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Spontaneously self-assembled micelles from poly(ethylene glycol)-*b*-poly(ε-caprolactone-*co*-trimethylene carbonate) for drug solubilization

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Di-block copolymers composed of polyethylene glycol (PEG) and a second block of (co)polyesters of ε-caprolactone (CL) and/or trimethylene carbonate (TMC) were synthesized and characterized. Tin octoate was used as catalyst and polymerization were completed over a period of 24 h with high conversion (> 95%). Self-assembling properties in water were evaluated. All di-block copolymers behave similarly except when PCL served as the second block. Stable crew-cut micelles of about 20 nm were obtained by direct dissolution of the liquid di-block copolymers in water at room temperature. When PCL was present as the second block, no solubilization occurred. Drug encapsulation of poorly water-soluble drugs belonging to biopharmaceutics classification system (BCS) class II (ketoprofen and furosemide) was evaluated. Experimental solubility for these two drugs shows a significant enhancement such that a maximum value of 23.4 mg/ml was obtained for ketoprofen in a 10% w/v micellar solution as compared to 0.14 mg in water. In the case of furosemide, the solubility increased from 0.04 mg/ml in water to about 3.2 mg/ml in a 10% w/v micellar solution. Enzymatic degradation of di-block copolymers was also studied in the presence of *Pseudomonas* lipase in a phosphate buffer solution (pH 7.4). Results indicated rapid degradation of copolymers containing relatively higher amounts of CL compared to TMC suggesting the potential *in vivo* degradation.

1. Introduction

Contemporary pharmaceutical pipelines tend to contain sophisticated synthetic compounds, as well as structurally complex therapeutics that are designed to bind to a chosen enzyme or specific site in the body. Over time studies have suggested that drug candidates are becoming more lipophilic, hydrophobic, and water-insoluble (Lipinski et al. 1997; Strickley 2004). The delivery of such potential therapeutic compounds can be quite challenging and can be rate limiting in the development of the compound. In some cases, standard techniques and excipients used for formulating oral and injectable dosage forms such as pH modifiers, water-soluble organic solvents, medium- and long-chain triglycerides, surfactants, cellulose-based polymers, cyclodextrins and phospholipids, are suboptimal in providing for a useable dosage form. Therefore novel drug delivery formulations have been explored.

In the recent years, polymeric micelles have attracted significant attention from the scientific community. It has been recognized that polymeric micelles can be an effective tool for drug delivery of poorly-water soluble drugs (Jones and Leroux 1999; Rosler et al. 2001; Kataoka et al. 1992). Polymeric micelles prepared from amphiphilic block copolymers are among the most actively studied of these systems (Kwon and Kataoka 1995; Jones and Leroux 1999; Croy and Kwon 2006; Torchilin 2007; Gaucher et al. 2005). Amphiphilic block copolymer molecules consist of hydrophobic and hydrophilic blocks that can spontaneously assemble in aqueous solutions at a certain concentration, the critical micelle concentration (CMC), to form micelles. In such structures, the hydrophilic blocks constitute the outer shell (corona) while the hydrophobic blocks form the core. Hence, the core is stabilized by the hydrophilic corona, which serves as an interface between the aqueous phase and the hydrophobic interior. One of the advantages of such a core-based construct is the ability of the micelle to accommodate/solubilize hydrophobic compounds that are sparingly water-soluble thereby enhancing their apparent solubility. With sizes ranging from a few nanometers to about 200 nm, micelles have emerged as interesting drug carriers because they can easily (when smaller than 100 nm) evade scavenging by the phagocyte system (Kwon and Okano 1996).

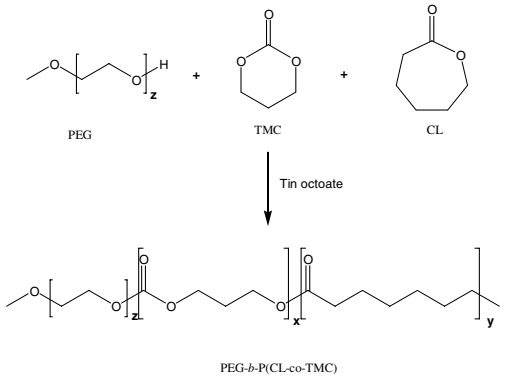
There are two principal methods for preparation of block copolymer micelles: the direct dissolution of copolymer in water and an indirect method based on dialysis and other techniques. The direct method is used for water-soluble copolymers such as Pluronics®. The indirect method is suitable for copolymers that are not readily soluble in water. Such copolymers are converted into micelles by dissolution in a water miscible organic solvent (e.g. tetra-

hydrofuran, acetonitrile, dimethylsulfoxide) followed by dialysis against an aqueous phase. Alternatively, drug loading is possible by the use of emulsion technology, solution casting as well as freeze-drying (Gaucher et al. 2005)

According to Allen et al. (1999), di-block copolymers that have a hydrophobic block which is longer than their hydrophilic component form crew-cut micelles. The formation of such aggregates has been explained by a force balance effect between the degree of stretching of the core-forming chains, the interfacial energy between the micelle core and the solvent, and the interaction between corona-forming polymeric chains (Zhang and Eisenberg 1995; Zhang et al. 1996; Zhang and Eisenberg 1996). Such micelles are prepared by long and tedious indirect methods. Therefore the use of these micelles is often not suited in the design of various formulations since many parameters of the micelles are sensitive to the manufacturing process.

This paper reports on a family of biodegradable di-block copolymers prepared by ring opening polymerization of ε-caprolactone (CL) and/or trimethylene carbonate (TMC) initiated by poly(ethylene glycol) (Latere Dwan'Isa et al. 2007; Ould-Ouali et al. 2005). The reaction (Scheme) is carried out with tin octoate [$Sn(Oct)_2$] as catalyst. This coordinated metal catalyst is highly efficient for polymerization of lactones such as CL and of TMC yielding almost complete conversion even at high monomer to catalyst ratios. The resulting di-block copolymer has a hydrophilic PEG block and a hydrophobic component (i.e., a copolyester of CL and TMC). TMC has been widely used in copolymers designed for biomedical applications because it enhances flexibility (Buchholz 1993).

Scheme



The synthesized di-block copolymers (Fig. 1) were designed to have longer hydrophobic blocks than hydrophilic chains. However, despite this structure, they spontaneously self-assemble in aqueous solution as crew-cut micelles. Such copolymers avoid the use of the long and tedious indirect method for micelle preparation and thus can be readily used for entrapment of poorly water-soluble compounds.

The use of biodegradable materials to prepare polymeric micelles is highly desirable. Indeed, such polymers can degrade in the body preventing adverse effects to the patients. In addition, polymeric micelles with a this type of "soft" design can add functionality to drug release, passive targeting via the epr mechanism and other features. The *in vitro* enzymatic degradation of PCL polymers in presence of lipase-type enzymes has been investigated (Fukuzaki et al. 1996; Mochizuki et al. 1995; Li et al. 1999). Results have shown that a highly crystalline PCL was totally degraded within 4 days in the presence of *Pseudomonas* lipase (Li et al. 1999). Zhu et al. (1991) reported on the *in-vivo* degradation of PTMC catalyzed by enzymes. We have previously assessed a number of copolymers derived from PEG, CL and TMC and have found that a 750 D based PEG containing a 50 : 50 ratio of CL and TMC was useful in drug solubilization and spontaneously emulsified in aqueous systems (Ould-Ouali et al. 2004). The current work expands on these polymeric systems by varying the hydrophobic component both in terms of polymer composition as well as by altering the CL to TMC ratios. Thus, we studied a series of novel di-block copolymers with an emphasis on drug solubilization as well as the effect of their structure on degradation in the presence of *Pseudomonas* lipase. The serum lipase concentration in healthy adults is in the range of 30–190 units/l (Burtis and Ashwood 1996).

2. Investigations, results and discussion

2.1. Synthesis and characterization of PEG-b-P(CL-co-TMC)

The di-block copolymers were synthesized by bulk polymerization of CL and/or TMC initiated by monomethoxy end-capped PEG in the presence of tin octoate as catalyst (Scheme). Polymerization proceeds via a coordination-insertion mechanism. In such processes, the hydroxyl end-group of PEG is converted into a tin alkoxide that initiates the polymerization of the lactone and/or the cyclic carbonate (Schindler et al. 1982). The first block of the resulting block copolymer consists of a PEG chain of molecular weight 750 g/mol linked to a (co)polyester block. This second block

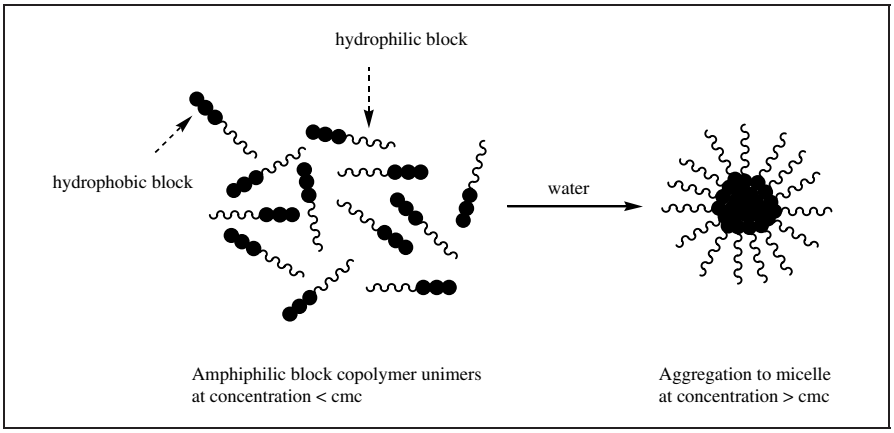


Fig. 1:
Self-assembly of PEG-based block copolymers in water

is either a copolyester of CL and TMC or a homopolymer of poly(caprolactone) (PCL) or poly(trimethylene carbonate) (PTMC). The ratio between the first and second block was kept constant at 13. Therefore the theoretical molecular weight of the latter block is about 1500 g/mol at complete conversion. The monomer to tin molar ratio used was as low as 50,000 to minimize the amount of catalyst present in the final di-block copolymer.

Table 1 shows the initial monomer feed and the chemical composition of the copolymers recovered. The composition was determined by proton NMR (Pêgo et al. 2003). Under the polymerization conditions used, the conversion of both monomers, i.e., CL and TMC, was above 97%. The obtained copolymer compositions are in good agreement with the ratio of monomers charged in the reaction vessel. At room temperature, block copolymers appeared either as waxy pastes [PEG-b-PCL, PEG-b-PTMC and PEG-b-P(CL-co-TMC) of CL/TMC molar composition of 90/10] or as viscous liquids (all other copolymers). Since PEG at a molecular weight of 750 g/mol is itself a viscous liquid, upon addition of the second block, i.e., the (co)polyester of either CL and/or TMC, the overall chain length is extended altering the aspect of the synthesized di-block copolymer. The nature of the second block is therefore crucial. Previous studies have shown that copolymerization of CL with TMC catalyzed by $Sn(Oct)_2$ lead to random copolyesters (Albersson and Eklund 1994).

Characterization of the copolymers by GPC using THF as the mobile phase indicated that chromatographic peaks were symmetric with Mn values ranging from 2400 to 3900 D based on polystyrene standards. The Mn decreased when the amount of CL is low or zero. The molecular weight distribution (polydispersity index) varied from 1.5 to 1.8 indicating that transesterification reactions had occurred but to a limited extent (Table 2) (Schindler et al. 1982). Such reactions are favored by high temperature (close to 200 °C) and long reaction time (> few days).

Table 1: Synthesis of PEG-based di-block copolymers

Entry	Initial CL/TMC molar ratio	Reaction conditions	CL/TMC molar ratio (^{1}H NMR)	Polymer aspect at 20 °C
1	100/0	8 h at 185 °C	–	Wax
2	90/10	24 h at 160 °C	89.6/9.9	Wax
3	70/30	24 h at 160 °C	72.6/26.8	Liquid
4	50/50	24 h at 160 °C	49.1/50.8	Liquid
5	30/70	24 h at 160 °C	30.3/69.0	Liquid
6	10/90	24 h at 160 °C	10.5/87.8	Liquid
7	0/100	18 h at 190 °C	–	Wax

Monomer to initiator (PEG) molar ratio = 1/13.3. Monomer-to-tin molar ratio ≈ 1/50000

Table 2: Molecular characteristics of di-block copolymers and their self-assembling properties

Entry	CL/TMC molar ratio	Mn (GPC)	Mw/Mn	Self-assemble	Size (nm) in 10% w/v
1	100/0	3900	1.5	No	–
2	90/10	3600	1.8	Yes	20.7
3	70/30	3500	1.7	Yes	20.6
4	50/50	3400	1.7	Yes	20.2
5	30/70	3200	1.6	Yes	20.2
6	10/90	3000	1.6	Yes	18.8
7	0/100	2400	1.5	Yes	22.1

GPC in THF (1ml/min) at 35 °C using polystyrene standards. Micelle size at 25 °C

2.2. Self-assembling of di-block copolymers

The direct method was applied to the preparation of micelles. Waxy block copolymers, i.e., PEG-b-PCL, PEG-b-PTMC and PEG-b-P(CL-co-TMC) of CL/TMC molar ratio of 90/10 were melted at 50 °C to generate a liquid and then dispersed in water while the copolymers which were liquid at room temperature were simply mixed with water. Solutions containing 10% w/v (100 mg/ml) of block copolymers were prepared. With the exception of the solution corresponding to entry 1 of Table 2 (PEG-b-PCL) all other systems were clear and isotropic. In the case of the PEG-b-PCL, the limited solubility combined with higher crystallinity of this di-block copolymer is likely the main factor for its poor miscibility. For the polymers assessed, dynamic light scattering revealed the presence of one population of micelles of ca. 19 to 22 nm at 25 °C (Table 2). These crew-cut micelles were stable (no change in micelle size or precipitation) for more than two months when the hydrophobic block is a copolyester of CL and TMC of molar compositions 70/30, 50/50, 30/70 and 10/90 but only for four days when PTMC served as the core and one week for P(CL-co-TMC) (90/10). The pH of the different solutions were checked and found to be constant at around 7.

2.3. Thermal properties

The thermal properties of the synthesized di-block copolymers were investigated by differential scanning calorimetry (DSC). The DSC curves obtained during the second heating run were analyzed and data are provided in Table 3. It was expected that the thermal properties of the prepared di-block copolymers should reflect those of the parent "homopolymers", i.e., PEG (first block) and (co)polyester (second block). PTMC is an amorphous, rubbery polymer with low tensile strength while PCL is a semicrystalline polymer melting at 60 °C (Zhu et al. 1991; Schindler et al. 1977). PEG samples of molecular weight lower than 1,000 g/mol are oligomers that show properties distinct from higher molecular weight homologs in terms of their thermo-mechanical properties. PEG (750 g/mol) is an oligomer that melts around 30 °C. PCL and PEG have comparable Tg values while PTMC manifests a Tg around −30 °C.

In the present study, all di-block copolymers show a single glass transition temperature (Tg) suggesting the presence of an amorphous phase (Table 3). Upon heating, the di-block copolymers corresponding to entries 3 to 7 of Table 3 showed a crystallization peak whose values increases with TMC content. Such a peak is not seen when PCL (entry 1, Table 3) or P(CL-co-TMC) (90/10) (entry 2, Table 3) constitutes the second block. Bogdanov et al. (1998) have indicated that when PCL and PEG are combined

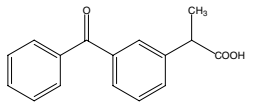
Table 3: Thermal properties measured by DSC of PEG-based di-block copolymers

Entry	CL/TMC molar ratio	Tg (°C)	Tc (°C)	Tm (°C)
1	100/0	−69	–	37/45
2	90/10	−69	–	20/32
3	70/30	−66	−49	17
4	50/50	−60	−35	18
5	30/70	−55	−24	17
6	10/90	−51	−10	18
7	0/100	−51	−10	19

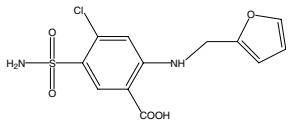
in di-block copolymer, PCL impedes or delays crystallization of PEG. Therefore, after being quickly quenched during the first heating cycle, PEG oligomeric chains are frozen and during the second heating cycle they crystallize to a certain extent as suggested by the observed crystallization temperature (Tc) (Graham et al. 1989). Decreasing amounts of CL leads to higher values of Tc (Tokiwa et al. 1979). The melting temperature (Tm) of the di-block copolymers is also strongly influenced by the crystallization. When CL is present in higher amounts (entries 1 and 2, Table 3) a double peak is observed which is possibly due to melting of small and/or imperfect PCL crystals. Well-formed PCL crystals melt at 60 °C but in the specific case of this study, polymer chains of entries 1 and 2 of Table 3 are short and therefore it is unlikely that such short chains could reach an ideal crystalline state. For the other di-block copolymers (entries 3 to 7 of Table 3), a single Tm is observed around 18 °C and is most likely due to melting of PEG crystals. Indeed, increasing the amount of TMC hinders crystallization of CL resulting in amorphous copolyesters of CL and TMC.

2.4. Solubilization of poorly water-soluble drugs in PEG-b-P(CL-co-TMC) di-block copolymers

In order to assess the possible use of these polymeric micelles as a solubilizing drug excipient, two commonly administered, poorly water-soluble drugs were selected: ketoprofen and furosemide. These agents belong to the Biopharmaceutic Classification Scheme (BCS) class II (low solubility and high gastrointestinal permeability) and 4 (low solubility and low gastrointestinal permeability), respectively.



Ketoprofen



Furosemide

Solubility of ketoprofen in 10% w/v polymeric micelles prepared from di-block copolymers of PEG-b-P(CL-co-TMC) of CL/TMC molar ratios of 90/10, 70/30, 50/50, 30/70 and 10/90 was measured in water at room temperature (Table 4). As compared to the solubility value in water (0.14 mg/ml), values higher than 14 mg/ml are obtained with a maximum solubility of 23.4 mg/ml, a 167-fold increase in the presence of the micelles. The lowest enhancement (100-fold) is observed in copolymers when the amount of CL is 30 mol%. The pH of various solutions remained constant in all experiments. As a general observation, lower amounts of CL in the copolymer tended to give lower solubilities. Dynamic light scattering performed on the drug-loaded micelles show that at saturation, the micelle size increased slightly to 30 nm from ~20 nm when the CL molar ratio varied from 90 to 70%. Micelles subsequently got larger with lower CL content, to reach values as high as 206 nm when the copolyester block contains 10 mol% of CL. Such large micelles are not desirable since they are close to the critical value of 200 nm (Allen et al. 1999).

Table 4: Solubilization of ketoprofen in polymeric micelles of PEG-P(CL-co-TMC)

Entry	CL/TMC molar ratio	Medium	Solubility (mg/ml)	pH	Size (nm) at saturation
1	90/10	10 % w/v Solution	23.41 ± 0.09	4.0	31.3 ± 0.5
2	70/30	10 % w/v Solution	19.22 ± 0.08	4.0	28.8 ± 0.2
3	50/50	10 % w/v Solution	19.61 ± 0.08	4.0	37.3 ± 0.5
4	30/70	10 % w/v Solution	14.03 ± 0.02	4.0	70.8 ± 1.2
5	10/90	10 % w/v Solution	16.96 ± 0.09	4.0	206.3 ± 4.1
6	–	Water	0.14 ± 0.01	4.2	–

Table 5: Solubilization of furosemide in polymeric micelles of PEG-P(CL-co-TMC)

Entry	CL/TMC molar ratio	Medium	Solubility (mg/ml)	pH	Size (nm) at saturation
1	90/10	10% w/v Solution	3.17 ± 0.02	4.0	10.1/142.8
2	70/30	10% w/v Solution	3.05 ± 0.02	4.0	10.8/144.2
3	50/50	10% w/v Solution	2.61 ± 0.01	4.0	10.6/170.5
4	30/70	10% w/v Solution	2.50 ± 0.01	4.0	11.5/136.6
5	10/90	10% w/v Solution	1.88 ± 0.01	4.0	11.0/200.0
6	–	Water	0.04 ± 0.01	4.4	–

Solubilization of furosemide in 10% w/v di-block copolymers of PEG-b-P(CL-co-TMC) was also studied (Table 5). The solubility of this poorly water-soluble drug increased from 0.04 mg/ml to a maximum value of 3.17 mg/ml, a 79-fold increase, when the copolyester block contains 90 mol % of CL. The lowest enhancement (47-fold) was observed when the copolyester block contains 10 mol% of CL. The pH of the micelle solutions was noted to be slightly more acidic than of the aqueous furosemide solution. Dynamic light scattering studies of the samples was carried out at 25 °C on filtered samples (0.1-μm Millipore). Results show two populations centered respectively around 10 nm and 140 to 200 nm for all samples.

2.5. In-vitro enzymatic degradation

Di-block copolymers exposed to *Pseudomonas* lipase were isolated after evaporation of water and analyzed by gel permeation chromatography (GPC). Chromatograms are presented in Fig. 2. Data showed a decrease of Mn and Mw and also an increase of the molecular weight distribution for di-block copolymers with higher molar ratio of CL, i.e. 90, 70 and 50% (Table 6). After 24 h of digestion time, a shift toward low molecular weight is observed with a non-symmetric chromatographic curve characterized by a shoulder. Also a peak is observed around 11 min in all three chromatograms. Hydrolytic degradation of PCL is known to produce smaller oligomer and ultimately the hydroxy-caproic acid (Tokiwa et al. 1979). Longer degradation times resulted in the disappearance of the main peak and only the presence of a small peak around 11.5 min. For those samples, i.e., with high CL amount, a precipitate occurred after 24 h and a clear, phase separation is observed in the vials. The precipitation is expected to occur over time due to the separation of the

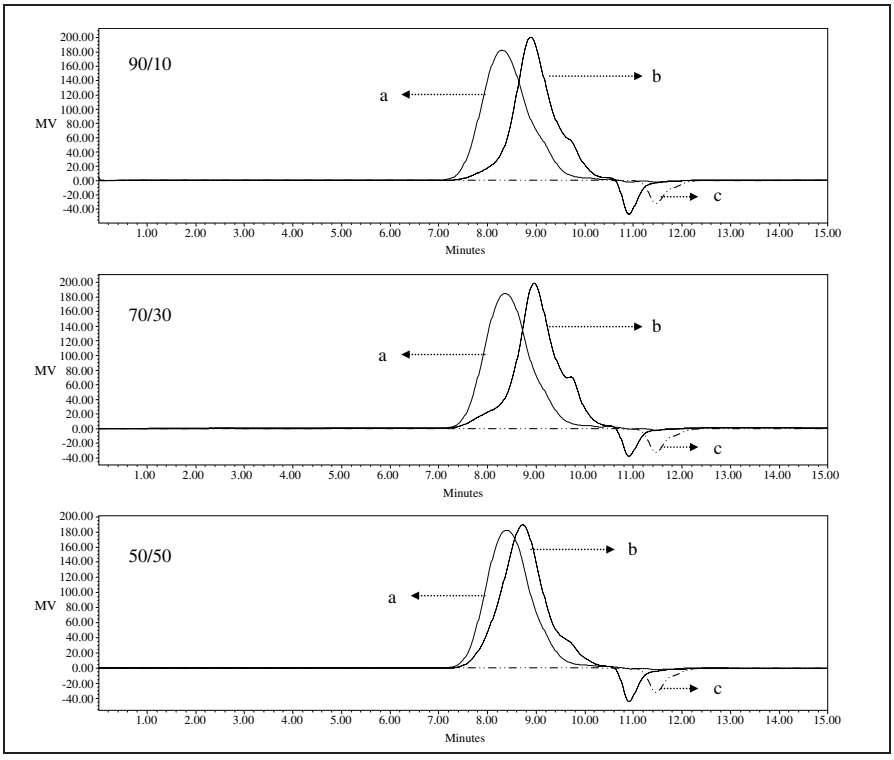


Fig. 2:
Aqueous Size Exclusion Chromatography for PEG-based block copolymers containing various CL to TMC ratios incubated in *Pseudomonas* lipase (9600 U/L) at time = 0 (a) and after 24 h (b). Peak c recurs in all degraded samples

Table 6: Enzymatic degradation of 10% w/v PEG-P(CL-co-TMC) copolymers in phosphate buffer solution (pH 7.4) containing *Pseudomonas* lipase (9600 units/L) at 37 °C

Entry	CL/TMC molar ratio	Time (days)	Mn (GPC)	Mw/Mn
1	90/10	0	3600	1.8
2	90/10	1	1400	1.8
3	90/10	3	ND	–
4	70/30	0	3500	1.7
5	70/30	1	1300	2.0
6	70/30	3	ND	–
7	50/50	0	3400	1.7
8	50/50	1	1900	1.9
9	50/50	3	ND	–
10	30/70	0	3200	1.6
11	30/70	1	3000	1.6
12	30/70	3	2800	1.6
13	10/90	0	3000	1.6
14	10/90	1	3000	1.6
15	10/90	3	2700	1.6
16	0/100	0	2400	1.5
17	0/100	1	2300	1.5
18	0/100	3	2200	1.5

GPC in THF at 35 °C (flow = 1 ml/min). Polystyrene standards were used for calibration. ND: no signal detected

hydrophilic PEG block and the hydrophobic copolyester block. When the amount of CL is small or zero, a very slow degradation is observed and no precipitation is observed. Light scattering analysis shows no substantial changes in micelle size. These results are not surprising since the *Pseudomonas* lipase can easily degrade aliphatic esters while carbonate bonds are more resistant. When the amount of TMC is high and the number of ester bonds low, *Pseudomonas* lipase does not induce substantial degradation and it is likely that only non-catalyzed hydrolytic degradation occurs (Zhu et al. 1991).

2.6. Conclusions

A series of low molecular weight di-block copolymers prepared by polymerization of CL and/or TMC initiated by PEG and catalyzed by tin octoate were prepared. Di-block copolymers were characterized and proved to be useful as drug solubilizers. Indeed, stable crew-cut type polymeric micelles are readily prepared (except when PCL is the second block) by the direct dissolution of copolymers in water. Efficient encapsulation of poorly water-soluble drug is possibly resulting in significant solubility enhancement in water. Such incorporation depends on the (co)polyester composition. Our results show that the model compounds studied, i.e. ketoprofen and furosemide, were better encapsulated when the CL component was high. The substantial solubility enhancement observed makes such polymeric micelles potential formulation candidates for poorly water-soluble drugs. The di-block copolymers with higher amount of CL proved to be degradable in presence of *Pseudomonas* lipase suggesting that *in vivo* degradation should be possible.

3. Experimental

3.1. Materials

The polymers were synthesized by J&J-Center for Biomaterials and Advanced Technologies (Somerville, NJ, USA). ε-Caprolactone (CAP) was purchased from Union Carbide (Danbury, CT, USA), and trimethylene carbonate (TMC) from Boehringer Ingelheim (Petersburg, VA, USA). Stannous octoate and toluene were from Aldrich (Milwaukee, WI, USA). Methoxy end-capped poly(ethylene glycol) (PEG) of molecular weigh 750 g/mol was purchased from Fluka (Milwaukee, WI, USA). Ketoprofen and furosemide were obtained from Sigma-Aldrich (Bornum, Belgium).

3.2. Synthesis of PEG-b-P(CL-co-TMC)

The diblock copolymers were synthesized by ring opening polymerization (ROP) in the presence of stannous octoate. The reaction is performed at 160 °C or 185 °C and the reaction times varied from 8 to 24 h. A typical polymerization was completed as follows: In the reaction flask, 7.6 μmol of stannous octoate solution in toluene (0.33 M), 187.5 mmol trimethylene carbonate (monomer), 187.5 mmol ε-caprolactone (monomer) and PEG-750 (initiator) in a molar ratio monomer to initiator of 13 to 1 were added

and heated to 160 °C for 24 h. After completion of the reaction, the polymer was heated under vacuum to devolatilize unreacted monomer.

3.3. Polymer characterization

The polymer composition and residual monomer content were analyzed by proton NMR. In theys procedure, the copolymers were dissolved in deuterated chloroform. Spectra were taken employing a Unity-Plus 400 NMR spectrometer. The ratio of the various monomers in the polymer were determined by integrating the methylene and methyl resonances in the 0 to 7.5 ppm spectral region and calculating the mol percent of each monomer in the polymer from the normalized peak area of the respective monomers (polymerized and monomer form). Gel permeation chromatography (GPC) was employed to determine the molecular weight and the polydispersity of the polymers. A Waters Alliance 2690 separation module equipped with a Waters 2414 Refractive Index Detector and Waters Styragel HR 3–4 columns was used. Polystyrene standards were used for calibration. HPLC grade tetrahydrofuran was used as solvent and mobile phase (flow = 1 ml/min) and the analysis temperature was 37 °C. Thermal properties were determined with a differential scanning calorimetry (DSC). Thermograms were obtained by analysis of the samples under a nitrogen flow (50 ml/min) using a TA Instrument apparatus (Q 100). The samples (5 to 10 mg) were sealed in aluminum pans, heated at a rate of 10 °C/min to 120 °C and then quenched to −90 °C and kept at that temperature for 2 min (run 1). This first run ensures identical thermal history for all di-block copolymer samples. The samples were then heated at a rate of 10 °C/min to 120 °C (run 2) to evaluate their thermal properties.

3.4. Self-assembling properties of PEG-b-P(CL-co-TMC)

Self-assembling properties were examined by adding water to the liquid block copolymers (10% w/v). The mixture was stirred for 10 min. Micelle formation was determined at 25 °C without filtering the solution with the size of the micelles of 10% w/v diblock copolymer solutions in water was determined by photon correlation spectroscopy using a Malvern Autosizer 4700.

3.5. Measurement of drug solubility in 10% w/v diblock copolymer micelles

The maximum drug solubility in micelles was determined as follows: In a glass flask, 0.5 g of copolymer and 0.1 to 0.15 g of the drug were weighed and then mixed at 50 °C for 10 min using a magnetic stirrer followed by addition of 5 ml of filtered water (0.1 μm Millipore). The solution (S) was stirred for 24 h at room temperature and then filtered through a 0.1 μm Millipore filter to remove non-solubilized drug. A Beers Law plot was obtained using a an ultraviolet (UV) spectrophotometer (Shimadzu UV-160, Japan). Absorbances (ranging from 0.08 to 0.9) were measured at 260 nm (ketoprofen) at 233 nm (furosemide). Solubility data are an average of at least three measurements.

3.6. Enzymatic degradation

Enzymatic degradation experiments were carried out at 37 °C in a phosphate buffer solution (pH 7.4) that contained enzyme (*Pseudomonas* lipase) at a concentration of 9600 units/L. Solutions were prepared by dissolving the copolymer (0.5 g) in 5 ml of the PBS/enzyme. At predetermined times, water is removed and 5 ml of THF was added to the residue. The reconstituted sample is then filtered through a 0.22 μm Millipore filter and analyzed by GPC. Polymer degradation was measured by monitoring the decrease in number- and weight-average molecular weight (Mn and Mw) and changes in polydispersity (Mw to Mn ratio).

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