Hydrolysis Induced Deterioration of Compressive Properties of Poly-ε-Caprolactone

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In this study solid, cylindrical samples manufactured from two poly-ε-caprolactone polymers having different average molecular weights were examined. Samples were conditioned in phosphate buffered saline at 37 °C (body temperature, "in vitro" conditions) and at 50 °C (accelerated test) for certain periods of time. DSC measurements established the degradation of amorphous regions and recrystallization in the 37 kDa samples at 37 °C, but it was not observed at 50 °C. SEC analyses clearly indicated the decrement of number average molecular weight; simultaneously compressive tests indicated the deterioration of mechanical properties. Elevated temperature accelerated the decrement of mechanical properties in both cases, which could be the result of faster diffusion and greater chain mobility, reactivity.

Keywords: *poly-ε-caprolactone, hydrolytic degradation, thermal properties, compressive mechanical properties.*

1. INTRODUCTION

During the last three decades numerous biodegradable polymers were synthesized, and applied in various fields of science, from agriculture to medicine. Degradable polymeric biomaterials are preferred candidates for medical devices such as sutures [1], coronary stents [2], bone plates and screws [3], controlled/sustained release drug delivery vehicles [4] and three-dimensional porous scaffolds for tissue engineering [5], which is one of the most rapidly developing fields of medicine. The aim of tissue engineering is to create tissues, organs and synthetic grafts under laboratory conditions to overcome the difficulties resulted from lack of donors or from currently available options; and to promote the tissue healing process additionally. Bone tissue engineering develops fast, too, and has a major importance, because bone is repaired frequently (in the 90's over 800000 grafting procedures were carried out annually in the US alone [6]). On the other hand, bone is a unique 'dynamic' tissue due to its continuous remodelling. Under mechanical load it builds up, but without stress it disintegrates. Therefore, in bone tissue engineering the mechanical properties (especially the compressive ones) of grafts are utmost important. To create a 'bone-mimic' scaffold from degradable materials not only the initial properties are essential. The degradation time must be also tuned in such way that the scaffold is able to support the *in vivo* stresses until the newly grown tissue takes it over from the synthetic support.

Both synthetic and natural polymers have been investigated as biomaterials. Synthetic polymers, however, have well determined properties compared to natural ones, thus they are easier to handle for certain applications. One of the widely investigated groups of synthetic, degradable polymers is the linear, aliphatic poly-α-esters. Poly-ε-caprolactone (PCL) belongs to this group, too. It is a semicrystalline polymer, and is of great interest as it is highly processible, soluble in a wide range of organic solvents, has a low melting point (55 – 65 °C) and glass transition temperature (– 60 °C), while having the ability to form blends with a wide range of polymers. The polymer undergoes hydrolytic degradation because of the presence of hydrolytically labile aliphatic ester linkages; however, the rate of degradation is quite slow, 24 – 36 months [7]. According to Hutmacher [8], the bone remodelling lasts for at least 6 months, thus the scaffold must retain its properties at any rate for this time. Since the total loss of mechanical properties occurs after 9 – 12 months in PCL, this material could be adequate for bone tissue engineering approaches.

Numerous studies were aimed to reveal the degradation kinetics of PCL. One of the first publications over the deterioration of PCL was written by Pitt [9]. In this study, kinetic equivalency was found between in vivo degradation in rabbits and *in vitro* tests at 37 °C in water (pH 7.4). Important observation was also that the degradation rate did not change despite 10-fold changes in surface-to-volume ratio of the implant; which is indicative of a bulk process. Additionally, linear change was observed in semilogarithmic plots of molecular weight versus time, until the number-average molecular weight (M_n) decreased to approximately 5000; regardless of the initial M_n. This behaviour was described, and evaluated as an autocatalyc process, where the liberated carboxylic end groups catalyze the further cleavage of ester groups. In a more recent study Sun et al. [10] analysed the *in vivo* degradation in rats for 3 years. They found that the implanted capsules, with an initial weight-average molecular weight (M_w) of 66000, remained intact in shape after 2 years, and only

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broke into low molecular weight pieces (M_w = 8000) at the end of the 30th month. Nearly the same linear relationship was found between log M_w and time as it was in Pitt's findings [9]. The distribution, absorption and excretion of PCL were also traced by radioactive labeling. They observed that after the degradation the low molecular weight PCL metabolized and ultimately excreted from body through urine and feces. No accumulation was found in any body organs.

Ye et al. [11] investigated the *in vitro* degradation of PCL, an other poly-α-ester: the polylactide (PLA) and their random block copolymers in phosphate buffered saline (PBS) for 28 days at 37, 45 and 55 °C, respectively. It was found that the intrinsic viscosity of PCL changed only slightly; regardless of the temperature. On the other hand, the incorporation of PLA accelerated the degradation significantly, and at 55 °C the apparent rate constant of the Mark-Kuhn-Houwink equation [12] was 4 to 10 times greater than at 37 °C.

Despite the importance of mechanical properties in load bearing applications such as bone or cartilage, this question is not addressed sufficiently during the degradation analyses. Therefore, the objective of the present study was to analyse the changes of polymer structure and morphology but also to examine the mechanical properties during hydrolysis. We aimed to find relation between the morphology and compressive modulus and reveal the effect of different experimental conditions.

2. MATERIALS AND METHODS

2.1 Materials

The analyzed PCL polymers with different nominal number-average molecular weight (namely: CAPA 6250, $M_n = 2.5 \cdot 10^4$ Da and CAPA 6400, $M_n = 3.7 \cdot 10^4$ Da) were obtained from Solvay Caprolactones, Solvay Interox Ltd.

Phosphate buffered solution was supplied by Semmelweis University, 1st Department of Pathology and Experimental Cancer Research according to the suggestion of ISO 13781:1997 standard. Chemical composition of the PBS was 0.2 g/dm^3 KCl, 0.2 g/dm^3 KH_2PO_4, 1.44 g/dm^3 $Na_2HPO_4 \cdot 2H_2O$ and 8 g/dm^3 NaCl; pH was set to 7.5, and the solution contained 0.16 g/dm^3 Gentamicin Sandoz as bactericide.

In this study PCL and distilled water were chemical grade, and all the other chemicals were biological grade.

2.2 Sample preparation

Before processing, polymers were dried at 35 °C over 24 hours. Solid, cylindrical samples (diameter: 6 mm, height: 7 mm) were hot pressed using a COLLIN P-200E-type compression moulding machine. The process was as follows: at first the granules were laid in a steel tool, which was pre-heated in a Heraus drying oven at 100 °C for 5 minutes. Following the pre-heating, samples were hot pressed at 120 °C temperature, 50 bar pressure for 10 minutes, followed by water-cooling. Finally, the specimens were put in containers (volume: 5 cm^3), were radiation sterilized (dose: 25 kGy), filled up with 4 ± 0.5 cm^3 PBS and laid in a Heraus drying oven at 37 ± 0.1 °C and 50 ± 0.1 °C, respectively. At certain intervals, samples were removed, washed in distilled water and dried at 40 °C under vacuum over 4 days.

2.3 Analytical techniques

The number-average molecular weight of the polymers was measured by size exclusion chromatography (SEC) in tetrahydrofuran (THF) at 35 °C with a Waters chromatograph equipped with an In-line Degasser, four gel columns (7 μm Ultrastyrolgel columns: 500, 10^3, 10^4, 10^5 Å), a Waters 600 Solvent Delivery System (HPLC pump) and Waters 410 Differential Refractometer detectors. Samples with concentration of 4 g/dm^3 were made of certain polymers by dissolution in distilled THF. After complete dissolution, samples were filtered through a 1 μm membrane filter, and measured. The measuring parameters were: solvent flow 1 cm^3/min, injection volume 0.1 cm^3, running time 55 minutes. The M_n values of the polymers were calculated from their chromatograms on the basis of a polystyrene calibration.

The mechanical properties of dried specimens were measured at room temperature using a Zwick Z020 universal testing machine at a crosshead speed of 2 mm/min. The test conditions were set and the results were also determined according to ISO 604:2002 standard. The compressive modulus values were calculated after linear regression by the following equation:

$$E = \frac{H}{A} \cdot \frac{F_{n+1} - F_n}{L_{n+1} - L_n}, \quad (1)$$

where E [MPa] is the compressive Young's modulus, H [mm] the initial sample height, A [mm^2] the initial samples cross-section, F_n [N] the force and L_n [mm] the gauge length.

The thermal properties of samples were studied by differential scanning calorimetry (DSC). The non-isothermal curves were measured with a Perkin-Elmer DSC 7 equipped with Perkin-Elmer Intercooler 2 cooling system, the purge gas was nitrogen (20 cm^3/min). Sample weights were between 3 and 5 mg, which were measured using a Sartorius RC 210 analytical balance (accuracy: ± 0.01 mg). The measurements were carried out between – 50 and 100 °C with a heating and cooling rate of 10 °C/min. The results were evaluated according to ISO 11357-3 standard.

3. RESULTS AND DISCUSSION

PCL is a hydrolytically labile polymer, which means that the rate of degradation is dependent on the rate of water diffusion. It was reported by Pitt [9], that the water-permeability depends on crystallinity; begins in the amorphous parts of polymer, and the crystalline regions are affected later. The degradation rate is reduced by the decrement in accessible ester bonds.

Table 1. Melting temperatures and enthalpies of PCL samples after hydrolysis at different temperatures

	37 °C						50 °C					
	CAPA 6250			CAPA 6400			CAPA 6250			CAPA 6400		
Time [week]	T_{mp} [°C]	ΔH_m [J/g]	X* [%]	T_{mp} [°C]	ΔH_m [J/g]	X* [%]	T_{mp} [°C]	ΔH_m [J/g]	X* [%]	T_{mp} [°C]	ΔH_m [J/g]	X* [%]
0	65	90.8	63.7	65	80.8	56.7	64	88.2	61.9	64	82.6	58.0
2	65	88.5	62.1	65	85.7	60.1	66	86.9	61.0	67	83.1	58.3
4	65	87.7	61.5	65	87.9	61.7	69	86.5	60.7	68	83.6	58.7

* Crystalline fraction estimated using a heat of fusion for 100 % crystalline material of ΔH_0 = 142.5 J/g, given by Crescenzi et al. [15].

The crystalline structure, which can be characterised by DSC, changes as a result of chain scission and temperature-induced recrystallization during the degradation. The enthalpy of fusion of the original material is inversely proportional to the number-average molecular weight (Tab. 1). With increasing molecular weight the crystalline amount and/or its completeness decreases; which corresponds to the findings of Pitt et al. [13]. This observation can be a result of greater segment mobility of shorter molecules during crystallisation, which leads to a more regular structure.

After hydrolysis the polymer was swollen and the amorphous parts started to degrade. Additionally, at 37 °C the chain-mobility of longer molecules of CAPA 6400 increased, and they were able to re-crystallise (annealing of the primary crystalline structure). On Figure 1 a second peak is visible which might be the result of the previously mentioned recrystallization. These two processes led to increment of the enthalpy of fusion and thus to the growing of crystalline proportion (Tab. 1). The lower molecular weight CAPA 6250 crystallizes faster [14] thus this kind of recrystallization was not observed in this material.

In comparison, at 50 °C recrystallization was not observed. The melting peak-temperatures improved slightly, which suggests an ordered crystalline structure, on the contrary the enthalpy of fusion did not increase significantly. This contradiction can be explained with higher segment and chain mobility, which resulted both in the hydrolytic degradation of the amorphous parts and in the disintegration of crystalline regions; thus in total, the crystalline proportion not increased with time.

In case of CAPA 6250 the enthalpy of fusion decreased after four weeks of hydrolysis both at 37 and 50 °C (Tab. 1). The highly crystalline structure of this polymer was not able to order further, thus it started to degrade. Figure 1 shows the influence of water and 37 °C temperature on the melting peaks of PCL samples after 49 days of hydrolysis. In case of CAPA 6250 the intensity and the area of peak decreased, while the melting temperature range became wider. These are indirect signs of polymer-degradation through hydrolysis.

The change of number-average molecular weight in samples was monitored with SEC, but only in case of hydrolysis at 37 °C. The initial, measured M_n values were 31800 and 39600 Da, respectively. The difference between of measured and nominal values resulted from the applied polystyrene calibration standards, but to draw a comparison these results are acceptable as well.

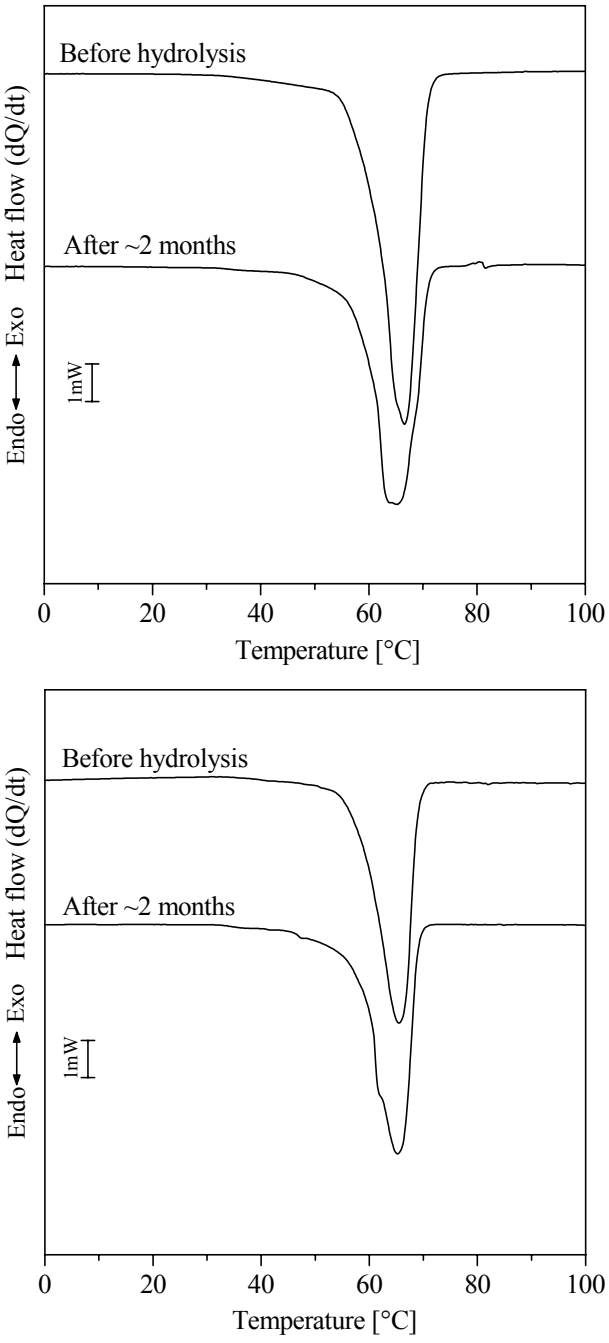

Figure 1. Calorimetric melting curves of CAPA 6250 (up) and CAPA 6400 (down) samples after hydrolysis at 37 °C (v_h = 10 °C/min)

Figure 2 shows the differences between the degradation of the two polymers during the first month. In this period CAPA 6400 degraded faster, and the M_n of

CAPA 6250 only slightly changed. As it was already mentioned CAPA 6400 had smaller crystalline proportion and/or it had worst completeness. Since the water-diffusion in the amorphous regions is faster, the lower crystallinity results in faster degradation, as it is shown below.

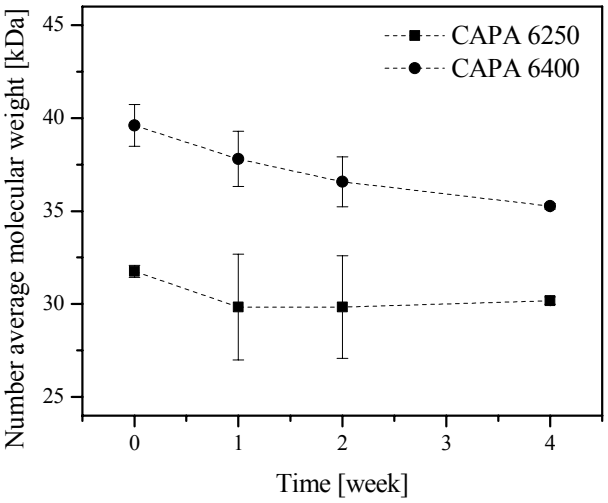

Figure 2. Change in the number average molecular weight of samples after hydrolysis at 37 °C

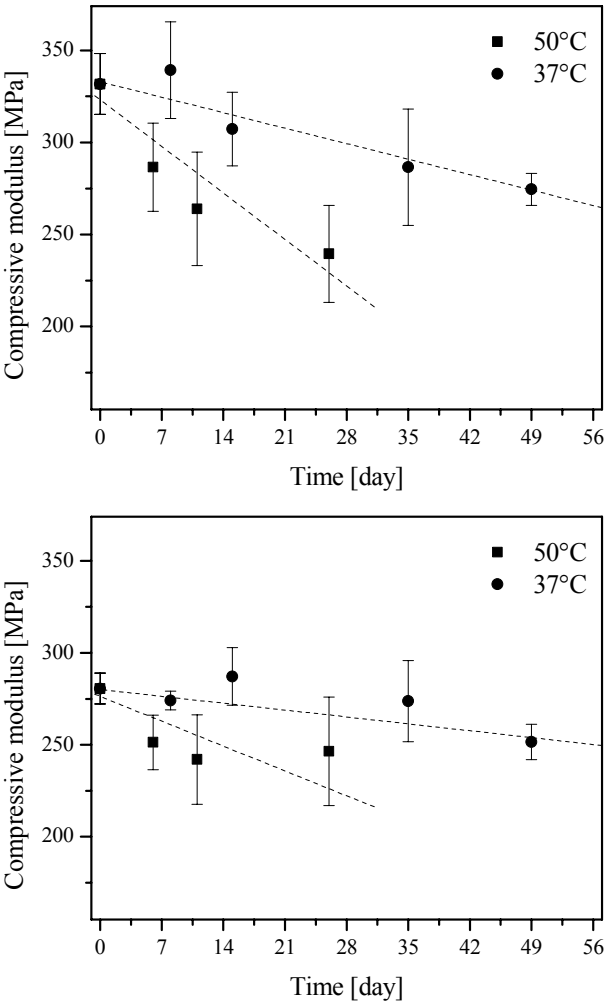

Figure 3. Time dependency of compressive modulus of CAPA 6250 (up) and of CAPA 6400 (down)

The mechanical properties of a polymer specimen depend on many factors, such as molecular chain length, crystallinity, orientation, etc. Figure 3 indicates that CAPA 6250, despite that it is build up of shorter molecules, shows better compressive modulus values. It is in contradiction to the common observation, that states that the longer the polymer chains the better the mechanical properties are [16]. DSC results showed that the CAPA 6250 material had possessed higher crystallinity than CAPA 6400, which resulted, due to more ordered structure, in better compressive properties, too.

The elevated temperature accelerated the decline of mechanical properties in both cases. At 50 °C, the polymer is close to the melting temperature, which increases the chain mobility and reactivity. On the other hand, the diffusion is also faster, since the temperature dependency of the diffusion coefficient is well predictable by an Arrhenius-type equation [16].

The deterioration of mechanical properties was more rapid in CAPA 6250 than in CAPA 6400, which is contradictory to the loss in the average molecular weight (Fig. 2). This phenomenon can be explained by the recrystallization process occurring in CAPA 6400. After hydrolysis the re-ordered crystalline structure resulted in higher enthalpy values (Tab. 1). This increased regularity of CAPA 6400 compensated the effect of faster deterioration of number average molecular weight. On the other hand, in CAPA 6250 the ordered structure started to decline and there were no processes which could slow down the degradation.

4. CONCLUSION

In this study, two commercially available, different number-average molecular weight (25 and 37 kDa) PCL samples were studied both at body temperature and at 50 °C. The higher temperature had an accelerating effect on the deterioration of properties, because the polymer chain mobility and the diffusion coefficient depend greatly on the temperature. In load bearing applications the compressive mechanical properties are principally important, therefore compressive tests were carried out. The compressive modulus of the lower molecular weight samples were better, which can be explained with the higher crystallinity and/or more regular crystalline structure. The change of molecular weight originated in the same reasons. However, the loss of mechanical properties was faster in the case of the lower molecular weight polymer, which is opposed to what is expected. SEC tests indicated that the higher crystallinity results in slower degradation; but the mechanical deterioration was in contrast with this. The performed DSC measurements showed that a recrystallization process had occurred in the higher molecular weight material. This process balanced the faster chain scission, and stabilized the degradation during the test time.

In summary, it is worth noting that although the crystallinity and molecular weight fundamentally govern the degradation and compressive properties, the thermal and mechanical history has a great influence on the degradation rate of PCL.

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ПОГОРШАЊЕ КАРАКТЕРИСТИКА НА ПРИТИСАК ПОЛИ-ε-КАПРОЛАКТОНА ИЗАЗВАНИХ ХИДРОЛИЗОМ

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Извршено је испитивање чврстих, цилиндричних узорака направљених од два поли-ε-капролактон полимера са различитим просечним молекулским тежинама. Узорци су потапани у одређеним временским интервалима у фосфатни пуферовани слани раствор на 37 °C (телесна температура, *in vitro* услови) и на 50 °C (убрзано испитивање). *DSC* мерењима је утврђено да долази до нарушавања аморфних површина и рекристализације код 37 kDa узорака на 37 °C , што се није дешавало на температури од 50 °C. *SEC* анализе су јасно показале да се смањује просечна молекулска тежина. Истовремена испитивања на притисак показала су погоршање механичких својстава. У оба случаја повећање температуре је убрзавало погоршање механичких својстава, што би могло да представља последицу убрзане дифузије и веће мобилности, тј. реактивности ланца.