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Development of biodegradable polyesters with various microstructures for highly controlled release of epirubicin and cyclophosphamide

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ABSTRACT

In this study, "predominantly isotactic", disyndiotactic, and atactic polylactides (PLAs) and poly(ε-caprolactone)s (PCLs) were loaded with anticancer agents, epirubicin (EPI) and cyclophosphamide (CYCLOPHO), to investigate their properties as highly controlled delivery devices. It was found that the kinetic release of drugs from the obtained polyester matrices tested *in vitro* at 37 °C and pH 7.4 was strongly dependent on average molecular weight (M_n) of the polymers as well as the PLAs' microstructure. EPI and CYCLOPHO were released from various obtained matrices according to the diffusion, diffusion-degradation, and degradation mechanisms in a rather regular and continuous manner. Importantly, in some cases, the kinetics of the EPI and CYCLOPHO release was nearly zero-order, suggesting predominantly polymer degradation. It is shown that the drug release profiles can be tailored by a controlled design of the microstructure and M_n of polyesters, allowing use of the synthesized matrices for the development of highly controlled biodegradable anticancer drug delivery systems.

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1. Introduction

In 2012, there were an estimated 14 million cases of cancer around the world. The majority of these deaths concerned lung, liver, stomach, colorectal, breast, and oesophageal cancers. Novel anticancer drug delivery systems (DDS) need to be obtained in order to substantially improve the efficiency of cancer therapy (Park et al., 2008). Various new types of anticancer drug carrier are available, such as liposomes, micelles, implants, microparticles, nanocapsules, and nanoparticles (Bajpai et al., 2008; Jagur-Grodzinski, 1999; Park et al., 2008). DDS should ideally deliver a drug to a specific site in a specific time and release pattern.

Epirubicin (EPI), the 4′-epimer of the anthracycline doxorubicin (DOX), is an anthracycline antineoplastic agent that inhibits DNA replication, transcription, and repair by binding to nucleic acids, which is commercially available for intravenous administration. EPI is an analogue of DOX and a safer alternative, with comparable clinical activities as well as reduced side effects at equivalent dose. EPI has a wide range of clinical applications in diverse types of malignancy, including acute leukaemia, breast cancer, gastric cancer, non-Hodgkin's lymphomas, ovarian cancer, pancreatic cancer, small-cell lung cancer, and soft-tissue sarcomas (Laurence et al., 2006).

Cyclophosphamide (CYCLOPHO) is an antineoplastic agent metabolized to active alkylating metabolites. CYCLOPHO is used in the treatment of chronic lymphocytic leukaemia, lymphomas, soft-tissue and osteogenic sarcomas, and solid tumours. It is given orally or intravenously. CYCLOPHO is inactive until metabolized by the liver (Laurence et al., 2006).

In modern cancer therapy, a large majority of patients require simultaneous administration of two or more anticancer drugs. Combinatorial therapy has remarkable potential to overcome the problems of conventional oncology treatments, since an enhanced therapeutic outcome can be obtained by co-administration of multiple bioactive molecules (Bonneterre et al., 2004; Hu et al., 2010; Saracchini et al., 2013).

In recent years, the utilization of polymeric carriers of EPI or CYCLOPHO has gained considerable attention. Encapsulation of drug molecules within polymeric particles protects them from efflux transporters, whereas the small size of particles facilitates entry across the biological membrane. Biodegradable or bioresorbable aliphatic polyesters have been widely exploited for encapsulation of anticancer drugs. Drug release rates, size, and loading can be easily manipulated to provide

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Table 1
Characterization of PCL matrices obtained in the presence of ZnEt$_2$/PGAc catalytic system.

No.	Molar ratio [Zn]$_0$:[CL]$_0$	Temp. (°C)	M_n^a [Da]	PD^a	MC^b (%)	M_v^c [Da]
MPCL-1	1/50	60	4300	1.58	5	4900
MPCL-2	1/100	60	9100	1.74	3	10,300
MPCL-3	1/200	80	17,500	2.01	0	19,100

Reaction conditions: reaction temperature - 60 °C, reaction time - 48 h;
[a] Determined by GPC (M_n corrected by a factor of *ca.* 0.47 (Zoltowska et al., 2015b));
[b] *MC* (macrocyclic content) determined by MALDI TOF MS;
[c] Determined by viscosity method (K = $1.94 \cdot 10^{-4}$ dL/g and α = 0.73) (Zoltowska et al., 2015b);

further control over drug delivery. Use of these polyester systems has been approved by the FDA (Conte et al., 2000; Hoste et al., 2004; Khandare and Minko, 2006; Sobczak et al., 2007; Uhrich et al., 1999).

Numerous new EPI delivery systems have been investigated up to now. They include the following: EPI-loaded self-assembled cholesterol-conjugated carboxymethyl curdlan nanoparticles (Wang et al., 2007); polypeptide and polyethylene glycol (PEG)-*block*-poly(L-glutamic acid) (Zhang et al., 2015); PEG-EPI conjugates (Canal et al., 2010); copolymers *rac*-lactic and glycolide (Tariq et al., 2015); poly(*N*-(2-hydroxypropyl)methacrylamide) copolymer-EPI conjugates (Rihova, 2009; Yang et al., 2015); poly(styrene-*co*-maleic acid) (Angelova and Yordanov, 2014); dextran (Marquez et al., 2002); dendritic PEG (Pasut et al., 2005); human monoclonal antibodies (Takahashi et al., 1999); and poly(sialic acid) (Greco et al., 2013). CYCLOPHO delivery systems have also been studied. There are known non- or pegylated liposome-encapsulated (Saracchini et al., 2013), poly(D,L-lactide-*co*-glycolide) microsphere (Abedin et al., 2015), and polyethylenimine (Cameron and Shaver, 2011; Jeong et al., 2010; Mansour et al., 2010) carriers of CYCLOPHO. Studies seeking to discover new carriers of these drugs are also underway. The main problem with the obtained carriers is probably the lack of control in the release of the mentioned anticancer agents. Therefore, biodegradable polyesters with various microstructures seem to be one of the most interesting and promising polymer groups for application in anticancer drug delivery systems. The preparation of novel carriers of EPI and CYCLOPHO which are characterized by a highly controlled drug release profile is being demanded both by the pharmaceutical industry and by medical practitioners.

In our previous papers, new and very effective diethylzinc/gallic acid (ZnEt$_2$/GAc) and diethylzinc/propyl gallate (ZnEt$_2$/PGAc) catalytic systems were used for the first time to synthesize and characterize various non-toxic biomedical polymeric matrices (Zoltowska et al., 2015a, 2015b). The present paper is the continuation of our previous work. The synthesized ZnEt$_2$/GAc and ZnEt$_2$/PGAc catalytic systems were used for the synthesis of the polymeric carriers with different microstructures to load anticancer agents. In our current work we prepared new biodegradable polyester drug delivery systems for high controlled release of EPI or CYCLOPHO. A significant novelty of our findings relies on using biodegradable polyester matrices with different

microstructures as an efficient solution for the modification of anticancer drugs-release properties. In our research, we showed for the first time that the microstructure of the polymer matrices is a crucial factor and played an important role on the EPI or CYCLOPHO controlled release rate.

We believe that the obtained polyester matrices, with well-defined microstructure, can be practically applied as "long-", "medium-", or "short-term" EPI and CYCLOPHO controlled delivery systems.

2. Materials and methods

2.1. Materials

rac-Lactide (3,6-dimethyl-1,4-dioxane-2,5-dione, 99%, *rac*-LA, Sigma-Aldrich, Co., Poznan, Poland) was purified by recrystallization from ethyl acetate solution and dried in a vacuum oven at room temperature. ε-Caprolactone (2-oxepanone, 99%, CL, Sigma-Aldrich, Co., Poznan, Poland) was dried with calcium hydride and distilled under argon atmosphere before use. Toluene (Sigma-Aldrich, Co., Poznan, Poland) was dried over potassium or phosphorus pentoxide. Epirubicin hydrochloride (4′-Epidoxorubicin hydrochloride, EPI, Sigma-Aldrich, Co., Poznan, Poland), cyclophosphamide (2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide, CYCLOPHO, Sigma-Aldrich, Co., Poznan, Poland), diethylzinc (ZnEt$_2$, solution 15 wt% in toluene, Sigma-Aldrich, Co., Poznan, Poland) and propyl gallate (3,4,5-trihydroxybenzoic acid propyl ester, ≥98%, PGAc, Sigma-Aldrich, Co., Poznan, Poland) were used as received from the manufacturer. Phosphate buffer solution (pH 7.4 ± 0.05, 0.1 M, PBS, potassium dihydrogen phosphate/disodium hydrogen phosphate, 20 °C, Avantor Performance Materials, Gliwice, Poland) was also used as received.

2.2. Synthesis of polyester matrices

A diethylzinc/propyl gallate (ZnEt$_2$/PGAc) catalytic system was freshly prepared under argon atmosphere at room temperature immediately before reactions, according to our previously described method (Zoltowska et al., 2015a, 2015b). The ROP of CL or *rac*-LA was carried out in a glass tube in the presence of ZnEt$_2$/PGAc as catalysts immediately before reactions, according to our procedure (Zoltowska et al., 2015a, 2015b). The 3 g of monomer and the required amount of ZnEt$_2$/PGAc were placed in a 20 mL glass ampoule under argon atmosphere. The reaction vessel was then kept standing in a thermostated oil bath at 40–80 °C for 16 or 48 h (Tables 1 and 2). When the reaction time was completed, a cold reaction product was dissolved in CH$_2$Cl$_2$ and precipitated from cold methanol with diluted hydrochloric acid (5% aqueous solution). The organic phase was separated, washed with distilled water and dried to a constant weight.

2.2.1. Spectroscopy data of PCLs
^{1}H NMR (CDCl$_3$, δ, ppm): 4.03 [2H, t, –CH$_2$CH$_2$CH$_2$CH$_2$**CH$_2$**OC(O)–], 2.27 [2H, t, –CH$_2$CH$_2$CH$_2$CH$_2$**CH$_2$**COO–], 1.61 [4H, m, –CH$_2$**CH$_2$**CH$_2$**CH$_2$**CH$_2$COO–],

Table 2
Characterization of PLAs matrices obtained in the presence of ZnEt$_2$/PGAc catalytic system.

No.	Molar ratio [Zn]$_0$/[*rac*-LA]$_0$	Temp. (°C)	Time (h)	M_n^a [Da]	PD^a	MC^b (%)	M_v^c [Da]	p_2	L_i	T
MPLA-1	1/100	40	16	8900	1.38	2	8700	0.90	2.22	0
MPLA-2	1/100	60	16	9200	1.57	3	9700	0.56	3.57	0
MPLA-3	1/100	60	48	9600	1.96	17	10,100	–	–	0.49
MPLA-4	1/200	40	16	16,500	1.63	2	17,700	0.92	2.17	0
MPLA-5	1/200	60	16	16,800	1.87	2	17,500	0.59	3.39	0
MPLA-6	1/200	60	48	18,200	2.28	15	17,100	–	–	0.53

p_2 - coefficient of stereoselectivity calculated from the equation presented in (Coudane et al., 1997; Kasperczyk, 1995; Kasperczyk and Bero, 2000).
T - transesterification coefficient (Coudane et al., 1997; Kasperczyk, 1995; Kasperczyk and Bero, 2000).
L_i = 2/p_2 - average length of lactyl units (Coudane et al., 1997; Kasperczyk, 1995; Kasperczyk and Bero, 2000).
[a] Determined by GPC; M_n corrected by a factor of *ca* 0.58 (Zoltowska et al., 2015a).
[b] *MC* (macrocyclic content) determined by MALDI TOF MS.
[c] Determined by viscosity method (K = $2.21 \cdot 10^{-4}$ dL/g and α = 0.77) (Zoltowska et al., 2015a).