Electron Beam Sterilization of the Agarose Gel Used for Electrophoresis

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Abstract

There are presented the results obtained by:

- **Electron beam (EB) sterilization of the plates with agarose gel used for human serum protein electrophoresis;**

- **Human serum protein electrophoresis performed with agarose gel plates irradiated at different EB doses, from 4 kGy to 20 kGy.**

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.

Introduction

- **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*.
- **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 *(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.**

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.

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.

Methods

The ionising radiation effects are related to the amount of energy deposited into a certain mass of tissue.

In chemistry and medicine, protein electrophoresis is a method of analysing a mixture of proteins by means a gel electrophoresis, mainly in blood serum.

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*.

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:

- **The alpha (α) band consisting of two parts, α1 and α2. The α1-protein fraction is comprised of α1-antitrypsin, thyroid-biding globulin and transcortin. The α2-protein fraction is comprised of α2-macroglobulin, ceruloplasmin and haptoglobulin;**
- **The beta (β) band consisting of two parts, β1 and β2. The β1 –protein is composed mostly of transferrin and β2 – 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.**

Serum proteins migrate toward the positive electrod in the order: *albumin, alpha globulins, beta globulins, and gamma globulins.*

There are two basic types of materials used to make gels: agarose and polyacrylamide. Agarose is a natural colloid extracted from seaweed and is usually used at concentrations between 1% and 3%.

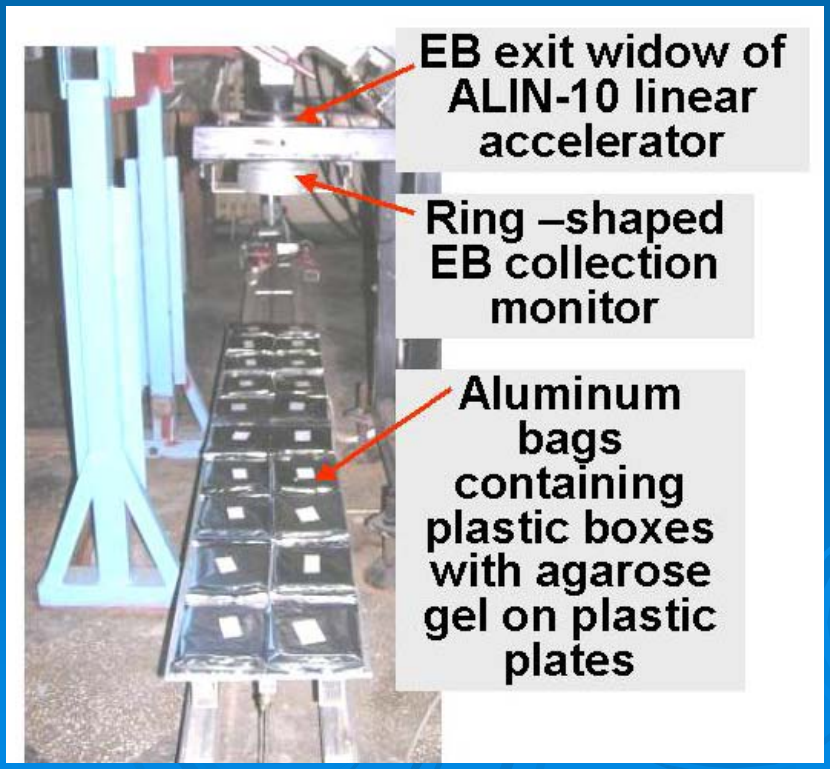
Agarose gel can be processed faster than polyacrylamide gels, but it is a favourable medium for the microorganisms' development, which is the cause for the degradation of the gel and of the results. Thus, the sterilization of the plates with agarose gels becomes important.

A disadvantage is that ionizing radiation degrades some plastic gels above a certain absorbed dose level.

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 NILPRP - 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. For demonstrative radiation processing at small-scale, the accelerated EB is spread by aluminium foils.



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).

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 $g \cdot cm^{-3}$. The maximum number of aluminum bags put in a layer to be simultaneous irradiated is 8.

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:

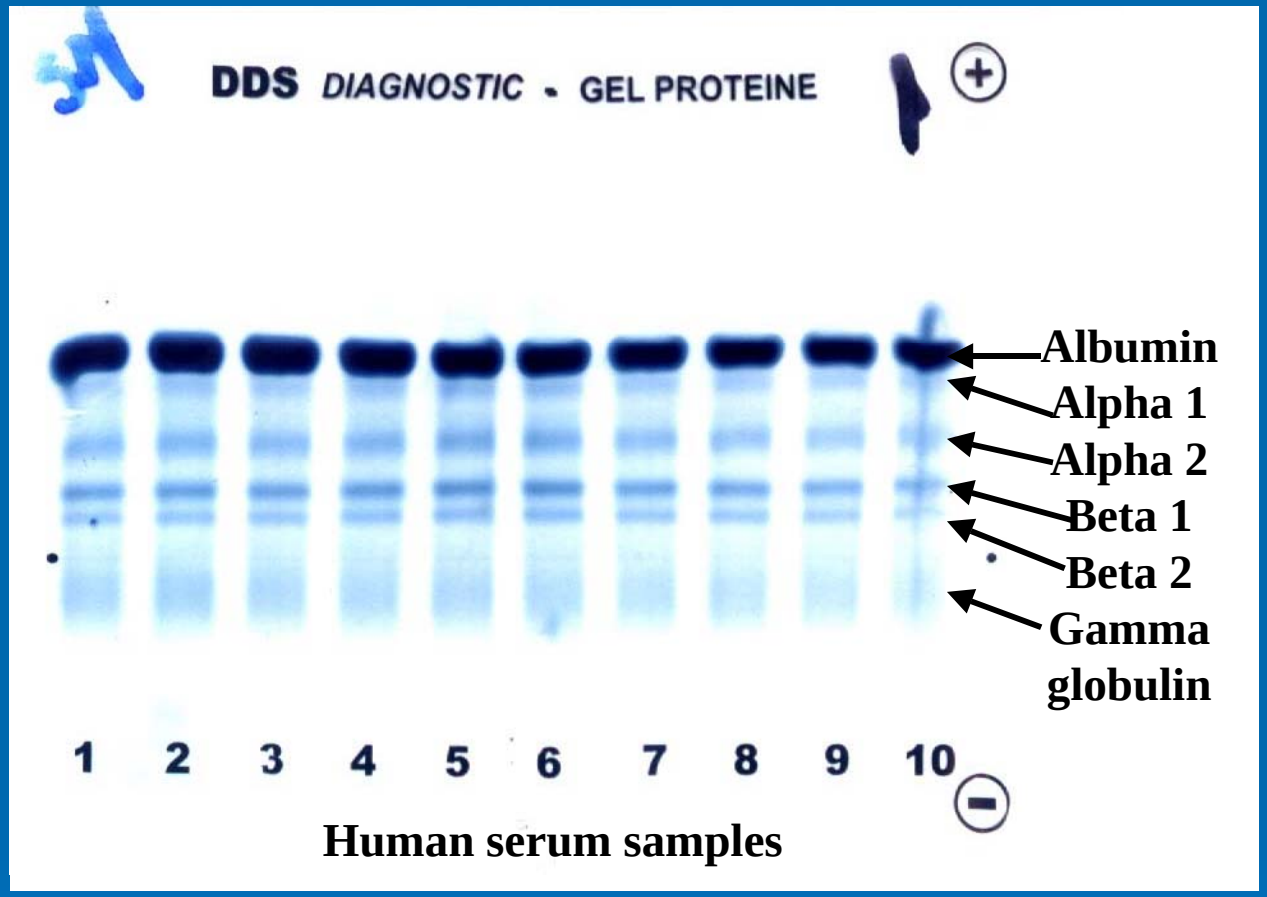
- The serum from a patient with the normal protein fractions (A samples);
- The serum from a patient with the increased values for alpha 1, alpha 2 and beta 2 fractions (B samples-patient with acute inflammatory process).

The separation of the serum proteins by electrophoresis was performed for both, irradiated and unirradiated agarose gel plates by applying a voltage of 100 V during 20 minutes. The used migration solution is Tris-barbital tampon pH 8.6.

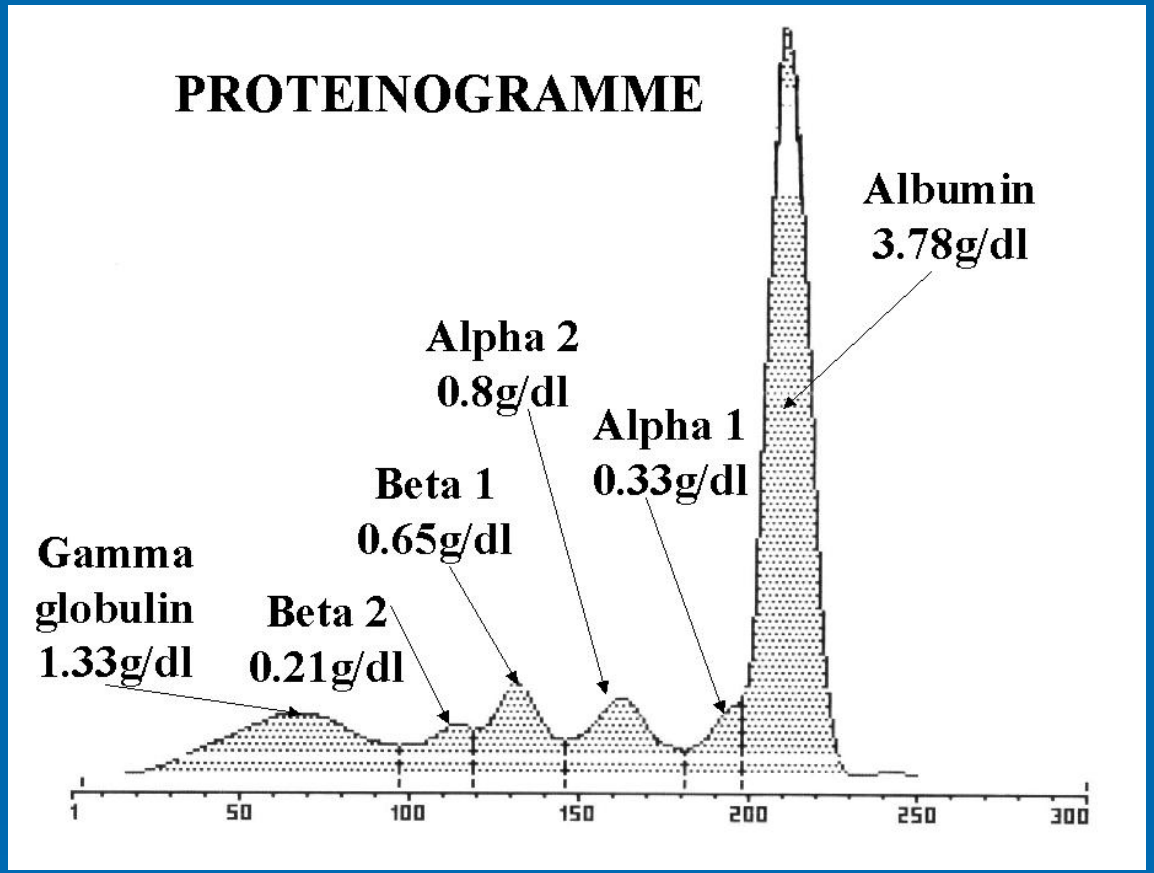
The investigation were focused on the concentration changes of the *albumin, alpha 1, alpha 2, beta 1, beta 2 and gamma globulin fractions.*

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;
- CLED Medium for the gram-negative germs.



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



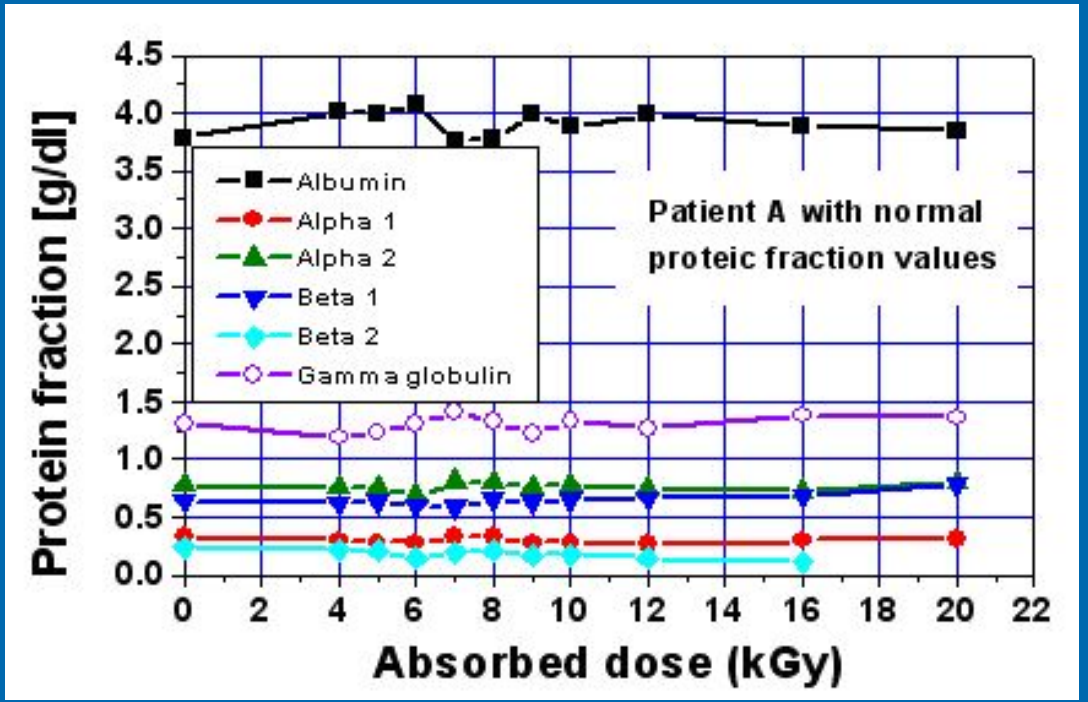
A typical proteinogramme obtained by using a plastic plate with agarose gel irradiated at 8 kGy

The microbiological results show that above 5 kGy the irradiated plates are sterile.

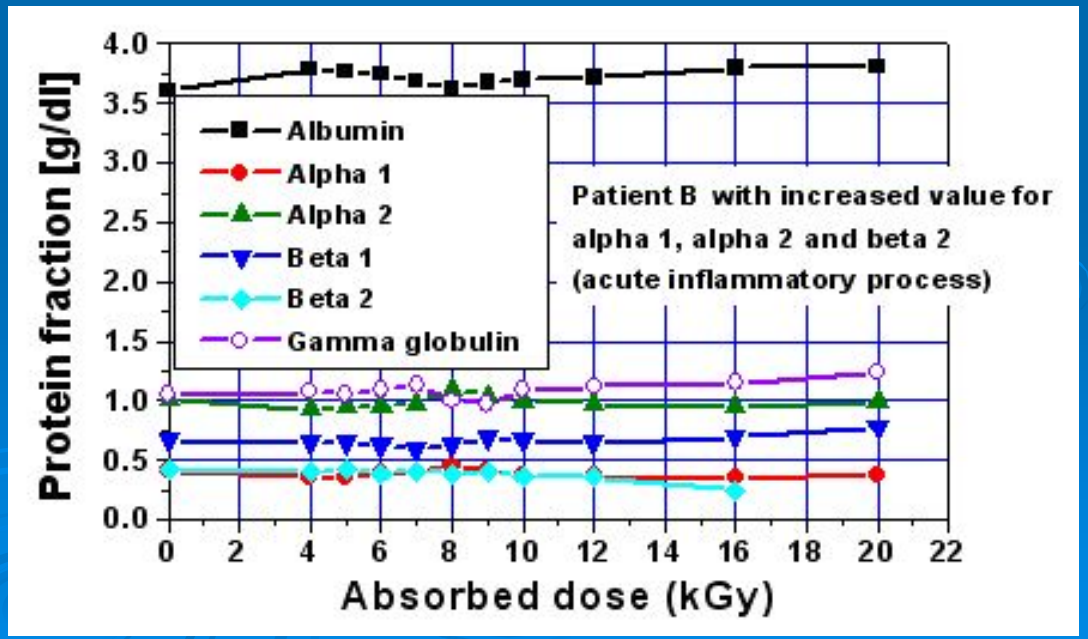
EB absorbed dose (kGy)	*Culture medium*		
	Gelose-blood	CLED	Sabouraud
0	3 CFU/24h		Positive/24h
4	2 CFU/24h	Sterile	Positive/48h
5	2 CFU/48h	Sterile	Sterile
6	Sterile	Sterile	Sterile
7	Sterile	Sterile	Sterile
8	Sterile	Sterile	Sterile
9	Sterile	Sterile	Sterile
10	Sterile	Sterile	Sterile
12	Sterile	Sterile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

The results for protein electrophoresis obtained with agarose plates irradiated at different EB doses

A type serum



B type serum



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.

It seems that the electron beam irradiation of the agarose gel plates in the dose range of 7-9 kGy improves the proteins separation ***(the protein fraction concentrations obtained by electrophoresis suffer little modification as compared with protein electrophoresis on the unirradiated agarose gel plates; also, the separation of protein fractions is very clear in this case).***

This is a new and very important result that could be verified by future investigations.