IONIZING RADIATION EFFECTS ON BIOGEL USED FOR SERUM PROTEIN ELECTROPHORESIS

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Irradiation with high energy electron beams allows direct sterilization of the plates with agarose gel packed in sealed aluminium foil bags and used for human serum protein electrophoresis. Human blood serum contains a large amount of protein (6.5 to 8.5 g/dL) and a greater diversity but, in terms of medical interest, the following six show better importance: albumin, alpha 1, alpha 2, beta 1, beta 2 and gamma globulin. The effects of electron beam irradiation upon this biogel and on the process of the proteic fraction separation were investigated at different absorbed doses in order to establish the optimum level. Avoiding dysfunction coming especially from changing of serum, dye or fixing solution or from mechanical defects (cracked gels on the support film) resulted in obtaining satisfactory results, as proved by tests made at 12 months after the agarose gel film making and irradiation. Microbiological decontamination of the agarose gel films irradiated in the dose range 3-12 kGy could be maintained for a period of a year. Also, agarose gel film can be microbiological decontaminated by irradiation with EB in the range 7-11 kGy without undergoing major changes in the electrophoretic properties for a storage period that can be appreciated to 12 months. There are two dose ranges where the irradiated films change more significant the separation of protein fractions: a low dose range between 4 and 6 kGy, and a range of doses between 12 and 20 kGy.

Keywords: electron beam, electrophoresis, agarose gel, proteic fractions

INTRODUCTION

Serum protein electrophoresis, using gels as a separation, migration and attachment medium is a fast and accurate method to obtain, by specific laboratory tests, extremely important information in diagnosing and monitoring patients treatment for a large number of clinical diseases in human medicine: multiple myeloma, chronic inflammation, autoimmune diseases, liver cirrhosis, chronic hepatitis, monoclonal gammapthies, Waldenström's macroglobulinemia, etc. (O'Connell *et al.*, 2005).

Gel serum protein electrophoresis with migration and separation in electric field, called "gel electrophoresis" is used as an analytical method because it allows clear separation of proteins from a protein blend, in well defined areas for each constituents, while maintaining intact their structural and functional properties. The advantage of this method is that separation zones or bands obtained by electrophoresis can be attached, viewed and stored for long periods, allowing the researcher or clinician to quickly assess the number, concentration and nature of a mixture of proteins and the degree to which that protein mixture differs from a standard protein mixture, e.g. a mixture of serum proteins in healthy patient or a monitored patient during treatment (Jacoby and Cole, 2000).

Proteins make up 6-8% of the human blood and they are macromolecular substances of polypeptide nature, fulfilling the basic functions, specific to living organisms. Serum is blood plasma without fibrinogen and other clotting factors. Medical and

biochemical research laboratories are constantly facing the problem of separation of serum proteins without disrupting their bio-physical and chemical properties. The separation's accuracy of protein fractions, on which depends the diagnosis and treatment of patients is mainly caused by the chemical and biochemical characteristics of the gel used as a medium for migration. Therefore, we aimed to achieve an electrophoresis kit with an improved gel, with features that allow rapid and accurate diagnosis of diseases investigated by electrophoresis of proteins isolated from human blood, simultaneously ensuring a long period of conservation of the kit.

Although very suitable for electrophoresis (Hoffman *et al.*, 2000; Ravel, 1995; Nauck, 1995), agarose-based gels is a favorable environment for microorganisms. This alters micro electrophoretic properties of gel, especially the resolution in electrophoretic separation of protein fractions. As a result, the development of germs of various microorganisms, but especially fungi on agarose films reduces accuracy in interpreting the results and the correct diagnosis and treatment of patients investigated on proteinographs. Also, agarose gels are easy to degrade under the action of light, dehydrate under heat action. The first important required measure is encapsulation of gel films in special cassettes to keep them away from contamination and degradation under the action of heat, humidity and light. Classical methodology applied to avoid contamination with microorganisms of the biogel films is their processing under special conditions of instruments and rooms sterility, which greatly increases the product cost.

This paper shows the solution of electron beam (EB) irradiation for decontaminating microbiological films of agarose gels, and its net benefits compared with other methods. EB sterilization (Burns *et al.*, 1996; Mehnert, 1996; Mondelaers, 1998), is able to keep the agarose gels advantages (speed, flexibility, lack of toxicity) and to eliminate the drawbacks (low conservation time) for this type of biogel. Irradiation with high energy electron beam allows direct sterilization of agarose films ready embedded in casettes and aluminized foil which protects them from moisture and light.

Also, the effects of the EB absorbed dose on the process of the protein fraction separation with irradiated agarose gel, have been investigated. Therefore, we aimed to achieve an electrophoresis kit with an improved gel, with features that allow rapid and accurate diagnosis of diseases investigated by electrophoresis of proteins isolated from human blood, simultaneously ensuring a long period of conservation of the kit. There is not any previous work in the literature examining the use of EB irradiation to the sterilization of the agarose gel put on plastic plates used for electrophoresis.

EXPERIMENTAL

The experimental setup with EB irradiation of agarose gel film is shown in Fig. 1, and mainly consists of the ALIN-10 electron linear accelerator of 6.23 MeV and 164 W maximum output power, built in Romania, National Institute for Lasers, Plasma and Radiation Physics, and the mobile platform on which aluminum bags containing plastic boxes with agarose gel plates are placed. EB irradiation was carried out in a very precise manner, regarding both electrical measurements like the absorbed dose rate D*, the absorbed dose D, the kinetic energy E_{EB} and geometric measurements of the distance H between the EB output window and the mobile surface on which irradiated samples are positioned, and the cross-section S of the EB field at the distance H. Dose distribution cannot be homogeneous across all cross section S of the EB field. In our case, we have imposed that the maximum non-uniformity to not exceed 15%. A post acceleration beam focusing and bending is utilized for ALIN-10 accelerator to project

EB at right angles to the accelerating structure. This allows a selection and a precise measurement of electron energy used for irradiation of biological systems.

An equation derived from the area through-put equation is a product line speed equation wherein the Linear Processing Coefficient, K, is commonly used in the low-energy EB area in relating average beam current to line speed (Cleland, 1984):

$$D\ (kGy) \cdot V_{platform}\ (cm/min) = K \cdot I_{EB}\ (\mu A) \quad (1)$$

wherein K is typically ~10 to 30 depending on the electron energy, the EB field width, window thickness and air gap between the window and product. Experimental measurement results made to determine the relationship between D, $V_{platform}$ and I_{EB} have shown that the relationship (1) can be used in our irradiation experiments, too.

The EB irradiation was applied on sealed bags made from aluminum foils, each bag containing a plastic box (0.123 m x 0.1 m x 0.005 m) with agarose gel on a plastic plate (0.1 m x 0.085 m x 0.001 m). In the experimental studies was used 1% agarose gel put on the plastic plates. 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.

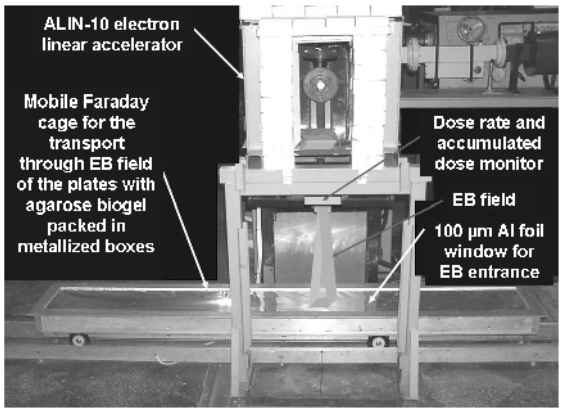

Figure 1. Frontal photo of the mobile Faraday cage containing boxes with agarose gel foils placed in sealed bags made from aluminum foils

RESULTS AND DISCUSSION

In order to evaluate the effects on the absorbed dose of parameters variations in the irradiation process, namely the EB average current and the platform speed from the relationship (1), were determined and plotted for an average factor K = 11.5, the characteristics showing the variation of dose D according to 7 different values, between 3.5 µA and 6.5 µA for I_{EB} and 15 different values, between 3.3 cm/min and 28.3 cm/min for $V_{platform}$. Also, we operated at large values of I_{EB} to benefit of the reducing of the effects of its variations on the dose and the possibility to use high speeds of the platform with samples and thus to obtain short times and high processing productivity. By irradiation on the conveyor belt of the packages containing foils coated with agarose, these, due to their continuous movement through the EB field, are exposed to

more uniform dose distribution compared with a static irradiation. It was calculated the useful penetration for E_{EB} = 6.23 MeV and the maximum number of packages stacked during irradiation. This information is important for processing productivity. Packages with agarose film cassettes were distributed on the mobile platform in 11 groups of 8 pieces placed one above the other.

In order to establish the optimum absorbed dose level for the sterilization of the plates with agarose gel, we studied the effect of different doses, from 4 kGy to 20 kGy. Regular tests were conducted over a period of 12 months, and were intended to identify the optimal dose of radiation for the microbiological decontamination while maintaining, and possibly improving, the electrophoresis properties of agarose biogel deposited on the support foils. Investigation was focused on changes that EB irradiation produced to agarose gel, compared with non-irradiated samples, in the electrophoresis of protein fractions. Human blood serum contains a large amount of protein (6.5 to 8.5 g/dL) and a greater diversity but, in terms of medical interest, the following six show better importance: albumin, alpha 1, alpha 2, beta 1, beta 2 and gamma globulin.

The results obtained from samples of a patient with normal proteic fraction values are presented in Fig. 2 and of a patient with acute inflammatory process are presented in Fig. 3. Relative protein concentrations within each fraction were determined as the optical absorbance percentage that is the ratio between the absolute quantity of each fraction (g/dl) and the total serum protein multiplied by 100. We mention that the values for each point on the characteristics of Figs. 2 and 3 are the result of the mediation of 10 different measurements made on each of the 10 positions on the same sheet, with the same serum from the same patient.

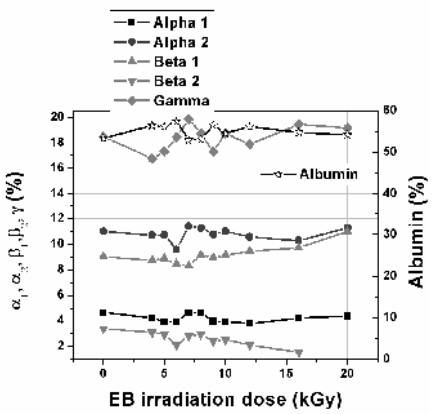

Figure 2. The results for serum protein electrophoresis obtained with agarose plates irradiated at different EB doses from a patient with normal proteic fraction values

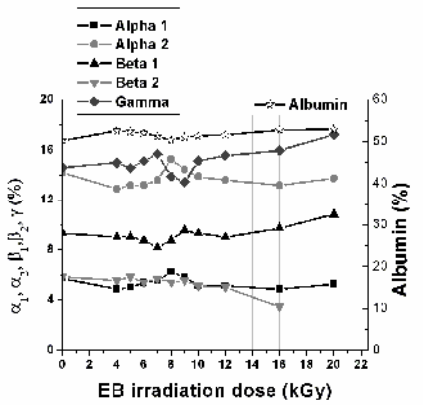

Figure 3. The results for serum protein electrophoresis obtained with agarose plates irradiated at different EB doses from a patient who had an acute inflammatory process

The analysis of experimental results led to the following conclusions:

a) Agarose films irradiated in the range 2-12 kGy differentially affects protein fractions: the smallest variations in relation to control values shows albumin protein fractions (+1.3% to -3.8%), alpha 1 (+7.4% and -4.5%) and beta 1 (0% to -4.9%) and

the highest variations are for the following protein fractions: alpha 2 (+13% to 0%), beta 2 (+21% with -0%) and gamma (+13% to 0%), beta 2 (+21% with -0%) and gamma globulin (+7.5% to -10.6%).

b) Separation of protein fractions alpha 2, beta 2 and gamma globulin shows more significant variations on agarose films irradiated with doses between 3kGy and 7kGy compared with values obtained from non-irradiated control films. However, the values obtained on foils irradiated with 8-9 kGy tend to return to the values obtained for the non-irradiated control films. It seems that certain doses of irradiation could improve the electrophoretic separation of protein fractions.

c) For each protein fraction there is an optimum doses range for which the separation of fraction is not substantially affected or tends to be very close to the values obtained for non-irradiated films. These ranges are: 8 - 12 kGy for albumin, 6 - 9 kGy for alpha 1; 8 - 9 kGy for alpha 2; 2 - 12 kGy for beta 1; 6 - 8 kGy for beta 2 and 11 - 12 kGy for gamma globulin.

d) The porous structure and the regularity and size of biogel's pores are essential in the process of migration, separation and attachment of protein bands obtained by electrophoresis. EB irradiation may affect this process, depending on the administered dose of radiation and the microscopic structure of agarose gel.

e) The irradiated and unirradiated agarose gel plates were microbiological tested on the following medium types: sabouraud medium for the fungi; gelose-blood for the gram-positive germs and CLED medium for the gram-negative germs. The microbiological results showed that above 5 kGy the irradiated plates are sterile.

Fig. 4 and Fig. 5 present a summary of the results obtained by investigating for a period of 12 months of a batch of 11 groups of 4 packages with agarose gel cassettes, at 11 different values of irradiation doses, in the range 2 – 12 kGy.

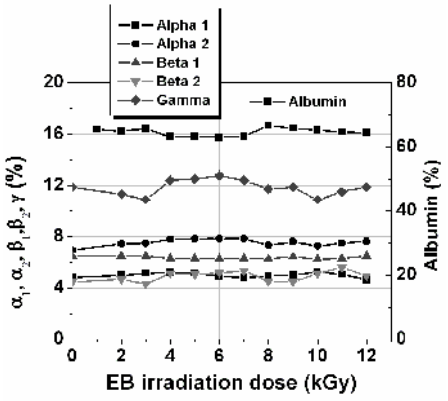

Figure 4. The synthesis graphic of the variations of protein fractions depending on the EB absorbed dose, determined at 4 days after irradiation

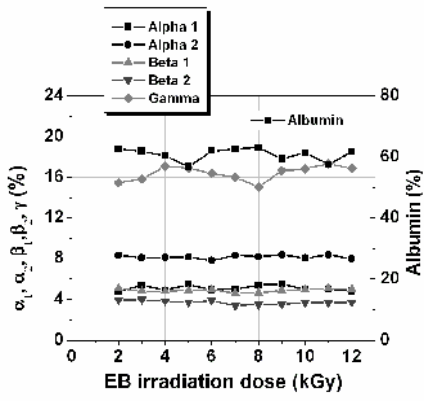

Figure 5. Variations of protein fractions depending on the EB absorbed dose, determined at 12 months after irradiation

Fig. 4 shows the synthesis graphic of the variations of protein fractions alpha 1, alpha 2, beta 1, beta 2, albumin and gamma depending on the EB irradiation dose, determined at 4 days after irradiation. Fig. 5 shows the synthesis graphic of protein

fractions variations determined after a year from irradiation that was made in the same day.

It was found that after a year, on the surface of the films irradiated with doses of 3-12 kGy, there was no development of molds that normally appears on the agarose gel and affects the most accurate electrophoretic separation of protein fractions. Otherwise, we found the non-irradiated control foils covered with mold. Also, in the 7-11 kGy range, we observed that agarose gel film can be decontaminated without major changes of microbiological electrophoretic properties. All irradiated films tested over 12 months gave, for all protein fractions, values showing less than 10% maximum deviations compared with the average values of each fraction. The most net and reproducible electrophoretic separations were observed at the irradiated films in the 7-11 kGy dose range. The described experimental setup with EB irradiation was used for electrophoresis kits treatment that were sent to medical clinics and hospitals.

CONCLUSIONS

Avoiding dysfunction coming especially from changing of serum, dye or fixing solution or from mechanical defects (cracked gels on the support film) resulted in obtaining satisfactory results, as proved by tests made at 12 months after the agarose gel film making and irradiation. Microbiological decontamination of the agarose gel films irradiated in the dose range 3-12 kGy could be maintained for a period of a year. Also, agarose gel film can be microbiological decontaminated by irradiation with EB in the range 7-11 kGy without undergoing major changes in the electrophoretic properties for a storage period that can be appreciated to 12 months. In addition, the separation of protein fractions in the 7-9 kGy range on the irradiated films, are better delineated than those of the same patient done on the unirradiated foil control.

The experimental setup presented in this paper ensure a sufficient productivity for a monthly service of a medium medical tests laboratory.

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