BIOCOMPATIBILITY OF PET-g-PHEMA GRAFTED COPOLYMER OBTNAINED BY IONIZING RADIATION

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ABSTRACT

The preparation of hemocompatible polymeric materials has been the subject of many investigations and the grafting of hydrophilic hydrogels onto polymeric matrices has allowed the obtention of surfaces with lower sorption of proteins which can elicit the blood coagulation. In this work the radiation grafting of 2-hydroxyethyl methacrylate (HEMA) onto poly(terephtalate ethylene) (PET) was achieved by the mutual irradiation in ^{60}Co source to strengthen the antithrombogenic property of the surface.The irradiation grafting parameters and the hydrophilicity of the copolymers were determined. The characterization of the copolymer was carried out by the IR spectroscopy. The biocompatibility of the radiation modified surfaces was evaluated by *in vitro* tests of cytotoxicity and hemocompatibility (platelets adhesion) as described in the ISO 10993. The grafted polymeric surfaces were not cytotoxic and the results pointed out for an improvement of the antithrombogenicity.

1. INTRODUCTION

Radiation technology has offered good tools for the preparation of polymeric biomaterials. The advantage of the radiation chemical methods compared to the traditional method is the purity of the materials (there is no need of addition of chemical initiators and catalysts)[1]. When a synthetic material is in contact with the blood, the rapid adsorption os plasma proteins and subsequent adherence os platelets lead to thrombus formation. Surface modification of biomaterials appear to be beneficial in the prevention of protein adsorption and platelet adhesion[2]. Grafting copolymerization is one of the most widely used method for the surface modification of polymeric biomaterials[3]. This work deals with the radiation grafting of 2-hydroxyethyl methacrylate (HEMA) onto poly(terephalate ethylen) (PET) films and the biocompatible characterization by tests of cytotoxicity and hemocompatibility.

2. EXPERIMENTAL

2.1- Modification of PET surface: Glass ampoules containing PET films (20μm thickness) and HEMA solution in dichloromethane (25% v/v) were irradiated by the mutual irradiation grafting method in a ^{60}Co source. The ampoules were connected to a vacuum system and evacuated by freeze-thaw cycles which was repeated five times. The samples were irradiated by gamma rays at doses rate of 73-366Gy/h and total doses of 18-40kGy. To extract the residual homopolymer the grafted tubes were placed in a Soxhlet extractor with methanol as solvent. The degree of grafting was determined gravimetrically by the Eq. (1), where m and m_g represent the weights of PET and copolymer, respectivelly.

$$\frac{(m_g - m)}{m} x100 \qquad (1)$$

2.2 – Infrared spectroscopy (FTIR): The spectra were obtained in the Fourier Transformed Infrared (FTIR) Bomem spectrophotometer (MB-100), in inert atmosphere at room temperature. The spectra were registered in the region from 600 to 4000 cm^{-1}, resolution of 4 cm^{-1} and 20 scans/min.

2.3- Citotoxicity assay: The citotoxicity test[4] was carried out with dilution of the extracts of grafting copolymers in contact with Chinese Hamster Ovary (CHO) cells culture, from ATCC. Phenol solution (0,02%) and high density polyethylene (HDPE) extracts were used as positive and negative controls, respectively. The extracts were prepared with about 1cm^2 superficial area of each sample of grafting copolymer and HDPE per mL of RPMI-FCS (RPMI 1640 culture medium suplemented with 10% calf fetal serum and antibiotics). For preparation of cell culture dishes 2mL of 1x10^2 CHO cells/mL suspension were seeded to each 60mm diameter assay culture dish and incubated for about 5h at 37ºC in a humidified 5% CO_2 air incubator, for cell adhesion. The medium then was removed and replaced with 5mL of fresh RPMI-FCS as control, undiluted and serial diluted extract of test materials, in triplicate. After 7 days incubation, the colonies were fixed with 10% formalin in 0.9% saline and stained with Giemsa. The amount of visible colonies on each dish was counted and compared with the result from CHO control dish.

2.4- Platelet adhesion: Blood compatibility of the polymeric surfaces was evaluated by the open-static adhesion test with human blood[5]. In this method, 10mL of whole human blood was collected in ACD (1mL blood : 0.25 ACD) and centrifuged for 15 minutes at 1000 rpm. The red cells were separated and a platelet rich plasma (PRP) was obtained. The samples were cut (25 mm^2) and adhered in a cover glass with an adhesive tape. The cover glass was placed in a 30 mm Petri dish, which was also placed in a 50 mm Petri dish with some water, to reach a suitable humidity during the test. After the specimens were kept for 10 minutes at 37ºC, the plasma (PRP) was poured into the small Petri dish and kept for 3 minutes at 37ºC and then saline was added to stop the reaction and wash the samples. Subsequently, the cover glass with the samples was immersed in glutaraldehyde (2.5%) for 10 minutes at room temperature and dehydrated with ethanol in the concentrations of 50%, 75% and 95%.

2.5 – Scanning Electron Miccroscopy
After the platelet adhesion, the PET samples were fixed in a metalic suport for the gold deposition and analysis in a PHILIPS (XL-30) scanning electron microscopy (SEM) with energy dispersive analysis (EDAX).

3. RESULTS AND DISCUSSION

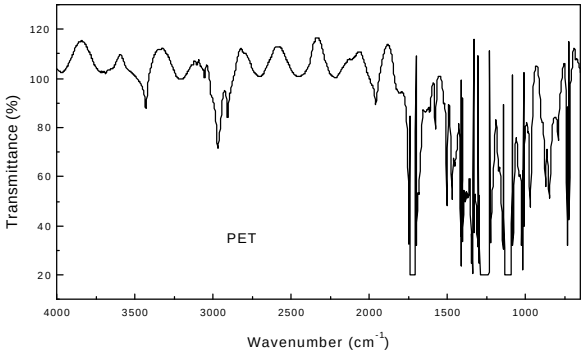
3.1- Grafted Surfaces
Tab. 1 shows the grafting irradiation parameters and the grafting yield and hydrophilicity of the copolymers. As it was expected the grafting of hydrophilic chains onto PET matrix caused an increasing in hydrophilicity. The degree of swelling was determined in water for 24 hours at 37ºC. The Tab. 1 shows that the percent of grafting become higher as the amount of incorporated hydrogel to the matrix is increased, which determines the water content in the copolymer.

Table 1. Experimental conditions for the grafting oh HEMA onto PET. Grafting level and water content of the grafted surfaces.

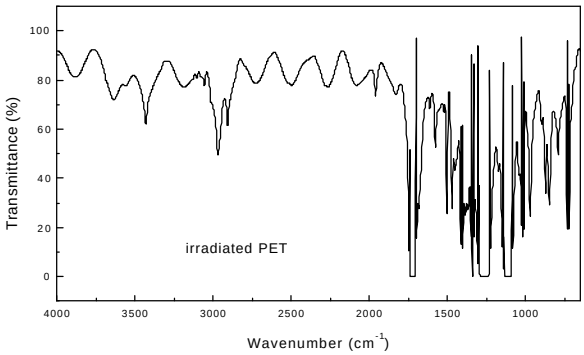
Dose	Dose rate	Grafting	H_2O
0	0	0	2.5% ± 1.0
18 kGy	73 Gy/h	25% ± 1	7.0% ± 1.3
18 kGy	73 Gy/h	35% ± 3	8.0% ± 2.2
40 kGy	366 Gy/h	24% ± 1	5.0% ± 1.0
40 kGy	366 Gy/h	55% ± 2	9.0% ± 1.8

3.2 – Infrared Spectroscopy (FTIR)
The presence of PHEMA in the PET matrices after the copolymerization was pointed out by the strong band in the region of 3500 cm^{-1} refering to the OH group , as can be observed in the Fig. 1c. Such band is not present in the spectra of Fig. 1a and 1b, related to the samples of original PET and irradiated PET in the absence of HEMA monomer, making evident the modification of PET matrix by the grafting of PHEMA.

3.3- Cytotoxicity
In the *cytotoxicity assay* the relative percentage of visible colonies number at different concentration of copolymer extracts was calculated and plotted in the graphic presented in Fig. 1. The concentration of extract necessary to kill half of the cell population is known as *cytotoxicity index* ($IC_{(50\%)}$). The negative control should not present toxic effect as observed with HDPE ($IC_{(50\%)}$ > 100) and the positive control should present cytotoxic effect, as phenol solution which kills 50% at the concentration of 45% ($IC_{(50\%)}$ = 45), as shown in Fig. 2.



(a)



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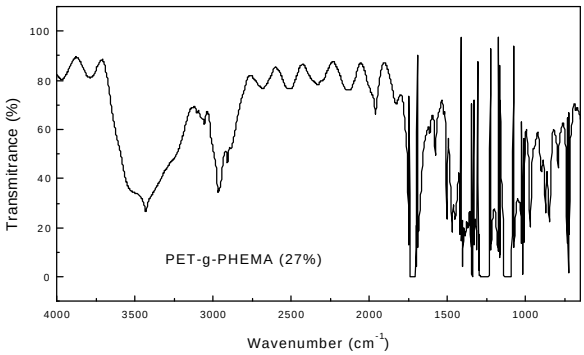


(c)

Figure 1. FTIR spectra of: a) PET (unmodified); b) irradiated PET; c) PET-g-PHEMA (27%). Irradiation condition: dose = 20kGy, dose rate = 3.6kGy.h^{-1}.

3.4 – Platelet adhesion

Among the blood cells (platelets, erythrocytes, leucocytes etc.) the platelets are the ones which perform an important role in the blood coagulation process. This happens because within these cells are present granules with contents of great importance in the blood coagulation mechanism[2].

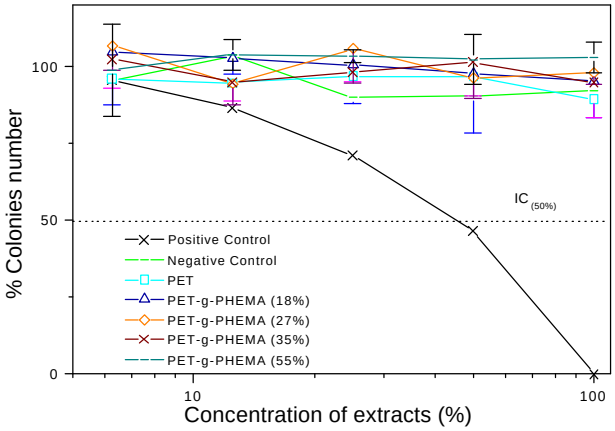


Figure 2. Colony suppression curves of the graft copolymers.

The picture 3 shows the SEM micrographs of PET, PET-g-PHEMA (38%) and PET-g-PHEMA (55%). As can be seen in the pictures, the grafted PET surface showed lower amount of adhered platelets compared to the original ungrafted PET, what pointed out a better antithrombogenic behavior. The Table 2 shows the number of adhered platelets on the surfaces of PET and PET grafted with PHEMA. According to Ratner[6] the low protein and cell adhesions onto the hydrogels are possibly correlated to the low free interfacial energy.

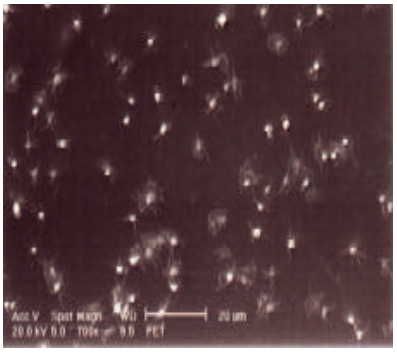
Table 2. Number of adhered platelets on the original PETand modified PET with PHEMA.

Sample	Adhered platelets ± standard deviation
PET	78 ± 5
PET-g-PHEMA (21%)	73 ± 18
PET-g-PHEMA (15%)	84 ± 8
PET-g-PHEMA (25%)	31 ± 8
PET-g-PHEMA (38%)	29 ± 6
PET-g-PHEMA (56%)	22 ± 2

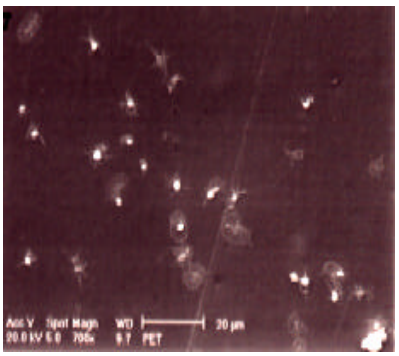
4. CONCLUSION

The grafting technique by the simultaneous irradiation method was very effective for the modification of PET films. The grafting of PHEMA increased the hydrophilicity of the PET polymer, and the amount of

the water adsorbed was higher as the grafting level increased. The SEM micrographs showed that the PHEMA grafted samples lowered the platelet adsorption, which means an improvement of the surface hemocompatible property. The biocompatibility was also shown by the absence of cytotoxicity in the tested materials.



(a)



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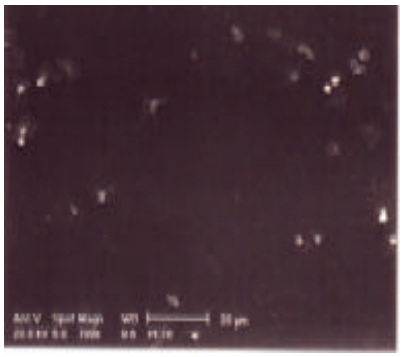

(c)

Figure 3. Platelet adhesion - SEM micrographs of : a) PET, b) PET-g-PHEMA (38%) and c) PET-g-PHEMA (55%). Increase: 700x, scale 20µm.

ACKNOWLEDMENTS

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