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# ESR investigations of gamma irradiated medical devices

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## HIGHLIGHTS

- The standardization of gamma sterilization conditions is proposed.
- GTR/GBR biomaterials can be very good candidates for the radiosterilization process.
- ESR is an appropriate spectroscopic technique in gamma radiation sterilization.
- ESR could be used in monitoring the gamma radiation sterilization of biomaterials.

## ARTICLE INFO

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## ABSTRACT

Guided tissue regeneration (GTR) and guided bone regeneration (GBR) biomaterials have been employed in recent years for periodontal procedures. In the present study, widely used dental GTR/GBR biomaterials (grafts: G1, G2, G3 and membranes: M1, M2, M3, M4) were exposed to gamma irradiation at an absorbed dose range of 0–50 kGy and the radiolytic intermediates that have been created in the samples upon irradiation were characterized in detail by Electron Spin Resonance (ESR) spectroscopy. We aimed to standardize the measurement conditions for practical applications of gamma radiation sterilization of GTR/GBR biomaterials. We investigated the characteristic features of free radicals in gamma irradiated GTR/GBR biomaterials and examined the stability of the induced radicals at room temperature and accelerated stability conditions with ESR spectroscopy including dose-response curves, microwave power studies, dosimetric features of the biomaterials, variations of the peak heights with temperature, and long term stabilities of the radical species. Long-term stability studies have shown that G1 is quite stable even in accelerated storage conditions. The signal intensities of graft-type GTR/GBR biomaterials stored in normal and stability conditions have decreased very rapidly even only a few days after gamma irradiation sterilization. Thus, those samples indicating relatively low stability features can be very good candidates for the radiosterilization process. The beta-tricalcium phosphate and PLGA containing G1 and M1 respectively have found to be the most gamma stable bone substitute biomaterials and be safely sterilized by gamma radiation. ESR spectroscopy is an appropriate technique in giving important detailed spectroscopic findings in the gamma radiation sterilization studies of GTR/GBR biomaterials.

## 1. Introduction

Tissue engineering has evolved from the use of biomaterials (Vats et al., 2003) which play an essential role by guiding new tissue growth both in vivo and in vitro (Griffith, 2002). A biomaterial was defined as "any substance or combination of substances, synthetic or natural in origin, which may be used for any period of time, as a whole or as a part of a system which treats, augments, or replaces any tissue, organ, or function of the body" (A Consensus Development Conference on the Clinical Applications of Bio-materials. National Institutes of Health, Bethesda, Maryland, USA) (Vats et al., 2003).

The biomaterial market is expanding rapidly, parallel to the growing demands for bone substitute biomaterials with better bulk and surface properties with better integration with host (Griffith, 2002; Kohane and Langer, 2008). Thus, the biomaterials market has focused on the development of biomimetic materials that are chemically and mineralogically identical to the inorganic part of bone and they evoke specific cellular responses and facilitate new bone formation and regeneration in a safe, reliable, economic, physiologically and esthetically acceptable manner for minimally invasive and less painful dental

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applications (Kohane and Langer, 2008; Shin et al., 2003; Kumar et al., 2013; Sadlo et al., 2012).

A wide range of polymeric biomaterials, nanoceramics–polymer composites, and bioactive materials such as calcium phosphates and bioglasses and ceramics such as hydroxyapatite (HAp) and tricalcium phosphate (TCP) and poly (lactic acid) (PLA) and poly (glycolic acid) (PGA) and poly (lactic-co-glycolic acid) (PLGA), have been used as bone substitute materials and for guided tissue regeneration (GTR) and guided bone regeneration (GBR) (Rezwan et al., 2006; Burg et al., 2000).

The bone substitute biomaterials used in GTR/GBR are in direct contact with tissues and due to their resorption in the body it is necessary to sterilize the whole product before administration to the patient. These bone substitute materials benefit from receiving regulatory approval and it is essential that these products meet the pharmacopoeia requirements of sterility (Croonenborghs et al., 2007).

Today, gamma radiation sterilization, has been accepted as a widely used method for the sterilization of single-use medical products due to its high penetrating power, low chemical reactivity, low measurable residues, and small temperature rise (Croonenborghs et al., 2007; Özer et al., 2013). However ionizing radiation may affect the performance of some of the biomaterials used in medical devices and this is directly related to the radiation dose (Dillow et al., 1999). Thus researchers need to know the doses these items have received and material compatibility is one of the most important issues to be examined before sterilization can be applied to medical devices (Özer et al., 2013; Turker et al., 2013).

Absorbed radiation dose is one of the most critical parameters in gamma radiation sterilization. A minimum absorbed dose of 25 kGy is regarded as adequate for the purpose of sterilizing pharmaceutical products without providing any biological validation (Özer et al., 2013; The use of ionizing, 1991; Dorati et al., 2008). However, certain undesirable chemical and physical changes may accompany and new radiolytic intermediates following the ionizing and excitation mechanisms during gamma radiation may produce, even with the traditionally applied dose of 25 kGy. The free radicals might have negative effect on patients` health due their potential to cause DNA damage and mutations leading to the development of cancer, which is one of the major problems of the gamma radiation sterilization (Croonenborghs et al., 2007; Türker et al., 2014, 2011; Todica et al., 2013).

A great deal of effort is therefore focused on characterizing the nature and extent of the induced radiolytic products. Dillow et al. Türker et al. (2014); Türker et al. (2011) also reported that the effect of degree of gamma sterilization observed is directly related to the radiation dose. So, material compatibility is one of the most important issues to be examined before industrial radiation sterilization can be applied to medical devices and researchers need to know the doses these items have received (Özer et al., 2013). As recommended by the International Organization for Standardization (ISO) the sterilization dose must be set for each type of product depending on its bioburden (Türker et al., 2014, 2011). Therefore, the principal problem in radiosterilization is to determine and to characterize these physical and chemical changes originating from high-energy radiation (Özer et al., 2013).

***Electron Spin Resonance (ESR) Spectroscopy,*** which is a very sensitive method for detection of radical intermediates induced in irradiated drugs and/or drug raw materials that appears to be well suited for their characterization as well as the detection of irradiated drugs (Colak and Korkmaz, 2003; Colak, 2016). Moreover, its high sensitivity, precision, ease and its non-destructive readout are the other important advantages of ESR spectroscopy (Morehouse and Desrosiers, 1993; Parlato et al., 2007; Onori et al., 1996). ESR yields both, qualitative and quantitative information so by this method it is possible to detect and to distinguish irradiated drugs from unirradiated ones (Özer et al., 2013; Turker et al., 2013; Türker et al., 2014, 2011). ESR can detect all kind of paramagnetic species, which possess one or more unpaired electrons, namely, free radicals, biradicals and triplet excited states and is used to measure the produced defects that are stable with time (Da Costa et al., 2004; Miyazaki et al., 1994; Desrosiers et al., 1991).

**Table 1**
GTR/GBR biomaterials used for gamma radiation sterilization processes.

| Code | Composition |
|---|---|
| G1 | TCP (500–1000 μm) |
| G2 | Bioglass (90–710 μm) |
| G3 | Equine bone tissue (500–1000 μm) |
| M1 | PLGA |
| M2 | PLA |
| M3 | Collagen |
| M4 | Collagen |

A number of different bone substitute biomaterials are currently available for clinical use. However, the structural modification of the different dental biomaterials under gamma irradiation with ESR has yet to be fully investigated, and this represents the aim of our work. A detailed analysis (mechanical, sterility and SAL, etc.) concerning the effects of gamma radiation sterilization of the materials used in this work has been published by this group elsewhere (Türker et al., 2014, 2011).

In the present study, we have used the technique of ESR, to characterize the free radical(s) induced upon exposure of different types of dental GTR/GBR biomaterials (grafts: G1, G2, G3 and membranes: M1, M2, M3, M4) (Table 1) in the gamma irradiation dose values of 5, 10, 25, and 50 kGy. We have also subsequently examined the stability of the induced radicals with long-term ESR studies for the stored samples that in different storage conditions. The main goal of the present work is to investigate the paramagnetic species in the irradiated bone substitute biomaterials by ESR in order to determine the suitable biomaterials for this purpose. In this work, the characteristic features of the radiolytic intermediates produced upon irradiation of G1, G2, G3, M1, M2, M3 and M4 samples were studied by ESR spectroscopy in detail, where the compositions of the samples are listed (Table 1).

## 2. Experimental procedures

### 2.1. Materials and methods

GTR/GBR biomaterials were non-sterile and all the chemicals used in the experiments were of analytical grade.

#### 2.1.1. Gamma radiation procedures

All irradiation procedures were performed under normal conditions (25 °C, 60% relative humidity), in dark, using a $^{60}$Co gamma cell (4523Ci, Hungary) supplying a dose rate of 1.28 kGy.hr$^{-1}$ as an ionizing radiation source at the Sarayköy Gamma Radiation Facility of Turkish Atomic Energy Agency in Ankara. Fricke dosimeters were placed to measure the actual absorbed dose during the irradiation process.

Unirradiated samples were used as control to detect changes resulting from the action of ionizing radiation. Even though commercial radiation applications do not normally exceed 25–30 kGy, lower radiation doses were also used in this study to determine the physical changes in the properties of the bone substitutes biomaterials, which are expected at lower radiation doses and 25 kGy is the recommended dose by pharmacopoeias, while 50 kGy is an illustration of the product safety. A value of 25 kGy is the minimum absorbed dose considered adequate for purpose of sterilizing pharmaceutical products without providing any biological validation (The use of ionizing, 1991; Dorati et al., 2008; Geze et al., 2001). This reason led us to consider these irradiation doses that are the most commonly used by the pharmaceutical industry.

ESR measurements of GTR/GBR biomaterials were performed using

**Table 2**
ESR spectrometer operating conditions adopted throughout the experiments.

| | |
|---|---|
| Central field | 350 mT |
| Sweep width | 20 mT |
| Microwave frequency | 9.85 GHz |
| Microwave power | 1 mW |
| Modulation frequency | 100 kHz |
| Modulation amplitude | 0.1 mT |
| Receiver gain | $6.3 \times 10^3$ |
| Sweep time | 83.89 s |
| Conversion time | 81.92 s |
| Time constant | 327.68 s |
| Number of scan | 10 |
| Temperature | Room temperature |

a Bruker EMX 131 X-band spectrometer operating at 9.5 GHz, equipped with a cylindrical cavity (ER 4119HS) after positioning samples in the microwave cavity by a quartz ESR tube of 3 mm inside diameter. The ESR spectrometer operating conditions adopted during the experiments are given in Table 2. The evolutions of the ESR signal with the applied microwave power, absorbed radiation dose, temperature dependency and storage time were followed from recorded experimental spectra by measuring the heights of the signals from spectra base lines. A DPPH (diphenylpicrylhydrazyl) sample was used as the standard material where the *g*-value of DPPH is 2.0036. A digital temperature control system (Bruker VT3000 ER4131-VT) is used for the temperature studies of the samples inside the microwave cavity.

Optimum ESR operating conditions have chosen depending on several important parameters such that both microwave power and modulation amplitude were selected to be far away from the saturation of the samples, receiver gain and scan time were chosen to give a good signal-to-noise ratio (Garcia et al., 2009).

Microwave power level of 1 mW was set during the experiments, as the ESR signals of the samples were not saturated at that value. Irradiated samples were subsequently transferred into a tube with an inner diameter of 4 mm and outer diameter of 5 mm for ESR analysis. The spectral parameters of the radical type(s) induced upon gamma irradiation, such as spectroscopic splitting factor (g-value) and peak-to-peak width ($\Delta H_{pp}$), were calculated for the recorded ESR spectra of the investigated samples. During the experiments, all the samples were normalized to the mass of the sample, signal intensity of the standard material (DPPH), and the receiver gain value used in the experiments (Colak and Korkmaz, 2003; Marrale et al., 2014).

Stability tests were performed under normal (252 °C, % 405 relative humidity) and accelerated (402 °C and %755 relative humidity) conditions over a period of 3 months. For the accelerated aging condition tests, the samples were stored in the climate chamber and aliquots were taken off for the ESR measurements. For comparison, unirradiated samples were used as negative control. So as to determine the stability features of the radical intermediates produced upon irradiation, the ESR spectra of the samples that were stored open to air for 3 months both at room condition (252 °C, % 405 relative humidity) and at accelerated aging condition (402 °C and %755 relative humidity) were recorded at predetermined time intervals and the ESR spectra recorded were compared.

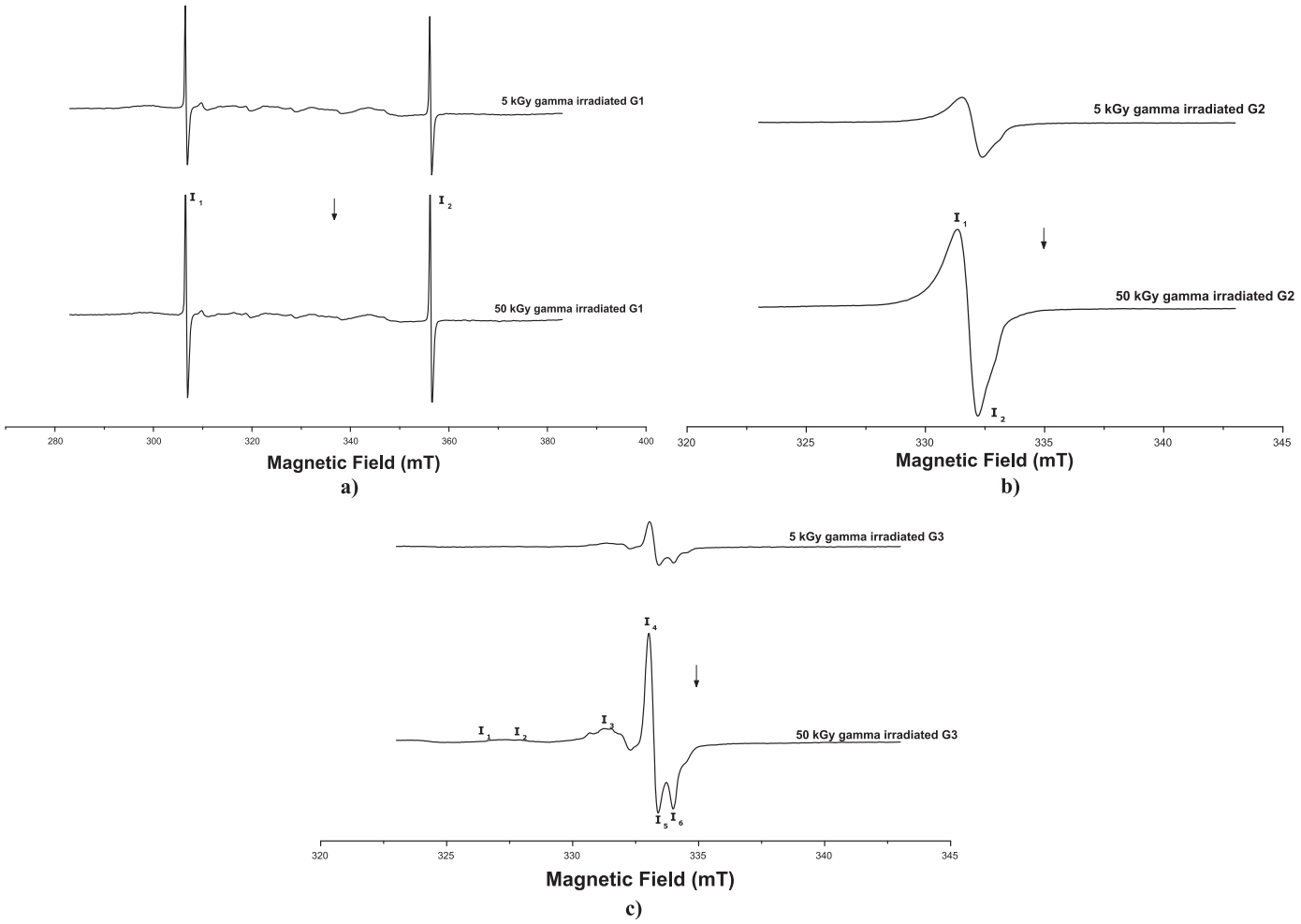


**Fig. 1.** Room temperature ESR spectra of gamma irradiated bone substitute biomaterials at different doses. **a)** G1, **b)** G2, **c)** G3. Arrow indicates the position of DPPH line.

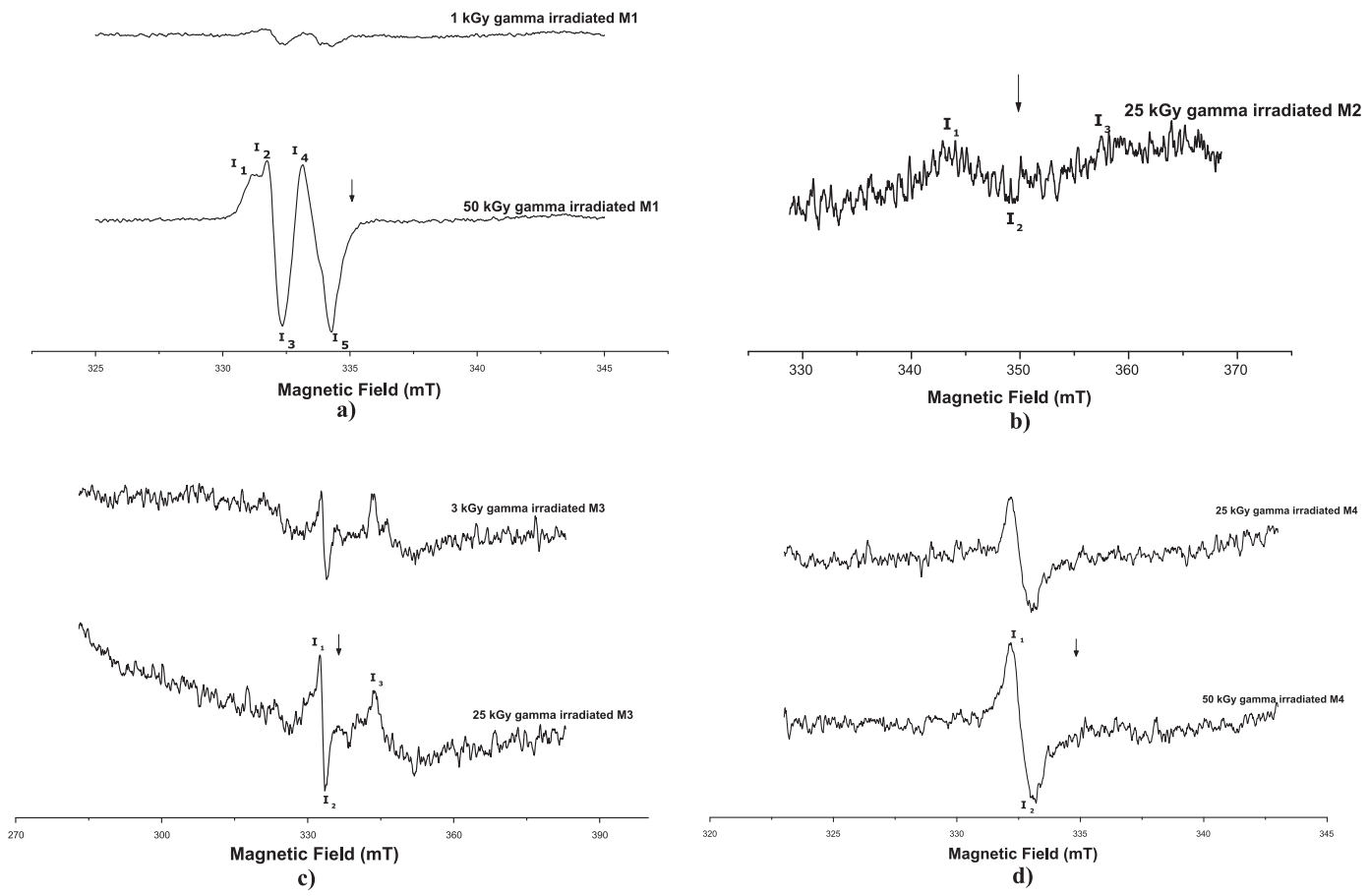


**Fig. 2.** Room temperature ESR spectra of bone substitute biomaterials irradiated at different doses. **a)** M1, **b)** M2, **c)** M3, **d)** M4. Arrow indicates the position of DPPH line.

## 3. Results and discussion

Beta-tricalcium phosphate (b-TCP), Hydroxyapatite (HA), collagen and poly (lactic acid) (PLA) and poly (glycolic acid) (PGA), as well as their co-polymers poly (lactic-*co*-glycolic acid) (PLGA) are the most popular biomaterials used in medicine and dentistry as bone substitute biomaterials (Griffith, 2002; Kohane and Langer, 2008). b-TCP is compositionally similar to the mineral phase of the bone and has been shown to have good biocompatibility, osteoconductivity and bone bonding, and bioresorption abilities followed by new bone formation in both animal and clinical studies (Shue et al., 2012; Wang et al., 2015). Bioglass that are used in bone regeneration applications in tissue engineering due to their ability to form a hydroxyapatite like layer, showed bioactivity and high mechanical strength in the body (Rahaman et al., 2011; Kokubo, 1991). Polymer membranes and especially PLGA have been widely used in GTR/GBR applications due to their excellent cell affinity, biodegradability, and biocompatibility (Wang et al., 2015). Collagen is the most widely distributed class of proteins in the human body, due to its being biodegradable, biocompatible, easily available and highly versatile properties that enable tissue regeneration in tissue defects (Neel et al., 2014). However, since collagen is a protein, it remains difficult to sterilize without alterations to its structure (Parenteau-Bareil et al., 2010; Zhang et al., 2013).

In our previous studies, we evaluated the effect of gamma radiation on physicochemical, regenerative, microbiological properties of GTR/GBR biomaterials with analytical, microbiological and histological examinations (Türker et al., 2014, 2011). Two different research studies were conducted on three different dental graft materials including b-TCP, bioglass, and equine bone tissue [G1, G2, and G3, respectively] and four different dental membranes including PLGA, PLA and collagen (M1, M2, (M3 & M4) respectively). According to physicochemical (organoleptic examination, FTIR, DSC, TGA, SEM), microbiological analysis (sterility, SAL determination, and apyrogenicity) and histopathological examinations, b-TCP containing G1 and PLGA containing M1 was selected as the most stable (optimum) dental graft materials for gamma radiation sterilization with minimum changes in chemical and physical properties in comparison with other bone substitute biomaterials. Animal experiments showed that G1 coded GTR/GBR biomaterials degrade completely in vivo with only mild histological responses, which indicated the osteoconductive properties of those dental GTR/GBR biomaterials. The results of the studies suggested that those biomaterials could be sterilized safely and time- and cost-effectively with validated radiation doses for the tissue engineering applications.

In the present study, we investigated the characteristic features of free radical(s) in gamma irradiated GTR/GBR biomaterials and examined the stability of the induced radicals at room temperature and accelerated stability conditions with ESR spectroscopy.

### 3.1. General features of the ESR spectra of GTR/GBR biomaterials

The ESR analysis showed that, unirradiated (G1, G2, G3 and M1, M2, M3, M4) exhibited no ESR signals but irradiated did. In addition, it was recorded that normal storage conditions did not cause any magnetic unit formations in the samples. Room temperature spectra recorded for the GTR/GBR biomaterials [(dental grafts (G1, G2 and G3)] and dental membranes (M1, M2, M3, M4)] at different doses of gamma irradiation are given in Fig. 1 and Fig. 2 with their assigned peak numbers.

As seen from the Fig. 1, ESR spectrum of G1 was spread over 55 mT magnetic field range having two basic characteristic resonance peaks with the magnetic field distance of 49.64 mT and four satellite peaks between these the related basic peaks with low signal intensities. The

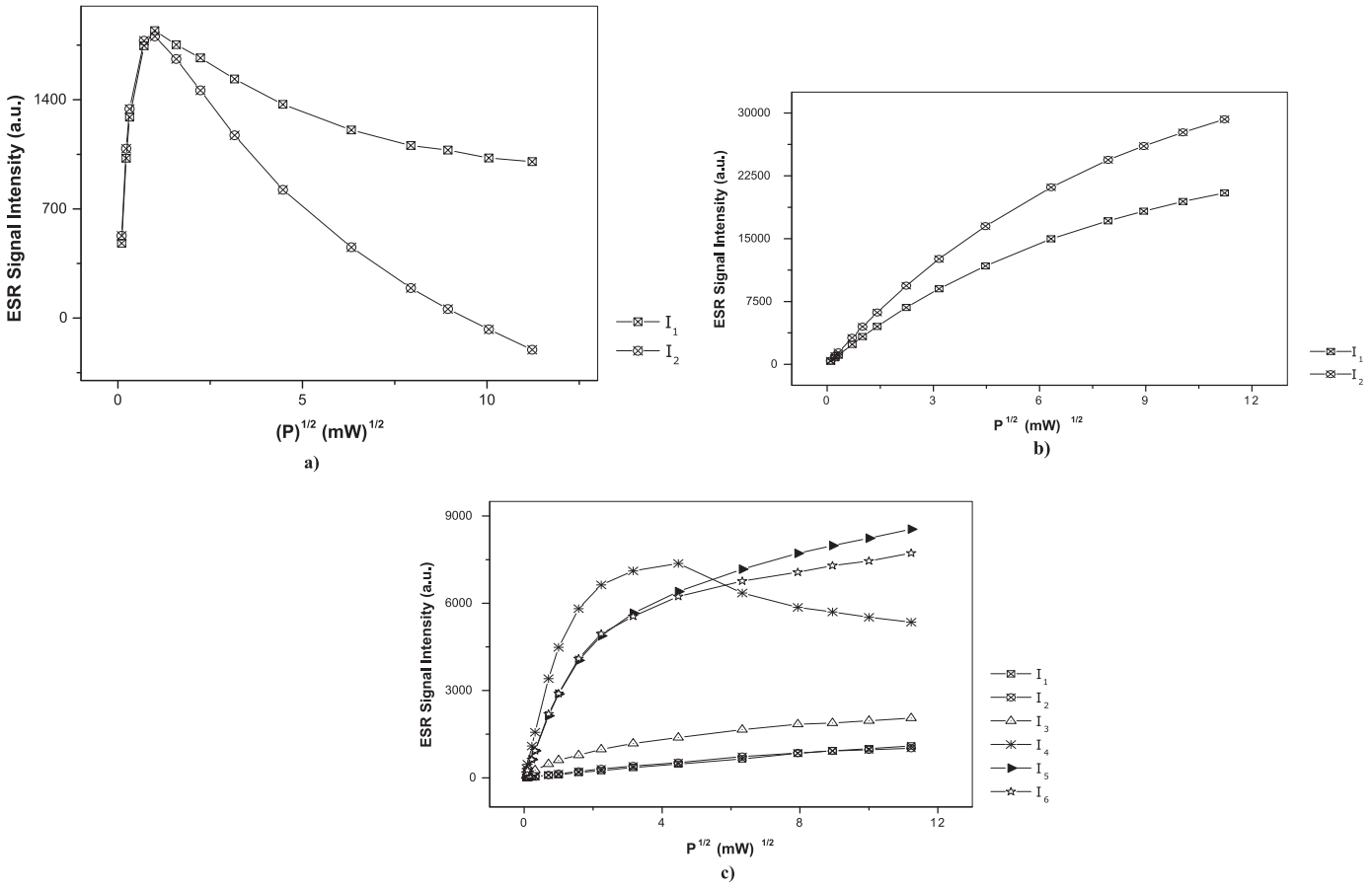


**Fig. 3.** Variations of the heights of assigned peaks with square root of applied microwave power at room temperature for the samples irradiated at dose of 50 kGy. **a)** G1, **b)** G2, **c)** G3.

spectral parameters of G1, such as g-factor and peak-to-peak width, were found to be $g_1 = 2.1899$, $g_2 = 1.8847$, $\Delta H_{pp,1} = 0.52$ mT and $\Delta H_{pp,2} = 0.41$ mT, respectively. ESR spectrum of G2 was extended over 6 mT magnetic field region with the spectral parameters of $g = 2.0238$ and $\Delta H_{pp} = 0{,}89$ mT. Six characteristic resonance lines were appeared for the ESR spectrum of G3 where it was spread over a 5 mT magnetic field region. The spectral parameters of the central resonance line ($I_4$ - $I_5$) was calculated to be $g_{(I4 - I5)} = 2.0161$ and $\Delta H_{pp,(I4 - I5)} = 0.38$ mT.

The ESR spectra of gamma irradiated dental membranes are presented in Fig. 2. Irradiated M1 sample had indicated ESR spectrum with five characteristic resonance peaks where the spectrum was extended to 6 mT magnetic field range. The spectral parameters of $I_4 - I_5$ resonance peak was found to be $g_{(I4 - I5)} = 2.0118$ and $\Delta H_{pp,(I4 - I5)} = 1.01$ mT, respectively. ESR spectrum of M2 sample was hardly distinguishable from noise where three resonance peaks (eg. $g_2 = 2.0098$) can be roughly related with the sample. Thus further studies were not performed for this dental biomaterial. ESR spectrum of M3 spread over 30 mT, with three characteristic resonance peaks, which were also hardly distinguishable from noise with the spectral parameters of $g_1 = 2.0206$, $g_2 = 2.0140$ and $g_3 = 1.9545$, $\Delta H_{pp,(I1 - I2)} = 1.07$ mT, respectively. M8 have showed ESR spectrum involving two characteristic resonance peaks with low intensity and spread over the magnetic field range of 5 mT. The spectral parameters of M8 were found to be $g_1 = 2.0221$, $g_2 = 2.0166$ and $\Delta H_{pp,(I1 - I2)} = 0.91$ mT. Increase in the absorbed dose only increased the ESR resonance peak intensities of the investigated samples but no pattern change was recorded in their ESR spectra.

### *3.1.1. Variations of GTR/GBR biomaterials peak heights with microwave power*

Variations in the line shapes and signal intensities with microwave power were studied for 50 kGy gamma irradiated GTR/GBR biomaterials at room temperature and at 130 K (for M2 and M4), in the microwave range of 0.01–126 mW. We performed microwave power studies on GTR/GBR biomaterials as the different types of radical species induced upon irradiation are expected to saturate with different rates, which could help to identify the radical species involved in the samples. The resonance line heights were measured with respect to base line and normalized to the receiver gain, the mass of the sample and the intensity of the standard sample (DPPH). Experimental results are presented in Fig. 3 and Fig. 4. The heights of the assigned peaks have increased rather linearly at low microwave powers and some of the resonance line intensities, that were selected to be presented in the graphs, saturated as homogeneously and/or in homogeneously broadened resonance lines depending on the samples.

According to the microwave power saturation studies held at room temperature, two basic ESR resonance peaks of G1 have indicated homogenously broadening (Fig. 3). Both resonance peaks have saturated after 1 mW microwave power value where the decreasing rate of $I_2$ was found to be higher than $I_1$, indicating that at least two types of radicals have been induced during the irradiation process of G2. The signal intensities of the satellite resonance lines in G1 sample, raised between the basic peaks, have increased continuously depending on the increasing rate of microwave power levels where this result is not given here to save space. For microwave saturation studies of G2, resonance peaks with inhomogenously broading character have been recorded for both $I_1$ and $I_2$. Except $I_4$, the other resonance peaks of G3 sample indicated inhomogenously broading character, representing that probably more than one type of radicals have also produced in G3 during the irradiation process.

ESR peak heights variations of M1, M3 and M4 measured with respect to spectrum base line with the applied microwave power were also studied in the range of 0.031–126 mW at room temperature

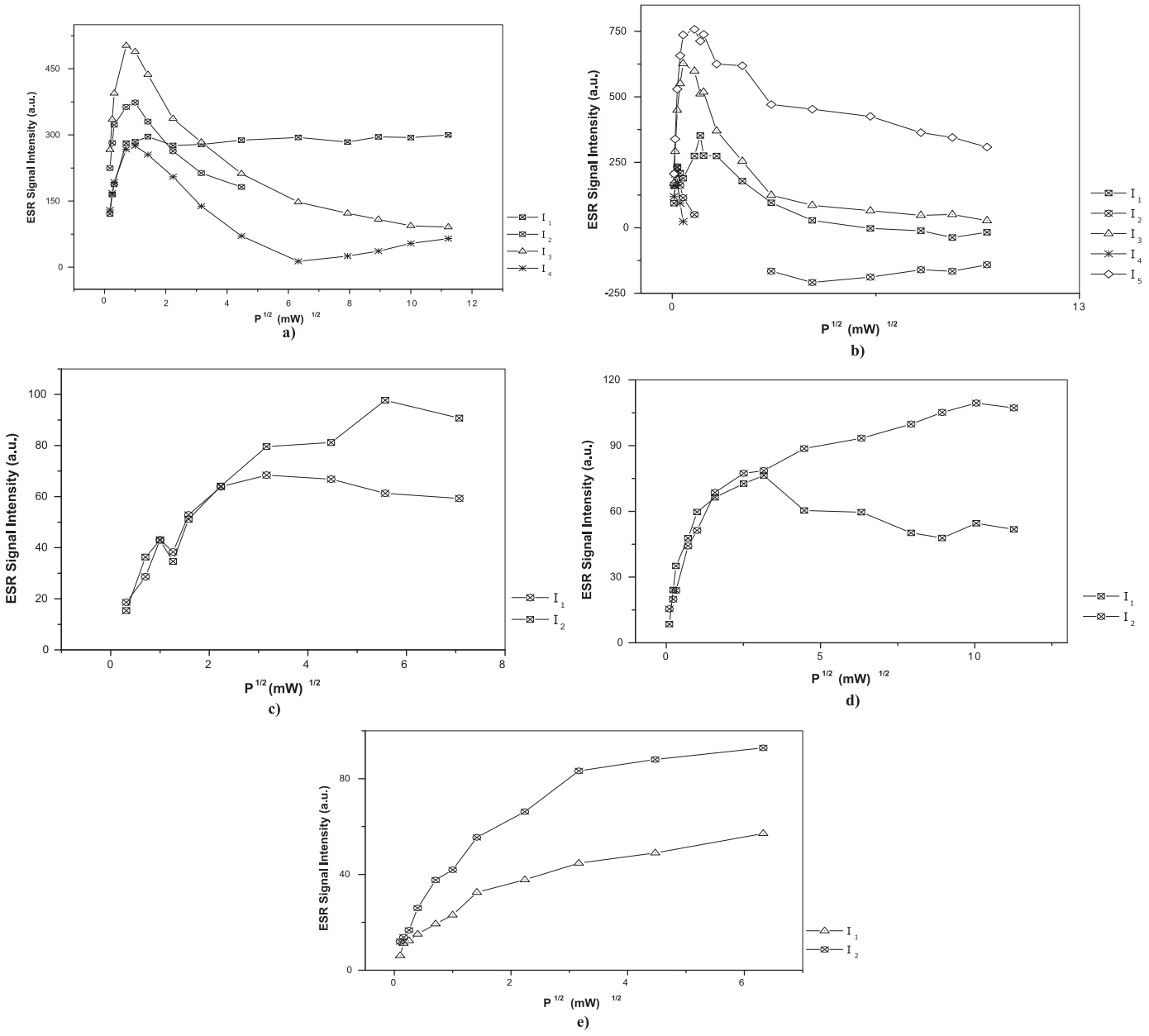


**Fig. 4.** Variations of the heights of assigned peaks with square root of applied microwave power at room temperature for the samples irradiated at dose of 50 kGy. **a)** M1 (room temperature), **b)** M1 (130 K), **c)** M3 (room temperature), **d)** M4 (room temperature), **e)** M4 (130 K).

(290 K) and at 130 K for only the samples M1 and M4 (Fig. 4). The resonance peaks of M1 were saturated at 2 mW at room temperature and ESR resonance peaks of M1 showed a homogenously broading character, except its $I_1$ resonance peak. ESR resonance peaks of M1 showed also a homogenously broading character at 130 K and but they saturated at lower microwave power (0.063 mW), as expected. These results indicated the existence of more than one type of radicals for 25 kGy gamma irradiated M1. As the recorded ESR signal intensity was very low for M2, the microwave saturation studies were not held for this sample. ESR resonance peak saturation curves of gamma irradiated M3 sample showed inhomogeneous feature broadening for its resonance peaks of $I_1$ and $I_2$. ESR data derived for M4 depending on the applied microwave power at room temperature indicated that while its $I_1$ resonance peak had inhomogeneous character, its $I_2$ resonance peak showed homogeneously saturation behaviour, confirming again the existence of more than one type of radical species in irradiated M4. Both resonance peaks of M4 were heterogeneously saturated for the microwave power studies held at low temperatures (130 K) and saturation occurred at lower microwave powers at low temperatures.

### 3.2. Dosimetric features of GTR/GBR biomaterials

The estimation of absorbed dose of irradiated samples can be done accurately by ESR spectroscopy. Generally radioresistive materials are accepted to be good candidates for radiation sterilization process. The organoleptic results, such as color change observed on the irradiated samples can also give an idea about the radiosensivity degrees of the irradiated samples. Selected resonance peak intensity variations with absorbed irradiation doses (5, 10, 25 and 50 kGy), in other words dose-response curves, are presented in Fig. 5 and Fig. 6. Increase in the absorbed dose only increased the ESR resonance line intensity of the samples but no pattern change was recorded in their ESR spectra. Dosimetric features of the samples were explored by fitting the experimental dose-response data to different mathematical functions in the

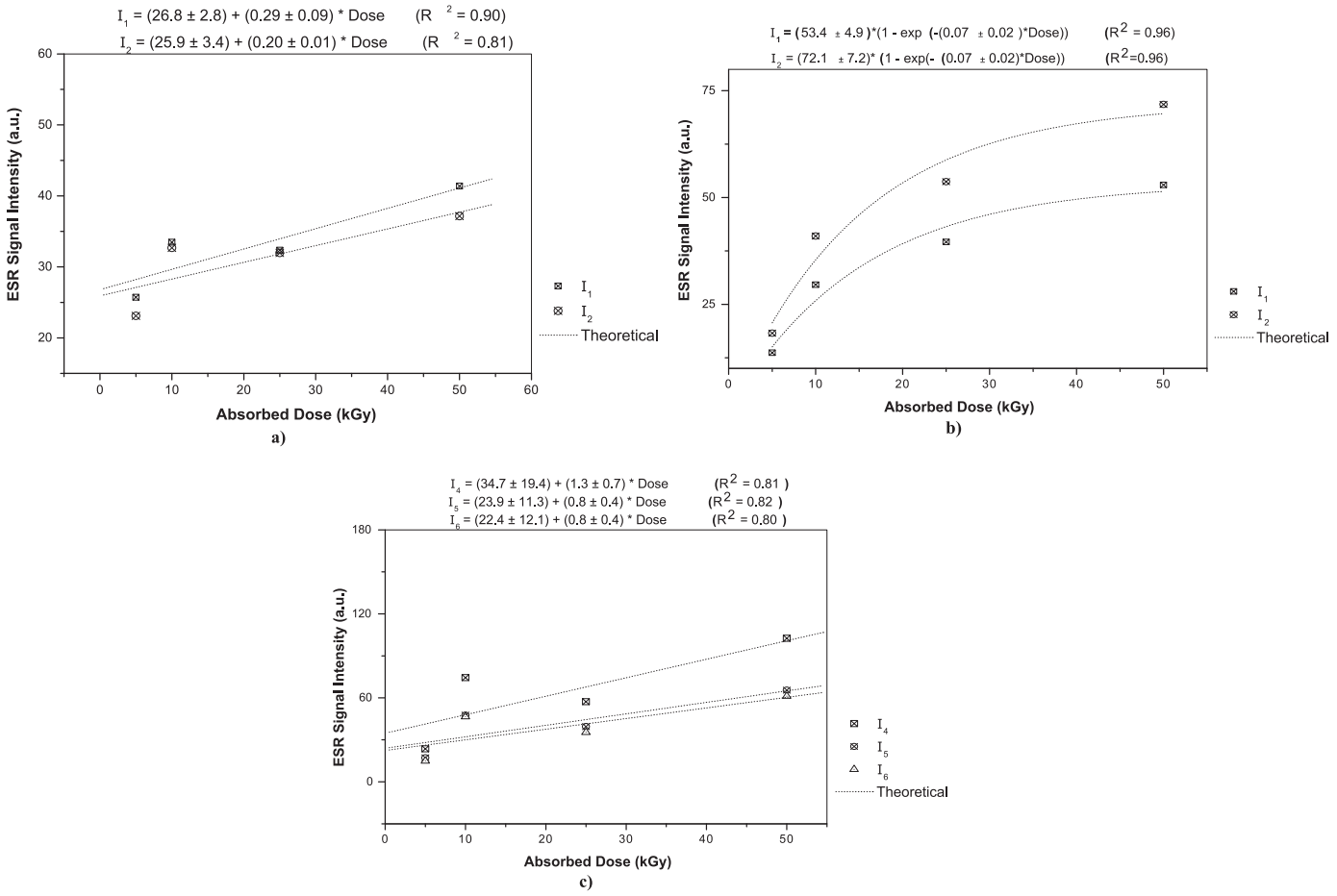


**Fig. 5.** Variations of peak heights with absorbed radiation dose of bone substitute biomaterials. **a)** G1, **b)** G2, **c)** G3. Symbols (experimental); solid and dashed lines (theoretical).

studied dose range. The mathematical functions with the calculated parameters by fitting procedures are presented in the dose-response curves of the investigated samples.

$I_1$ and $I_2$ basic resonance peaks of G1 indicated nearly a linear [I = a + b*Dose] dose-response curve (Fig. 5) where I is the ESR peak intensity and Dose stands for applied dose in kGy. As the slope of $I_1$ is found to be higher than the slope of $I_2$, $I_1$ resonance peak of M1 is better to be preferred in dose measurements (Fig. 5). The radiation yield of the satellite peaks found in G1 ESR spectra was observed to be significantly low. G2 showed a dose-response curve obeying a dose growth mathematical formula, such as [$I = I_o (1 - e^{-a*Dose})$] where a is the growth parameter and Dose is the absorbed dose. It is found that G2 cannot be accepted as a good candidate for dosimetric measurements especially at low absorbed doses. The resonance peaks ($I_4$, $I_5$, $I_6$) of G3 indicated nearly a linear [I = a + b*Dose] dose-response curve (Fig. 5) both including the experimental data and the theoretical functions with the best fitted parameters replaced.

Dose-response curves of the resonance peaks of M1 (Fig. 6) was fitted to exponential growth mathematical functions [$I = I_o (1 - e^{-a*Dose})$] and it is observed that $I_5$ resonance peak will be best to be used in dose measurements. As the radical yield of M2 was low, no meaningful dose depended signal intensity variations could be observed, thus this graph is not given here to save space. M3 and M4 did not indicate good dosimetric properties, thus the peak-to-peak intensity [$I_{pp} = I_1 + I_2$] of the irradiated samples depending on the absorbed dose are preferred to be used in Fig. 6 with the best fitted linear mathematical equation.

### 3.3. Variations of the peak heights with temperature

For only M1 and M4, a variable temperature study relative to the variations of the selected resonance peaks with temperature, in the range of 290 → 130 K and 290 → 390 K were also performed. Cooling M1 down to room temperature did not create any pattern change in the spectra of 25 kGy irradiated sample except slight and reversible decreases in the resonance peak intensities. This behaviour indicated that M1 was already saturated during the experiments. Cooling M4 down to room temperature again did not create any pattern change in the spectra except only slight and reversible increases in the resonance peak intensities. This result was related with the classical paramagnetic behaviour of the contributing radical species (Curie's Law), as expected. Heating the sample above room temperature produced no significant change in the heights of the selected resonance lines up to 320 K for M1 and M4, but the resonance peak intensities, especially $I_3$ and $I_5$ of M1, decreased sharply and irreversibly above 320 K (Fig. 7), indicating that the structure of samples can be destroyed at high temperatures. From this point of view, the storing temperature conditions of the irradiated M1 and M4 can also be determined.

### 3.4. Long term stabilities of the radical species

Long time stability studies of the radical(s) produced upon irradiation for the irradiated materials are another important concept for the identification of the radiosterilized samples. Therefore, this feature of the radicals produced in GTR/GBR biomaterials irradiated at 25 kGy was also studied. Samples irradiated at different radiation doses and stored over a period of 90 days at normal (atmospheric humidity, room temperature) and stability conditions (75% relative humidity, 40 °C)

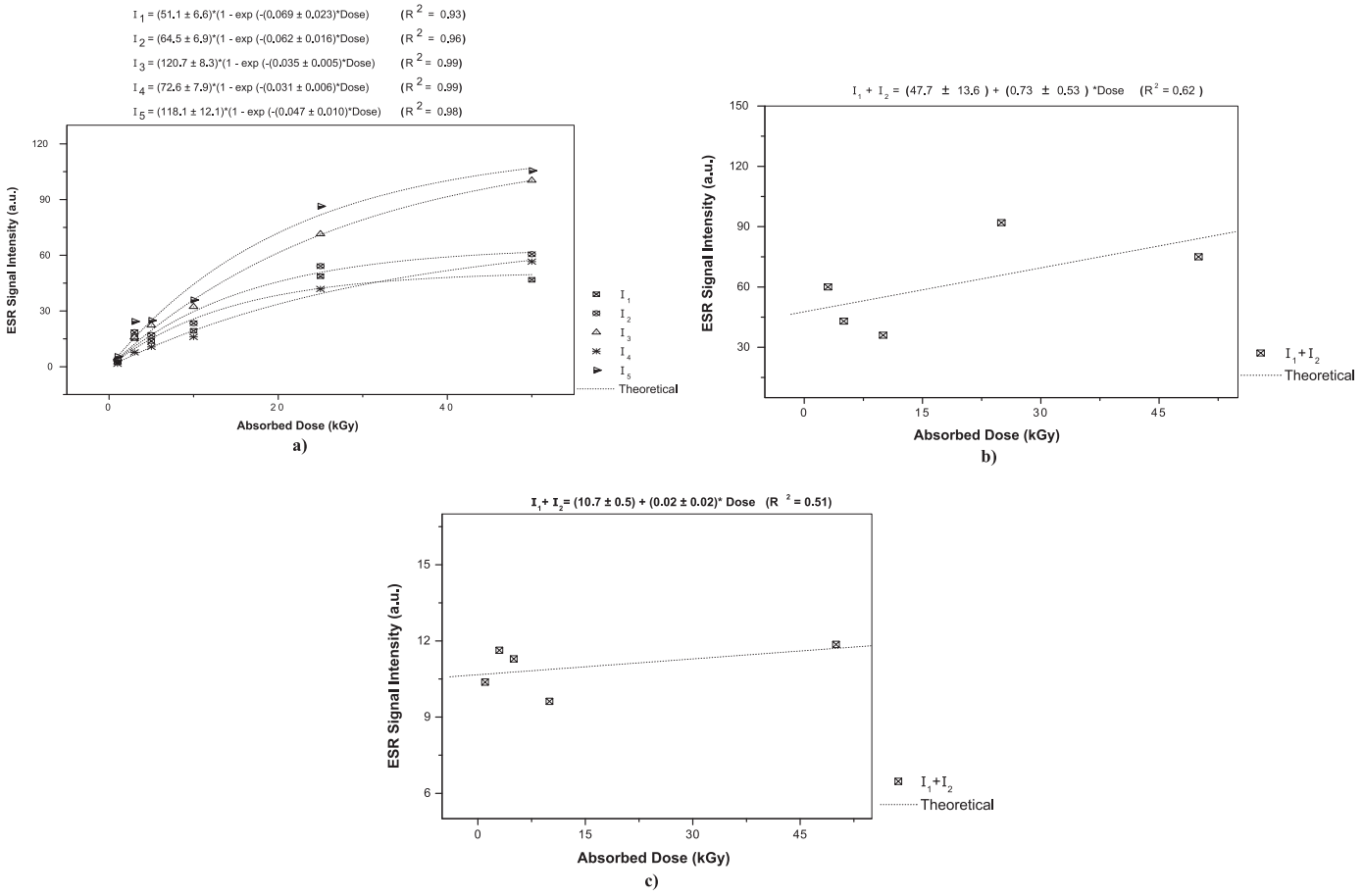


**Fig. 6.** Variations of peak heights with absorbed radiation dose of bone substitute biomaterials. **a)** M1, **b)** M3, **c)** M4. Symbols (experimental); solid and dashed lines (theoretical).

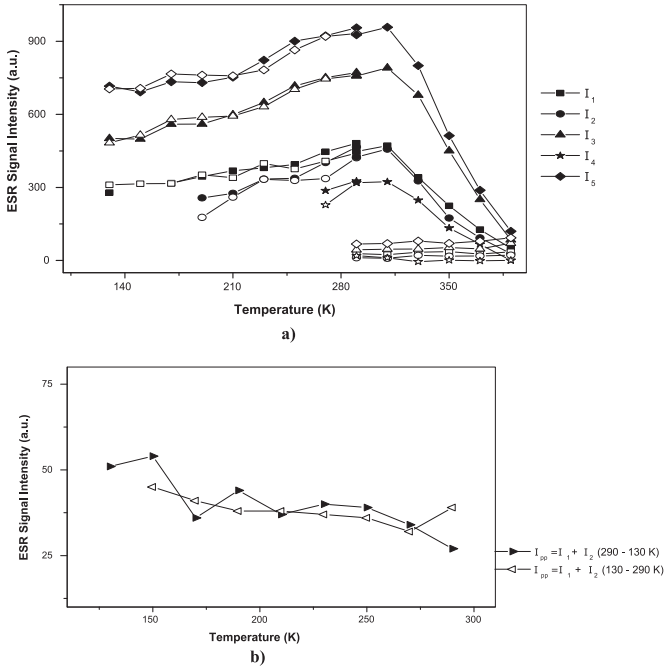


**Fig. 7.** Variations of the peak heights with temperature in the range of 130–390 K. **a)** M1 **b)** M4.

were used to reach this purpose. The long term stability results of G1, G2 and G3 are presented in Fig. 8 with fitting the recorded data to the first order exponentially decay equation. The variations of the resonance line heights of the samples irradiated at different doses and stored at normal and stability conditions were found to be independent from the irradiation dose. Resonance peak intensities of the samples were found to decrease very fast in the first 30 storage days in normal conditions and was found to be faster (10 days) in stability conditions. As is expected, the decay rates of the contributing radical species induced in the samples, which were related with the selected resonance peaks, were faster under stability conditions. Long-term stability studies have shown that G1 is quite stable even in the accelerated storage conditions. Data recorded for G2 indicated that its peak-to-peak intensity were decreased to 37% for the sample stored at room temperature and decrease to 83% for the sample stored in stability conditions. G3 was also detectable for a long time of storage, after the irradiation process.

It had been observed that the signal intensities of M1, M2, M3 and M4, that were stored in normal and stability conditions, have decreased very rapidly and the resonance peaks of these samples were hardly be distinguishable from noise even only a few days after irradiation. The signal intensity of $I_2$ resonance peak of M1, which was 25 kGy gamma irradiated, have decreased to 60% in the first storage day and to 78% in the second day of storage duration in normal conditions. The resonance peaks of M2 and M3 were also nearly disappeared in the first day of storage in the normal conditions. The signal intensities of M4 have decreased to 67% in the first day of it storage and to 78% in the second day of its storage, in normal conditions. Thus these samples, indicating relatively low stability features can be very good candidates for the radiosterilization process.

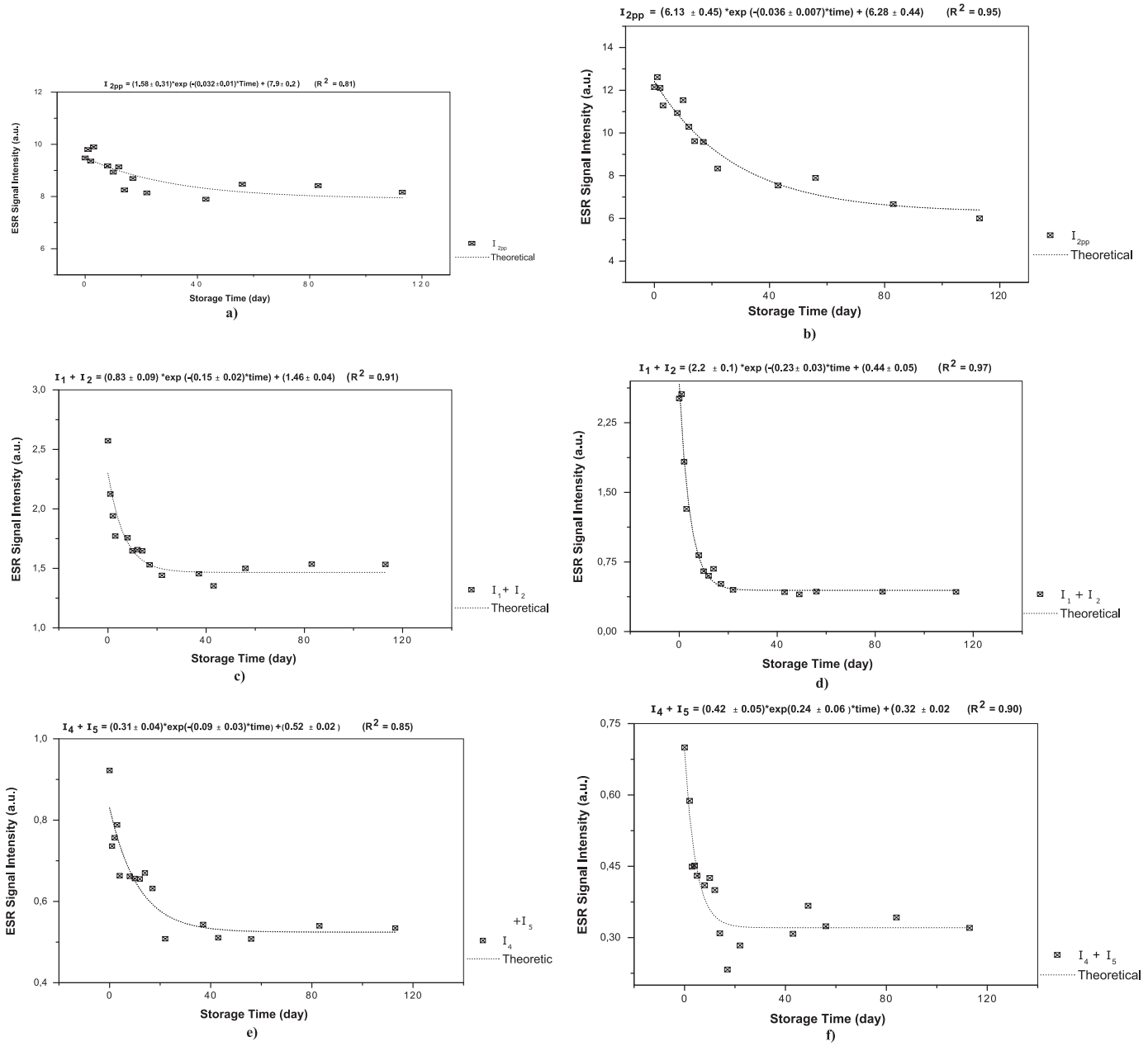


**Fig. 8.** Variations in the peak heights of the samples irradiated at a dose of 25 kGy stored in normal and stability conditions. **a)** G1 (normal conditions), **b)** G1 (stability conditions), **c)** G2 (normal conditions), **d)** G2 (stability conditions), **e)** G3 (normal conditions), **f)** G3 (stability conditions).

## 4. Conclusions

In the present study, widely used dental GTR/GBR biomaterials (grafts: G1, G2, G3 and membranes: M1, M2, M3, M4) were exposed to gamma irradiation and the radiolytic intermediates that have been created in the samples upon irradiation were characterized in detail by ESR spectroscopy. For G1, G2 and G3, although the radiosensitivity of these samples to gamma radiation were not high enough, the detection and discrimination of unirradiated samples from the irradiated ones were possible even at low radiation doses (5 kGy, 10 kGy) and for a relatively long storage period (several months). Especially G1 have found to be the most stable graft sample in this study. Thus the radical identification of these samples can also be done in further studies. The radical yield of M1, M2, M3 and M4 were very low and the radical decay data obtained for them which were stored over a period of three months, at normal and stability conditions, indicated that these samples were not stable. Thus the investigated membranes were accepted to be good materials for radiation sterilization process. It is concluded that the G1, G2, and G3 grafts and M1, M2, M3, M4 membranes can be sterilized by gamma radiation and ESR spectroscopy is an appropriate technique in giving important detailed spectroscopic findings in the radiation sterilization studies of them.

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