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Poly(vinyl chloride) catheters modified with pH-responsive poly(methacrylic acid) with affinity for antimicrobial agents

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ABSTRACT

Radiation-grafting of pH-responsive methacrylic acid (MAA) onto poly(vinyl chloride) (PVC) was carried out by the pre-irradiation method using gamma rays, which demonstrated to be an efficient and fast procedure for obtaining PVC-g-MAA copolymers. The influence of preparation conditions, such as absorbed dose, monomer concentration, reaction time, and reaction temperature on the grafting yield was studied. The grafting of MAA onto PVC catheters was confirmed by means of Fourier transform infrared spectroscopy (FT-IR), thermogravimetry analysis (TGA), and differential scanning calorimetry (DSC). The pH-responsiveness of the grafted copolymers (critical point 8.5) was measured by swelling under cyclic changes in the pH of the medium. Interestingly, PVC-g-MAA showed enhanced capability to immobilize benzalkonium chloride and, particularly, ciprofloxacin and to sustain the release this antimicrobial agent at both acid and alkaline pH. Tests carried out with *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* point out that the developed functionalized catheters may play a role in the prevention/management of urinary tract infections.

1. Introduction

Medical devices play an important role in diagnosis and therapeutic procedures of current medical care. The increasing availability of synthetic materials, advances in polymer engineering, techniques in surgery and medical treatments, as well as methods of increasingly effective sterilization, have helped to develop a variety of biomaterials suitable as medical devices (Joung et al., 2013; Teo et al., 2016).

Poly(vinyl chloride) (PVC) is a common component of medical devices due its biocompatibility, chemical stability, versatile mechanical properties in terms of flexibility and softness, transparency, and relative low cost (Bigot et al., 2013; Hasan et al., 2015). Flexible PVC is extensively used for the production of urine bags, urine catheters, and transfusion tubing (Pradeep and Sailas, 2012). Unfortunately, these polymeric medical devices are frequently susceptible to be colonized by microorganisms causing infections, with a strong negative impact on patient health and sanitary costs (Lin et al., 2013; Pascual et al., 2011; Zhang, 2011; Herrero et al., 2006; Trautner and Darouiche, 2004). Several strategies are being tested to overcome microorganisms adhesion and biofilm formation (Kameda et al., 2011; Alvarez-Lorenzo et al., 2016). Anti-adhesion surfaces can be created by tuning the hydrophobicity/hydrophilicity properties of the polymer surface (Zhang et al., 2006; Triandafillu et al., 2003). In this sense, physicochemical properties of PVC can be tuned by grafting of stimuli-responsive polymers (Meléndez-Ortiz et al., 2016). A few attempts of preparing blends of PVC and poly(methacrylic acid) (PMAA) or comb-like copolymers of PVC and PMAA applying controlled radical polymerization, with the use of suitable chemical initiators, have been already reported (Fang et al., 2015). The main drawback of this later approach is the laborious removal of the remaining initiators, particularly when copper is used and a biomedical application is pursued.

Radiation-induced grafting techniques comprise the pre-irradiation, the mutual and the simultaneous methods; the energy source being either gamma ray, UV or electrons (Bucio and Burillo, 2009). The preparation of graft copolymers by using gamma radiation has been proved to be a simple process that does not require the addition of chemical initiators (Muñoz-Muñoz et al., 2012). The grafting percentage is adjusted by tuning variables such as temperature, reaction time,

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monomer concentration, dose and dose rate, solvent, etc. (Alvarez-Lorenzo et al., 2010). Grafting of stimuli-sensitive polymers has been shown already useful to endow medical devices with ability to host and control the release of a variety of drugs, and to regulate adhesion of proteins and cells (Cole et al., 2009; Alvarez-Lorenzo et al., 2016; Cross et al., 2016). The combination of drugs with medical devices can mutually reinforce the specific functions of each component in separate, exhibiting improved performance in the treatment and prophylaxis of infections (Wu and Grainger, 2006).

The aim of this work was to prepare pH-responsive PVC catheters grafted with poly(methacrylic acid) (PVC-g-PMAA) by means of a gamma-ray pre-irradiation method, systematically studying the effects of absorbed dose, monomer concentration, reaction time, and temperature on the grafting percentage. It is expected that PMAA could not only tune the overall surface hydrophilicity of PVC catheters to regulate bacterial adhesion but also to endow the catheters with a pH-responsive surface able to immobilize/release different antimicrobial agents.

2. Materials and methods

2.1. Materials

Medical-grade catheters of PVC (diameter 3 mm, thickness 0.5 mm; Biçakcilar, Turkey) were washed with ethanol for one day and then dried under vacuum oven. Methacrylic acid (MAA) was from Sigma Aldrich (St. Louis MO, USA) and distilled under reduced pressure before use. Benzalkonium chloride was from Sigma Aldrich (St. Louis MO, USA) and ciprofloxacin hydrochloride Ph. Eur. was from Fagron Iberica S.A.U. (Barcelona, Spain). Toluene and ethanol (reagent grade) were from J.T. Baker (Mexico). Bi-distilled water has been used for all experiments.

2.2. Preparation of PVC-g-MAA

Pieces of PVC catheters (previously weighed) in glass ampoules were exposed to ^{60}Co γ-source (Gammabeam 651PT, Nordion International Inc., Ottawa, Canada) for irradiation (10–80 kGy) in the presence of air at dose rate of 9.2 kGy h^{-1}. Then, a solution of MAA (10–70 vol%) in toluene was added to the ampoules containing the pre-irradiated catheters, and oxygen was removed by freeze-thaw cycles (5 times). The ampoules were sealed and heated at different temperatures (between 50 and 90 °C) to initiate the grafting process. The grafted catheters were washed with ethanol (two times) and finally extracted with bi-distilled water overnight in order to remove the residual monomer and the homopolymer produced during grafting process. Then, the catheters were dried overnight in a vacuum oven at 50 °C and the grafting percentage was calculated as follows:

$$\text{Grafting}\ (\%) = 100[(W_g - W_o)/W_o] \quad (1)$$

where W_o and W_g represent the weights of the catheter before and after grafting, respectively.

2.3. Characterization of PVC-g-MAA

FTIR-ATR (attenuated total reflection) spectra were recorded using a Perkin-Elmer Spectrum 100 spectrometer (Perkin Elmer Cetus Instruments, Norwalk, CT, USA). For determination of swelling, weighed grafted catheters were immersed into bi-distilled water at 25 °C for 3 h. The weight of the catheters was recorded every 15 min. The pH-responsiveness was obtained by measuring the percentage of swelling of PVC-g-MAA catheters in boric acid (0.2 M)/$Na_3PO_4{\cdot}12H_2O$ (0.1 M) and citric acid (0.05 M), buffer solutions of pH ranging were between 2 and 12 for 3 h. When the equilibrium was achieved, the surface of the grafted catheters was wiped with paper and then the swollen catheters were weighed.

Swelling (%) was calculated from the weights of the swollen (W_s) and dried (W_d) catheters by using Eq. (2):

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

where W_s and W_d are the weights of the swollen and the dried grafted catheter respectively.

Thermogravimetric measurements were carried out under nitrogen atmosphere using a TGA Q50 (TA Instruments, New Castle, DE) from room temperature to 800 °C at 10 °C min^{-1}. Differential scanning calorimetry runs were recorded using a DSC 2010 calorimeter (TA Instruments, New Castle, DE) between room temperature and 300 °C at 10 °C min^{-1} in nitrogen atmosphere. To obtain SEM images, the catheters were first swollen in phosphate buffer pH 8, washed with water, frozen by immersion in liquid nitrogen, and freeze-dried. Surface and cross-sectional areas of catheters were sputter coated with gold and then visualized in a ZEISS EVO LS 15 SEM apparatus (Oberkochen, Germany).

2.4. Loading/release of antimicrobial agents and antimicrobial efficiency

Pieces of PVC and PVC-g-MAA catheters (1 cm length; 150–200 mg) were individually placed in vials containing either benzalkonium chloride (1 mg mL^{-1}; 10 mL) or ciprofloxacin hydrochloride (0.015 mg mL^{-1}; 10 mL) aqueous solution. Before immersion in ciprofloxacin solution, the catheters were swollen in phosphate buffer pH 8 for 3 h, and then immediately transferred to the loading medium. The loading was monitored for 4 days at room temperature, under static conditions. At given time intervals, aliquots (2 mL) of the loading medium were taken, and the absorbance measured at 262 nm for benzalkonium chloride or 275 nm for ciprofloxacin (Agilent 8453, Agilent Technologies, USA). The samples were immediately returned to the corresponding vials. The amount loaded was calculated from the decrease in the amount of drug in solution, using the corresponding calibration curve. Experiments were carried out with 6 replicates.

After the loading, 4 replicates were used for release experiments and 2 replicates were challenged against microbial growth. Release experiments were carried out in phosphate buffer pH 5.8 and pH 8.0 (5 mL) under static conditions. At given time periods, samples (1 mL) of release medium were taken and their absorbance was spectrophotometrically recorded at 262 or 275 nm for benzalkonium chloride or ciprofloxacin quantification, respectively. Each sample was immediately returned to the corresponding vial.

Microbial tests were carried out by placing drug-soaked catheters on Petri plates containing Müller-Hinton agar, previously seeded with *Staphylococcus aureus* ATCC25923 ($0.19{\cdot}10^9$ FCU/mL), *Escherichia coli* CECT434 ($3.1{\cdot}10^9$ FCU/mL), and *Pseudomonas aeruginosa* CECT110 ($2.2{\cdot}10^9$ FCU/mL). Pieces of the same catheter that were not exposed to the drug solutions were used as controls. The plates were kept at 37 °C for 24 h and then the zones of inhibition were measured.

3. Results and discussion

3.1. Preparation of PVC-g-MAA

Radiation-grafting is particularly suitable for functionalization of medical devices as gamma-radiation based methods avoid the use chemical initiators and prevent microbial growth during processing. The proposed mechanism for obtaining the PVC-g-MAA copolymer is depicted in Scheme 1. First, PVC was irradiated in air in order to generate peroxides and hydroperoxides on the polymeric catheter (Boughattas et al., 2016). The principal reaction is based on the generation of grafting sites on the carbon atom containing the chloride group. The loss of chlorine atoms generates free radicals that react with