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Elaboration of Small-Diameter Vascular Prostheses—Selection of Appropriate Sterilisation Method

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ABSTRACT: The aim of study is the elaboration of semi-biodegradable, multilayered tubular structures as substitutes for the reconstruction of small diameter vascular prostheses (<6 mm). The inert external layer of the prostheses will be fabricated via the melt electrospinning of poly (L-lactide-*co*-glycolide) (PLGA). The middle layer will be constructed from polypropylene (PP); the first prototype will be produced via melt electrospinning and the second using the melt blowing technique. The general aim of this stage of the research is the selection of a sterilisation technique that is appropriate for semi-biodegradable, multilayered tubular structures. For this purpose, single tubular structures created via the melt electrospinning of PLGA or PP and melt blown tubular structures of PP were elaborated. The influence of steam, ethylene-oxide (EO), and radiation sterilisation techniques on the elaborated microstructure of tubular structures was analyzed during this study. The effect of each sterilisation technique was evaluated using differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy/energy-dispersive X-ray spectroscopy analysis (SEM/EDS). The changes in average molecular weight (M_w) and crystallinity index (CI) of the PLGA tubular structures after EO and steam sterilisation were evaluated. The EO and steam sterilisation resulted in the complete destruction of PLGA tubular structures. Only the radiation sterilisation (accelerated electrons) did not influence on PLGA tubular structures morphology as well as thermal and chemical properties. FTIR and SEM/EDS analysis indicated that no changes in the chemical properties of PP tubular structures after each sterilisation occurred. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40812.

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INTRODUCTION

Synthetic vascular prostheses made of polyethylene terephthalate (PET) or polytetrafluoroethylene (PTFE) serve as the "gold standard" in the reconstruction of large peripheral arteries.[1,2] There is no suitable textile material for the design of small diameter vascular prostheses with diameters smaller than 6 mm because of the significant difficulties caused by inconsistencies in mechanical compliance, the associated thrombus formation in the short term and intimal hyperplasia (stenosis) in the long term.

Our proposed solution to this challenge is the design of three-layered semi-biodegradable vascular prostheses fabricated using solvent-free nonwoven techniques, including melt electrospinning and melt blowing.[3–6]

The concept of the multilayered vascular implant design is described in previous publication.[3] The outside layers of graft will be fabricated using the biodegradable polymer poly(L-lactide-*co*-glycolide) (PLGA). The biodegradable layers should resorb after implantation, effecting a reduction in the mass of the prostheses and promoting the enhancement of a porous structure for natural tissue ingrowth. This design will allow the control of the tissue growth to ensure the intraoperative surgical tightness of the implant. The inner resorbable layer also can act as a carrier for endothelialization. The middle layer of the prostheses, made of the nonbiodegradable polymer polypropylene (PP), provides the high strength structure of the prostheses.

During an earlier stage of research, the appropriate polymer and manufacturing technique for the single, nonbiodegradable, and resorbable layers were selected.[4,5] Based on this previous research, two prototype vascular prostheses were tested, in which the biodegradable inside and outside layers were fabricated using the PLGA melt electrospinning technique, and the middle layers were formed using the PP melt electrospinning or

melt blowing technique. Additionally, PLGA melt electrospun tubular structures were thermally stabilisation. This process strongly influenced the fibre melting and the formation of bonds among the fibres, resulting in an insignificant reduction in the physical and increase of the mechanical properties of the melt electrospun structures.

Medical devices that originate from biodegradable polymers are commonly sterilized using ethylene oxide (EO) or radiation processes.[7] The EO sterilisation technique is used because of its bactericidal effectiveness at low temperature and high penetration.[8] Porous scaffolds, because of their large surface areas, can contain sufficiently large quantities of EO residues to pose a toxicity risk, even for humans.[8,9] However, the ethylene-oxide treatment offers the benefits of small molecular weight loss and a possible cross-linking effect.

EO sterilisation can be used for materials that are sensitive to temperatures higher than 60°C, such as polylactide or poly(lactide-*co*-glycolide), and for materials that are sensitive to radiation sterilisation.[10]

The effect of EO on PLGA[7,9–11] and PP[10,12,13] has been studied. The impact of EO sterilisation on 3-D PLGA scaffolds was also presented.[14] A less damaging effect on the molecular weight of PLGA in scaffolds after EO sterilisation was found with respect to products sterilised using gamma irradiation.

A study of the influence of EO sterilisation on polypropylene meshes has been conducted by Serbetci et al.[12] The EO sterilisation resulted in insignificant changes in the mechanical properties of the PP meshes.

Sterilisation using γ irradiation is typically applied for the sterilisation of gaseous, liquid, and solid materials and both homogeneous and heterogeneous systems.[15] The energy of the γ rays traverses the material structure and destroys pathogens by damaging their DNA.[10,15] Irradiation can achieve a large depth of penetration into a nonwoven structure, but the rate of interaction is rather low for biodegradable polymers.

γ irradiation is an accepted and validated method for the sterilisation of products fabricated using PLA and PLGA.[11,15–17] Nevertheless, this method may cause radiolysis and cross-linking of the polymer, but it does not result in a toxic effect.

Polypropylene is widely used for the manufacture of medical devices. However, structural (crystallinity, morphology, and/or topography) and physic-chemical changes have been observed in PP materials sterilized using γ irradiation because of the oxidative degradation of the PP.[13,15]

A gentler sterilisation technique for polypropylene medical devices is the steam technique. Steam sterilisation is performed in the temperature range of 120–130°C.[13] This process has a less destructive influence on PP structures than does the irradiation method because of its low impact on the molecular structure. The steam sterilisation of PP materials causes post-crystallization and physical aging of PP.[18,19] However, it can lead to both greater stiffness and brittleness of the treated material.[13]

For biodegradable polymers such as PLA, PLGA, or their copolymers, the steam method can result in deformation or even degradation because of the low melting temperatures and hydrolytic degradation mechanisms of these materials.[7,20,21]

Therefore, in our research, an analysis has been performed of the thermal stabilisation process and the influence of the EO, steam, and radiation sterilisation techniques on the morphology and physical and mechanical properties of PLGA and PP tubular structures as a preliminary study for the selection of the appropriate sterilisation technique for future semi-biodegradable tubular structures.[22]

A significant influence of the thermal stabilisation process on the physical properties of PLGA tubular structures was observed. An increase in the apparent density of the biodegradable prototypes by over 300% and a reduction in its porosity by ~15% with respect to the PLGA melt electrospun tubular structure with no thermal stabilisation were found. The stabilisation caused a decrease in the wall thickness of the PLGA tubular structures from 1.15 ± 0.17 mm to 0.15 ± 0.02 mm. The thermal stabilisation of the PLGA tubular structures led to a significant increase in the maximal load of the prototypes in both investigated directions (longitudinal and circumferential) compared with the properties of the initial structures.[22]

An increase in the mechanical parameters (Young's modulus, stress at maximal load, and elongation at maximal load) was also observed.[22] This phenomenon is most likely related to the thermal cross-bonding of the fibres and to changes in the fibrous microstructures caused by the thermal stabilisation process. In our studies, EO and steam sterilisation caused the complete deformation of biodegradable (PLGA) tubular fibrous structures. The deformation of the nonwoven fibrous structures depended on the EO sterilisation process conditions, such as temperature, pressure, and exposure time.[9,23] The temperature of the EO sterilisation process was near the glass transition temperature of PLGA. Therefore, the EO sterilisation conditions were found to be unsuitable for the treatment of biodegradable materials because of the structural deformation they caused. The high temperature (121°C) used during steam sterilisation had, rationally, an even more drastic effect on the PLGA tubular structures than did the EO treatment technique because of the low melting point of PLGA.[22]

However, the radiation sterilisation technique did not cause any macroscopic changes in the external layers of the biodegradable prototype prostheses. In addition, the radiation sterilisation process did not significantly alter the physical properties of the PLGA tubular structure. It was the one method that allowed the effective sterilisation of the biodegradable prototype without any macro-structural disintegration.[22]

Nonbiodegradable tubular prototypes, which were fabricated using the melt electrospinning and melt blowing techniques, exhibited no macroscopic changes after steam, radiation or EO treatment.[22]

A decrease in wall thickness and surface mass was observed for the PP melt electrospun tubular structures after steam sterilisation without any accompanying changes in the apparent density and graft porosity. These changes in the physical properties of the PP melt electrospun tubular structures may

have been caused by the high temperature of the steam sterilisation. The main result of the changes is the flatting of the final structure. The opposite effect was observed for the PP melt electrospun structures after EO or radiation sterilisation. These two sterilisation processes caused an increase in the surface mass of the nonbiodegradable melt electrospun prototypes without exerting any significant influence on other physical parameters.[22]

All applied sterilisation techniques, including EO, steam, and radiation, had insignificant influence on the physical properties of the PP melt blown tubular structures. The various effects of the sterilisation techniques used for the sterilisation of the PP electrospun samples were dependent on the differences in the morphologies of the initial fibrous structures. The tubular structures that were fabricated via melt electrospinning were characterised by a diameter of several dozen micrometres, whereas the structures that were fabricated using the melt blowing technique had diameters of several hundred nanometers.[22]

The radiation sterilisation of the biodegradable tubular structures led to a decrease in their mechanical properties. The maximal load was reduced in both directions by ~50%, whereas the elongation at maximal load was reduced in both directions by ~35%. The reduction in the maximal load and the greater stiffness of the PLGA tubular structures observed after radiation sterilisation were related to degradation effects.[22]

The radiation sterilisation of PP melt electrospun tubular structures did not appear to influence the maximal load in the longitudinal direction. However, the maximal load for these structures was reduced by ~50%. The sterilisation agents led to higher stiffness of the PP melt electrospun tubular structures. Both steam and radiation sterilisation reduced the elongation at maximal load in the longitudinal direction, while the elongation at maximal load was reduced in the circumferential direction following both EO and radiation sterilisation.[22]

The prototype vascular prostheses that were obtained using the melt blowing technique were characterised by mechanical properties that were incomparable to those obtained for the PP melt electrospun tubular structures. All sterilisation agents caused a reduction of the maximal load, stress and elongation measured in the longitudinal direction. Moreover, no changes in the abovementioned mechanical properties were detected in the circumferential direction, with the exception of an increase in the maximal load (~19%) in the circumferential direction after radiation sterilisation. In the case of radiation sterilisation, the sterilisation agents were found to have a low impact on the mechanical properties of PP melt blown tubular structures in both investigated directions.[22]

The aim of the present research, as an element of the larger study aimed at the elaboration of multilayered small diameter vascular prostheses, was to select the appropriate sterilisation technique for semibiodegradable structures. Single tubular structures of PLGA and PP were elaborated and exposed to EO, steam, or radiation sterilisation. A stringent analysis of the effects of EO, steam, and radiation sterilisation on the structural properties of the elaborated PLGA and PP tubular structures was performed.

Table I. The Thermal Analysis of the Native PP and PLGA Used in This Study[22]

Raw materials	Melt temperature T_m (°C)	Glass transition temperature T_g (°C)	Crystallinity index CI (%)
PP	166	-	34
PLGA	Amorphous	54	Amorphous

MATERIALS AND METHODS

Materials

The material used to fabricate the nonbiodegradable tubular fibrous structures was Bormed HF840MO PP polypropylene (Borealis/Austria; MFR = 19 g/10 min at 230°C under a piston loading of 2.16 kg). The polypropylene was characterised in terms of melt flow rate (MFR) in accordance with the ISO 1133:2011 standard. The melt temperature (T_m) and crystallinity index (CI) of Bormed HF840MO PP were determined via differential scanning calorimetry (DSC) in accordance with the PN-EN ISO 11357:2009 standard (Table I).

The resorbable tubular fibrous structures were elaborated using PLGA (molar ratio of 84 : 16). PLGA was purchased from the Centre of Polymer and Carbon Materials of the Polish Academy of Sciences (Zabrze, Poland). The polymer was characterised in terms of its number average molecular weight, M_n = 127 kDa.

The thermal parameters of PLGA were determined via DSC in accordance with the PN-EN ISO 11357:2009 standard (Table I).The PLGA granulate did not exhibit any melt temperature or crystallinity index because of its amorphous nature. The PLGA exhibited only a seal initiation temperature of 69°C (in accordance with the ASTM F88:2009 standard).

Methods

The melt electrospun PLGA and PP tubular structures and the melt blown PP tubular structures were elaborated using a Mini-Lab twin-screw extruder (Haake, Germany). The processing system and parameters used for the elaboration of the tubular structures were presented in previous publication.[4,22]

The extruder processing parameters for PLGA tubular structures melt electrospinning were as follow:

- head temperature: 200°C,
- spinning voltage: 37 kV.

The extruder processing parameters for PP tubular structures melt electrospinning were as follow:

- head temperature: 270°C,
- spinning voltage: 25 kV.

The twist of extruder screws and distance between collector and spinneret were the same for elaboration melt electrospinning tubular structures of PLGA or PP, and amounted 1 rpm and 8 cm, respectively.

The extruder processing parameters for PP tubular structures melt blowing were as follow:

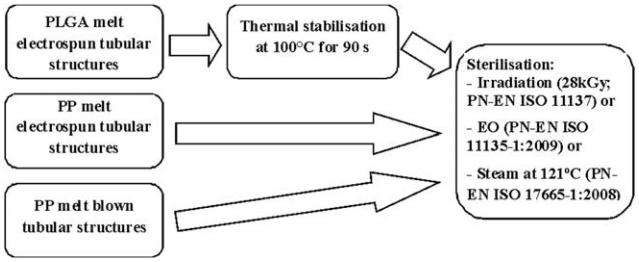

Figure 1. The schematic representation of the finishing steps applied for the PLGA and PP tubular structures.

- head of temperature: 270°C,
- air flow rate: 20 Nm/h,
- twist of screws: 1 rpm,
- distance between collector and spinneret 15 cm.

Collector processing parameters were the same for each used technique (speed of spindle: 11 rpm; speed of oscillation 30 rpm.)

In the next stage, the obtained PLGA and PP tubular structures were exposed to EO, steam, or radiation sterilisation. The preparation process was performed according to the scheme presented in Figure 1.

The radiation sterilisation was performed at the Institute of Nuclear Chemistry and Technology (Poland). A radiation dose of 28 kGy, under conditions consistent with the PN-EN ISO 11137-1/2/3 standard, was used.

The ethylene oxide (EO) sterilisation was performed at CSP Technochemia (Poland) in accordance with the PN-EN ISO 11135-1:2009 standard, whereas the steam sterilisation performed at the sterilisation plant of TZMO S.A. (Poland) at 121°C under conditions consistent with the PN-EN ISO 17665-1:2008 standard.[22]

Microscopic and macroscopic views of the elaborated structures at various stages of the finishing process are shown in Figure 2.

ANALYTICAL METHODS

Differential Scanning Calorimetry (DSC)

The melting behavior of the PLGA and PP tubular structures before and after EO, steam and radiation sterilisation was characterised via DSC using a Q2000 device (TA Instruments, USA). The calibration was performed using indium. A heating rate of 10°C/ min and a temperature range of 0–200°C were used. The glass transition temperature (T_g), the cold crystallization temperature (T_c), and the melting temperature (T_m) were determined.

The crystallinity index (CI) of each sample was calculated using the following equation:

$$\text{Crystallinity index} = \left[\frac{\Delta H_m - \Delta H_c}{\Delta H_f}\right] \cdot 100, [\%] \quad (1)$$

where

ΔH_m is the measured enthalpy of melting,

ΔH_c is the measured enthalpy of crystallization, and

ΔH_f is the theoretical heat of fusion for a 100% crystalline material.

The theoretical value of 100% crystalline PP is ~207 J/g.[24] Published reports indicate that the ΔHf of PLGA (85/15) is similar to that of pure PLLA and is calculated to be ~87 J/g.[25,26]

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was used to investigate the chemical structures of the initial PLGA and PP tubular structures and after the application of each sterilisation agent. The spectroscopic measurements were performed using a Nicolet FTIR Spectrometer (Thermo Scientific, USA). The collection and primary analysis of the data were accomplished using ACD/SpecManager (Thermo Scientific, USA). FTIR spectra in absorbance mode were collected in a wavelength range of 4000 cm^{-1} to 600 cm^{-1}.

Gel Permeation Chromatography (GPC)

The changes in average molar mass (M_n) of the initial, thermally stabilised and sterilized PLGA tubular structures were estimated via GPC using a Water 2695 gel chromatograph (Harlow Scientific, USA) with a viscometer, a refractive index detector and a PLgel MIXED-C column. The experiments were conducted in dichloromethane solution at a flow rate of 0.8 mL/min. The system was calibrated based on polystyrene standards. The influence of radiation on the formation or breaking of PLGA bonds via intermolecular cross-linking (G_x) and chain scission (G_s) was estimated. These two parameters were calculated according to eqs. (2) and (3).[27–29]

$$\frac{1}{M_w} = \frac{1}{M_{w,0}} + \left(\frac{G_s}{2} - 2G_x\right) D_x * 1.038 * 10^{-6} \quad (2)$$

$$\frac{1}{M_n} = \frac{1}{M_{n,0}} + (G_s - G_x) D_x * 1.038 * 10^{-6} \quad (3)$$

where:

$M_{w,o}$ and $M_{n,o}$are the molecular weights of nonirradiated samples,

M_w and M_n are the molecular weights of irradiated samples,

D_x is the irradiation dose (kGy),

G_x is the cross-linking parameter,

G_s is the chain-scission parameter.

A G_s/G_x ratio of greater than 4 would means that chain scission is more significant than cross-linking.[29] Sen et al. have reported that (2) can be used for any molecular-weight distribution of the initial samples, whereas (3) can be used for samples that are characterised by a M_w/M_n value of ~2.

Scanning Electron Microscopy/Energy-Dispersive X-Ray Spectroscopy Analysis

The chemical and morphological analysis of the PLGA and PP tubular structures before and after sterilisation were performed using a Nova Nano-SEM 230 scanning electron microscope (FEI Company, the Netherlands) with an X-ray microanalyser for energy-dispersive X-ray spectroscopy (FEI Company, the Netherlands) using the methodology described by Stawski et al.[30]

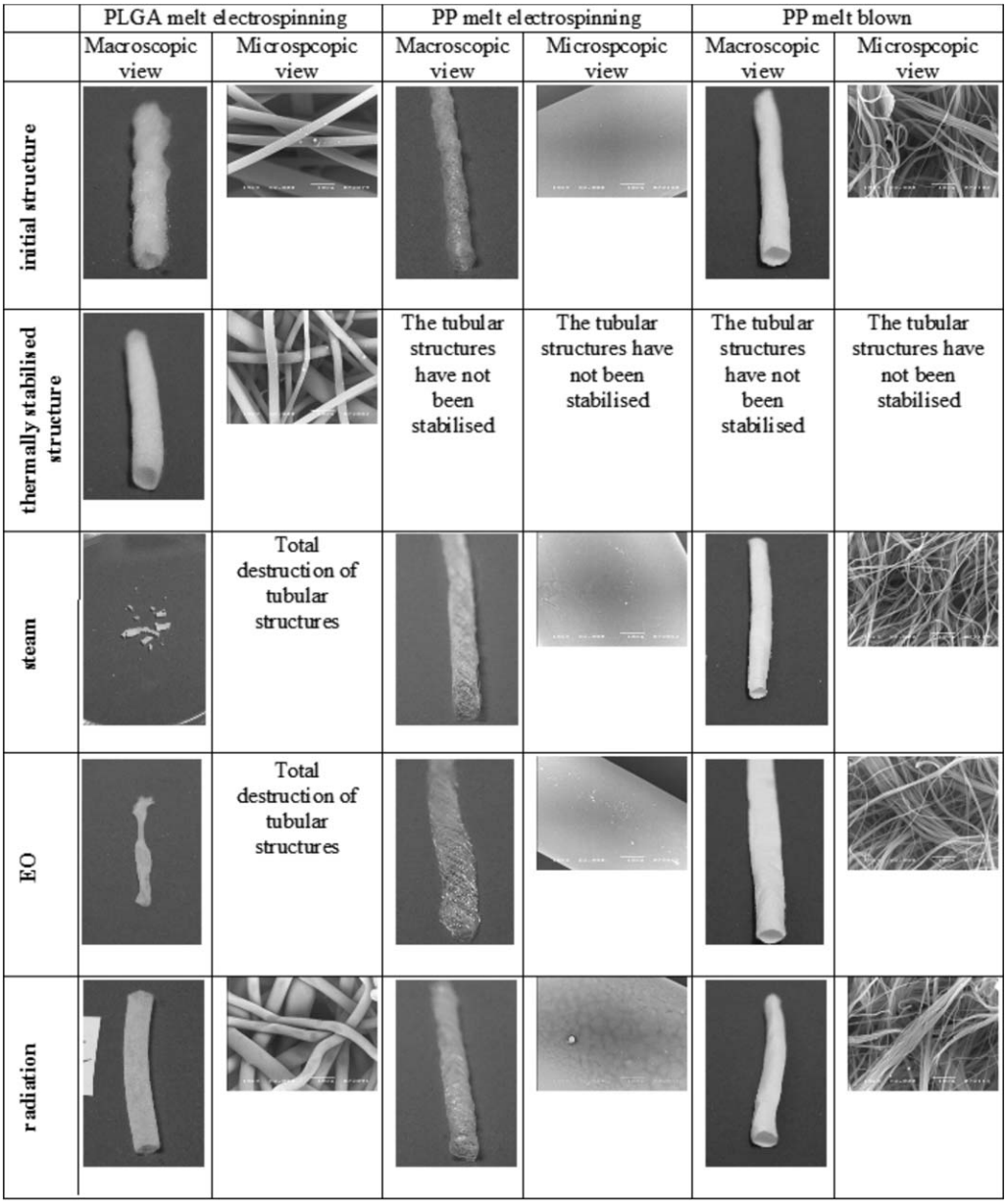

Figure 2. Macroscopic views and SEM microphotographs of representative structures (magnification ×2000) of elaborated PLGA and PP tubular fibrous structures: initial, after thermal stabilisation and after steam, EO or radiation sterilisation.

RESULTS AND DISCUSSION

Tubular Structures Morphology

The microscopic views of the elaborated biodegradable and nonbiodegradable prototypes were made for analysis of sterilisation techniques impact on their surface (Figure 2). The SEM micrographs at magnification 2000 were performed. At microscopic view, the fiber connection points after thermal stabilisation process of PLGA tubular structures were observed. The steam and EO sterilisation effected the total destruction of biodegradable prototypes. Therefore, the microscopic analysis only for PLGA tubular structures sterilized by radiation was performed. The surface changes for biodegradable tubular structures after radiation sterilisation were not detected. All tested sterilisation agents did not influence on the microscopic changes at melt blown and melt electrospun PP tubular structures surface.

The Thermal Properties Analysis

A thermal analysis was performed of the initial PLGA and its melt electrospun tubular structures before and after the sterilisation: radiation, EO, and steam. The results of the thermal analysis are presented in Figure 3(a). The melt behavior relations of the initial PP resin and the tubular fibrous structures elaborated via the melt electrospinning and melt blowing techniques before and after the application of the various sterilisation agents are presented in Figure 3(b,c). Table II presents the comparison of the T_m and CI for each studied PLGA sample.

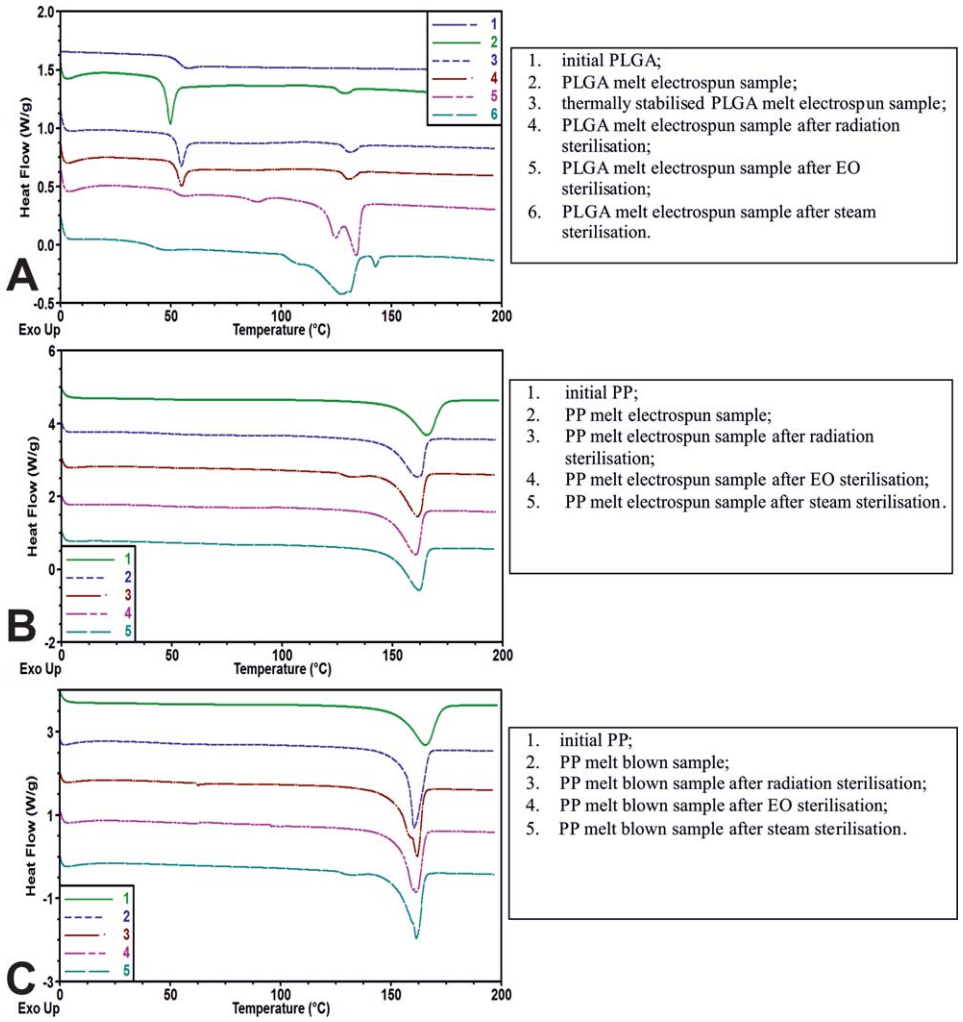

Figure 3. DSC thermographs after each sterilisation of tubular structures fabricated using: (a) PLGA and melt electrospinning, (b) PP and melt electrospinning, and (c) PP and melt blowing. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The PLGA resin had an amorphous nature and exhibited only a glass transition temperature (T_g) at ~54°C. The melt electrospinning process resulted in a decrease in the T_g temperature and the appearance of a melt temperature endothermic peak (T_m). The high temperature applied during melt electrospinning and the slow fibre cooling after extrusion caused chain scission, which resulted in less entanglement and an increase in chain mobility. Amorphous chains have sufficient energy to reorient and promote crystal growth.[28,31]

The thermal stabilisation process of the PLGA tubular structures also induced an increase in T_g of ~10% and a small increase in CI. The thermal energy supplied through the stabilisation process was sufficient for the movement and reorientation of the polymer chains. This process resulted in the formation of a

Table II. Comparison of the Glass Transition Temperature, Melting Temperature, and Crystallinity Index for the Initial PLGA and Melt Electrospun Tubular Fibrous Structures After Thermal Stabilisation and After Each Sterilisation: EO, Steam, and Radiation

Processing stage; parameters	T_g (°C)	T_m (°C)	CI (%)
Initial PLGA	53.88	Amorphous polymer	0.00
PLGA melt electrospun sample	48.78	128.43	1.84
thermally stabilised PLGA melt electrospun sample	53.74	131.03	2.67
PLGA melt electrospun sample after radiation sterilisation	53.33	130.60	2.65
PLGA melt electrospun sample after EO sterilisation	52.11	134.09	27.70
PLGA melt electrospun sample after steam sterilisation	42.33	142.93	26.53

Table III. Comparison of the Glass Transition Temperature, Melting Temperature, and Crystallinity Index for the Initial PP, Melt Electrospun, or Melt Blown Tubular Fibrous Structures and Before or After Each Sterilisation: EO, Steam, and Radiation

Processing stage; parameters	T_m (°C)	CI (%)
Initial PP	165.63	35.03
PP melt electrospun sample	161.85	32.07
PP melt electrospun sample after radiation sterilisation	160.78	32.54
PP melt electrospun sample after EO sterilisation	162.15	31.92
PP melt electrospun sample steam sterilisation	160.78	32.66
PP melt blown sample	160.50	42.38
PP melt blown sample after radiation sterilisation	161.86	38.43
PP melt blown sample after EO sterilisation	161.30	38.44
PP melt blown sample steam sterilisation	161.38	36.73

close-packed structure in the crystalline regions and an increase in the degree of crystallinity Table II.[28,31]

A larger area of the relaxation peak was observed for the PLGA melt electrospun specimens before and after thermal stabilisation and for the PLGA melt electrospun tubular structures after radiation sterilisation. This effect was the consequence of the molecules exhibiting a longer overall relaxation time.[4,32]

EO and steam sterilisation also caused the CI to increase by ~900%. The increase in CI was caused by the EO and steam sterilisation conditions. The temperatures used for EO sterilisation (40–60°C), for a duration of several hours, and for steam sterilisation (121°C) caused the chain scission of the polymers and the reorganization of their structure. Unfortunately, these sterilisation processes also led to the total disintegration of the tubular structures (Figure 2). The CI of the PLGA melt electrospun samples after thermal stabilisation and radiation sterilisation showed no significant changes.

The DSC curve of the initial PP indicates a T_m of ~165°C (Table III). Changes in the endothermic peaks were recorded for all studied samples with respect to the initial PP. However, the greatest reduction in T_m was only 3% (for the PP melt electrospun tubular structures after EO sterilisation and for the initial PP melt blown tubular structures). No significant differences in the endothermic peak values between the initial polymers and the melt electrospun or melt blown tubular structures before or after EO, steam or radiation sterilisation were found.

The thermographs of the PP melt blown tubular structures after EO or steam sterilisation exhibited two melting peaks. This phenomenon can be explained by the broadening of the melting range caused by the presence of a biphasic system (α and β phases) in which the two phases melt at different temperatures or the partial adhesion of the sample to the heating holder.[33]

The CI values of the examined samples were estimated from the peak area and were found to exhibit no significant differences between the PP melt electrospun tubular structures before and after the application of the various sterilisation agents, or between the initial and sterilized PP melt blown tubular structures. An ~13% reduction in CI was observed for the PP melt blown tubular structures after steam sterilisation.

The FTIR Analysis

FTIR analysis was applied for the verification of the degrees of surface modification of the PLGA or PP tubular structures caused by the EO, steam and radiation sterilisation.

Figure 4 shows the FTIR spectra of the native PLGA and the melt electrospun tubular structures before and after the various sterilisation. Figure 4(b) presents the FTIR spectra of the native PP and the melt electrospun tubular fibrous structures before and after each sterilisation, whereas Figure 4(c) presents the corresponding comparison for the PP melt blown structures.

The characteristic bands for ester carbonyl stretching (C=O) at a wavelength of 1749 cm^{-1}, C-O stretching at a wavelength of 1128 cm^{-1} and the C-O-C group wavelength of 1038 cm^{-1} were observed for the PLGA melt electrospun tubular structures before and after each applied sterilisation.[34,35]

Table IV. The Molecular Weights and Polydispersity of PLGA After Melt Electrospinning, Thermal Stabilisation, and Various Sterilisations

Processing stage	M_w (Da)	M_n (Da)	M_w/M_n
Initial polymer	206,800	127,400	1.62
PLGA melt electrospun sample	24,600	10,100	2.44
thermally stabilised PLGA melt electrospun sample	39,950	21,590	1.85
PLGA melt spunelectrospun sample after EO sterilisation	48,800	25,200	1.94
PLGA melt electrospun sample after steam sterilisation	16,400	8,570	1.91
PLGA melt electrospun sample after radiation sterilisation	37,490	20,000	1.87

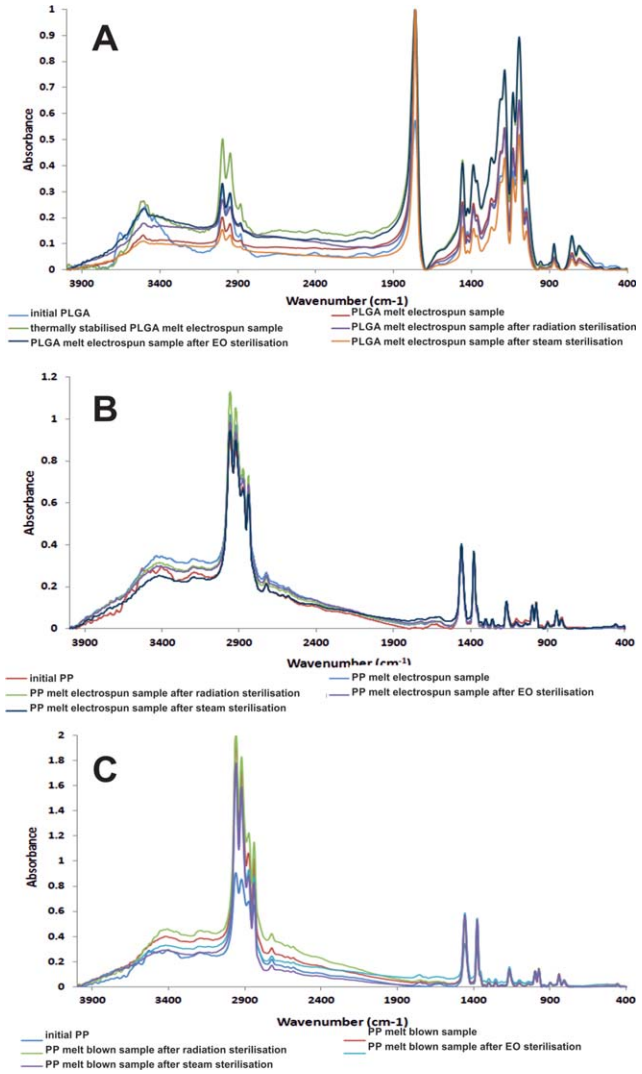

Figure 4. Absorbance FTIR spectra collected before and after each sterilisation for initial polymers and tubular structures fabricated using (a) PLGA and melt electrospinning, (b) PP and melt blowing, and (c) PP and melt electrospinning. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The collected FTIR spectra suggest that the melt electrospinning process, thermal stabilisation and EO, steam and radiation sterilisation did not significantly influence the chemical structure of the PLGA.

This lack of effect can be attributed to the fact that the FTIR spectra of polyester are inherently broad and weak. It is difficult to detect minor changes occurring on the polymer chain.[36]

The dose of 28 kGy applied during the radiation sterilisation was not sufficient to effect significant degradation in the PLGA. The FTIR analysis was not sufficiently sensitive to observe any polymer degradation that might have been caused by the applied sterilisation agents.[36]

The most important absorption bands for polypropylene are those detectable in the wavelength range of 2700–3000 cm^{-1}, which are associated with the symmetric and asymmetric stretching of the CH, CH_2, and CH_3 groups; the peak that extends from 1430 to 1450 cm^{-1}, which is attributable to the bending of CH_3 and CH_2; the peaks attributed to bending, rocking, wagging, twisting, and stretching that range between 807 and 1375 cm^{-1} and the absorption bands that indicate the helicoidal conformation of PP.[37]

Considering,[13] it is well known that the radiation sterilisation process influences the PP properties more drastically than EO or steam sterilisation. Irradiation may cause the formation of radicals in the polymer and could result in oxidation degradation. Free radicals are unstable and decompose into carbonyl and hydroxyl compounds, which can cause chain destruction.[38] The oxidation effect causes the formation of peaks in the wavelength ranges of 3600 to 3300 cm^{-1} and 1800 to 1600 cm^{-1}.[33] E-beam sterilisation is seeing increasing use because of its very short exposition time (because of the high dose rate applied) and the reduction of the potential degradation of the polymer.[39]

All curves collected for the studied PP specimens were similar to those obtained for native PP. No significant increase in absorbance at wavelengths of 3600–3300 cm^{-1} or 1800–1600 cm^{-1} was recorded. This lack of effect may have been caused by the low irradiation dose applied (only 28 kGy), which did not affect the PP properties.

As observed in the FTIR spectra, neither EO nor steam sterilisation effected significant changes in the PP chemical structure.

Changes in the Molecular Weight

Table IV illustrates the effects of the melt electrospinning process, thermal stabilisation, and EO, steam and radiation sterilisation on the average molecular weight of PLGA and on the polydispersity (M_w/M_n).

The melt electrospinning process caused a considerable decrease in M_n and M_w without significantly affecting the M_w/M_n ratio.

A polymer with a relatively high molecular weight is characterised by longer polymer chains and takes more time to degrade than a polymer of low molecular weight. However, the degradation rate is directly proportional to CI. Polymers of higher CI degrade more slowly.[40,41] In this study, PLGA granulates of high molecular weight but an amorphous nature was used. This fact influenced the high degradation rate of the PLGA during the melt electrospinning process.

The next stage of the preparation of the prototype small-diameter vascular prostheses was the thermal stabilisation of the PLGA melt electrospun tubular structures at 100°C. This process caused an increase in the PLGA molecular weight that originated from transesterification.[21,27] The increase in Mw observed for the PLGA melt electrospun tubular structures after thermal stabilisation was ~60%.

The various influences of EO, steam and radiation sterilisation on the molecular weight of PLGA formed into melt electrospun tubular structures were observed. Steam sterilisation caused a significant decrease in M_w of more than 50%. This phenomenon resulted from the thermo oxidative degradation of the PLGA. The steam sterilisation was carried out at 121°C for ~2 h. As a

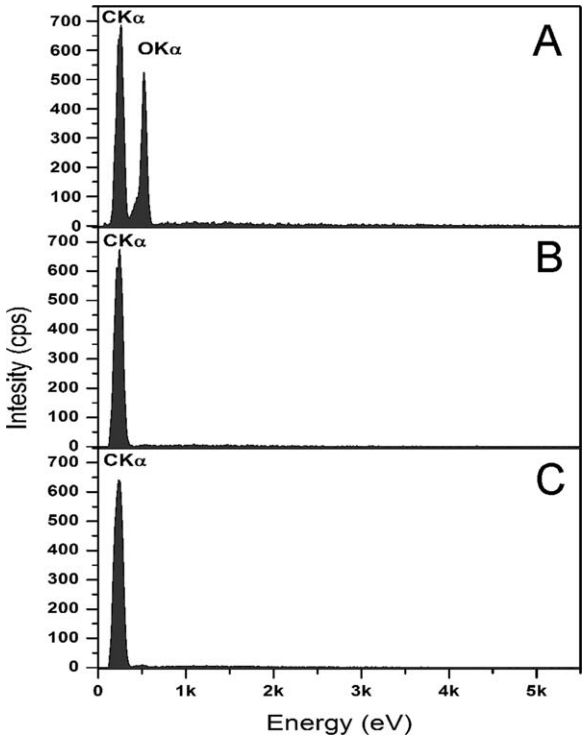

Figure 5. The X-ray spectra for (a) PLGA melt electrospun, (b) PP melt electrospun, and (c) PP melt blown tubular structures.

result, the tubular structures were melted; the seal initiation temperature of the PLGA used in this study was ~69°C.[21,27]

EO sterilisation caused an increase in the M_w of the PLGA because of the process conditions that were used. The temperature used was above the glass transition temperature of PLGA and led to their combination of the PLGA structure and an increase in CI and molecular weight. On the other hand, the cross-linking effect of highly reactive EO should be taken into consideration.

The lowest impact on the PLGA molecular weight was exerted by the radiation sterilisation. The sterilisation was performed at room temperature and at a radiation dose of 28 kGy. These conditions did not significantly reduce M_w and M_n.

The effect of radiation sterilisation was also assessed by calculating the cross-linking (G_x) and chain-scission (G_s) parameters. The resulting G_s and G_x values were 0.13 and 0.005, respectively. It follows that the G_s/G_x ratio was equal to 26. This result indicates that the influence of chain scission was more significant than cross-linking reactions.[28,34]

The applied sterilisation agents did not significantly influence the PLGA polydispersity with respect to the melt electrospun and thermally stabilised tubular structures.

Energy-Dispersive X-Ray Spectroscopy Analysis

Figure 5(a) shows an example of an X-ray spectrum obtained for a PLGA sample. The maximum, located at 0.2 keV and characterised as CKα, originates from carbon. The next strongest peak, located at 0.5 keV, is associated with the oxygen characteristic line OKα. Quantitative analysis established the presence of carbon, in the amount of 50.72 at%, and oxygen, in the amount of 49.28 at%. There were no significant changes in the chemical composition of the PLGA after EO, steam or radiation sterilisation when compared with the initial PLGA melt electrospun tubular structure.

The spectra obtained for the PP tubular structures before and after the application of the various sterilisation techniques exhibit only the carbon characteristic line CKα, which is typical for a polyolefin macromolecule. The EO, steam, and radiation treatments did not induce any composition changes in the PP melt electrospun or melt blown tubular structures, especially in the case of the oxidation effect. An example spectrum is presented in Figure 5(b).

CONCLUSIONS

The PLGA and PP melt electrospun and PP melt blown tubular structures before and after EO, steam, and radiation (accelerated electrons) sterilisation were investigated.

The melt electrospinning process caused a significant reduction in the PLGA molecular weight and an increase in the degree of crystallinity. However, the thermal stabilisation of resorbable tubular structures caused an increase in the molecular weight and insignificant change of thermal properties. The melt electrospinning and melt blown process did not influence at structural properties of PP.

The high temperature (121°C) during steam sterilisation has resulted on PLGA tubular structures melting and consequently caused the chain scission as well as reorientation of the molecular chains. The EO sterilisation, which was conducted at a temperature near the PLGA glass transition temperature, led to an increase in molecular weight and CI but also to dimensional and structural changes. Only irradiation with accelerated electrons had slight damaging effect on PLGA tubular structures.

However, the EO, steam and radiation (accelerated electrons) sterilisations did not significantly influenced either the PP tubular structures morphology or their thermal and chemical properties.

As the result of this research, irradiation with accelerated electrons was identified as the optimal sterilisation technique for future semibiodegradable tubular structures.

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REFERENCES

1. Rabkin, E.; Schoen, F. *Cardiovasc. Pathol.* **2002**, *11*, 305.
2. Hoerstrup, S. P.; Zünd, G.; Sodian, R.; Schnell, A. M.; Grünenfelder, J.; Turina, M. I. *Eur. J. Cardiothoracic Surg.* **2001**, *20*, 164.

3. Mazalevska, O.; Struszczyk, M. H.; Chrzanowski, M.; Krucińska, I. *Fibres Text. Eastern Europe* **2011**, *19*, 4 (87), 46.
4. Chrzanowska, O.; Struszczyk, M. H.; Krucińska, I. *J. Appl. Polym. Sci.* **2013**, *129*, 2, 779.
5. Mazalevska, O.; Struszczyk, M. H.; Krucińska, I. *J. Appl. Polym. Sci.* **2013**, *129*, 2, 770.
6. Krucinska, I.; Struszczyk, M. H.; Mazalewska, O. Patent application RP No. P3998860, 2012.
7. Hofman, S.; Stok, K. S.; Kohler, T.; Meinel, A. J.; Műller, R. *Acta Biomaterialia*, **2014**, *10*, 1, 308.
8. Dimitrievska, S.; Petit, A.; Doillon, C. J.; Epure, L.; Ajji, A.; Yahia, L. H.; Bureau, M. N. *Macromol. Biosci.* **2011**, *11*, 13.
9. Kim, H. L.; Lee, J. H.; Lee, M. H.; Kim, H. H.; Kim, J.; Han, I.; Park, B. J.; Kim, J. K.; Han, D. W.; Kim, S. H.; Lee, S. J.; Park, J. C. *Tissue Eng. Regenerative Med.* **2011**, *8*, 3, 320.
10. Hsiao, C. Y.; Liu, S. J.; Ueng, S. W. N.; Chan, E. C. *Polym. Degrad. Stabil.* **2012**, *97*, 715.
11. Lee, J. S.; Chae, G. S.; Khang, G.; Kim, M. S.; Cho, S. H.; Lee, H. B. *Macromol. Res.* **2003**, *11*, 5, 352.
12. Serbetci, K.; Kulacoglu, H.; Devay, A. D.; Hasirci, N. *Am. J. Surg.* **2007**, *194*, 375.
13. Gahleitner, M.; Wolfschwenger, J.; Fiebig, J. 2003 9th Europeane Place Conference. 2003, Available at: http://www.tappi.org/content/enewsletters/eplace/2004/11-4gahleitner.pdf (accessed 14.12.2013).
14. Holy, C. E.; Cheng, C.; Davies, J. E.; Shoichet, M. S. *Biomaterials* **2001**, *22*, 25.
15. da Silva Aquino, KA.: Gamma Radiation, 2012 available at: http://cdn.intechopen.com/pdfs/32842/InTech-Sterilization_by_gamma_irradiation.pdf (accessed 14.12.2013).
16. Yapar, E. A.; Baykara, T. *J. Fac. Pharm* **2008**, *37*, 1, 1.
17. Yaman, A. *Current Opin. Drug Dis. Dev.* **2001**, *4*, 6, 760.
18. O'Kane, W.; Young, R. J.; Ryan, A. J. *J. Macromol. Sci.-Phys.* **1995**, *B34*, 4, 427.
19. Fiebig, J.; Gahleitner, M.; Paulik, C.; Wolfschwenger, J. *Polym. Test.* **1999**, *18*, 4, 257.
20. Shearer, H.; Ellis, M. J.; Perera, S. P.; Chaudhuri, J. B. *Tissue Eng.* **2006**, *12*, 2717.
21. Gogolewski, S.; Mainil-Varlet, P. *Biomaterials* **1997**, *18*, 251.
22. Chrzanowska, O.; Struszczyk, M. H.; Krucińska, I. *Med-Tex13*, **2013**, Raleigh, NC, USA.
23. Fries, W.; Schlapp, M. *Eur. J. ParmBiopharm.* **2006**, *63*, 176.
24. Cheng, S. Z. D.; Janimak, J. J.; Zhang, A. Q.; Hsieh, E. T. *Polymer* **1991**, *32*, 6, 648.
25. Fischer, E. W.; Sterzel, H. J.; Wegner, G. *Koll-Z Z Polym.* **1973**, *251*, 978.
26. Koegler, W. S.; Griffith, L. G. *Biomaterials* **2004**, *25*, 2819.
27. Gogolewski, S.; Mainil-Varlet, P. *Biomaterials* **1997**, *18*, 251.
28. Loo, J. S. C.; Ooi, C. P.; Boey, F. Y. C. *Biomaterials* **2005**, *26*, 1359.
29. Sen, M.; Uzun, C.; Kantoglu, O.; Erodgan, S. M.; Deniz, V.; Guven, O. *Nucl. Instrum. Methods Phys. Res. B* **2003**, *208*, 480.
30. Stawski, D.; Sarkar, A. K.; Połowiński, S.; Banerjee, A.; Ranganath, A.; Puchalski, M.; Stanczyk, K. *J. Text. Inst.* **2013**, *104*, 8, 883.
31. Loo, S. C. J.; Ooi, C. P.; Wee, S. H. E.; Boey, Y. C. E. *Biomaterials*, **2005**, *26*, 16, 2827.
32. Sichina, W. I. *Measurement of Tg by DSC*. 2000, Available at: http://www.metrotec.es/metrotec/WWW_DOC/PETech-09.pdf (accessed April 6, 2013).
33. Nistico, R.; Faga, M. G.; Gautier, G.; Magnacca, G.; D'Angelo, D.; Ciancio, E.; Piacenza, G.; Lamberti, R.; Martorana, S. *Appl. Surf. Sci.* **2012**, *258*, 7889.
34. Jo, S. Y.; Park, J. S.; Gwon, H. J.; Shin, Y. M.; Khil, M. S.; Nho, Y. C.; Lim, Y. M. *Radiat. Phys. Chem.* **2012**, *81*, 846.
35. Jose, M V.; Thomas, V.; Dean, D R.; Nyairo, E. *Polymer* **2009**, *50*, 3778.
36. Kodama, Y.; Batista de Lima, N.; Giovedi, C.; Machado, L. D. B.; Aparecido, W.; Calvo, P.; Oishi, A.; Nakayama, K. *J. Phys. Sci. Appl.* **2012**, *2*, 4, 80.
37. Reinhold, I.; Kalkis, V.; Zicans, J.; Meri, R. M.; Elksnite, I. *Mater. Sci. Appl. Chem.* **2012**, *25*, 16.
38. Sevil, U. A.; Gűven, O. *Radiat. Phys. Chem.* **1995**, *46*, 4, 875.
39. Silindir, M.; Özer, A. Y.; *FABAD J. Pharm. Sci.* **2009**, *34*, 43.
40. Makadia, H. K.; Siegel, S. J. *Polymers* **2011**, *3*, 1377.
41. Liggins, R. *Int. J. Pharm.* **2001**, *222*, 19.