



Complex Time Dependence of the EPR Signal of Irradiated L- α -alanine

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(Received 9 February 1996; in revised form 26 March 1996)

Measurements of the EPR signal amplitude of γ -irradiated L- α -alanine with use of an adjacent reference sample have revealed variations in the signal intensity within hours and days after irradiation. The character of the time dependence of the amplitude varies with dose and the amplitude changes reach 1–1.5%. This observation favors the hypothesis that irradiated alanine contains several paramagnetic centers. Usefulness of adjacent reference samples in alanine dosimetry is also demonstrated. Published by Elsevier Science Ltd

Introduction

The alanine system is now used for a transfer dosimetry in primary and secondary standards laboratories and as a routine tool in some industrial applications (Regulla *et al.*, 1993; Nette *et al.*, 1993; ASTM, 1995). Improvements of its accuracy and precision were so successful that, at present, even errors below 1% are of importance. Under these conditions, uncontrollable long-term drifting of electron paramagnetic response (EPR) spectrometers, which can be neglected in most other EPR applications, becomes an important component of inaccuracies. In fact, even sophisticated modern EPR spectrometers typically do not provide a long-term stability of a signal with variations in intensity less than 0.5%, and signal variations as large as several percent occasionally occur.

In order to further improve alanine dosimetry, it is necessary to take these instabilities into account. An effective method to do this has been proposed long ago, when instabilities of EPR spectrometers were much greater. It consists of placing a second, immobile, paramagnetic sample with a different EPR signal into the microwave cavity to serve as a reference during the measurements (Anderson *et al.*, 1959; Singer, 1959, 1961). Variations in the intensity of the signal of the reference sample reflect instrument instabilities, and, if this signal is recorded

each time immediately after the signal of a test sample, the ratio of the signals in question will provide a characteristic of the test sample intensity that is free (or nearly free) of errors due to long-term drift and other uncontrollable changes in the system sensitivity.

Upon applying this technique to alanine dosimetry, we uncovered a time dependence of the signal of irradiated alanine during hours and for several days after irradiation, which is significant for precise dose measurements. These effects are especially important in transfer dosimetry where several days can elapse between exposure and instrumental evaluations.

Experimental

Alanine pellets* (4.9 mm diameter, 2.7 mm height) containing 90% (by mass) L- α -alanine (Aldrich, 99 + %) and 10% polyethylene (Polysciences, MW = 700, 60 μ m) as a binder, were irradiated with ^{60}Co γ -rays to absorbed doses from 0.01 to 100 kGy under electron equilibrium conditions and controlled temperature.

The pellets were prepared by the following procedure. The bulk alanine crystals were ground with a centrifugal mill (Brinkmann) fixed with a 0.5 mm ring sieve. Alanine crystals in the 53–125 μ m range were selected with a vibrating sieve (Brinkmann). Appropriate weights of sieved alanine and polyethylene were blended in a powder blender (Paterson-Kelly). The mixture was pressed into pellets with a Manesty hand-tabletting press to produce alanine dosimeters 4.9 mm in diameter with an average height of 2.7 mm. The dosimeters were placed in a 130°C oven for 30 min, followed by 5 min

*The mention of commercial products throughout this paper does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that products identified are necessarily the best available for this purpose.

in an 85°C oven. The dosimeters were stored in the dark space at ambient temperature under controlled humidity conditions ($45 \pm 5\%$ r.h. For replicate checking, irradiation of samples of the pure alanine powder used in preparing the pellets has been performed under the same conditions.

EPR measurements were taken at room temperature and relative humidity 55–60% with a Bruker ECS106 spectrometer equipped with a 4108TMH/8909 cavity. Because this cavity has no additional holes in the side walls, the exposure of alanine samples to light during measurements was negligible. In the course of measurements, an alanine sample was kept intact in the cavity, which also contained an immobile small single crystal of synthetic ruby (Al_2O_3 containing about 0.05% Cr^{3+}). The spectra of both samples were recorded at time intervals ranging from 7 min to 1.5 h depending on the rate of change in the alanine signal intensity.

The ruby signal was recorded each time immediately after the alanine measurement at exactly the same settings of instrumental variables, except the Zeeman field range and, in most cases, the receiver gain. In each experiment, receiver gains for alanine and ruby remained unchanged during the whole measurement session. The microwave power was at the level of 0.25 mW, which does not cause saturation of either of the two signals. Modulation amplitude of 1.4 mT was used in all of the measurements to provide a good signal-to-noise ratio for the lower-dose samples and comparability of results for different doses. Prior to the measurement period, the spectrometer was kept operating at the same parameters (including the modulation amplitude) as used in the experiment, for at least several hours. First-derivative amplitudes were used as characteristics of intensity for both signals. For alanine, the central line in the powder spectrum was monitored, whereas, for ruby, the line with the greatest intensity at a selected crystal orientation was used.

Field sweep rate and time constant were selected to meet the manufacturer's guideline (time constant ten times less than the peak-to-peak sweep time).

Results and Discussion

Time dependencies of the amplitude of the central line in the irradiated alanine spectrum normalized by the intensity of the ruby signal are presented in Fig. 1. The data show that the intensity of the signal varies with time and absorbed dose. At any dose, we observed a sharp decrease in the signal intensity for the first 1.5–2.5 h after irradiation. Because it took several minutes to deliver a sample from the radiation source to the EPR spectrometer and make the necessary adjustments of the spectrometer, signal evolution could not be observed from the very beginning. Furthermore, because we tried to start measurements as soon as possible in each case, the initial moment of the measurement start was not

exactly the same in all the experiments, and one should not directly compare the magnitudes of the initial drops on the plots.

In the case of the 10 Gy sample, the overall drop observed following the 13th minute after irradiation is about 1.2%. Reasoning from the general principles of chemical kinetics, one should assume an even steeper signal decrease at the very initial period after irradiation. The total percentage magnitude of the dip seems to decrease with increasing dose, although it is difficult to draw a firm conclusion on this point without values of the signal just at the end of irradiation.

In the dose range 10–1000 Gy, the signal remains virtually stable after this initial drop, at least for 1–2 days. However, at the doses above 1 kGy, a subsequent increase in the intensity occurs. At 2 kGy, the signal seems to increase steadily, at least for the first 4 days. In the range 5–20 kGy, the signal dependence on time exhibits a plateau beginning at about the 70th hour (3 days after irradiation). The signal remains constant for at least 3 more days. The greatest observed magnitude of the increase from the dip to the saturation level is about 1.5%.

The situation becomes even more complicated at higher doses: after the drop and the increase, the signal begins to fade again. The time for the maximum seems to depend on the dose. At 25 kGy, it is observed at about the 80th hour and at 100 kGy at about 40 h. The decrease in 250 h is about 0.55%.

The described dependencies of the signal were observed both for pellets containing polyethylene as a binder and for pure alanine powders without additives.

The results clearly indicate that the widespread concept of the irradiation product of alanine as a unique and stable radical is overly simplified. Obviously, there are chemical processes occurring in alanine after irradiation that differ with absorbed dose. This is consistent with the results of the other studies that suggest that several paramagnetic centers are present in the irradiated alanine (Callens *et al.*, 1996; Pilbrow *et al.*, 1996; Desrosiers *et al.*, 1995). Therefore, we conclude that the radicals present in freshly irradiated alanine samples are not a unique chemical species and that the system is much more dynamic than was previously assumed.

Practical conclusions from these observations are straightforward. When precise dose determinations are needed, one should not conduct any measurements during the first 2–3 h after irradiation; this is true for any dose. For measuring doses below 1–2 kGy, the period 3–24 h after the end of irradiation is favorable. For doses in the range 5 kGy to 20 kGy, it is better to perform measurements 3 days after irradiation, that is, in the plateau region. Finally, to determine precisely doses above 20–25 kGy, it is recommended that measurements be performed in the region of the maximum; it occurs at 60–120 h for the dose 25 kGy and at 30–70 h for

the dose 100 kGy. These peculiarities should be taken into consideration in designing accurate dose measurement and calibration procedures.

In conclusion, we consider use of an adjacent reference sample in general. This old technique has proved to be excellent in its applicability and usefulness to us in alanine dosimetry. In fact, although the EPR spectrometer we used in this study was relatively stable (variations in the ruby signal

intensity were typically below 1% for periods of several days), we would not have been able to measure the time dependencies in a statistically significant form without simultaneously monitoring the ruby signal. As an illustration, we present the plots of the intensities of the alanine and ruby signals along with their ratios in Fig. 2. It can be also seen from Fig. 1 that, using this technique, one can achieve reproducibility in measuring alanine signal

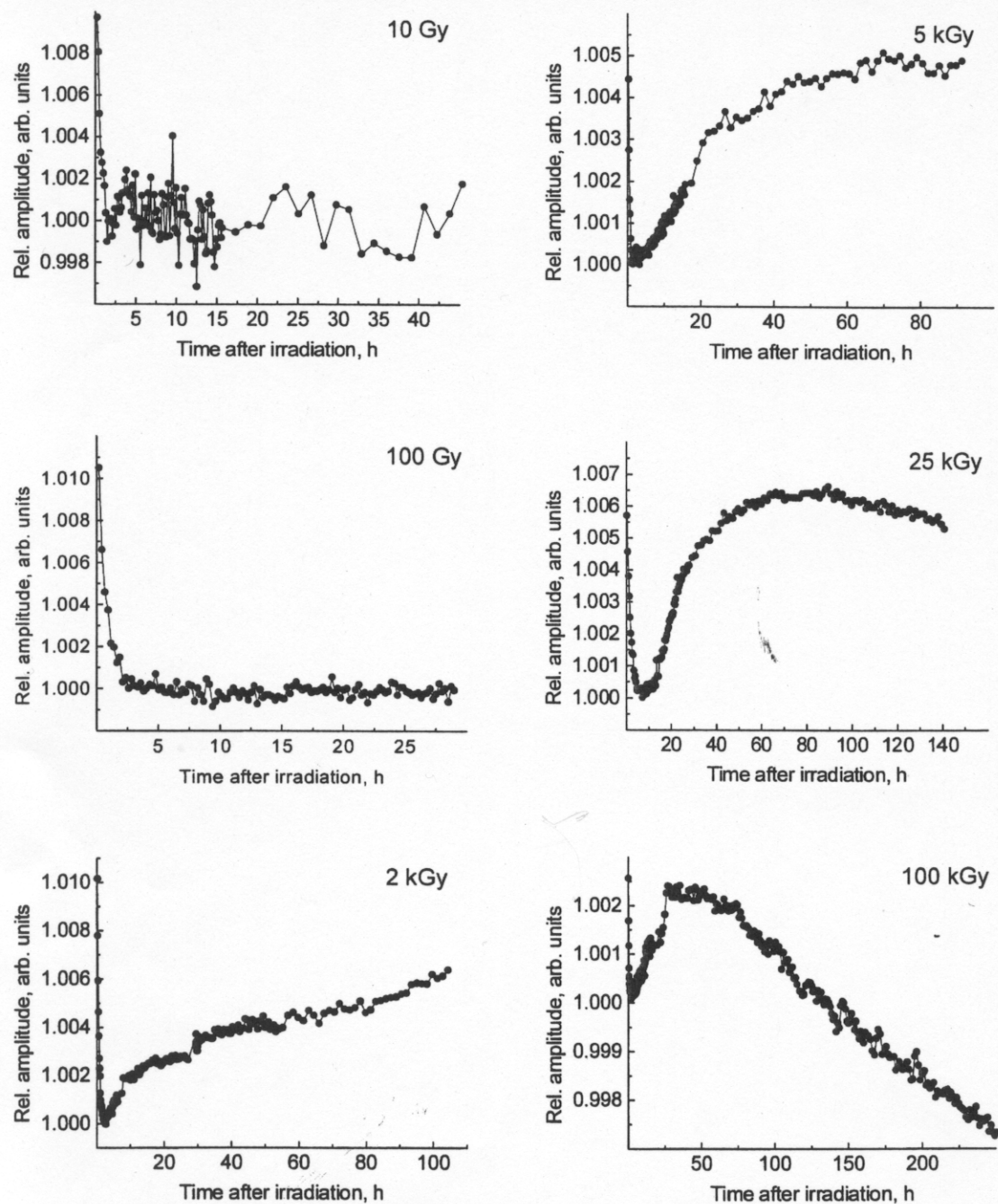


Fig. 1. Time dependence of the amplitude of the central line in the EPR spectra of irradiated polycrystalline L- α -alanine. The plotted relative amplitude is the result of the division of the alanine signal peak-to-peak amplitude by the peak-to-peak amplitude of the $\text{Cr}(3+)$ signal in the adjacent reference sample. In each experiment, the averaged relative amplitude at the first minimum was taken to be 1.000.

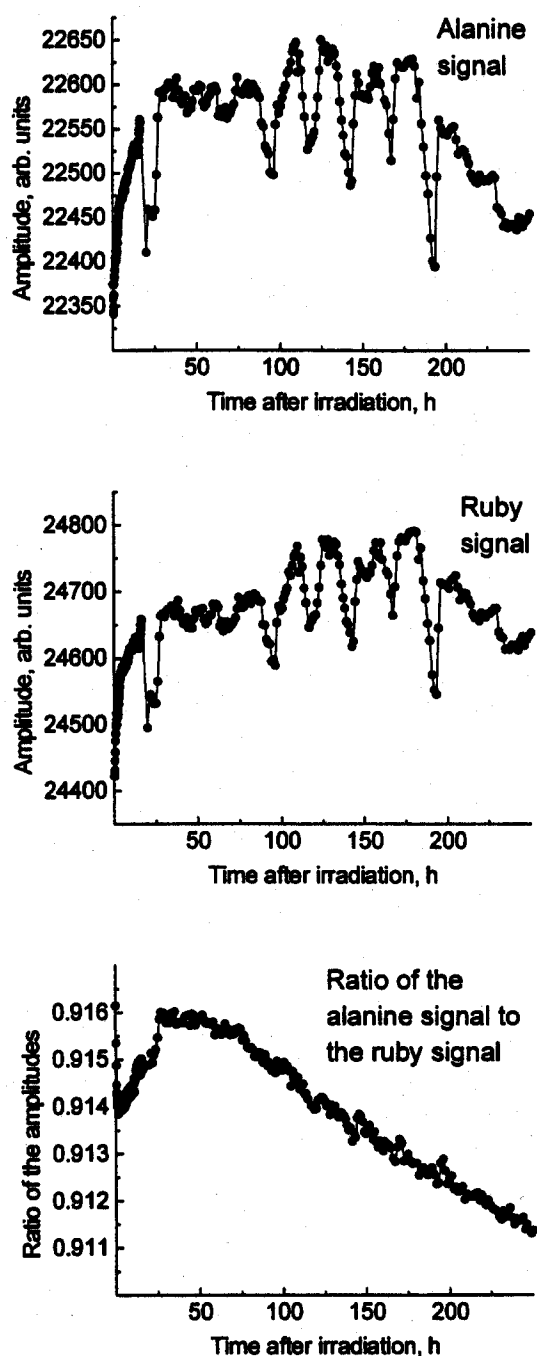


Fig. 2. Effect of the compensation of the EPR spectrometer instability by means of an adjacent ruby reference sample (data for the 100 kGy sample).

amplitudes with relative standard deviations as small as 0.1% or even better for doses above 100 Gy. Although the quoted value refers to immobile alanine pellets, this approach should also improve precision of dosimetry techniques using uniform exchangeable pellets.

In principle, any material with stable paramagnetic centers and a non-overlapping EPR signal can

be used as an adjacent reference sample. Ruby is particularly convenient because, in addition to being highly stable, its EPR signal is strongly anisotropic, and one can, by means of rotating the crystal, locate a narrow chromium(3+) line almost at any place in the spectrum. For best results, the microwave power and modulation amplitude used must be within the strictly linear ranges of the dependencies of the amplitudes of *both* the signals on these parameters. Additionally, the spectrum sweep time should be minimized such that the interval between recording the two spectra is as short as possible.

An adjacent reference sample should be reliably fixed in the cavity. There are several commercial cavities equipped with a special insert for such a sample in the front wall. Unfortunately, they do not cover all of the frequently used microwave modes. A convenient assembly bearing a rotatable ruby crystal for the Varian V4531 cavity has previously been described (Thompson and Waugh, 1965). The commercial removable ruby-bearing front wall for the Varian E-231 cavity is also convenient. The use of the second hole of a dual cavity to insert an adjacent standard results in noticeably poorer reproducibility. Most probably, this is because of the switching of the current between the two pairs of modulation coils, which causes permanent thermal instability of the cavity.

Although, theoretically, the adjacent standard technique may not provide complete correction of errors under conditions of very large variations of cavity Q -values (Nagy, 1994), it is well-suited for EPR dosimetry, where both the shapes and the dielectric characteristics of the samples under comparison are exactly the same.

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