Electron beam sterilization of implantable rods with risperidone and with 17-β-estradiol: a structural, thermal and morphology study

JUSTYNA WILIŃSKA[1], ARTUR TUREK[1]*, ALEKSANDRA BORECKA[2], JAKUB RECH[1], JANUSZ KASPERCZYK[1, 2]

[1] School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Katowice, Chair and Department of Biopharmacy, Sosnowiec, Poland.
[2] Centre of Polymer and Carbon Materials, Polish Academy of Sciences, Zabrze, Poland.

Purpose: Poly(L-lactide-co-glycolide-co-trimethylene carbonate) rods with risperidone and 17-β-estradiol were sterilized by electron beam irradiation. The aim of the study was to assess electron beam irradiation impact on terpolymer composition, chain microstructure, glass transition temperature, molecular weight and the morphological features of rods. *Methods*: Hot melt extrusion in the formulation of rods was applied. Sterilization of the rods was performed by electron beam in an electron beam accelerator (10 MeV, 360 mA, 25 kGy). The following methods in the development of rods were applied: nuclear magnetic resonance, differential scanning calorimetry, gel permeation chromatography and scanning electron microscopy. *Results*: Sterilization influenced only glass transition temperature in blind rods and rods with risperidone. As for the other parameters, no significant changes were observed as far as a sterilization effect is concerned. However, some changes were noted after introducing drug substances and after extrusion. *Conclusions*: Electron beam irradiation of rods with risperidone and rods with 17-β-estradiol is an adequate method for sterilizing implantable drug delivery systems.

Key words: *electron beam sterilization, implantable rods, poly(L-lactide-co-glycolide-co-trimethylene carbonate), risperidone, 17-β-estradiol*

1. Introduction

Schizophrenia is one of several neuropsychiatric disorders that manifest a complex of syndromes, e.g., positive, negative and cognitive deficits. In the last six decades, intensive progress was observed in this area in both pharmacotherapy and drug technology.

The introduction of typical neuroleptics, i.e., chlorpromazine and haloperidol, was a significant breakthrough in the treatment of schizophrenia, and many novel drug substances have been developed since then. Currently, atypical neuroleptics, such as risperidone (RSP), are the most commonly used to treat the symptoms of schizophrenia.

It is known that schizophrenia is also considered to be a neurodegenerative disease. Previously, it was proved that 17-β-estradiol (E2) possesses neuroprotective properties against Parkinson's and Alzheimer's disease. E2 inhibits dopamine action, improves neuronal regeneration, neuroplasticity and cognitive functions. However, RSP may reduce the level of endogenous E2 [12]. For these reasons, a complex therapy with RSP and E2 should be considered.

Nowadays, RSP is administered via various oral formulations like solutions, tablets, disintegrating tablets and by intramuscular microparticles as an aqueous injection. However, novel propositions of the solid implantable drug delivery systems with RSP are also developed [27], [28]. According to the literature data, poly(lactide-co-glycolide) (PLGA) copolymers are the most often proposed as carriers for prolonged RSP release [1]. Many issues should be solved at the stage of formulation design [11], [13], [26], and one of them

is sterilization of the drug carrier. Beta irradiation (electron beam (EB)) and gamma irradiation are the routine sterilization methods of PLGA copolymers [7]. It is worth noting that EB processing is characterized by a low exposure time (less than 1 min) and a high dose rate and low penetration. Gamma sterilization, on the other hand, is connected with a longer exposure time (6–24 h), a lower dose rate and higher penetration [4], [7], [25] than EB sterilization.

According to the literature, the irradiation process may significantly influence the properties of the drug formulations and medical devices based on polymers (composition, chain microstructure, thermal properties, molecular weight (M_n) and morphology) [4], [17], [19], [23]. Changes in polymer features occurring during irradiation may be the result of the cross-linking and chain scission [5], [7], [9], [14], [16], [21], [24], [30].

It should be noted that EB or gamma irradiation is also routinely applied to sterile drug substances (e.g., E2 and RSP) and medicinal products (e.g., Rispolept Consta®, Risperdal Consta® with RSP) to keep maintained stability and biological activity [18], [29].

The aim of this study was to assess the EB irradiation impact on terpolymer composition, chain microstructure, glass transition temperature (T_g), M_n and morphology of poly(L-lactide-co-glycolide-co-trimethylene carbonate) (P(L-LA:GA:TMC)) extruded rods with RSP (P(L-LA:GA:TMC)-RSP) and rods with E2 (P-(L-LA:GA:TMC)-E2).

2. Materials and methods

2.1. Terpolymer

P(L-LA:GA:TMC) (57:19:24) (59000 Da) was synthesized in bulk with the use of a low-toxic initiator zirconium (IV) acetylacetonate (Sigma-Aldrich) at the Centre of Polymer and Carbon Materials of the Polish Academy of Sciences in Zabrze, according to previously developed method [3].

2.2. Extrusion process

P(L-LA:GA:TMC) was used to prepare three kinds of rods: (i) a P(L-LA:GA:TMC) rod, (ii) a P(L-LA:GA:TMC) rod with 10% w/w of RSP (Teva, Kutno) (P(L-LA:GA:TMC)-RSP) and (iii) a P(L-LA:GA:TMC) rod with 10% w/w of E2 (Sigma, USA) (P-(L-LA:GA:TMC)-E2) by hot melt extrusion. Before the process, the terpolymer was dried under a vacuum and subjected to

grinding at a temperature of –196 °C in a cryogenic mill (6870 SPEX, USA). E2 or RSP was mixed with milled raw terpolymer. The mixtures were vortexed and fed to an extruder cylinder heated to 105 °C. This process was carried out in a co-rotating twin screw extruder (Minilab, Thermo-Haake, GE) using a plasticizing screw rotational speed of 20 rpm. The molten mixture was extruded through a 0.7 mm diameter die. The molded rods were received on a chilled roll. Afterwards, rods 1 mm in diameter and 10 mm long were formulated.

2.3. Sterilization process

Sterilization of the rods was performed by electron beam in an EB accelerator (10 MeV, 360 mA, 25 kGy). The process was conducted at the Institute of Nuclear Chemistry and Technology at the Radiation Research and Technology Center (Certificate No. 625/2017/E).

2.4. Terpolymer composition and chain microstructure study

The composition and chain microstructure study were determined by nuclear magnetic resonance spectroscopy (NMR). Spectra were recorded using a Bruker-Avance II Ultrashield Plus spectrometer operating at 600 MHz (^{1}H) and 150 MHz (^{13}C) using DMSO-d6 as a solvent, with a 5 mm sample tube. ^{1}H NMR spectra were obtained with 32 scans, 11 μs pulse width and 2.65 s acquisition time. ^{13}C NMR spectra were obtained with 20 000 scans, 9.4 μs pulse width and 0.9 s acquisition time. Signals observed in ^{1}H and ^{13}C NMR spectra were assigned to appropriate sequences in the polymer chain. The molar percentages of lactidyl (F_{LL}), glycolidyl (F_{GG}) and carbonate (F_{TMC}) units as well as the average length of lactidyl (l_{LL}), glycolidyl (l_{GG}) and carbonate (l_{TMC}) blocks were calculated according to a previously described procedure [8].

2.5. Thermal study

The study of the thermal properties of the analyzed rods was carried out by the differential scanning calorimetry (DSC) method. The measurements were performed with a TA DSC 2010 apparatus (TA Instruments, New Castle, DE) calibrated using standards of indium and gallium with high purity and worked under a nitrogen atmosphere (a flow rate of 50 ml/min). The rod samples were heated to 190 °C during the first heating run, then the melted samples were rapidly

cooled to –30 °C. During the second heating run, the rods were heated within a range of –30 °C to 190 °C. The T_g was determined as the midpoint of the heat capacity change of the amorphous sample from the second heating run. DSC measurements were performed according to previously described procedure [28].

2.6. Molecular weight and molecular weight distribution study

M_n and molecular weight distribution (D) of the samples were determined by gel permeation chromatography (GPC) using a Viscotek Rimax chromatograph with two Viscotek 3580 columns and a Shodex SE 61 detector. The process was performed with a flow rate of 1 ml/min and with chloroform used as a solvent. The M_n value was calibrated with polystyrene standards.

2.7. Morphology study

The surface and cross-section of the rods were characterized by scanning electron microscopy (SEM)

(Quanta 250 FEG, FEI Company, USA) operating under low vacuum conditions (80Pa) and an acceleration voltage of 5 kV from secondary electrons collected by a large field detector.

Heterogeneity, circularity and solidity of the structures were developed by using software ImageJ® version 1.49e (National Institutes of Health, Bethesda, MD, USA). Appropriate magnification of the SEM images was a length scale calibrated to a known distance and converted to binary. Solidity (value of the monolithic area), circularity (a value of 1.0 indicates a perfect circle) and heterogeneity (percentage of the elevation area compared to the total area) were determined.

3. Results

3.1. Terpolymer composition and chain microstructure changes

Figures 1 and 2 present the ^{1}H NMR spectrum and ^{12}C NMR spectrum, respectively. In Table 1 the NMR
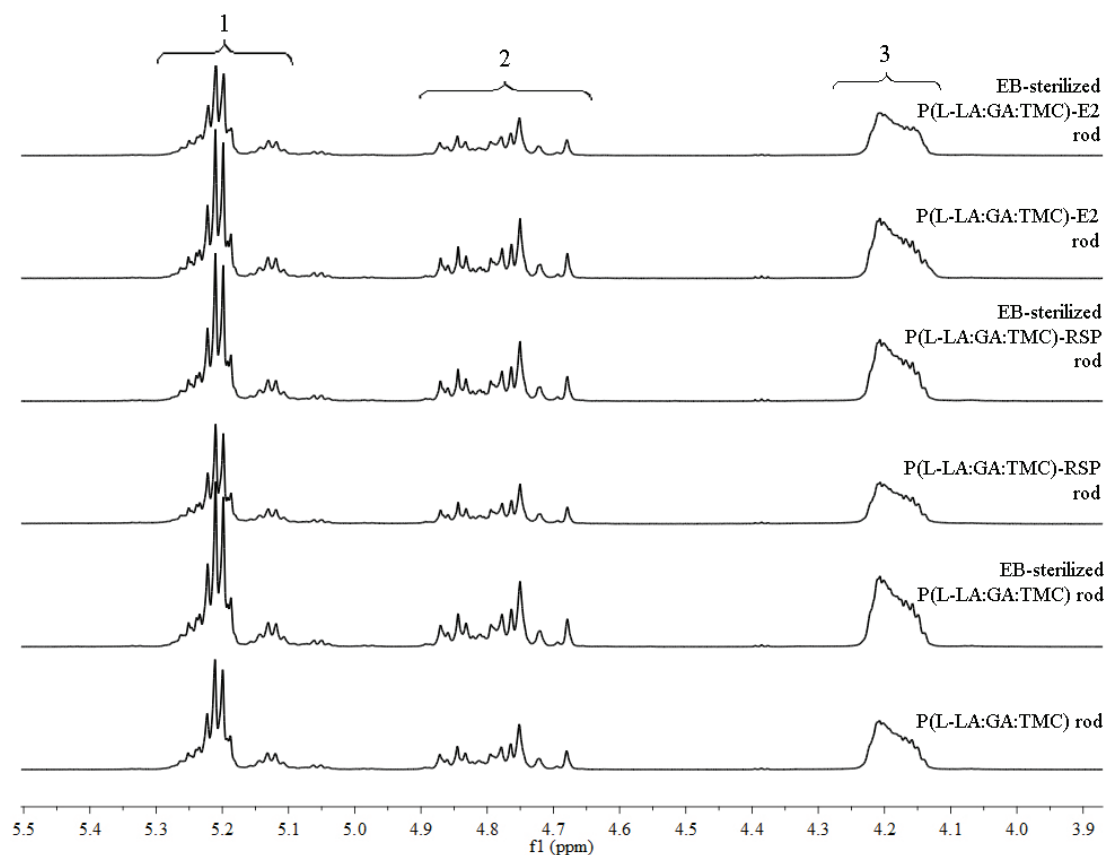

Fig. 1. ^{1}H NMR spectra of the P(L-LA:GA:TMC) rod, EB-sterilized P(L-LA:GA:TMC) rod, P(L-LA:GA:TMC)-RSP rod, EB-sterilized P(L-LA:GA:TMC)-RSP rod, P(L-LA:GA:TMC)-E2 rod and EB-sterilized P(L-LA:GA:TMC)-E2 rod (600 MHz, DMSO-d6).
1 – Methine proton region of the lactidyl units CH; 2 – methylene proton region of the glycolidyl units CH$_2$;
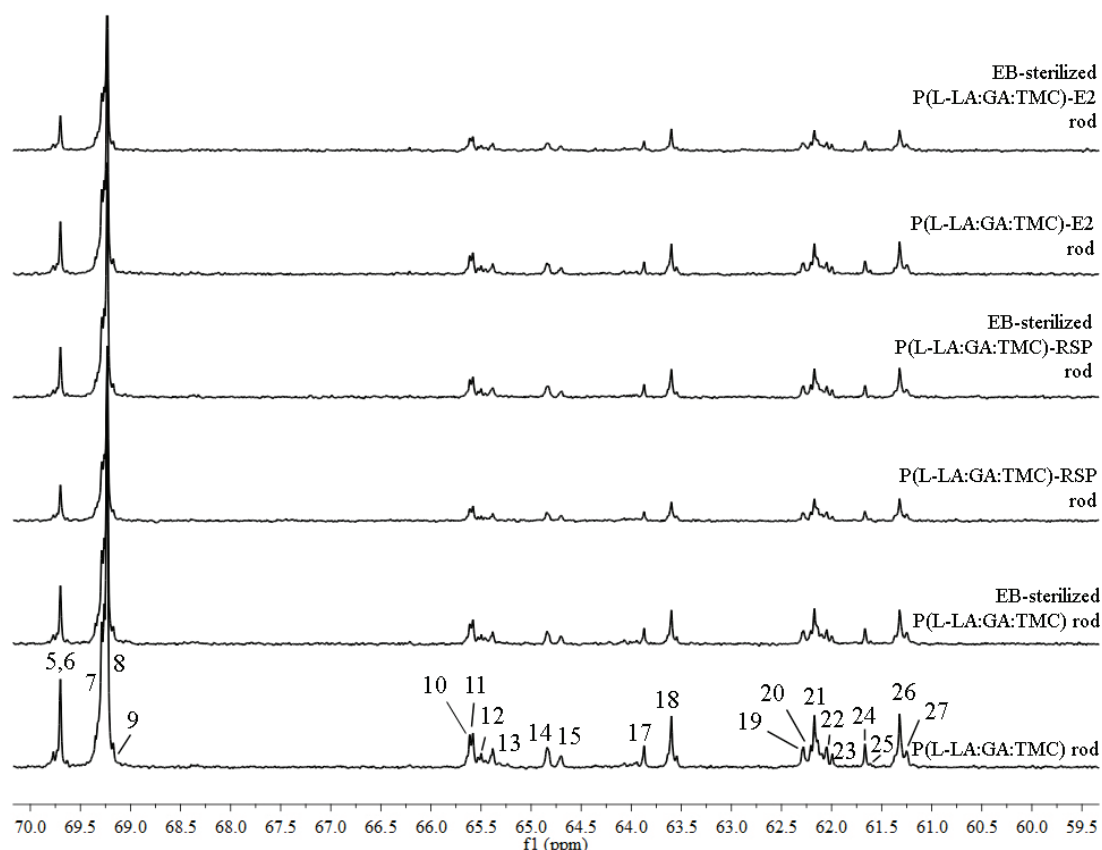3 – methylene proton region of the carbonate units (3) CH$_2$

Fig. 2. ^{13}C NMR spectra of the P(L-LA:GA:TMC) rod, EB-sterilized P(L-LA:GA:TMC) rod, P(L-LA:GA:TMC)-RSP rod,
EB-sterilized P(L-LA:GA:TMC)-RSP rod, P(L-LA:GA:TMC)-E2 rod and EB-sterilized P(L-LA:GA:TMC)-E2 rod (150 MHz, DMSO-d6).
5-GLLT+GLLLT, 6-TLLLL+LLLLT, 7-LLLL, 8-LLGG, 9-GLG, 10-TT'G+GGT'GG+TGT'GG, 11-GGTT, 12-GGT'GT+GT'GT,
13-GGT'GT+TT'L+LTT', 14-TT'T+TTT'+TT''G, 15-TT'GG, 17-TGT+TGGL+TGGT, 18-LGGGT+TGGGT+GGGGT,
19-TT'L+LT'T, 20-LT'L+LT''L, 21-GGT'T, 22-GGT''GT+GGT''GG, 23-TGT''GG+TGT'GT,
24-TGGGG+TTGG, 25-TGGT, 26-GGLL, 27-GGGG

Table 1. Composition and chain microstructure of the P(L-LA:GA:TMC) rod, EB-sterilized P(L-LA:GA:TMC) rod,
P(L-LA:GA:TMC)-RSP rod, EB-sterilized P(L-LA:GA:TMC)-RSP rod, P(L-LA:GA:TMC)-E2 rod
and EB-sterilized P(L-LA:GA:TMC)-E2 rod

Rod	F_{LL} [mol%]	F_{GG} [mol%]	F_{TMC} [mol%]	l_{LL}	l_{GG}	l_{TMC}
P(L-LA:GA:TMC)	57.3	18.2	24.5	4.5	1.1	1.5
EB-sterilized P(L-LA:GA:TMC)	57.0	18.2	24.7	4.3	1.1	1.5
P(L-LA:GA:TMC)-RSP	57.6	18.2	24.23	4.5	1.1	1.5
EB-sterilized P(L-LA:GA:TMC)-RSP	57.4	18.2	24.7	4.4	1.1	1.5
P(L-LA:GA:TMC)-E2	58.0	17.7	24.3	4.5	1.1	1.5
EB-sterilized P(L-LA:GA:TMC)-E2	57.5	17.6	24.9	4.4	1.1	1.5

F_{LL} – molar percentage of lactidyl units in terpolymer, F_{GG} – molar percentage of glycolidyl units in terpolymer, F_{TMC} – molar percentage of carbonate units in terpolymer, l_{LL} – average length of lactidyl blocks, l_{GG} – average length of glycolidyl blocks, l_{TMC} – average length of carbonate blocks.

parameters, i.e., terpolymer composition (F_{LL}, F_{GG}, F_{TMC}) and chain microstructure (l_{LL}, l_{GG}, l_{TMC}) are shown. The analyses of the spectra and data revealed no significant changes as a result of the presence of drug substances and EB sterilization.

3.2. Thermal properties study

The P(L-LA:GA:TMC) rods shows T_g at 40.7 °C. The introduction of both RSP and E2 into the rods did

not significantly influence changes in these parameters, i.e., 40.5 °C and 41.0 °C, respectively.
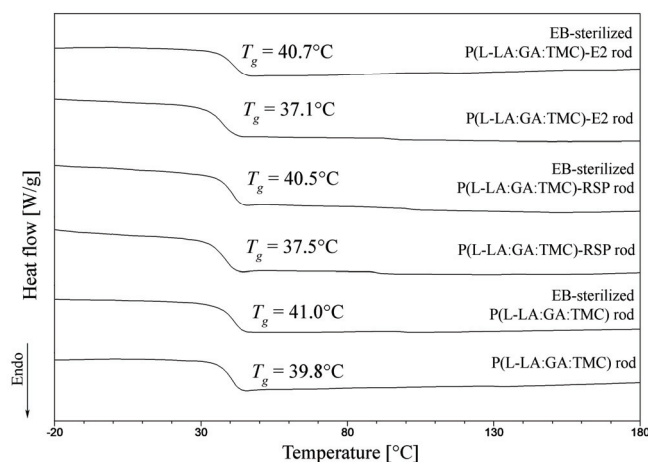

Fig. 3. DSC second heating run curves of the P(L-LA:GA:TMC) rod, EB-sterilized P(L-LA:GA:TMC) rod, P(L-LA:GA:TMC)-RSP rod, EB-sterilized P(L-LA:GA:TMC)-RSP rod, P(L-LA:GA:TMC)-E2 rod and EB-sterilized P(L-LA:GA:TMC)-E2 rod

In turn, EB sterilization resulted in a decrease in T_g for the blind rods and rods with RSP, i.e., 37.1 °C and 37.5 °C, respectively. Sterilization of rods with E2 did not influence T_g significantly (Fig. 3).

3.3. Molecular weight and molecular weight distribution study

The addition of RSP to P(L-LA:GA:TMC) revealed a decrease in M_n, i.e., 52%. No changes were observed for rods with E2. The sterilization process did not significantly influence M_n and D for any of the rods (Table 2).

Table 2. Molecular weight and molecular weight distribution of the P(L-LA:GA:TMC) rod, EB-sterilized P(L-LA:GA:TMC) rod, P(L-LA:GA:TMC)-RSP rod, EB-sterilized P(L-LA:GA:TMC)-RSP rod, P(L-LA:GA:TMC)-E2 rod and EB-sterilized P(L-LA:GA:TMC)-E2 rod

Rod	M_n [kDa]	D
P(L-LA:GA:TMC)	45.5	2.2
EB-sterilized P(L-LA:GA:TMC)	45.1	2.0
P(L-LA:GA:TMC)-RSP	23.7	2.2
EB-sterilized P(L-LA:GA:TMC)-RSP	23.3	2.4
P(L-LA:GA:TMC)-E2	45.8	1.9
EB-sterilized P(L-LA:GA:TMC)-E2	45.5	2.0

M_n – molecular weight (kDa), D – molecular weight distribution.

3.4. Morphology study

The surface of P(L-LA:GA:TMC) rods, P(L-LA:GA:TMC)-RSP rods and P(L-LA:GA:TMC)-E2 rods revealed no porosity. Circularity was in the range of 0.010–0.014. RSP and E2 loading resulted in no changes of this parameter. Solidity also showed no differences. However, the presence of RSP and E2 resulted in an increase in surface heterogeneity, i.e., 14.4% and 14.2%, respectively. EB sterilization did not influence the above-mentioned parameters (Fig. 4, Table 3).

The cross-sections of the P(L-LA:GA:TMC) rods, P(L-LA:GA:TMC)-RSP rods and P(L-LA:GA:TMC)-E2 rods also showed no porosity. Circularity was in the range of 0.003–0.051. RSP and E2 loading resulted in insignificant changes. Solidity did not show any differences. However, the presence of RSP and E2 resulted in an increase in surface heterogeneity, i.e., 36.25% and 36.51%, respectively. EB sterilization did not influence the above-mentioned parameters (Fig. 4, Table 4).

4. Discussion

4.1. Terpolymer composition and chain microstructure changes

Terpolymer composition and chain microstructure are important factors that affect the degradation rate of drug carriers, including PLGA copolymers and other aliphatic polyesters [27], [29].

In this study, the analyses of ^{1}H NMR spectra and ^{12}C NMR spectra revealed no significant changes in the composition and chain microstructures of all kinds of EB-processed rods (Figs. 1 and 2, Table 1).

This issue has been briefly developed and discussed in the literature. Only Plikk and co-workers pointed to the influence of EB sterilization (25 kGy) on the composition and chain microstructure of scaffolds based on various kinds of copolymers, i.e., (i) 1,5-dioxepan-2-one (DXO): L-lactide (L-LA), (ii) DXO: ε-caprolactone (CL) and (iii) L-LA: CL with various contents of co-monomers. The composition changes after EB irradiation for co-polymers with a higher content of lactide, an increase in l_{LL} and decrease in l_{CL} were noted for the analyzed copolymers [21]. It is difficult to expect significant changes when sterilization is the last stage of processing. Therefore, the changes may be due to another background. In this study, no significant changes were observed in terpolymer composition and chain microstructure (Table 1), which was in line with expectations.

Surface   Cross-section
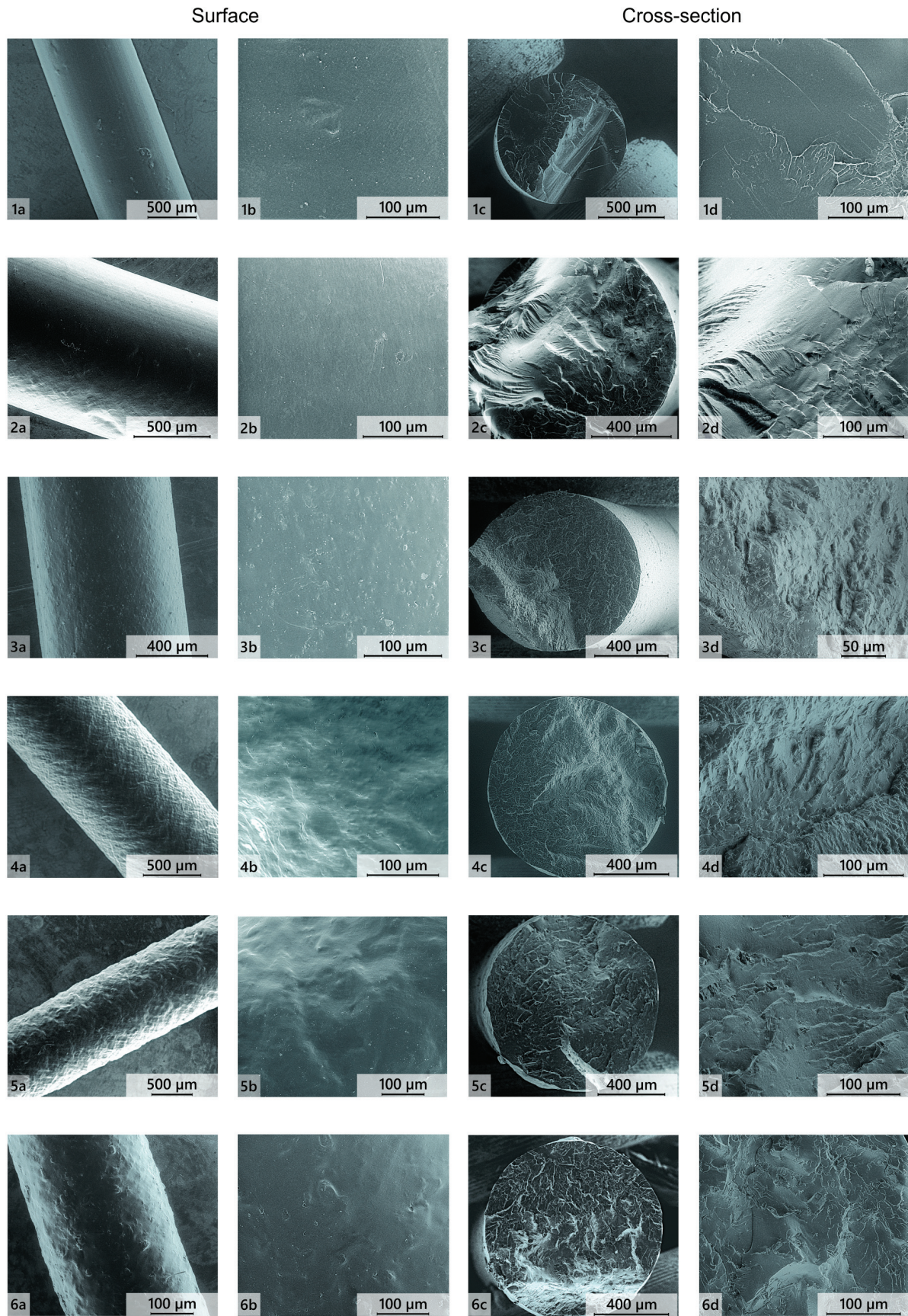

Fig. 4. SEM images of the P(L-LA:GA:TMC) rod (1a–1d), EB-sterilized-P(L-LA:GA:TMC) rod (2a–2d),
P(L-LA:GA:TMC)-RSP rod (3a–3d), EB-sterilized-P(L-LA:GA:TMC)-RSP rod (4a–4d),
P(L-LA:GA:TMC)-E2 rod (5a–5d), EB-sterilized-P(L-LA:GA:TMC)-E2 rod (6a–6d)

Table 3. Circularity, solidity and heterogeneity of surfaces of the P(L-LA:GA:TMC) rod (magnification 1036×), EB-sterilized P(L-LA:GA:TMC) rod (magnification 1130×), P(L-LA:GA:TMC)-RSP rod (magnification 196×), EB-sterilized P(L-LA:GA:TMC)-RSP rod (magnification 1006×), P(L-LA:GA:TMC)-E2 rod (magnification 598×) and EB-sterilized P(L-LA:GA:TMC)-E2 rod (magnification 734×), as calculated by ImageJ®

Rods	Area [μm]	Circularity	Solidity	Heterogeneity [%]
P(L-LA:GA:TMC)	15347.8	0.014	0.91	32.8
EB-sterilized P(L-LA:GA:TMC)	2070.3	0.083	0.87	34.8
P(L-LA:GA:TMC)-RSP	19177.5	0.017	0.89	47.2
EB-sterilized P(L-LA:GA:TMC)-RSP	18135.5	0.054	0.86	48.0
P(L-LA:GA:TMC)-E2	4910.3	0.010	0.91	47.0
EB-sterilized P(L-LA:GA:TMC)-E2	2797.9	0.002	0.91	47.3

Area – total area of image after set scale; Circularity – a value of 1.0 indicates a perfect circle; Solidity – value of the monolithic area; Heterogeneity – percentage of the elevation area compared to the total area.

Table 4. Circularity, solidity and heterogeneity of cross-sections of the P(L-LA:GA:TMC) rod (magnification 1131×), EB-sterilized P(L-LA:GA:TMC) rod (magnification 1131×), P(L-LA:GA:TMC)-RSP rod (magnification 1037×), EB-sterilized P(L-LA:GA:TMC)-RSP rod (magnification 1131×), P(L-LA:GA:TMC)-E2 rod (magnification 1037×) and EB-sterilized P(L-LA:GA:TMC)-E2 rod (magnification 1037×), as calculated by ImageJ®

Rod	Area [μm]	Circularity	Solidity	Heterogeneity [%]
P(L-LA:GA:TMC)	35611.1	0.032	0.89	13.9
EB-sterilized-P(L-LA:GA:TMC)	34883.9	0.048	0.90	13.8
P(L-LA:GA:TMC)-RSP	3845.8	0.003	0.85	36.3
EB-sterilized-P(L-LA:GA:TMC)-RSP	7371.6	0.012	0.89	36.5
P(L-LA:GA:TMC)-E2	37481.2	0.051	0.84	39.2
EB-sterilized-P(L-LA:GA:TMC)-E2	645.7	0.010	0.89	40.4

Area – total area of image after set scale, Circularity – a value of 1.0 indicates a perfect circle, Solidity – value of the monolithic area, Heterogeneity – percentage of the elevation area compared to the total area.

4.2. Thermal properties

The analyses of T_g for P(L-LA:GA:TMC) rods, P(L-LA:GA:TMC)-RSP rods and P(L-LA:GA:TMC)-E2 rods pointed to the lack of either antiplastification or plastification effects caused by the presence of the drug substances. A decrease in T_g for the blind rods and rods with RSP as a sterilization effect was also observed (Fig. 3). According to the literature data, this effect may be related to chain scission [7], [16], [22], [23]. It is known that the value of T_g for drug carriers should be higher than the body temperature because of the transition from a vitreous to an elastic state [8], which was shown in this study.

Additionally, the lack of any significant changes after sterilization for rods with E2 may result from E2's properties. It may suggest that E2 protects the polymer chains against radiolysis via free radical scavenger properties. The same effect was also revealed by Mohrr and co-workers in a study on D,L-PLGA (75:25) microparticles with E2 sterilized by gamma irradiation [18]. This phenomenon was also confirmed in biochemical studies in other areas [15].

On the other hand, the lack of any significant changes in T_g in the case of rods with E2 may have also resulted from terpolymer cross-linking or the presence of E2-terpolymer interactions. However, a decrease in T_g was observed for blind rods, therefore a significant cross-linking effect is not possible. Moreover, the lack of interactions between E2 and P(L-LA:GA:TMC) was shown by the transmission technique of Fourier--transform infrared spectroscopy as previously described [30]. Therefore, the lack of a T_g decrease may have resulted from E2's antioxidant properties. Moreover this data reflected previous study on thermal properties of EB-sterilized P(L-LA:GA:TMC) matrices [23].

4.3. Molecular weight and molecular weight distribution study

In this study, a significant decrease was revealed in M_n in rods with RSP (Table 2). This effect resulted from thermal degradation during hot melt extrusion. According to the literature data, significant decrease

in M_n, which may be caused by the presence of moisture and oxygen, the formulation temperature and period residence of the polymer in the extruder, the shear stress exerted on the polymer, random chain scission and the autocatalytic degradation process [6], [27] was noted.

For rods with E2, no decrease in M_n was revealed (Table 2), which may have resulted from the free radical scavenger properties of E2 [3], [15], as described in the section with thermal properties.

In this study, the sterilization process did not have an influence on M_n (Table 2). The influence of EB sterilization on the M_n of PLGA, poly(trimethylene carbonate) (PTMC) or poly(ε-caprolactone) (PCL) was relatively well developed. Chain scission and cross-linking may take place during this process and result in either a decrease or increase in M_n, respectively [7], [10], [20], [22], [24]. However, these results did not reflect previously study on P(L-LA:GA:TMC) matrices, in which a decrease of M_n was observed. This effect might result from formulation, shape and material composition [23]. The lack of M_n changes may result from the balance of these phenomena or from a non-significant impact of the applied dose.

4.4. Morphology study

The loading of RSP and E2 influenced an increase in rod heterogeneity (Fig. 4, Tables 3 and 4). This was shown in a previous study, in which changes in the morphology of wafers and rods were noted [8], [29]. Other parameters, e.g., circularity and solidity, remained at the same level.

According to the literature data, structure disintegration, pores and cracks, microcavities and deformation as a result of high energy action and radiolysis during EB sterilization were observed [2], [5], [9], [23]. In this study, no changes in morphology as a result of EB irradiation were revealed. These results were also presented in a study by Cairns and co-workers [2] who revealed the lack of morphological changes of P(L-LA) sheets after EB irradiation at 150 kGy and 500 kGy. This may suggest the possibility of applying higher doses for rods based on P(L-LA:GA:TMC) if necessary.

5. Conclusions

In summary, EB sterilization (25 kGy) of P(L-LA:GA:TMC) rods with RSP and E2 revealed insignificant changes in tested properties. A decrease was noted only for T_g. However, the values of T_g did not decrease below body temperature, which may aggravate the quality of the medicinal product. Moreover, negligible changes in polymer composition, chain microstructure, M_n and morphological features were noted. Therefore, EB sterilization is an adequate method in the processing of rods with RSP and E2 based on P(L-LA:GA:TMC).

Acknowledgements

Conflict of interests

The authors declare that there is no conflict of interest.

References

[1] AMANN L.C., GANDAL M.J., LIN R., LIANG Y., SIEGEL S.J., *In vitro–in vivo correlations of scalable PLGA-risperidone implants for the treatment of schizophrenia*, Pharm. Res, 2010, 27(8), 1730–1737, DOI: 10.1007/s11095-010-0152-4.

[2] CAIRNS M.L., DICKSON G.R., ORR J.F., FARRAR D., HARDACRE C., SA J., LEMOINE P., MUGHAL M.Z., BUCHANAN F.J., *The potential of electron beam radiation for simultaneous surface modification and bioresorption control of PLLA*, J. Biomed. Mater. Res. A, 2012, 100 (9), 2223–2229, DOI: 10.1002/jbm.a.34156.

[3] DOBRZYNSKI P., KASPERCZYK J., SMOLA A., PASTUSIAK M., SOBOTA M., Patent No. EP 2647656 A2, Munich, Germany, European Patent Office.

[4] DORATI R., COLONNA C., SERRA M., GENTA I., MODENA T., PAVANETTO F., PERUGINI P., CONTI B., *γ-Irradiation of PEGd, lPLA and PEG-PLGA multiblock copolymers: I. effect of irradiation dose*, AAPS PharmSciTech, 2008, 9 (2), 718–725, DOI: 10.1208/s12249-008-9103-3.

[5] DORATI R., PATRINI M., PERUGINI P., PAVANETTO F., STELLA A., MODENA T., GENTA I., CONTI B., *Surface characterization by atomic force microscopy of sterilized PLGA microspheres*, J Microencapsul, 2006, 23 (2), 123–133, DOI: 10.1080/02652040500435220.

[6] ELLÄ V., NIKKOLA L., KELLOMÄKI M., *Process-induced monomer on a medical-grade polymer and its effect on short-term hydrolytic degradation*, J. Appl. Polym. Sci., 2011, 119 (5), 2996–3003, DOI: 10.1002/app.33027.

[7] FRIESS W., SCHLAPP M., *Sterilization of gentamicin containing collagen/PLGA microparticle composites*, Eur. J. Pharm. Biopharm., 2006, 63 (2), 176–187, DOI: 10.1016/j.ejpb.2005.11.007.

[8] GĘBAROWSKA K., KASPERCZYK J., DOBRZYŃSKI P., SCANDOLA M., ZINI E., LI S., *NMR analysis of the chain microstructure of biodegradable terpolymers with shape memory properties*, Eur. Polym. J., 2011, 47 (6), 1315–1327, DOI: 10.1016/j.eurpolymj.2011.02.022.

[9] HOLY C.E., CHENG C., DAVIES J.E., SHOICHET M.S., *Optimizing the sterilization of PLGA scaffolds for use in tissue engineering*, Biomaterials, 2000, 22 (1), 25–31, DOI: 10.1016/S0142-9612(00)00136-8.

[10] JOZWIAKOWSKA J., WACH R.A., ROKITA B., ULAŃSKI P., NALAWADE S.P, GRIJPMA D.W., FEIJEN J., ROSIAK J.M., *Influence of electron beam irradiation of physicochemical properties of poly(timetylene carbonate)*, Polym. Degrad. Stab., 2011, 96 (8), 1430–1437, DOI: 10.1016/j.polymdegradstab. 2011.05.010.

[11] KOBIELARZ M., GAZIŃSKA M., TOMANIK M., STĘPAK B., SZUSTAKIEWICZ K., FILIPIAK J., ANTOŃCZAK A., PEZOWICZ C., *Physicochemical and mechanical properties of CO2 laser-modified biodegradable polymers for medical applications*, Polym. Degrad. Stabil., 2019, 165, 182–195, DOI: 10.1016/j.polymdegradstab.2019.05.010.

[12] KONARZEWSKA B., WOŁCZYŃSKI S., SZULC A., GALIŃSKA B., POPŁAWSKA R., WASZKIEWICZ N., *Effect of risperidone and olanzapine on reproductive hormones, psychopathology and sexual functioning in male patients with schizophrenia*, Psychoneuroendocrino, 2009, 34 (1), 129–139, DOI: 10.1016/j.psyneuen.2008.08.015.

[13] KOTLARZ M., JORDAN R., WEGENER E., DOBRZYŃSKI P., NEUNZEHN J., LEDERER A., WOLF-BRANDSTETTER C., PAMUŁA E., SCHARNWEBER D., *One step 3D printing of surface functionalized composite scaffolds for tissue engineering applications*, Acta Bioeng. Biomech., 2018, 20 (2), 35–45, DOI: 10.5277/ABB-01131-2018-02.

[14] LEONARD D.J., PICK L.T., FARRAR D.F., DICKSON G.R., ORR J.F., BUCHANAN F.J., *The modification of PLA and PLGA using electron-beam radiation*, J. Biomed. Mater. Res. A, 2009, 89 (3), 567–574, DOI: 10.1002/jbm.a.31998.

[15] LIU Y., SHIMIZU I., OMOYA T., ITO S., GU X.S., ZUO J., *Protective effect of estradiol on hepatocytic oxidative damage*, World J. Gastroenterol., 2002, 8(2), 363–366, DOI: 10.3748/wjg.v8.i2.363.

[16] LOO S.C.J., OOI C.P., BOEY Y.C.F., *Influence of electron-beam radiation on the hydrolytic degradation behaviour of poly(lactide-co-glycolide) (PLGA)*, Biomaterials, 2005, 26 (18), 3809–3817, DOI: 10.1016/j.biomaterials.2004.10.014.

[17] MARTINEZ-SANCHO C., HERRERO-VANRELL R., NEGRO S., *Study of gamma-irradiation effects on aciclovir poly(D,L-lactic-co-glycolic) acid microspheres for intravitreal administration*, J. Control Release, 2004, 99(1), 41–52, DOI: 10.1016/j.jconrel.2004.06.004.

[18] MOHR D., WOLFF M., KISSEL T., *Gamma irradiation for terminal sterilization of 17β-estradiol loaded poly-(D,L-lactide-co-glycolide) microparticles*, J. Control Release, 1999, 61 (1–2), 203–217, DOI: 10.1016/S0168-3659(99)00118-2.

[19] MONTANARI L., COSTANTINI M., SIGNORETTI E.C., VALVO L., SANTUCCI M., BARTOLOMEI M., FATTIBENE P., ONORI S., FAUCITANO A., CONTI B., GENTA I., *Gamma irradiation effects on poly(DL-lactictide-co-glycolide) microspheres*, J. Control Release, 1998, 56(1–3), 219–229, DOI: 10.1016/S0168-3659 (98)00082-0.

[20] PARK P.I.P., JONNALAGADDA S., *Predictors of glass transition in the biodegradable poly-lactide and poly-lactide-co-glycolide polymers*, J. Appl. Polym. Sci., 2006, 100 (3), 1983–1987, DOI: 10.1002/app.22135.

[21] PLIKK P., ODELIUS K., HAKKARAINEN M., ALBERTSSON A.C., *Finalizing the properties of porous scaffolds of aliphatic polyesters through radiation sterilization*, Biomaterials, 2006, 27 (31), 5335–5347, DOI: 10.1016/j.biomaterials.2006.06.024.

[22] ROSARIO-MELÉNDEZ R., LAVELLE L., BODNAR S., HALPERIN F., HARPER I., GRIFFIN J., UHRICH K.E., *Stability of a salicylate-based poly(anhydride-ester) to electron beam and gamma radiation*, Polym. Degrad. Stabil., 2011, 96 (9), 1625–1630, DOI: 10.1016/j.polymdegradstab.2011.06.005.

[23] RYCHTER P., ŚMIGIEL-GAC N., PAMUŁA E., SMOLA-DMOCHOWSKA A., JANECZEK H., PROCHWICZ W., DOBRZYŃSKI P., *Influence of radiation sterilization on properties of biodegradable lactide/glycolide/trimethylene carbonate and lactide/glycolide/ε-caprolactone porous scaffolds with shape memory behavior*, Materials (Basel), 2016, 9 (1), E64, DOI: 10.3390/ma9010064.

[24] SILINDIR M., OZER Y., *The effect of radiation on a variety of pharmaceuticals and materials containing polymers*, PDA J. Pharm. Sci. Technol., 2012, 66 (2), 184–199, DOI: 10.5731/pdajpst.2012.00774.

[25] SINTZEL M.B., MERKLI A., TABATABAY C., GURNY R., *Influence of irradiation sterilization on polymers used as drug carriers-a review*, Drug. Dev. Ind. Pharm., 1997, 23 (9), 857–878, DOI: 10.3109/03639049709148693.

[26] SZEWCZENKO J., KAJZER W., GRYGIEL-PRADELOK M., JAWORSKA J., JELONEK K., NOWIŃSKA K., GAWLICZEK M., LIBERA M., MARCINKOWSKI A., KASPERCZYK J., *Corrosion resistance of PLGA-coated biomaterials*, Acta Bioeng. Biomech., 2017, 19 (1), 173–179, DOI: 10.5277/ABB-00556-2016-04.

[27] TUREK A., BORECKA A., JANECZEK H., SOBOTA M., KASPERCZYK J., *Formulation of delivery systems with risperidone based on biodegradable terpolymers*, Int. J. Pharm., 2018, 548 (1), 159–172, DOI: 10.1016/j.ijpharm.2018.06.051.

[28] TUREK A., KASPERCZYK J., JELONEK K., BORECKA A., JANECZEK H., LIBERA M., GRUCHLIK A., DOBRZYŃSKI P., *Thermal properties and morphology changes in degradation process of poly(L-lactide-co-glycolide) matrices with risperidone*, Acta Bioeng. Biomech., 2015, 17 (1), 11–20, DOI: 10.5277/ABB-00210-2014-02.

[29] TUREK A., OLAKOWSKA E., BORECKA A., JANECZEK H., SOBOTA M., JAWORSKA J., KACZMARCZYK B., JARZĄBEK B., GRUCHLIK A., LIBERA M., LIŚKIEWICZ A., JĘDRZEJOWSKA-SZYPUŁKA H., KASPERCZYK J., *Shape-memory terpolymer rods with 17-β-estradiol for the treatment of neurodegenerative diseases: an in vitro and in vivo study*, Pharm. Res., 2016, 33(12), 2967–2978, DOI: 10.1007/s11095-016-2019-9.

[30] VETTEN M.A., YAH C.S., SINGH T., GULUMIAN M., *Challenges facing sterilization and depyrogenation of nanoparticles: effect on structural stability and biomedical application*, Nanomedicine, 2014, 10(7), 1391–1399. DOI: 10.1016/j.nano.2014.03.017.