International Baccalaureate

BIOLOGY

Effect of Different gamma-irradiation Doses on Blood Compatible Property of Polycarbonate Membranes Prepared with Chloroform

Extended Essay

Candidate Name: Rüyet Sefercioğlu
Candidate Number: 001129 - 0028

SEFERCİOĞLU
001129 - 0028

Effect of Different gamma-irradiation Doses on Blood Compatible Property of Polycarbonate Membranes Prepared with Chloroform

Abstract:

In 21st century, medical treatments become one of the most essential thing for survival of patients. So, scientists have developed several synthetic materials to resolve problems in medical field. These materials include prosthesis, implements and needle catheters which are originated from polycarbonate membranes. Most of these materials are blood compatible however it is known, from patient to patient the effect of blood compatibility isn't efficient: The aim of this study is to increase blood compatibility of polycarbonate membranes.

The research question of this study is determined as *"How do different γ-irradiation doses affect roughness and protein adsorption properties of polycarbonate membranes prepared by chloroform indicated by AFM and competitive adsorption measurements of different blood plasma proteins by batch-wise reactor?"*.

To deduce the effect of γ-irradiation on surface roughness and protein adsorption features of polycarbonate membranes, polycarbonate membranes prepared by solvent-casting technique from chloroform are tested in "Atomic Force Microscopy Studies" and "Competitive Adsorption of Blood Proteins from Plasma studies". The experiment was conducted at Hacettepe University. At 130kGy irradiation dose, mean surface roughness of polycarbonate membranes is calculated 42.880 nm whereas untreated membranes' mean surface roughness are calculated 28.881 nm. Protein adsorption experiments carried out with blood proteins (Serum Albumin, Fibrinogen and γ-globulin) demonstrated that protein adsorption significantly increased by increasing irradiation dose. Therefore It is deduced, polycarbonate membranes γ-irradiated at 130kGy, has a mean of competitive absorption 110.783 ng/cm^2 for Serum Albumin , 60.003

ng/cm^2 for fibrinogen, 49.630 ng/cm^2 for γ-globulin. ANOVA test ($p = 1.56x10^{-25}$ for surface roughness, $p=1.5510^{-39}$ for adsorption of Serum Albumin, $p=1.23x10^{-49}$ for adsorption of Fibrinogen, $2.56x10^{-36}$ for adsorption of γ-globulin) proved the sanity of data as the p values are smaller than 0.05. ANOVA test concluded; irradiation dose statistically changes surface roughness as well as irradiation dose statistically changes adsorption of different blood proteins.

Word count:300

Table of Contents

BACKGROUND INFORMATION

My mother had influenced me a lot to choose the topic of "blood compatibility of polycarbonate membranes". Doctors found out that my mother had AML M2 (Acute Myeloblastic Leukemia) at 6 June 2006. She had treated in Ankara Cebeci Hemotology Hospital for 378 days. During this process, she had some blood compatibility issues with needle catheters. After I made my research about needle catheters, I found out needle catheters are made of biomaterials. Thus, I realized that I should narrow the scope of subject to blood compatibility property of biomaterials.

A biomaterial is a substance used in medical devices designed for contact with the living body for the intended method of application and for the intended period. Biomaterials can be either synthesized in laboratory with certain materials and techniques or can be derived from nature. Applications of biomaterials have a wide range from implants to supporting materials such as joints, needle catheters, mammary prosthesis. Whether biomaterial is synthetic or derived from nature their health care and quality should be evaluated since biomaterials are such effective and considerable part of medical applications. Specifically, they form the parts of many products like hemodialysis, blood-oxygenation, intra-veneous lines, needle catheters and blood-bags. Furthermore, tissue engineering is another growing area which is highly related to biomaterials in 21st century and it is opened to new researches about polycarbonates' features for good.

The biomaterials that are used in these medical applications must meet certain criteria and regulatory requirements and be biocompatible. If the biomaterial used in medical application fails to comply with the criteria, the time and efforts to save patients from diseases would be unnecessary. Those conditions may lead to low quality and health care and cause possible infections. Recent studies showed that the surface properties of biomaterials are the determinant factors for biocompatibility. For the materials which come in contact with blood, the first event is the adsorption of blood proteins at the solid-liquid interface. Thereafter processes like, adsorption

of blood proteins depending on the composition and the conformation of the adsorbed protein layer[1]. The composition of the adsorbed protein layer usually changes as a function of exposure time. To command on the relation between the character of the polymer surface and it's blood compatibility, protein adsorption has to be studied with polymers that have well characterized surface structures[2].

Although there are countless class of biomaterials, polycarbonates are the most well-known and common class of biomaterials. It's preferred for its features like consistent clarity, creep resistance, dimensional stability, heat resistance, perfect impact strength, light and inert response to blood and body tissue[3]. Thus, polycarbonates ease the disposable medical devices like dialyzer, oxygenator, infusion and bypass filters etc.

It is stated polycarbonates have many superior features, however, their health care properties are not so "trustable". In order to increase the health care of polycarbonates, radiation sterilization could be used to increase its resistance to adhesion of bacteria and viruses. This method is the most effective way to sterilize medical devices or biomaterials made from polycarbonate by γ-rays without causing any breaking down of biomaterials[4].

This study is carried to understand the effect of different γ-irradiation dose on surface roughness and adsorption of blood proteins of polycarbonate membranes prepared with chloroform. For this purpose, polycarbonate membranes prepared with chloroform are γ-

[1] Kuwahara, T., Markert, M., Wauters, J.P., Artif. Organs, 13 (1989(427.
[2]http://www.researchgate.net/publication/11348783_Competitive_adsorption_of_blood_proteins_on_gamma-irradiated-polycarbonate_films
[3]http://www.researchgate.net/publication/11348783_Competitive_adsorption_of_blood_proteins_on_gamma-irradiated-polycarbonate_films
[4] Ishigaki, I., Yoshii, F., Radiat. Phys. Chem., 39 (1992) 527.

irradiated at different doses and their competitive adsorption of blood proteins from plasma onto the surfaces are examined. To sum up, the research question is determined as *"How do different γ-irradiation doses affect roughness and protein adsorption properties of polycarbonate membranes prepared by chloroform indicated by AFM and competitive adsorption measurements of different blood plasma proteins by batch-wise reactor?"*.

HYPOTHESIS

There are several factors that affect the blood compatibility of biomaterials such as concentrations of polymers, surface morfology and bulk structure, interaction, competitive plasma protein adsorption and γ-irradiation. In this study, surface roughness of polycarbonate membranes prepared with chloroform and competitive plasma protein adsorption are only discussed with the influence of γ-irradiation.

While I was doing my research about surface structure of biomaterials, I found out that; as the three-dimensional pore structure, fine pore size of biomaterial CPP were increased, controllable degradability and reasonable compressive strength properties of CPP were advanced. Thus, modified CPPs usage is allowed in tissue engineering (in the application of loaded bone implant.).[5] Since "Many tissue engineering applications require a bioactive and biocompatible material for building a tissue-scaffold construct."[6], I realized the criteria and regulatory requirements for blood compatibility and tissue engineering are nearly same. Thus, I assumed that as the porosity of polycarbonate membranes prepared from chloroform increases, their blood compatibility would also increase. It is worth to notice that porosity and roughness of polycarbonate membranes are directly proportional terms.

Moreover, I found out *γ*-irradiation also increases the porosity of biomaterials[7] besides its sterilization property.

On the other hand; while I was reading an article about blood compatility of biomaterials, I found out "The presence of porosity greatly increases the surface area of materials and improves

[5]http://www.google.com.tr/url?sa=t&rct=j&q=&esrc=s&source=web&cd=5&ved=0CEYQFjAE&url=http://www.ceramics-silikaty.cz/2011/pdf/2011_01_43.pdf?origin=publication_detail&ei=_aN1VJbKFYrmaPCKgfAH&usg=AFQjCNH-DlhuaXeIAEtN_PA4MAB1FFr9JA
[6] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2440513/
[7] https://www.google.com.tr/?gws_rd=ssl#q=γ-irradiation increases roughness

the protein adsorption of biomaterials."[8]. It is worth to notice protein adsorption increases the blood compatibility properties of biomaterials.

Due to given reasons above, it is hypothesized "as *γ-irradiation dose increases, roughness and protein adsorption properties of polycarbonate membranes prepared with chloroform increases"*.

[8] "A Review of Protein Adsorption on Bioceramics." *Home*. Web. 26 Nov. 2014. <http://rsfs.royalsocietypublishing.org/content/early/2012/03/21/rsfs.2012.0012.full>.

METHOD DEVOLOPMENT AND PLANNING

From the very beginning of my study, I was determined to advance blood compatibility of needle catheters due to the side effects arised from its interaction with my mother's body. For this purpose, I chose to explore the polycarbonates blood compatibility rather than any kind of biomaterials such as ceramics and collagen.

First of all, I searched for a suitable laboratory. The laboratory should have necessary equipments and chemicals to perform my experiment. Also, I needed scientific advice about how to use laboratory tools. For these reasons, I performed my experiment in Hacettepe University Biochemistry Major Laboratory. Fortunatelly, Prof. Adil Denizli accepted to help me in laboratory and gave me some advice about which materials to use in my experiment.

To achieve my goal, I decided to prepare my polcarbonate membranes with chloroform. The main reason why I chose chloroform is its actions in chemical reactions are rapid, complete and persistent. Therefore, it is being required in less quantity, its odor is not unpleasant and it is relatively cheap when it is compared to 1.4-dioxane, cyclohexane and 1.2-dicholoroetane[9].I prepared chlorofrom membranes with dry solvent casting method, since my instructor in laboratory told me it is the easiest method which can be performed by a high school student. On the other hand; to increase the surface roughness of polycarbonate membranes prepared with chloroform, γ-irradiation studies were performed with Issledovatelj self protected type ^{60}Co γ-irradiator. The main reason for using this method was a variety of polymer based medical devices including those made from polycarbonate are currently being sterilized with γ-irradiation. There

[9] "Chloroform." *Chloroform*. Web. 26 Nov. 2014. <http://www.general-anaesthesia.com/misc/chloroform.html>.

are arguments about whether ethylene oxide should be used in sterilization rather than γ-irradiation. However, Ethylene oxide is well known to be dangerous, toxic, carcinogenic, with mutagenic effects on living organisms[10]. In comparison, safety of workers at the radiation processing facilities is trustable and easily controlled, and no traces of radioactivity are introduced in irradiated products[11]. Another benefit of radiation is, It allows products to be sterilized after packaging, thus avoiding problems of recontamination.

γ- irradiation was performed in Hacettepe University Biochemistry Major Laboratory. My instructor at laboratory, Professor Denizli, informed me about radiation doses. He stated that above 200 kGy γ-irradiation, biomaterials structures tend to be broken. Thus, Irradiation dose is changed in the range of 0-200 kGy. Polycarbonate membranes prepared with chloroform were exposured to 7 different irradiation dose selected randomly: 0, 5, 15, 25, 35, 60 and 130 kGy . I chose irradiation doses increasingly to see if there is a direct relationship between γ-irradiation and roughness property of membranes.

[10] Ishigaki, I., Yoshii, F., Radiat. Phys. Chem., 39 (1992) 527.

[11] http://www.researchgate.net/publication/11348783_Competitive_adsorption_of_blood_proteins_on_gamma-irradiated-polycarbonate_films

There are many methods to evaluate the roughness of polycarbonates membranes. I had accessed following options to choose from:

Method	Advantages	Disadvantages
Conventional Microscopies	• Very direct • Easy to set up and construct • Relatively cheap	• Not very sensitive, surface of biomaterials cannot be seen • Only small amounts of sample can be used
Atomic Force Microscopies	• Very high magnification and resolution • Ability to obtain different views of the sample from a single data collection[12] • Very accurate	• Relatively expensive • Not easy to access

Table-1 Advantages and disadvantages of different methods for evaluating the surface roughness of polycarbonate membranes prepared with chloroform

Competitive adsorption of blood protein from plasma is examined because the adsorption of plasma proteins to polymer materials profoundly affects the interaction of blood cells with polymer materials. When blood is placed in contact with any foreign surface, a spontaneous competitive adsorption of proteins and glycoproteins occurs at the surface and forms a complex protein coating on the surface[13]. These adsorptions greatly depend on the surface characteristics of polymers, which affect their blood-compatibility [14]. Fibronogen, Serum Albumin and γ-globulin adsorption are examined due to their high surface activity[15].

[12] Atomic Force Microscopy. (n.d.). Retrieved November 26, 2014, from http://www.springer.com/life sciences/biochemistry & biophysics/book/978-1-58829-094-6
[13] http://onlinelibrary.wiley.com/doi/10.1002/app.1557/full
[14] Andrade, J.D., ASAIO J, 10 (1987) 75.
[15] Kim, S.W., and Jacobs, H., Blood Purification, 14 (1996) 357.

To sum up, I performed 3 competitive adsorption experiments (fibronogen, Serum Albumin and γ-globulin) for 7 different γ-irradiated polycarbonate membranes prepared with chloroform. 3 trials are performed for each case to increase accuracy of collected data and decrease the random errors in the experiment.

METHOD

Materials

- Ciba Corning Albumin Reagent
- Commercially available polycarbonate (Lexan®)
- a batch-wise reactor
- Chloroform
- Bovine serum albumin(from Sigma)
- Fibrinogen (from Sigma)
- a precision micrometer (**Mituyoto**, Japan).
- Issledovatelj self protected type ^{60}Co γ-irradiator
- an electronic balance (Sartorius, $\pm$ 1.10^{-4} g)
- an AFM (Topometrix TMX 2000 Explorer, AFM in contact mode in air)
- Fibrinogene-Kit (Ref No: 68452 and 68582, bioMerieux Laboratory Reagents and Instruments, Marcy-l'Etoile, France)
- a round glass mould (8 cm in diameter)

Procedure

- ***Step-1* Preparation of Polycarbonate Membranes**

  The standard method used in *Step-1* is attached as *Appendix-1.*

- ***Step-2* γ-Irradiation**

  The standard method used in *Step-2* is attached as *Appendix-2.*

- **Step-3 Atomic Force Microscopy Studies**

1. Cut 40x40 mm^2 area from each film with a perforator.
2. Fix the films onto the metal sample holders of AFM by a solventless glue.

3. Change AFM settings to 130 micron tripod and pyramidal type
4. Set AFM resolution to 200x200 pixels from the settings
5. Take atomic force micrographs by using an AFM (in contact mode in air in order to observe the surface topography of the untreated and γ-irradiated membranes).

- ***Step-4*** **Competitive Adsorption of Blood Proteins from Plasma**

Adsorption of blood proteins (serum albumin, γ-globulin and fibrinogen) from sigma on the untreated and γ-irradiated polycarbonate membranes are examined in batch-wise reactor.

1. Centrifuge the blood samples of 500 gram obtained from sigma for 35 minutes at 25^{O}C to separate the plasma.
2. Incubate 10 mL of the freshly separated plasma containing serum albumin (38.3 mg/mL), fibrinogen (2.6 mg/mL) and γ-globulin (17.7 mg/mL) with 3 circular pieces (1.2 cm in diameter) of the untreated and γ-irradiated polycarbonate membranes for 1.5 hours.
3. Measure total protein concentration by using the total protein reagent (Ciba Corning Diagnostics Ltd, Halstead, Essex, England; Catalog Ref. No: 712076) at 540 nm, based on Biuret reaction [16].
4. Perform Chronometric determination of fibrinogen by using Fibrinogene-Kit.
5. Determine serum albumin concentration by using Ciba Corning Albumin Reagent based on bromocresol method [17].(γ-globulin concentration is determined from the difference).

- Repeat steps 1-4 for 3 times to increase the accuracy of the experiment.

[16] Textbook of Clinical Chemistry, WB Saunders Comp., N.W. Tietz, Philadelphia, 1986.
[17] Clauss, A., Acta Haemat., 17 (1957) 237.

DATA ANALYSIS

A.Results

Irradiation Dose (kGy)	Roughness of polycarbonate membranes (±0.001 nm)		
	Trial -1	*Trial-2*	*Trial-3*
0	28,811	29,000	28,831
5	29,112	29,110	29,152
15	29,134	29,131	29,144
25	29,101	29,142	29,165
35	29,150	29,183	29,212
60	31,971	31,830	31,757
130	42,751	42,890	43,000

Table -2 Raw data table of effect of γ-irradiation dose on surface roughness of polycarbonate membranes prepared with chloroform at 25^{o}C in Issledovatelj self protected type ^{60}Co γ-irradiator at a dose rate of 3.5 kGy/h.

Competitive adsorption of Serum Albumin onto polycarbonate membranes prepared by chloroform: Serum Albumin concentration: 38.3 mg/mL			
Irradiation Dose (kGy)	Absorbed Serum Albumin (±0.001ng/cm2)		
	Trial-1	*Trial-2*	*Trial-3*
0	76.219	76.244	76.232
5	81.376	81.374	81.363
15	83.003	82.997	83.001
25	85.265	85.268	85.194
35	86.387	86.369	86.378
60	97.142	97.147	97.146
130	110.782	110.785	110.783

Table-3 Raw data table of effect of γ-irradiation dose on competitive adsorption of Serum Albumin onto polycarbonate membranes prepared with chloroform: Serum Albumin concentration: 38.3 mg/mL

Competitive adsorption of Fibrinogen onto polycarbonate membranes prepared by chloroform: Fibrinogen concentration: 2.6 mg/mL			
Irradiation Dose (kGy)	**Absorbed Fibrinogen (±0.001ng/cm²)**		
	Trial-1	***Trial-2***	***Trial-3***
0	**37.778**	**37.774**	**37.769**
5	**42.003**	**42.004**	**42.006**
15	**46.312**	**46.314**	**46.310**
25	**50.001**	**49.998**	**50.001**
35	**55.075**	**55.071**	**55.072**
60	**58.063**	**58.065**	**58.062**
130	**60.002**	**60.003**	**60.005**

Table-4 Raw data table of effect of γ-irradiation dose on competitive adsorption of Fibrinogen onto polycarbonate membranes prepared with chloroform: Fibrinogen concentration: 2.6 mg/mL

Competitive adsorption of gamma-globulin onto polycarbonate membranes prepared with chloroform: gamma-globulin concentration: 17.7 mg/mL			
Irradiation Dose (kGy)	**Absorbed gamma-globulin (0.001ng/cm²)**		
	Trial-1	***Trial-2***	***Trial-3***
0	20.743	20.742	20.740
5	24.328	24.327	24.327
15	34.212	34.220	34.219
25	44.457	44.458	44.554
35	45.110	45.117	45.115
60	48.234	48.233	48.330
130	49.630	49.631	49.629

Table-5 Raw data table of effect of γ-irradiation dose on competitive adsorption of γ–globulin onto polycarbonate membranes prepared with chloroform: γ-globulin concentration: 17.7 mg/mL

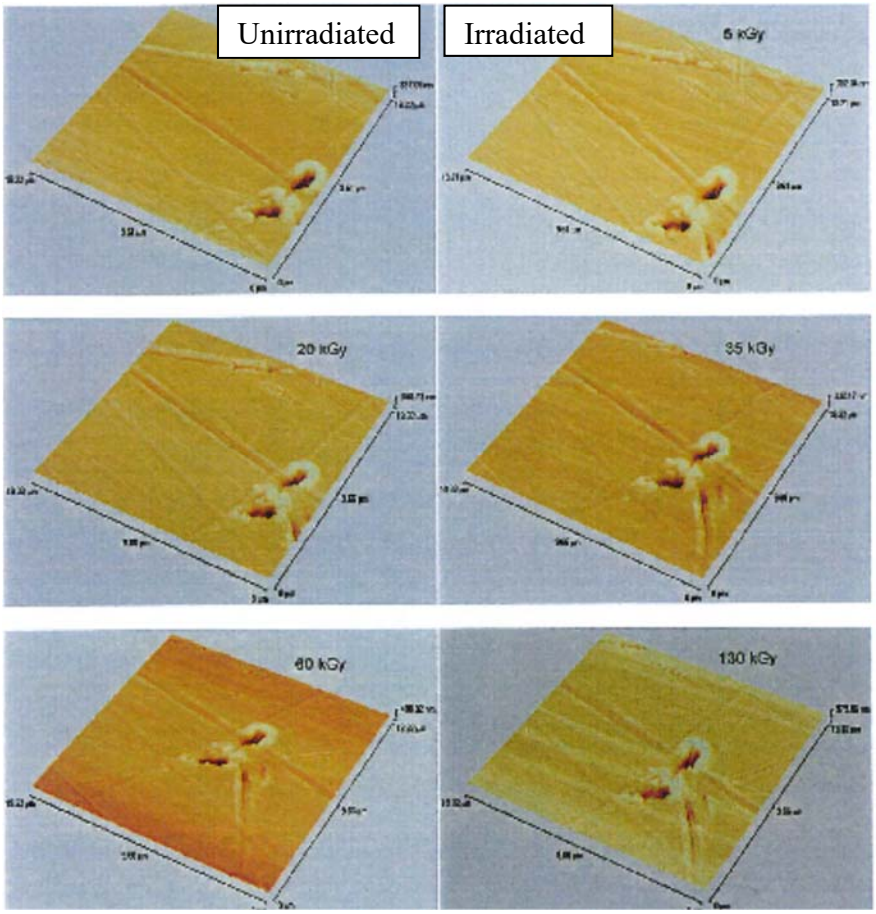


Image-1 AFM images of polycarbonate membranes prepared with chloroform at 0 kGy, 35 kGy, 130kGy irradiation doses

B. Statistical Analysis

1. Mean:

$$\overline{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

Where;

n = number of trials

x_i = results obtained from each trial

2. Variance

$$s^2 = \frac{1}{(N-1)} \sum_{i=1}^{N} (x_i - \bar{x})^2$$

Where;

n = number of trials

x_i = results obtained from each trial

$\bar{x}$ = mean

3.Standard Deviation

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

Where;

n = number of trials

x_i = results obtained from each trial

$\bar{x}$ = mean

4.Standard Error

$$SE_{\bar{x}} = \frac{s_x}{\sqrt{n}}$$

Where;

S_x= Standard Deviation

n = number of trials

- **Surface Roughness of polycarbonate membranes prepared with chloroform**

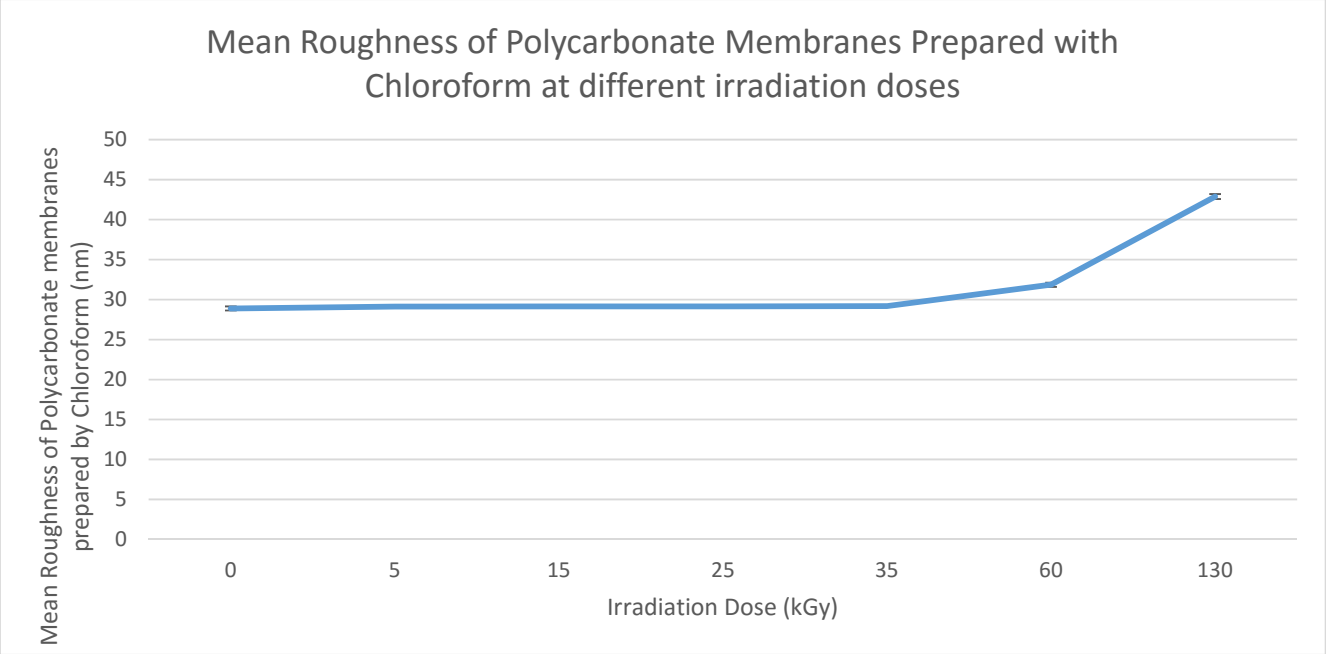
	Irradiation Dose (kGy)						
	0	*5*	*15*	*25*	*35*	*60*	*130*
Mean	28.88067	29.12467	29.13633	29.136	29.18167	31.85267	42.8803
Standard Error	0.059945	0.013679	0.00393	0.018717	0.01791	0,.062807	0.07204
Standard Deviation	0.103828	0.023692	0.006807	0.032419	0.031021	0.108786	0.12478
Variance	0.01078	0.000561	4.63E-05	0.001051	0.000962	0.011834	0.01557
Count	3	3	3	3	3	3	3
Confidence Level(95,0%)	0.257924	0.058855	0.016909	0.080534	0.077062	0.270239	0.30997354

Table-6 Statistical analysis of effect of irradiation dose on surface roughness of polycarbonate membranes prepared with chloroform

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	476.0281	6	79.33802	13609.91	**1.56E-25**	2.847726
Within Groups	0.081612	14	0.005829			
Total	476.1097	20				

Table-7 ANOVA results of calculated values of surface roughness of polycarbonate membranes prepared with chloroform



Graph-1 Mean roughness of polycarbonate membranes prepared with chloroform at different γ-irradiation doses

*Error bars are neglected due to very low standard error in graph-1

- **Competitive Absorption of Serum Albumin onto polycarbonate membranes chloroform: Serum Albumin concentration: 38.3 mg/mL**

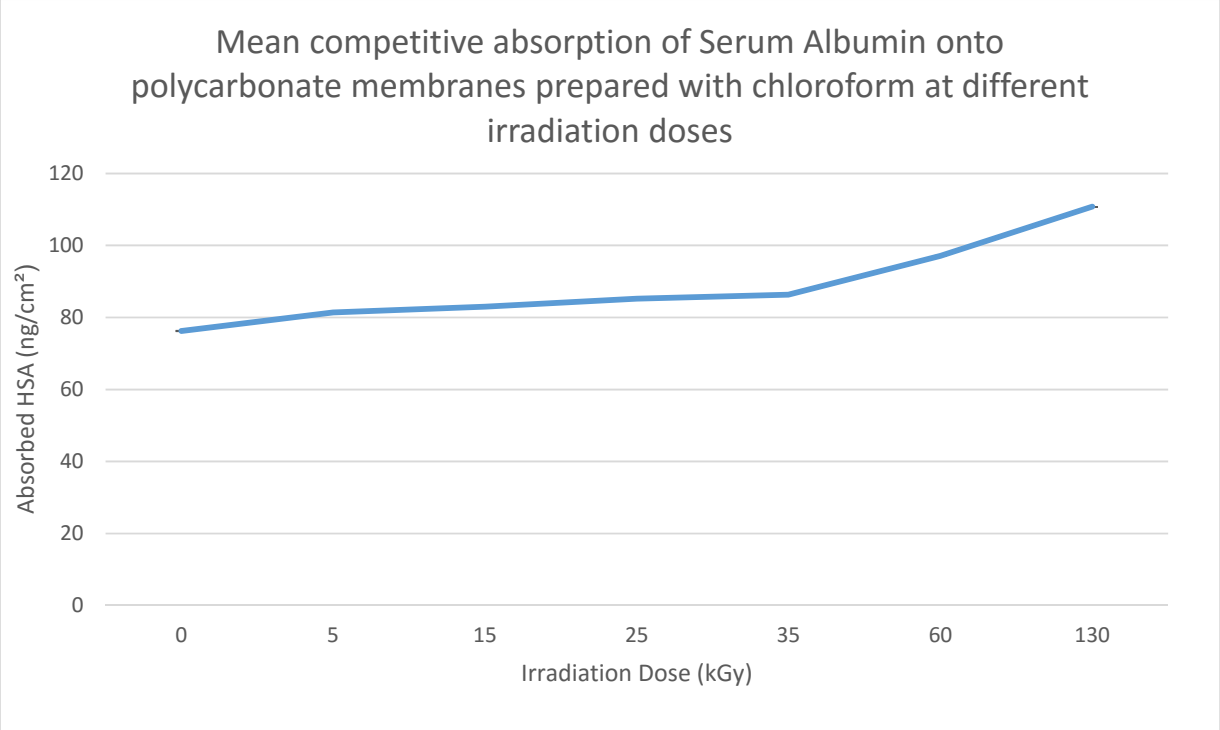
	Irradiation Dose (kGy)						
	0	*5*	*15*	*25*	*35*	*60*	*130*
Mean	76.23067	81.371	83.00033	85.24233	86.378	97.145	110.7833
Standard Error	0.00811	0.004041	0.001764	0.024182	0.005196152	0.01528	0.000882
Standard Deviation	0.014048	0.007	0.003055	0.041885	0.009	0.002646	0.001528
Variance	0.000197	4.9E-05	9.33E-06	0.001754	8.1E-05	7E-06	2.33E-06
Count	3	3	3	3	3	3	3
Confidence Level(95,0%)	0.034896	0.017389	0.007589	0.104048	0.022357239	0.006572	0.003795

Table -8 Statistical analysis of effect of irradiation dose on competitive absorption of Serum Albumin onto polycarbonate membranes prepared with chloroform: Serum Albumin concentration: 38.3 mg/mL

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2453.83	6	408.9716	1363022	**1.55E-39**	2.847726
Within Groups	0.004201	14	0.0003			
Total	2453.834	20				

Table-9 ANOVA results of calculated values of competitive absorption of Serum Albumin onto polycarbonate with chloroform: Serum Albumin concentration: 38.3 mg/mL



Graph-2 Mean competitive absorption of SerumAlbumin onto polycarbonate membranes prepared with chloroform: Serum Albumin concentration: 38.3 mg/mL at different γ-irradiation doses

*Errors bar are neglected due to very low standard error in graph-2

- **Competitive adsorption of Fibrinogen onto polycarbonate membranes prepared with chloroform: Fibrinogen concentration: 2.6 mg/mL**

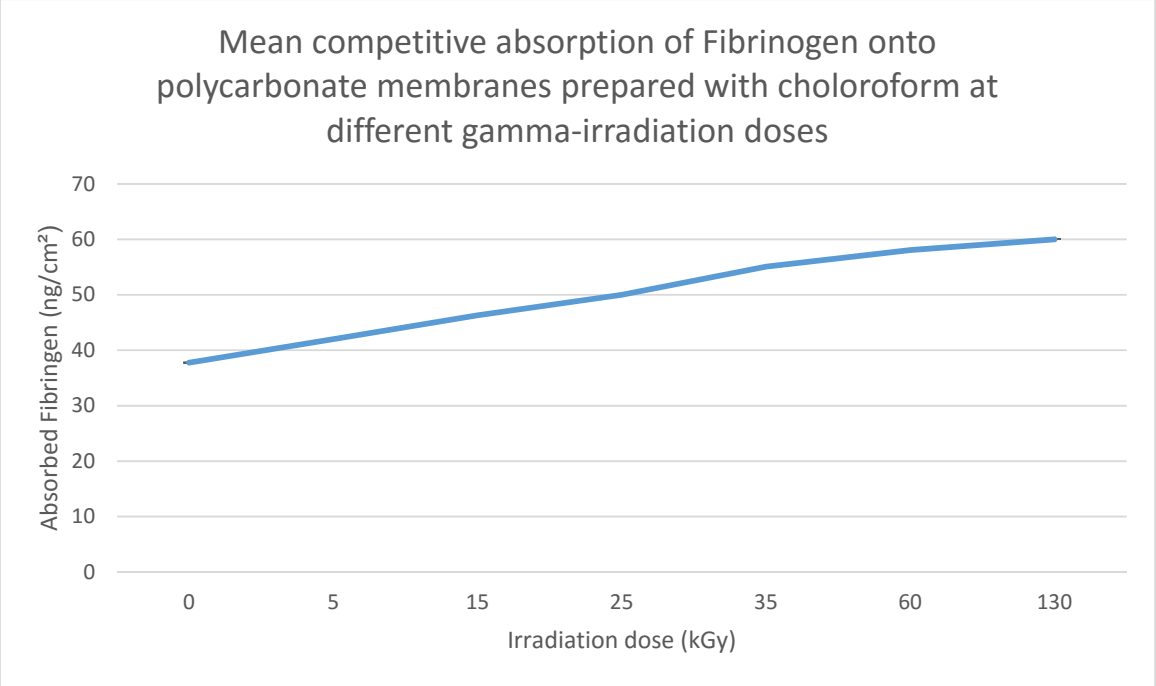
	Irradiation Dose (kGy)						
	0	*5*	*15*	*25*	*35*	*60*	*130*
Mean	37.77367	42.00433	46.312	50	55.07267	58.06333	60.00333
Standard Error	0.002603	0.000882	0.001155	0.001	0.001202	0.000882	0.000882
Standard Deviation	0.004509	0.001528	0.002	0.001732051	0.002082	0.001528	0.001528
Variance	2.03E-05	2.33E-06	4E-06	3E-06	4.33E-06	2.33E-06	2.33E-06
Count	3	3	3	3	3	3	3
Confidence Level(95,0%)	0.011202	0.003795	0.004968	0.004302653	0.005171	0.003795	0.003795

Table -10 Statistical analysis of effect of irradiation dose on competitive absorption of Fibrinogen onto polycarbonate membranes prepared with chloroform: Fibrinogen concentration: 2.6 mg/mL

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1253.239	6	208.8732	37813250	**1.23E-49**	2.847726
Within Groups	7.73E-05	14	5.52E-06			
Total	1253.239	20				

Table- 11 ANOVA results of calculated values of competitive absorption of Fibrinogen onto polycarbonate membranes prepared with chloroform: Fibrinogen concentration: 2.6 mg/mL



Graph-3 Mean competitive absorption of Fibrinogen onto polycarbonate membranes prepared with chloroform: Fibrinogen concentration: 2.6 mg/mL at different γ-irradiation doses

*Error bars are neglected due to very low standard error in graph-3

- **Competitive adsorption of γ-globulin onto polycarbonate membranes prepared with chloroform: γ-globulin concentration: 17.7 mg/mL**

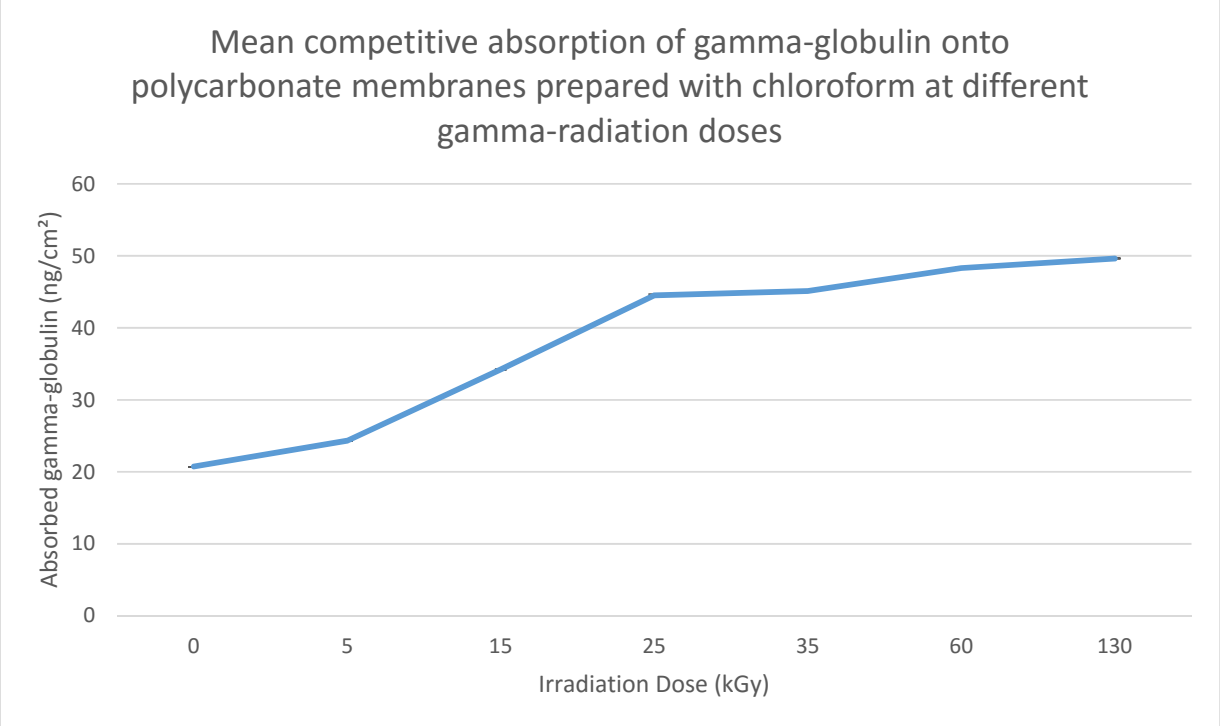
	Irradiation Dose (kGy)						
	0	*5*	*15*	*25*	*35*	*60*	*130*
Mean	20.74167	24.32533	34.217	44.523	45.114	48.29566667	49.63
Standard Error	0.000882	0.001333	0.002517	0.032512	0.002082	0.031381169	0.000577
Standard Deviation	0.001528	0.002309	0.004359	0.056312	0.003606	0.054353779	0.001
Variance	2.33E-06	5.33E-06	1.9E-05	0.003171	1.3E-05	0.002954333	1E-06
Count	3	3	3	3	3	3	3
Confidence Level(95,0%)	0.003795	0.005737	0.010828	0.139886	0.008957	0.135022273	0.002484

Table -12 Statistical analysis of effect of different irradiation dose on competitive adsorption of γ-globulin onto polycarbonate membranes prepared with chloroform: γ-globulin concentration: 17.7 mg/mL

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2500.414	6	416.7357	473102.5	**2,56E-36**	2.847726
Within Groups	0.012332	14	0.000881			
Total	2500.427	20				

Table-13 ANOVA results of calculated values of competitive adsorption of γ-globulin onto polycarbonate membranes prepared with chloroform: γ-globulin concentration: 17.7 mg/mL



Graph-4 Mean of competitive absorption of γ-globulin onto polycarbonate membranes prepared with chloroform: γ-globulin concentration: 17.7 mg/mL at different γ-irradiation doses

*Error bars are neglected due to very low standard error in graph-2

CONCLUSION AND EVALUATION

A. Evaluation

In the present study, the influence of different γ-irradiation doses on surface roughness and competitive adsorption of blood proteins of polycarbonate membranes prepared with chloroform are examined. The significant results answered the research question and proved my hypothesis: "*as γ-irradiation dose increased, roughness and protein adsorption properties of polycarbonate membranes (prepared with chloroform) increased"*. Thus, increased surface roughness and higher mean competitive absorption of blood proteins onto polycarbonate membranes verified, polycarbonate membranes prepared with chloroform became more blood compatible at the end of this study.

With reference to Graph-1; It is observed that as the γ-irradiation dose is increased, the surface roughness of the membranes are drastically increased. Mean surface roughness of polycarbonate membranes irradiated at 130kGy is calculated 42.880 nm whereas non-irradiated membranes' mean surface roughness is calculated 28.881 nm. It has been found that protein adsorption experiments carried out with blood proteins (Serum Albumin, Fibrinogen and γ-globulin) demonstrated that protein adsorption significantly increased with increased irradiation dose (see Table-6, Table-8, Table-10 and Table 12; graph-1, graph-2, graph-3 and graph-4). Besides, error bars in all graphs are neglected due very low standard error. For instance, γ-irradiated polycarbonate membranes prepared with chloroform at 130kGy, has a mean of competitive absorption 110.783 ng/cm^2 for Serum Albumin, 60.003 ng/cm^2 for fibrinogen, 49.630 ng/cm^2 for γ-globulin. Furthermore, sanity of the collected data are proved with very low standard deviation and variance values. The standard deviation of surface roughness of γ-irradiated

polycarbonate membranes prepared with chloroform at 60kGy is calculated 0.108786 whereas standard deviation is calculated 0.002646. Such a low standard deviation value and variance value confirms the precision of the date collected. It is worth to restate, error bars in all graphs are neglected due very low standard error.

The collected data prove that there is a meaningful difference of surface roughness at different irradiation doses. Likewise, the collected data also prove that there is a meaningful difference of adsorption of blood proteins (Serum Albumin, Fibrinogen, gamma-globulin) onto polycarbonate membranes at different irradiation doses. The null hypothesis was that for $\alpha = 0.05$, the difference between the groups are statistically insignificant. Since the p value of the data given by the ANOVA test is obviously less than the α value in all studies. P value is calculated $1.56x10^{-25}$ for surface roughness. For the blood proteins; P value is calculated 1.5510^{-39} in Serum Albumin adsorption; P value is calculated $1.23x10^{-49}$ in fibrinogen adsorption whereas P value is calculated $2.56x10^{-36}$ in gamma-globulin adsorption. Thus, the results rejects the null hypothesis and further proves my hypothesis. Moreover, ANOVA tests verified; surface roughness of polycarbonate membranes prepared with chloroform statistically changed at different irradiation doses. The means at different irradiation doses given in *Table-6* suggests a direct proportion between surface roughness and irradiation dose. Likewise, ANOVA test for each blood protein statistically demonstrated, blood protein adsorption onto polycarbonate membranes prepared with chloroform varies statistically with different irradiation doses. Just the same, mean values at different irradiation doses given in *Table-8* suggests a direct proportion between serum albumin adsorption and irradiation dose; mean values at different irradiation doses given in *Table-10* suggests a direct proportion between fibrinogen adsorption and irradiation dose; mean values at different irradiation values given in *Table-12* suggests a direct proportion between gamma-

globulin and irradiation dose. To sum up, ANOVA test concluded that the standard error, standard deviation and confidence level (95.0%) values for surface roughness and competitive adsorption of blood proteins (Serum Albumin, Fibrinogen, γ-globulin) are relatively too low (see Table-7, Table-9 ,Table-11 and Table-13) for the results to be 100% accurate. Such a significant amount of preciseness and accuracy is favored with the usage of profoundly equipment and techniques which were used in the experiment.

B. **Conclusion**

Although, my hypothesis is proved and I achieved my goal of making polycarbonate membranes prepared with chloroform more blood compatible, there are still things to modify the current experiment. As it is stated from the beginning, there are several factors that affect blood compatibility rather than surface roughness and protein adsorption. These factors are included type of solutions used to prepare polycarbonate membranes, hydrophilicity (water uptake qualities), interaction, activation of intrinsic coagulation, adhesion and aggregation of platets.

In order to modify this study, It is strongly suggested to examine effect of γ-irradiation on hydrophilicity of polycarbonate membranes .The hydrophilicity deal with the water uptake which is a determining factor for polycarbonate membranes to be biocompatible and blood compatible. For this reason, contact angle measurements could be done since it is accepted as a very precise method[18] to examine both biocompatibility and blood compatibility. To get more deep insight of blood compatibility of polycarbonate membranes, their composition and the conformation of the

[18] "Contact Angle." *Wikipedia*. Wikimedia Foundation, 29 Nov. 2014. Web. 3 Dec. 2014. <http://en.wikipedia.org/wiki/Contact_angle>.

absorbed protein layer should be studied extensively. For this purpose, polycarbonate membranes can be prepared from different solutions. 1,2 dichloroetane, tetrahyrofuran, 1,4-dioxane and cyclohexane could be studied since they are easy to access and found in lots of medical devices. Therefore, these biomaterials are relatively cheap for students to afford. Furthermore; APTT and PT tests can be carried out with untreated and irradiated membranes to estimate the blood compatibility of polycarbonate membranes and to manifest their bioactivity of intrinsic blood coagulation factors and extrinsic blood coagulation factors [19].

[19] Competitive adsorption of blood proteins on gamma-irradiated-polycarbonate films. (n.d.). Retrieved January 20, 2015, from http://www.researchgate.net/publication/11348783_Competitive_adsorption_of_blood_proteins_on_gamma-irradiated-polycarbonate_films

Appendices

Appendix-1

- ***Step-1* Preparation of Polycarbonate Membranes**

1. Prepare 21 polycarbonate membranes with chloroform structure by dry solvent-casting method[20]. (The chloroform solutions (6.0%))
2. Pour the chloroform solutions (6.0%) into a round glass mould (8 cm in diameter) and place them in a temperature-controlled chamber at 25°C until it becomes dry.
3. Ish the membranes obtained 5 times with distilled water
4. Cut the ished membranes into squares (0.4 cm x 0.4 cm) with a perforator.
5. Measure thickness of the membranes between 40 μm and 45 μm with a precision micrometer (**Mituyoto**, Japan).

Appendix-2

- ***Step-2* γ-Irradiation**

1. Irradiate the polycarbonate membranes in air at room temperature (at 25°C). in Issledovatelj self protected type ^{60}Co γ-irradiator at a dose rate of 3.0 kGy/h.
2. Irradiate the polycarbonate membranes in air at room temperature (at 25°C). in Issledovatelj self protected type ^{60}Co γ-irradiator at a dose rate of 3.5 kGy/h.
3. Irradiate three piece of polycarbonate membranes prepared with chloroform at 0 kGy
4. Irradiate three piece of polycarbonate membranes prepared with chloroform at 5 kGy

[20] http://www.google.com.tr/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CCAQFjAA&url=http://www.springer.com/cda/content/document/cda_downloaddocument/series_2882_vol130_p1.pdf?SGWID=0-0-45-173854-p173622957&ei=tUB2VNTfEab5ywOtxYLADQ&us

5. Irradiate three piece of polycarbonate membranes prepared with chloroform at 15 kGy
6. Irradiate three piece of polycarbonate membranes prepared with chloroform at 25 kGy
7. Irradiate three piece of polycarbonate membranes prepared with chloroform at 35 kGy
8. Irradiate three piece of polycarbonate membranes prepared with chloroform at 60 kGy
9. Irradiate three piece of polycarbonate membranes prepared with chloroform at 130 kGy

Bibliography

1. Kuwahara, T., Markert, M., Wauters, J.P., Artif. Organs, 13 (1989(427.
2. Competitive adsorption of blood proteins on gamma-irradiated-polycarbonate films. (n.d.). Retrieved January 20, 2015, from http://www.researchgate.net/publication/11348783_Competitive_adsorption_of_blood_proteins_on_gamma-irradiated-polycarbonate_films
3. Competitive adsorption of blood proteins on gamma-irradiated-polycarbonate films. (n.d.). Retrieved January 20, 2015, from http://www.researchgate.net/publication/11348783_Competitive_adsorption_of_blood_proteins_on_gamma-irradiated-polycarbonate_films
4. Ishigaki, I., Yoshii, F., Radiat. Phys. Chem., 39 (1992) 527.
5. "THE EFFECT OF POROSITY ON THE STRUCTURE AND PROPERTIES OF CALCIUM POLYPHOSPHATE BIOCERAMICS." School of Chemistry and Environment, Beihang University, Beijing 100191, P. R. China *College of Polymer Science and Engineering, Sichuan University, Chengdu 610065, P. R. China, 12 June 2010. Web. 26 Nov. 2014. <http://www.google.com.tr/url?sa=t&rct=j&q=&esrc=s&source=web&cd=5&ved=0CEYQFjAE&url=http://www.ceramics-silikaty.cz/2011/pdf/2011_01_43.pdf?origin=publication_detail&ei=_aN1VJbKFYrmaPCKgfAH&usg=AFQjCNH-DlhuaXeIAEtN_PA4MAB1FFr9JA>.
6. EVELYN K.F. YIM,, Ph.D., I-CHIEN LIAO, and KAM W. LEONG. "Tissue Compatibility of Interfacial Polyelectrolyte Complexation Fibrous Scaffold: Evaluation of Blood Compatibility and Biocompatibility." NIH Public Access, 26 June 2008. Web. 26 Nov. 2014. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2440513/>.
7. "MORPHOLOGICAL CHANGES OF ELECTRON-BEAM IRRADIATED PMMA SURFACE." Rashi Nathawat*, Anil Kumar, Y. K.Vijay Department of Physics, University of Rajasthan, Jaipur-302004, India. Web. 26 Nov. 2014. <https://www.google.com.tr/?gws_rd=ssl#q=γ-irradiation increases roughness>.
8. "A Review of Protein Adsorption on Bioceramics." *Home*. Web. 26 Nov. 2014. <http://rsfs.royalsocietypublishing.org/content/early/2012/03/21/rsfs.2012.0012.full>.
9. "Chloroform." *Chloroform*. Web. 26 Nov. 2014. <http://www.general-anaesthesia.com/misc/chloroform.html>.
10. Ishigaki, I., Yoshii, F., Radiat. Phys. Chem., 39 (1992) 527.
11. Competitive adsorption of blood proteins on gamma-irradiated-polycarbonate films. (n.d.). Retrieved January 20, 2015, from

http://www.researchgate.net/publication/11348783_Competitive_adsorption_of_blood_proteins_on_gamma-irradiated-polycarbonate_films

12. "Atomic Force Microscopy." Web. 26 Nov. 2014. <http://www.springer.com/life sciences/biochemistry & biophysics/book/978-1-58829-094-6>.
13. Adsorption of blood proteins on glow-discharge-modified polyurethane membranes. (n.d.). Retrieved January 20, 2015, from http://onlinelibrary.wiley.com/doi/10.1002/app.1557/full
14. Andrade, J.D., ASAIO J, 10 (1987) 75.
15. Kim, S.W., and Jacobs, H., Blood Purification, 14 (1996) 357.
16. Textbook of Clinical Chemistry, WB Saunders Comp., N.W. Tietz, Philadelphia, 1986.
17. Clauss, A., Acta Haemat., 17 (1957) 237.
18. "Contact Angle." *Wikipedia*. Wikimedia Foundation, 29 Nov. 2014. Web. 3 Dec. 2014. <http://en.wikipedia.org/wiki/Contact_angle>.
19. Competitive adsorption of blood proteins on gamma-irradiated-polycarbonate films. (n.d.). Retrieved January 20, 2015, from http://www.researchgate.net/publication/11348783_Competitive_adsorption_of_blood_proteins_on_gamma-irradiated-polycarbonate_films
20. "Dry Solvent Casting Method." Web. 26 Nov. 2014. <http://www.google.com.tr/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CCAQFjAA&url=http://www.springer.com/cda/content/document/cda_downloaddocument/series_2882_vol130_p1.pdf?SGWID=0-0-45-173854-p173622957&ei=tUB2VNTfEab5ywOtxYLADQ&us>.