Postprint of: Brzeska J., Janeczek H., Janik H., Kowalczuk M., Rutkowska M., Degradibility in vitro of polyurethanes based on synthetic atactic poly[)R,S)-3-hydroxybutyrate, Bio-Medical Materials and Engineering, Vol. 25, Iss. 2 (2015), pp. 117-125, DOI: 10.3233/BME-151262

Degradability in vitro of polyurethanes based on synthetic atactic poly[(*R,S*)-3-hydroxybutyrate]

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Abstract

The aim of the present study was to determine the degradability of aliphatic polyurethanes, based on a different amount of synthetic, atactic poly([R,S]-3-hydroxybutyrate) (a-PHB), in hydrolytic (phosphate buffer) and oxidative ($H_2O_2/CoCl_2$) solutions. The soft segments were built with atactic poly([R,S]-3-hydroxybutyrate) and polycaprolactone or polyoxytetramethylenediols, whereas hard segments were the reaction product of 4,4'-methylenedicyclohexyl diisocyanate and 1,4-butanediol.

The selected properties - density and morphology of polymer surfaces – which could influence the sensitivity of polymers to degradation processes - were analyzed.

The analysis of molecular mass (GPC), thermal properties (DSC) and the sample weight changes were undertaken to estimate the degree of degradability of polymer samples after incubation in environments studied.

Investigated polyurethanes were amorphous with the very low amount of crystalline phases of hard segments.

The polyurethane synthesized with a poly([R,S]-3-hydroxybutyrate) and polyoxytetramethylenediol at a molar ratio of NCO:OH=3.7:1 (prepolymer step) appeared as the most sensitive for both degradative solutions. Its weight and molecular mass losses were the highest in comparison to other investigated polyurethanes.

It could be expected that playing with the amount of poly([R,S]-3-hydroxybutyrate) in polyurethane synthesis the rate of polyurethane degradation after immersion in living body would be modeled.

Keywords: polyurethane, atactic poly([R,S]-3-hydroxybutyrate), biomaterial, hydrolytic degradation, oxidative degradation

1.1. Introduction

Synthetic polymers are commonly used in medicine because of their high biocompatibility and easy forming into different shapes. Temporary or a long term implants, drug carriers or scaffolds for tissue regeneration are good examples of their application in medicine.

Polyurethanes (PUR) are very versatile materials in comparison to the range of other polymers. They can be tailored made by changing the components and their ratio. So far hydroxyterminated polycaprolactone (PCL) and polyoxytetramethylene (PTMG) are well known oligodiols used for the synthesis of medical grade polyurethanes [1-

3]. Recently the synthetic, telechelic polyhydroxybutyrate (a-PHB) was used to obtain polyurethanes as well [4-6].

It is known that polyhydroxybutyrate (PHB), belonging to the family of polyhydroxyacides, is naturally synthesized by many microorganisms as carbon and energy resource. A product of its degradation – 3-hydroxybutyric acid - a common metabolite in human blood, is produced in ketone bodies of mammals during the prolonged starvation and as short-chain fatty acids reveals antibacterial activity [7]. Natural PHB is partly crystalline. Whereas the chemically synthesized substitute of natural PHB - atactic poly([R,S]-3-hydroxybutyrate) (a-PHB) - is almost amorphous, which is close to the original state of PHB in the living cell. Thus the incorporation of a-PHB into polyurethane structure ought to let to obtain biocompatible material, which is easy degradable under conditions of body environment.

It is obvious that the hydrolysis (both chemical and enzymatic) is a very important process of polymer degradation in the environment. Degradability of polymers is dependent on many parameters, like presence of hydrolysable bonds in macrochain or density and crystallinity. The model environment for testing of a chemical hydrolysis process in polymers samples is phosphate buffer solution [8].

It is also well known that the reactive oxygen species released by adherent macrophages and foreign body giant cells take a very important part of the material biodegradation. The *in vivo* degradation can be reproduced *in vitro* using $H_2O_2/CoCl_2$ system. Christenson et al. [9] showed that the changes in polyesterurethanes and in polycarbonates samples, after the 24 days of incubation in oxidative solution, were similar to changes of polymer samples implanted into rat body for 1 year. Hydrogen peroxide and cobalt(II) ion undergo the Habere Weiss reaction [10] and produce reactive hydroxyl radicals. Hydroxyl radicals induce series of oxidative reactions

when they contact with polyurethane. It results in chain scission and/or crosslinking of the polymers [10].

In this study the degradability of novel polyurethanes for medical application in mind, obtained with the use of a-PHB besides of typical oligodiols (PCL and PTMG) in soft segments, was estimated in hydrolytic (phosphate buffer) and oxidative solutions ($CoCl_2/H_2O_2$). As it was presented in our previous work [11] introducing of atactic poly([R,S]-3-hydroxybutyrate) into polyurethane structure caused the acceleration of degradation processes in these both environments.

In our previous studies we found that novel polyurethanes have not affected whole blood parameters but restricted of pathogenic microorganisms growth what suggested that they could be useful for medical application [12,13].

The morphology, thermal properties, density and weight and molecular mass loss were investigated for the estimation of degradability of synthesized polyurethanes in hydrolytic and oxidative conditions.

1.2. Experimental

1.2.1. Materials

Four kinds of polyurethanes differing in soft segments structure and in soft to hard segments ratio were tested.

Table 1

Samples from series A contained synthetic a-PHB (Mn 2000) and PTMG (Mn 2000) in soft segments, with 14 and 17.5% w/w of a-PHB in PUR-A14 and PUR-A17.5 samples, respectively. Whereas soft segments of the next two polyurethanes (PUR-B14 and PUR-B17.5) were built with a-PHB (Mn 2000) and PCL (Mn1920)

with14 and 17.5% of a-PHB in polyurethane bulk, respectively.

Hard segments of all polyurethanes were the reaction product of 4,4'-methylenedicyclohexyl diisocyanate (H_{12}MDI) and 1.4-butanediol (1.4-BD). PURs were obtained by two-step reaction, as described previously [14], with a molar ratio of NCO:OH=3.7:1 (in prepolymer) for PUR-A14 and PUR-B14 and NCO:OH=2:1 for PUR-A17.5 and PUR-B17.5.

1.2.2. Methods

Microscopy

The original surfaces and surfaces revealed after breaking of obtained PU samples in liquid nitrogen (cryogenically broken samples) were tested by Transmission Electron Microscopy (TEM) by two step replica. Poly(vinyl alcohol) was the replicating material. After replication the PVA matrix taken from PU surfaces was shadowed with Pt and covered with a carbon. After dissolving of a PVA matrix the Pt/C replica was observed under TEM BS 540 operated at 90 kV.

Density

Density of polyurethanes was estimated using analytical balance equipped with the density determination kit. The measurements were repeated three times for each polymer.

Differential scanning calorimetry (DSC)

Thermal properties of polyurethane samples before and after incubation in buffer and oxidative solutions were determined using of DuPont 9900 thermal analyser. Indium metal was used for calibration. The degraded polyurethane samples were dried to constant weight before DSC measurements. The specimens were sealed in aluminium pans and scanned from -80 ºC to 200ºC with heating rate of 10 ºC/min. All

experiments were done in a flow of dry N_2.

Degradation in buffer solution

Hydrolytic degradation of polyurethane samples was carried out for 4, 12, 24 and 36 weeks, using phosphate buffer solution (PBS, pH=7.4), containing sodium azide (0.02%) as bacteriostatic agent. The “pseudodynamic” degradation mode was applied in the study. The pH of the ageing medium was checked every 2 weeks and the solution was replaced if the pH dropped more than 0.5 [15].

Degradation in oxidative solution

Oxidative degradation of polyurethane samples was carried out for 2, 4, 12, and 16 weeks, using oxidative solution of 20% w/w hydrogen peroxide in 0.1M cobalt chloride solution [9]. The solution was changed every week to maintain a constant concentration of radicals. One week was selected as an appropriate interval to replace the solution since the half-life of hydrogen peroxide at 37ºC was measured to be about 7 days [10].

1.3. Results and discussion

The examples of TEM observations of polyether-esterurethane samples are presented in Table 2.

Table 2

The roughness of a polymer surface and its cryogenically fractured surface were comparable for all investigated samples. The only difference between the samples was the presence of small entities in the surface of cryogenically broken samples of PUR-A17.5 and sharper edges in rough figures of sample PUR-A14 cryogenically broken.

Table 3

According to the pictures presented in Table 3 there was no clear evidence of crystalline texture on both free and cryogenically revealed surfaces of polyesterurethanes. The free surface of PUR-B17.5 was rougher than the others.

In Table 4 the densities of the samples are shown. Density of polyether-esterurethanes was lower than polyesterurethanes.

Table 4

The weight and molecular mass changes of polyurethane samples, synthesized with a-PHB after incubation in a phosphate buffer solution are presented in Table 5 and Table 6 respectively.

Table 5

Table 6

Surprisingly polyether-esterurethane PUR-A14 (with higher amount of hard segments and with oligoether in the structure) was the most sensitive to degradation in phosphate buffer solution although the faster hydrolysis of ester bonds in polyesterurethanes (in PUR-B14 and PUR-B17.5) was more expected. After 36 weeks of incubation in buffer solution the molecular mass of PUR-A14 was reduced to 20% of its initial value while molecular mass reduction of the rest of the samples was much smaller. The rate of degradation of PUR–A17.5 (also obtained with PTMG and a-PHB, but with higher amount of soft segments) was similar to degradation of polyesterurethanes. It was a consequence of lower density and a bit higher water sorption [14] of these polymers in comparison to PURs based on PCL (Table 3).

According to GPC chromatograms (especially in case of PUR-A14 and PUR-A17.5) the degradation of studied polyurethanes was a two stage process: at first macrochains were randomly cut and then - when the molecular mass was under 10,000 (after 24 weeks) – the weight loss of samples was noticed.

Figure 1

Samples of PUR-A14 were degraded gradually and quite slowly during the first 24 weeks of incubation in buffer solution but after that time the rate of degradation accelerated and the weight loss was 60 % (Tab. 5) whereas the molecular mass decreased from 13668 to 2744 after 36 weeks.

PUR–A17.5 synthesized with higher value of soft segments than PUR-A14 degraded in different way (Fig. 1a and Fig.1b). There was seen a split of elution curves on GPC chromatograms of PUR-A17.5 after incubation and its molecular mass was reduced to 36 % of initial value after 36 weeks of incubation. It could be explained by fact that ester bonds of a-PHB and urethane cleavages between hard segments and a-PHB were hydrolysed first. This caused the formation of short chains, built with a-PHB and hard segments, whereas chains of PTMG oligoethers were unchanged.

For series B the higher weight loss for PUR-B17.5 in comparison to PUR-B14 was observed. Moreover in our previous studies [11] on polyurethanes built with PCL, H_{12}MDI and 1.4-BD the weight changes of these samples after incubation in a buffer were not noticed. It was supposed that ester linkages were hydrolysed but because of insolubility of PCL products they were not rinsed polymer bulk. The lower weight loss of samples PUR-B14 and PUR-B17.5 than PURs of series A was noticed.

The introduction of almost amorphous a-PHB into the structure increased the degradability of polyurethanes based on PCL. The similar influence was observed by Hong [16] for polyurethanes obtained via ring opening polymerization of Ɛ-caprolactone and β-butyrolactone.

The weight changes of polyurethane samples during incubation in oxidative solution are presented in Table 7 [11]. Taking into account the results showed in Table 6 and Table 7, it was clear that the ester cleavages were sensitive to hydrolysis whereas the ether cleavages to oxidative reactions.

Table 7

As it was expected the rate of oxidative degradation was higher for polyurethane based on PTMG oligoether than on PCL oligoester.

The degradation process of PUR–A14 samples (with PTMG and a-PHB) was much faster in oxidative than in hydrolytic solution. It was confirmed previously by microscopic observation of polymer samples after incubation [11].

The highest weight loss was noticed for PUR-A14. The weight loss of its samples was 70.9% after 16 weeks of incubation in oxidative solution.

Thermal properties of investigated polyurethanes are presented in Table 8 and Table 9.

Table 8

Table 9

Thermal analysis of all polyurethanes before degradation showed a clear glass transition temperature (Tg) proper for soft segments at -55 and -61.5°C for PTMG based PURs and at -32.4 and -29.0°C for PCL based PURs.The crystallinity of soft segments of polyurethanes was very low if we compare the data of melting enthalpy (ΔH) of pure oligomerols and soft segments (ΔH_1) in PUR samples. These data were according to microscopic observations.

After degradation in hydrolitic or oxidative environments the glass transition temperature of soft segments of PUR-A14 and PUR-B17.5 shifted to the higher temperatures. For these samples the highest mass loss was observed. The changes of Tg for those samples were very noticable what means that the systems changed their internal structure very much.

Unsignificant changes of the glass transition temperature value or even decreasing of Tg after degradation in buffer and oxidative solutions observed for PUR-A17.5 and

PUR-B14 were probably a consequence of acting of water in both environments as plasticizers for those samples.

As it was said before the polyurethanes contained a-PHB in soft segments were almost amorphous. The lack of crystallinity of soft segments of polyurethanes synthesized with PTMG and a-PHB (PUR-A14 and PUR-A17.5) after the incubation in both solutions was also observed . The crystallinity of hard segments in PUR-B14 increased after incubation especially in PBS, as ΔH_2 increased almost twice (Table 8). It indicated that the amorphous phase of hard segments of polyether-esterurethanes degraded in the first stage.

There were completely different observations for polyurethanes based on PCL and a-PHB (Table 9). Changes in ΔH of soft segments were closely related to degradation degree of soft segments, which takes place mostly in an amorphous phase. It was visible especially for PUR-B17.5, containing a higher amount of soft segments than PUR-B14, where ΔH_1 increased for about 30 J/g after incubation in both environments.

1.4. Conclusion

The degradability of aliphatic polyurethanes, based on synthetic, atactic poly([R,S]-3-hydroxybutyrate) and commercial oligomers, in hydrolytic and oxidative solutions was investigated.

The differences in degradation rates of obtained polyurethanes could be attributed to the differences between the soft segment chemistry of these polymers. The structure of the soft segment built with an atactic poly([R,S]-3-hydroxybutyrate), polycaprolactonediol or polyoxytetramethylenediol played a decisive role in

determination of the degradation rates, since the hard segment chemistry of obtained polyurethanes was of the same type.

Polyurethane based on poly([R,S]-3-hydroxybutyrate) and polyoxytetramethylenediol obtained with NCO:OH=3.7:1 ratio in prepolymer step, appeared as the most sensitive to conditions of both degradative solutions (the higher weight loss and molecular mass reduction were noticed). This sample is the most suitable for designing future biodegradable material - tissue scaffolds - used in medicine.

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Table 1. Composition of the obtained polyurethanes

PUR	Soft segments	Hard segments	Amount of a-PHB [% w/w]	Molar ratio of NCO:OH in prepolymer
PUR-A14	a-PHB+PTMG	H_{12}MDI+1.4-BD	14	3.7:1
PUR-A17.5			17.5	2:1
PUR-B14	a-PHB+PCL		14	3.7:1
PUR-B17.5			17.5	2:1

Table 2.Microscopic observations of polyether-esterurethane surface

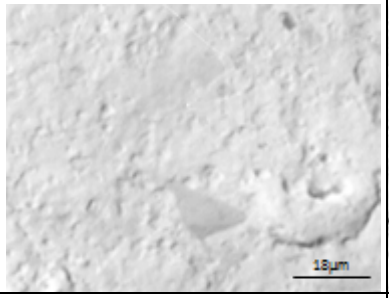
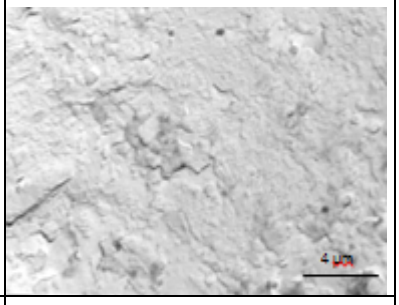
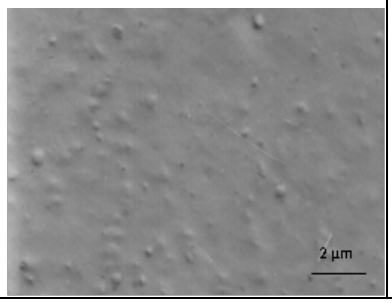
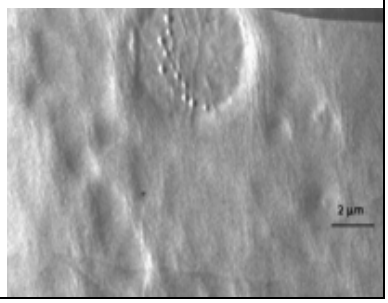
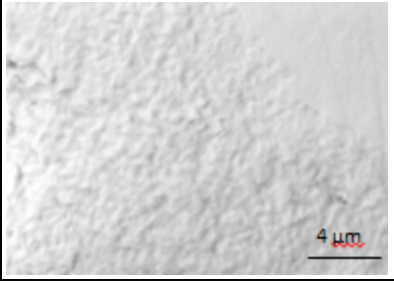
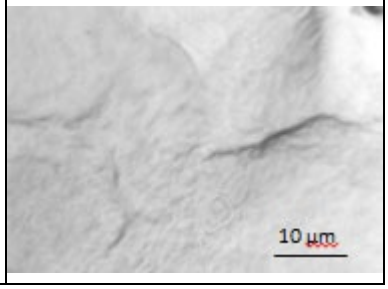
Sample	Sample surface (called original)	Surface of cryogenically fractured samples
PUR-A14		
PUR –A17.5		

Table 3. Microscopic observations of polyesterurethane surface

Sample	Sample surface (called original)	Surface of cryogenically fractured samples
PUR-B14		

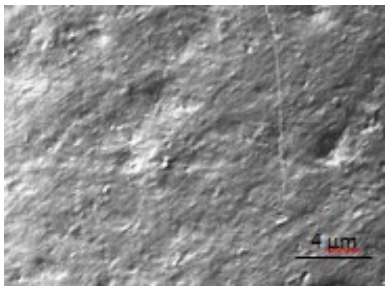
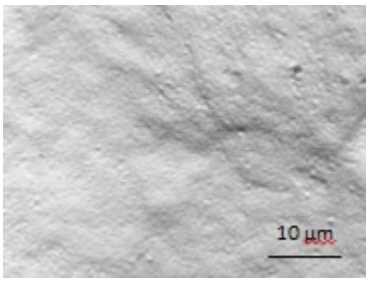
PUR –B17.5		

Table 4. Density (±SD)* of polyurethanes

PUR	Density [g/cm^3]
PUR-A14	1.081±0,002
PUR-A17.5	1.060±0,001
PUR-B14	1.152±0,008
PUR-B17.5	1.148±0,001

*(±SD): mean standard deviation

Table 5. The weight changes (±SD) of polyurethane samples after incubation in a phosphate buffer solution [11]

PUR	Incubation time [weeks]			
	4	12	24	36
PUR-A14	0.7±0.1	-0.1±0.2	-11.2±1.1	-60.3±13.2
PUR-A17.5	1.4±0.1	1.0±0.1	-3.2±0.2	-5.2±0.9
PUR-B14	2.0±0.7	0.4±0.3	0.3±0.2	-5.2±0.1
PUR-B17.5	1.6±0.1	1.5±0.2	-1.6±0.2	-9.7±0.4

Table 6. Molecular mass changes (percent of initial mass) of polyurethane samples after incubation in a phosphate buffer solution

PUR	Incubation time [weeks]			
	0	12	24	36
PUR-A14	13668	11497 (84%)	5337 (39%)	2744 (20%)
PUR-A17.5	18360	13400 (73%)	9300 (51%)	6630 (36%)
PUR-B14	7930	9900 (125%)	8656 (109%)	5520 (70%)
PUR-B17.5	16040	12400 (77%)	10400 (65%)	6990 (44%)

Table 7.The weight changes (±SD) of polyurethane samples after incubation in oxidative solution [11]

PUR	Incubation time [weeks]			
	2	4	12	16
PUR-A14	-7.5±3.5	-25.6±3.1	-42.0±4.1	-70.9±1.7
PUR-A17.5	-3.3±0.5	-7.8±1.0	-11.3±1.2	-14.2±0.7
PUR-B14	-0.3±0.0	-1.6±0.3	-8.2±0.9	-11.2±0.4
PUR-B17.5	-0.2±0.1	-1.9±0.1	-9.3±1.2	-15.8±3.3

Table 8.Thermal properties of polyurethanes based on PTMG and a-PHB before and after incubation in hydrolytic and oxidative solutions

Sample		T_g [°C]	T_{m1} [°C]	ΔH_1 [J/g]	T_{m2} [°C]	ΔH_2 [J/g]
PTMG	Pure oligomer	-73.1	30.7	101.6	-	-
PUR-A14	Before degradation	-55.3	37.8	0.7	107.4; 135.9; 157.5	2.5; 4.0; 2.2
	12 weeks of incubation in PBS	-28.5	-	-	152.5; 188.8	29.5; 4.0
	36 weeks of incubation in PBS	-9.7	-	-	138.7; 198.4	28.6; 4.3
	4 weeks of incubation in oxidative solution	-62.3	-	-	126.1; 192.7	61.7; 3.9
	16 weeks of incubation in oxidative solution	-5.7	-	-	121.2; 186.0	40.9; 0.5
PUR-A17.5	Before degradation	-61.5	-	-	126.6	27.1
	12 weeks of incubation in PBS	-67.2	-	-	118.1; 195.5	12.8; 0.5
	36 weeks of incubation in PBS	-67.3	-	-	113.1; 200.9	9.9; 0.9
	4 weeks of incubation in oxidative solution	-65.1	-	-	113.6; 172.6	22.7; 0.6
	16 weeks of incubation in oxidative solution	-65.2	-	-	114.1; 197.6	42.5; 2.2

Table 9.Thermal properties of polyurethanes based on PCL and a-PHB before and after incubation in hydrolytic and oxidative solutions

sample		T_g [°C]	T_{m1} [°C]	ΔH_1 [J/g]	T_{m2} [°C]	ΔH_2 [J/g]
PCL	Pure oligomer	-67.7	53.6	69.0	-	-
PUR-B14	Before degradation	-32.4	50.7	3.6	90.2; 138.1	4.7; 9.1
	12 weeks of incubation in PBS	-34.8	52.1	10.0	131.9; 192.3	13.9; 2.9

	36 weeks of incubation in PBS	-31.8	52.8	12.8	127.7; 191.6	20.0; 1.2
	4 weeks of incubation in oxidative solution	-35.9	-	-	120.8; 161.6	20.9
	16 weeks of incubation in oxidative solution	-33.6	52.9	6.1	119.6; 154.5	10.2; 3.2
PUR-B17.5	Before degradation	-29.0	-	-	70.1; 114.9	26.1; 12.8
	12 weeks of incubation in PBS	-13.7	55.6	30.3	109.0; 187.9	8.7; 2.6
	36 weeks of incubation in PBS	-14.2	55.3	35.6	107.3; 191.5	5.9; 0.5
	4 weeks of incubation in oxidative solution	-11.6	55.3	30.3	104.7	13.9
	16 weeks of incubation in oxidative solution	-15.8	50.1	27.1	97.1	13.2

Fig.1. GPC chromatograms of PUR-A14(a),PUR-A17.5(b) and PUR-B17.5(c) after incubation in buffer solution. The molecular mass estimated before degradation (black lines) and after 12 weeks (green lines), 24 weeks (blue lines), 36 weeks (red lines) of incubation.