Radiation Physics and Chemistry 97 (2014) 298–303

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Radiation-grafting of thermo- and pH-responsive poly (N-vinylcaprolactam-co-acrylic acid) onto silicone rubber and polypropylene films for biomedical purposes

Caroline C. Ferraz [a], Gustavo H.C. Varca [a], Juan-Carlos Ruiz [b], Patricia S. Lopes [c], Monica B. Mathor [a], Ademar B. Lugão [a], Emilio Bucio [b,*]

[a] Instituto de Pesquisas Energéticas e Nucleares – IPEN/CNEN-SP, Av. Prof. Lineu Prestes, 2242, Cidade Universitaria, 05508-000 São Paulo, Brazil
[b] Departamento de Química de Radiaciones y Radioquímica, Instituto de Ciencias Nucleares, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, México DF 04510, Mexico
[c] Universida de Federal de São Paulo – UNIFESP, Rua São Nicolau, 210, Centro, 09913-030 Diadema, SP, Brazil

HIGHLIGHTS

- Graft copolymers synthesized by simultaneous polymerization and grafting under distinct conditions: irradiation and grafting reactions.
- Simultaneous grafting of NVCL-co-AAc from PP and SR achieved via a γ-ray pre-irradiation method.
- Grafted NVCL-co-AAc onto PP or SR substrates led to thermo and pH stimuli responsive properties.

ARTICLE INFO

Article history:
Received 4 September 2013
Accepted 20 December 2013
Available online 30 December 2013

Keywords:
Radiation-grafting
Stimuli responsive film
Acrylic acid
N-vinylcaprolactam
Cytotoxicity
Fibroblast BALB/c 3T3

ABSTRACT

This work focuses on the effects of gamma-ray irradiation conditions on the stimuli-responsiveness of polypropylene (PP) films and silicone (SR) rubber substrates grafted with N-vinylcaprolactam (NVCL) and acrylic acid (AAc). PP films and SR rubber were modified by simultaneous polymerization and grafting of NVCL and AAc, using pre-irradiation oxidative method at a dose rate of 12.23 kGy h^{-1} and doses ranging from 5 to 70 kGy. NVCL and AAc solutions (1/1, v/v) at 50% monomer concentration (v/v) in toluene were added to the sample substrates, degassed, sealed and heated at 60 and 70 °C for 12 h. After grafting, the samples were soaked in ethanol and distilled water for 24 h successively, followed by drying under vacuum. Samples were characterized by FTIR-ATR, DSC and swelling measurements. Critical points (pH critical or LCST) of grafts were obtained in a pH-environment (pH ranges from 2.2 to 9) and in a thermo-environment (temperature ranges from 22 to 50 °C). Cytotoxicity evaluation was performed using fibroblast BALB/c 3T3 cells. The relationship between NVCL-co-AAc grafting and radiation dose was different for each substrate, PP and SR. At 50% NVCL/AAc concentration in toluene, grafting values were higher for SR than for PP. Despite the fact that PP-g-(NVCL-co-AAc) membrane presented a cytotoxic profile at the highest experimental concentration assayed, cytotoxicity evaluation revealed noncytotoxic profiles for the membranes synthesized highlighting their applications for biomedical purposes.

1. Introduction

In recent years there has been remarkable growth in the research and development of synthetic polymers for biomedical, microelectronics, and other advanced technological applications (Rosiak et al., 2003). In this context radiation processing technology represents a useful tool for the modification of polymeric materials including grafting of monomers onto polymers (Bucio and Burillo, 2007; Ferreira et al., 2012; Alvarez-Lorenzo et al., 2010; Grasselli et al., 2003). Radiation grafting specifically allows the modification of the surface composition of polymeric biomaterials without changing their mechanical properties.

Apart from those properties, radiation processing holds advantages over conventional methods including no need for catalysts or additives and possibility of simultaneous sterilization (ISO/TS 13409, 1996). Grafting techniques normally include the pre-irradiation as well as the mutual or simultaneous method, the energy source being gamma rays, UV or electrons (Bucio and Burillo, 2009; Bettini et al., 2013; Song et al., 2012).

Radiation-induced copolymerization and crosslinking of copolymers have been increasingly used for creation of novel biomaterials (Estrada-Villegas and Bucio, 2012). Polymers are an important class of

* Corresponding author. Tel.: +52 55 56224674.
E-mail address: ebucio@nucleares.unam.mx (E. Bucio).

0969-806X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.radphyschem.2013.12.027

materials for pharmaceutical and biotechnological applications. There are many examples of how and where to take advantage of macromolecules from controlled drug delivery to carriers for cell and tissue transplantation (Contreras-García et al., 2008). Enhanced biocompatibility and prophylaxis against infections are the most pursued aims of altering the surface structure/composition (Anderson et al., 2008; Subramanyam and Kennedy, 2009).

Radiation synthesis and fabrication techniques gear up the research on bio-medical applications in the early 1960s and at present are in full swing. Charlesby and Chapiro (Charlesby, 1960; Chapiro, 1962) are the pioneers of this area. In this particular field, bio-compatibility is the most essential aspect to be considered regarding its importance for bio-medical materials due to their direct contact with biological components such as blood and tissues (Bhattacharya, 2000).

The number of applications using polypropylene (PP) is increasing due to its low elastic modulus, superior biocompatibility and enhanced corrosion resistance when compared to materials that are mostly used. It is also frequently used due to its antimicrobial properties and resistance to environmental changes such as pH and temperature (Morales-Wiemer et al., 2013).

Acrylic acid (AAc) is one of the most popular monomers that has been grafted onto different polymeric matrices. Its derived polymers or copolymers with pH sensitive response attributed to carboxylic groups from poly(acrylic acid) (PAAc) have ability to undergo further chemical reaction in order to acquire or generate new functional groups (Lazo and Burillo, 2010).

Recent studies have incorporated different monomers such as N-vinylpyrrolidone (NVP) and amino acid derivatives as spacers between the carboxylic acid groups (Arlyapov et al., 2013). This modification increased the polymeric backbone flexibility and allowed greater access for acid/base reactions. Water-soluble polymers with lower critical solution temperature (LCST) have attracted a great deal of attention in recent years, due to their potential applications in biomedicine and biotechnology. Among them, poly(N-vinylcaprolactam) (PNVCL) stands out based on the fact that it is not only nonionic, water-soluble, nontoxic and thermo-sensitive but also biocompatible. Moreover, the LCST of PNVCL (32–34 °C) is near the range of physiological temperature (Cheng et al., 2002). Temperature- and pH-responsive delivery systems have drawn much attention because some diseases induce changes in body temperature and/or pH (Zhang and Wu, 2004). Thus a relevant application is directed towards their use as delivery devices based on a novel, squeezing concept, utilizing specific swelling–deswelling characteristics of temperature or temperature/pH (Gotowska et al., 1997).

In this context the advantage of the binary graft of NVCL-co-AAc in a two-step method is related to the formation of branches of the NVCL in the grafted chains of AAc; as reported by Yoshida et al. (1995) such systems give fast response to temperature or pH changes related to the grafted ordinary copolymer.

In this paper γ-ray modifications of PP or SR with the NVCL thermo-responsive or pH sensitive of AAc were studied, as well as the optimum conditions for grafting and final properties of the membrane. In a more specific way this work aimed to γ-ray graft temperature- and pH-responsive copolymers systems containing NVCL-co-AAc onto PP or SR, commercially used for the manufacture of implantable devices, in order to endow the surface with antibiofouling features while attempting to maintain their mechanical and biocompatibility properties. The final purpose is to develop a specific material capable of avoiding the risk of bacterial contamination upon medical insertion while maintaining the original mechanical and biocompatibility properties. Both PP and SR are widely used as components of medical devices and are frequently colonized by microorganisms (Gottenbos et al., 2002; De Prijck et al., 2010; Engelsman et al., 2010; Nava-Ortiz et al., 2010). Cytocompatibility tests were also carried out in order to provide an overview of the potential of PP-g-(NVCL-co-AAc) and SR-g-(NVCL-co-AAc) as components of medical devices.

2. Materials and methods

2.1. Materials

Polypropylene films and silicone rubber (1 mm in thickness) were purchased from Goodfellow (Huntingdon, UK); N-vinyl-caprolactam and acrylic acids were acquired from Sigma-Aldrich Co. (St. Louis, MO, USA). Toluene and ethanol (Analytical grade) were acquired from Mallinckrodt Baker (Ecatepec de Morelos, Mexico). BALB/c 3T3 were acquired from ATCC CCL 163 (USA); Dulbecco's Modified Eagle Medium (DMEM, no. 11965-092), Donor Bovine Serum (no. 11765-054), L-glutamine (no. 25030-122), penicillin, streptomycin (no. 15140-122) and amphotericin (no. 10452-075) were acquired from GIBCO (USA); Tryspsin (no. T 4799/ E6758) was purchased from Sigma-Aldrich (Brazil); 3-(4, 5-Dimethyl-2-thiazolyl)-2 and 5-diphenyl-2H-tetrazolium bromide (MTS) were purchased from Promega Corporation, cat. Cell Titer 96® (Brazil).

2.2. Grafting

PP films and SR rubber were weighed and placed into glass ampoules and exposed to ^{60}Co γ-source (Gammabeam 651 PT, MDS Nordion) in the presence of air at room temperature at a dose rate of 12.23 kGy h^{-1} and a dose between 5 and 70 kGy. Chemical dosimetry was performed by the modified Fricke method prior to samples' irradiation. Samples were irradiated at a fixed position adjacent to the source. NVCL and AAc solutions (1/1, v/v) at 50% monomer concentration (v/v) in toluene were added to the samples. The ampoules were degassed by repeated freeze-thaw cycles (5 times per 20 min) and sealed. Afterwards, the ampoules were heated at 60 or 70 °C at a reaction time of 12 h. To extract the residual monomer and homopolymer formed during the grafting, the samples were soaked in ethanol for 24 h and then in distilled water, followed by drying under vacuum to constant weight. The grafting yield (Y_g) was calculated as follows:

$$Y_g(\%) = 100[(W_g - W_o)/W_o] \quad (1)$$

where W_o and W_g are weights of the initial and grafted films, respectively. PP film blanks were in contact with toluene at 70 °C for 12 h and changes neither in weight nor chemistry, followed by FTIR, were present.

2.3. Physicochemical properties

Samples were characterized according to their physicochemical properties by FTIR-ATR, and thermal profile by differential scanning calorimetry (DSC). Membrane properties were evaluated by means of water absorbency equilibrium, i.e. swelling equilibrium. The lower critical solution temperature and pH critical point were obtained by swelling measurements and DSC.

2.3.1. Infrared

FTIR-ATR spectra were taken using a Perkin-Elmer Spectrum 100 spectrometer (Perkin-Elmer Cetus Instruments, Norwalk, CT) with 16 scans.

2.3.2. Water absorbency equilibrium

Samples were immersed into distilled water from 15 to 240 min. The excess of solution on the copolymer films was

removed with a filter paper, and the swollen samples were weighed. The swelling ratio was determined as follows:

$$\text{Swelling}\ (\%) = [(W_s - W_d)/W_d] \times 100 \quad (2)$$

where W_s and W_d are weights of the swollen and initial films respectively.

2.3.3. Lower critical solution temperature (LCST)

The determination of thermodynamic transitions was performed by differential scanning calorimetry (DSC). Runs were recorded under nitrogen atmosphere using a DSC 2010 calorimeter (TA Instruments, USA) starting at room temperature. The LCST of the grafted films was determined by DSC from 21 to 50 °C at 1 °C min^{-1} with the swollen samples in distilled water. LCST was also calculated with swelling measurements in water at temperatures from 26 to 34 °C defined as the inflection point of the swelling vs. temperature plot (Boltzmann function fitting).

2.3.4. pH critical point

The pH critical point was determined from the swelling values of films placed for 2 h at 25 °C in buffer solutions of pH ranging from 2.2 to 9, prepared by mixing adequate volumes of boric acid (0.2 M) and citric acid (0.05 M) solution with trisodium phosphate dodecahydrate (0.1 M) solution. For example, mixtures of both solutions at 50:50 vol/vol ratio rendered a buffer system of pH 7.0 (Muñoz-Muñoz et al., 2012). The pH critical point was defined as the inflection point of the swelling vs. pH plot (Boltzmann function fitting).

2.3.5. Cytotoxicity evaluation

The tests were performed under aseptic conditions, in a controlled environment, using sterilized materials and reagents.

Membrane extraction. The membranes were sterilized by UV exposition for 20 min (10 min/side) using bactericidal wavelength (λ=200–280 nm) and kept in 3 mL extraction media – culture medium – for 72 h at 37 °C (ISO 10993/EN 30993 1992). After such period aliquots were taken in order to achieve eight dilutions of the extraction media with concentrations ranging from 100% to 0.78% (v/v) (C_I – 0.78; C_{II} – 1.56; C_{III} – 3.125; C_{IV} – 6.25; C_V – 12.5; C_{VI} – 25; C_{VII} – 50 and C_{VIII} – 100%). The dilution was performed using a culture medium.

Cytotoxicity assay. Cytotoxicity assays were performed using BALB/c 3T3 cells. The fibroblasts were keep in an incubator under controlled atmosphere at 37 °C, 95% relative humidity and 5% CO_2, in bottles (175 cm^2) using the DMEM medium containing 10% Donor Bovine Serum (v/v) supplemented with L-glutamine (4 mM L^{-1}), penicillin (100 UI mL^{-1})/streptomycin (100 μg mL^{-1}) and amphotericin (0.25 μg mL^{-1}). After reaching a subconfluent stage of 70–80%, the cells were submitted to trypsinization using trypsin solution (0.05/ EDTA 0.02% (w/v)) and then cultured overnight in a 96 well microplate containing 1.5×10^4 cells/well. The cells were then exposed to the samples for 24 h and quantified using a vital die – MTS/PMS. Absorbance was evaluated using a UV/vis microplate reader (Multiskan EX 355, Thermo Electron Corporation) at 490 nm. Control cells unexposed to extraction media, cultured and evaluated under the same conditions were taken as 100% cell viability.

3. Results and discussion

PP films and SR rubber were submitted to gamma irradiation in air at different pre-irradiation doses prior to grafting in order to create hydroperoxides and peroxides. The films were then placed into toluene solutions of NVCL-co-AAc and reacted under various conditions. The grafting efficiency was assessed gravimetrically after extensive washing and drying of reacted films. It is expressed by the grafting yield Y_g, as defined in the experimental section (Eq. (1)).

The effect of pre-irradiation dose on the grafting yield was examined by performing graft polymerizations for a reaction time of 12 h at 60 or 70 °C. The relationship between NVCL-co-AAc grafting and radiation dose was different for PP (Fig. 1) and SR (Fig. 2). For a fixed NVCL-co-AAc (1/1, v/v) at 50% monomer concentration in toluene, the values of grafting percentage reached higher levels for SR than for PP. For example, at 50 kGy (T=60 °C), grafting percentages for PP-g-NVCL-co-AAc (Fig. 1) and SR-g-NVCL-co-AAc (Fig. 2) corresponded to ≈6% and 21%, respectively; and at 30 kGy (T=70 °C), grafts yielded ≈15% on PP and 26% on SR. The higher grafting efficiency for SR than for PP was probably related to the ability of the pristine polymeric matrix to take up the solvent. PP and SR were able to swell in toluene up to 13% and 134% (w/w), respectively, making penetration of dissolved NVCL and AAc in the SR matrix easier than that in PP. Therefore, NVCL-co-AAc is more likely to reach more sites to react with and to grow from in the SR matrix, following similar grafting/polymerization mechanisms (Ruiz et al., 2007). The increase in grafting yield as a function of temperature for PP and SR samples pre-irradiated at doses from 5 to 70 kGy (Figs. 1 and 2) indicated a general tendency followed by an expected increase of efficacy for the grafting process when the temperature was increased from 60 to 70 °C. Gamma pre-irradiation performed in air-saturated films resulted in peroxidation of the amorphous domains, with

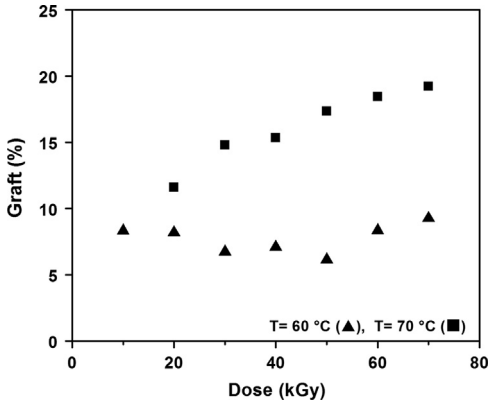

Fig. 1. Graft percentages of PP-g-(NVCL-co-AAc) as a function of absorbed dose (kGy) for two different temperatures: 60 and 70 °C. Constant conditions: NVCL/AAc concentration=1/1 (v/v), monomers/toluene concentration=1/1 (v/v), I=12.23 kGy h^{-1}, and reaction time=12 h.

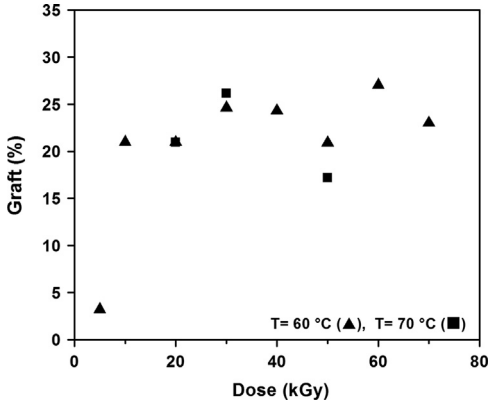

Fig. 2. Graft percentages of SR-g-(NVCL-co-AAc) as a function of absorbed dose (kGy) for two different temperatures: 60 and 70 °C. Constant conditions: NVCL/AAc concentration=1/1 (v/v), monomers/toluene concentration=1/1 (v/v), I=12.23 kGy h^{-1}, and reaction time=12 h.

restricted contribution of cross-linking or degradation (Ivanov, 1992). By comparing the reactions conducted at 60 and 70 °C, the higher levels of grafting yield observed at 70 °C might be explained by thermal activation of peroxyl groups with typical activation energy (Bach et al., 1996). Monomer diffusion into the amorphous regions of the material as well as into the peripheral zones of the crystallites is also enhanced at higher temperatures.

Notable differences in the infrared spectra of the original and modified PP and SR were recorded (Fig. 3). The characteristic bands of PP appeared at 840 and 1154 cm^{-1} due to –CH(CH_3)–C– at 2949–2838 cm^{-1} caused by CH_3, CH_2, and CH stretching, and at 1454–1375 cm^{-1} due to the CH_3 out of plane bending vibrations and the symmetric bending vibration of CH_2. While the SR rubber peaks of –Si–CH_3 are shown at 2963 and 1258 cm^{-1} which correspond to C–H in CH_3, 1006 cm^{-1} corresponds to stretching vibration of the Si–O–C bond. A distinctive band of AAc was 2659 cm^{-1} from overtones and combinations of the C–O stretch band at 1200–1315 cm^{-1} and from in-plane deformation of C–O–H at 1431–1294 cm^{-1}. NVCL monomer showed peaks at 2929 and 2858 cm^{-1} for C–H, CH_3 at 1360 cm^{-1}, CH_2 at 1470 cm^{-1}, and N–H at 1621 cm^{-1}; additional peaks of NVCL lactam ring were observed from 1421 to 1478 cm^{-1} characteristic signals for NVCL. The peak at 1715 cm^{-1} can be assigned to the stretching mode of carbonyl groups, as its presence in the spectra of PP-g-(NVCL-co-AAc) and SR-g-(NVCL-co-AAc) confirmed grafting of PAAc. The NH peak of PNVCL is clearly present in PP-g-(NVCL-co-AAc), confirming binary graft copolymerization, but not in SR-g-(NVCL-co-AAc). Silicon-based polymers present peaks of Si–O at 1006 cm^{-1} and of Si–CH_3 at 1258 cm^{-1}, which are very intense and any modification with NH or CHx groups is difficult to observe; this peak-intensity-comparison can be observed when plasma polymerized hexamethyldisiloxane is modified (Chifen et al., 2007; Grundmeier et al., 2003). The main difference of grafting in both substrates (PP and SR) is the grafting percentage, considering that both NVCL and AAc monomers follow same grafting/copolymerization mechanisms. Therefore the binary graft copolymers for SR-g-(NVCL-co-AAc) can be confirmed by swelling measurements in different environments.

The water uptake of PP-g-(NVCL-co-AAc) and SR-g-(NVCL-co-AAc) as a function of time, the swelling of selected samples at different absorbing times in distilled water, was quantified (Fig. 4). The swelling rate indicated that the initial swelling process was attributed primarily to the water penetrating into the copolymer film through capillary and diffusion. Then the penetrated water is absorbed by hydrophilic groups. The swelling is fast during the first 30 min and gradually slows down until the equilibrium swelling is reached at 120 min.

The typical swelling behavior of AAc grafted onto PP and SR films was determined at distinct pH values. As expected, the

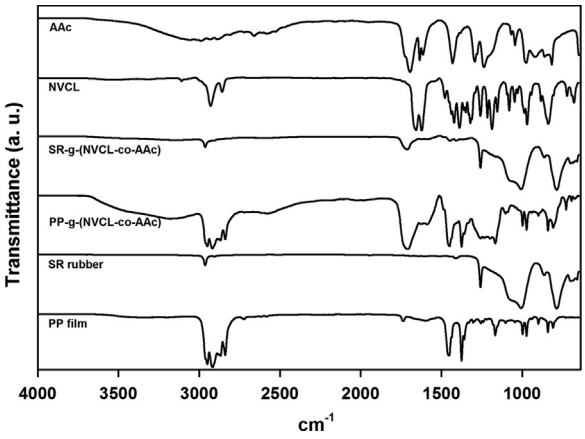

Fig. 3. FTIR-ATR spectra of PP, SR, AAc, NVCL, PP-g-(NVCL-co-AAc) (11% graft), and SR-g-(NVCL-co-AAc) (24% graft).

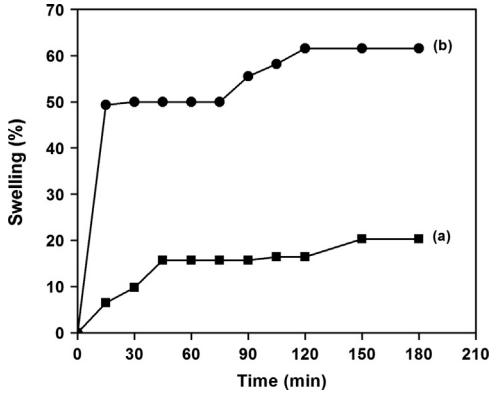

Fig. 4. Swelling, in water, as a function of time for 21% grafted on SR (a) and 42% grafted on PP (b) samples modified with NVCL-co-AAc.

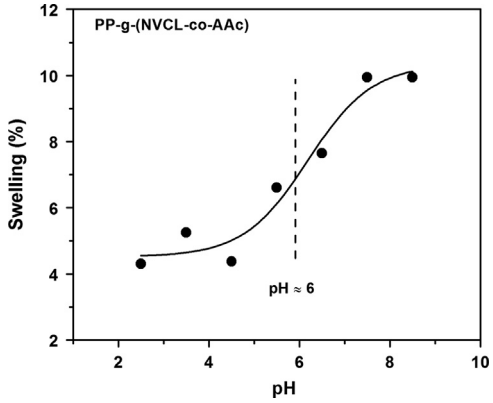

Fig. 5. 15% graft PP-g-(NVCL-co-AAc) swelling degree as a function of pH in buffer solutions at 25 °C. The dashed line indicates the pH critical point.

swelling ratios of PP-g-(NVCL-co-AAc) and SR-g-(NVCL-co-AAc) were significantly higher at pH above 6.5 (beyond the pH critical point of ≈ 6), where the hydrogen bond interactions between two AAc units and between AAc and NVCL were destroyed, and the system presents hydrophilic behavior if compared to the lower pH (below the pH critical point) with a hydrophobic behavior (Fig. 5).

Fig. 6 exhibits the DSC thermograms of the 8% grafted PP-g-(NVCL-co-AAc), which was swollen in distilled water and the determination was performed at 1 °C min^{-1}. The onset point of the endothermal peak, determined by the intersecting point of two tangent lines from the baseline and slope of the endothermal peak, was used to establish the LCST. PP-g-(NVCL-co-AAc) shows an LCST of ≈ 30.8 °C. The obtained LCST by DSC is similar to the one obtained by swelling measurements. Fig. 7 shows the SR-g-(NVCL-co-AAc) thermo-sensitivity through swelling measurements in water for 24% and 27% of grafting, presenting LCSTs of ≈ 30.5 °C. This LCST value is alike to LCST of PNVCL. This sensitivity of SR-based grafts confirms PNVCL presence.

In order to provide an approach into the biocompatibility of the films produced, fibroblast cells were exposed to membrane extraction media at distinct concentrations to evaluate the influence of polymer and possible residual solvents or monomers over cell growth. Both SR and PP (control) films lack effects over cell viability highlighting good cytocompatibility of the involved polymers (Fig. 8). SR-g-(NVCL-co-AAc) films did not lead to any significant changes with regard to the cytotoxicity profile, providing evidence that the grafting process and the monomer addition did not alter this characteristic in this case. However, regarding PP-g-(NVCL-co-AAc) films a distinct profile was observed. Specifically, a concentration dependent cytotoxic effect was observed, where the highest assayed concentration (≈ 100%) led to very low cell

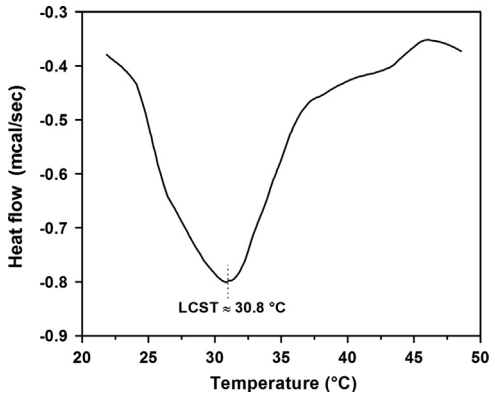

Fig. 6. DSC thermograms of swelled PP-g-(NVCL-co-AAc) with distilled water at a heating rate of 1 °C min^{-1} from 21 to 50 °C. LCST is indicated.

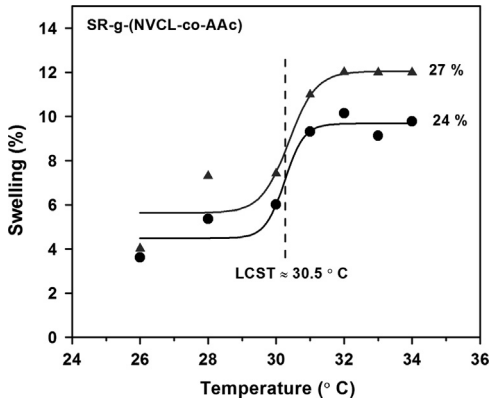

Fig. 7. Effect of temperature on swelling behavior of SR-g-(NVCL-co-AAc) in water for two different graft yields: (●) 24 and (▲) 27% graft. The dashed line indicates the LCST.

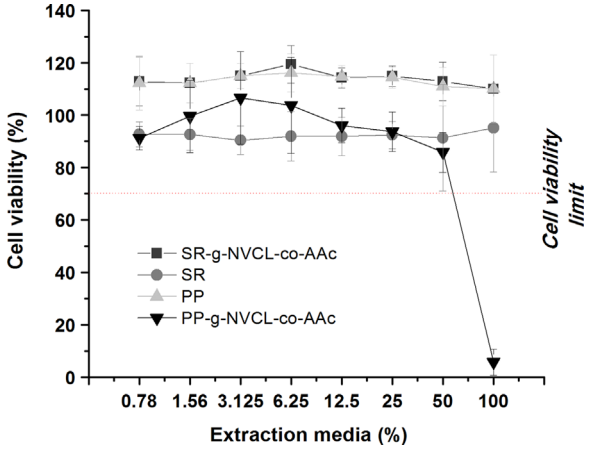

Fig. 8. In vitro cytotoxicity evaluation of membranes produced: PP, SR, PP-g-(NVCL-co-AAc), and SR -g-(NVCL-co-AAc).

viability (Fig. 8). This information provides an in vitro evidence of the presence of remaining monomers, indicating a limitation of this specific system, which highlight the need for a careful washing process or some modifications in the system in order to assure proper cytocompatibility of the material.

4. Conclusion

Grafting of NVCL-co-AAc onto polymeric PP and SR films has been achieved by the use of pre-irradiation oxidative method as confirmed by infrared analysis and changes in weight. Grafting efficiency increased as a function of pre-irradiation absorbed dose and reaction temperature increase. The graft copolymer films presented pH- and thermo-sensitivity properties as determined by swelling and DSC measurements. Radiation grafting corresponded to a suitable method to obtain the binary grafting involving thermosensitive NVCL and pH sensitive AAc monomers. It is relevant to note that stimuli sensitivity properties were preserved after grafting processing. The films presented acceptable cytocompatibility, except for PP-g-(NVCL-co-AAc) film, specifically at the highest assayed concentration.

Acknowledgment

This work was supported by DGAPA-UNAM Grant IN202311 and CONACYT-CNPq Project 174378 (Mexico), 490200/2011-7 (Brazil), and the Ibero-American Programme for Science, and Technology and Development of CYTED ("Red iberoamericana de nuevos materiales para el diseño de sistemas avanzados de liberación de fármacos en enfermedades de alto impacto socio-económico" RIMADEL). The authors thank M. Cruz and J. Rangel from ICN-UNAM and H. Perez and M.J.A. Oliveira from CQMA-IPEN/CNEN-SP for technical assistance.

References

Alvarez-Lorenzo, C., Bucio, E., Burillo, G., Concheiro, A., 2010. Medical devices modified at the surface by γ-ray grafting for drug loanding and delivery. Expert Opin. Drug Del. 7, 173–185.
Anderson, J.M., Rodriguez, A., Chang, D.T., 2008. Foreign body reaction to biomaterials. Semin. Immunol. 20, 86–100.
Arlyapov, V.A., Yudina, N.Y., Asulyan, L.D., Alferov, S.V., Alferov, V.A., Reshetilov, A.N., 2013. BOD biosensor based on the yeast Debaryomyceshansenii immobilized in poly(vinyl alcohol) modified by N-vinylpyrrolidone. Enzyme Microb. Technol. 53, 257–262.
Bach, R.D., Ayala, P.Y., Schlegel, H.B., 1996. A reassessment of the bond dissociation energies of peroxides. An ab initio study. J. Am. Chem. Soc. 118, 12758–12765.
Bettini, S.H.P., de Mello, L.C., Muñoz, P.A.R., Ruvolo-Filho, A., 2013. Grafting of maleic anhydride onto polypropylene, in the presence and absence of styrene, for compatibilization of poly(ethylene terephthalate)/(ethylene–propylene) blends. J. Appl. Polym. Sci. 127, 1001–1009.
Bhattacharya, A., 2000. Radiation and industrial polymers. Prog. Polym. Sci. 25, 371–401.
Bucio, E., Burillo, G., 2007. Radiation grafting of pH and thermosensitive N-isopropylacrylamide and acrylic acid onto PTFE films by two-steps process. Radiat. Phys. Chem. 76, 1724–1727.
Bucio, E., Burillo, G., 2009. Radiation-induced grafting of sensitive polymers. J. Radioanal. Nucl. Chem. 280, 239–243.
Chapiro, A., 1962. Radiation Chemistry of Polymeric Systems. Interscience, New York.
Charlesby, A., 1960. Atomic Radiation and Polymers. Pergamon Press, New York.
Cheng, S.C., Feng, W., Pashikin, I.I., Yuan, L.H., Deng, H.C., Zhou, Y., 2002. Radiation polymerization of thermo-sensitive poly (N-vinylcaprolactam). Radiat. Phys. Chem. 63, 517–519.
Chifen, A.N., Jenkins, A.T.A., Knoll, W., Förch, R., 2007. Adhesion improvement of plasma-polymerized maleic anhydride films on gold using HMDSO/O_2 adhesion layers. Plasma Processes Polym. 4, 815–822.
Contreras-García, A., Burillo, G., Aliev, R., Bucio, E., 2008. Radiation grafting of N,N-dimethylacrylamide and N-isopropylacrylamide onto polypropylene films by two-step method. Radiat. Phys. Chem. 77, 936–940.
De Prijck, K., De Smet, N., Coenye, T., Schacht, E., Nelis, H.J., 2010. Prevention of Candida albicans biofilm formation by covalently bound dimethylaminoethyl-methacrylate and polyethylenimine. Mycopathologia 170, 213–221.
Engelsman, A.F., Saldarriaga-Fernandez, I.C., Nejadnik, M.R., van Dam, G.M., Francis, K.P., Ploeg, R.J., Busscher, H.J., van der Mei, H.C., 2010. The risk of biomaterial associated infection after revision surgery due to an experimental primary implant infection. Biofouling 26, 761–767.
Estrada-Villegas, G.M., Bucio, E., 2012. Temperature- and pH-responsive behavior of a novel copolymer of (PP-g-DMAEMA)-g-AAc. J. Radioanal. Nucl. Chem. 292, 1–6.
Ferreira, H.P., Parra, D.F., Lugao, A.B., 2012. Radiation-induced grafting of styrene into poly(vinylidene fluoride) film by simultaneous method with two different solvents. Radiat. Phys. Chem. 81, 1341–1344.
Grasselli, M., Carbajal, M.L., Yoshii, F., Sugo, T., 2003. Radiation-induced GMA/DMAA graft copolymerization onto porous PE hollow-fiber membrane. J. Appl. Polym. Sci. 87, 1646–1653.

Gotowska, A., Bark, J.S., Kwon, I.C., Bae, Y.H., Cha, Y., Kim, S.W., 1997. Squeezing hydrogels for controlled oral drugs delivery. J. Controlled Release 48, 141–148.
Gottenbos, B., van der Mei, H.C., Klatter, F., Nieuwenhuis, P., Busscher, H.J., 2002. In vitro and in vivo antimicrobial activity of covalently coupled quaternary ammonium silane coatings on silicone rubber. Biomaterials 23, 1417–1423.
Grundmeier, G., Brettmann, M., Thiemann, P., 2003. In situ spectroscopic and corrosion studies of ultra-thin gradient plasma polymer layers on zinc. Appl. Surf. Sci. 217, 223–232.
ISO/TS 13409, 1996. Sterilization of health care products – radiation sterilization – substantiation of 25 kGy as a sterilization dose for small or infrequent production batches, International Organization for Standardization.
IS0 10993/EN 30993, 1992. Biological evaluation of medical devices. Tests for cytotoxicity: in vitro methods, International Organization for Standardization, Geneve, Switzerland.
Ivanov, V.S., 1992. Radiation Chemistry of Polymers (New Concepts in Polymer Science). VSP, Utrecht.
Lazo, L.M., Burillo, G., 2010. Novel comb-type hydrogels of net-[PP-g-AAc]-g-4VP synthesized by gamma radiation, with possible application on Cu^{2+} immobilization. Radiat. Phys. Chem. 79, 1–8.
Morales-Wiemer, E.A., Macossay, J., Bucio, E., 2013. Radiation grafting of N,N'-dimethylacrylamide and 2-hydroxyethylmethacrylate onto polypropylene films by one step method. Radiat. Phys. Chem. 84, 166–169.
Muñoz-Muñoz, F., Ruiz, J-C., Alvarez-Lorenzo, C., Concheiro, A., Bucio, E., 2012. Temperature- and pH- sensitive interpenetrating polymer networks grafted on PP: cross-linking irradiation dose as a critical variable for the performance as vancomycin-eluting systems. Radiat. Phys. Chem. 81, 531–540.
Nava-Ortiz, C.A.B., Burillo, G., Concheiro, A., Bucio, E., Matthijs, N., Nelis, H, Coenye, T., Alvarez-Lorenzo, C., 2010. Cyclodextrin-functionalized biomaterials loaded with miconazole prevent Candida albicans biofilm formation in vitro. Acta Biomater. 6, 1398–1404.
Rosiak, J.M., Janik, I., Kadlubowski, S., Kozicki, M., Kujawa, P., Stasica, P., Ulanski, P., 2003. Nano-, micro- and macroscopic hydrogels synthesized by radiation technique. Nucl. Instrum. Methods Phys. Res. 208, 325–330.
Ruiz, J.C., Burillo, G., Bucio, E., 2007. Interpenetrating thermo and pH stimuli-responsive polymer networks of PAAc/PNIPAAm grafted onto PP. Macromol. Mater. Eng. 292, 1176–1188.
Song, B., Meng, L., Huang, Y., 2012. Surface modification of PBO fiber through oxygen plasma induced vapor phase grafting of acrylic acid. Mater. Lett. 83, 118–120.
Subramanyam, U., Kennedy, J.P., 2009. PVA networks grafted with PDMS branches. J. Polym. Sci.: Polym. Chem. 47, 5272–5277.
Yoshida, R., Uchida, K., Kaneko, Y., Shakai, K., Kikuchi, A., Sakura, Y., Okano, T., 1995. Comb-type grafted hydrogels with rapid deswelling response to temperature changes. Nature 374, 240–242.
Zhang, K., Wu, X.Y., 2004. Temperature and pH-responsive polymeric composite membranes for controlled delivery of proteins and peptides. Biomaterials 25, 5281–5291.