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THE INFLUENCE OF PACLITAXEL ON HYDROLYTIC DEGRADATION IN MATRICES OBTAINED FROM ALIPHATIC POLYESTERS AND POLYESTER CARBONATES

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Abstract: Biodegradable polymers have become common materials used in pharmacy and medicine due to their properties such as mechanical strength, biocompatibility and non-toxic degradation products. Different compositions of copolymers and also their chain microstructure may have an effect on matrices degradation and thus on the drug release profile. In our study, we aimed at the influence of paclitaxel content on hydrolytic degradation process of terpolymeric matrices. Hydrolytic degradation of three kinds of matrices (with 5 or 10% of paclitaxel and drug free matrices) prepared from three types of terpolymers was performed *in vitro* at 37°C in phosphate buffer solution (PBS, pH 7,4). The ^{1}H and ^{13}C NMR spectra of terpolymers were recorded. Thermal properties were monitored by differential scanning calorimetry (DSC). Molecular weight dispersity (D) and molecular weight were determined using gel permeation chromatography (GPC). The surface morphology was studied by means of the scanning electron microscopy (SEM). The most significant degradation was observed in case of poly(L-lactide-co-glycolide-co-ε-caprolactone) 44:32:24. Weight loss and water uptake were similar in the event of the same type of matrices obtained from the two poly(L-lactide-co-glycolide-co-TMC). Decelerated paclitaxel release in case of matrices with 51:26:23 molar ratio was noticed and it can be connected with higher content of carbonate units. Knowledge of paclitaxel influence on hydrolytic degradation process may contribute to receive valuable information about its release mechanisms from biodegradable terpolymers.

Keywords: paclitaxel, biodegradable terpolymers, hydrolytic degradation, restenosis, controlled drug delivery

In 1977 Andreas Grunzig carried out first percutaneous transluminal coronary angioplasty (PTCA) and in 1986 Jacques Puel and Ulrich Sigwart implanted the first coronary metal stent to prevent the vessel collapse during PTCA (1). This phenomenon began a new era in the treatment of coronary artery disease, however, in-stent restenosis still remains the main limitation of stent placement. Restenosis is caused by proliferation of blood vessel cells and leads to restriction in blood flow after percutaneous coronary intervention (PCI) (2). Drug-eluting stents (DES) have become promising solution of this problem as they release different kinds of immunosuppressive drugs. Cypher™ with sirolimus was the first DES approved by the American Food and Drug Administration in 2003 and TAXUS™ with paclitaxel has been available one year later, however, both of them are made of non-resorbable polymers (3).

Paclitaxel (Taxol®) is one of the diterpenoid pseudoalkaloid isolated from *Taxus brevifolia (*Pacific Yew). It stabilizes microtubule causing arrest of mitosis in G2/M phase. Therefore, taxol has cytostatic instead of cytotoxic properties and is used as anti-restenotic drug (4). There are attempts to prepare fully biodegradable polymeric matrices which may be employed as stents or stent coatings.

Synthetic polymers belong to materials which are very important in biomedical field. They are used as implants, scaffolds and drug delivery systems due to their excellent properties. One of the most important advantages of polymeric devices is their degradation to non-toxic products inside the human body, that eliminates the necessity of second surgery in order to remove the implants (5). The most common materials are those obtained from glycolide, lactide, ε-caprolactone and trimethylene carbonate (TMC). They differ with regard to their

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crystallinity, solubility in organic solvents, tensile strength, degradation time, etc. and therefore, it is crucial to design polymer with appropriate composition and also polymer chain microstructure.

In this study different types of matrices were prepared and the influence of paclitaxel on hydrolytic degradation of terpolymer was determined.

EXPERIMENTAL

The studied matrices were prepared from different types of terpolymers synthesized by ring-opening polymerization with Zr(Acac) as the non-toxic initiator at the Centre of Polymer and Carbon Materials, Polish Academy of Sciences in Zabrze. Three selected materials were used to prepare matrices with 5% and 10% of paclitaxel (LC laboratories): poly(L-lactide-co-glycolide-co-ε-caprolactone) (44:32:24) – TER1 and 2 poly(L-lactide-co-glycolide-co-TMC) with various composition (51:26:23 – TER2 and 62:27:11 – TER3). Thin films were obtained from mixed solutions of terpolymer in methylene chloride and solution of the appropriate drug amount. Drug-free matrices were also prepared. Solutions were cast by means of a standard casting device on a glass plate, evaporated at ambient temperature and dried under reduced pressure.

The 1.2 cm diameter specimens of each terpolymeric matrice were weighted and placed in closed flasks filled with phosphate buffer solution (PBS, pH 7,4). The flasks were incubated at 37°C and constantly shaked. At defined time points, the PBS was renewed. Every three weeks, one sample of each kind of matrices was withdrawn, washed with distilled water, weighted, vacuum dried and investigated. Water uptake and weight loss were defined from the following equations:

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

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

where W_{wet} is weight after wiping, W_{dry} – weight after vacuum drying and W_0 – initial weight.

The ^1H and ^{13}C NMR spectra of terpolymers were recorded with AVANCE II Ultra Shield Plus, Bruker 600 MHz apparatus. Dried DMSO-d_6 and $CDCl_3$ were used as solvents and tetramethylsilane as an internal reference. The initial amount of incorporated drug was confirmed by NMR.

The thermal properties of matrices and pure paclitaxel were characterized by analysis of the differential scanning calorimetry thermograms (TA DSC 2010, TA Instruments, New Castle, DE). Weighted samples were placed in aluminium pans and scanned at constant temperature ramp of 20°C/min from –20°C to 220°C. The second scan was performed for glass-transition temperature (T_g) determination. The samples were rapidly cooled to –20°C, and then heated again at 20°C/min to 220°C. Gel permeation chromatography measurements (molecular weight dispersity (D) and number average molecular weight (M_n)) were carried out with Physics SP 8800 chromatograph (Spectra Physics). Chloroform was used as the solvent at a flow rate of 1 mL/min.

Scanning electron microscope (Quanta 250 FEG, FEI Company, USA) was employed to study surface morphology of matrices. Prior to observation, the samples were sputter coated with a 3 nm

Table 1. Microstructure of matrices containing paclitaxel (F_{LL}, F_{GG}, F_{Cap}, F_T – the percentage content of lactidyl, glycolidyl, caproyl, carbonate units; Mn – number average molecular weight; D – molecular weight dispersity; T_g – glass transition temperature; I/M – initiator to monomer ratio).

Name of terpolymer		**TER1**	**TER2**	**TER2**
Kind of terpolymer		poly(L-lactide-co-glycolide -co-ε-caprolactone)	poly(L-lactide-co -glycolide-co-TMC)	poly(L-lactide-co -glycolide-co-TMC)
F_{LL}		44	51	62
F_{GG}		32	26	27
F_{Cap}		24		
F_T			23	11
M_n (Da)		22000	26800	36000
D		2.6	2.6	2.4
T_g (°C)		25	34	45
Copolymerization condition	I/M	1/1000	1/1000	1/1000
	Temp.	120°C	120°C	120°C

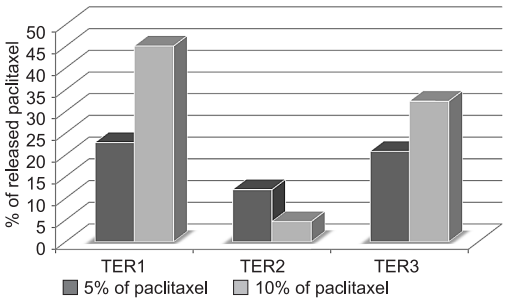

Figure 1. The percentage amount of paclitaxel released from different kind of terpolymers during 12 weeks

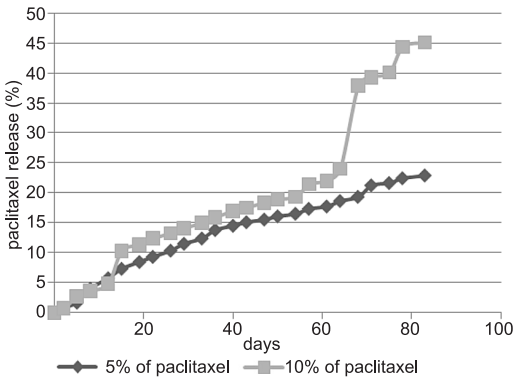

Figure 2. Cumulative release of paclitaxel from poly(L-lactide-co-glycolide-co-Í-caprolactone) – TER1

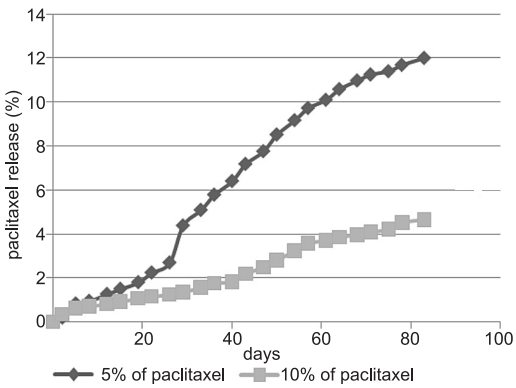

Figure 3. Cumulative release of paclitaxel from poly(L-lactide-co-glycolide-co-TMC) – TER2

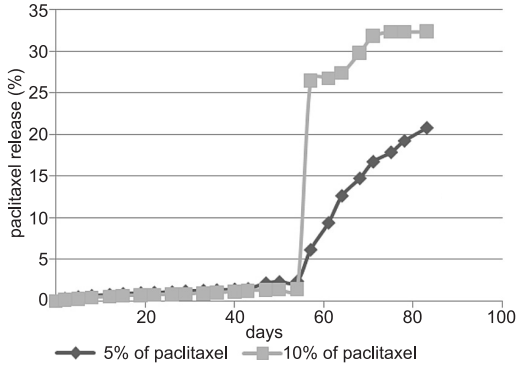

Figure 4. Cumulative release of paclitaxel from poly(L-lactide-co-glycolide-co-TMC) – TER3

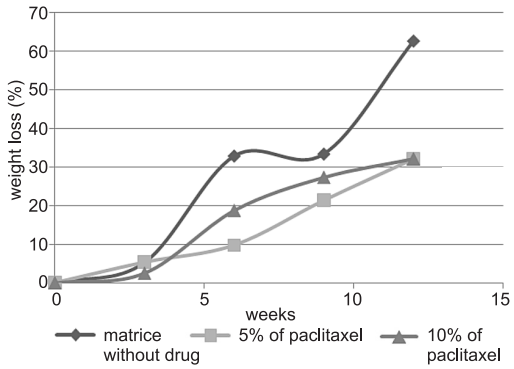

Figure 5. Weight loss during degradation of matrices obtained from TER2

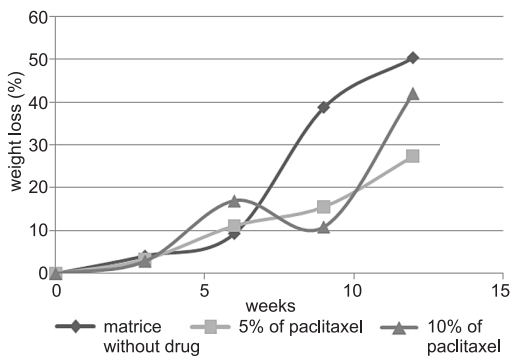

Figure 6. Weight loss during degradation of matrices obtained from TER3

layer of gold and investigated at low vacuum. The accelerating voltage was 5 kV during scanning.

Release media of PBS were retained for HPLC analysis (VWR-Hitachi LaChrom Elite®) in order to assess paclitaxel concentration. 0.4 mL of medium was mixed with 0.2 mL preservative solution (99.7%, v/v acetonitrile and 0.3% v/v glacial acetic acid) and stored at 4°C until analyzed (6). The LiChrospher® RP-18 (250 × 4 mm, 5 µm pore size) column was used and the mobile phase of acetonitrile:water (60:40, v/v) was delivered at a flow rate of 1 mL/min. Paclitaxel was detected at 227 nm and

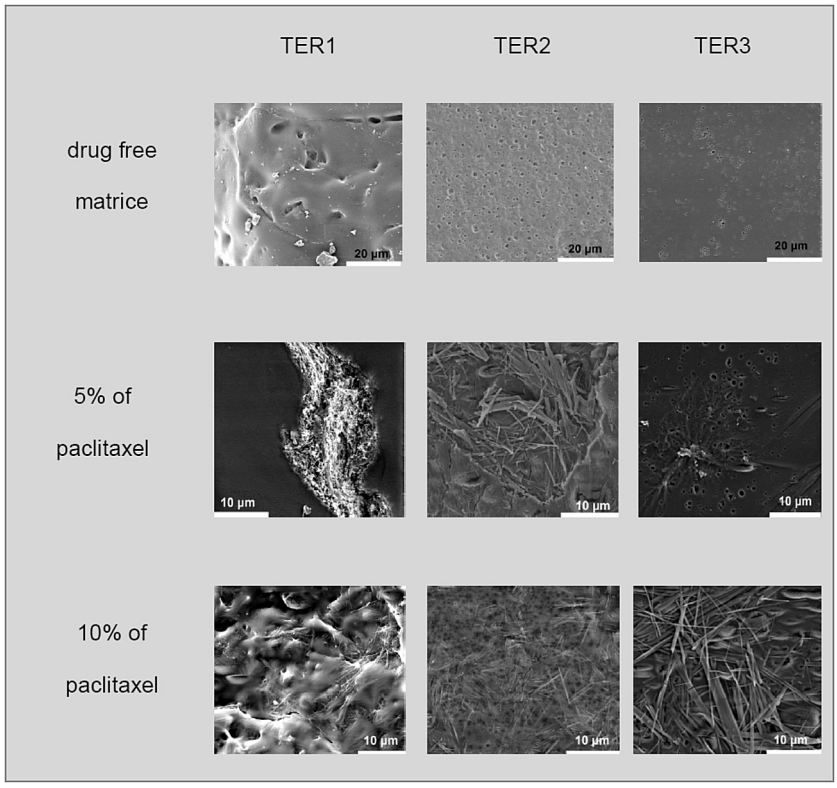

Figure 7. SEM pictures of matrices after 3 weeks of degradation

docetaxel (LC laboratories) was used as an internal standard.

RESULTS AND DISCUSSION

In this study, the influence of paclitaxel on hydrolytic degradation of terpolymeric matrices was studied. Three kinds of terpolymers were used to prepare matrices with 5% and 10% of paclitaxel and drug free matrices. Characterization of the terpolymers is presented in Table 1. All of the matrices were amorphous and had similar molecular weight as well as molecular mass dispersity. Before degradation there were no differences in chain microstructure, monomer ratio and the glass transition temperatures (T_g) between matrices of the same terpolymer type, regardless the paclitaxel loading. After 3 weeks of incubation, all matrices became semicristalline. Release profile of paclitaxel from three different terpolymeric matrices was studied, too. The amount of the released drug and drug release profiles were determined by means of HPLC analysis and are presented in Figures 1–4. The most stable and also the slowest release profile of paclitaxel was observed from poly(L-lactide-co-glycolide-co-TMC) (51:26:23 – TER2) matrices.

Degradation of matrices obtained from TER1 proceeded very fast. Small amount of material remained after three weeks of degradation that enabled to perform all of the physicochemical analyses. However, NMR study indicated the same composition changes in paclitaxel containing matrices and drug free matrices. Lactidyl units content increase and glycolidyl units decrease was observed. Those matrices released the largest amount of paclitaxel in comparison with matrices prepared from two other terpolymers. Matrices obtained from TER1 characterized the lowest T_g and molecular weight. The glass transition tempearture of TER1 was 25^{o}C, so it was in elastomeric state under the experiment conditions, thus more susceptible to water inflow because of increased polymer chains' mobility. This phenomenon may be one of the factors influencing rapid degradation of TER1 and also the higher amount of paclitaxel released.

Matrices obtained from TER2 and TER3 showed the same changes of polymer composition during degradation process – the decrease of lactidyl and glycolidyl units content with increasing TMC units. After 3 weeks of incubation, a significant decrease of molecular weight was observed, afterwards the loss of molecular mass was slower. Water uptake and weight loss after 6 weeks was observed in all kinds of matrices obtained from TER2, however, this effect was the most significant in drug free matrices (Fig. 6). This occurrence was also observed 3 weeks later in case of TER3 (Fig. 7). The analysis of changes in terpolymer composition described above indicates that the weight loss was caused by erosion of segments composed of glycolidyl and lactidyl units. Acceleration of drug release was also observed at that time. TER2 matrice with 5% of paclitaxel released more drug in comparison with TER3 matrice, nevertheless, this relation is reversed in case of matrices with 10% drug loading (Fig. 7). The presence of higher content of carbonate units may explain slower paclitaxel release from TER2. It was demonstrated that PTMC undergoes very slow degradation *in vitro* (7).

Surface morphology of matrices was observed using SEM. All the materials before degradation had non-porous and smooth surface. Paclitaxel was not observed on the surface of drug loaded matrices. Differences between drug free matrices and matrices with paclitaxel appeared after 3 weeks of incubation. All of the drug free matrices exhibited porous surface with large pores in case of TER1 or medium size pores in case of TER2 and TER3. The matrices with drug presented porous structure with visible deposits of paclitaxel on the surface (Table 2).

The presented study demonstrates, that different amount of paclitaxel was released from each kind of terpolymer. The highest amount of drug was liberated from TER1, which characterized the lowest molecular weight. Moreover, it contained caproyl units, which undergo faster degradation than the carbonate ones. The received results showed that the highest is carbonate units content, the lowest is paclitaxel release rate. However, it should be mentioned that different drug release profile may be obtained also from polymer of the same composition but with various chain microstructure. Polymer chain microstructure influences degradation (8) and thus kinetics of released drug.

CONCLUSION

The presented study demonstrates the influence of terpolymer composition on paclitaxel release rate. Slower drug release was determined for polymer with the highest carbonate units content. Slightly slower degradation was observed in case of matrices with drug, but it proceeded in very similar way – changes of physicochemical properties of drug free and drug loaded matrices did not differ significantly.

The results of the study may be helpful in developing of controlled paclitaxel release systems. Synthesis of terpolymer with suitable comonomer composition and chain microstructure may allow to obtain the tailored paclitaxel release.

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