

# Determination of methemoglobin in human blood after ionising radiation by EPR

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**Abstract.** In the present work presents results of investigations of radiation influence on blood of patients examined by radio-isotopes diagnosis ( $Tc^{99m}$ ), blood of Chernobyl clean-up workers and human blood irradiated by LINAC using Electron Paramagnetic Resonance (EPR). The EPR spectroscopy reveals information on electronic states of transition metal ions, particularly  $Fe^{3+}$  in different spin states. It is shown that EPR spectra of blood of patients before examination has signal from metal-protein transferrin ( $g=4.3$ ) and after administration of radio-isotope proves signal of  $Fe^{3+}$  (methemoglobin) in the high spin state ( $g=6.0$ ). The EPR spectra of Chernobyl liquidator display number of signals including low and high state of ion  $Fe^{3+}$  ( $g = 2.0$  and  $g = 6.0$ ), and transferrin ( $g=4.3$ ). The EPR spectra of irradiated human blood by LINAC (linear accelerator) have only signal  $Fe^{3+}$  (methemoglobin) in low-spin state with  $g = 2.0$ .

## 1. Introduction

Paramagnetic centres in human blood include primarily the molecular complexes containing iron  $Fe^{3+}$  (transferrin, methemoglobin) or copper  $Cu^{2+}$  ions (ceruloplasmin) and free radicals [1]. EPR spectroscopic studies of iron and copper proteins have been published by Taiwo [2]. The unique ability of in vivo EPR to measure clinically significant exposures to ionizing radiation has been demonstrated by group of scientists [3]. Studies [4-10] were dedicated to explore radiation damage to blood and hemoglobin. In these studies, various physical techniques have been used to determine structural variations of hemoglobin after irradiation: EPR spectroscopy [4, 10], Mossbauer [9] and IR spectroscopy [10], absorption spectroscopy [11]. The new data on structural changes of oxyhemoglobin under irradiation have been investigated by Mössbauer spectroscopy to estimate the probability of protein degradation products resulting from the effect of external factors on the oxyhemoglobin and scheme of oxyhemoglobin radiolysis process proposed [9]. Confocal micro-Raman and FT-IR spectroscopies have been used for detection of radiation influence of hemoglobin of patients examined by radio-isotopes diagnosis ( $Tc^{99m}$ ). After irradiation some tiny changes of the Raman scattering bands which connected with out-of-plane porphyrine bending vibrations have been observed. Additionally, several scattering bands attributed latter to methemoglobin have been detected [11]. It was assumed that radiation exposure of blood leads to transition from hemoglobin ( $Fe^{2+}$ ) to methemoglobin ( $Fe^{3+}$ ) with a delocalization of iron from porphyrine ring plane [11]. In the present work results of EPR studies of radiation influence on blood of patients examined by radio-isotopes



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diagnosis ( $Tc^{99m}$ ), of Chernobyl clean-up workers and irradiated human blood by LINAC are presented.

## 2. Materials and Methods

Three different ways of blood irradiation and follow-up studies have been performed. Part of the blood samples has been received from the P. Stradins Clinical University Hospital of Latvia, where patients underwent bone diagnostics by  $Tc^{99m}$  radioisotope. Venous blood has been donated by consenting patients before and after radioisotope administration and collected freely in air in the glass tubes containing anticoagulant (heparin or EDTA). Administered activity of  $Tc^{99m}$  to the patient had 600-1000 MBq range individually prescribed. Blood without any anticoagulant was also tested and confirmed no difference to EPR spectra with respect to EDTA or heparin in blood. Activity of injected isotope  $Tc^{99m}$  to the patient has been measured by dose calibrator "Curiemontor 4" Physikalisch-Technische-Werkstätten (PTW). The calibrator has been proved against the Physikalisch Technische Bundesanstalt (PTB) traceable standard with uncertainty less than 5%.

The other part of blood samples have been collected from Chernobyl liquidators undergoing medical examination at the Centre of Occupational and Radiological Medicine of P. Stradins Clinical Hospital of Latvia. Some liquidators have documented individual dose in the dose register, some having individual dose reconstructed based on beta activity of the teeth [12].

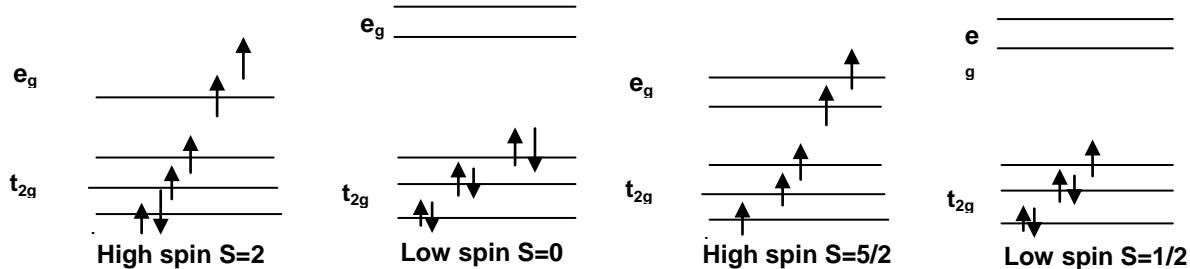
Few blood samples have been voluntarily donated by people from our laboratory group. These samples have been irradiated on LINAC with distinctive doses 0.5 Gy, 1.0 Gy, 2.0 Gy, 5.0 Gy, 10.0 Gy of 6 MV x-rays (photon beam). The absorbed dose values are traceable to BIPM (Bureau International des Poids et Mesures).

The EPR spectra of blood have been measured on a BRUKER EMX-6/1 spectrometer equipped with an Aspect 2000 data system. The spectra have been recorded at microwave power 6.2 mW, applying magnetic field modulation of 100 kHz and amplitude 1 mT. The g-factors of EPR signals have been determined by reference to the external magnetic field value measured by a BRUKER ER 035 Gaussmeter and of the microwave frequency measured by a SYSTRON DONNER 6235A frequency counter. EPR spectra of blood have been measured at temperatures 60 - 200 K using Dewar mounted to the X-Band waveguide type cavity, by fully remote operated control system via the acquisition software WinEPR of the EMX spectrometer. The Digital Temperature Control System, ER 4141VT makes use of gaseous nitrogen as coolant. Additionally, the EPR spectra of fresh blood have been measured at room temperature using the "AquaX" cell. The EPR signal intensities in blood have been measured against standard EPR signals with known spin number using the MgO ( $Cr^{3+}$ ) standard crystal placed in the resonant cavity.

## 3. Results and Discussion

The ion of iron in the blood commonly occurs as ferrous  $Fe^{2+}$  with electronic configuration Ar (3d<sup>6</sup>) in one of two different spin states (see Fig.1). When ion of iron  $Fe^{2+}$  in porphyrin ring of hemoglobin binds the oxygen it changes the spin state. Actually ion of iron  $Fe^{2+}$  is in high spin state (S=2) in deoxyhemoglobin (venous blood) and low spin state (S=0) in oxyhemoglobin (arterial blood) (see Fig.1).

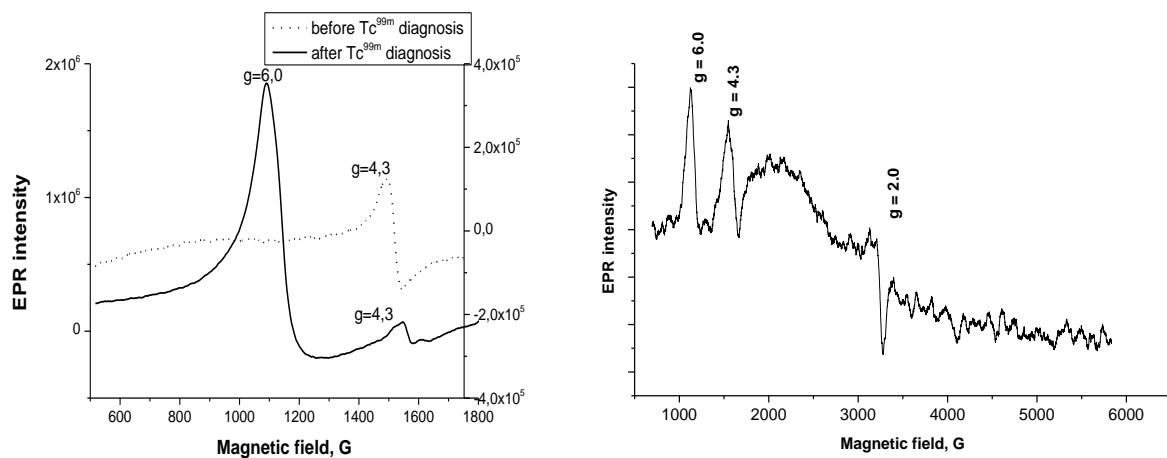
Ionizing radiation changes valence of iron from  $Fe^{2+}$  to  $Fe^{3+}$ . Ions of  $Fe^{3+}$  have electronic configuration Ar (3d<sup>5</sup>) in the high S=5/2 and low S=1/2 spin states (see Fig.2). Human blood contains number of non-heme ions of iron e.g. blood plasma glycoprotein transferrin, which is unable to transport oxygen but used as iron depot in the body.



**Figure 1.** Electronic configuration of  $\text{Fe}^{2+}$  (ferrous)  $[\text{Ar}]3\text{d}^6$  in low and high spin states in hemoglobin.

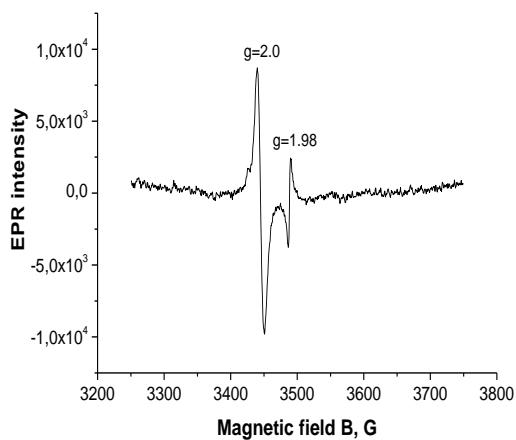
**Figure 2.** Electronic configuration  $\text{Fe}^{3+}$  low and high spin state  $\text{Fe}^{3+}$  (ferric)  $[\text{Ar}]3\text{d}^5$ .

The EPR measurements have been done before and after  $\text{Tc}^{99m}$  administration for bone diagnosis (see figure 3.). Taking into account the activity (600- 1000 MBq) and type of nuclide injected ( $\text{Tc}^{99m}$ ) one could estimate the dose absorbed by the blood [13]. The assessed blood absorbed dose was in the range 50 – 100 mGy. The EPR spectra before  $\text{Tc}^{99m}$  diagnosis have signal with g-factor 4.3 related to  $\text{Fe}^{3+}$  ion in high spin state (transferrin). Our investigations showed that not all samples of human blood of none-irradiated blood have EPR signal with g-factor 4.3. Elevated amount of transferrin could be attributed to the individuals having iron deficiency in their organisms. The EPR measurements of samples after  $\text{Tc}^{99m}$  diagnosis showed none difference in EPR signal with g-factor 4.3 related to transferrin, but demonstrates very high rise in signal with g-factor 6.0 related to  $\text{Fe}^{3+}$  ion in high spin state ( $S=5/2$ , originated from dioxyhemoglobin).

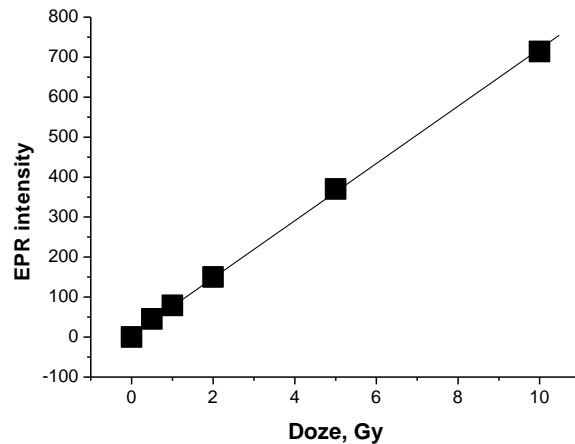


**Figure 3.** EPR spectra of human blood before and after  $\text{Tc}^{99m}$  diagnosis.

**Figure 4.** EPR spectrum of blood of Chernobyl clean up worker. (Documented dose in register 0.211 Gy.  ${}^{90}\text{Sr}/{}^{90}\text{Y}$  activity measured on teeth in 1997 - 80 Bq/g).



**Figure 5.** EPR spectrum of human blood after radiation 1 Gy by LINAC.



**Figure 6.** Dependence of EPR signal ( $g=2.00$ ) intensity of irradiated blood by LINAC.

The EPR spectra for blood samples from Chernobyl clean-up workers have been obtained as well (some of the spectra see on Fig. 4). The blood of Chernobyl clean-up workers with increased level of methemoglobin have been selected for further studies. It is necessary to point out, that obtained EPR spectra have several signals with g-factors 2.0 (methemoglobin and free radical), 4.3 (transferrin), 6.0 (methemoglobin). Overview of results of the measurements let us to assume that some of Chernobyl clean-up workers have methemoglobin content ( $\text{Fe}^{3+}$  in high spin state) higher than average population. The case of individual presented on Figure 4 suffered both on iron deficiency (high amount of transferrin) and at the same time on anaemia (high amount of methemoglobin). The supposition raised during the studies was that ion  $\text{Fe}^{2+}$  in hemoglobin is oxidized to the  $\text{Fe}^{3+}$  in heme by internal radiation exposure. The internal irradiation is resulted from  ${}^{90}\text{Sr}/{}^{90}\text{Y}$  and probably other radionuclides incorporated in tooth and further calcified tissues during clean-up activities in Chernobyl. Correlation between EPR signal of methemoglobin and measured activity of the  ${}^{90}\text{Sr}/{}^{90}\text{Y}$  in tooth of Chernobyl clean-up workers has been observed. The excess of methemoglobin is reported to the other Chernobyl clean-up workers having high documented doses in dose register [12, 13]. Part of human blood samples have been irradiated by LINAC with 6 MV x-rays (photon beam). Following doses have been delivered to the samples 0.5 Gy, 1.0 Gy, 2.0 Gy, 5.0 Gy, 10.0 Gy. The EPR spectra of irradiated samples have only one notable signal with g-factor 2.0 (see Fig. 5), free radical or delocalized electron. The EPR signal with g-factor 2.0 attributed to  $\text{Fe}^{3+}$  ion with electronic configuration at low spin state ( $S=1/2$ , methemoglobin), which was originated from oxyhemoglobin. This fact could be explained that human venous blood has been collected in the sample tubes containing air for future irradiation by LINAC. During sample collection and transport venous methemoglobin bounded the oxygen in the tube, becoming an oxyhemoglobin, which under irradiation turned again to methemoglobin. Additional irradiation of blood samples indicated good linear dependency of EPR signal intensity from the dose received by the blood (see Fig. 6).

#### 4. Conclusion

The samples of human blood before and after three different ways of irradiation have been studied. Blood samples donated by patient involved in  $Tc^{99m}$  bone diagnostic have notable EPR signal due to  $Fe^{3+}$  ion at high spin states in methemoglobin. Very weak EPR signal of  $Fe^{3+}$  ion at low spin states in methemoglobin of patient diagnosed using  $Tc^{99m}$  is subject for further investigation. EPR signal at  $g=4.3$  attributed to blood plasma glycoprotein transferrin has been detected as well.

The EPR spectra of Chernobyl clean-up workers have numerous signals related to  $Fe^{3+}$  ion in high and low spin states, transferrin and unpaired electron or free radical. The observed correlation between level of methemoglobin in blood of Chernobyl clean-up workers, documented individual dose and/or activity of  $^{90}Sr/^{90}Y$  content in teeth provided certain physical background for explanation of diseases prevalence among Chernobyl liquidators compare to the general population [12,14].

The EPR spectra of irradiated blood on LINAC with different doses of 6 MV megavoltage x-rays have signal of  $Fe^{3+}$  ion at the low-spin state in methemoglobin. Linear dependence between EPR signal intensity and blood expose has been observed.

Comparing results on EPR spectra intensity of  $Fe^{3+}$  ion before and after irradiation one can conclude the different types of radiation could lead to the same effect, enhanced level of methemoglobin after exposure. Ionizing radiation can cause change of hemoglobin to methemoglobin or in other words ion  $Fe^{2+}$  heme in hemoglobin is oxidized to the  $Fe^{3+}$  in heme by radiation. Iron ion changes the valence due to influence of ionizing radiation, but retains spin state configuration.

#### Acknowledgments

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