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Research paper

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

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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.

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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

standards prior to use. Unless otherwise stated a heating and cooling rate of 10 °C/min was applied with a temperature program that involved a heating cycle followed by a cooling cycle and a final heating cycle. The temperature was initially raised from ambient to 75 °C and held for 10 min at that temperature. This was followed by the cooling of the sample from 75 to −120 °C with a hold time of 10 min. Finally, the sample temperature was increased back to 75 °C. The crystallization temperature (T_c) was measured from the cooling cycle and was taken as the onset of crystallization. The glass transition temperature, T_g, was taken as the inflection point of the second-order transition. The melting point, T_m, was measured from the second heating cycle and was taken to be the onset of melting. ΔH_f and ΔH_c were evaluated using the areas of melting and crystallization, respectively. CL is the only monomer responsible for the presence of crystallinity in CL homo-oligomers and its co-oligomers with TMC. Thus, the percentage of crystallinity (%X) for these samples was calculated by comparing their heats of fusion to the heat of fusion of 100% crystalline PCL (ΔH_f°) which is known to be 139.5 mJ/mg (Eq. 1) [21].

$$\%X = \frac{\Delta H_f}{\Delta H_f^\circ}. \quad (1)$$

The DSC was also used to obtain the crystallinity fraction at various temperatures. Initially, DSC measurements were conducted at a cooling and heating rate of 10 °C/min as outlined in the above temperature program. This was then followed by the integration of the oligomer endotherms with respect to temperature. To obtain the percent crystallinity of an oligomer at a specific temperature ($\%X_T$) Eq. (2) was used. %X represents the total percent crystallinity of the oligomer and $RelX_T$ represents the relative crystallinity at temperature T.

$$\%X_T = (RelX_T) \times (\%X). \quad (2)$$

Melt viscosity measurements were conducted using a Rheologica ViscoTech-controlled stress rheometer, which provided a temperature control within 0.1 °C. A parallel plate stainless steel fixture with a diameter of 20 and a 0.5 mm plate gap was used. Measurements were conducted at temperatures of 37 and 40 °C. The viscosities reported are zero shear rate viscosities.

2.3. In vitro degradation studies

A degradation study of homo- and co-oligomers of CL and TMC was carried out in isotonic phosphate-buffered saline (PBS) at pH 7.4 in the presence of sodium azide (0.2% w/v). 0.5 g of the preheated oligomers was injected into the bottom of preheated (37 °C) one dram vials using a 1-ml syringe equipped with an 18 gauge, 1.5 in. needle. The exact mass of oligomers injected in each vial was recorded. Since the oligomers possessed different thermal properties, they were heated to different temperatures prior to injection. The oligo(ε-caprolactone) (OCL) and 10.5:1.5 CL:TMC molar ratio oligo(ε-caprolactone-co-1,3-trimethylene carbonate) (OCLTMC 10.5/1.5) were initially heated at 60 °C for 30 min. This was followed by an additional 30 min heating at 45 °C prior to injection. The other oligomers were preheated to 40 °C for 30 min prior to injection. Following oligomer injection, the vials were filled with 3 ml of preheated PBS (37 °C) and placed in a shaker bath at 37 °C. For every different oligomer 30 vials were aliquoted from a master preparation for 10 different time points, in triplicate. The PBS was replaced every 2 weeks. At every time point for every oligomer type, three vials were removed from the shaker bath and the PBS was decanted. The oligomer was then washed with water to remove any residual PBS. After draining the water from the vial the samples were placed under vacuum at room temperature to dry. The mass of the remaining oligomer was then measured and percent mass loss was calculated.

2.4. In vitro release studies

In vitro release studies similar to degradation studies were conducted in isotonic PBS at pH 7.4 in the presence of sodium azide (0.2% w/v). Vitamin B12 (VB12) was ground and sieved to a diameter less than 25 μm. A mixture of VB12 and the oligomers was prepared at 1% w/w concentration. A homogeneous distribution of VB12 throughout the oligomer was obtained by the dissolution of the mixture in THF followed by solvent evaporation at 60 °C under constant stirring. To ensure the complete removal of residual solvent, samples were dried at a vacuum of 20 in. Hg at room temperature for 24 h. The VB12-loaded oligomers were then transferred into 1 ml syringes equipped with 18 gauge, 1.5 in. hypodermic needles. 0.1 g of the preheated mixture was then injected into the bottom of preheated (37 °C) HPLC vials to completely fill the bottom of the vial to a depth of approximately 3 mm. The exact mass of the mixture for each vial was recorded. Following injection, the vials were filled with 1 ml of preheated (37 °C) PBS and placed in a shaker bath at 37 °C. For every different oligomer, three vials were aliquoted from the syringe containing the master preparation as outlined above. As controls, oligomer-only samples were also used. At sampling times, 0.5 ml of the release medium was collected and replaced with fresh PBS. The collected release medium was then analyzed using a microplate spectrophotometer at a single wavelength of 380 nm. The concentration of VB12 in the release medium was measured as a function of time using calibration curves of known concentrations of VB12 varying from 0 to 0.5 mg/ml in PBS. In the case of trehalose incorporation, VB12 was co-lyophilized with trehalose, in a 50:50 mass ratio, from water using a Modulyo D freeze dryer (Thermosavant, USA) at 80 μbar and −50 °C for 48 h. The lyophilized powders were ground using a mortar and pestle and sieved through a Tyler sieve to yield particles of less than 25 μm diameter. These particles were distributed throughout oligo(trimethylene carbonate) (OTMC) at the same 1 w/w% loading as done without trehalose and released into PBS as described above.

2.5. VB12 and trehalose osmotic activity

The osmotic pressure (Π) of a saturated solution of VB12 in water and trehalose in water at 37 °C was calculated by measuring the melting point depression of each solution. This was done by measuring the melting point of pure water, saturated VB12 solution and saturated trehalose solution using calorimetry. The temperature profile was comprised of a cooling cycle from ambient temperature to −30 °C at a cooling rate of 10 °C/min and a hold time of 15 min. This was followed by a heating cycle with a heating rate of 0.5 °C/min which increased the temperature from −30 °C back to ambient temperature. To ensure accuracy, for every solution, this experiment was conducted three times and the melting points (midpoint) were recorded. The osmotic pressure of each sample was then calculated using Eq. (3), where ΔT_m represents the melting point depression, T is the temperature of interest (37 °C), ΔH_f is the latent heat of fusion of water, V_1 is the molar volume of water at STP and T_m^* is the melting point of the saturated solution [22].

$$\Pi = \frac{\Delta H_f \Delta T_m T}{V_1 \, T_m T_m^*}. \quad (3)$$

3. Results and discussion

3.1. Oligomer polymerization kinetics

Before measuring the thermal and rheological properties of the oligomers, it was important to determine their monomer composition. Trimethylene carbonate has been reported to polymerize at a slower rate than ε-caprolactone [23,24], which may lead to a blocky structure wherein portions of the oligomer chain are predominantly a single monomer. This in turn could result in crystallinity in the products which would increase the oligomer viscosity. The monomer conversion was therefore followed with time by quenching a polymerizing sample at a given time point, then determining the monomer composition of the quenched product via ^{1}H NMR. Fig. 1 shows an example of the NMR spectra obtained and indicates how the TMC monomer conversion was followed by using the intensity of the –$CH_2CH_2CH_2$-peak associated with the TMC monomer ($\delta = 2.05$ ppm) and the same group in the growing polymer ($\delta = 1.92$ ppm), and the CL monomer conversion was followed by using the intensity of the –CH_2CO–O– peak associated with the CL monomer ($\delta = 2.32$ ppm) and in the growing polymer ($\delta = 2.65$ ppm). The conversion rates thus calculated are illustrated in Fig. 2.

Fig. 2 shows that the CL monomer does polymerize more rapidly than the TMC monomer, although not dramatically so. Moreover, the monomer conversion was independent of the initial monomer compositions. CL and TMC polymerized at essentially the same rate in the presence or absence of the other monomer. This result indicates that monomer insertion into the growing chain does not depend on the nature of the monomer at the terminus. CL conversion was essentially complete after 2 h, while TMC conversion was complete after 4 h. Additionally, the copolymers comprised of equimolar TMC and CL are random copolymers since at virtually every time point, there is both TMC and CL conversion. However, there is

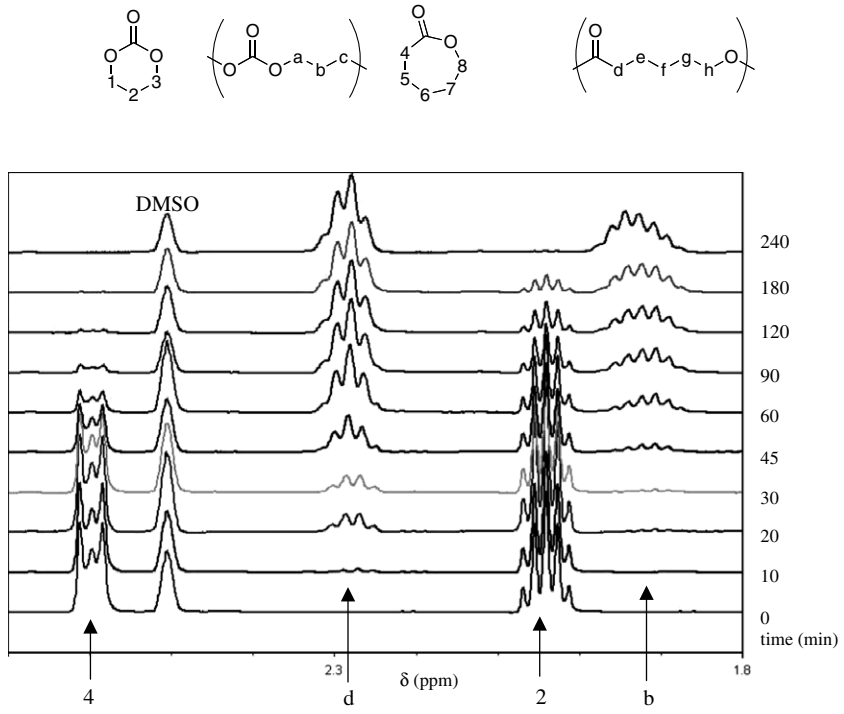

Fig. 1. ε-Caprolactone and trimethylene carbonate monomer conversion with time during copolymerization as determined by ^{1}H NMR.

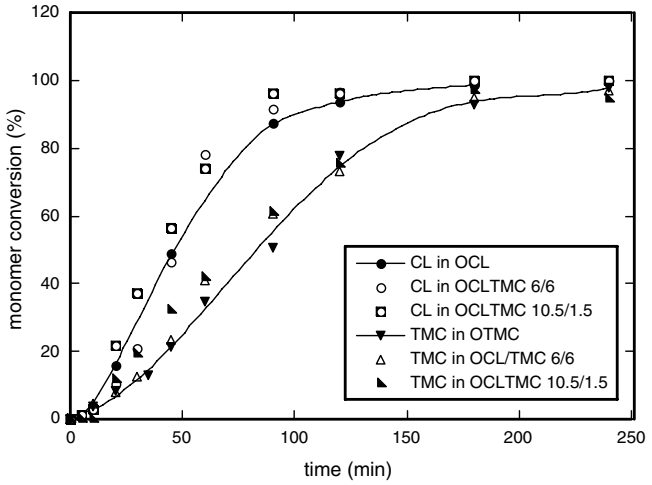

Fig. 2. Monomer conversion versus time for homopolymerization of ε-caprolactone (CL) and trimethylene carbonate (TMC), and for their copolymerization at 120 °C. The solid lines have been drawn to show the trend in the data.

slight monomer blockiness in this oligomer as the chain ends are virtually exclusively TMC.

The ^{1}H NMR spectra were also used to calculate the copolymer molar compositions and number average molecular weight via end-group analysis. These data are provided in Table 1, along with the number average and weight average molecular weight and polydispersity index as measured via GPC with a light-scattering detector. The monomer compositions in the oligomers were very close to the initial molar ratios in the pre-polymerized mass, as expected from the conversion results. The M_n obtained from ^{1}H NMR end-group analysis and from GPC are in good agreement. The polydispersity indices indicate that the TMC-containing oligomers have a broader molecular weight distribution than the CL homo-oligomer, likely as a result of their differing reactivities.

3.2. Oligomer thermal properties

As mentioned in Section 1, a low melting point (T_m) and crystallization temperature (T_c) are required to reduce pain upon injection. In addition, crystallinity of the oligomer is expected to influence its degradation rate and the drug release kinetics. Thus, thermal analysis was performed on the oligomers and a summary of the obtained data is given in Table 2. It should be noted that once heated above the melting point range, all crystals in the material are destroyed. Upon cooling, crystals do not reform immediately and do so at temperatures below the melting point. This is the crystallization temperature, T_c, reported in Table 2. Thus, the viscosity of the oligomer can be measured at a temperature within the melting point range, if it is first heated above the melting point range, then cooled down.

Table 1
Molar composition and molecular weights of homo- and co-oligomers of ε-caprolactone and 1,3-trimethylene carbonate

Sample	^{1}H NMR		GPC		
	CL:TMC	M_n	M_n	M_w	PDI
OCL	11.3:0	1425	1480 ± 25	1980 ± 20	1.4
OCLTMC 10.5/1.5	10.0:1.5	1375	1550 ± 25	2810 ± 75	1.8
OCLTMC 6:6	5.6:5.6	1345	1400 ± 50	2525 ± 15	1.8
OTMC	0:11.6	1315	1470 ± 20	2520 ± 35	1.7

An initial total monomer to 1-octanol initiator ratio of 12:1 was used in preparing the oligomers.

The CL homo-oligomer (OCL) is highly crystalline, and has a relatively high onset of melting at 42 °C and a broad melting point range of 21 °C. Conversely, the TMC homo-oligomer (OTMC) is amorphous. The copolymerization of just 12.5 mol% TMC with CL (OCLTMC 10.5/1.5) decreased the melting point of the resulting oligomer to 25 °C, however, the oligomer still had a broad melting point range of 25 °C. The inclusion of the TMC in this oligomer also reduced the % crystallinity at 37 °C from that of the OCL, from 49% to 17%. As expected, the OCLTMC 6/6 oligomer was amorphous. Blending OCLTMC 10.5/1.5 and the OTMC in equal proportions was also done to modify the properties of the injectable delivery vehicle. Blending had little influence on the melting point, which remained at around 25 °C, but reduced the degree of crystallinity from 17% to 5% at 37 °C. The decrease in the degree of crystallinity to less than half that of the OCLTMC 10.5/1.5 indicates that the presence of the TMC does more than just dilute the crystallizable CL blocks, it also reduces the capacity of the CL blocks to crystallize.

The melt viscosity of a linear polymer is closely related to its glass transition temperature. The glass transition temperature of the OCL is very low at −69 °C, and that of the OTMC is somewhat higher at −41 °C. Accordingly, the melt viscosity of the OCL is lower than that of the OTMC at 45 °C. Due to the high melting temperature of the OCL, its melt viscosity could not be measured at 40 °C, and it is not a likely candidate for use as an injectable drug delivery carrier. All oligomers containing TMC had melt viscosities that were measurable at 37 °C, and thus appear suitable as potential injectable vehicles for localized drug delivery. As the amount of TMC copolymerized with CL increased, the T_g increased as did the melt viscosity. Additionally, the blend exhibited a single T_g that was intermediate to that of the individual oligomers comprising the blend. This result indicates that these two oligomers are miscible. This result was expected, as PTMC and PCL in higher molecular weights have also been shown to be miscible [24].

3.3. In vitro degradation

In vitro degradation studies were performed to gain an indication of whether oligomer hydrolysis plays a significant role in drug release. Higher-molecular-weight PTMC

Table 2
Thermal and rheological properties of homo- and co-oligomers of ε-caprolactone and 1,3-trimethylene carbonate

Sample	Melting range	T_c (°C)	T_g (°C)	%X[a] (20 °C)	%X (37 °C)	η (Pa s) (37 °C)	η (Pa s) (40 °C)
OCL	42–63	29	−69	49	49	nd	nd
OCLTMC 10.5/1.5	25–50	12	−67	33	17	1.12	0.96
OCLTMC 6/6	n/a	n/a	−62	n/a	n/a	2.72	2.28
Blend of OCLTMC 10.5/1.5 and OTMC	24–47	10	−49	14	5	3.08	2.56
OTMC	n/a	n/a	−41	n/a	n/a	16.8	13.10

[a] X, degree of crystallinity.

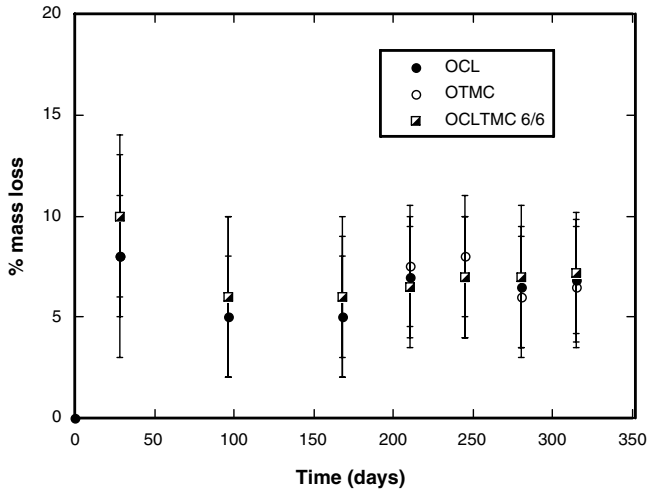

Fig. 3. *In vitro* mass loss of the oligomers in pH 7.4 PBS at 37 °C.

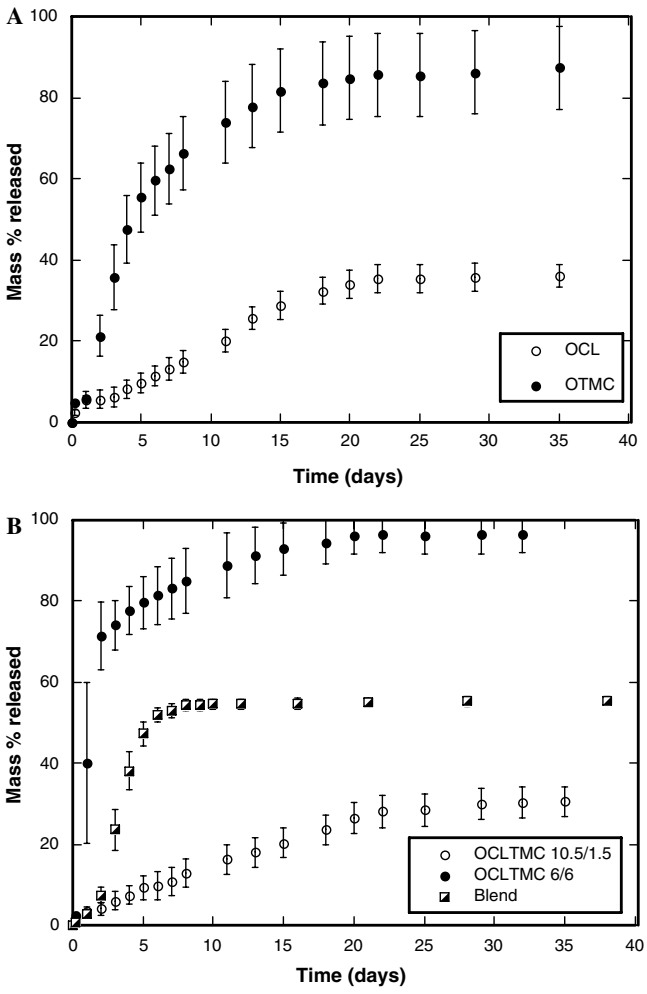

Fig. 4. VB12 release from (A) oligo(ε-caprolactone) (OCL) and oligo(trimethylene carbonate) (OTMC) and (B) oligo(ε-caprolactone-co-trimethylene carbonate) of various comonomer composition, and a 50:50 w/w% blend of the OTMC and OCLTMC 10.5/1.5.

and poly(trimethylene carbonate-co-ε-caprolactone) (PCLTMC) have been demonstrated to undergo insignificant hydrolysis *in vitro* for prolonged periods of time [15,25]. For example, Zhu et al. found that PTMC of an initial M_n of 14,000 decreased in molecular weight by only 1000 g/mol over a 30-week period in PBS [15] while Pego et al. report that PTMC-CL of 10–82% TMC content and M_n from 150 to 200,000 showed no significant mass loss up to 2 years in PBS [25]. Our oligomers are of much lower molecular weight, and would theoretically require fewer bond cleavages to be reduced to a water-soluble fraction. However, the mass loss over a period of 320 days was very low (5–8 wt%) and was the same for each oligomer studied (see Fig. 3). Thus, it is unlikely that oligomer degradation would influence the release kinetics. It should be noted, however, that PTMC and its copolymers have been found to degrade significantly faster *in vivo* [16,17,26]. This increased degradation rate has been attributed to oxidative cleavage due to polymorphonucleocyte release of oxidizing species [17].

3.4. In vitro release

The *in vitro* release of VB12 from the various oligomers is shown in Fig. 4. In each case, release began with a slight burst (<5 w/w%) due to dissolution of surface resident particles, followed by a lag period. The lag period was relatively short for formulations prepared from the amorphous oligomers (less than 1 day), but significantly longer for formulations prepared from oligomers possessing crystallinity at 37 °C. For example, for OCL formulations, which possess a crystallinity of 49% at 37 °C, the lag period was 3 days. Following the lag period, the release was nearly constant, but generally leveled within 20 days. The total fraction of VB12 released decreased as the crystallinity of the oligomer increased (Fig. 5). This result indicates that the mechanical/rheological properties of the oligomer play a major role in the release mechanism. Complete VB12 release from the more crystalline oligomers was not

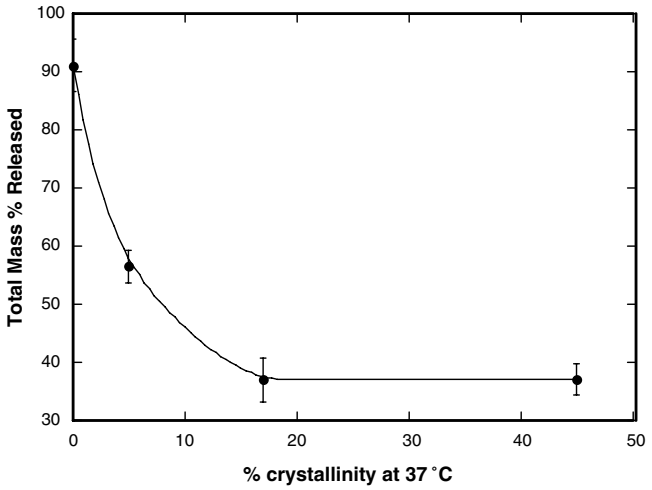

Fig. 5. Influence of percent crystallinity of the oligomer matrix on total mass fraction of VB12 released.

observed because this would require degradation of the oligomer, which did not occur to a significant extent over the time frame of the release study. For the formulations prepared using the amorphous oligomers, the release was approximately linear for a substantial portion of the release period, and the release rate increased as the melt viscosity of the oligomer decreased (Fig. 6). Fig. 6 includes a linear regression over the portion of the release profile that exhibits nearly zero-order release (solid lines). The release rate thus calculated from the OTMC formulation ($\eta_{37°C} = 16.8$ Pa s) was 10.6 ± 0.8 mass fraction/day over 6 days (correlation coefficient$^2 = 0.971$), while that of the OCLTMC 6/6 formulation ($\eta_{37°C} = 2.72$ Pa s) was 37.3 ± 2.7 mass fraction/day over 2 days (correlation coefficient$^2 = 0.989$). This finding is again indicative that the rheological properties of the oligomer were instrumental in governing release. From these release profiles, it can be concluded that, although a small amount of crystallinity may be desired to maintain form stability of the depot at the injection site, crystallinity slows down the release process and total release requires significant degradation of the oligomer delivery vehicle. For these reasons, amorphous delivery vehicles may be more suitable for *in vivo* use.

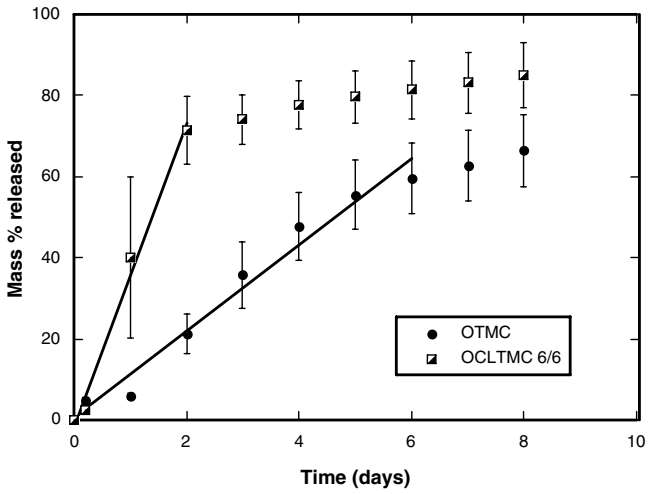

Fig. 6. Comparison of VB12 release from the amorphous oligomer matrices. The lines represent linear regressions to the data within the range indicated.

Various mechanisms govern the release of distributed water-soluble drug particles throughout solid polymers. These include dissolution of interconnected particles forming pores in the polymer through which the dissolved drug diffuses to the surface [27,28], degradation of the polymer to generate a porous network [29], and osmotic pressure-driven release wherein water is drawn into the polymer surrounded drug particle capsules within the matrix leading to swelling of the capsules and ultimately resulting in microcrack formation within the polymer [30–32]. This latter mechanism depends to a large extent on the mechanical properties of the polymer. For the dissolution and diffusion mechanism to dominate and lead to nearly total drug release, the loading of the particles in the polymer must be at or near the percolation threshold, or the polymer must degrade rapidly. The percolation threshold is generally 20–30 v/v% for solid polymers, which is much higher than the given situation. As total VB12 release is observed for the amorphous oligomers, release cannot be driven solely by dissolution and diffusion. Moreover, the oligomers do not degrade significantly over the release period.

The fact that the oligomer rheological properties are influential in determining the release kinetics and total fraction released led us to hypothesize that VB12 release was

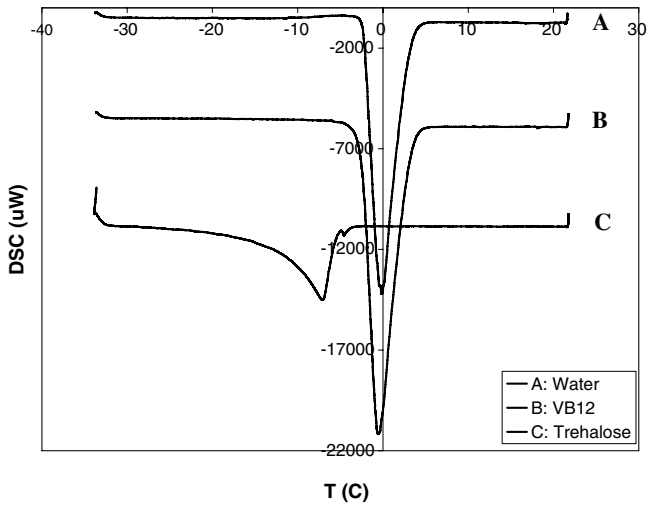

Fig. 7. DSC thermograms of (A) water, (B) saturated VB12 solution, and (C) saturated trehalose solution.

Table 3
Osmotic pressures, Π, for saturated VB12 and trehalose solutions

Sample	Solubility	T_m (°C)	Π (atm)
Water		0.0 ± 0.1	0.0
Saturated VB12 solution	12.5 mg/ml [36]	-0.5 ± 0.1	6.9
Saturated trehalose solution	50 mg/ml [37]	-6.6 ± 0.1	92.1

driven by an osmotic pressure mechanism. To test this hypothesis, VB12 was co-lyophilized with a more osmotically active excipient, trehalose, in a 50:50 mass ratio, ground and sieved to less than 25 μm, and distributed throughout OTMC at the same 1 w/w% loading as done without trehalose, and released into PBS. The osmotic pressure of a saturated trehalose and a saturated VB12 solution was calculated from the freezing point depression of water measured via differential scanning calorimetry as described in Section 2. The resulting thermograms are illustrated in Fig. 7, and the calculated osmotic pressures are given in Table 3. Trehalose is approximately 13 times more osmotically active than VB12 at 37 °C. If release is driven by osmotic pressure, then the inclusion of a more osmotically active excipient, i.e., trehalose, in the incorporated particles should result in a faster release rate. This has been demonstrated previously for drug particles distributed throughout solid polymer matrices [33,34]. In agreement with this prediction, VB12 was released at a faster rate for the formulations containing trehalose in the particles (Fig. 8). The release rate obtained from a regression of the nearly zero-order portion of the release for the formulation containing 50 w/w% trehalose in the particles was 36.5 ± 0.9 mass fraction/day (correlation coefficient$^2 = 0.999$), which is more than triple that of the formulation without trehalose. Furthermore, the formulation containing trehalose also released a greater total fraction of the VB12 (100 w/w%) than the formulation without trehalose (92 w/w%). This enhanced total fraction released upon the inclusion of a more osmotically active excipient has also been observed with the osmotic release mechanism in solid polymers [35].

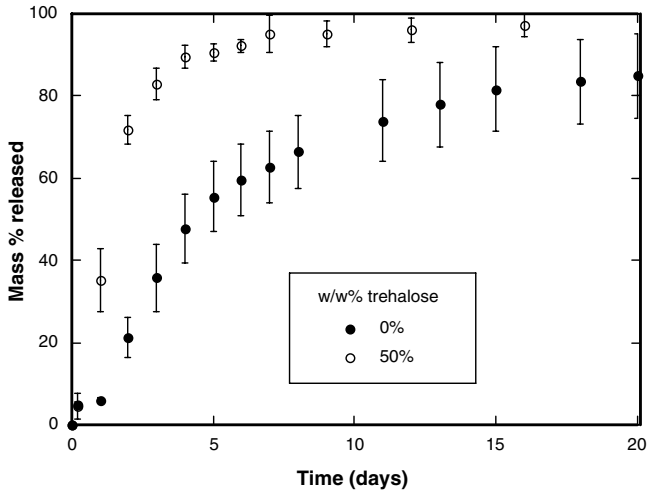

Fig. 8. Influence of trehalose co-lyophilized with VB12 on release of VB12 from oligo(trimethylene carbonate) matrices.

The release mechanism was not visually observed, but based on the physical evidence, is considered to proceed as follows (Fig. 9). After the dissolution of surface resident

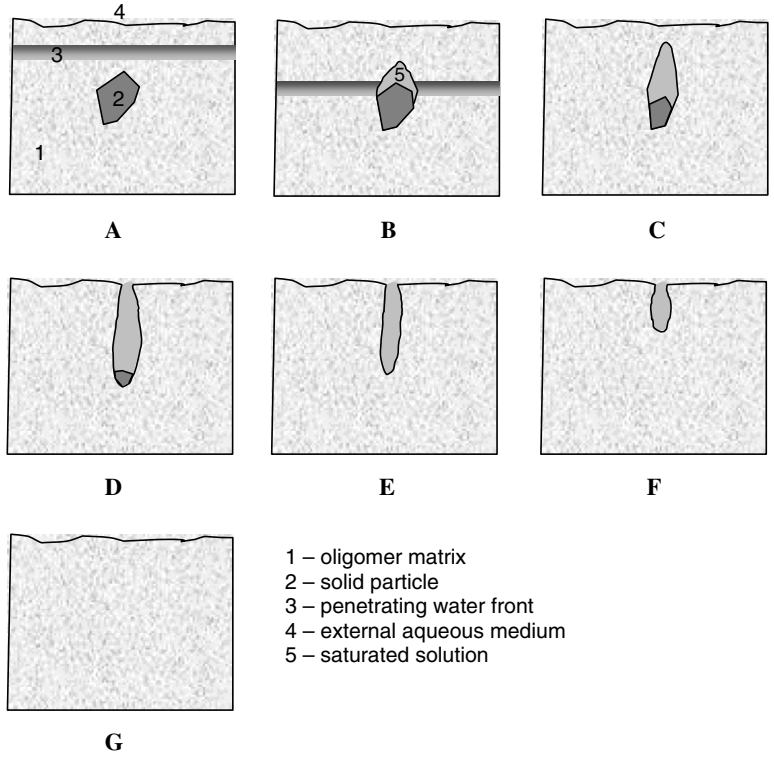

Fig. 9. Schematic of the proposed osmotic pressure-driven release mechanism. (A) Upon immersion into an aqueous medium, water dissolves in and diffuses through the oligomer as a moving front. (B) After reaching an incorporated particle, the water dissolves the solid at the water/oligomer interface producing a saturated solution. (C) The chemical potential gradient between the external solution and the capsule solution draws in water, which pushes against the oligomer, causing it to flow. (D) The internal pressure forces a channel to the surface through which dissolved drug gets released. (E–G) Once the solid completely dissolves, the internal pressure decreases and the channel begins to collapse as a result of oligomer flow.

particles and their diffusion into the bulk surrounding medium, water diffuses into the oligomer matrix surrounding the distributed particles. Once the water reaches a particle and dissolves a portion at the particle/oligomer interface, a saturated solution is formed and water is drawn in more quickly under the enhanced chemical potential gradient. The incoming water collecting at the particle site exerts a pressure on the surrounding oligomer. In this case, which is different than for a solid polymer that would resist elastically, the oligomer resistance to the osmotic pressure is a result of its viscosity. The oligomer begins to flow in the direction of least resistance, i.e., to the surface. In this manner, a liquid filled pore is created that ultimately reaches the surface and thus releases the drug.

4. Conclusions

This work demonstrates that it is feasible to obtain prolonged delivery of a water-soluble compound from oligomers prepared from ε-caprolactone and 1,3-trimethylene carbonate. The amorphous oligomers exhibited better release characteristics in that nearly complete release was obtained and an approximately constant release rate was observed for a substantial portion of the release period. The release of water-soluble compounds is proposed to be driven by an osmotic pressure mechanism, as the release rate was governed by both the viscosity of the oligomer and the osmotic activity of the incorporated solids. Of the amorphous oligomers examined, oligo(1,3-trimethylene carbonate) appears the most promising as it possessed the best combination of release duration, lack of crystallinity, and total fraction released. However, the degradation time of this oligomer needs to be assessed *in vivo* as the *in vitro* degradation time is too long for reasonable use.

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