Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 65 (2007) 336–345
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

In vitro release of a water-soluble agent from low viscosity biodegradable, injectable oligomers

Soroor Sharifpoor, Brian Amsden *

Department of Chemical Engineering, Queen's University, Kingston, Canada

Received 14 June 2006; accepted in revised form 20 September 2006
Available online 28 September 2006

Abstract

Low-molecular-weight poly(ε-caprolactone-co-1,3-trimethylene carbonate) and poly(1,3-trimethylene carbonate) are potential vehicles for the regio-specific delivery of water-soluble agents. In this paper, the characteristics and the mechanism governing the *in vitro* release of a model water-soluble drug, vitamin B12, from these polymer vehicles was determined. The loading of vitamin B12 was kept to 1 w/w%. The oligomers examined ranged from amorphous, high viscosity to crystalline but low viscosity. The oligomers did not degrade appreciably *in vitro*. The total fraction of vitamin B12 released increased as the crystallinity of the oligomers decreased, reaching nearly total release only for the completely amorphous oligomers. The rate of release was fastest for the amorphous oligomers and dependent on their viscosity. Inclusion of a more osmotically active agent, trehalose, into the vitamin B12 particles through co-lyophilization resulted in enhanced total fraction released and a faster release rate. The results are consistent with an osmotically driven release mechanism.

Keywords: Vitamin B12; Biodegradable thermoplastic; Injectable; Local delivery; Trimethylene carbonate; ε-Caprolactone; Osmotic release

1. Introduction

Localized delivery of a therapeutic agent within a polymer vehicle has long been recognized as a beneficial strategy. To accomplish this, *in situ* forming thermoplastic semi-solid or viscous liquid depots are potentially appealing because the agent can be incorporated into the polymer through a straightforward mixing process, sustained release can be achieved, and the polymer can be designed to degrade in the biological environment. These polymer vehicles are meant to be warmed to above their melting point, and injected in liquid form. Within the tissue site, they cool to body temperature. Many different approaches to achieving this goal have been examined (see [1,2] for reviews). Key parameters of such an injectable formulation include melting point, crystallization temperature, melt viscosity and degree of crystallinity. To reduce pain upon injection, a low crystallization temperature is required, and to prevent therapeutic agent degradation, a low melting point is necessary. The polymer carrier should possess a sufficiently low viscosity to be easily injected through standard clinical needles and a suitably high viscosity to remain at the site of implantation. Moreover, crystallinity in the polymer will influence drug release rates, increase the polymer degradation time [3], and potentially result in a late-term inflammatory response [4,5].

Injectable biodegradable thermoplastics have been prepared from low-molecular-weight co- and terpolymers of ε-caprolactone and either lactide, glycolide or trimethylene carbonate [6–12]. Although factors influencing the thermal and rheological properties of these oligomeric thermoplastics and the release of hydrophobic drugs from these oligomers have been examined [10,11], to date there have been no reports on the factors influencing the release of water-soluble compounds from such delivery vehicles.

* Corresponding author. Department of Chemical Engineering, Queen's University, 19 Division Street, Kingston, Ont., Canada K7L 3N6. Tel.: +1 613 533 3093; fax: +1 613 533 6637.
E-mail address: amsden@chee.queensu.ca (B. Amsden).

0939-6411/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2006.09.008

Moreover, there have been no reports on the use of low-molecular-weight poly(trimethylene carbonate) as a drug delivery vehicle.

It was the objective of this paper to examine the effect of the degree of crystallinity and viscosity at 37 °C on the *in vitro* release rate and total fraction releasable of a water-soluble drug analog, vitamin B12, from a series of homo- and copolymers of ε-caprolactone and 1,3-trimethylene carbonate. Vitamin B12 (VB12) was incorporated into these polymer vehicles as solid particles at a low loading of 1 w/w%. These monomers were chosen for the following reasons. The homopolymer of ε-caprolactone (PCL) is semi-crystalline with a melting point (T_m) of approximately 60 °C, and a low glass transition temperature (T_g) of approximately −60 °C, depending on molecular weight [13], that results in a low melt viscosity. In addition, PCL has been demonstrated to be biocompatible [14]. Poly(1,3-trimethylene carbonate) (PTMC) is a linear amorphous aliphatic polymer, possessing a T_g that ranges from −26 to −15 °C as the molecular weight increases from 7000 to 42,000 [15]. In addition, PTMC has been reported to be degradable both *in vitro* and *in vivo* by hydrolysis, enzymatic action, and the reactive oxygen species produced by inflammatory cells [16,17]. PTMC has also been demonstrated to be biocompatible [16,18].

2. Materials and methods

Unless otherwise stated, all materials were used as received from the manufacturer. 1-Octanol (anhydrous, 99% purity), anhydrous tetrahydrofuran (THF, 99.9% purity), Dulbecco's phosphate-buffered saline (PBS), sodium azide (anhydrous), vitamin B12 (cyanocobalamin, 99% purity) and D(+)-trehalose dihydrate (from *Saccharomyces cerevisiae*), calcium hydride powder (purity 90–95%) and stannous 2-ethylhexanoate (95% purity) ($SnOct_2$) were used as received from Sigma–Aldrich Canada. ε-Caprolactone (99% purity) was purchased from Lancaster Synthesis Inc. It was dried and distilled under reduced pressure and over calcium chloride dihydrate. Trimethylene carbonate (99% purity) was used as received from Boehringer Ingelheim Corporation. Chloroform-D ($CDCl_3$, 99.8% purity) and dimethyl sulfoxide-d_6 (DMSO, 99% purity) were obtained from Cambridge Isotope Laboratories Inc. Type-1 water was obtained from a Millipore Milli-Q Plus Ultra-Pure Water System.

2.1. Oligomer synthesis

All polymerization reactions were carried out by ring-opening in the bulk at 120 °C. 1-Octanol was used as an initiator, while $SnOct_2$ was used as a co-initiator/catalyst. The monomer to initiator ratio and the catalyst to monomer ratio were set to 12:1 (mol:mol) and 10^{-3}:1 (mol:mol), respectively [10,19,20]. A mixture of the monomer(s) and the initiator was placed in a flame-dried glass ampoule. The mixture was heated to 120 °C and the resulting melt was vortex mixed and placed under vacuum for 3–4 min. $SnOct_2$ was then added and the resulting mixture was mixed and purged with nitrogen gas for 3–4 min. Finally the ampoule was heat-sealed under vacuum and transferred into a preheated oven at 120 °C for 24 h. Unless otherwise stated, after the completion of the polymerization reaction, the contents of the ampoule glass were transferred into a four dram vial and stored in a dessiccator under vacuum.

2.2. Oligomer characterization

To confirm the structure and the purity of the oligomers and to provide preliminary measurements of monomer conversion and number average molecular weight, ^{1}H NMR spectroscopy was carried out on a Bruker Avance-400 MHz spectrometer. Samples of CL and TMC monomers and homo-oligomers were prepared in $CDCl_3$ and the co-oligomers of CL and TMC were prepared in DMSO-d_6. The samples were run at a concentration of approximately 30 mg/ml at room temperature and the resulting peaks were compared to the solvent peak(s), relative to tetramethylsilane (TMS) reference.

The rate of CL and TMC monomer conversion was used to verify the sequence distribution of co-oligomers and assessing whether they are block or random in nature. For every different oligomer, approximately 10 pre-polymerization reaction mixtures were prepared in glass ampoules. The samples were placed in the oven at 120 °C simultaneously. At various reaction times the samples were removed from the oven and immediately placed within the freezer at a temperature of −20 °C to terminate the polymerization reaction. In order to obtain monomer conversion as a function of reaction time, all these samples were analyzed using ^{1}H NMR. These experiments were performed in triplicate.

Oligomer number average molecular weight (M_n), weight average molecular weight (M_w) and the polydispersity index (PDI) were obtained via gel permeation chromatography. The GPC consisted of a Waters 1525 Binary HPLC pump and a Precision Detectors Enterprise^{MDP} PD2100 Series equipped with refractive index and light-scattering detectors. Samples were prepared in anhydrous THF in concentrations of 30 mg/ml. They were then filtered (0.45 μm) and injected (100 μl) into an Ultrastyragel column (500A° THF, 7.8 × 300 mm) at 1.0 ml/min and a temperature of 30 °C. Data acquirement and processing was conducted using Precision Detectors' Precision Aquire32 and Discovery32 software programs. The increment of refractive index (dn/dc) used in the molecular weight calculations was measured using a Wyatt Optilab rEX. Samples were prepared in anhydrous THF in six different concentrations between 5 and 20 mg/ml and were then injected into the device in volumes of approximately 10 ml at 1 ml/min.

Thermal characterization of the oligomers was achieved using a Seiko 5200 differential scanning calorimeter (DSC). The calorimeter was calibrated with indium and gallium