Acta Poloniae Pharmaceutica – Drug Research, Vol. 67 No. 6 pp. 722–724, 2010 ISSN 0001-6837
Polish Pharmaceutical Society

COMPARISON OF ACCELERATED AND REAL-TIME CYCLOSPORINE A RELEASE TESTING FROM POLY(L-LACTIDE-CO-TRIMETHYLENE CARBONATE) MATRICES

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Keywords: cyclosporine A, PLATMC, *in vitro* release, accelerated degradation, controlled drug delivery

Cyclosporine A (CyA) is a cyclic undecapeptide, used in prophylaxis and therapy of graft rejection in all types of solid organ and bone narrow transplantations, as well as in treatment of a number of autoimmune diseases. However, prolonged repeated treatment with CyA may cause many side effects like nephrotoxicity, gingival hyperplasia and neurological disorders (1). Several controlled delivery systems of CyA has been studied so far as microspheres and nanospheres based on biodegradable aliphatic polyesters including poly(lactide-co-glycolide), polylactide, and poly(ε-caprolactone) (1, 2). Copolymers of TMC with L-lactide may be interesting in developing alternative delivery systems of cyclosporine A. PTMC degrades by surface erosion without acidic products, that could allow to obtain zero order drug release kinetics as well as protection of labile drug molecules (3, 4). Poly(L-lactide) (PLLA) is a crystalline polymer which undergoes slow hydrolytic degradation *via* the bulk erosion mechanism by random scission of ester bonds (3). Since slowly degradable polymers are appropriate as carriers of cyclosporine A, the process of real-time release rate estimation may last even several months. Therefore, accelerated CyA release testing is very desirable in early research for fast screening purpose – selection of polymers or establishment of drug/polymer ratio. It can be achieved by the increase in polymer degradation *via* acid or alkali catalyzed hydrolysis, addition of surfactants to enhance drug diffusion or an increase in temperature, which enhances polymer chains' mobility and drug diffusion (5). There are few literature reports on accelerated drug release testing from biodegradable carriers. It was demonstrated, that elevated temperature accelerated release testing was not suitable for PLGA microsphere systems where release was diffusion-controlled. On the other hand, elevated temperature accelerated testing was predictive of real-time release where erosion appeared to be the dominant mechanism of release (6). The aim of this study was to evaluate the usefulness of accelerated CyA release testing obtained by elevated temperature. Drug release profile and ratio was analyzed as well as changes of physicochemical properties of copolymer.

EXPERIMENTAL

Matrices were obtained from poly(L-lactide-co-TMC) synthesized at the Centre of Polymeric and Carbon Materials, Polish Academy of Sciences in Zabrze, using of $Zr(Acac)_4$ as non toxic initiator of copolymerization reaction. Drug free matrices and matrices with 2, 5 and 10 weight-% of cyclosporine A were prepared by solution casting method. After solvent evaporation at ambient temperature, the matrices were dried under reduced pressure. The samples with 1 cm diameter and 0.5 mm thickness were immersed in phosphate buffer

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Table 1. Microstructure of poly(L-lactide-co-TMC) used to produce matrices with cyclosporine A (M_n – number-average molecular weight; D – molecular-weight dispersity; F_{LL}, F_T – percentage content of lactidyl and carbonate units, respectively; l_{LL}, l_T – the average length of lactidyl and carbonate units, respectively; R – randomization ratio; T_g – glass transition temperature).

poly(L-lactide-co-trimethylene carbonate) 74 : 26							
M_n (kDa)	D	F_{LL}	F_T	l_{LL}	l_T	R	T_g (°C)
35.3	1.9	74	26	4.8	1.7	0.7	42

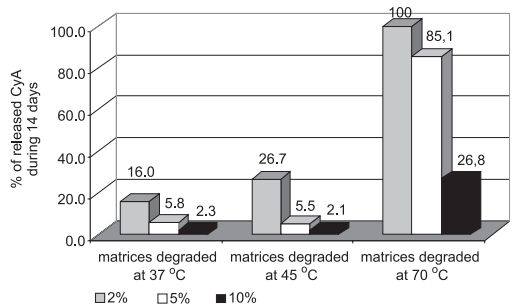

Figure 1. Cumulative release profile of CyA release from PLATMC 74 : 26 matrices incubated at 37°C, 45°C and 70°C during 14 days

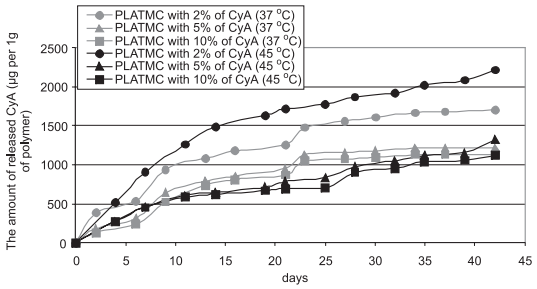

Figure 3. Release profile of cyclosporine A from PLATMC 74 : 26 matrices degraded for 42 days at 37°C and 45°C

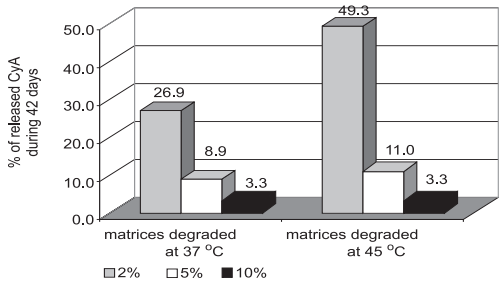

Figure 2. Cumulative release profile of CyA release from PLATMC 74 : 26 matrices incubated at 37°C and 45°C during 42 days

saline (PBS; pH = 7.4) and incubated at 37°C (the real-time degradation). The accelerated degradation was conducted in the same medium, but under elevated temperatures: at 45°C (the temperature around the T_g of copolymer) and at 70°C (the temperature above the T_g of copolymer). At regular time periods (every third or fourth day), the solution was renewed and the drug concentration was determined. The concentration of released drug during the 42 days' period was determined by means of UV- VIS spectroscopy (Spectrophotometer V-570, UV-VIS – NIR – JASCO).

All kinds of matrices were characterized before degradation and after 21 and 42 days of degradation process performed at 37°C and 45°C. Copolymer microstructure was analyzed by means of high resolution NMR spectroscopy (AVANCE II Ultra Shield Plus, Bruker 600 MHz) (7). $CDCl_3$ was used as a solvent. The ^{1}H NMR spectra were obtained at 28°C with 32 scans, 3.74 s acquisition time and 7 µs pulse width. The molecular weight (M_n) and molecular weight dispersion (D) were determined by GPC (Physics SP 8800 chromatograph), with chloroform as eluent and the flow rate was 1 mL/min. The molecular weights were calibrated with polystyrene standards. The thermal properties were examined by differential scanning calorimetry (DSC) with a TA DSC 2010 apparatus (TA Instruments, New Castle, DE) calibrated with high purity indium and gallium. The samples were scanned from –20°C to 220°C at a heating rate of 20°C/min and then quenched into liquid nitrogen.

RESULTS AND DISCUSSION

Characterization of PLATMC 74 : 26 used to obtain drug free matrices and matrices with cyclosporine A is presented in Table 1. The copolymer had random structure (R = 0.72). Thermal analysis showed that the used PLATMC was amorphous with a glass transition temperature (T_g) at 42°C. According to the literature, initially amorphous copolymers can remain amorphous during degradation due to the lack of long lactidyl sequences in the chain structure (8).

Drug release from PLATMC 74 : 26 matrices was evaluated under real-time (37°C; the temperature below T_g of copolymer) and accelerated release testing conditions temperature: 45°C (close to the T_g of copolymer) and 70°C (copolymer was in elastomeric state under these conditions).

The drug release analysis conducted at different temperatures revealed the same phenomena – the highest amount of drug was released from matrices containing 2% of cyclosporine A and the lowest from matrices with the highest initial drug loading (10%) (Fig. 1 and 2). According to expectations, very fast degradation of PLATMC 74 : 26 matrices at 70°C was observed. All cyclosporine was released until 14 days from matrices initially containing 2% of CyA and 85% from matrices with 5% of drug (Fig. 1). Comparison of matrices degraded at 37°C and 45°C revealed almost twice faster cyclosporine A release at higher temperature after 14 and 42 days. These differences were not observed in case of matrices with higher drug content (5% and 10%). This suggests, that accelerated cyclosporine A release studies may be performed at temperature close to T_g of copolymer only in case of matrices with low drug content. Aso et al. (9) investigated the effect of temperature on drug release from PLA microspheres and discs and reported that no significant release occurred below the T_g over the experimental period. At temperatures above the T_g, drug release rates increased with an increase in temperature.

However, CyA release testing from PLATMC matrices under elevated temperature seems to be useful only for designing of controlled drug release system purposes e.g., establishment of drug/polymer ratio. It revealed the influence of drug loading on the release rate and profile, however, more detailed analysis is needed for explanation of this phenomenon. This method does not allow prediction of drug release profile. Similar cyclosporine A release profiles were determined only in case of matrices with 2, 5 and 10% of drug degraded at 37°C and matrices with 5 and 10% of drug degraded at 45°C (Fig. 3). CyA release profile was completely different in case of matrices releasing drug with much higher rate (matrices with 2% of drug degraded at 45°C) (Fig. 3).

Analysis of copolymer composition and chain microstructure performed by means of NMR spectroscopy did not reveal any changes in case of all kind of matrices degraded at 37°C and 45°C for 42 days. Regular degradation process caused very uniform drug release without burst effect. The differences in copolymer composition were noticed in matrices degraded at 70°C – a decrease of carbonate units was determined in drug free matrices and an increase of carbonate units in case of drug loaded matrices.

CONCLUSIONS

The conducted study revealed, that accelerated cyclosporine A release testing under elevated temperature can be a useful method of rapid polymer screening and design of controlled drug release system, e.g., establishment of drug/polymer ratio. The influence of drug loading on the release rate and profile was demonstrated, because the CyA release analysis conducted at different temperatures showed the same phenomena – the highest amount of drug was released from matrices containing 2% of cyclosporine A and the lowest from matrices with the highest initial drug loading (10%) However, this method does not allow predicting the drug release profile.

Acknowledgment

This study has been financially supported by MEMSTENT (Grant No. UDA-POIG.01.03.01-00-123/08-02).

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