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Thermal properties and morphology changes in degradation process of poly(L-lactide-co-glycolide) matrices with risperidone

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Determining thermal properties and morphology seems to be useful in the analysis of release and degradation processes form polymeric materials. Risperidone is available in the formulation of a long-acting injection based on poly(D,L-lactide-co-glycolide). Currently, alternative solutions are also offered, i.e., nano- and microparticles or implants, including copolymers of lactide and glycolide. The effect of risperidone content on the properties of poly(L-lactide-co-glycolide) matrices was determined. The study also involved an assessment of the changes during degradation. Risperidone free matrices and the matrices with risperidone were obtained by solvent casting. Thermal characteristics were tested by means of differential scanning calorimetry, and the morphology was evaluated using a scanning electron microscope.

Risperidone did not change significantly semi-crystalline structure of poly(L-lactide-co-glycolide) matrices. The decrease in crystallization temperature and glass transition temperature during degradation was observed. Many pores and their deformation, the widening of pore area, cracks and slits because of degradation were observed. The analysis of thermal properties and morphology allowed us to explain degradation process. Matrices exhibited stable process of degradation, which may be advantageous for development of prolonged risperidone release systems.

Key words: *risperidone, drug carriers, degradation, poly(L-lactide-co-glycolide), thermal properties, morphology*

1. Introduction

Risperidone (RSP) is a drug substance which is currently often used in the treatment of mental diseases. It is available in various formulations. However, it can be said that it is the most commonly used in orally administrated medicinal products. In the last two decades most studies comparing bioavailability and treatment efficiency of various formulations with neuroleptics (*inter alia* RSP) were performed [1]–[4].

The current trend favors prolonged release parenteral formulations. In the case of long-acting RSP, drug substance in medicinal product is administered as aqueous suspension of poly(D,L-lactide-co-glycolide) (D,L-PLGA) 75:25 microspheres [5]. However, alternative solutions, i.e., nano- and microparticles or implants (e.g., rods) containing copolymers of lactide and glycolide of various comonomer content were also developed [6]–[8].

Degradation is one of the important factors influencing drug release from PLGA matrices. Many factors are known to influence the biodegradation rate of a polymer (e.g., polymer chemistry, molecular architecture, molecular weight, morphology, area, geometry, porosity and a method of preparing) also the surrounding conditions (e.g., pH and temperature) or additives [9]–[12]. Regular degradation may provide

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uniform drug release. However, it should be noted that degradation is negligible in the very initial phase for high molecular weight polymers. Nevertheless, this period is very important considering the risk of burst effect. This may be caused by adsorption of drug substance on the surface of the matrix, which causes its rapid initial release [13]. Therefore, a surface may also play an important role in drug release. Lately, this topic has been receiving more attention [14], [15].

The choice of appropriate methods for monitoring degradation-related changes is crucial for development of novel drug formulation based on aliphatic polyesters. Apart from conventional methods used for the analysis of the degradation process, also combined analysis of thermal properties and morphological features seems to be equally important in formulations designed for prolonged drug release. Thermal properties, e.g., the melting temperature (T_m) and crystallization temperature (T_c) with enthalpy of melting (ΔH_m) and crystallization (ΔH_c), respectively, and the glass transition temperature (T_g) may be determined by means of differential scanning calorimetry (DSC). The comparison of these parameters for a native matrix (i.e., non-degraded) and a degraded matrix may be useful in the interpretation of changes in a matrix caused by the degradation process.

The analysis of T_m and ΔH_m during degradation allows us to track the changes in a crystalline phase of polymeric matrices. It is important for PLGA controlled drug delivery systems because it was reported that even initially amorphous copolymers containing larger amounts of lactidyl units can crystallize during degradation due to the presence of relatively long L-LA blocks [16]. This may cause irregular drug release or fluctuations after degradation of regions surrounding accumulated drug molecules. Therefore, even drug release may be provided by copolymers with random copolymer chain structure that do not crystallize during degradation [17]. Consequently, it may be easier also to explain and control drug release process. The T_g influences polymer chain mobility. A polymer undergoes slower degradation in vitreous state (below T_g) than in elastic state (above T_g) [18]. A decrease in T_g is one of the thermal parameters which give evidence to degradation changes of polymer matrix [19], [20].

Scanning electron microscope (SEM) is a one of the tools used commonly in the determination of degradation changes of PLGA drug carriers. It allows many morphological features to be observed, which may influence drug release i.e., homogenous vs. heterogenous surfaces, solid vs. porous, flat or undulating, etc. [19]–[21]. Therefore, combining such methods as DSC and SEM may be advantageous for the analysis of changes in matrix properties. The complex analysis allows not only a matrix surface to be observed, which is important in the first phase of drug release, but also a polymer matrix in further phases of degradation process [19]–[21]. The degradation of polymers may lead to an increase in matrix porosity, which may accelerate drug release and degradation due to improved water absorption. Small pores may grow during degradation, coalesce with neighboring pores to form fewer but larger pores. However, the pores may also be closed due to the decrease in T_g, which leads to increased polymer chain mobility [18], [22].

The aim of this study is to determine the effects of 5%-wt content of RSP on the thermal properties and morphology of poly(L-lactide-co-glycolide) (L-PLGA) matrices. The study also involved an assessment of changes occurring in the degradation process of matrices with RSP.

2. Materials and methods

2.1. Preparation of matrices

The matrices (10 mm in diameter) were obtained from L-PLGA 85:15 (100 000 Da). In the degradation study, samples containing RSP (5%-wt) (TevaKutno S.A., Kutno, Poland) prepared with the use of the solution casting method were used. The copolymer and the drug were dissolved in methylene chloride. To assess RSP influence on L-PLGA thermal properties, drug free matrices were also prepared.

L-PLGA was synthesized at the Centre of Polymer and Carbon Materials of the Polish Academy of Sciences in Zabrze in bulk with the use of $Zr(Acac)_4$ as a low toxic initiator according to the previously developed methodology [23], [24].

2.2. Degradation process

The matrices were incubated in a PBS buffer (pH 7.4) for 294 days under constant agitation (240 rpm) at 37 °C. The samples were analyzed after 0, 7, 14, 48, 58, 105 and 294 days of degradation.

Before measurements, the matrices were air dried at room temperature in the laminar box and then under reduced pressure.

2.3. Thermal properties of matrices

Thermal characteristics of L-PLGA were assessed with DSC, using the TA DSC 2010 apparatus (TA Instruments, New Castle, DE, USA) at a heating rate of 20 °C/min, in range from –20 to +200 °C, under the nitrogen atmosphere (flow = 50 mL/min). The instrument was calibrated with high purity indium and gallium.

The T_m, T_c and the values of ΔH_m and ΔH_c were obtained from the first heating run. The T_m was taken as the peak temperature maximum of melting endotherm from the first heating run, whereas T_c as the peak temperature maximum of crystallization exotherm. T_g was obtained from the second heating run for the amorphous samples, which were obtained by quenching from the melt. The T_g was obtained by heating at 20 °C/min in which this is the only phenomenon revealed. It was taken as the midpoint of heat capacity change for amorphous samples.

For pure RSP, DSC analysis was performed analogously.

2.4. Morphology of matrices

The matrices' surface morphology was assessed with a SEM (Quanta 250 FEG, FEI Company, Oregon, USA). The micrographs were obtained under low vacuum with an acceleration voltage 5 kV (80 Pa) or 10 kV (60 Pa) from secondary electrons, which were collected by Large Filed Detector (LFD). The samples were stuck to the microscopic stubs with a double-sided adhesive carbon tape. Micrographs analysis was carried out using ImageJ software.

3. Results

3.1. DSC measurements

The comparison of the thermograms obtained for the native L-PLGA matrix with RSP, the native L-PLGA matrix and pure RSP for the first heating run

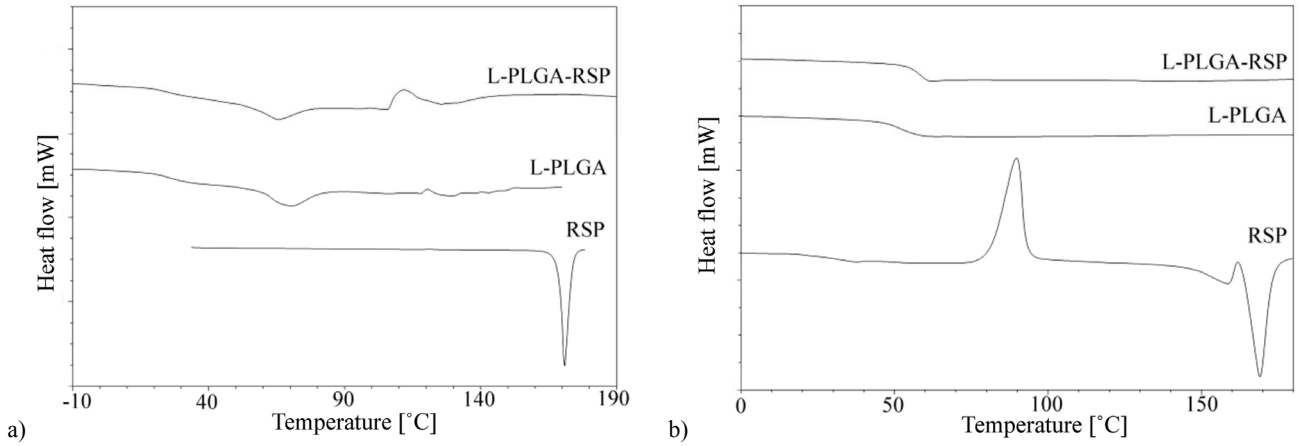

Fig. 1. The curves representing the first heating run (a) and second heating run (b) of DSC measurements obtained for the native L-PLGA matrix with RSP (L-PLGA-RSP), the native L-PLGA matrix and pure RSP

Table 1. Thermal properties of the native L-PLGA matrix with RSP (L-PLGA-RSP), the native L-PLGA matrix and pure RSP

Sample	First heating run (20 °C/min)						Second heating run (20 °C/min)				
	T_{m1} [°C]	ΔH_{m1} [J/g]	T_{m2} [°C]	ΔH_{m2} [J/g]	T_c [°C]	ΔH_c [J/g]	T_g [°C]	T_c [°C]	ΔH_c [J/g]	T_m [°C]	ΔH_m [J/g]
L-PLGA-RSP	65.5	7.6	125.6	3.5	111.0	3.0	57.0	ND	ND	ND	ND
L-PLGA	70.1	8.7	130.0	3.3	120.0	0.5	52.0	ND	ND	ND	ND
RSP	171.0	118.0	ND	ND	ND	ND	29.0	90.0	89.8	169.0	96.2

T_m and T_c – melting and crystallization temperature with ΔH_m and ΔH_c – enthalpy of melting and crystallization; T_g – glass transition temperature; ND – not detected.

(Fig. 1a) and for the second heating run (Fig. 1b) revealed many thermal effects.

The first heating run for matrices revealed two endotherms, which corresponded to the melting process. For L-PLGA matrix with RSP, melting points were observed at 65.5 °C (ΔH_{m1} = 7.6 J/g) and 125.6 °C (ΔH_{m2} = 3.5 J/g). The higher maximum for endotherms was observed for the free RSP sample, i.e., 70.1 °C and 130.0 °C, respectively (Table 1). The analysis of the endotherms revealed higher value for the first endotherm (ΔH_{m1} = 8.7 J/g) and lower for the second endotherm (ΔH_{m2} = 3.3 J/g) (Fig. 1a, Table 1) for RSP free matrices. Moreover, one exotherm corresponding to cold crystallization between these endotherms was also observed, i.e., for L-PLGA matrix with RSP exotherm with maximum at 111 °C (ΔH_c = 3.0 J/g) and for L-PLGA matrix exotherm with maximum at 120.0 °C (ΔH_c = 0.5 J/g) (Fig. 1a, Table 1). The analysis of the second heating run revealed an increase in T_g after introduction of RSP into the matrix from 52.0 °C to 57.0 °C (Fig. 1b, Table 1).

The analysis of the first (Fig. 1a) and the second (Fig. 1b) heating runs of pure RSP revealed endothermal peaks at 171.0 °C (ΔH_{m1} = 118.0 J/g) and 169.0 °C ($\Delta H_{m.}$ = 96.2 J/g), respectively (Table 1). In the second heating run, the T_g at 29.0 °C was noted. Moreover, T_c at 90.0 °C with ΔH_c = 89.8 J/g was observed (Fig. 1b, Table 1).

The differences between thermograms obtained from the first heating run (Fig. 2a, Table 2) and the second heating run (Fig. 2b, Table 2) for the samples with RSP degraded for 0, 7, 14, 48, 58, 105, 294 days were observed. It was found that the changes depended on degradation time (Fig. 2a and b, Table 2). For the first heating run endothermic and exothermic events were observed in all the curves. Analogously to the findings presented above (Fig. 1a and b, Table 1), the first one corresponded to melting, whereas the second one – to the cold crystallization (Fig. 2a and b, Table 2). For matrices degraded within 7, 14, 48, 58, 105 and 294 days, two endotherms were observed (Fig. 2a, Table 2) as described above for the native sample (Fig. 1a, Table 1). However, the sample degraded for 0, 7 and 14 days revealed the widest endothermal area. The endotherms were the least marked (Fig. 2a). Furthermore, one exotherm was observed between two endotherms for the samples degraded for 0, 7, 14, 48, 58 and 105 days (Fig. 2a, Table 2). For the sample degraded for 294 days, the findings involved the first slightly marked endotherm and the second strongly marked endotherm without exotherm (Fig. 2a, Table 2).

It should be pointed out that during the first heating run in all the curves no additional and significant endothermic and exothermic events were observed (Figs. 1 and 2, Tables 1 and 2).

The changes in the values of endothermal peaks were noted. Differences were found in the value of the maxima of first endotherm between the native samples (65.5 °C) and the samples degraded for 7, 14, 48, 58, 105 and 294 days (81.2, 83.5, 85.4, 84.3, 80.3 and 80.4 °C, respectively). For the second endotherm, differences observed between native samples (125.6 °C) and the samples degraded for 7, 14, 48, 58, 105 and 294 days (127.7, 132.3, 133.0, 131.0, 130.4 and 108.6 °C, respectively) (Table 2) were lower.

The changes in enthalpies for the degraded samples were also observed. For the first endotherm no clear decreasing or increasing trend was observed

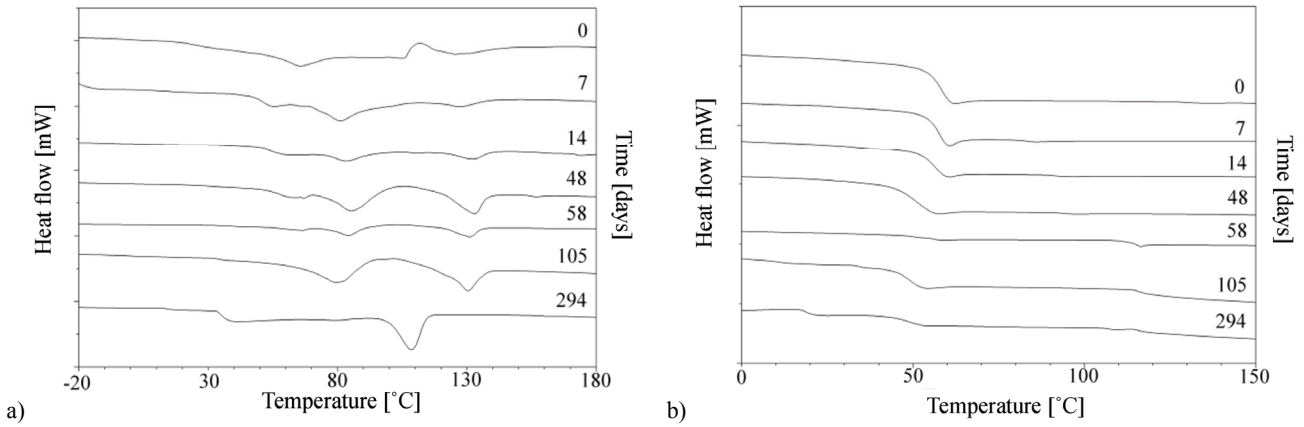

Fig. 2. The curves representing the first heating run (a) and the second heating run (b) of DSC measurements obtained for L-PLGA matrices with RSP degraded within 0, 7, 14, 48, 58, 105 and 294 days

Table 2. Thermal properties of L-PLGA matrices with RSP degraded for 0, 7, 14, 48, 58, 105 and 294 days

Degradation period [days]	First heating run (20 °C/min)						Second heating run (20 °C/min)	
	T_{m1} [ºC]	ΔH_{m1} [J/g]	T_{m2} [ºC]	ΔH_{m2} [J/g]	T_c [ºC]	ΔH_c [J/g]	T_g [ºC]	
0	65.5	7.6	125.6	3.5	111.0	3.0	ND	57.0
7	81.2	11.0	127.7	1.8	109.8	1.6	ND	56.3
14	83.5	4.8	132.3	4.6	103.7	2.4	ND	55.4
48	85.4	13.4	133.0	10.3	106.0	10.3	ND	49.3
58	84.3	13.2	131.0	13.3	104.3	12.5	ND	49.2
105	80.3	21.0	130.4	20.6	101.8	13.2	10.8	47.5
294	80.4	2.7	108.6	51.0	ND	ND	19.5	47.8

T_m and T_c – melting and crystallization temperature with ΔH_m and ΔH_c – enthalpy of melting and crystallization respectively; T_g – glass transition temperature; ND – not detected.

during degradation. However, for ΔH_{m2} increasing trend was noted (Table 2).

There was a decrease of T_c from 111.0 °C to 101.8 °C in the period of analysis (0–105 days). It should be noted that the values of ΔH_{m2} corresponded mostly to the values of ΔH_c (Table 2).

The T_g decreased within the degradation (0–294 days) from 57.0 °C to 47.8 °C. However, for the sample degraded for 105 and 294 days, the second additional T_g (i.e., at 10.8 °C and 19.5 °C, respectively) appeared.

3.2. SEM imaging

The microscopic study revealed changes in morphological features between the native matrix (Fig. 3a and b) and degraded samples (Fig. 3c–p). The comparison of all SEM images can show the surfaces (Fig. 3a–f, i) and cross-sections (Fig. 3g, h, j–m, o and p) of the L-PLGA matrices with RSP during degradation. Therefore, outer and inner morphology may be observed, respectively. The picture showing both the surface and a cross-section is presented in Fig. 3n.

Surface of native sample showed two various areas with different morphological features. The dominant type of surface presented as a solid cladding covered by the elements similar to fish scales in which small pores were observed (Fig. 3a). The area of scales was in the range from 3.5 μm^2 to 17.4 μm^2. There was also a porous surface with pore area in the range from 0.8 μm^2 to 9.2 μm^2 (Fig. 3b).

Morphology analysis of degraded samples was performed after 0, 7, 14, 48, 58, 105, 294 days of incubation in PBS. Differences were observed in the outer and inner morphology. The widening of pores was evident in most cases. For outer morphology of the samples collected after 7, 48 and 58 days of degradation pore area was in the range 1.2 μm^2–6.1 μm^2, 1.1 μm^2–12.8 μm^2 and 1.6 μm^2–24.1 μm^2, respectively (Fig. 3c, e and i). Moreover, the samples collected after 48 and 58 days of degradation also showed pore deformation (Fig. 3e and i).

Outer morphology showed mainly porous character (Fig. 3b–f and i), however inner morphology revealed both solid and porous structure (Fig. 3g, h, k–m and p). Generally, pores visualized in the cross-section had a significantly smaller area than in outer morphology. Moreover, the progress of degradation resulted in an increase of pore size areas, i.e., for the samples degraded for 48, 105 and 294 days, the area was in the range of 0.2 μm^2–4.7 μm^2, 0.3 μm^2–3.5 μm^2 and 0.6 μm^2–4.9 μm^2, respectively (Fig. 3h, l and p).

Degradation caused formation of more pores on the surface (Fig. 3d) and appearance of new morphological features. After 7 and 48 days of degradation, there were visible new pores on the bottom layer of the porous area (Fig. 3c, e and f). Cracks and splits appeared in the sample after 48 and 105 days of degradation (Fig. 3g, h, k and m). Formation of crystal-like forms occurred in the samples, which were subjected to degradation for 58 and 294 days (Fig. 3j, n and o).

4. Discussion

4.1. Characteristic of RSP formulations based on PLGA

RSP is a neuroleptic drug used and proposed as implantable and biodegradable formulations [2], [6]–[8].

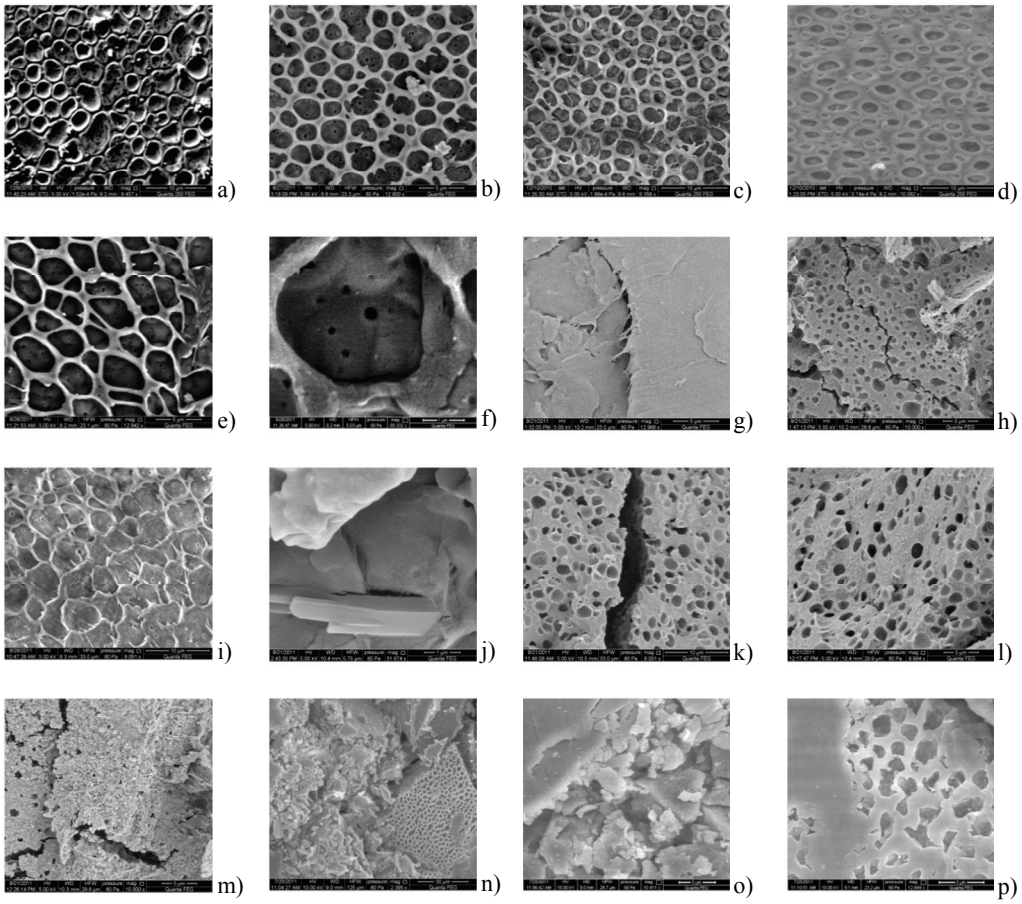

Fig. 3. SEM images of L-PLGA matrices with RSP degraded for 0 days (a – magnification ×8457, b – magnification ×12800), 7 days (c – magnification ×9556), 14 days (d – magnification ×10082), 48 days (e – magnification ×12942, f – magnification ×59332, g – magnification ×12968, h – magnification ×10000), 58 days (i – magnification ×9051, j – magnification ×51874), 105 days (k – magnification ×9051, l – magnification ×9984, m – magnification ×10000) and 294 days (n – magnification ×2395, o – magnification ×10411, p – magnification ×12849)

In the designing of novel medicinal products based on aliphatic polyesters, it is crucial to determine their thermal and morphological features. This fact is confirmed by many authors [16]–[21]. In this study, L-PLGA with comonomer ratio of 85:15 was used. According to the literature, the composition of polymeric matrix, i.e., the content of lactidyl and glycolidyl segments, the configuration of lactide and drug type, influence the thermal properties of the whole system [25]–[27]. It may reflect morphological properties. For example, it was reported that the T_g has a great influence on the surface segregation of methyl groups, i.e., polymer with lower T_g revealed larger extent of surface restructuring in comparison with polymers with higher T_g [28]. Therefore, thermal properties may affect morphological properties.

In this work, thermal properties and morphology of the native L-PLGA matrix with RSP and of L-PLGA matrices during degradation process were analyzed to confirm their usefulness in the development of long-term release system of RSP. In should be emphasized that in chronic diseases sustained, controlled and prolonged release of drug is important. It would be an interesting solution to administer a drug substance from an implantable formulation for as long as possible. If necessary, daily doses released from the implant (e.g., rod) could be supplemented with oral administration. On the other hand, in the emergency events implantable formulations may be explanted.

4.2. Thermal properties in degradation process

The analysis of curves and thermal parameters, i.e., T_m and T_c with ΔH_m and ΔH_c, respectively, and T_g, for RPS free matrices and matrices with RSP revealed the same thermal effects. All of the curves with one exception had endotherm, exotherm and endotherm in sequence (Fig. 1a, Table 1). It should be explained that presence of endotherms may indicate various

effects, i.e., T_m, relaxation processes and the evaporating of solvent and/or solution residues from polymeric matrices. However, considering the fact that these thermal effects are present in all degraded samples (Fig. 2a, Table 2), it may clearly indicate the double melting process. Additionally, overall analysis of ΔH_{m1}, ΔH_c, ΔH_{m2} for all samples (Tables 1 and 2) confirms the hypothesis that melting, cold crystallization and melt-recrystallization took place because the fraction which crystallized allowed melt-recrystallization. Therefore, it can be said that L-PLGA shows crystalline polymorphism, the presence of many small crystals and/or crystallographic defects occur. The L-PLGA analyzed is homogenous and semi-crystalline. Considering the fact that the period of degradation was 294 days (Fig. 2, Table 2), semi-crystalline L-PLGA can provide prolonged matrix degradation and RSP releasing.

In this study, the comparison of thermal parameters, i.e., T_m and T_c with ΔH_m and ΔH_c, respectively, and T_g revealed the differences after the introduction of RSP into L-PLGA matrix (Fig. 1a and b, Table 1). It should be noted that thermal analysis of RSP indicated the crystalline character of RSP with T_m 171 °C (Fig. 1, Table 1). The obtained results correspond to the literature data on thermal properties of RSP [29], [30]. Undoubtedly, the nature of RSP may influence the changes in the semi-crystalline character of L-PLGA during obtaining a polymeric matrix, which was observed in such parameters as T_m and T_c with ΔH_m and ΔH_c, respectively, and T_g (Table 1).

The drug–polymer interactions are an interesting issue concerning drug release. The aim of this work was not to find interactions between RSP and L-PLGA. However, some issues should be clarified. There are various opinions on the presence of drug–polymer interactions. Siegel et al. (2006) proved that using the same polymer matrix and drug loading (20% by weight) but various kinds of drug may cause a different rate of polymer degradation and the drug release profile. Therefore, it was concluded that the design of biodegradable polymeric drug carriers with high drug loadings must account for the effect of the drug on the polymer degradation and drug release rate [31]. In this study, drug content in matrix was lower, i.e., 5%-wt. The low drug content may explain the lack of T_m arising from RSP in thermograms obtained after the first heating run (Fig. 1a, Table 1). Moreover, the lack of additional peaks, i.e., exothermic and/or endothermic, after incorporation of RSP into matrix in the first heating run may evidence two various results, i.e., the lack of interactions between L-PLGA and RSP or an insufficient amount of RSP to create these interactions. This phenomenon does not point unreservedly to the lack of interactions. It must be confirmed in another study. However, it may be suggested that releasing process of RSP from L-PLGA matrix with the content of 5%-wt of RSP may take place despite these interactions.

The second heating run of native samples revealed an increase in T_g value after the introduction of RSP, which may suggest the anti-plastification effect. However, another fact is important (Fig. 1b) – the polymer possessed the T_g above human body temperature (57 °C), which is appropriate for prolonged degradation of L-PLGA and prolonged release of RSP.

Thermal analysis of the matrix with RSP, RSP free matrix and pure RSP showed the influence of RSP on L-PLGA matrix. It cannot be stated, however, that the character of L-PLGA matrix was significantly changed.

Degradation study provided interesting information regarding the slow degradation process of L-PLGA, which is advantageous for prolonged release of RSP. Analogous thermal effects were observed in the degradation study (Fig 2, Table 2), similarly to the study on the influence of introduction of RSP into L-PLGA matrix (Fig. 1, Table 1).

The differences were also observed in the values of the thermal parameter, i.e., T_m and T_c with ΔH_m and ΔH_c, respectively, and T_g because of degradation (Fig. 2, Table 2).

For the native matrix with RSP and degraded for 7 and 14 days, relatively wide endothermal areas were visible in the first heating run of DSC. They were also very small (Fig. 2a). Therefore, these areas may indicate solvent (i.e., methylene chloride) evaporation, which might have remained in the matrix structure in trace amounts due to the preparation method. This would suggest changing the preparation methods for, e.g., extrusion or injection molding. However, for the sample degraded for 48, 58 and 105 days (Fig. 2a), the endotherms were visible, which may indicate the changes in crystallinity of L-PLGA. It was supposed that crystalline RSP may influence semi-crystalline L-PLGA. However, no clear decreasing and increasing trend of values in enthalpies for the first heating run was observed, which may indicate crystallinity changes.

It was found that the T_c decreased during degradation. It is known that the degradation contributes to a decrease in molar mass and therefore the length of polymer chains. The polymer chains can be easily rearranged in spherulites, since the mobility is enhanced by the availability of more free volume and the crystallization takes place at lower temperatures [32].

Changes in thermal parameters were also observed after 294 days, probably due to the decrease in RSP amount in the matrices. This, however, is only applicable to the sample degraded for 294 days. The disproportion between endothermal peaks was visible. Only one endotherm was clearly visible (Fig. 2a). This may indicate the loss of one component from the matrix during hydrolytic degradation. It is known that for PLGA copolymers faster loss of glycolidyl blocks may occur because of their higher hydrophilicity.

4.3. Morphology in degradation process

The SEM analysis revealed the presence of pores in each stage of degradation, which confirmed the results obtained by means of DSC. As mentioned above, pore closing could be expected after a decrease in T_g and an increase in polymer chain mobility [18], [22]. In this case, although a decrease in T_g was observed during degradation (Fig. 2), the polymer was still in a vitreous state even after 294 days of degradation that is advantageous for long delivery system designed for long term release. 294 days of degradation significantly affected T_g (Fig. 2b). However, in the first two weeks slight changes took place, which may indicate the lack of significant degradative changes in the structure of L-PLGA matrices with RSP. It can be suggested that in this period slow release of RSP may be observed (Fig. 2a). On the other hand, the lack of significant changes in the values of T_g for samples degraded for 48, 58, 105 and 294 days may indicate the stability of the matrix, which may ensure a stable degradation process (Fig. 2b).

Moreover, it is interesting that in the samples degraded for 105 and 294 days an additional T_g effect was noted. This may suggest formation of low molecular fraction of degraded L-PLGA (Fig. 2b, Table 2).

A microscopic study of the native matrix revealed a solid area (Fig. 3a) and a porous surface in outer morphology (Fig. 3b). However, only porous surface was visible from the 7th day of degradation (Fig. 3c, d, e, i). The appearance of pores is a result of the opening of pores rather than their formation. First, in the native matrix, pores were revealed already within the small area. Secondly, it is known that the degradation of such high molecular polymers as L-PLGA used is not so rapid. Moreover, also in DSC results of the sample after 7 days of degradation, no significant changes in the T_g were observed, i.e., 0.7 °C (Table 2). The differences in T_g of 1.6 °C (Table 2) in the sample degraded for 14 days also revealed the changes in morphology. The range of the pore size areas observed increased. This may suggest that many novel pores developed (Fig. 3d). However, the formation of smaller pores created within greater pore may also point to the beginning of changes facilitating the degradation process. This was observed in the samples degraded for 7 and 48 days (Fig. 3d, e and f).

In outer morphology pore area widening observed on the primary surface of the sample degraded for 7, 48 and 58 days may evidence the progress of degradation (Fig. 3c, e and i). The same conclusion results from the deformation of oval pores observed for samples degraded for 48 and 58 days (Fig. 3e and i).

Significant changes in the value of T_g were observed after 48 days of degradation (Table 2). Simultaneously, morphological features changed significantly. As observed in the cross-sections cracking and delamination took place due to degradation. Inner morphology could by characterized *ipso facto* (Fig. 3g, h, k, l, m, p). Moreover, the creation of pores and their widening in progress of degradation were also observed in the inner structure (Fig. 3h, l, p).

The greatest differences in morphological features were observed in the samples degraded for 105 and 293 days. The primary surface virtually disappeared. Moreover, slits and cracks were observed as well (Fig. 3g, h, k, m).

The changes in T_g after 105 and 294 days of degradation were the most significant. The heterogeneous character of images of the samples degraded for 105 and 294 days may be reflected by two T_g effects. The results of SEM and DSC studies suggest the presence of various fractions (Fig. 2b, Table 2, Fig. 3, m, n and o).

Moreover, the analysis of curves and the values of thermal parameters representing the first heating run for these samples revealed the changes in comparison with the native matrix. In particular, the changes in the ΔH_{m1} and ΔH_{m2} may evidence the changes in crystallinity. In SEM images crystall-like forms were visible in the samples degraded for 58 and 294 days (Fig. 3j, n, o). However, this is not a direct evidence and it would be difficult to formulate an unambiguous interpretation in this way.

5. Conclusions

From thermal properties and morphological features it can be confirmed that combining the analysis of DSC and SEM is a valuable approach in the interpretation of the influence of drug introduction into the polymeric matrix and monitoring of degradation changes. It has

been proven that this method can be complementary in the interpretation of degradation results for drug carriers. Moreover, the DSC and SEM analysis can be helpful in preliminary screening analysis and prediction of drug release.

In this study, the thermal analysis indicated the following facts: (i) RSP did not change significantly semicrystalline character of L-PLGA; (ii) the decrease in T_g showed stable degradation process during observation, which was confirmed by morphological features.

The obtained data may suggest slow degradation that is advantageous for prolonged release of RSP from L-PLGA matrices.

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References

[1] GUTIERREZ R., LEE P.I., HUANG M.L., WOESTENBORGHS R., *Risperidone: effects of formulations on oral bioavailability*, Pharmacotherapy, 1997, 17(3), 599–605.

[2] BAI Y.M., CHEN T.T., WU B., HUNG C.H., LIN W.K., HU T.M., LIN C.Y., CHOU P., *A comparative efficacy and safety study of long-acting risperidone injection and risperidone oral tablets among hospitalized patients: 12-week randomized, single-blind study*, Pharmacopsychiatry, 2006, 39(4), 135–141.

[3] CITROME L., *A systematic review of meta-analyses of the efficacy of oral atypical antipsychotics for the treatment of adult patients with schizophrenia*, Expert Opin. Pharmacother, 2012, 13(11), 1545–1573.

[4] HUANG M., SHEN-TU J., HU X., CHEN J., LIU J., WU L., *Comparative fasting bioavailability of dispersible and conventional tablets of risperidone: a single-dose, randomized-sequence, open-label, two-period crossover study in healthy male chinese volunteers*, Clin. Ther., 2012, 34(6), 1432–1439.

[5] *Food and Drug Administration*, 2003, http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021346_s31_s35_s38_s39lbl.pdf. Accessed 1 August 2014.

[6] RABIN C., LIANG Y., EHRLICHMAN R.S., BUDHIAN A., METZGER K.L., MAJEWSKI-TIEDEKEN C., WINEY K.I., SIEGEL S.J., *In vitro and in vivo demonstration of risperidone implants in mice*, Schizophr. Res., 2008, 98(1–3), 66–78.

[7] MUTHU M.S., RAWAT M.K., MISHRA A., SINGH S., *PLGA nanoparticle formulations of risperidone: preparation and neuropharmacological evaluation*, Nanomedicine, 2009, 5(3), 323–333.

[8] SU Z.X., SHI Y.N., TENG L.S., LI X., WANG L.X., MENG Q.F., TENG L.R., LI Y.X., *Biodegradable poly(D, L-lactide-co-glycolide) (PLGA) microspheres for sustained release of risperidone: Zero-order release formulation*, Pharm. Dev. Technol., 2011, 16(4), 377–384.

[9] ALBERTSSON A.Ch. (ed.), *Degradable Aliphatic Polyesters*, Springer-Verlag, 2002.

[10] SWARBRICK S. (ed.), *Encyclopedia of Pharmaceutical Technology*, Informa Healthcare, 2006.

[11] BARTKOWIAK-JOWSA M., BĘDZIŃSKI R., KOZŁOWSKA A., FILIPIAK J., PEZOWICZ C., *Mechanical, rheological, fatigue, and degradation behavior of PLLA, PGLA, PDGLA as materials for vascular implants*, Meccanica, 2013, 48(3), 721–731.

[12] STĘPAK B., ANTOŃCZAK A.J., BARTKOWIAK-JOWSA B., FILIPIAK J., PEZOWICZ C., ABRAMSKI K.M., *Fabrication of a polymer-based biodegradable stent using a CO_2 laser*, Arch. Civ. Mech. Eng., 2014, 14(2), 317–326.

[13] WANG J., WANG B.M., SCHWENDEMAN S.P., *Characterization of the initial burst release of a model peptide from poly(D,L-lactide-co-glycolide) microspheres*, J. Control Release, 2002, 82(2–3), 289–307.

[14] DANGI R., HURKAT P., JAIN A., SHILPI S., JAIN A., GULBAKE A., JAIN S.K., *Targeting liver cancer via ASGP receptor using 5-FU-loaded surface-modified PLGA nanoparticles*, J. Microencapsul, 2014, 31(5), 479–487.

[15] ZHANG H., XU J., *Enhanced oral bioavailability of salmeterol by loaded PLGA microspheres: preparation, in vitro, and in vivo evaluation*, Drug Deliv., 2014, 14, 1–6.

[16] HUA J., GEBAROWSKA K., DOBRZYŃSKI P., KASPERCZYK J., WEI J., LI S., *Influence of chain microstructure on the hydrolytic degradation of copolymers from 1,3-trimethylene carbonate and L-lactide*, J. Polym. Sci. A Polym. Chem., 2009, 47(15), 3869–3879.

[17] JELONEK K., KASPERCZYK J., LI S., DOBRZYNSKI P., JARZĄBEK B., *Controlled poly(l-lactide-co-trimethylene carbonate) delivery system of cyclosporine A and rapamycine-the effect of copolymer chain microstructure on drug release rate*, Int. J. Pharm., 2009, 414(1–2), 203–209.

[18] FREDENBERG S., WAHLGREN M., RESLOW M., AXELSSON A., *The mechanisms of drug release in poly(lactic-co-glycolic acid)-based drug delivery systems – a review*, Int. J. Pharm., 2011, 415(1–2), 34–52.

[19] NIKKOLA L., VIITANEN P., ASHAMMAKHI N., *Temporal control of drug release from biodegradable polymer: multicomponent diclofenac sodium releasing PLGA 80/20 rod*, J. Biomed. Mater Res. Part B Appl. Biomater., 2009, 89(2), 518–526.

[20] KLOSE D., SIEPMANN F., WILLART J.F., DESCAMPS M., SIEPMANN J., *Drug release from PLGA-based microparticles: effects of the "microparticle: bulk fluid" ratio*, Int. J. Pharm., 2010, 383(1–2), 123–131.

[21] TUREK A., JELONEK K., WÓJCIK A., DZIERŻEWICZ Z., KASPERCZYK J., DOBRZYŃSKI P., MARCINKOWSKI A., TRZEBICKA B., *Surface properties of poly(L-lactide-co-glycolide) matrices with risperidone and their changes after two weeks of degradation*, Eng. Biomat, 2010, 13(96–98), 117–120.

[22] YAMAGUCHI Y., TAKENAGA M., KITAGAWA A., OGAWA Y., MIZUSHIMA Y., IGARASHI R., *Insulin-loaded biodegradable PLGA microcapsules: initial burst release controlled by hydrophilic additives*, J. Control. Release, 2002, 81(3), 235–249.

[23] DOBRZYŃSKI P., BERO M., KASPERCZYK J., *Sposób wytwarzania bioresorbowalnych polimerów*, PL 191846 B1, 2000.

[24] DOBRZYŃSKI P., KASPERCZYK J., JANECZEK H., BERO M., *Synthesis of biodegradable copolymers with the use of low toxic zirconium compounds. 1: Copolymerization of glycolide with L-lactide initiated by $Zr(Acac)_4$*, Macromolecules, 2001, 34(15), 5090–5098.

[25] YANG Y.Y., CHIA H.H., CHUNG T.S., *Effect of preparation temperature on the characteristics and release profiles of PLGA microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method*, J. Control Release, 2000, 69(1), 81–96.

[26] DOBRZYŃSKI P., LI S., KASPERCZYK J., BERO M., GASC F., VERT M., *Structure-property relationships of copolymers obtained by ring-opening polymerization of glycolide and epsilon-caprolactone*. Part 1. *Synthesis and characterization*, Biomacromolecules, 2005, 6(1), 1483–1488.

[27] LI S., DOBRZYŃSKI P., KASPERCZYK J., BERO M., BRAUD C., VERT M., *Structure-property relationships of copolymers obtained by ring-opening polymerization of glycolide and epsilon-caprolactone*. Part 2. *Influence of composition and chain microstructure on the hydrolytic degradation*, Biomacromolecules, 2005, 6(1), 1489–1497.

[28] THANKI P.N., DELLACHERIE E., SIX J.L., *Surface characteristics of PLA and PLGA films*, Appl. Surf. Sci., 2006, 253(5), 2758–2764.

[29] RAHMAN Z., ZIDAN A.S., KHAN M.A., *Non-destructive methods of characterization of risperidone solid lipid nanoparticles*, Eur. J. Pharm. Biopharm., 2010, 76(1), 127–137.

[30] SILVA A.C., GONZÁLEZ-MIRA E., GARCÍA M.L., EGEA M.A., FONSECA J., SILVA R., SANTOS, D., SOUTO E.B., FERREIRA D., *Preparation, characterization and biocompatibility studies on risperidone-loaded solid lipid nanoparticles (SLN): high pressure homogenization versus ultrasound*, Colloids Surf B Biointerfaces, 2011, 86(1), 158–165.

[31] SIEGEL S.J., KAHN J.B., METZGER K., WINEY K.I., WERNER K., DAN N., *Effect of drug type on the degradation rate of PLGA matrices*, Eur. J. Pharm. Biopharm., 2006, 64(3), 287–293.

[32] RYDZ J., WOLNA-STYPKA K., MUSIOŁ M., SZELUGA U., JANECZEK H., KOWALCZUK M., *Further evidences of polylactide degradation in paraffin and selected protic media. A thermal analysis of the eroded PLA films*, Polym. Degrad. Stab., 2009, 98(8), 1450–1457.