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An in vitro L-band EPR study with whole human teeth in a surface coil resonator

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Abstract

L-band EPR measurements were done in vitro on extracted human teeth with the objective to evaluate the feasibility of retrospective in vivo EPR dosimetry. In a recent study, the relative contributions of individual tooth components (enamel, crown dentin and root) to the total response of a whole tooth inside an L-band surface coil resonator was investigated. In the present work, the gamma-dose response curves were evaluated for different EPR signal evaluation methods, using 35 whole teeth with absorbed doses in the range 1–100 Gy. The paper reports on the first attempt to deconvolute the single composite L-band EPR line in components due to CO_2^- and native radicals. The L-band EPR spectrum of teeth could be approximated by combining powder-simulated spectra of orthorhombic and quasi-axial signals of the CO_2^- radical and an isotropic signal of the native radicals. Among the applied EPR signal evaluation methods, the evaluation by spectrum deconvolution revealed the lowest detection limit for absorbed dose. A detection limit of about 0.5 Gy was estimated for the currently available L-band equipment.

Keywords: L-band EPR; Tooth enamel; Gamma irradiation; Retrospective *in vivo* dosimetry; Spectrum deconvolution

1. Introduction

X-band (~ 10 GHz) EPR spectroscopy with tooth enamel powder is the leading method for retrospective assessment of absorbed doses for individuals exposed to radiation (Wieser et al., 2000). But X-band EPR is limited for the application to extracted teeth and does not have the potential to measure whole teeth inside the mouth. This will be possible by L-band (~ 1 GHz) EPR due to its low microwave frequency. However, it is linked with a substantial loss in sensitivity, which can be partly compensated because a larger sample volume is measured. L-band spectroscopy offers a unique potential for in vivo measurements of absorbed radiation doses. In the present state of research, in vitro experiments are still necessary to check on the feasibility and reliability of the method with the available spectrometers and resonators (Miyake et al., 2000; Zdravkova et al., 2002).

Tooth enamel is well suited for reconstruction of accumulated dose during lifetime because it is not remodelled after formation in childhood. It has a high content of inorganic matter (96% by weight carbonated hydroxyapatite, 3% water, 1% organic matter) and an average density 2.92 g/cm^3 (Driessens and Verbeeck, 1990). At the crown, tooth enamel covers dentine (average density 2.51 g/cm^3) containing 70% inorganic and 20% organic matter, and 10% water. Dentine is a vital tissue that is deposited also after tooth eruption throughout the whole life. Thus, the use of dentine for dose reconstruction during lifetime is complicated.

In a recent study, the relative contributions of the individual tooth components, enamel, crown dentine and root, to the

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total L-band EPR signal of a whole tooth were investigated (Zdravkova et al., 2002). In using a surface coil resonator, enamel was found to contribute 88% of the total EPR signal, when the surface coil was placed on top of the tooth. This type of resonator was identified as the best choice for in vivo measurements. The EPR spectrum of tooth enamel is resulting principally from radiation generated CO_2^- (corresponding to the "dosimetric signal") and native radicals. In L-band EPR spectroscopy, the individual signals of these radicals are poorly resolved and the spectrum is reduced to a single composite line.

In the present work, various methods for evaluating the composite L-band EPR line were compared. The gamma-dose response curves of double integral and amplitude of the composite line, as well as the amplitudes of the deconvoluted signals were determined.

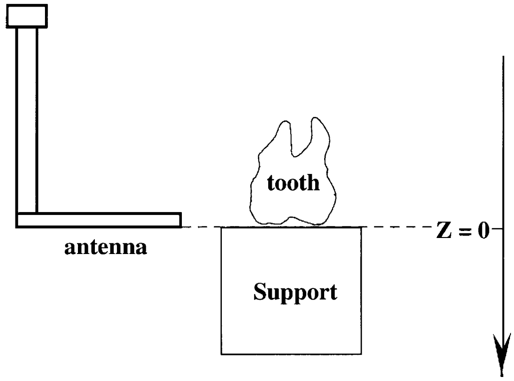

Fig. 1. Normal position of a tooth on its Plexiglas support. The top of the crown lies in the $z = 0$ plane, inside the surface coil resonator (antenna) drawn separately for clarity.

2. Experimental

Thirty-five permanent third molars were used in the experiments. The teeth were extracted for orthodontic reasons from 15 to 42 years old persons in the years 1997–2001 (half of them in 1999) at the UCL Dentistry School and stored in a 1% chloramines solution at room temperature.

The irradiation of teeth was done at GSF with a ^{60}Co beam source (Type Eldorado) inside of a Plexiglas phantom box with 0.5 cm thick walls. Groups of five molars were irradiated simultaneously, each group applied with an absorbed dose in hydroxyapatite of 1, 2, 4, 7, 10, 20 or 100 Gy. The phantom to source distance was 100 cm and the air kerma rate was 34 mGy/min at the surface of the phantom. For this irradiation set up, the air kerma rate was converted to absorbed dose rate in hydroxyapatite using a factor 0.975. This conversion factor is valid for single side irradiation and is the average value for two opposite enamel layers of a tooth with 1 cm in diameter, as calculated by Monte Carlo simulation (Wieser et al., 2000). The conversion factors are 0.996 and 0.954 for the enamel layer next to the source and the opposite layer, respectively. The lower conversion factor for the opposite layer is caused by a decrease of 1.8% in the air kerma rate due to further distance from the point source and by 2.6% due to absorption by dentine and enamel.

EPR measurements were carried out at UCL using an L-band Magnettech EPR spectrometer (Berlin, Germany) equipped with a low-frequency microwave bridge operating at 1.2 GHz, a frequency counter (CUB RF minicounter, Optoelectronics, USA) and an extended loop resonator. The resonator with a surface coil of 12.5 mm inner diameter and 2 mm thickness was designed and constructed by T. Walczak at Dartmouth Medical School, Hanover, NH, USA (Nilges et al., 1989). The teeth were positioned on top of a Plexiglas support with the surface coil surrounding the top of the crown (see Fig. 1). The tooth axis was shifted out of the surface coil axis into the most sensitive area of the coil, opposite to the wave guide, which resulted in a gain of about 30% in the EPR signal intensity. The EPR spectra were recorded only with one tooth orientation because rotational effects were found to be negligible in L-band measurements (Zdravkova et al., 2002). It is known that tooth enamel is a partially ordered system. From X-band studies of enamel fragments (block samples), the simultaneous presence of at least two different CO_2^- (bulk axial and surface orthorhombic) radicals was proposed (Callens et al., 2002; and references quoted therein). These two radicals are expected to exist in comparable concentrations but only one of them, the bulk species, showed anisotropy upon rotation of the block in the magnetic field. However, rotational effects were hardly visible in L-band measurements of whole teeth. This experimental result is probably related to the fact that the masticatory surface with its crater-shaped pit in the centre can be considered as a conglomeration of disordered enamel blocks.

A lithium phthalocyanine (LiPC: $g = 2.0015, \Delta H_{pp} = 0.024$ mT) reference sample was used for magnetic field calibration, signal intensity normalization and as reference for g-value evaluation. All spectra were recorded with a resolution of 1024 data points. The settings of EPR parameters for measurement of teeth were: 23 mW input microwave power, 100 kHz magnetic field modulation frequency, 0.173 mT modulation amplitude, 3 mT magnetic field sweep, 60 s scan time and 0.48 s time constant. The LiPC spectra were recorded with 0.0173 mT modulation amplitude and 0.06 s time constant.

The resonator has an intrinsic EPR signal that may interfere with the tooth signal. Position, shape and intensity of the intrinsic signal are varying with time and the intensity may become comparable to that of non-irradiated teeth. Therefore, the EPR spectrum of the resonator without tooth sample was recorded before and after each tooth spectrum for subtraction. After warming-up of the spectrometer for 1.5 h, the following procedure for measurement of each tooth sample was used:

(a) First measurement of the empty resonator + Plexiglas support (resonator signal) using the same recording conditions as for the tooth spectra, 20 scans.
(b) Measurement of the tooth, 40 scans.
(c) Measurement of the LiPC reference sample added near the tooth, four scans.
(d) Second measurement of the resonator signal, as in (a).

In a previous study, signal fading of about 20% within 90 days after irradiation was observed in whole teeth irradiated at high absorbed dose (Zdravkova et al., 2002). Therefore, the 35 teeth of this study were repeatedly measured in three sets: two weeks, two months and four months after irradiation.

Spectrum analysis in dependence on absorbed dose was done with the cumulated spectra of each five tooth samples per dose. Before, the cumulated spectrum of first and second measurement of the resonator signal was subtracted from each tooth spectrum (all spectra were normalized to 1 scan). The individual spectra were adjusted in magnetic field position and signal intensity relative to the measurement of the LiPC reference sample.

Spectrum manipulations as well as evaluation of double integral and amplitude of the single composite L-band EPR line were done with the WINEPR software package (Bruker). Deconvolution of the composite EPR line was done with the DOSIMETRY software package developed by GSF and IMP. This software is a modification of an earlier version (Koshta et al., 2000) used at GSF for X-band EPR-dosimetry with tooth enamel. In the new version, EPR spectra can be deconvoluted either by linear combinations of Gaussian functions or/and by simulated powder spectra, which need to be imported from external simulation programmes. The powder spectra used in this work were simulated by the SimFonia software package (Bruker). The simulated spectra were normalized to unit spectrum intensity.

3. Results and discussion

The g-value of the single composite L-band EPR line was evaluated with four samples from Zdravkova et al. (2002) that were irradiated at an absorbed dose of 875 Gy, 9 months ago. The line was located at $g = 2.0009 \pm 0.0005$. The peak-to-peak width was broadening with decreasing absorbed dose from 0.26 mT at 875 Gy, 0.30 mT at 100 Gy to 0.60 mT at 1 Gy. Amplitude and double integral of the EPR signal in dependence on absorbed dose of the 35 teeth of this study were not significantly different for the three sets of measurements performed 2 weeks, 2 months and 4 months after irradiation (t-significance test at the 5% level). EPR signal fading was thus not observable within the present uncertainties. This is in agreement with results of Miyake et al. (2000) who found that the EPR signal intensity of 10 Gy irradiated teeth was stable for at least 100 days. The fading observed at an absorbed dose of 875 Gy (Zdravkova et al., 2002) was possibly the consequence of the presence of other less stable radicals, like CO^-, generated at high absorbed dose.

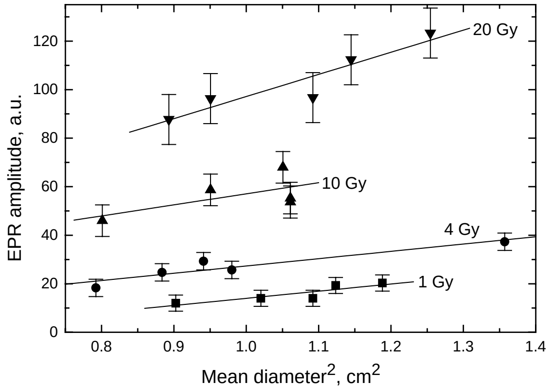

Fig. 2. The amplitude of the composite L-band signal in dependence on the square of tooth diameter for various applied doses. Mean values and standard deviations (σ) for three measurements performed 2 weeks, 2 and 4 months after irradiation are shown.

The calibration of radiation sensitivity of tooth samples for in vivo dosimetry cannot be done by additional in vivo irradiation of samples. Recent investigations have shown that the variability in radiation sensitivity of tooth enamel was below 10% for teeth from different individuals (Wieser et al., 2001). The moderate variation in radiation sensitivity is justifying the use of a dose calibration curve for evaluation of absorbed dose. Meanwhile, dose calibration curves are widely used in in vitro X-band EPR dosimetry with tooth enamel (Wieser et al., 2000). In this work, calibration curves for L-band EPR dosimetry were evaluated with the cumulated spectra of a set of five teeth for each value of absorbed dose.

The EPR amplitude of the composite L-band EPR signal was found to be dependent on tooth size. Fig. 2 shows the EPR signal amplitude in dependence on the square of the mean tooth diameter, for four of the seven sets of each five samples with the largest differences in tooth diameter. The amplitude increased equally well linearly with both, tooth diameter as suggested by Miyake et al. (2000) and the masticatory surface area (square of diameter). In case of homogeneous irradiation, flat surfaces of the tooth crown with equally thick enamel layer, and constant resonator sensitivity all over its plane, the EPR signal amplitude would be expected to be proportional to the mass of enamel and hence the masticatory surface area. In reality, the masticatory surface has an asymmetric crater-shaped pit in its centre, the thickness of the enamel is varying and the resonator sensitivity is not constant over its plane. These might counteract a dependence of the EPR signal amplitude on size of the masticatory surface.

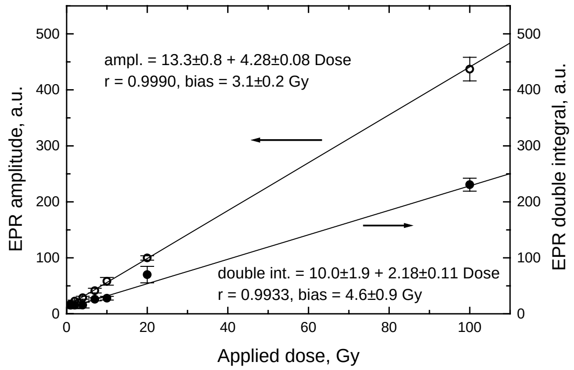

Fig. 3. Dose response behaviour obtained for the double integral (solid circles) and amplitude (open circles) of the single composite L-band EPR line. The evaluation was done with cumulated spectra of five teeth per dose. Mean values and standard deviations (σ) for three measurements performed 2 weeks, 2 and 4 months after irradiation are shown.

The dose response curves of the double integrals and amplitudes of the composite L-band EPR line are shown in Fig. 3. The given error bars correspond to the standard deviation (σ) for measurements performed 2 weeks, 2 and 4 months after irradiation. The relative standard deviations of double integral measurements, in the range of 5–41%, were larger than for amplitude measurements, in the range of 4–24%. The correlation with applied dose was slightly better for amplitude measurements ($r = 0.999$) than for double integral measurements ($r = 0.993$). We defined the bias of measurements as dose equivalent of the signal intensity of unexposed samples. It was obtained by division of the regression line intercept with the slope of the line. The bias was 4.6 ± 0.9 and 3.1 ± 0.2 Gy for double integral and amplitude measurement, respectively. The detection limit of a signal evaluation method is not primarily dependent on the value of the bias itself (because it can be subtracted), but by the uncertainty of the bias. We defined the detection limit as two standard deviations of the bias, yielding values of 1.8 and 0.4 Gy for double integral and amplitude measurements, respectively. The difference in the bias of the two evaluation methods might be an indication that all of the various types of radicals existing in tooth enamel are contributing to the double integral, while the amplitude is mainly dependent, on only a few most dominant types of radicals. As shown below, the bias was further decreased if only the EPR signals from CO_2^- radicals were considered by spectrum deconvolution (see Fig. 6).

The deconvolution of the composite L-band line into its different components is important, because only the signals of the CO_2^- radical are qualified for dosimetry. The protocols of GSF and ISS (Istituto Superiore di Sanita, Rome) for X-band EPR dosimetry with tooth enamel currently employ in deconvolution an axial CO_2^- signal with line widths of 0.3–0.4 mT (Onori et al., 2000). However, these line widths are too large and were not able to reconstruct the experimental L-band signal. The composite L-band signal of a highly irradiated tooth (875 Gy) recorded a few months after irradiation has a line width of 0.26 mT. This value can be taken as a good approximation of the line width of the CO_2^- species, which should be predominant after such high irradiation.

The attempt of deconvolution presented in this work is based on the results obtained by Vanhaelewyn et al. (2001, 2002). According to these authors, the X-band spectrum of the CO_2^- radicals in enamel can be deconvoluted into two components, including an orthorhombic signal with Lorentzian line shape ($g_x = 2.0032$, $g_y = 1.9972$, $g_z = 2.0019$ and with line widths of 0.20, 0.21 and 0.2 mT, respectively) from radicals located near the surface of the enamel micro crystallites and a quasi-axial signal with Gaussian line shape ($g_x = 2.0027$, $g_y = 1.9972$, $g_z = 2.0025$ and with line widths of 0.46, 0.38 and 0.22 mT, respectively) from radicals in the bulk of the micro crystallites. The quasi-axial signal is in fact composed of two signals with nearly identical parameters from two species of CO_2^- radicals replacing a hydroxyl or phosphate site in the hydroxyapatite crystal lattice. The EPR spectrum from the native radicals was found as isotropic signal with Gaussian line shape at $g = 2.0045$ with a line width of 0.78 mT. These values were obtained with a maximum likelihood common factor analysis (MLCFA) on teeth, which were gamma-irradiated up to 10 Gy. The results obtained with MLCFA do not depend much on the line shape used for the CO_2^- radical. This could be related to the actual presence of the two aforementioned bulk and surface CO_2^- components, which strongly overlap, each with its own line shape.

The narrow Lorentzian orthorhombic and the wide Gaussian quasi-axial CO_2^- signal are approximately equally well suited to reproduce the real line shape in the X-band spectrum, which is not the case in the L-band spectrum. The spectrum simulation for the L-band of both species yielded each a single line of 0.23 mT in width for the orthorhombic and 0.33 mT in width for the quasi axial signal. These two values surround the experimental line width of 0.26 mT. A hypothetical 0.26 mT wide CO_2^- line could actually be constructed by adding both orthorhombic and quasi-axial signals with an intensity ratio of 3:2. The same hypothetical construction yielded also for the simulation of the X-band spectrum to an improved approximation of the experimental spectrum (see Fig. 4). This empirical approach of a hypothetical line was considered as a plausible solution and used for deconvolution in this work. In spite of the fact that the parameters of both CO_2^- components are currently not known exactly and a g-strain effect could also possibly be involved to account for a line decrease of the quasi-axial CO_2^- signal when going from X- to L-band (Schramm et al., 1998).

Experimental and simulated L- and X-band spectra of tooth samples that were irradiated with 10 Gy are shown in Fig. 4. The simulated spectra include signals from native radicals, and optionally contributions of Lorentzian

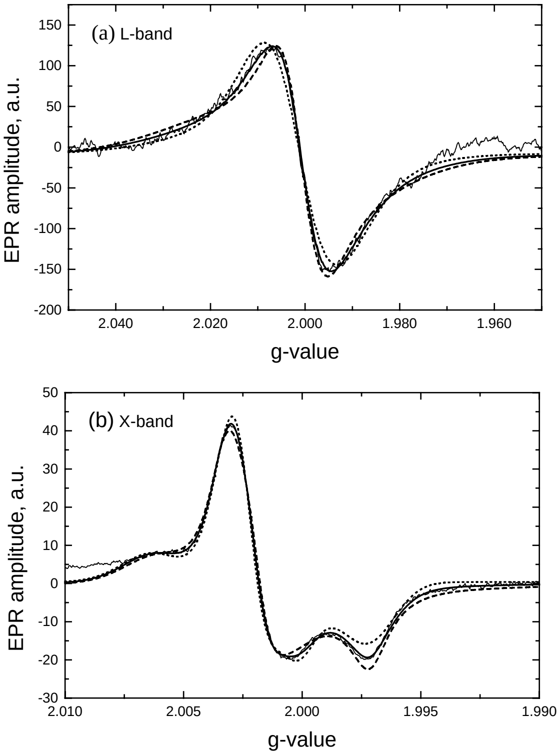

Fig. 4. Experimental (solid line) and simulated (a) L-band spectra of whole teeth and (b) X-band spectra of tooth enamel powder, both irradiated with 10 Gy. Shown are simulations with orthorhombic Lorentzian (dashed line), quasi-axial Gaussian (dotted line) and the combination of both signals (bold solid line) with an intensity ratio of 3:2. The simulated spectra include signals from native radicals as described in the text and powder simulated spectra of CO_2^- radicals.

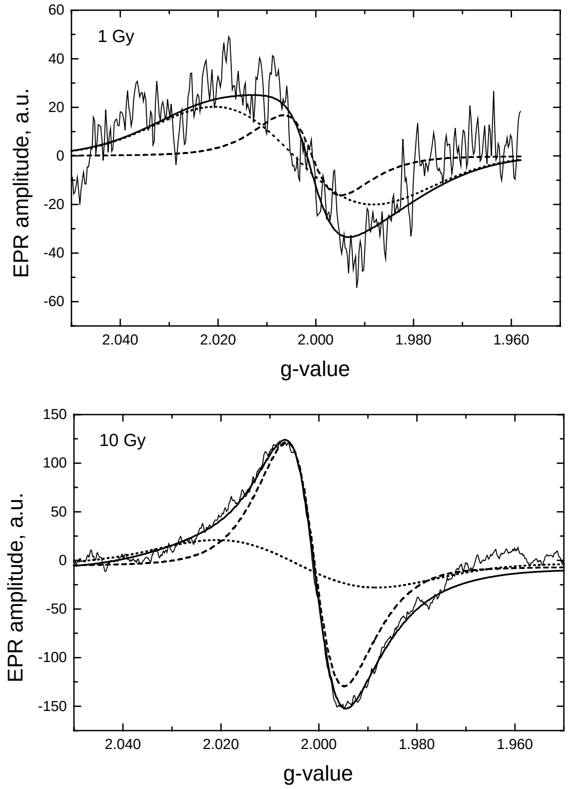

Fig. 5. Experimental (solid line) and simulated (bold solid) L-band spectra of whole teeth irradiated with 1 and 10 Gy. The individual contributions of the CO_2^- signal (dashed line) and native signal (dotted line) as obtained by spectrum deconvolution are also shown.

orthorhombic, Gaussian quasi-axial CO_2^- signals or both mixed in a ratio 3:2. In the X-band spectrum, the native signal was approximated best by an axial Gaussian signal represented in the DOSIMETRY programme as the sum of two lines at $g = 2.0051$ (line width 0.49 mT) and $g = 2.0035$ (line width 0.42 mT), with an amplitude ratio of 0.26. In the L-band spectrum, the native signal could be approximated best by an isotropic signal at $g = 2.0045$ with 0.78 mT line width as obtained by Vanhaelewyn et al. (2000). Examples of the simulation of experimental L-band spectra from 1 and 10 Gy irradiated teeth are shown in Fig. 5.

The amplitudes of the CO_2^- and native signals obtained by spectrum deconvolution in dependence on applied dose are shown in Fig. 6. The standard deviation (error bars) was in the range of 6–22% for the CO_2^- amplitude and in the range of 29–53% for the amplitude of the native signal. The native signal was found to be dependent on absorbed dose. Thus, in agreement with the work of Vanhaelewyn et al. (2001), the native radicals are radiosensitive. Removing the data at 100 Gy had an influence on the regression line of the native

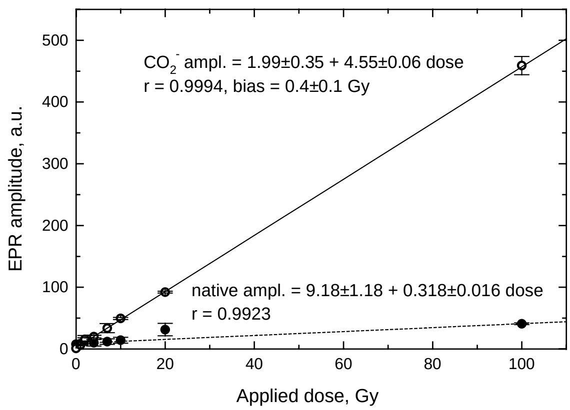

Fig. 6. Dose response curves obtained from cumulated spectra of five teeth per dose, after deconvolution in CO_2^- (open circles) and native (solid circles) components up to an applied dose of 100 Gy with the corresponding linear fits equations. Shown are mean values and standard deviations (σ) for three measurements performed 2 weeks, 2 and 4 months after irradiation.

signal amplitude. The slope increased by a factor of 2 and the intercept decreased about 10 times. This is indicative of a probable dose saturation effect for the native radicals for absorbed doses above 20 Gy. In comparison to double integral and amplitude measurements of the composite L-band EPR line, the amplitude of the deconvoluted CO_2^- signal had shown the best correlation ($r = 0.9994$) with applied dose. The bias of the CO_2^- amplitude was 0.4 ± 0.1 Gy. This results in a detection limit of 0.2 Gy.

The biases of all three signal evaluation methods were determined alternatively directly from measurements of 25 teeth before applying radiation. For all methods, the values obtained from regression lines of dose response curves and from direct measurement were in agreement within the limits of errors. The biases obtained for each method from direct measurements were in average smaller but with larger standard deviation, namely 1.5 ± 4.0, 1.6 ± 0.5 and 0.21 ± 0.27 Gy for evaluation by double integral and amplitude of the composite signal, and spectrum deconvolution, respectively. This results in a detection limit of 8.0, 1.0 and 0.54 Gy for the three methods, respectively. On the basis of these two alternative evaluations, the detection limit was found to be lowest for deconvolution of the CO_2^- signal and not worse than about 0.5 Gy with the present spectrometer and resonator.

The contributions from native radicals to the L-band spectrum of teeth were found to complicate considerably the determination of absorbed dose, especially in the range below 10 Gy. In X-band, big efforts (chemical treatments) are done for eliminating, as much as possible, its contribution to the enamel spectrum. In in vivo L-band dosimetry teeth cannot be chemically treated and the differences in microwave saturation behaviour of CO_2^- and native radicals are currently considered as the only possibility for reduction of contributions from native radicals. The EPR signals of the native radicals are saturated at a microwave power close to 2 mW, while the signals of the CO_2^- radicals do not saturate at a power below 100 mW (Ignatiev et al., 1996). For the measurements of this study a power of 23 mW was set on the spectrometer, but the tooth in the resonator was actually exposed to only 1 or 2 mW. This loss in microwave power was estimated by comparing the saturation behaviour of reference samples (LiPC and DPPH) in X- and L-band. Other limiting factors for analysing spectra of teeth with low absorbed dose are the existence of an intrinsic resonator signal and noise.

4. Conclusions

Dose response curves with double integrals and amplitudes of the single composite L-band EPR line and the deconvoluted CO_2^- line could be obtained with 35 molars, gamma-irradiated in the 1–100 Gy range. No significant fading of the EPR signals could be measured within 4 months after irradiation. Deconvolution of the experimental spectra in two components due to CO_2^- and native radicals were performed. The L-band EPR spectrum of irradiated teeth was simulated by a hypothetical CO_2^- signal, combined out of orthorhombic Lorentzian and quasi-axial Gaussian signals with an intensity ratio of 3:2. With the present spectrometer and resonator, a detection limit of about 0.5 Gy was found by deconvolution of the dosimetric CO_2^- signal.

Limitations are imposed by considerable contribution of native radicals at low doses. In order to reduce this influence, an optimization of the L-band system is planned that allow for measurements at higher microwave power. Other limitations are related to the presence of an intrinsic fluctuating resonator signal, and the noise inherent in low signal measurements. Improvements can be obtained by longer and repeated measurements. Technical improvements (optimization of AFC, stability of microwave output, shape of resonator and its coupling to microwave source) are necessary and planned for improvement of measurement precision and detection limit.

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