The use of human hair as biodosimeter

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ABSTRACT

Purpose: To investigate the potential use of human hair samples as biologic dosimeter by electron spin resonance (ESR) spectroscopy.
Subjects and methods: The hair samples were obtained from female volunteers and classified according to the colour, age and whether they are natural or dyed. Natural black, brown, red, blonde and dyed black hair samples were irradiated at low dose (5-50 Gy) and at high doses (75-750 Gy) by gamma source giving the dose rate of 0.25 Gy/s in The Sarayköy Establishment of Turkish Atomic Energy Authority.
Results: While the peak heights and g-values (2.0021 -2.0023) determined from recorded spectra of hair were color dependent , the peak-to-peak line widths were varied according to natural or dyed hair (ΔHpp: 0.522- 0.744 mT).In all samples, the linear dose-response curves at low doses was going to saturate after ~ 300 Gy. In black hair samples taken from different individuals, the differences in the structure of the spectrum and signal intensities were not observed. The ESR signal intensities of samples stored at room temperature for 22 days fell to their half values in 44 hour in black hair, 41 hours in blonde and brown hairs, 35 hours in dyed black hair and in 17 hour in red hair. The activation energies of samples annealed at high temperatures for different period of time were correlated well with those obtained in the literature.
Conclusions: The conditions in which hair samples can be used as a biological dosimeter were found. The dose estimation with human hair by ESR technique should be done within 1-2 days after irradiation.

Key Words: *Ionizing radiation; Biologic dosimeter; Hair; Electron spin resonance.*

Introduction

Application of ionizing radiation in many different fields is constantly increasing, including the use for energy and medical purposes, so it becomes very important to monitor people exposed to radiation. In case exposed people do not wear a personal dosimeter, a rapid and accurate method to meet the needs for effective and efficient triage after a large-scale radiation exposure event is required. This paper describes the potential use of human hair as physically-based biodosimetry method that uses electron spin resonance spectroscopy (ESR) for the emergency medical response to a large scale radiological/nuclear event. The usefulness of this method has been several times reported in the case of serious accidents (Clairand et al. 2006; Schauer et al. 1993, 1996; Desrosiers 1991) In ESR technique, depending on the material, a single measurement can take between some minutes up to a few hours. The readout is non-destructive, allowing for repeated measurements on the same sample. The ESR signal intensity is directly proportional to the amount of free radical specifically generated by ionizing radiation. But in case of soft a sample which has fast radical decaying after irradiation (Müller and Streffer 1991), the ESR method should be applied immediately on them. The biological tissue that have been proposed for ESR dosimetry should have some criteria such as ubiquity, non invasiveness and easiness of sample collection, presence of a post-irradiation ESR signal, negligible background signal, linearity of dose-response relationship, minimum detection limit and post-irradiation signal stability (Trompier et al. 2009a).

Human hair has a broad background signal, not negligible, due to melanin content of hair (Herrling et al. 2008). The signal stability is unfortunately limited several hours (Çolak and Özbey 2011). Nevertheless in detailed study the conditions of human hair to provide alternative biologic dosimeter in case of an emergency overexposure was investigated (Tepe Çam 2011). There are few studies investigated the dosimetric potential use of human hair have been reported

(Nakajima 1982; Trivedi and Greenstock 1993; Kudynski et al 1994; Alexander et al. 2007; Çolak and Özbey 2011) and still, there is a need to study dose response curves, activation energies, decay constants and biological variability for each colors of hair samples. In this sense in the present investigation, we provided more systematic information on radiation-induced radicals in hair than has previously been available. ESR technique was applied for all colors of hair samples for the issues mentioned above and for the first time the dyed hair samples were investigated in detail in this study.

Materials and Methods

Hair samples

The hair samples were obtained from young female volunteers (20-30 years old) and classified according to the color and whether they are natural or dyed. The samples used without any treatment except one cut sample and all were stored in small locked bags in the dark. Measurements were done on natural dark (three sample; DS N1, DS N4 and cut sampled N7), brown, red, blonde and dyed black hair (BS) samples obtained each from the same donors. The ESR spectra were measured prior to irradiation at room temperature and within 10 min after the irradiation. The samples were bent by hand, insert the ESR tubes along the active cavity region and fixed by using iron filled rod from top of the tube.

Irradiation

The samples were irradiated with ^{60}Co gamma rays at ambient conditions using gamma cell with a dose rate of 0.25 Gy/s at the Sarayköy Establishment of the Turkish Atomic Energy Authority in Ankara. The radiation doses between 5-50 Gy were named as low doses and doses between 75-750 Gy were named as high doses in order to get dose-response curve. The uncertainty in

radiation doses was nearly 3%. The absorbed dose at the sample location was checked by Fricke chemical dosimeter. An unirradiated hair (control) sample was also prepared for comparison purposes. The samples were protected from light during irradiation and transport to the measurement laboratory and then stored in the closed bags in the dark.

ESR measurements

ESR measurements were carried out using a Bruker e-scan X-band ESR spectrometer operating at 9.8 GHz. Samples were placed in standard pyrex tubes with inner diameter 4.0 mm not exhibiting any ESR signal. The ESR spectra were recorded at room temperature (open to air) under the spectrometer operating conditions of: sweep width 10 mT, microwave power 0.5-1 mW, modulation frequency 86 kHz, modulation amplitude 0.3 mT, and gain $1.5x10^2$. Each measurement was repeated three times. Signal intensities were calculated from the first derivative spectra and were normalized to sample mass. A strong pitch sample was used as a standard sample to determine the g-factors.

All ESR measurements were done at normal laboratory conditions (about 21 ± 2 °C and 25 ± 3 % relative humidity) about 10 min after the irradiation to observe the radiation-induced free radicals. For each color, the sample was placed in the cavity three times and at each time the ESR spectra of the sample were recorded separately. The mean value of the signal intensities were taken in order to minimize the error from the cavity-filling factor. A long term radical decay feature at room temperature was performed over a storage period of 22 days using a sample irradiated at a dose of 12 kGy. Decay kinetics of the radiation-induced radicals at three different temperatures [313, 333 and 353 K] was performed by using the samples irradiated at a dose of 12 kGy. The kinetic experiments were begun immediately after the irradiation to avoid

the radical decay at room temperature. The hair samples were transferred to water baths at temperatures mentioned above, then their ESR spectra were recorded regularly over a time interval of 0-90 min after cooling them to room temperature following the predetermined heating times (3, 6, 10, 15, 20, 25, 30, 35, 45, 55, 65, 75, 90 min). The activation energies of the involved radical species were calculated from Arrhenius plots.

Results and Discussion

ESR spectra of unirradiated human hair samples

The background ESR spectra of human hair samples that have not been washed, irradiated or mechanically damaged except one cut sample were recorded. To determine the optimum sample mass to be used in ESR measurements, the variation in the ESR peak height (I_{pp}) with sample mass was examined. The ESR peak height (Figure 1) varied linearly up to 30 mg and then, began to saturate above this mass value. Hence, in all experiments, the sample mass falling into this linear region was used.

Untreated hair samples, except dyed dark, were observed to exhibit a sharp ESR singlet. For dyed dark sample, a weak resonance line was also appeared at right side of the central resonance signal (~3465 G) (Figure 2). As shown in Table I, the background spectra recorded for hair samples indicated that the g-factor and peak-to-peak line width are color and structure (natural or dyed) dependent.

The origin of the ESR spectra of background human hair is known mainly due to the presence of melanin which is a pigment that determines the color of both human skin and hair (Commoner et al. 1954; Swartz et al. 1972). The characteristic of melanin is the presence of free radicals in their structure. Due to the presence of cystein found in melanin, human hair has free radicals which contribute to the background ESR spectrum. Thus, there is a background ESR

signal in all types and color of hair (Nakajima 1982; Trivedi and Greenstock 1993). The type of melanin varies in hair samples depends on its color (Bilinska 2001). There is eumelanins in brown-dark hairs and pheomelanins in blonde-red-light colors and these melanin types exhibit differences in their physical and chemical properties (Potten et al. 1996), it is probably the reason for the different ESR spectrum pattern and signal intensities recorded for each color. The experimental results showed that while dark hair sample has the highest ESR signal intensity, the blond hair has the lowest one. It is explained as follows; the melanin concentration in darker hair samples is greater than the less melanized (lighter) samples. Therefore, the intensity of ESR peaks for darker hair samples is expected to be correspondingly higher. The variation of the peak height of the background ESR spectrum of hair samples with storage time was investigated in literature (Çolak and Özbey 2011; Commoner et al. 1954) and they reported that the signal for untreated hair is very stable and no significant decay was observed in ambient conditions (room temperature and humidity) which identified biological free radicals in human hair and nail, such as melanin, and found them to be extraordinarily stable.

Irradiated hair ESR spectrums

Irradiation produced a significant increase in ESR resonance signal intensities of hair samples. Room temperature ESR spectra of hair samples irradiated at a dose of 625 Gy were given in Figure 3. The range of y-axis was kept same for each sample in order to compare the signal intensity dependency to the color and structure of the hair samples. As shown in Figure 3, the ESR signal intensities of irradiated natural dark (DS N1) and blonde hair samples are the greatest. While the blonde hair sample has the lowest background, after the irradiation, the increase in signal intensity is more severe than the other samples except the black hair sample. This indicates that the blonde hair sample has the high radiation sensitivity.

In irradiated samples the spectral parameters such as g-factors and line-widths did not change. However, the increases in signal intensities were observed for all the samples. This can be concluded that the radiation-induced radical or radicals are the same origin with the radicals in untreated samples. The g-factors and peak-to-peak line-widths of the ESR spectra in figure 3 are given in Table I. Similar to the background ESR spectra, they all were varied according to the color and structure (natural or dyed hair) of the hair samples. The spectrum of dyed hair sample compared with of natural hair samples, it was observed that the ESR signal intensity was less, the g-value and line-width were not changed in a large extend. It is in agreement with the study reported that when both pheo and eumelanin chelate paramagnetic metal ions such as Cu(II), Mn(II) or Fe(III), the apparent intensity of the observable ESR signal of the metal-doped melanin can be dramatically reduced, compared with the melanin with no paramagnetic metal ions (Sarna et al. 1981). It has been concluded that the loss of the melanin ESR signal amplitude (without apparent changes in the signal line-width) is purely magnetic in nature and does not involve any chemical reaction of the melanin radicals. It was declared that metal ions are connected to phenolic, hydroxyl and amine parts of the melanin pigment. For these reasons, we suggested more radicals generation for dyed hair sample than naturals after irradiation in the following sections.

The g-value of two natural dark hair samples (DS N1 and DS N4) and brown hair sample before irradiation was calculated as 2.0013. However, the g-value of the light-color samples (blonde and red hairs) and also the dyed black hair was 2.0028. For the sample DS-N7, the g-value was about 2.0023. The hair samples with large g-values had also the large line-widths, too. It was explained with the different types of melanin as eumelain in dark samples and pheomelanin in light samples. In DS-N7 sample, there can be a mixture of both melanin types in different percents. Using ESR spectroscopy, it has been shown that the comproportionation

equilibrium determines both the amount and type of free radicals present in different melanins (Felix et al. 1978a,1978b; Sealy 1982, 1984). Ortho-semiquinone and ortho-semiquinonimine radicals reflect the characteristics of eumelanins and pheomelanins, respectively. The ESR signal of pheomelanins, exhibits a distinct immobilized nitroxide like features with the hyperfine coupling ($2A_{zz}$) of about 3.0 mT (Sealy et al. 1982). The observed hyperfine coupling of pheomelanin ESR signal is due to a partial localization of the unpaired electron on the nitrogen atom of the ortho-semiquinonimine form of 1,4-benzothiazine subunits. That is why ESR spectrums of red and blonde hair samples spread over the wide magnetic field range compared to dark hair samples.

Variations of the peak heights with microwave power

Variations of the peak heights of the samples irradiated at a dose of 625 Gy with the applied microwave power were studied at first in the range of 0.1–6 mW. The results are given in Figure 4. The measured peak heights exhibited the characteristics of inhomogeneously broadened resonance lines. The signal intensities of resonance lines increased rapidly up to a microwave power of ~1.5 mW and then the increase slowed down for all the samples. This result was considered as a manifestation of the presence of similar radical species in gamma-irradiated hair samples. To keep the microwave power in unsaturated region is a general rule for ESR measurements. In the present study, the microwave power of 0.5 and 1 mW were adopted to avoid any saturation effects during the experiment.

Room temperature microwave saturation characteristics of the ESR singlet of unirradiated (control) hair samples were earlier investigated (Çolak and Özbey 2011). A decrease in signal intensity was observed above the microwave power of 4 mW. It points to the homogenously broadening nature of the observed resonance lines. The distinction between the microwave

saturation features of the background resonance signal and the radiation-induced resonance signal can be used to determine the number of radicals contributing to the resonance signal. This method based on the difference in microwave saturation behaviour was used for the detection of irradiated foods by Yordanov et al. 2005. In their work, typical curves of saturation of unirradiated and irradiated dry plants versus power were different.

Dose –response curve

The dosimetric features of hair samples were explored in the dose ranges of 5- 50 Gy (low doses) and 75-750 Gy (high doses), respectively. The samples were irradiated at doses of 5, 10, 20, 30, 40 and 50 Gy for low dose ranges to achieve this goal. The signal intensity variations with the applied radiation dose were constructed for the monitored resonance signals. The results are presented in Figure 5. Several mathematical functions such as linear, quadratic and power were tried to fit the experimental dose-response data without forcing the functions to pass through origin. A linear function of irradiation dose typed as $I = a+bD$ was found to describe well the dose-response curve for all hair samples. In this function I and D stand for ESR signal intensity and absorbed radiation dose in Gy. The parameter a represents the signal intensity of the unirradiated sample and the parameter b represents the rate of radical production or radiation yield upon irradiation. The calculated parameters (a and b) from fitting procedure are given in Table II. The theoretical dose-response curves calculated by using the parameter values given in Table II were also presented in Figure 5 (dashed lines). Linear dose-response behavior of the samples is a desired feature in terms of biological dosimeter. In order to investigate mechanical effect on dose response data, the natural dark hair sample cut in variable lengths (1-2 cm) was irradiated at low doses range (10-50 Gy) and their ESR spectrum were recorded. The use of cut hair samples did not changed the ESR spectrum shape. While the signal intensity of the

unirradiated sample (DS N7) was higher (% 20) compare to other unirradiated natural dark sample, after irradiation it was lower at all low doses (i.e. 67% lower compare to DS N1 at 50 Gy dose). However, the previous studies examined the mechanical effect on unirradiated hair and nail samples reported that it created more sulfur radicals, so increased the signal intensity (Symons et al. 1995; Chandra and Symons 1987).

The mathematical function described before were also used to fit the experimental dose-response data constructed for high doses (75-750 Gy). A power function of the applied dose typed as $I = a + bD^c$ was found to describe well the dose-response data of natural dark, red and brown hair samples. The parameter b and c represent the rate of radical production or radiation yield upon irradiation. However, an exponential function typed as $I = a+c[1-\exp(-bD)]$ was the best for dyed dark and brown hair samples. In the later, the parameter c represents the maximum number of radical produced. The calculated parameters (a and b) from fitting procedure are presented in Table III. Theoretical dose-response curves derived using the parameter values given in Table III for the power and exponential functions were also represented in Figure 6 to give an idea about the degree of agreement between experimental and theoretical dose-response data. The dose response curves in the dose range of 75- 750 Gy are in agreement with few studies in literature (Trivedi and Greenstock 1993; Kudynski et al. 1994; Dalgarno and McClymont 1989)

In order to verify the utility of the mathematical functions used, back-projected doses were calculated by entering the measured peak heights in the right equation according to hair color described in Table III. It was found that the applied dose could be determined with an accuracy of better than ±10% in the dose range of 75–750 Gy. The back projection procedure was applied for hair samples where dose-response curves were get long after irradiation. It was found that the applied doses could be estimated with an uncertainty of better than 30% even long after irradiation (Çolak and Özbey 2011).

Long-term decays of the peak heights at normal conditions

The changes in the peak heights with time were observed when the samples were stored at normal laboratory conditions (about 21 ± 2°C, 25 ± 3 % humidity) in the dark without any special conditioning before and after irradiation. This fading study covers records of ESR spectra over ~ 22 days storage period using the samples irradiated at a dose of 12 kGy. Fig. 7 shows the fading behaviour of the resonance line for all samples. After 22 days storage at laboratory conditions signal intensity decreased to about 30% of the initial value (recorded 10 min. after the irradiation) for natural dark hair, 31% for brown, 54% for red and dyed dark, and 29% for blonde hair samples . The ESR signal intensities of resonance lines for all samples were found to decrease very rapidly in the first few hours of storage, then the rate of decrease became slowly after ~200 hours. The ESR signal intensities of the samples stored at room temperature for 22 days fell to their half values in 44 hours in black hair, 41 hours in blonde and brown hairs, 35 hours in dyed black hair and in 17 hours in red hair.

Room temperature decay data were described through a model based on the assumption of the presence of two different radical species (for natural dark, brown, red and blonde) and three radical species for dyed dark hair sample having different decay constants. Hence, a function such as $I = \sum_{i=1}^{2} (I_{0i} e^{-k_i t})$, which consists of the sum of two/three different exponential functions each representing different contributing radical species and exhibiting first-order decay kinetics, was used to fit the experimental long-term decay data obtained for studied peak heights (Fig. 7). In this function, I_{0i} ‘s and k_i ‘s are the contribution weights of the radical species to the interested peak height and decay constants, respectively, of the involved radical species, and t is the time elapsed after stopping irradiation. Calculated parameters from fitting procedure, describing well the experimental decay data, are presented in Table IV. Decay constants and percent weights given in Table IV were used to calculate the theoretical decay data. The results are also given in

Figure 7 as dashed lines with their experimental counterparts for comparison. From the inspection of Figure 7, it can be seen that the model based on the presence of two and three different radical species with different decay characteristics describes fairly well the experimental long-term decay data. The decay constants were varied between $6.4x10^{-6}$- $16x10^{-6}$ s^{-1} according to hair color. The decay constant for brown hair sample was found $4.67x10^{-6}$ s^{-1} for brown and blonde hair samples. In literature, the decay constant for brown hair sample was found as $1.5x10^{-4}$ s^{-1} (Kudynski et al. 1994; Çolak and Özbey 2011). However, it should be noted that the signal intensity decay depends on various parameters such as humidity, temperature, etc.

Annealing study results and activation energies of contributing species

The decays in signal intensities at high temperatures (313, 323 and 333 K) were also investigated. Radical species decay faster at high temperatures due to increase in the molecular motions. To test this idea and to determine the decay constants and activation energies of the contributing radical species, annealing studies were performed by using the water baths at normal laboratory conditions. The natural dark and blonde and dyed dark samples irradiated at 12 kGy were annealed at three different temperatures (313, 323 and 333 K) for predetermined times between 3 and 90 min. Although the samples were annealed at predetermined temperatures, all the ESR spectra were recorded at room temperature after cooling the samples down to room temperature. The ESR signal intensities were determined at each annealing time for each annealing temperature and it was plotted versus the annealing time. The results are shown in Figure 8. As is seen from this figure, the signal intensities decay fast for all the samples with increasing temperature. The experimental decay data at each annealing temperature were fitted to the sum of two/three first-order decay functions, as in the case of long-term decay at room temperature. A

sum of two first-order decay functions represented radical types A and B for natural dark and blonde hair samples and three ones represented radical types A, B and C for dyed dark hair sample were found to describe best the experimental decay data. The rate constants (k_A, k_B and k_C) of the contributing radical species were calculated from the fitting for each isotherm (Table V) and the theoretical decay data calculated by using these rate constant values were also given in Figure 8 as dashed lines. As it is seen from this figure, the agreement between experimental and theoretical decay data is fairly well.

The rate constant k is expected to exhibit an exponential dependence on the temperature of the type [$k(T) = k_0 \exp(-E/RT)$], where E is the activation energy, R the gas constant, k_0 the frequency factor and T the absolute temperature. If so, the $\ln(k) - 1/T$ plot should give a straight line whose slope is proportional to the activation energy. The reaction activation energy values for the contributing radical species were calculated from these plots (Fig.9). The activation energy values for radical types A and B were found as 60.0 and 27.3 kJ/mol, respectively, for natural dark sample; the values of 52.3 and 27.6 kJ/mol were found, respectively, for blonde sample. In case of dyed dark sample, we proposed a third radical of type C in addition to the radical types A and B. The activation energies of the radical types A, B and C were found as 55.3, 29.3 and 37.3, respectively, for dyed dark sample. The activation energy values for melanin pigments were reported as 0.49-0.76 eV (47.3-73.3 kJ/mol) (Jastrzebska et al. 1995) and 0.1-0.78 eV (9.6-75.3 kJ/mol) (Strzelecka 1982). These results indicate that the activation energies calculated for the proposed radical types, in the present study, are in good agreement with the previous results.

Conclusion

- It was determined that unirradiated and irradiated samples could be distinguished even at the minimum radiation dose of 5 Gy used in the present study. In few studies, the minimum detectable dose was found as 3 Gy and 6 Gy (Trivedi and Greenstock 1993; Çolak and Özbey 2011). However, the melanin signal is so strong that it is unlikely that hair could be suitable at the dose levels needed for triage (2 Gy). Some significant instability in the radiation-induced signal would also impact its use for triage.
- The dose-response curves of the hair samples studied in the present work were found to be described well by a linear function at low doses. However, they were going to saturate after the irradiation dose of ~300 Gy at high doses. The absorbed radiation dose can be determined from back extrapolation technique with the accuracy better than 10% just after irradiation. Thus, hair samples can be accepted as good candidates for biological dosimeter basing on these results.
- Natural dark and brown hair samples have higher radiation yield as expected.
- There were not observed any differences in ESR spectrum characteristics of the black hair samples taken from different individuals. It shows the independence of dose-response curve to he biological differences of individuals for black hair sample.
- The ESR signal intensity of the samples stored at room temperature for 22 days fell to their half values in 44 hours for DS, 41 hours for yellow and brown colors, 35 hours for BS and 17 hours for red hair. Therefore, the dose estimation by ESR technique with the hair samples should be done within 1-2 days after irradiation. However, the measurements can be made from immediately up to several weeks after exposure using nails and indefinitely using teeth

after the event during which triage and assessment would be pertinent (Black and Swarts 2010; Desrosiers and Schauer 2001; Symons et al. 1995; Trompier et al. 2009b).

- The activation energy of the radical type A is higher than the others (radical type B and C). The species which have high activation energy are more stable than the others. This result shows that the fast decreases in the first few days are responsible from the decays of the radical types B and C.

We showed the limitations of ESR on hair as a method for triage, but we are providing more systematic information on radiation-induced radicals in hair than has previously been available.A protocol for hair sample can also be written including the sample collection, storage conditions and ESR measurements in emergency situations as done for fingernail samples (Trompier et al. 2007; Wilcox et al. 2010; Alexander et al. 2007). To prepare a biological dosimetry protocol, it is required that so many studies should be performed on the same kind of samples, thus the generalized findings could be get for each individual. In this sense, the present work will contribute significantly to the literature.

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Declaration of interest

The authors report no declarations of interest.

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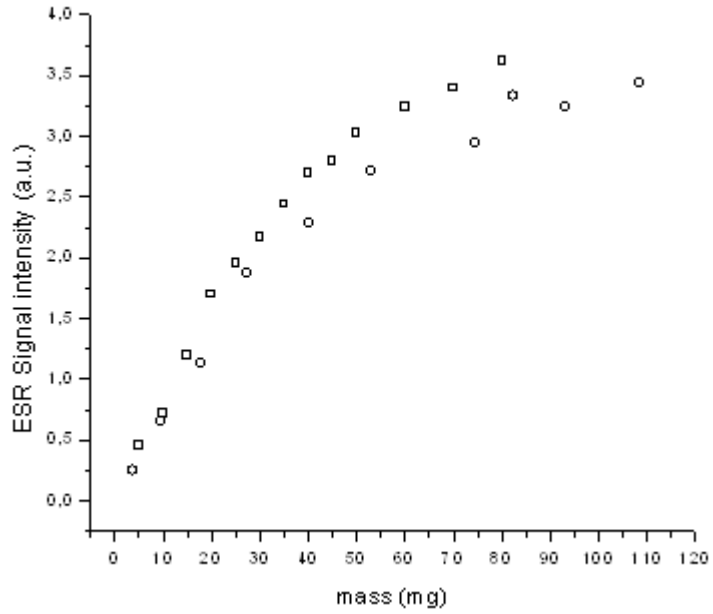


Figure 1. Variations of ESR signal intensity with sample mass: □ (natural dark); ○ (brown).

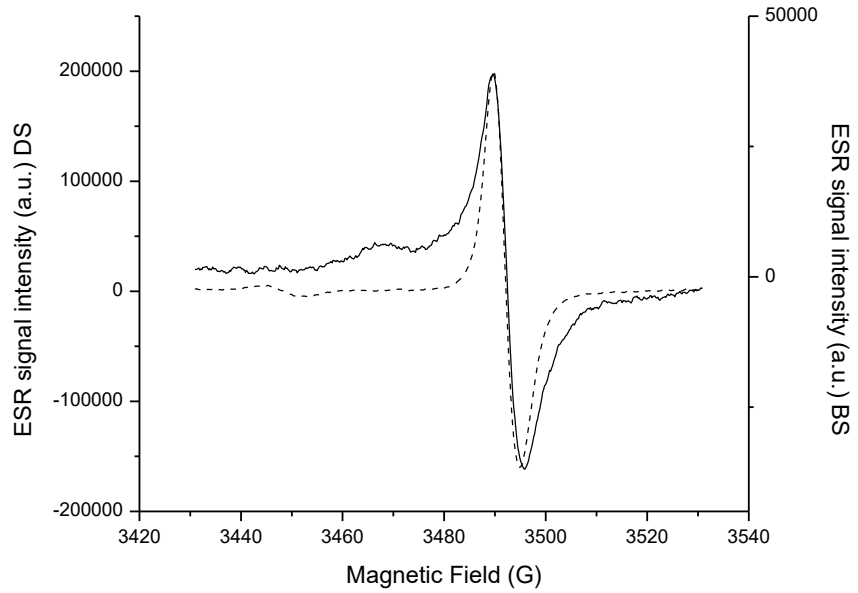


Figure 2. ESR Spectrums of unirradiated natural dark (DS N1) (---) and dyed dark (BS) (—) hair samples.

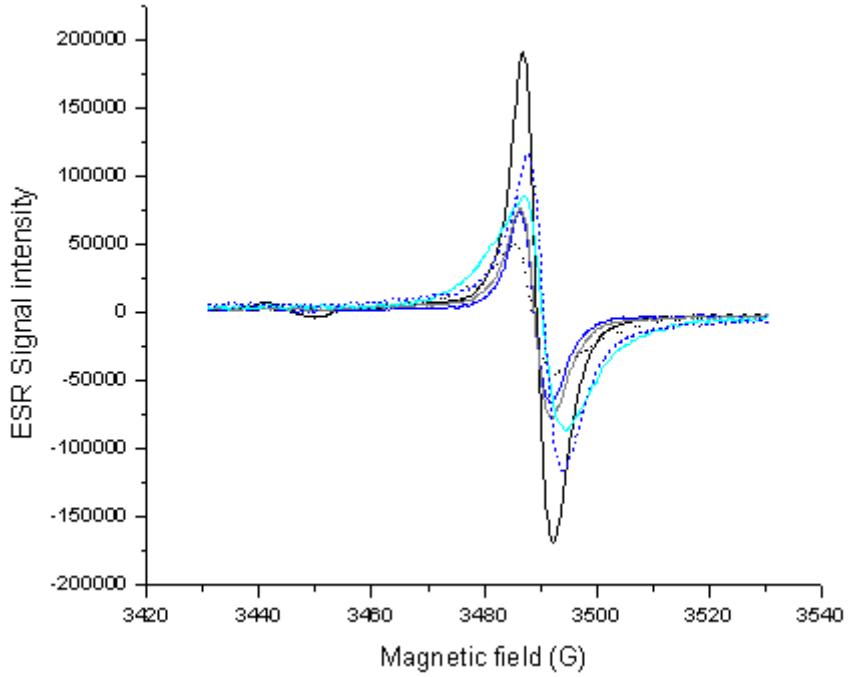


Figure 3. Irradiated hair ESR spectrums; (—) DS N1; (—) DS N4; (…) BS; (—) brown; (—) red ; (---) blonde

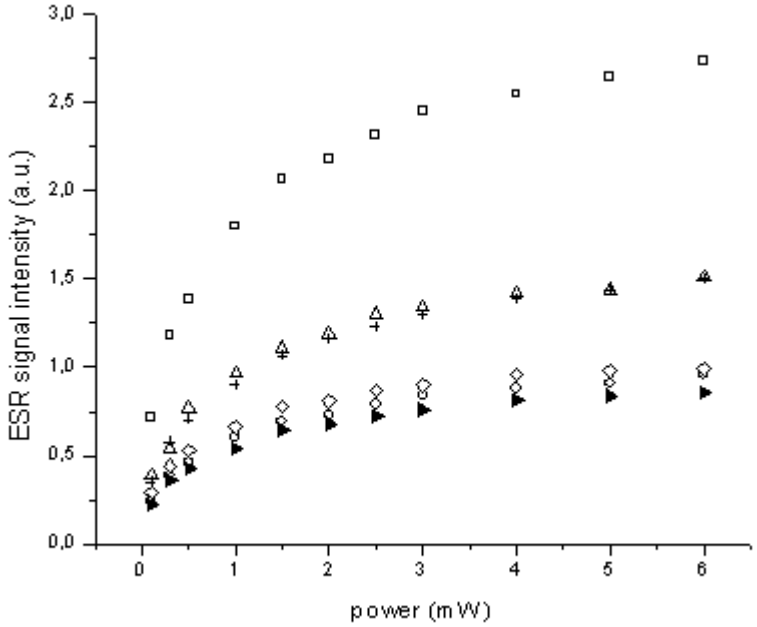


Figure 4. Variations of ESR signal intensity with microwave power. Samples were irradiated at 625 Gy dose [(□) DS; (Δ)red; (+) DS N4; (◊) blonde; (○) BS; (►) brown]

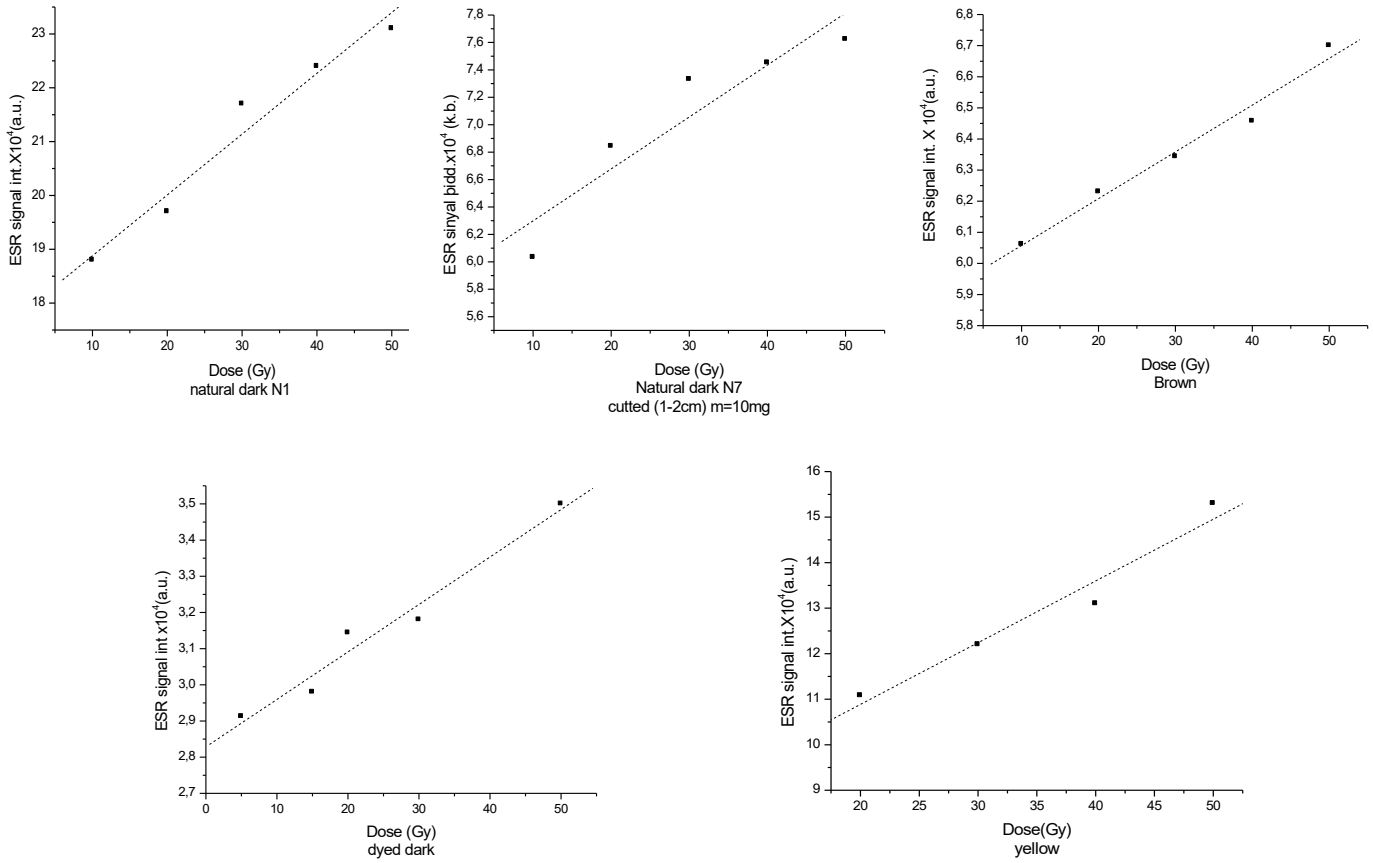


Figure 5. Variations of signal intensities with applied radiation doses in the dose range of 5-50 Gy. [(n) experimental; (---) theoretical]

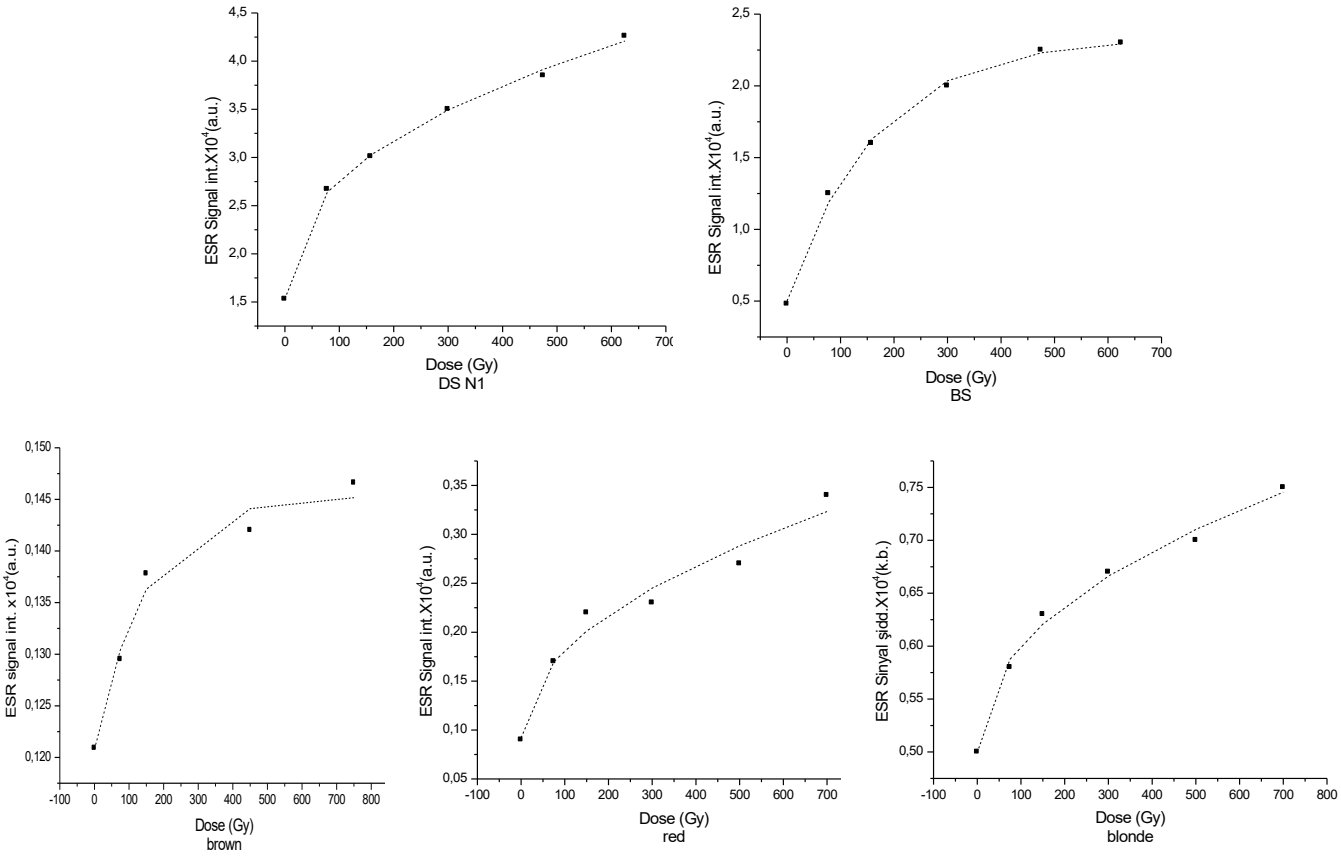


Figure 6. Variations of signal intensities with applied radiation doses in the dose range of 0-750 Gy. [(n) experimental; (---) theoretical]

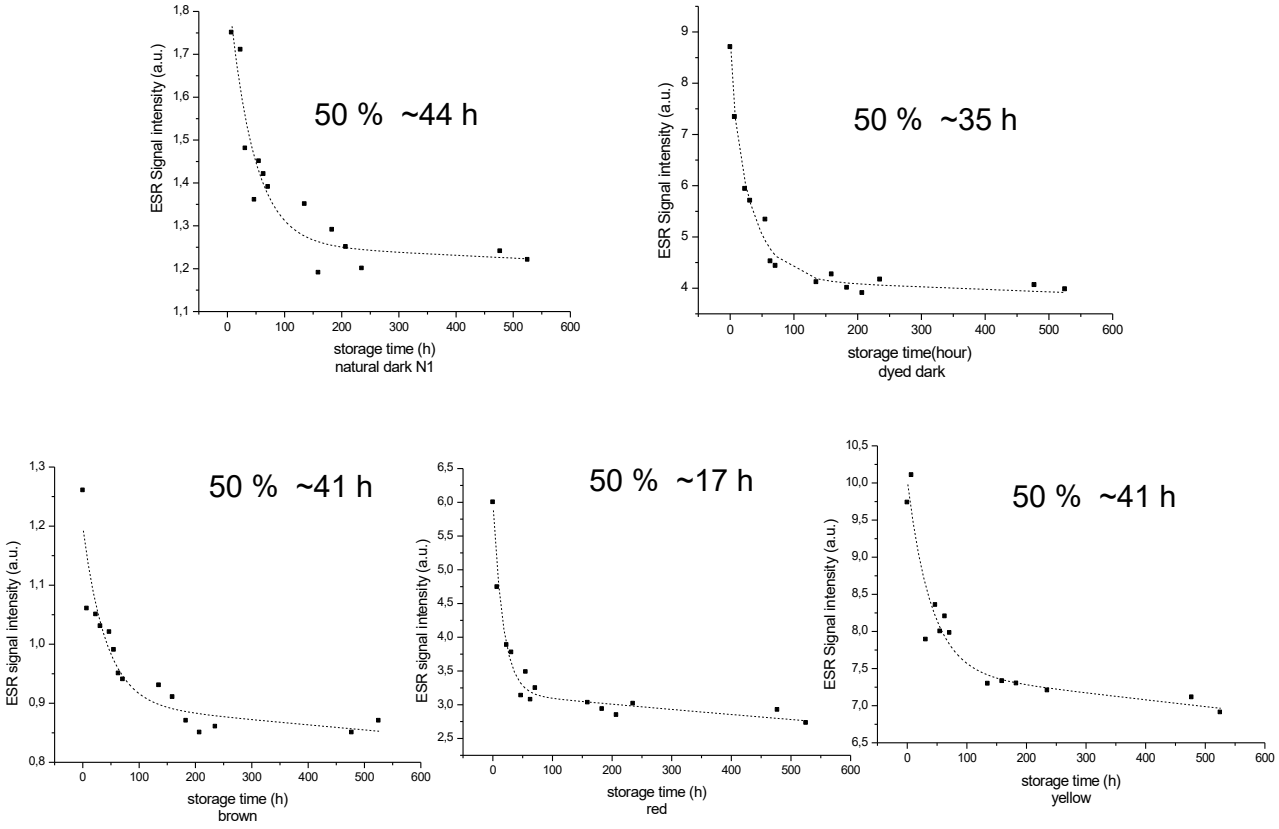


Figure 7. ESR signal decaying at room conditions

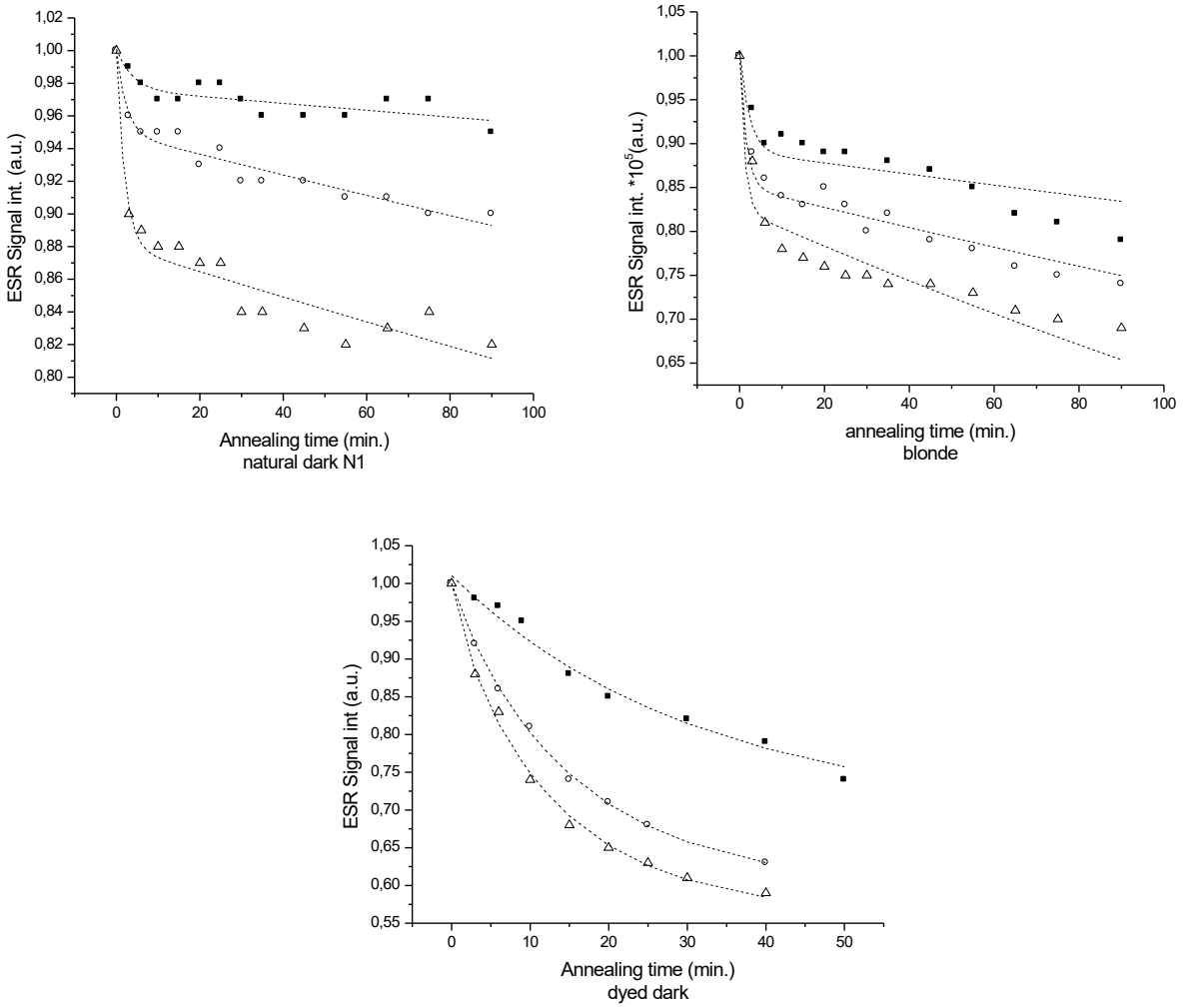


Figure 8. Variations of ESR signal intensity with annealing time at high temperatures

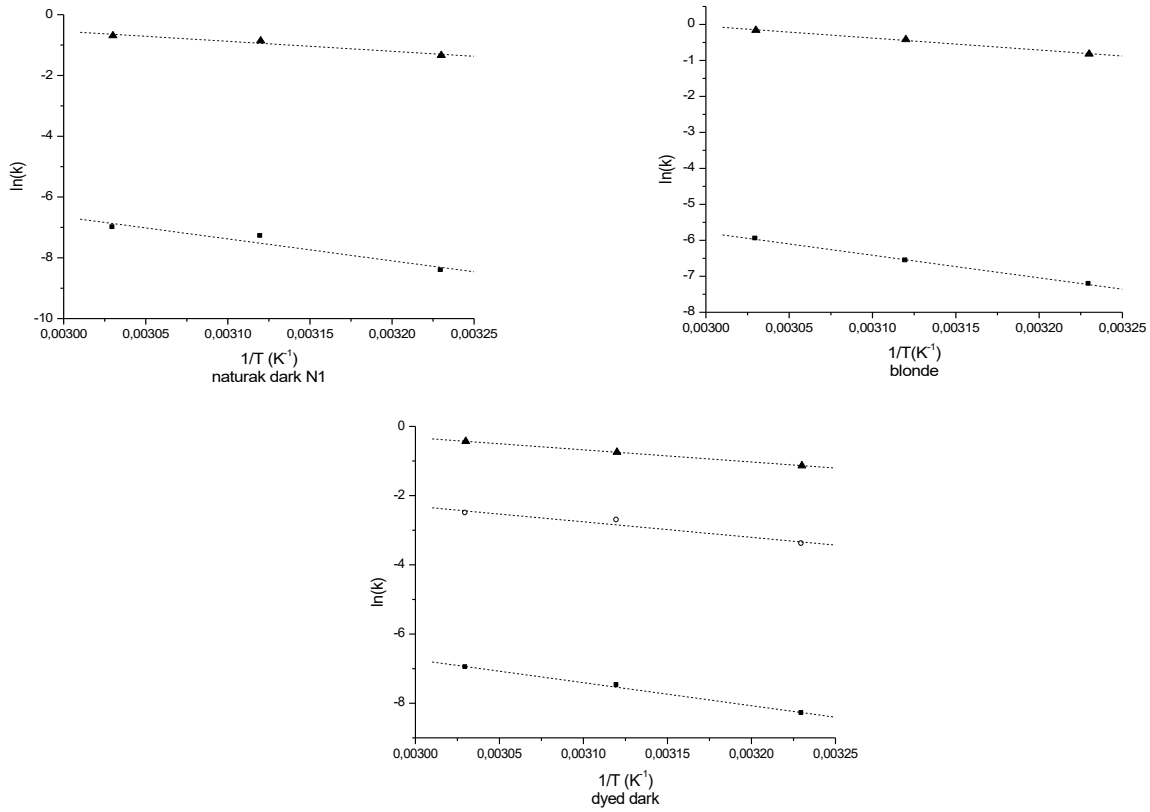


Figure 9. Variation of reaction rate constants with temperature (Arrhenius plot). Symbols (experimental), dashed lines are the best fitting lines.

Table I g-factors and peak-to-peak line widths (ΔHpp) of unirradiated and irradiated hair samples at a dose of 625 Gy.

Sample	Before irradiation		After irradiation	
	g	ΔH_{pp}(G)	g	ΔH_{pp}(G)
DS N1	2.0013	5.1	2.0021	5.4
DS N4	2.0013	5.2	2.0021	5.4
DS N7	2.0023	5.2	2.0023	5.2
BS	2.0028	6.2	2.0021	7.3
brown	2.0013	5.1	2.0020	5.7
red	2.0028	6.1	2.0023	7.4
blonde	2.0028	7.2	2.0023	6.2

Table II The calculated parameters from fitting the dose-response data of the hair samples. The tried function in fitting procedure: I = a+bD.

Sample	Parameters		
	a	b	r^2
DS N1	17.75	0.113	0.9801
DS N7	5.92	0.038	0.9340
BS	2.83	0.013	0.9826
Brown	5.91	0.015	0.9890
red	1.75	0.023	0.9612
blonde	8.17	0.136	0.9735

Table III The calculated parameters from fitting the dose-response data of the hair samples.

Function		**Natural dark**	**Dyed dark**	**N.brown**	**N. red**	**N.blonde**
$I = a+bD^c$	a	1.5344	0.4696	0.1207	0.0921	0.4994
	b	0.1741	0.1568	0.0022	0.0095	0.0122
	c	0.4244	0.3890	0.3720	0.4875	0.4589
	r^2	0.9983	0.9900	0.9691	0.9671	0.9928
$I = a+c[1-\exp(-bD)]$	a	1.6312	0.5013	0.1208	0.1073	0.5074
	b	0.0049	0.0060	0.0067	0.0030	0.0042
	c	2.5879	1.8332	0.0245	0.2426	0.2394
	r^2	0.9761	0.9973	0.9770	0.9284	0.9782

Table IV Decay constants for time variations of irradiated human hair samples stored at room conditions

Hair Colour	Decay constants (h^{-1})			Relative Contribution			r^2
	k_A (x10^{-2})	k_B(x10^{-4})	k_C(x10^{-2})	I_{0A}	I_{0B}	I_{0C}	
Natural dark	2.297	0.5		0,611	1,257		0,9002
	2.797	1.2	2.021	3,648		4,170	0,9650
Dyed dark	2.434	1.0		1,202			0,9010
Brown	2.515	1.3		0,300	0,899		0,9140
Blonde	5.865	2.6		2,589	7,457		0,9751
Red				2,875	3,170		

Table V Decay constants relevant to the proposed radicals at high temperatures.

Radical	**Temperature**	**Decay constant k (min^{-1})**		
		Natural dark	**Blonde**	**Dyed dark**
A	313	0.00022	0.00073	0.00025
	323	0.00068	0.00141	0.00056
	333	0.00091	0.00258	0.00094
B	313	0.26090	0.44010	0.32152
	323	0.42308	0.65890	0.47500
	333	0.50060	0.85260	0.65121
C	313			0.03345
	323			0.06664
	333			0.08101