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PRELIMINARY STUDY OF ALANINE-POLYMER RODS, MANUFACTURED IN THE INCT, IRRADIATED WITH GAMMA RAYS

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Irradiation of crystalline aminoacids generates paramagnetic centres which can be detected by the electron paramagnetic resonance (EPR) method at room temperature. Paramagnetic centres generated in α -alanine are extremely stable.

In the early sixties the α -alanine/EPR system was investigated and suggested as a new type of relative dosimeter. In the eighties the α -alanine/EPR dosimeter was carefully reinvestigated and proposed for transfer dosimetry. The International Dose Assurance Service (IDAS) initiated in 1985 by the International Atomic Energy Agency is based on an alanine/EPR dosimeter [1]. ASTM and ISO standards dealing with alanine/EPR dosimetry were published lately [2,3].

In this paper we present first results obtained for one of our compositions of alanine-polymer rods, in which a very low concentration of the cheap racemic form of α -alanine was used. This composition has much better mechanistic properties than the previously used alanine/escoren system. It enables us to obtain rods with the same (3 mm ±2%) diameter and good homogeneity of constituents. These two factors are important if we want to use all the cavity length and abandon weighing procedure. 3 mm diameter of the rods fits the dimentions of our EMS-104 spectrometer tubes. (Smaller diameters are also acceptable.) The EPR signals do not depend on the length, 1, of the rods for $1 \ge 35$ mm (whole cavity length is utilized) and on their rotation around the axis, if irradiated in homogeneous field.

The EPR signal of irradiated α -alanine is shown in Fig.1. It is a little different from that observed in neat microcrystalline alanine. The reason of the differences is actually investigated. The amplitude of the central peak is taken for the dose evaluation.

Our rods, containing few per cents of D,L- α -alanine in a polymer mixture, 3 mm in diameter, 40 mm long, were irradiated at room temperature in a Co-60 gamma source ISSLEDOVATIEL at the doses of: 1.3; 2.7; 4.1 and 12.2 kGy. The EPR signals were measured on an EMS-104 Bruker EPR spectrometer - a device specially dedicated to dosimetric applications. The signals were measured im-



Fig.1. EPR signal of gamma irradiated α -alanine - polymer rods. The samples were irradiated with Co-60 gamma rays at 298 K and measured on EMS-104 Bruker EPR spectrometer at room temperature. The measurements were done in the INCT.





Fig.2. Dependence of EPR signal of D,L-a-alanine - polymer rods irradiated in Co-60 gamma source, on the storage time, for various doses.

mediately after irradiation, as well as during the storage time up to 72 h (one sample - up to 94 h). The influence of the storage time on the amplitude of EPR signals is shown in Fig.2.

It can be seen from Fig.2, that the signals decrease somewhat during the first few hours after irradiation, especially those in the samples irradiated with higher doses, and then they are stable for a long time. We suppose, that the decay is connected mainly with unstable polymer radicals created in the alanine-polymer mixture or alanine originated radicals situated on the grain boundaries.

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EFFECT OF STORAGE CONDITIONS AND GAMMA IRRADIATION OF MEAT ON DNA ANALYZED BY "COMET" ASSAY

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DNA "comet" assay has been developed to analyze the extent of DNA damage in irradiated cells, mainly single and double strand breakage [1,2]. Cells embedded in agarose on microscopic slides were subjected to lysis and electrophoresis. Upon staining this technique enables visualization of DNA fragmentation in single cell. Hence, it was named Single Cell Gel Electrophoresis (SCGE) [3]. This technique found its practical application in toxicology, cancer research, radiobiology, genetics, biomonitoring and detection of irradiated food [2,4]. Fragmentation of DNA cannot be ascribed solely to the effect of radiation. Other factors like heat, enzyme action, repeated freezing and thawing play a role in this event.

Here we present results showing that storage conditions can have a significant effect on the outcome of DNA "comet" assay used for detection of irradiated meat. The method used in this investigation was adopted from that described in the literature [2-4] and in many aspects was identical with that proposed recently in a procedure for screening of irradiated food [5]. It will be published elsewhere [6].

Visual evaluation of cells was made using a common transmission microscope for slides stained with a silver or fluorescent microscope for slides stained with 4,6-diamidino-2-phenylindole (DAPI). According to the shape and stain intensity, DNA "comets" were classified arbitrarily to 4 classes (Fig.). Class I: images of apparently unchanged cells; class II: cells with a dye expanded slightly beyond the core (nucleous) border; class III: classic "comet" image, and class IV: almost all dye in the tail of the "comet". Objective evaluation of "comet" images stained with DAPI was made using an automatic scanning device controlled by a computer program (Comet V3). As most suitable for this investigation a tail moment was chosen, which is a product of "comet" tail length and DNA content (stain intensity) in the tail.

The results obtained are summarised in Tables. Table 1 shows that in the beef fresh and stored up to 48 h at 4° C or up to 4 days at -21° C the cells with images of class I and II are within the range of

