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X- and *Q*-band EPR study on dosimetric biomaterials

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Abstract. Electron paramagnetic resonance (EPR) spectroscopy (*X*- and *Q*-band) was used for identification of stable radicals in: compact bone powder, shell of *arcidae* mollusc and crystalline alanine. The aim of our investigation was to analyze the complex EPR spectra of these materials and to identify paramagnetic species contributing to it. The most successful results were obtained in the EPR study (*X*- and *Q*-band) on deproteinized and irradiated bone powder and *arcidae* shell at room temperature.

Key words: EPR • *X*- and *Q*-band • dosimeters • bone powder and *arcidae* shell • radicals

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Introduction

Electron paramagnetic resonance (EPR) spectroscopy is a method suitable for radiation dosimetry due to its accuracy, sensitivity and fast measuring procedure. Materials in which stable paramagnetic species are produced by irradiation can be used as EPR dosimeters for radiation research and radiation technologies. When the relationship between EPR signal intensity of stable paramagnetic center and the dose is of linear character, the material can be used as a dosimeter of absorbed radiation. Depending on sensitivity and range of linearity, the dosimeters can be used for different purposes as geological and archeological dating, standard and accidental dosimetry, detection of irradiated food [1–3].

The best material for radiation dosimetry is the one in which only a single radical signal with a linear signal--to-dose dependence is stabilized or at least one signal distincly dominates. However, usually the EPR spectra in dosimetric materials are complex. The EPR spectrum of synthetic alanine commonly used in EPR dosimetry consists, for example, of two signals with similar intensity derived from alanine, CH_3-$^{\bullet}$CH-COOH and NH_2-$^{\bullet}$C-(CH_3)-COOH radicals trapped in different structural sites [4].

In the paper we present the results of testing the possibility to use compact bone powder in natural form or after deproteinization and mollusc shell samples for dosimetry of ionizing radiation. It was found [7] that irradiation of bone tissues at room temperature results in the formation of two types of paramagnetic species: free radicals in bone collagen and paramagnetic centers induced in the main constituent of bone mineral--hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$. In the presence of air collagen radicals disappear completely after few

days. In contrast, the EPR signal of radiation-induced paramagnetic centres in bone hydroxyapatite is long-lived and did not show any noticeable changes of the shape and intensity for years [2].

It has been found that EPR spectra of irradiated synthetic apatites consist of several signals due to paramagnetic species derived mainly from carbonate impurities, substituting hydroxyl groups (so-called A site) or phosphate groups (B site) or those located on the surface of microcrystallites. The species which have been identified, for example, CO_3^{-3}, CO_3^-, CO_2^-, O^- are of differrent molecular structure and charge [1, 2].

In irradiated samples of tooth enamel which contains 98% of carbonate hydroxyapatite and in synthetic hydroxyapatities with carbonates substituting hydroxyl or phosphate groups, a few EPR signals had been identified in the EPR and ENDOR studies. It was shown that the most intensive signal derived from CO_2^- radical is overlapped by the signals of CO_3^{-3} and CO_3^- radical anions and O^- radical [1, 10]. It was also suggested earlier that the EPR spectra of irradiated *arcidae* shell which consists mainly of aragonite are complex with dominanting CO_2^- radical component [9].

In the present study we apply *X*- and *Q*-band EPR spectroscopy in order to identify paramagnetic centers which are responsible for EPR signal stable at room temperature. For EPR signals characterized by *g* anisotropy the resolution of *g* components is better at higher microwave frequency.

Experimental

The powdered human bone samples and deproteinized, both in natural form were obtained from the National Centre of Tissue and Cell Banking, Warsaw, Poland. Bone samples were deproteinized according to the procedure described elsewhere [6, 8]. Shell of sea water *arcidae* mollusc were collected from the Mediteranian Sea and powdered in the laboratory.

All samples were irradiated at room temperature at the Institute of Nuclear Chemistry and Technology (INCT) Warsaw with a dose of 5 kGy in a ^{60}Co gamma source "Issledovatel".

The EPR measurements were carried out at room temperature in *X* (9.5 GHz frequency) and *Q* (34 GHz frequency) bands. The *X*-band spectra have been recorded with a Bruker ESP-300 spectrometer, whereas the *Q*-band measurements were carried out with a Bruker ELEXSYS E-500 spectrometer. In both bands a wide range of microwave powers and modulation amplitudes were tested in order to optimize the detection conditions.

The measurement parameters were the following: for *X*-band-modulation frequency 100 kHz, modulation amplitude 0.1mT and microwave power within the range 1–10 mW, and for *Q*-band-modulation amplitude between 0.01–0.1 mT, microwave power in the range 0.1–1 mW. A standard polycrystalline DPPH sample ($g = 2.0036$) was used for accurate *g*-tensor determination.

Our *X*-band spectrometer is equipped with precise frequency counters (Hewlett Packard and Bruker gaussmeters enabling the precise determination of *g* tensor.

The powdered samples were placed in thin-wall spectrosil quartz tubes with outer diameter of 4.00 mm for *X*-band and of 2.00 mm for *Q*-band.

Results and discussion

Bone powder

The EPR measurements of bone samples were carried out about one month after γ-irradiation in order to be sure that all signals derived from organic radicals decayed.

The EPR spectrum of compact bone powder recorded in *X*-band presented in Fig. 1a is a complex one with a dominant singlet of axial anisotropy with: $g_\perp = 2.003$ and $g_{II} = 1.997$ representing CO_2^- radical anion (orthorombic). That signal was earlier observed in different matrices, i.e. in carbonate hydroxyapatite, calcium carbonate and tooth enamel [1, 2]. The *g* parameters of less intensive lines partly overlapped with CO_2^- signal represent most probably carbonate radical anions: CO_3^{3-} $g = 2.004$ and CO_3^- $g = 2.009$.

Q-band spectrum of the same sample (Fig. 1b) is much better resolved revealing sharp lines hidden in *X*-band spectrum. The detailed analysis of *Q*-band spectrum allows us to identify the following signals in γ-irradiated compact bone, all stable at room temperature:

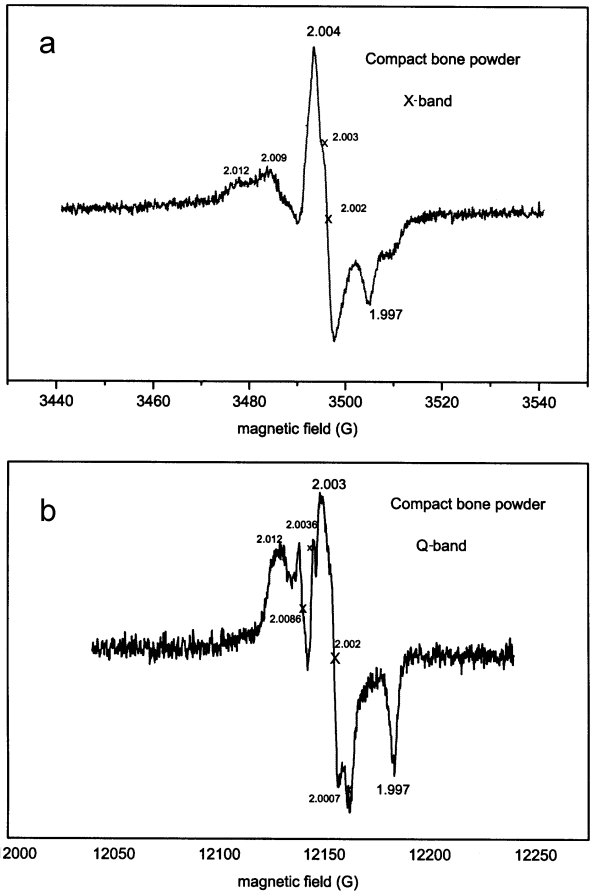

Fig. 1. Experimental EPR spectra of compact bone powder, γ-irradiated at room temperature, with a dose of 5 kGy at *X*-band (a), *Q*-band (b): (CO_3^- $g_{av} = 2.012$, CO_3^- $g_{av} = 2.0086$, CO_3^{-3} $g_{av} = 2.0036$, CO_2^- (iso) $g_{iso} = 2.0007$, CO_2^- (ortho) $g_\perp = 2.003$, $g_{II} = 1.997$).

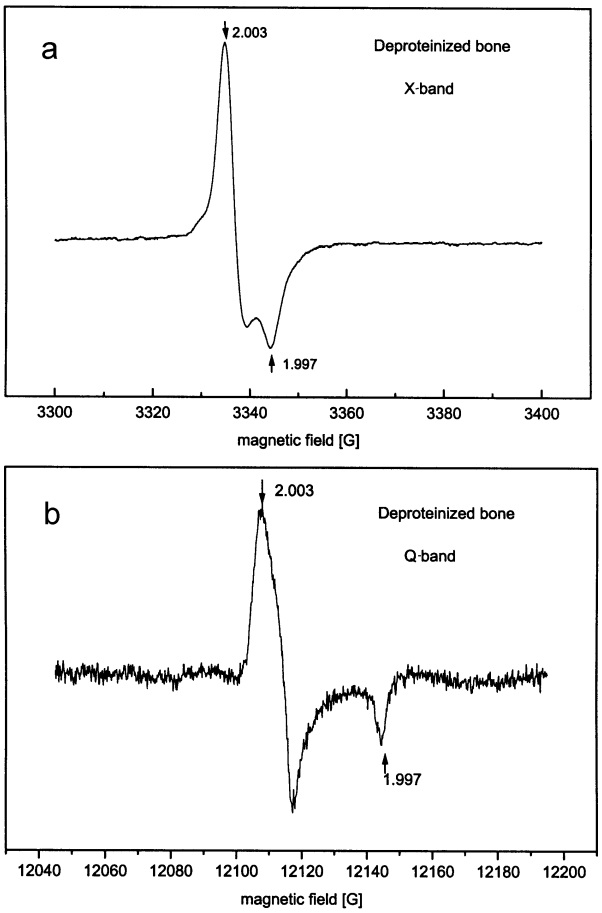

Fig. 2. Experimental EPR spectra of deproteinized compact bone powder, γ-irradiated at room temperature with a dose of 5 kGy: *X*-band (a), *Q*-band (b): (CO_2^- (ortho) $g_\perp = 2.003$, $g_{II} = 1.997$).

CO_2^- : $g_\perp = 2.003, g_{II} = 1.997$ (orthorombic),
CO_2^- : $g_{iso} = 2.0006$ on the surface of hydroxyapatite crystallites,
CO_3^- : $g_{iso} = 2.012$ occluded water,
CO_3^- : $g_{av} = 2.0086$ on the surface,
CO_3^{-3} : $g_{av} = 2.0036$ trapped in site A, substituting hydroxyl groups.

Q-band spectrum of the irradiated compact bone shows clearly that at least two other species, besides CO_2^- contribute to the intensity of the highest spectral line. Their dose responses may be different disqualifying that material for EPR dosimetry.

The reason why we decided to examine deproteinized bone by EPR was the expectation of the increase of dose responses by about 30% in the material consisted mostly of hydroxyapatite. It is since collagen is about two third by weight of compact bone [6, 7]. The *X*-band showed a nice shape of CO_2^- anisotropic singlet: $g_\perp = 2.003$, $g_{II} = 1.997$ (Fig. 2a). Although *Q*-band measurements reveal a small distortion of central EPR (Fig. 2b) we can conclude that the contribution of additional signal to the total signal intensity is meaningless [8].

Arcidae shell

The *X*-band EPR signal of γ-irradiated powdered *arcidae* mollusc shells (Fig. 3a) shows also rather an undisturbed CO_2^- anisotropic signal with: $g_\perp = 2.003, g_{II} = 1.997$, however, the top of *g* component is not sharp. The *Q*-band spectrum clearly reveals the complex character of that signal (Fig. 3b). The spectrum consists of two signals, the well resolved signal of CO_2^- radical anion with orthorombic *g* tensor $g_1 = 2.003, g_2 = 2.002, g_3 = 1.997$, and *a* dominant isotropic singlet with $g_{iso} = 2.0006$ which probably represents CO_2^- radical trapped in the environment allowing molecular dynamics. It was proposed earlier that CO_2^- associated with ion vacancy occupied by H_2O molecule is able to rotate freely and thus to produce in EPR an isotropic singlet [1]. The present *Q*-band measurements proved that *arcidae* mollusc shell can be used for EPR dosimetry. The dominant signal of free rotating CO_2^- radical anion is overlapped to a small extent by the second signal of CO_2^- trapped at different site [5, 9, 10].

It is known that the EPR spectrum of irradiated alanine consists of two different signals derived from alanine radicals localized in slightly different environments [4]. Thus, we proceeded *X*- and *Q*-band experiments with γ-irradiated alanine, too. However, in that case *Q*-band spectra did not reveal any additional features in comparison to *X*-band spectra what could be helpful to analyze quantitatively the ratio of two alanine radicals.

The *Q*-band measurements allow us to adapt deproteinized bone and *arcidae* molluscs shells as the biomaterials potentially useful for EPR dosimetry. However, to propose them for common use we have to validate the dose dependence of both biomaterials.

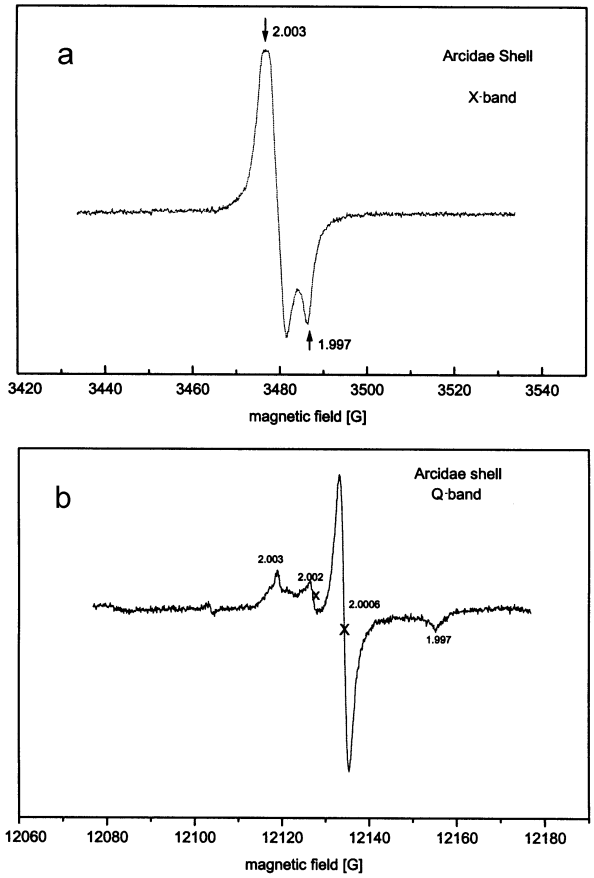

Fig. 3. Experimental EPR spectra of sea shell *arcidae*, γ-irradiated at room temperature with a dose of 5 kGy: *X*-band (a), *Q*-band (b): (CO_2^- (iso) $g_{iso} = 2.0006$, CO_2^- (ortho) $g_\perp = 2.003, g_{II} = 1.997$).

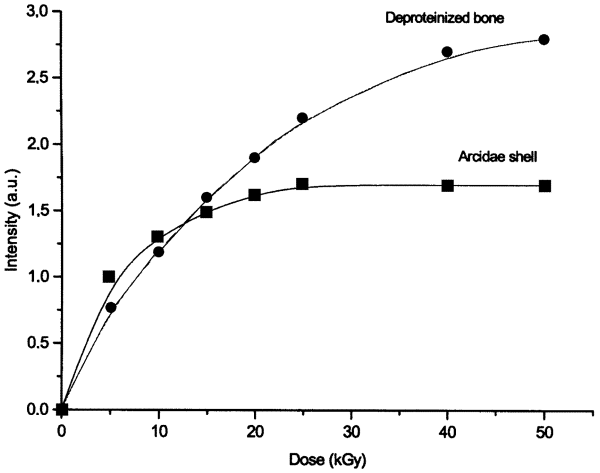

Fig. 4. The dose dependence of CO_2^- signal intensity for deproteinized compact bone and *arcidae* shell.

The dependence of EPR signal intensity vs. dose for γ-irradiated deproteinized bone and *arcidae* shell in *X*-band is presented in Fig. 4. For deproteinized bone the dependence on EPR intensity with dose is almost linear and quite steep untill 20 kGy. For higher doses, saturation effect is observed but even in the range 50–60 kGy the EPR signal intensity markedly increases with increasing dose.

For *arcidae* shell, the EPR intensity up to 10 kGy increases strongly with dose, similarly as for deproteinized bone. At higher doses, however, the relationship reaches plateau which makes impossible dose measurements.

The results clearly show that *arcidae* shells cannot be used as an EPR dosimeter to control radiation sterilization for which usually the doses in the range 25–35 kGy are applied (Fig. 4).

Conclusions

The comparative EPR measurements at *X*- and *Q*-band made it possible to test two type of EPR biodosimeters – *arcidae* shell and deproteinized compact bone, where after γ-irradiation only one stable EPR signal is recorded.

However, the dose dependence curves are quite different for two materials. In the range up to 5 kGy the dependence curves are steep for both deproteinized bone and for mollusc shell. For doses below 2 kGy, the EPR response is even stronger for mollusc shell than for bone, indicating that this material is more sensitive at low doses. For doses higher than 15 kGy, both dependences become distinctly different. For deproteinized bone, the intensity of CO_2^- signal still increases with dose up to 50 kGy. However, the relationship looses slowly the linear character. Nevertheless, the intensity changes with the range of technological doses (25–35 kGy) are important enough to propose that material as dosimeter to control the sterilization of tissue grafts.

In contrast, the EPR signal intensity vs. dose curve for molluscs shell reaches its plateau at 20 kGy what disqualifies that material as dosimeter for radiation sterilization.

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