

Experimental Model for Retrospective Assessment of X-Ray Exposures in Dento-Maxillary Radiology Measured by Electron Paramagnetic Resonance in Tooth Enamel

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Abstract: Electron paramagnetic resonance (EPR) dosimetry of human tooth enamel has been widely used in measuring radiation doses in various scenarios. For experimental purposes in X-ray diagnostic or therapy human persons can not be involved. For such cases we have developed an EPR dosimetry technique making use of enamel of molars extracted from pigs. The method can evaluate doses and dose-profiles of irradiated teeth at low level as 50 – 100 mGy (in air). EPR-spectra acquisition, data processing and dose assessment were done using non-dedicated equipment, devices and software.

Keywords: Dosimetry, Electron paramagnetic resonance (EPR), Dental enamel, X-rays.

Introduction

It is stated that while available data clearly shows ionizing radiation can result in biological damage if delivered in sufficiently high dose, it is not clear that radiation doses required for dental radiography present any risk. However, neither is it clear that such small doses are entirely free from risk [1]. Many studies revealed that individuals receiving high-dose accidental or therapeutic radiation to the head or neck have been shown to have an increased risk of developing a meningioma or glioma [2]. Other studies imply that the radiation exposure obtained from diagnostic dental panoramic radiographs could be associated with meningioma risk, but with a long latency period of at least two decades [3].

International Atomic Energy Agency (IAEA, Vienna) recognizes in 2007 that “medical ionizing radiation sources provide by far the largest contribution to the population dose from artificial sources and most of this contribution comes from diagnostic X rays (above 90%)” and elaborates an International Code of Practice for dosimetry in diagnostic radiology [4]. This Code can not be soon effective because various examination techniques are used in X ray diagnostics and, in some cases, expensive dosimeters and specialized personnel are required.

Even so, X-ray exposures in cranial region can be retrospectively assessed by electron paramagnetic resonance (EPR) spectrometry on tooth enamel. A large number of investigations have used this biodosimetric method many years after external exposure to reconstruct doses received from accidents, from occupational exposures, from environmental releases, and from medical exposures [5].

The method is based on the fact that in dental enamel carbonate impurities, which are incorporated into or attached to the surface of hydroxyapatite crystals during formation, are converted to CO²⁻ radicals through absorption of ionizing radiation. The concentration of radicals increases with absorbed dose. The intensity of the resultant EPR absorption is a measure for the absorbed dose. Because the EPR signal is generally stable with time it remains as possibly the strongest technique for individual retrospective dose assessment [6,7].

Every free radical species has a unique EPR spectrum, which is distinguished by its linewidth (ΔB), shape (symmetric or asymmetric) and resonant field (B); the latter value being proportional to the more characteristic Landé g -factor.

In dental enamel are of interest native background signal (BGS) and radio-induced signal (RIS) or “dosimetric” signal. Native signal is a symmetric signal at $g=2.0045$ with $\Delta B=0.8-1.0$ mT likely derived from the organic component of enamel and practically insensitive to radiation exposure. It can be found from the unirradiated enamel. Dosimetric signal is an asymmetric signal caused by stable radiation-induced radicals (centers). The main contribution to dosimetric signal is due to axial-symmetric CO_2^- radio-induced orientated centers at $g_{\perp}=2.0018$, and $g_{\parallel}=1.9971$, with the maximum at $g=2.0032$ and the minimum at $g=1.9971$ [6].

In the experimental EPR spectrum (ES) of irradiated tooth enamel BGS and RIS overlap each other. The spectra deconvolution for RIS is performed according to the spectrum subtraction method in which the EPR spectrum of a non-irradiated reference sample (BGS) is subtracted from the spectrum of the irradiated sample (ES).

An evident weakness of the method is that the measurement is performed *ex vivo*, on extracted or exfoliated teeth. Moreover, any experimental radiation exposures of humans can not be done. In such conditions, for research purposes would be useful a model that uses easy available teeth.

In a recent study [8] were used cow molars. For such teeth significant variations of sensitivity of the radiation-induced ESR signal to radiation were found. For dento-maxillary X-ray exposures we considered pig teeth to be more appropriate. Pig oral maxillofacial region is more similar to that of humans in anatomy, development, physiology, pathophysiology, and disease occurrence [9]. For EPR dosimetry the use of healthy permanent molars with same position, recollected from same animal without an irradiation history, eliminates many sources of errors [10-12].

Because the necessary instrumentation, e.g., the EPR spectrometer and sample preparation tools, is expensive and requires well-trained and skilled operators, the applicability of the method is considered limited to a small number of expert laboratories [5].

The aim of this paper is to describe an experimental model for the study of X-ray dental exposures of pig molars using non-dedicated equipment, devices and software, taking into account the necessity of a lot of extra work.

Material and Method

It should be stressed that presently no single standard EPR technique exists. Combination of particular solutions determines unique protocols, which are practiced in each individual laboratory [13]. Moreover, any version of EPR dosimetric technique is subject of continuous reevaluation and improvement [10,12,14-17].

Enamel Sample Preparation

Permanent molars M1 from both jaws were extracted from pig crania purchased on food market. To avoid age-dependent variations [10] only the teeth from single animal were included in one series of measurements. The enamel separated from two molars (from one side) was used for measurement of BGS and the enamel from other two for measurements of ES after orientated X-ray exposure. The teeth were irradiated with their vestibular faces toward the incident beam and placed between two Plexiglas plates 8 mm thick [13], as a tissue substitute (phantom). The results presented in this paper are obtained with a Siemens – “Polimobil Plus” X-ray machine after a single exposure having the following parameters: 81 KVp; 160 mAs; total inherent filtration 2.7 mm Al; focus to vestibular surface 21.5 mm. The estimated dose in soft tissue for this exposure is close to 100 mGy [18,19], value considered at the lower limit of reliable EPR dose assessment [16,17].

The crowns were cut from the roots and each crown was cut in its vestibular and lingual halves using dental diamond disk, at low speed and cooled with water. The halves were separated in four groups: unirradiated vestibular, unirradiated lingual, irradiated vestibular and irradiated lingual. The dentine was removed manually using water cooled hard alloy dental drill. The enamel was crushed

by a pair of nippers to chips sized 0.5-1.5 mm [14] and thereafter rinsed twice in distilled water and dried in an oven for at least 10 hours at 60°C [6].

The spectra measurements were done not less than ten days after irradiation and sample preparation, so all transient signals should have faded [6,14].

EPR Spectra Measurement

The measurements were performed at room temperature with an old but high sensitivity X-band (~9.1 GHz) spectrometer ART-6 (IPRS Baneasa, 1979) equipped with a general purpose data acquisition 12-bit interface Pasco 750 driven by Science Workshop program (Pasco Scientific, Roseville, CA, USA) [20]. The following spectra recording conditions and parameters were used: microwave power 8.5 mW, modulation frequency 100 KHz, modulation amplitude 0.3 mT, receiver time constant 100 ms, receiver amplification 500 and sweep width 130 mT. Interface was set to 10 samples per second acquisition frequency resulting digitized spectra with 1300 data points. The spectrum of each scan was recorded individually for further processing. The number of spectra scans was 30 corresponding to spectra accumulation time of 110-120 min.

The same quartz sample tube of 5-mm inner diameter was used for all measurements. The tube was loaded with precisely weighted 150-200 mg enamel powder and positioned in the cavity so that the center of the sample coincided with the center of the cavity. A Mn²⁺: CaO powder sample, inserted in tube at the sample bottom, was used as a reference for g-value and for signal intensity normalization [10,16,17,21].

In order to secure spectra g-factor accuracy a supplementary calibration of the field was accomplished with the resonance of polycrystalline DPPH (diphenyl picryl hydrazyl) with g=2.0036.

Data Processing

To obtain the spectra of every sample, the digitized scans recorded as *.sws (ScienceWorkshop) files were inspected for obvious errors caused mainly by supply voltage fluctuations. Those without such errors were exported and processed with the numerical program MS-Excel. First stage was to align the graphs so that 3rd and 4th lines of Mn²⁺ overlap for all. Second, was to average point-by-point at least 25 scans to increase signal-to-noise ratio by a factor of 5. The final steps were to correct the base-line of all enamel sample spectra so that have the same slope and to normalize all by intensity of 4th line of Mn²⁺ and by 100 mg enamel mass.

RIS spectra were obtained subtracting point-by-point normalized BGS spectrum from the ES normalized spectra. For vestibular and lingual irradiated enamel radioinduced signals were calculated as RIS_v = ES_v – BGS and RIS_l = ES_l – BGS, respectively.

Spectra calibration was necessary because the spectrometer we used has no output for either values of g-factor or magnetic field B. Calibration in terms of g-factor was made by establishing the linear correlation between the rank of recorded points and g-factors of signals Mn²⁺(3), Mn²⁺(4) and DPPH. For magnetic field B, calibration was performed using following equation: B_(mT) = 7.14455(v(GHz)/g); where v stands for microwave frequency (9,1425 GHz in this case) and g for g-factor [22].

Expert laboratories use dedicated computer codes which simulate ES and BGS spectra. The program guesses the best fit of given theoretical spectrum with recorded spectra [8,12-14]. RIS spectra issued from such procedure are the difference of two mathematical functions and absolutely noiseless. Our RIS spectra are “classical” (experimental) [23] and contain a lot of high-frequency noise which can be attenuated by smoothing the graphs. A “local” (over nine points) averaging function was applied to each experimental RIS spectrum. The final spectra were checked comparing their parameters and shape with generally accepted data [6].

Dose Evaluation and Dose Profile

The peak-to-peak amplitude R of RIS is related to the radiation-induced radical yield and therefore, to the absorbed dose. At an orientated exposure, as are skull radiographs or intraoral dental radiographs, vestibular enamel absorbs a higher dose than lingual one. Consequently the

signal intensity in vestibular enamel (Rv) is higher than in lingual (Rl). This difference is quantified by “dose profile”, is present in any X-ray diagnostic exposure and any good technique must evidence and measure an acceptable value of dose profile [24].

The uncertainty of a final EPR spectrum was evaluated calculating signal-to-noise ratio. The signal-to-noise ratio (R/I_N) of the EPR spectrometer, at a given dose, is defined by the ratio of “maximal intensity of EPR signal”, R , to the “maximal intensity of low-frequency noise”, I_N . Low-frequency noise results in a statistical uncertainty of the EPR signal intensity, $\sigma_N = 30 \times (I_N/R) \%$ [6].

Results

Separation of Enamel

The total amount of granulated tooth enamel obtained from one molar half was in the range 0.5–0.7 g, which is enough to make 2-3 aliquots of 200 mg for EPR measurement or further experimental procedures.

Spectra Acquisition

The results from steps of individual spectra acquisition and processing are shown in Figure 1.

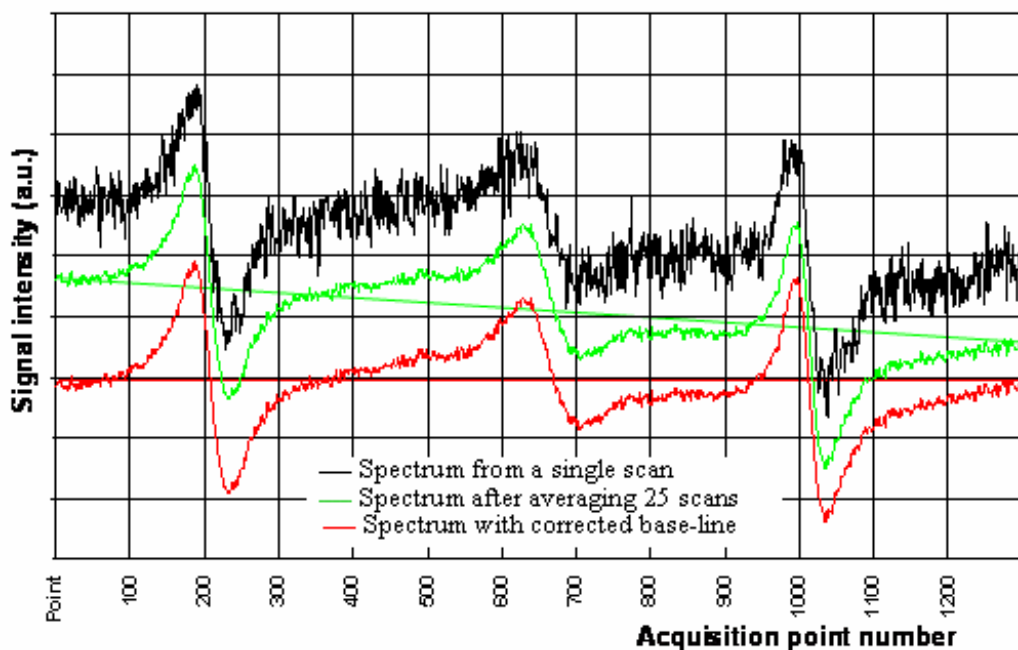


Figure 1. Results from acquisition and primary processing of a BGS spectrum. For clarity the spectra were shifted vertically to avoid superimposition (a.u. = arbitrary units)

Spectra Calibration

The calibration, valid for all aligned signals, done in respect of both EPR spectral parameters is shown in Figure 2. The values of the parameters found for BGS are $g=2.0044$ and $\Delta B \approx 0.8\text{mT}$.

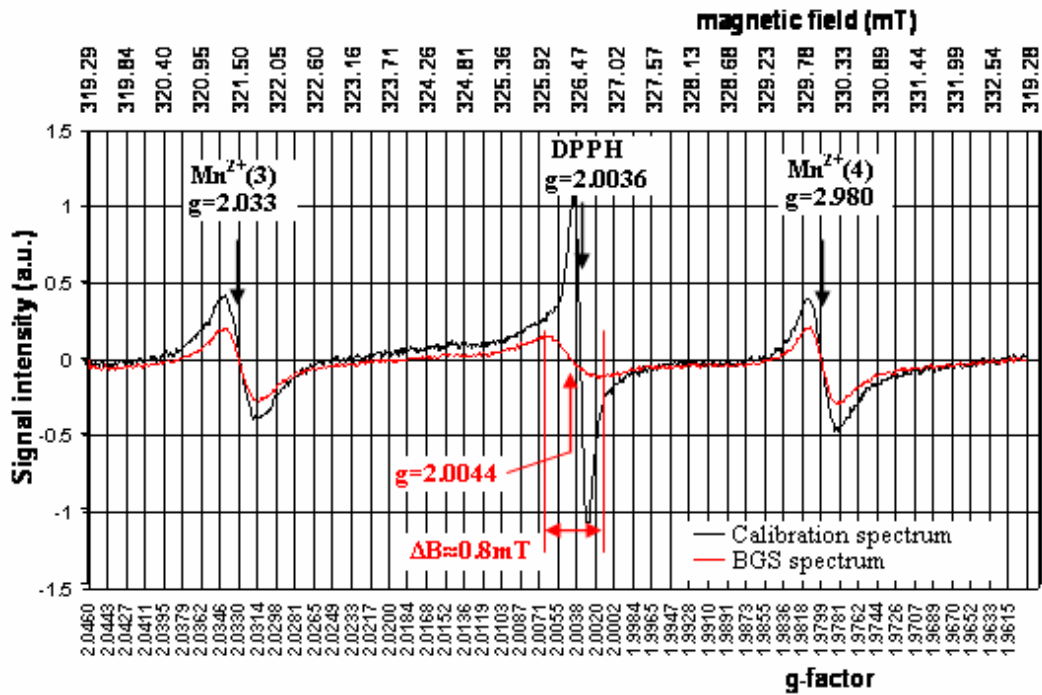


Figure 2. The graphs of calibration spectrum (black) and BGS spectrum (red) (a.u.= arbitrary units)

Spectra Subtraction and RIS Graph Smoothing

The results of "classical" version of protocol including manual spectra manipulation and subtraction are shown in Figure 3.

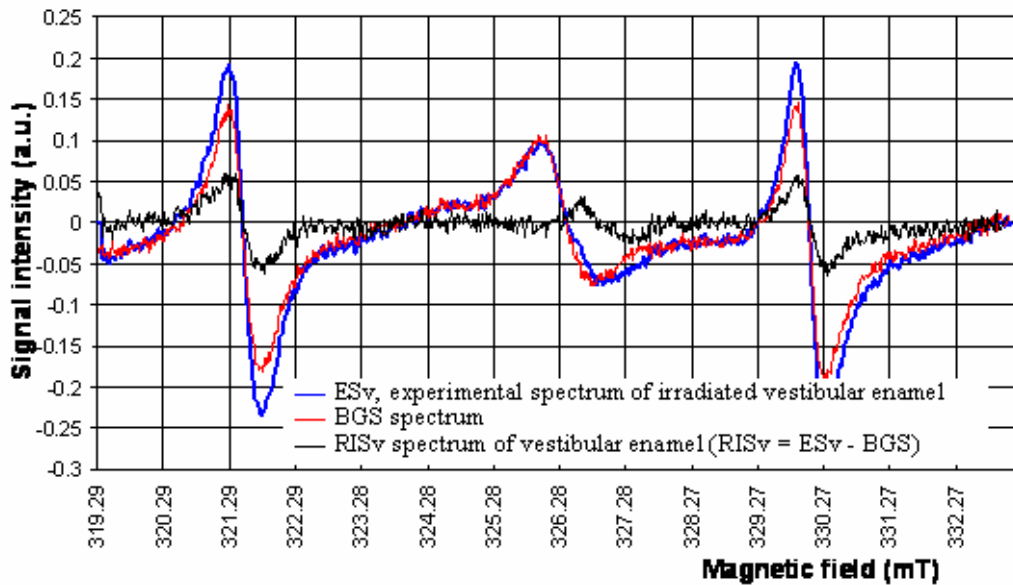


Figure 3. Results of spectra processing and subtraction (a.u. = arbitrary units)

The amplitude of RIS spectra resulted from subtraction are smaller than corresponding ES and BGS but the noise (i.e. errors) increases by a factor of $2^{1/2}=1.41$.

The high frequency noise reduction of the RISv spectrum, achieved by smoothing (local averaging), can be seen in Figure 4.

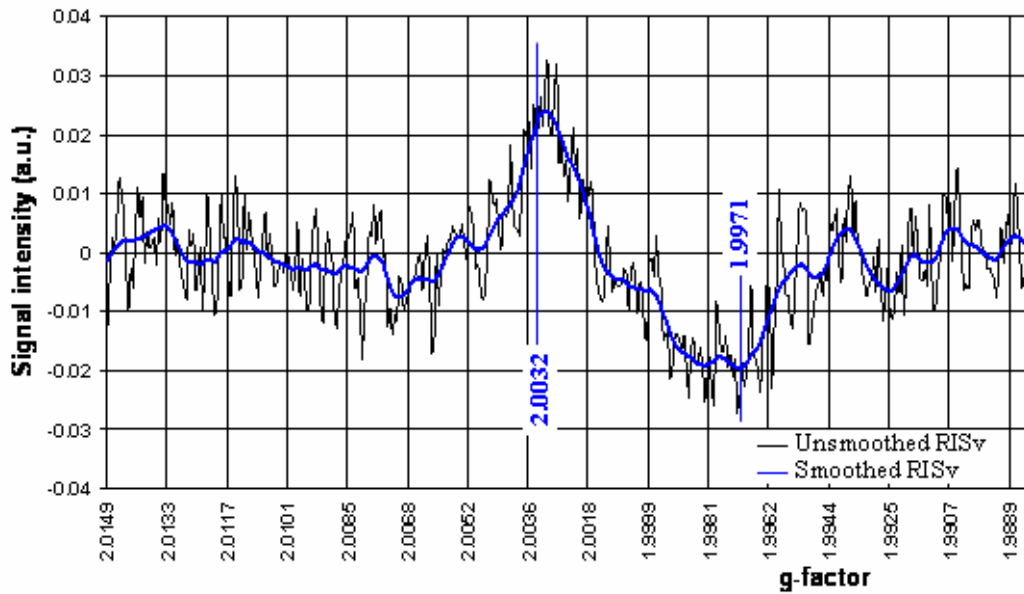


Figure 4. Unsmoothed and smoothed RISv graphs. Highlighted g-values are for accepted positions of signal maximum and minimum

Dose Evaluation and Dose Profile

The recorded radioinduced signals RISv and RISl are shown in Figure 5. Their intensities (peak-to-peak heights) Rv and Ri are proportional to absorbed doses on vestibular and lingual enamel, respectively. The calculated depth dose profile ratio is $D_p = R_l/R_v = 45\%$. In terms of doses the lingual enamel was exposed to only around 50 mGy, in air.

The signal-to-noise ratio and corresponding uncertainty calculated for dosimetric signals of vestibular and lingual enamel are listed in Table 1.

Table 1. Signal-to-noise ratios and uncertainties ($\pm 1\sigma$) of dosimetric signals

	Vestibular	Lingual	Dose profile (D_p)
Quantity	$R_v/I_n = 4.4$	$R_l/I_n = 2.0$	$(R_l/R_v)\% = 45\%$
Uncertainty (%)	$\sigma_{N_v} = 7$	$\sigma_{N_l} = 15$	$\sigma_{N_{D_p}} = 17$

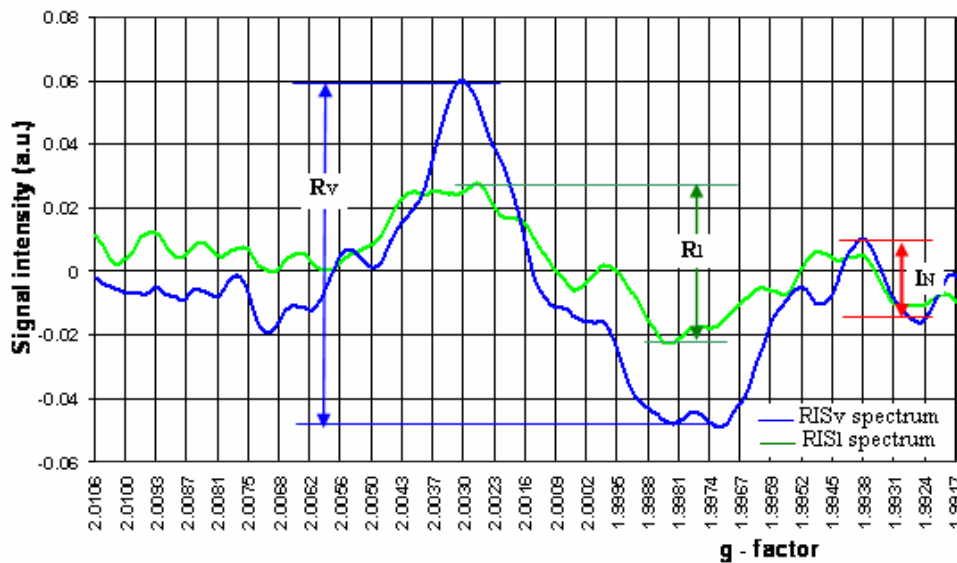


Figure 5. Radiation induced signal intensities in vestibular (Rv) and lingual (Rl) enamel. I_n – maximum intensity of low-frequency noise

Discussion

After processing, the shape of BGS signal and its signal-to-noise ratio (Figure 1) are the same with those published for a well purified enamel, correct sample preparation, spectra acquisition and data processing [12,25]. The EPR spectral parameters of native signal (BGS, shown in Figure 2) and dosimetric signal (R, shown in Figure 4) are also in very good agreement with those generally accepted [6].

The value of dose profile found by us falls between the limits found in expert laboratories who measured an attenuation between first and last tooth layers for low-energy beams in the range from 0.28 to 0.57 [24]. The numbers describing uncertainty at low doses prove the good quality of EPR spectrometer used and the validity of its parameter settings [6].

These results confirm that method fulfill the requirements for correct evaluation of X-ray exposures. We consider that there are two key steps in obtaining good results: first is a very good mechanical cleaning of the enamel and the second resides in individual inspection and alignment of every scan of every spectrum. Both imply time consuming work of two professionals - a dentist and an EPR spectroscopy technician. For good enamel mechanical separation a partial loss of enamel is assumed but this loss is covered by dimension and availability of pig teeth.

Since an experiment includes teeth from a single animal, method avoids the uncertainty due to individual variation in the sensitivity of radiation-induced signal to dose. This variation is 12% in humans [13], 12-25% in dogs [26] and around 80% in cows [8]. Individual variation of enamel radiation sensitivity of pig teeth is still unknown and must be determined if is intended to use in one experiment teeth from different animals.

As it is, method can assess correctly the ratios of different doses deposited in teeth as a function of position and orientation in X-ray beam. The absolute doses can be only roughly approximated on basis of literature data. Together with a proper calibration against an ionization-chamber can be approached in systematic way dose responses prompted by different types of X-ray examination including different geometry, X-ray apparatus and dose per examination [25]. Because of high level of doses used for radiotherapy their measurement can be done without difficulty.

It was shown that teeth enamel radiation sensitivity of large mammals is similar to that of humans [26,27]. The degree of similarity between enamel radiation-sensibility of pig teeth and that of human teeth must be stated in statistical terms and make the subject of our next work.

Conclusions

With the proposed model can be detected and evaluated low level X-ray exposures by EPR spectroscopy method using enamel of pig molars, general purpose data-acquisition equipment and non-dedicated software.

Enamel of pig molar has EPR native and dosimetric signals with same parameters as enamel of human teeth. Pig molars can be a valuable alternative for human teeth in experimental studies on X-ray diagnostic and therapeutic procedures.

If is adopted classical (experimental) way to find dosimetric signal, the program MS-Office Excel can be successfully used for data processing, spectra manipulation, numerical calculations and graphic representations.

The relation between X-ray radiation sensitivity of the enamel of pig molars and the enamel of human teeth is not exactly known and must be established by further comparative studies.

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