Acta Poloniae Pharmaceutica – Drug Research, Vol. 71 No. 6 pp. 1023–1028, 2014 ISSN 0001-6837
Polish Pharmaceutical Society

BIORESORBABLE COPOLYMER OF L-LACTIDE AND ε-CAPROLACTONE FOR CONTROLLED PACLITAXEL DELIVERY

MONIKA MUSIAŁ-KULIK[1]*, KATARZYNA GĘBAROWSKA[1], JANUSZ KASPERCZYK[1,2], MAŁGORZATA PASTUSIAK[1], HENRYK JANECZEK[1] and PIOTR DOBRZYŃSKI[1,3]

[1]Centre of Polymer and Carbon Materials, Polish Academy of Sciences, Skłodowska-Curie St. 34, 41-819 Zabrze, Poland
[2]Medical University of Silesia in Katowice, School of Pharmacy and Division of Laboratory Medicine, Department of Biopharmacy, Jedności 8, 41-200 Sosnowiec, Poland
[3]Faculty of Mathematics and Natural Science, Jan Dlugosz University, Al. Armii Krajowej 13/1, 42-200 Częstochowa, Poland

Abstract: Bioresorbable, aliphatic polyesters are known in medicine where serve as orthopedic devices (e.g., rods, pins and screws) or sutures and staples in wound closure. Moreover, such materials are extensively studied as scaffolds – three-dimensional structures for tissue engineering but also drug delivery systems (DDS). The aim of this study was to determine the release profile of paclitaxel, one of the anti-inflammatory, antiproliferative and anti-restenotic agent, from biocompatible copolymer of L-lactide and ε-caprolactone that seems to be very attractive especially for minimally invasive surgery due to its potential shape-memory property. The influence of drug on copolymer hydrolytic degradation was also analyzed. Three types of matrices (3%, 5% of PTX and without drug) were prepared by solvent-casting method and degraded *in vitro*. The physicochemical changes of copolymer were analyzed by means of nuclear magnetic resonance spectroscopy (NMR), gel permeation chromatography (GPC) and differential scanning calorimetry (DSC). The amount of drug released into media was monitored with the use of high-pressure liquid chromatography (HPLC). Similar drug release profiles were obtained for matrices with paclitaxel. The drug-containing matrices degraded slightly slower than drug free matrices, regardless PTX content. Results of this work may be helpful in designing new bioresorbable paclitaxel delivery system applied in anti-cancer therapy or drug-eluting stents technology.

Keywords: paclitaxel, drug delivery systems, bioresorbable polymers, poly(L-lactide-co-ε-caprolactone), hydrolytic degradation

Bioresorbable, aliphatic polyesters are known in medicine where serve as orthopedic devices (e.g., rods, pins and screws) or sutures and staples in wound closure. These materials are extensively studied as scaffolds – three-dimensional structures for tissue engineering but also drug delivery systems. They possess many desirable features as biodegradation to non-toxic products, biocompatibility and easy manufacturing/processing (1). Furthermore, it was established that some of aliphatic polyesters and polyestercarbonates have the ability to recover from temporary to permanent shape upon external stimulus such as temperature or irradiation. Shape-memory effect may be utilized in minimally invasive surgery when deformed device is implanted through natural orifices or small incisions, e.g., cardiovascular stents (2, 3).

Controlled drug delivery systems (CDDS) possess many advantages when compared to conventional dosage forms. They provide even, local or systemic, release profile of drug for a determined period of time, help to reduce or avoid side effects and, what is more important, increase patient comfort during the treatment (4).

Paclitaxel (Taxol™, PTX), a plant-derived anti-cancer and anti-restenotic agent, has been originally isolated from the bark of *Taxus brevifolia*. Its anti-proliferative activity is due to the inhibition of microtubule depolymerization, which results in a suppression of cell cycle and leads to apoptosis. PTX is widely used as an antineoplastic agent against breast, ovarian, lung, head, colon cancer and Kaposi's sarcoma but also as an anti-restenotic drug eluted from cardiovascular stents (Taxus™, Boston

* Corresponding author: e-mail: mmusial@cmpw-pan.edu.pl

Scientific). Highly hydrophobic nature of paclitaxel promotes its cellular uptake but presents substantial formulation challenges, especially concerning duration and rate of drug release (5, 6). Copolymers of L-lactide and ε-caprolactone may be interesting as alternative materials for paclitaxel delivery.

Poly(L-lactide) (PLLA) constitutes one of the most intensively studied polymers for a wide range of applications, including packaging, agriculture and disposable materials as well as medical devices or scaffolds for tissue regeneration (7). PLLA characterizes good mechanical properties but even though the polymer loses its strength in approximately 6 months when degraded *in vitro*. Therefore, in order to modify its mechanical features and also rate of degradation, PLLA is often blended with other polymers as poly(ethylene glycol) (PEG), collagen, chitosan or copolymerized with e.g., D,L-lactide or glycolide. Poly(ε-caprolactone) (PCL), a semicrystalline polyester with high drug permeability and good organic solvent solubility, is known as a polymer with slow degradation rate. It was applied as a contraceptive implant delivering levonorgestrel (Capronor®). PCL has a glass transition temperature of –54°C, low tensile strength and an extremely high elongation at breakage (4700%) (8). Copolymers of L-lactide with ε-caprolactone are obtained to meet requirements, dependent on application, for physicochemical and mechanical properties that influence degradation and drug release rate. It was reported that some of poly(L-lactide-co-ε-caprolactone)s (PLACap) possess shape-memory property and may be also considered as drug delivery systems (9, 10).

The aim of this study was to determine the release profile of paclitaxel from biocompatible PLACap that seems to be very attractive especially for minimally invasive surgery due to its potential shape-memory property. The influence of drug on copolymer hydrolytic degradation was also analyzed.

EXPERIMENTAL

Poly(L-lactide-co-ε-caprolactone) was synthesized with the use of $Zr(Acac)_4$ as a low toxic initiator of the ring-opening polymerization. The reaction was performed at 150°C for 25 h with the initiator to monomer molar ratio (I/M) of 1/1100. Paclitaxel (PTX) was purchased from LC Laboratories®.

Appropriate amount of PLACap and drug were dissolved separately in methylene chloride. Then, the two solutions were mixed, degassed under reduced pressure and cast on Teflon plates. All films were dried at ambient temperature and then under reduced pressure. Ten millimeters discs were cut from the films. The matrices without drug were obtained analogously.

Three kind of matrix: 0, 3 and 5% of PTX was hermetically packed and irradiated with the use of electron beam. The weighted matrices were immersed in phosphate buffered saline (PBS, pH 7.4) and incubated at 37°C under constant shaking for 15 weeks. Medium was renewed once a week and collected for HPLC analysis. At predetermined time points, each type of matrix was withdrawn, washed with distilled water, weighted then dried under reduced pressure until constant weight. Water uptake and weight loss were calculated according to the following equations:

$$\text{Water uptake } (\%) = [(W_{wet} - W_{dry})/W_{dry}] \times 100 \quad (1)$$

$$\text{Weight loss } (\%) = [(W_0 - W_{dry})/W_0] \times 100 \quad (2)$$

where W_{wet} = the weight of wet sample after withdrawn, W_{dry} = the weight of dried sample, W_0 = the initial weight of sample before degradation.

NMR spectroscope (AVANCE II Ultra Shield Plus, 600 MHz, Bruker) was employed to record ^1H spectra of copolymers in order to characterize comonomers composition and chain microstructure. The number average molecular weight (M_n) and molecular mass dispersity (D) were defined by means of gel permeation chromatograph (GPC, Physics SP 8800 chromatograph). The glass-transition temperature T_g, melting temperature T_m and melting enthalpy ΔH_m were determined with the use of differential scanning calorimetry (DSC, TA DSC 2010, TA Instruments, New Castle, DE). The matrices were scanned from of –50°C to 200°C with the heating rate of 20°C/min) then quenched to –100°C in liquid nitrogen and scanned again. DSC was calibrated with high purity gallium and indium standards.

High-performance liquid chromatography (VWR-Hitachi LaChrom Elite®) was used to assess amount of paclitaxel released into PBS. Measurements were carried out with the use of LiChrospher® RP-18 column (250 × 4 mm, 5 μm) and guard column LiChrospher® RP-18 column (4 × 4 mm, 5 μm). The mobile phase consisted of acetonitrile and water (60 : 40, v/v) with the flow rate of 1 mL/min. Paclitaxel was detected at 227 nm in the presence of internal standard – docetaxel (LC Laboratories®).

RESULTS AND DISCUSSION

Copolymer of L-lactide and caprolactone (PLACap) was synthesized in order to prepare matrices for controlled paclitaxel delivery as well as

drug free matrices. Table 1 presents molecular characteristic of PLACap. The comonomer molar ratio was 87 : 13 with the average length of lactidyl and caproil blocks of 5.6 and 3.0, respectively. M_n was 38600 g/mol with the dispersity index (D) of 2.3. PLACap was semicrystalline, the glass transition temperature T_g = 35.5°C and melting temperature T_m = 134°C (ΔH_m = 20 J/g). Signals shown in ^{1}H-NMR spectrum (Fig. 1) were assigned to the lactidyl and caproil units.

Degradation process of matrices with and without paclitaxel was monitored by measuring water uptake and weight loss. It was established that water uptake is one of the initial step during hydrolytic degradation. Water molecules penetrate amorphous regions and lead to cleavage of ester bonds in the polymer chains (11). The water uptake of all matrices was almost the same during 12 weeks. After 15 weeks of incubation, drug free matrix exhibited the highest water uptake of 31%, while water uptake of

Table 1 Characterization of copolymer applied to obtain matrices.

Poly(L-lactide-co-*Í*-caprolactone)	F_{LL}	F_{Cap}	l^e_{LL}	l^e_{Cap}	Tg (°C)[1]	T_m (°C)[2]	ΔH_m (J/g)[2]	M_n (g/mol)	D
	87	13	5.6	3.0	35.5	139	20	38 600	2.3

F_{LL}, F_{Cap} – the percentage content of lactidyl and caproil units; l^e_{LL}, l^e_{Cap} – the average length of lactidyl and caproil blocks; T_g – glass transition temperature; T_m – melting temperature; $\ddot{A}H_m$ – melting enthalpy; M_n – number average molecular mass; D – dispersity index
[1] – data obtained from the second DSC scan; [2] – data obtained from the first DSC scan.

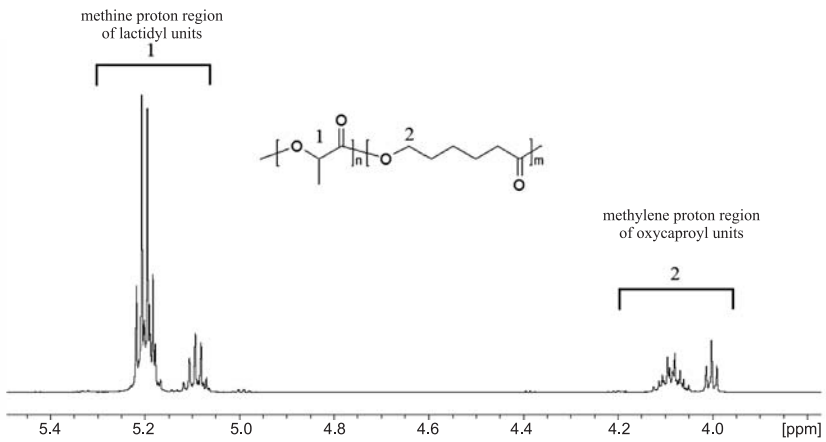

Figure 1. ^{1}H-NMR spectrum of poly(L-lactide-co-ε-caprolactone) recorded in $CDCl_3$

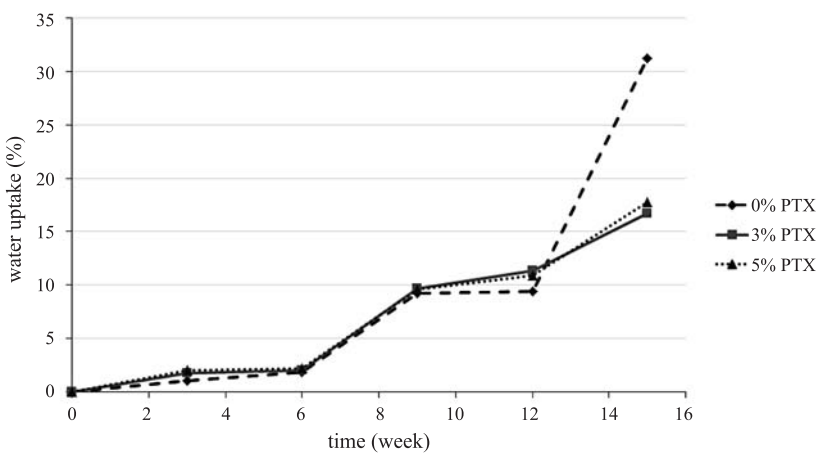

Figure 2. Water uptake of matrices during degradation

matrices with PTX reached 17 and 18% for matrix with 3 and 5% of PTX, respectively (Fig. 2). Weight loss of matrices is an effect of water-soluble oligomers and monomers release from the polymer bulk (12). The matrices with 3% and 5% of PTX lost their weight steadily to attain 29% after 15 weeks (Fig. 3). For matrix without drug, the weight loss profile was more unstable. Two rapid increases of weight loss was noticed after 9 weeks (from 3 to 18%) and 15 weeks (from 20 to 43%).

^{1}H-NMR spectra of PLACap were recorded in order to assess comonomer composition as well as the average length of lactidyl (l^e_{LL}) and caproil (l^e_{Cap}) blocks. The lactidyl units content increased and caproil units content decreased along with degradation. However, those changes were relatively small

Table 2. Changes in T_g of matrices during degradation.

Type of matrix	Degradation time (week)				
	3	6	9	12	15
0% PTX	32.5	28	24	26	35
3% PTX	34.5	28	25	25	29
5% PTX	33	28	27	24	30

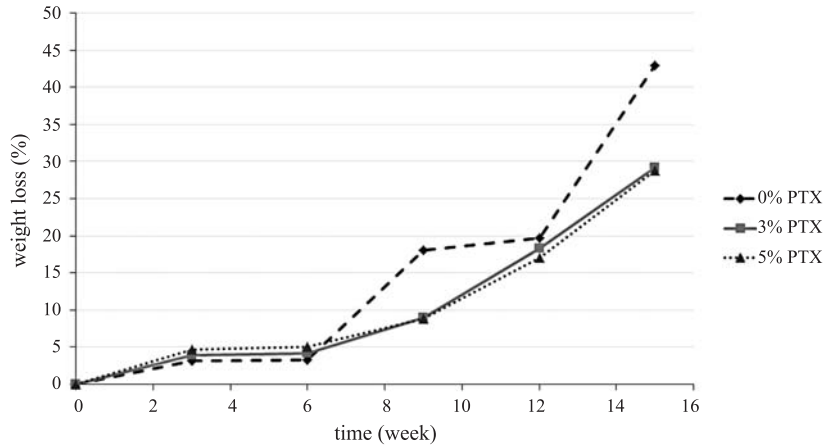

Figure 3. Weight loss of matrices during degradation

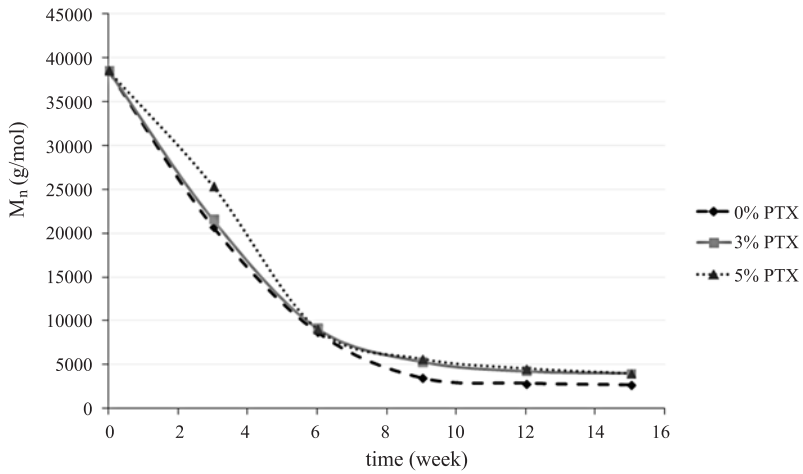

Figure 4. Changes of number average molecular weight M_n of matrices with and without PTX during degradation

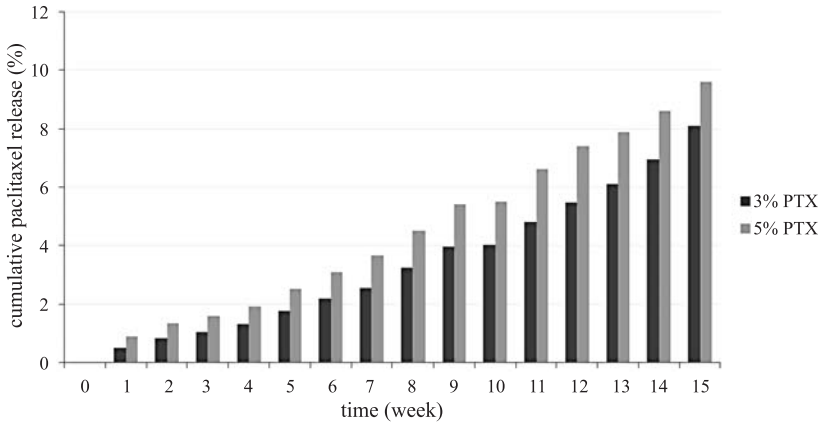

Figure 5. Cumulative release profiles of PTX from the matrices during degradation

since after 15 weeks comonomer molar ratio reached 90 : 10 (LA : Cap) for all matrices. The average lengths of caproil blocks remained stable during incubation. The l^e_{LL} decreased after 3 weeks, then increased gradually until 12 week in case of drug-containing matrices or 15 week for drug free matrix. A decrease of l^e_{LL} was observed at 15 week for both matrices with drug.

The changes in M_n and dispersity index (D) during degradation were followed by means of GPC. The highest M_n decrease was observed after 3 and 6 weeks in case of all types of matrices. After 15 weeks, M_n of drug free matrix was slightly lower than matrices with paclitaxel (Fig. 4). Dispersity index decreased after 3 weeks from initial 2.3 to 1.9, then increased to 2.0 after 6 weeks and decreased steadily to reach 1.9 (drug free matrix) and 1.7 (both matrices with PTX) after 12 weeks. It was previously reported that D decrease arises from scission of the polymer chains while its increase from release of soluble degradation products (13).

Thermal properties of matrices were analyzed with DSC. Table 2 shows changes of the glass transition temperature during degradation. In case of PTX containing matrices, T_g decreased steadily for 12 weeks and then increased to reach 29°C (3% PTX) and 30°C (5% PTX). T_g of drug free matrix started to increase after 6 weeks and attain 35°C. The increase of T_g may be attributed to the release of degradation products what corresponded to D decrease. First DSC scan revealed also melting temperature T_m that slightly decreased to 130°C after 15 weeks. It was assumed that PLACap matrices contained one type of crystalline form composed mainly of lactidyl units. The melting enthalpy ΔH_m increased from 20°C to 29°C for all matrices. This findings may be due to degradation of chains within amorphous regions and following crystallization, which was reported elsewhere (14). The ΔH_m increase was related with the l^e_{LL} and probably resulted from crystallization of long lactidyl blocks and degradation of short ones.

HPLC allowed to evaluate the amount of paclitaxel released from PLACap matrices. Both types of matrices provided even PTX release during degradation. It was noticed that PLACap matrices with 3% of PTX released less drug than matrices with 5% of PTX. After 15 weeks, 8.1% and 9.6% of PTX was released from matrices with 3% and 5% of PTX, respectively (Fig. 5).

The conducted study revealed regular degradation of matrices, regardless paclitaxel content, although degradation of drug-containing matrices was slightly slower than matrices without drug. Results of this work demonstrate the advantages of bioresorbable copolymer as material for controlled paclitaxel delivery.

CONCLUSION

The matrices of poly(L-lactide-co-ε-caprolactone) were degraded *in vitro* and studied for changes in physicochemical properties and paclitaxel release. The most noticeable was higher values of water uptake and weight loss for drug free matrices than matrices with PTX. Although paclitaxel did not affect significantly the physicochemical properties of PLACap during degradation when compared to drug free matrices, it seemed that PTX slightly slowed degradation rate of copolymer. Different

percentage content of PTX in PLACap matrices may be applied to design paclitaxel delivery systems tailoring specific clinical indications.

Acknowledgments

This study has been financially supported by a Grant from Polish Ministry of Science and Higher Education (N N405 682 340) and Centre of Polymer and Carbon Materials (Grant for Young Scientists 2013).

REFERENCES

1. Ulery B.D., Nair L.S., Laurencin C.T.: J. Polym. Sci. B Polym. Phys. 49, 832 (2011).
2. Zini E., Scandola M., Dobrzynski P., Kasperczyk J., Bero M.: Biomacromolecules 8, 3661 (2007).
3. Yu X., Wang L., Huang M. Gong T., Li W., Cao Y., Ji D. et al.: J. Mater. Sci. Mater. Med. 23, 581 (2012).
4. Bhowmik D., Gopinath H., Kumar B.P., Duraivel S., Kumar K.P.S.: J. Pharm. Innov. 1, 24 (2012).
5. Martin D.M., Boyle F.J.: Med. Eng. Phys. 33, 148 (2011).
6. Singla A.K., Garg A., Aggarwal D.: Int. J. Pharm. 235, 179 (2002).
7. Lopes M.S., Jardini A.L., Filho M.R.: Procedia Eng. 42, 1402 (2012).
8. Ulery B.D., Nair L.S., Laurencin C.T.: J. Polym. Sci. B Polym. Phys. 49, 832 (2011).
9. Lu X.L., Sun Z.J., Cai W., Gao Z.Y.: J. Mater. Sci. Mater. Med. 19, 395 (2008).
10. Jelonek K., Kasperczyk J., Li S., Dobrzynski P., Janeczek H., Jarzabek B.: BioMed. Res. Int. 2013, 607351 (2013).
11. Hofmann D., Entrialgo-Castańo M., Kratz K., Lendlein A.: Adv. Mater. 21, 3237 (2009).
12. Engineer C., Parikh J., Raval A.: Trends Biomater. Artif. Organs 25, 79 (2011).
13. Schliecker G., Schmidt C., Fuchs S., Wombacher R., Kissel T.: Int. J. Pharm. 266, 39 (2003).
14. Hua J., Gebarowska K., Dobrzynski P., Kasperczyk J., Wei J., Li S.: J. Polym. Sci. A Polym. Chem. 47, 3869 (2009).