Electron Beam Sterilization of the Agarose Gel Used for Electrophoresis

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Abstract. The results obtained by electron beam (EB) sterilization of the plates with agarose gel used for human serum protein electrophoresis are presented. Also, the results obtained by human serum protein electrophoresis performed with agarose gel plates irradiated at different EB doses, from 4 kGy to 20 kGy, are presented. The microbiological results demonstrate that above 5 kGy the irradiated agarose plates are sterile. The EB irradiation of the agarose gel plates in the dose range of 7-9 kGy gives the best results for both, sterilization and protein fraction separation processes.

1. Introduction

Electron beam (EB) sterilization is based on its ability to kill pathogenic microorganisms. It is applied to a broad range of disposable medical products such as syringes, needles, surgical sutures, transplant kits, inhalation and dialysis equipment, blood-handling equipment, wound and burn dressings, gloves, masks, gowns, Petri dishes and pipettes [1. 2]. By this work we intend to extend the EB irradiation to the sterilization of the agarose gel put on plastic plates. The agarose gel is used for serum protein electrophoresis that is used to identify patients with multiple myeloma and other serum protein disorders [3]. Serum is blood plasma without fibrinogen and other clotting factors. Electrophoresis separates proteins based on their physical properties, and the subsets of these proteins are used in interpreting the results [4]. Proteins make up 6-8% of the human blood. Gel electrophoresis advantage is that proteins can be visualized as well as separated, permitting to a specialist to estimate quickly the number of proteins in a mixture or the degree of purity of a particular protein. The proteins are stained, and their density is calculated electronically to provide graphical data on the absolute and relative amounts of the various proteins [3]. There is not any previous work in the literature examining the use of EB irradiation to the sterilization of the agarose gel used for electrophoresis. EB application to the sterilization of agarose gel plate designed for serum protein electrophoresis is developed by the project "Preparation of biogels for serum protein electrophoresis with applications in diseases diagnostic and therapy", in the framework of the "New materials, Micro & Nano-Technologies Program" (MATNANTECH in Romanian), within the National Program for Research, Development and Innovation, supported by the Education and Research Ministry.

2. Methods

The essence of the biological effects of ionizing radiation is the fact that the irradiation with electrons, ions or photons can modify the physical, chemical and biological properties or a combination of them. Both, the photons and the electrons, lose their energy mainly by collisions with orbital electrons leading to ionisation and excitation. Finally a great variety of

intermediates are formed: free radicals, ions and excited molecules or atoms [1, 2]. The chemical reactivity of positive and negative ions is not high, but if they recombine with other ions they can form free radicals. Especially the free radicals, electrically neutral molecules or atoms with an unpaired electron in the outer shell, are very reactive at inducing chemical reaction in the irradiated matter. Since cells consist of more than 70% water, most of the energy is absorbed in water. The free radicals and the reactive agents such as hydrogen peroxide will attack all cell constituents. The ionising radiation effects are related to the amount of energy deposited into a certain mass of tissue. The dose is the total energy deposited by radiation per unit mass of the medium. It is expressed in Gray (1 Gy = 1 Joule/kg). In chemistry and medicine, protein electrophoresis is a method of analysing a mixture of proteins by means a gel electrophoresis, mainly in blood serum. The relative proportion of plasma proteins can vary in certain diseases and electrophoretic tracings showing changes can be useful diagnostic aid. Thus, protein electrophoresis can be used to screen for blood abnormalities. Serum is preferred for many tests as the anticoagulants in plasma can sometimes interfere with the results. There are two large classes of serum proteins: albumin and globulin. They are generally equal in proportion, but albumin is much smaller and lightly negatively charged, leading to an accumulation of albumin on the electrophoretic gel. At pH 8.6, which is commonly used, all proteins are negatively charged, but some more strongly than others. As the current flows, the serum proteins move toward the positive electrode. The stronger the negative charge on a protein, the faster it migrates. After a time (typically 20 min), the current is turned off and the proteins stained to make them visible. The separated proteins appear as distinct bands. The most prominent of these and the one that moves closest to the positive electrode is serum albumin. The other proteins are the various globulins such as alpha globulins, beta globulins, and gamma globulins. Serum proteins migrate in the order: albumin, alpha globulins, beta globulins, and gamma globulins. Gamma globulins are the least negatively charged serum proteins. They are so weakly charged, in fact, that some are swept in the flow of buffer back toward the negative electrode. The globulins are classified by their banding pattern (with their main representatives) as follows [3]:

- The alpha (α) band consisting of two parts, α_1 and α_2. The α_1-protein fraction is comprised of α_1-antitrypsin, thyroid-biding globulin and transcortin. The α_2-protein fraction is comprised of α_2-macroglobulin, ceruloplasmin and haptoglobulin;
- The beta (β) band consisting of two parts, β_1 and β_2. The β_1 –protein is composed mostly of transferrin and β_2 – fraction contains beta-lipoprotein. IgA, IgM, and sometimes IgG, along with complement proteins, also can be identified in the beta fraction.
- The gamma (γ) globuluin band. Because immunoglobulins migrate in to this band much of clinical interest is focused on the gamma region.

There are two basic types of materials used to make gels: agarose and polyacrylamide [5-9]. Agarose is a natural colloid extracted from seaweed. Agarose is usually used at concentrations between 1% and 3%. Agarose gels are formed by suspending dry agarose in aqueous buffer, then boiling the mixture until a clear solution forms. This is poured and allowed to cool to room temperature to form a rigid gel. It is very fragile and easily destroyed by handling. Agarose gels have very large "pore" size and are used primarily to separate very large molecules with a molecular mass greater than 200 kdal. Agarose gel can be processed faster than polyacrylamide gels, but the agarose gel is a favourable medium for the microorganisms' development, which is the cause for the degradation of the gel and of the results. Thus, the sterilization of the plates with agarose gels becomes important. A disadvantage is that ionizing radiation degrades some plastic gels above a certain absorbed dose level [10]. In view of these important aspects the disinfection of the agarose gel that is put on plastic plates, by electron

beam irradiation has been studied. Also, the effects of the EB absorbed dose on the process of the protein fraction separation with irradiated agarose gel have been investigated.

3. Apparatus

Experiments and demonstrations to small-scale operation were carried out using an electron linear accelerator, ALIN-10 of 6.23 MeV and 164 W maximum output power, built in Romania, National Institute for Lasers, Plasma and Radiation Physics, Electron Accelerator Laboratory-Bucharest. ALIN-10 is of travelling-wave type, operating at a wavelength of 10 cm. It is driven by a tunable S-band magnetron (EEV M 5125 type), delivering 2 MW of power in 4 μs pulses. The first portion of the accelerating structures is a variable phase velocity buncher and the remainder has a uniform section. As a laboratory installation, initially designed for fundamental research, ALIN-10 was located in a horizontal configuration. A post acceleration beam focusing and bending is utilized for ALIN-10 to project EB at right angles to the accelerating structure. In the present work, we have used a sampling method involving a ring-shaped electron collection monitor and its associated instrumentation for monitoring absorbed dose rate and accumulated absorbed dose during the irradiation process. This monitor which intercepts only a fraction of the scanned electron beam gives a relative value of the absorbed dose rate: it has been first calibrated by several chemical systems (such as the Ceric dosimeter) placed at the position of the samples to be irradiated. For demonstrative radiation processing at small-scale, the accelerated EB is spread by aluminium foils. In the experimental studies are used 1% agarose gel put on the plastic plates, supplied by DDS DIAGNOSTIC-Romania (Romanian firm that made electrophoresis kits for different hospitals). The used migration solution is Tris-barbital tampon pH 8.6. Electrophoresis of serum proteins on agarose gel was performed by the applied a voltage of 100 V during 20 minutes. The EB irradiation was applied on sealed bags made from aluminum foils, each bag containing a plastic plates (0.1 m x 0.085 m x 0.001 m) with agarose gel put into a plastic box (0.123 m x 0.1 m x 0.005 m). Fig. 1 shows the photograph of the EB irradiation facility for the sterilization at small-scale level of the sealed aluminum bags containing plastic boxes with agarose gel plates Fig. 2 shows the photograph of a sealed aluminum bag with a plastic box containing a plastic plate with agarose gel. The average density of the packages to be irradiated is 0.46 g · cm^{-3}. The maximum number of aluminum bags put in a layer to be simultaneous irradiated is eight.

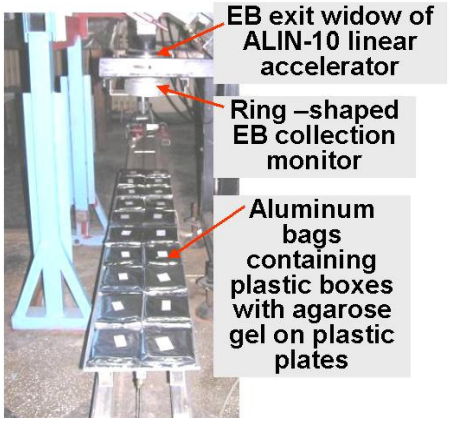

FIG. 1.Photograph of the EB irradiation facility for the sterilization at small-scale level of aluminium bags with plastic boxes containing plastic plates with agarose gel

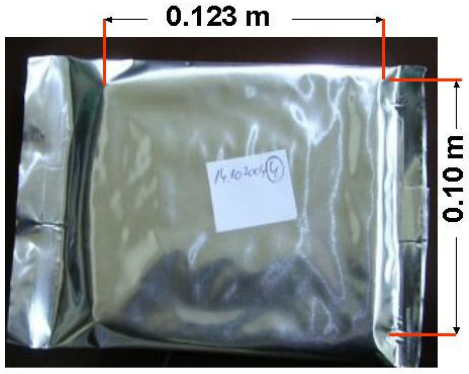

FIG. 2. Photograph of a sealed aluminium bag with a plastic box (0.123 m x 0.10 m x 0.005 m) containing a plastic plate with agarose gel

4. Results

In order to establish the optimum absorbed dose level for the sterilization of the plates with agarose gel, we studied the effect of different absorbed doses, from 4 kGy to 20 kGy. As biological material were used two serum types:

- The serum from a patient with the normal protein fractions (A samples);
- The serum from a patient with the increased values for alpha 1, alpha 2 and beta 2 fractions (B samples-patient with acute inflammatory process).

The separation of the serum proteins by electrophoresis were performed for both, irradiated and unirradiated agarose gel plates. The investigation were focused on the concentration changes of the albumin, alpha 1, alpha 2, beta 1, beta 2 and gamma globulin fractions separated by electrophoresis on the agarose gel plates (AGP) irradiated at different EB doses as compared with the results obtained on the unirradiated AGP. Also, the irradiated and unirradiated AGP were microbiological tested on the followed medium types:

- Sabouraud medium for the fungi;
- Gelose-blood for the gram-positive germs;
- CLED Medium for the gram-negative germs.

Fig. 3 presents the photograph of an agarose gel plate irradiated at 8 kGy that was used for protein electrophoresis of 10 human serum samples. This photograph shows the following six protein fractions obtained by electrophoresis: albumin (lies closed to the positive electrod), alpha 1, alpha 2, beta 1, beta 2 and gamma globulin (lies closed to the negative electrod). Fig. 4 presents a typical proteinogramme obtained by using a plastic plate with agarose gel irradiated at 8 kGy. The protein fraction values are determined with DS2-SEBIA-France density-meter. The results obtained for the A sample type are presented in Fig. 5 and for the B sample type in Fig. 6.

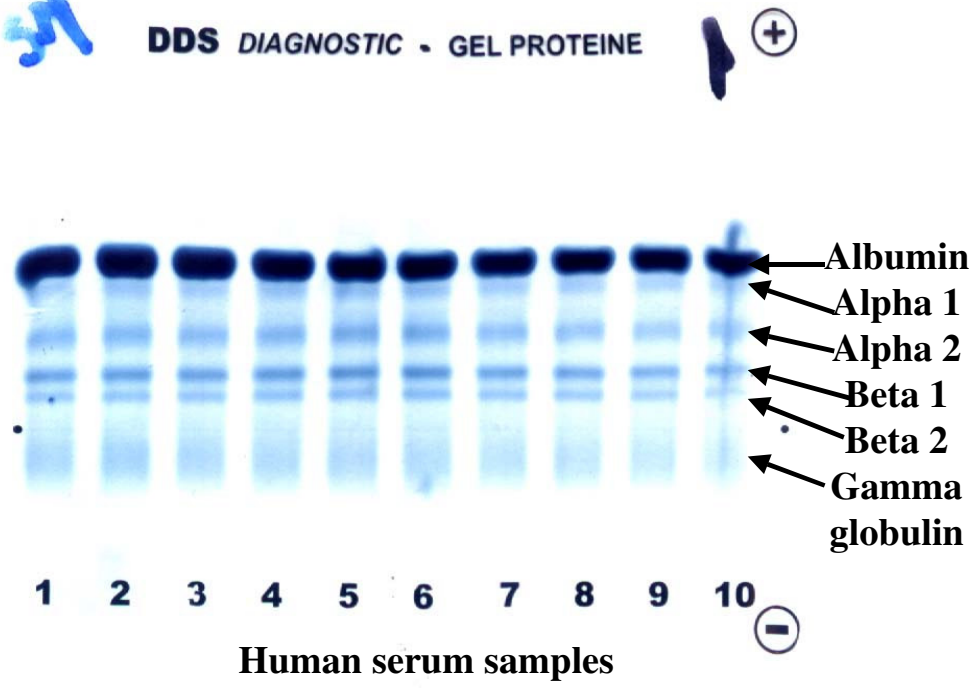

FIG. 3. Photograph of an irradiated agarose gel plate at 8 kGy used for protein electrophoresis of 10 human serum samples.

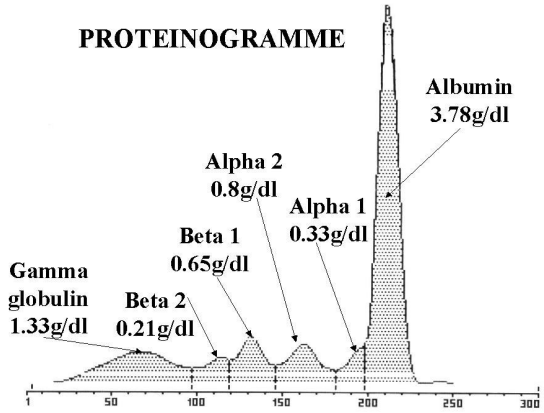

FIG.4. A typical proteinogramme obtained by using a plastic plate with agarose gel irradiated at 8k Gy

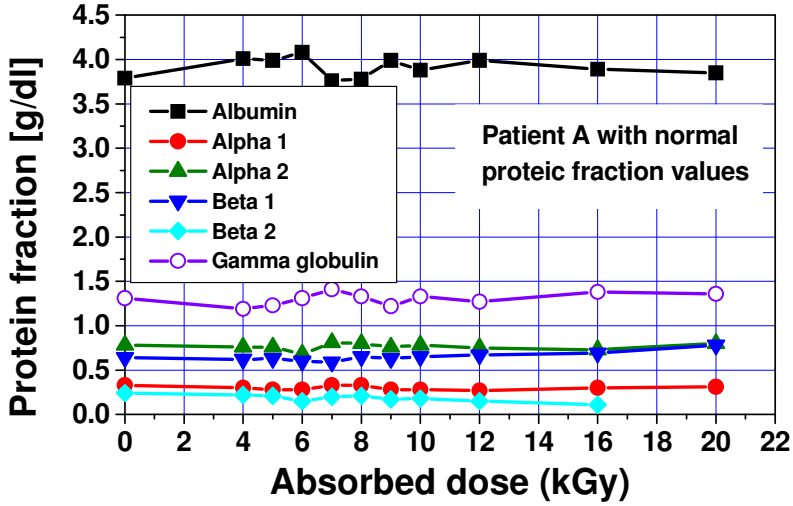

FIG. 5. The results for A type serum protein electrophoresis obtained with agarose plates irradiated at different EB doses

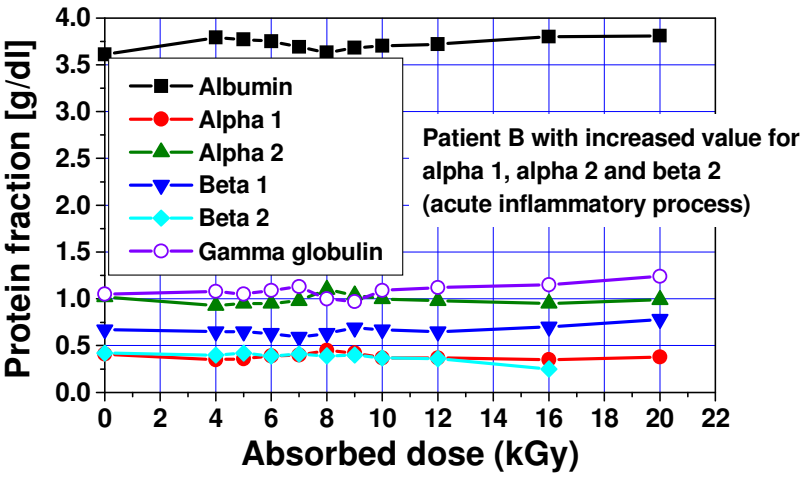

FIG. 6. The results for B type serum protein electrophoresis obtained with agarose plates irradiated at different EB doses

The comparative studies concerning the protein fraction values separated on the irradiated and unirradiated plates with agarose gel show that EB irradiation especially affects the separation of beta 1 and beta 2 protein fractions. Thus, the experimental results show that the beta 2 fraction decreases on the agarose gel plates irradiated above 10 kGy level after then, for the AGP irradiated at the doses above 16 kGy, the separation of this fraction stops (Fig. 5 and Fig. 6). The beta 1 fraction increases lightly on the AGP irradiated above 8 kGy and finishes by the inclusion of the beta 2 fraction for the AGP irradiated at doses above 16 kGy. On the other hand, it was observed that for the AGP irradiated above the dose of 9 kGy, the albumin fraction increases. It is important to note that the albumin fraction also increases on the agarose gel plates irradiated in the dose range of 4-6 kGy after then decreases and obtains, on the agarose gel plates irradiated in the dose range 7-9 kGy, the same values as those obtained on the unirradiated plates. The alpha 1 and alpha 2 fractions do not exhibit important modifications, only a small decreasing on the AGP irradiated in the dose range of 4-6 kGy and the dose range of 9-20 kGy. The AGP irradiated in the dose range of 7-9 kGy give the best results: the protein fraction concentrations obtained by electrophoresis suffer little modification as compared with protein electrophoresis on the unirradiated AGP. Also, the separation of protein fractions is very clear in this case. It seems that the EB irradiation of the AGP in the dose range of 7-9 kGy improves the proteins separation. This is a new and very important result that could be verified by future investigations. The microbiological results presented in Table 1 show that above 5 kGy the irradiated plates are sterile.

TABLE I: MICROBIOLOGICAL RESULTS

EB absorbed dose (kGy)	**Culture medium**		
	Gelose-blood	CLED	Sabouraud
0	3 CFU/24h		Positive/24h
4	2 CFU/24h	Sterile	Positive/48h
5	2 CFU/48h	Sterile	Sterile
6	Sterile	Sterile	Sterile
7	Sterile	Sterile	Sterile
8	Sterile	Sterile	Sterile
9	Sterile	Sterile	Sterile
10	Sterile	Sterile	Sterile
12	Sterile	Sterile	Sterile
14	Sterile	Sterile	Sterile
16	Sterile	Sterile	Sterile
18	Sterile	Sterile	Sterile
20	Sterile	Sterile	Sterile

5. Conclusions

The most important conclusion is that the electron beam irradiation of the agarose gel used for serum protein electrophoresis could become a good sterilization method if the irradiation dose is optimised and controlled. Also, it seems that the agarose gel irradiation in the dose range of 7-9 kGy improves the proteins separation. This result will be verified by future investigations.

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