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Manufacture of non-thrombogenic polymer surfaces by gamma irradiation to induce simultaneous grafting and heparinization of thin PVC films

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Abstract

Investigations regarding alternative methods for producing polymeric materials with hydrophilic properties have increased considerably. In this context, polymeric biomaterials with hemocompatible surface properties have been successfully obtained by grafting hydrophilic monomers onto commercial polymer films by simultaneous irradiation processes. In this study, simultaneous irradiation and grafting were used to produce a copolymer PVC-co-DMAEMA-co-heparin with hemocompatible surface properties. Characterization by FTIR of the graft copolymer indicates that the increase in monomer grafting levels inhibits the bonding sites to heparin. FTIR-PAS analyses of the graft copolymers showed that the highest graft levels were obtained for the irradiated samples containing 45% of monomer. Heparin, however,

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could only be detected in the irradiated samples containing 30% of DMAEMA. The analysis of the micrographs, on the other hand, showed that increasing the monomer concentration enhances surface roughness of the graft copolymers. Roughness however decreased with heparin addition. It was possible to verify that an excess of surface roughness of the graft copolymers inhibits anticoagulant properties of heparin, triggering thrombus formation. Platelet adhesion, on its turn, was not significantly affected by the presence of heparin when PVC-co-DMAEMA and PVC-co-DMAEMA-co-heparin, obtained from the systems containing 45% of monomer, are compared. The addition of heparin in the systems containing 30% of DMAEMA resulted in fewer thrombogenic surfaces.

Keywords
Polyvinylchloride, gamma irradiation, platelet adhesion, blood compatibility, copolymer

Introduction

Investigations regarding alternative methods for producing polymeric materials with hydrophilic properties have increased during the last few years. These properties confer improved smoothness and lubricity to the surfaces of medical devices such as catheters, drainage, and feeding tubes and thereby reduce physical trauma and possible infections in patients.[1–3]

In this context, many polymeric materials are being studied such as PVC (polyvinyl chloride), PP (polyethylene), and polyester. These materials have been extensively used for biomedical applications, mainly after introducing functional groups or after performing graphitization processes.[4–6]

Introducing specific functional groups, such as SO_3, C=O, and COOH into the polymer matrix modifies its physical, chemical properties, and surface properties by increasing hydrophilicity. Mechanical and thermal properties are also affected.[5–8]

The success of many intra-arterial medical devices depends on the biocompatibility of the blood in contact with surfaces.[2,9] Interaction between artificial surfaces and blood often results in protein deposition, platelet adhesion, and activation and deactivation of the intrinsic coagulation pathway.[10–12] Therefore, important investigations have been carried out to develop thromboresistant polymers for short duration implants (72 h or less).[13]

Hemocompatibility of polymer surfaces can be improved by immobilizing substances on the polymer surface such as heparin, endothelial cells and albumin, or by modifying the polymer structure by means of hydrophilic monomer grafting.[2,13,14]

Polymer biomaterials with blood compatible surface properties have been synthesized by grafting hydrophilic monomers onto commercial polymers by means of ionizing radiation using, for example, UV, gamma-ray, and laser irradiation. This technique is a simple, easily controlled, and reproducible process.[15–17] Ionizing radiation induced grafting is potentially an alternative method for polymer surface modification.[18]

Grafted copolymers may be obtained through three main techniques: pre-irradiation, where the polymer matrix is activated and subsequently immersed in the monomer solution; peroxidation, where the polymer matrix is irradiated in the presence of oxygen and thereby forms hydroperoxides which decompose after exposure to room temperature; and simultaneous irradiation, where the polymer matrix is immersed in the monomer solution and both are irradiated.[19,20]

In this work, a technique of simultaneous irradiation was used to obtain a graft copolymer PVC-co-DMAEMA-co-heparin with hemocompatible surface properties.

Experimental

Materials

Commercial plasticized PVC from OPP-Triken S/A was used to prepare thin films. *N,N*-dimethylaminoethyl methacrylate was obtained

from Fluka Chemika. Tetrahydrofuran was purchased from Merck S/A. Heparin sodium salt from porcine intestinal mucosa low molecular weight was acquired from Fluka Chemika.

Preparation of PVC thin films

All PVC films were prepared by slowly evaporating a 5% tetrahydrofuran (THF) polymer solution, so as to obtain uniform films with no pressure and heat effects. The polymer solution was prepared by weighing PVC powder samples in an AL 500 Marte analytical balance. It was transferred to a 250-mL volumetric flask with emery polished stopper and afterwards THF with 99.9% purity was added in a sufficient amount to yield a 5% solution.[21]

Complete dissolution of the polymer was accomplished at 85°C (±1°C) under continuous stirring for 4 h. The solution then remained at rest for 1 h to eliminate bubbles. Subsequently, the solution was cast on Petri dishes placed in a previously leveled dry chamber. The air inside the chamber was displaced by a continuous flow of dry nitrogen from White Martins and the sides of the chamber were sealed off with adhesive tape. Complete drying of the films occurred in 15 days.

Afterwards, the films were cut in strips of 7 mm × 30 mm × 0.07 mm, washed with neutral soap in running water and rinsed with distilled water. Next, the films were dipped in methanol and vacuum dried using 10^{-3} mmHg for 5 h to remove possible solvent residues.

Samples preparation

To investigate the effect of heparin on the properties of the graft copolymers, PVC-co-DMAEMA, and PVC-co-DMAEMA-co-heparin, two sample series were prepared; one in the absence of heparin and the other in the presence of heparin.

For this, to make the materials in the absence of heparin, aqueous isopropanol solutions ($0.02\,mol{\cdot}L^{-1}$) were prepared and a sufficient amount of DMAEMA was added to obtain solutions containing 30% and 45% of monomer.[21,22]

Of these solutions, 7 mL of aliquots were transferred to Pyrex glass sample holders. PVC films were prepared and immersed in these solutions which were burbled with nitrogen gas for 5 min. Next, the sample holders were closed and remained still for 24 h before irradiation to promote diffusion of the monomer into the polymer matrix.[23] To prepare the material in the presence of heparin, the solutions were made using the same methodology, adding 0.25% of sodium heparin.

Irradiation of the samples

All samples were irradiated with gamma rays from a ^{60}Co panoramic source from Yoshizawa Kiko Co. Ltd at a dose rate (DR) from 0.5 to 0.67 kGy h^{-1}. The samples used for characterizing PVC, DMAEMA and heparin by infrared spectroscopy were irradiated with doses between zero and 16 kGy.

Doses of 2.5 and 5.0 kGy were used to irradiate samples in the systems containing 45% and 30% DMAEMA, in the presence and in the absence of heparin, respectively. These values were predetermined experimentally and reflect the highest grafting levels obtained under these conditions. Afterwards, the irradiation samples were left to rest for 24 h to obtain thermodynamic equilibrium.

Extraction

After irradiation, the grafted films were removed from the sample holders and washed with running water until the surface-adhered homopolymer was removed. The films were then washed with neutral soap and rinsed in running water and distilled water. Next, the samples were placed in a thermostatic bath at room temperature with continuous stirring for 24 h using distilled water to extract residual homopolymer and monomer. Afterwards, the extraction samples were rinsed in methanol for 5 (five) seconds and vacuum dried using 10^{-3} mmHg for 5 h. The samples were then placed in a vacuum desiccator to avoid moisture uptake prior to analysis.[24]

Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopy allows identifying alterations in specific functional groups resulting from the chemical reactions involved in the grafting and heparinization of the PVC films.

Preliminary investigations of the samples by transmission mode FTIR (KBr pellets), attenuated total reflectance (ATR), diffuse reflectance infrared fourier transform (DRIFT), FTIR microscopy and photoacoustic spectroscopy (PAS) were carried out and the best results were obtained by ATR and PAS. In this work, only the results obtained by ATR and PAS are presented. All the constituents of the grafting system and graft copolymers were analyzed by FTIR, which is a comparative technique.

The analysis of the samples by attenuated total reflectance (ATR) used a Perkin-Elmer 2000 spectrophotometer between 4,000 and 500 cm^{-1}, with Gain B; resolution of 4 cm^{-1}, and 80 scans. The samples were placed only on one side of the salt plate.

However, for the analyses by photoacoustic spectroscopy (PAS), we used a BOMEM spectrophotometer with flow of helium gas at a rate of 0.1 cm/s, with a resolution of 8 cm−1 and 30 scans to analyze the samples, with the exception of PVC-co-DMAEMA (45%) and PVC-co-DMAEMA-co-Heparin (45%), which presented better definition of important bands when a rate of 0.05 cm/s was used.

Scanning Electron Microscopy (SEM)

SEM was used to assess surface alterations of the sample caused by gamma radiation to induce grafting and heparinization, as well as platelet adhesion on the modified surfaces. The analyses were carried out in a JSM-T300 JEOL SEM with EDS (Energy Dispersive Spectroscopy).

To analyze the surface morphology, commercial PVC films (80 mg), PVC-co-DMAEMA 30% and 45%, and PVC-co-DMAEMA-co-heparin were used. For the SEM-EDS tests, the samples were irradiated with doses of 1.5, 2.5, 5.0 kGy, using 0.53 kGy h^{-1} dose rate.

To assess platelet adhesion on the polymer surfaces, non-irradiated PVC films and heparinized and non-heparinized graft copolymer films were cut to 5 mm × 5 mm and fixed with double-sided tape to glass slides, which were placed on a 50 mm Petri dish.

This material was placed on a larger Petri dish lined with cotton moistened with distilled water and kept at 37°C for 30 min. Meanwhile, 10 mL of fresh human blood was placed into a test tube containing a freshly prepared ACD solution (anticoagulant) at a proportion of 1 mL of blood to 0.25 mL of ACD. The glass slides, after removal from the oven, were covered with the blood-ACD mixture and placed back into the oven at 37°C for an additional 10 min.

Next, the slides containing the samples were carefully washed with a salt solution (0.2 $mol \cdot L^{-1}$) and immersed in glutaraldehyde (2.5%) for 10 min, at room temperature, to adhere the platelets to the surface. Afterwards, the samples were dehydrated with ethanol at the following concentrations: 50%, 75%, and 95% for 5, 10, and 15 min, respectively.[28]

Results and discussion

Infrared spectroscopy allowed characterization of the graft copolymers from alterations in characteristic absorptions of the functional groups present in the polymer, monomer, and heparin. Each constituent of the mixture as well as the graft copolymers were analyzed in order to assess the effects of irradiation in the specific functional groups based on the alterations observed in the infrared spectra.

Alterations in surface roughness and platelet adhesion were assessed by scanning electron microscopy to verify the effects of grafting and heparinization in the polymer matrix.

Characterization of PVC

A comparison between the Fourier Transform Transmission Spectra (FTIR) of the non-irradiated and irradiated PVC films, not submitted to plasticizer extraction (Figure 1(a)) shows an absence of bands above 3,000 cm^{-1} and at

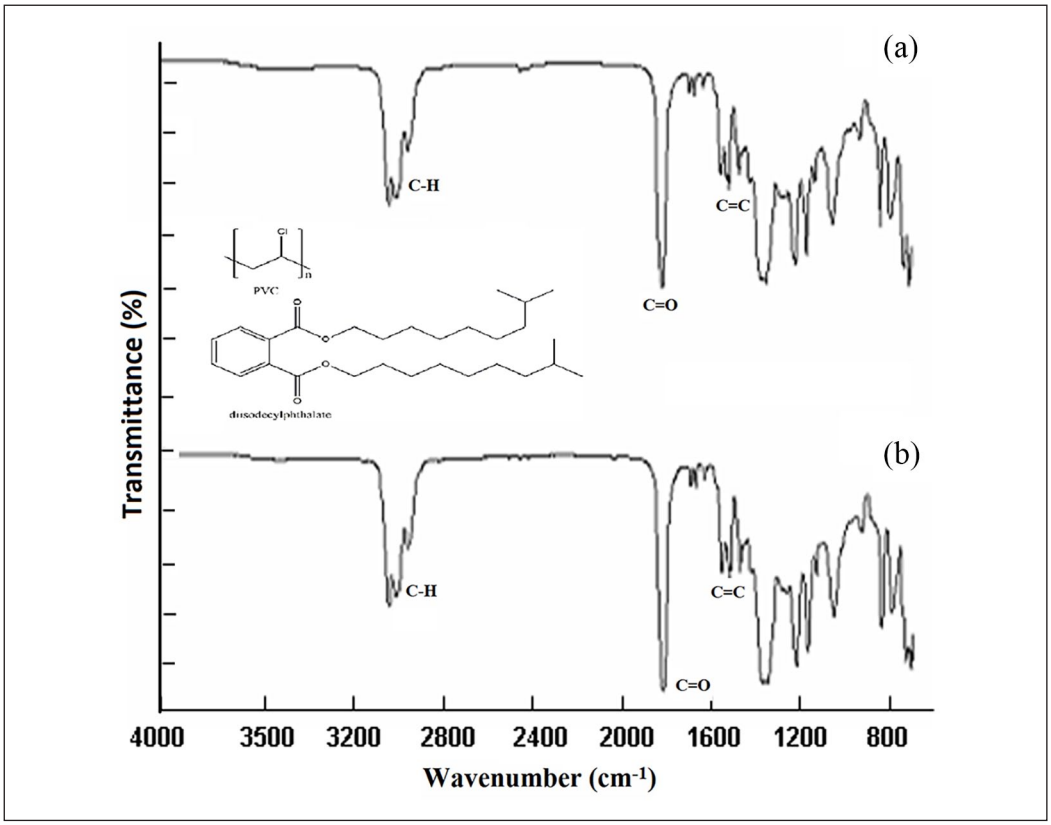

Figure 1. FTIR spectra: (a) non-irradiated PVC film and (b) irradiated PVC film (16 KGy).

1650 cm^{-1}, indicating an absence of unsaturation in the polymer chain as well as N-H and O-H bonds. The absorption bands observed at 1,539 cm^{-1}, 1,579 cm^{-1}, and 1,600 cm^{-1} were attributed to the aromatic C=C stretch of the phthalic ester plasticizer diisodecylphthalate. The presence of this plasticizer is also confirmed by the 1,731 cm^{-1} absorption band, characteristic of the C=O stretch of the carbonyl ester.[28]

The FTIR spectrum of the irradiated PVC film (Figure 1(b)) showed no significant alteration in the absorption bands. However, investigations have shown that irradiation of PVC leads to dehydrocloration reactions with release of hydrogen chloride and an increase in conjugated unsaturations.[25,26,28] The latter would lead to the appearance of an absorption band in the region between 1,680 cm^{-1} and 1,620 cm^{-1}. This, however, was not observed, despite the yellowish color presented by the polymer after irradiation, typical of increases in conjugated unsaturations.

These observations indicate good radiolitic protection of the polymer considering doses up to 16 kGy, likely due to the presence of additives and plasticizers. To improve the evaluation of the characteristic bands of PVC, without the interference of plasticizers and other additives, an extraction was carried out with ether.[27]

A comparison with spectra obtained in the literature of several phthalic esters showed that the absorption bands agree with those of the plasticizer diisodecylphthalate. This is an attribution of the main characteristic absorption bands of the PVC sample.[25,27]

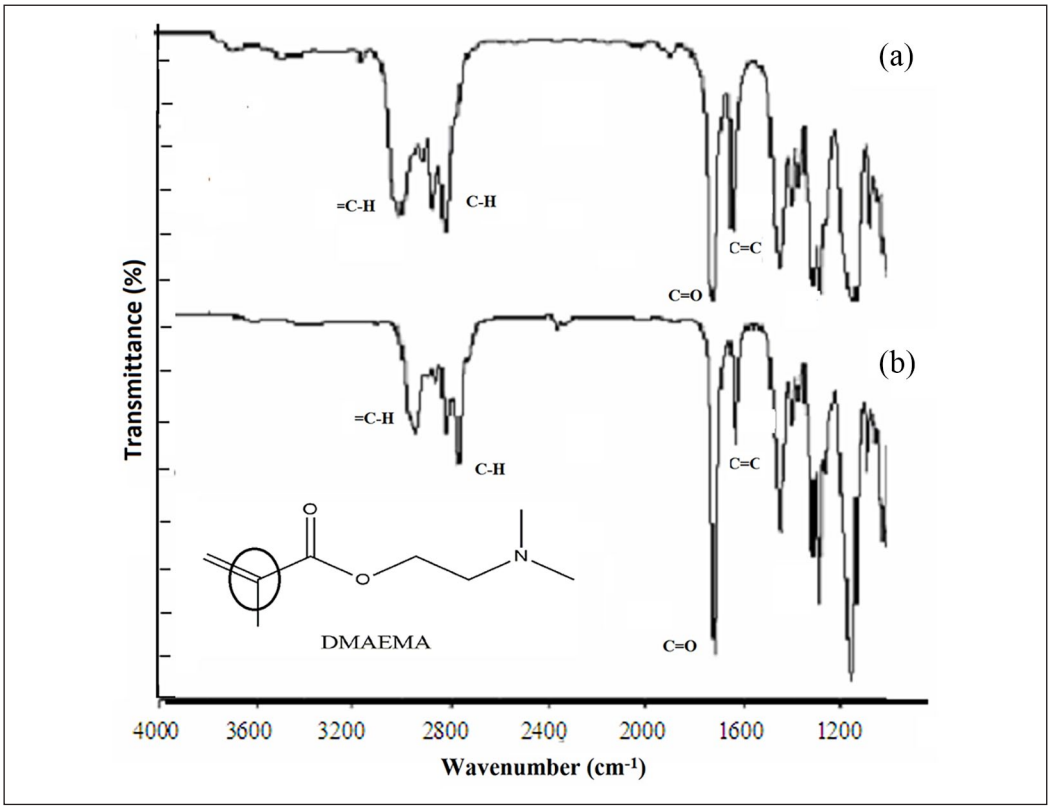

Figure 2. FTIR spectra: (a) non-irradiated DMAEMA and (b) irradiated DMAEMA.

Characterization of DMAEMA

By comparing the FTIR spectra of *N,N*-dimethylaminoethyl methacrylate (DMAEMA) Non-irradiated (Figure 2(a)) and irradiated (Figure 2(b)), it was possible to observe a reduction in band intensity at 1,638 cm^{-1} in the irradiated DMAEMA, which may be attributed to the addition of radicalar hydrogen to the alpha carbon insaturation, generating a tertiary radical, responsible for the graft and homopolymerization reactions.[28]

Characterization of heparin

A comparison between the FTIR-PAS spectra of the heparin samples (Figure 3) shows a variation in the region between 750 cm^{-1} and 800 cm^{-1}, characteristic of the S=O stretch modes and in the regions between 1,100 cm^{-1} and 1,230 cm^{-1}, characteristic of the C-N, C-O, and HSO_3^- stretch modes.[28,29] This variation can indicate the occurrence of desulfonation.

Heparin's anticoagulant properties were attributed to the sulfate groups in their chemical structures.[30] Bisio et al.[11] observed that the gamma-irradiated low-molecular-weight heparin aqueous solutions of doses up to 5.6 kGy did not present reduction in biologic activity of heparin as anti-thrombin (AT) and anti-factor Xa activities, despite desulfonation of the chemical structure of heparin.[1,31] Chawla et al.[23,24] observed a reduction in anticoagulant activity of $<10\%$ when aqueous sodium heparin solutions were irradiated, despite the reduction observed in the molecular weight of heparin.

These observations are in agreement with the hypothesis that gamma radiation does not

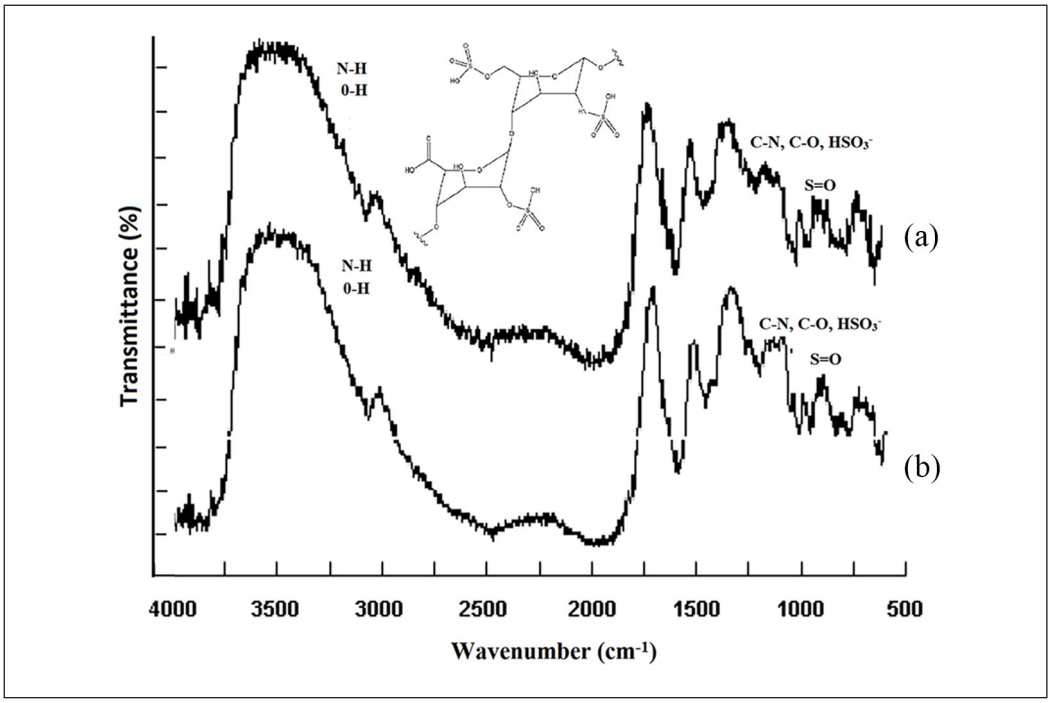

Figure 3. FTIR-PAS spectra of heparin: (a) non-irradiated sample and (b) irradiated sample (7.3 kGy).

promote random chain scission in heparin macromolecules, but rather affects low sulfated sites generating shorter sequences which do not contain binding sites with AT. These regions account for heparin's anticoagulant characteristics.[1,32]

Characterization of the graft copolymers

In an attempt to remove the plasticizer and improve visualization of absorption bands of PVC, monomer and heparin, the plasticizer was extracted in ether from the grafted and heparinized PVC films. The PAS-FTIR technique allows a variation of analysis conditions, which was used to analyze the samples.

The absorptions observed are basically from PVC and an ester compound. The band in the 1,150 cm^{-1} region was attributed to the DMAEMA monomer. According to Urbanski et al.,[33] this is one of the characteristic absorptions of methacrylates.

The presence of bands at approximately 3,300 cm^{-1} and 1,600 cm^{-1} reveals the possibility to form OH and/or NH groups. This suggests that the incorporation of DMAEMA might be associated with a reaction or interaction between groups from PVC and DMAEMA, since not all DMAEMA bands can be observed and the spectra of the separate samples of PVC and DMAEMA present differences in some regions.

Since the absorption bands of the graft copolymers were observed under different analysis conditions, this suggests that the incorporation of DMAEMA into the samples occurred on different depth levels.

Compared to transmission techniques, which did not show satisfactory results and therefore were not presented, FTIR-PAS spectra measured with deep sampling (micrometers and 100s of micrometers) show that DMAEMA or

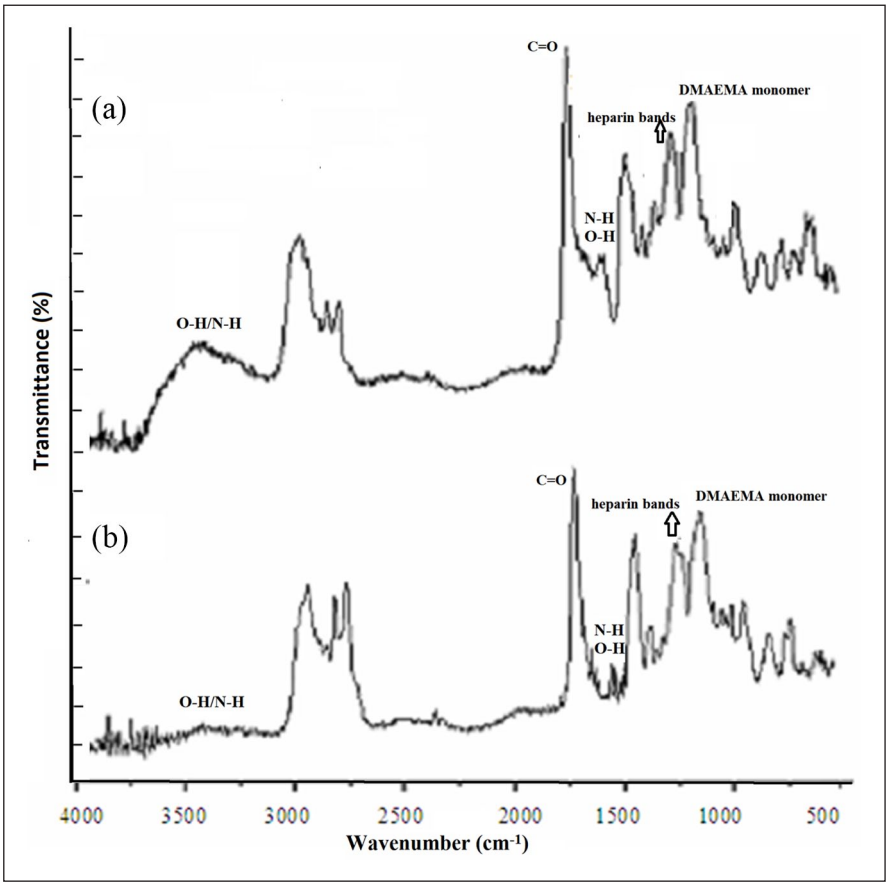

Figure 4. FTIR-PAS spectra: (a) PVC-co-DMAEMA-co-heparin (30%) and (b) PVC-co-DMAEMA-co-heparin (45%).

its reaction derivative is located closer to the surface of the sample.

A comparison of the FTIR-PAS spectra of the PVC-co-DMAEMA-co-heparin films (Figure 4(a) and (b)) shows that the characteristic heparin bands at 1,230 cm^{-1} and 796 cm^{-1} due to S=O vibrations are not very clear. This probably happens due to overlapping C-O and C-N bands of the monomer and C-Cl bands of PVC.[28,33,34]

However, an intensification of the band near 1,600 cm^{-1} and widening of the band near 800 cm^{-1} indicate a modification of the investigated material in relation to the samples with no heparin. A comparison of the PVC-co-DMAEMA spectra clearly shows characteristic plasticizer absorption bands, indicating lower degree of grafting for this sample.

Morphology characterization by Scanning Electron Microscopy (SEM)

To verify alterations in surface topography of each sample and to assess antithrombogenic characteristics, SEM analyses were performed and the results are presented next.

SEM is a technique that allows assessing morphological modifications of the sample by irradiating it with electrons. The absorbed electrons provide a signal which in many cases is the exact complement of the reflected electrons at the sample thickness ratio. This provides information on the chemical composition of the surface of the sample.

The photomicrographs obtained by SEM were used to study the alterations in surface texture of

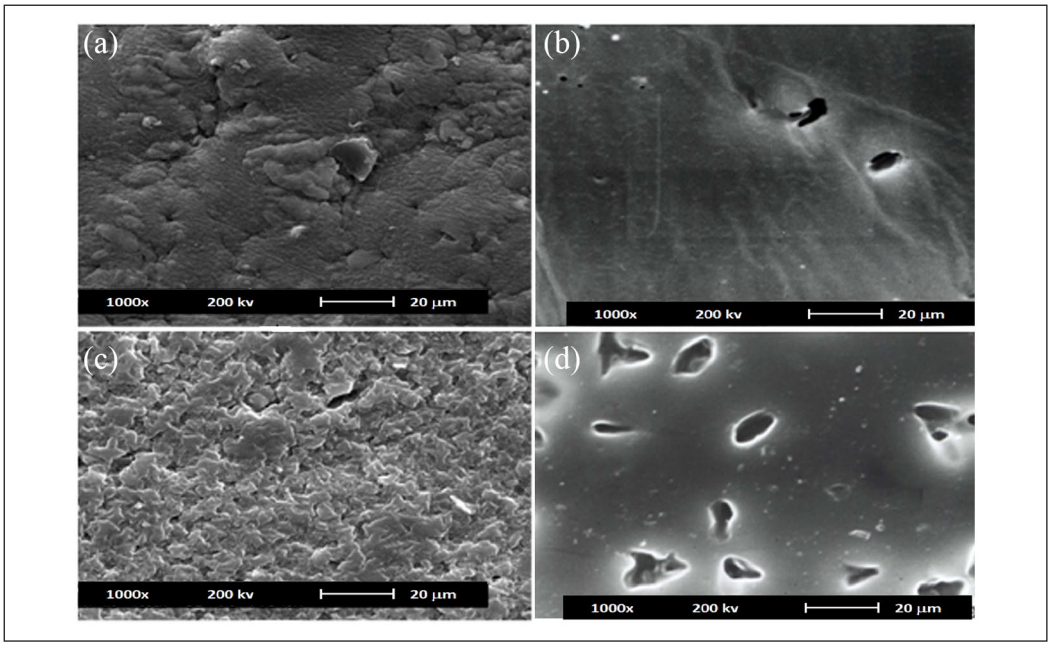

Figure 5. SEM photomicrographs of the graft copolymers: (a) PVC-co-DMAEMA (dose: 5.0 KGy; DMAEMA 30%), (b) PVC-co-DMAEMA-co-heparin (dose: 5.0 KGy; DMAEMA 30%; heparin 0.25%), (c) PVC-co-DMAEMA (dose: 2.5 KGy; DMAEMA 45%), and (d) PVC-co-DMAEMA-co-heparin (dose: 2.5 KGy; DMAEMA 45%; heparin 0.25%); Magnification of 1000×.

the samples after grafting. A comparison of the photomicrographs of the non-heparinized graft copolymers (Figure 5(a) and (c)) and of the heparinized graft copolymers (Figure 5(b) and (d)) shows significant changes on the surface with samples with 30% DMAEMA, showing more homogeneous surfaces than those containing 45% DMAEMA (Figure 5(b)).

A material is considered non-thrombogenic when it manages to control the mechanism that triggers coagulation, hence avoiding thrombus formation.[35,36] Non-thrombogenic materials have an inert surface when in contact with physiological fluids of the blood and do not trigger the mechanism of thrombus formation at the artificial surface interface in the blood. One of the tests carried out to evaluate non-thrombogenic properties of biomaterials is the platelet adhesion test.

A comparison of the SEM photomicrographs of PVC (Figure 6(a)) and the graft copolymers (Figure 6(b) and (c)) shows that the increase in grafting levels did not render the surface non-thrombogenic, which is in agreement with the observations by Otsuhata et al. (1985).[3] Since surface change increases with increasing grafting levels, this effect can be related to the catalysis where the enzymatic reactions take place, culminating in the generation of thrombin and fibrin formation, which is the main constituent of the thrombus.[3,37]

In the absence of heparin, both the PVC film (Figure 6(a)) and the graft copolymers (Figure 6(b) and (c)) presented ruptured platelets adhered to the surface. Figure 6(a) to (c) show platelet adhesion, demonstrating a thrombogenic characteristic of the surface. Platelet adhesion increases with increasing DMAEMA concentration.

The addition of heparin to the system led to a more homogeneous distribution of the grafted chains, culminating in a smoother surface (Figure 7(a) and (b)). As a consequence, weakly adhered platelets are seen on the polymer's

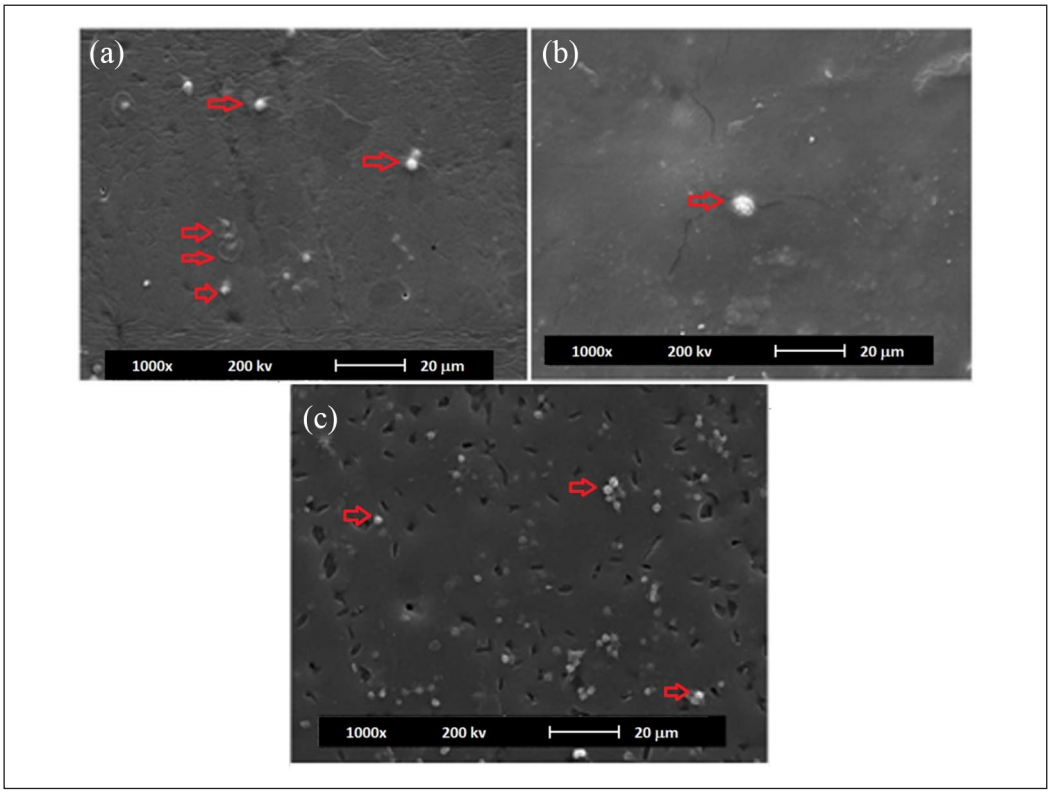

Figure 6. Comparison of the SEM photomicrographs of the non-heparinized polymer surfaces, after complete contact with blood: (a) PVC, (b) PVC-co-DMAEMA (30%), and (c) PVC-co-DMAEMA (45%).

surface. Assays were carried out in duplicate and photographs of at least four different regions of the sample were taken, showing no thrombus at all. These results confirm that irradiation of aqueous heparin solutions does not significantly inhibit its anticoagulant properties. Figure 7(a) shows the presence of weakly adhered platelets as a consequence of a non-thrombogenic surface. Conversely, Figure 7(b), which reveals an excess of DMAEMA, presents platelets adhered with the formation of thrombi.

Therefore, the less thrombogenic characteristics of the obtained graft copolymers justify simultaneous irradiation in aqueous medium as an alternative method for obtaining polymeric materials with non-thrombogenic properties. Its use as a biomaterial, however, requires complementary investigations, which may give birth to a novel segment within the area of biotechnology and polymer biomaterials.

Conclusions

FTIR-PAS spectra measured with deep sampling (micrometers and 100s of micrometers) allow to conclude that DMAEMA or its reaction derivative is located close to the surface of the sample. Characterization of the graft copolymer by FTIR indicates that the increase in monomer grafting levels inhibits the bonding sites to heparin.

The addition of heparin to the system led to smoother and less thrombogenic surfaces. An excess of surface roughness of the graft copolymers inhibits anticoagulant properties of heparin, triggering thrombus formation.

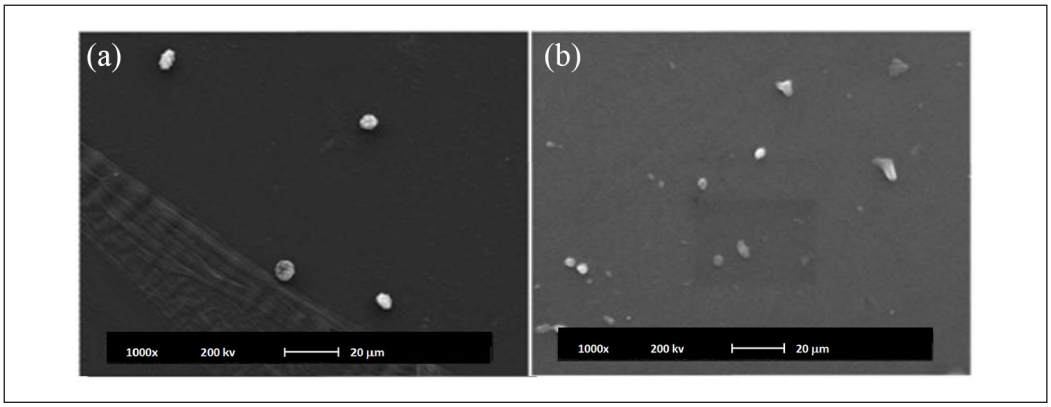

Figure 7. Comparison of the SEM photomicrographs of the heparinized graft copolymers, after contact with blood: (a) PVC-co-DMAEMA-co-heparin (30%) and (b) PVC-co-DMAEMA-co-heparin (45%).

Grafting with 30% DMAEMA and 0.25% heparin leads to smoother surfaces and despite the lower grafting levels, it was possible to observe less platelet adhesion and no thrombus formation. Gamma irradiation induced simultaneous grafting and heparinization of PVC is an efficient method for obtaining fewer thrombogenic surfaces by means of covalent bonding.

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