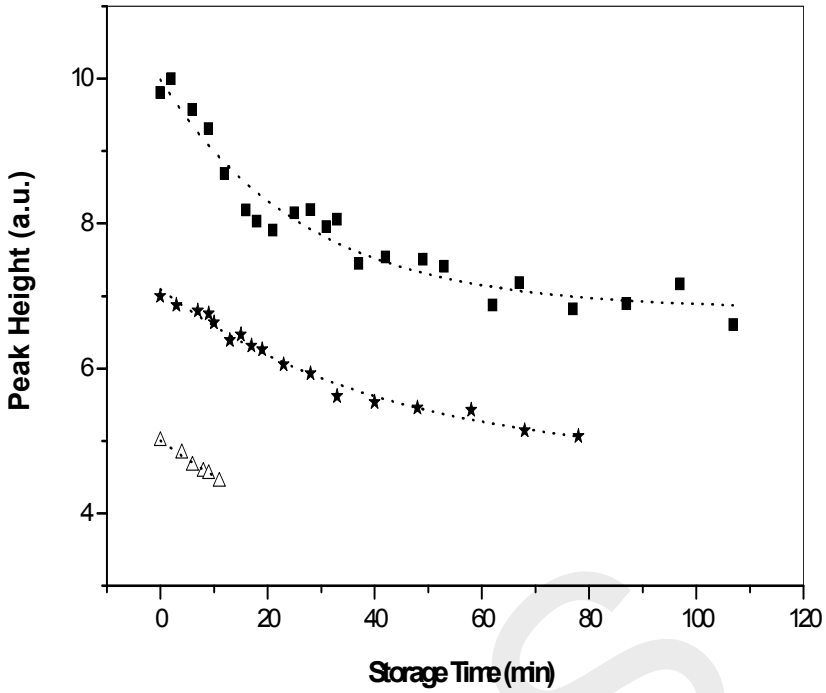
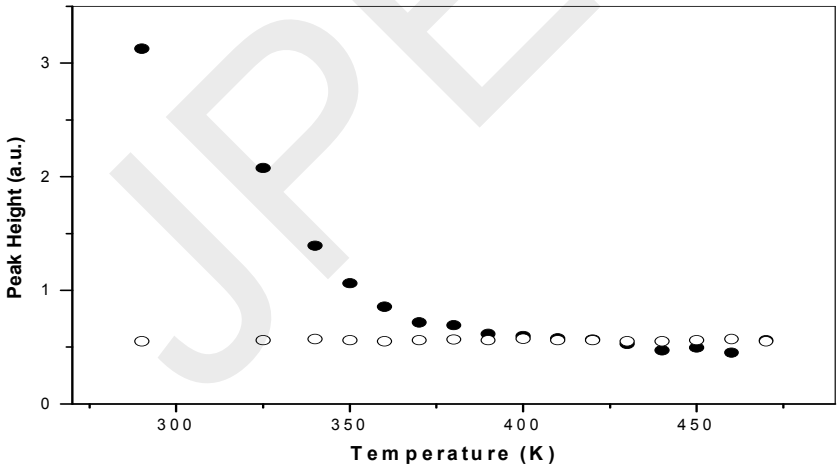


Figure 7. Variations of the I_5+I_7 height with temperature in the range of 290–470 K. Bolds: heating; open: cooling.



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