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European Journal of Pharmaceutics and Biopharmaceutics 65 (2007) 379–387
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

pH-responsive polymeric micelles of poly(ethylene glycol)-*b*-poly(alkyl(meth)acrylate-*co*-methacrylic acid): Influence of the copolymer composition on self-assembling properties and release of candesartan cilexetil

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Received 17 July 2006; accepted in revised form 26 September 2006
Available online 7 October 2006

Abstract

The objective of the present study was to investigate the influence of chemical structure and molecular weight of pH-sensitive block copolymers on their self-assembling properties, the loading and the release of candesartan cilexetil (CDN). Block copolymers of poly(ethylene glycol) and *t*-butyl methacrylate, *iso*-butyl acrylate, *n*-butyl acrylate or propyl methacrylate were synthesized by atom transfer radical polymerization. pH-sensitivity was obtained by hydrolysis of *t*-butyl groups. The poorly water-soluble drug CDN was incorporated in the micelles and the *in vitro* drug release was evaluated as a function of pH. The critical aggregation concentration of hydrolyzed copolymers ($pK_a = 6.2$–6.6) was higher compared to the unhydrolyzed ones. Dynamic light scattering studies and atomic force microscopy images revealed uniform size micelles with aggregation numbers ranging from 60 to 160. The entrapment efficiency of CDN was generally found to be above 90%, with drug loading levels reaching ~20% (w/w). Differential scanning calorimetry studies showed the amorphous nature of entrapped CDN. The release of CDN from pH-sensitive micelles was triggered upon an increase in pH from 1.2 to 7.2. These findings suggest that the PEG-*b*-poly(alkyl(meth)acrylate-*co*-methacrylic acid)s can self-assemble to form micelles which exhibit high loading capacities for CDN and release the drug in a pH-dependent fashion.

Keywords: pH-sensitivity; Polymeric micelles; Candesartan cilexetil; Atom transfer radical polymerization; Critical aggregation concentration; Solvent evaporation; Oral delivery; Poly(ethylene glycol)-*b*-poly(alkyl(meth)acrylate-*co*-methacrylic acid); Drug solubilization

1. Introduction

The oral route is the most common route of drug administration in view of its convenience and patient acceptance, even more so in the case of chronic therapies [1]. Many existing and new therapeutic entities are characterized by a low degree of water solubility leading to poor and erratic oral bioavailability [2]. In order to overcome this problem, several strategies such as micronization, complexation, formation of solid solutions, microemulsification and novel drug delivery systems, including nanoparticles, lipid-based vesicles and micelles, have been proposed [3–10]. Amongst these approaches, polymeric micelles (PM) hold promise because of their nanoscopic size, ability to solubilize hydrophobic drugs in large amount and achieve site-specific delivery [11].

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doi:10.1016/j.ejpb.2006.09.012

Amphiphilic block copolymers with biodegradable and/or biocompatible chains can self-assemble into micelles, which can be used for the targeted drug delivery. Several block copolymers such as poly(ethylene glycol) (PEG)-*b*-poly(α-ester)s [12] and PEG-*b*-poly(amino acid)s [13] have been reported to enhance the solubility of hydrophobic drugs through micellization and change their pharmacokinetic pattern after parenteral injection. Recently, block copolymer micelles have been investigated for the oral delivery of poorly water-soluble compounds. Ould-Ouali et al. showed that PEG-*b*-poly(ε-caprolactone/trimethylene carbonate) micelles increased risperidone permeation across Caco-2 monolayers [14]. Elsewhere, PEG-*b*-(ε-caprolactone) micelles were reported to reduce the efflux of rhodamine across Caco-2 cell monolayers by inhibition of P-glycoprotein pump upon release of individual polymer chains [15].

On the other hand, PM can be designed to exhibit mucoadhesive properties [16] or release their cargo at a specific site of the gastro-intestinal tract in response to an external trigger. Indeed, micelles [17] or micelle-like systems [18], which exhibit a pH-dependent dissociation profile can increase the bioavailability of hydrophobic drugs. Such micelles minimize the initial burst release and possible precipitation in the stomach by releasing small amounts of their cargo at acidic pH. At the intestinal pH (pH > 5.0), they partially or completely dissociate, thereby liberating the remaining entrapped drug in a molecularly dispersed form in the upper GI region where absorption is maximal. Recently, our group reported pH-sensitive polymers composed of PEG-*b*-poly(alkyl acrylate-*co*-methacrylic acid) [PEG-*b*-P(AlA-*co*-MAA)] with an aggregation pH of 4.7 [19], which were capable of enhancing the oral bioavailability of fenofibrate in rats [17]. With this in mind, it was decided to explore the potential of pH-responsive micelles in improving the solubility of candesartan cilexetil (CDN). CDN is the esterified prodrug of candesartan, a non-peptide angiotensin II type 1 (AT_1) receptor antagonist used in the treatment of hypertension. A daily dose of CDN (2–32 mg) significantly reduces the systolic and diastolic blood pressure of patients with mild to moderate hypertension. Though CDN exhibits good efficacy as an antihypertensive agent, it displays poor water solubility ($<5\times10^{-5}$ g/L) and low oral bioavailability (~15%) [20,21]. The absorption of CDN occurs mainly in the small intestine and to a smaller extent in the stomach and large intestine [22]. Hence, encapsulating CDN in pH-responsive block copolymer micelles may prove to be a useful strategy in enhancing its oral bioavailability.

The aim of the present study was to systematically investigate the effect of molecular weight and chemical structure on the self-assembling properties, drug loading and release behavior of PEG-*b*-P(alkyl(meth)acrylate-*co*-methacrylic acid) (PEG-*b*-P(Al(M)A-*co*-MAA)) micelles. Block copolymers of PEG, *tert*-butyl methacrylate (*t*BMA) or methyl methacrylate (MMA) and *iso*-butyl acrylate (*iso*BA), *n*-butyl acrylate (*n*BA) or propyl methacrylate (*Pr*MA) were synthesized and subsequently hydrolyzed to obtain the pH-sensitive copolymers. The different copolymers were characterized with respect to their self-assembling properties in water and in simulated gastric fluid (SGF). CDN was then incorporated in the micelles and its release kinetics was studied as a function of pH.

2. Materials and methods

2.1. Materials

All products were purchased from Sigma Aldrich (Milwaukee, WI). Copper(I) bromide (99.99% grade), 2-bromoisobutyryl bromide, anhydrous triethylamine and *N*,*N*,*N*′,*N*′,*N*″pentamethyldiethylenetriamine (PMDETA) were used without further purification. PEG monomethyl ether (MeO–PEG–OH, M_n 2000 and 5000) was dried with toluene by azeotropic distillation before use. MMA, *n*BA, *iso*BA, *Pr*MA and *t*BMA served as vinyl monomers and were purified before polymerization by passing through an inhibitor remover column. CDN was obtained from the Hangzhou HETD Pharm and Chem Corp. (Hangzhou, Zhejiang, P.R. China), ^{3}H-CDN (1 mCi/mL) was procured from American Radiolabeled Inc. (Saint Louis, MO, USA). Prior to use, tetrahydrofuran (THF) was distilled over sodium, with benzophenone as the drying indicator. PEG macroinitiator, *i.e.* α-(2-bromoisobutyrylate bromide)-ω-methyl PEG, was synthesized using PEG and 2-bromoisobutyryl bromide, as reported elsewhere [23]. All other chemicals were of reagent grade and used without further purification.

2.2. Copolymer synthesis and characterization

Atom transfer radical polymerization (ATRP) of alkyl(meth)acrylate [Al(M)A] and *t*BMA was carried out as reported previously [19]. Two series of copolymers with different molecular weights ($M_n \sim 6500$ and 14,000) were synthesized. Briefly, required amounts of α-(2-bromoisobutyrylate bromide)-ω-methyl PEG (2000 or 5000) (1 eq), copper(I) bromide (0.65 eq), PMDETA (1.5 eq), monomers (17 or 35 eq for each), and 2 mL THF were placed in a dry round-bottom flask. The flask was sealed with a rubber septum, degassed, back-filled with nitrogen three times and left under nitrogen. The solution was stirred for 15 min and the flask was then placed overnight in an oil bath thermostated at 65 °C. Pure polymers were recovered as white powder [19]. The transformation of *t*BMA into MAA was achieved by cleaving the PEG-*b*-P(Al(M)A-*co*-*t*BMA) in concentrated HCl and dioxane, as described previously [23]. The synthesized copolymers are abbreviated as follows: PEG_X-*b*-P(Al(M)A_Y-*co*-tBMA_Z) and PEG_X-*b*-P(Al(M)A_Y-*co*-MAA_Z), where subscript *X*, *Y* and *Z* correspond to the number of units of ethylene oxide, Al(M)A and *t*BMA or MAA moieties, respectively.

2.3. Copolymer characterization

2.3.1. NMR analysis and molecular weight determination

^{1}H NMR spectra were recorded on a Bruker AMX 300 spectrometer (Milton, ON, Canada) in $CDCl_3$ or DMSO-d_6 at 25 °C. M_n and polydispersity indices (PI) were determined by size exclusion chromatography (SEC) with an Alliance GPCV 2000 system (Waters, Midford, MA), using high sensitivity refractive index detector as reported earlier [23]. Monodisperse PEG standards served to establish the calibration curve.

2.3.2. Determination of critical aggregation concentration (CAC)

The CACs were measured by the steady-state fluorescence method using pyrene as a probe. The copolymers were dissolved in methanol (4% w/v) and water or SGF (82 mM HCl, 34 mM NaCl, pH 1.2) was slowly added under constant stirring. After the addition of water or SGF, stirring was continued for 36 h in open air to complete the self-assembling process. The volume was then adjusted with water or SGF to obtain a copolymer concentration of 2.5 mg/mL. A series of copolymer solutions (0.05–1250 mg/L) containing 2×10^{-7} M pyrene were prepared by diluting the stock solution with distilled water or SGF. The solutions were heated (68–72 °C) for 3 h under slow stirring and cooled overnight at room temperature. The volume of cooled solutions was adjusted to the desired concentration, and the excitation spectrum of pyrene was recorded at 390 nm and 20 °C using an Aminco Bowman Series 2 Luminescence spectrometer (Spectronic Instruments, Rochester, NY). The CAC was determined from the intersection of two straight lines (the horizontal line with an almost constant value of the ratio I_{335}/I_{333} and the tangential line with a steady increase in the ratio value) on the plot of the intensity ratio I_{335}/I_{333} of pyrene against the log of the copolymer concentration.

2.3.3. pK_a Determination

The copolymer was dissolved in 0.01 N NaOH at a concentration of 1 mg/mL. The solution was titrated against standardized 0.01 N HCl. Changes in pH values were monitored following incremental addition of HCl. The pK_a of the copolymer corresponds to the pH value at which the ionization degree (α) was 0.5.

2.4. Micelle characterization

2.4.1. Micelle size measurement

The hydrodynamic diameter and particle size distribution of micelles prepared in water and in SGF were determined by dynamic light scattering (DLS) with a Malvern Autosizer 4800 (Malvern, Worcestershire, UK). The measurements were carried out at a scattering angle of 90° and at 25 °C. CONTIN program was used to extract size distributions from the autocorrelation functions.

2.4.2. Determination of the aggregation number

An accurately weighed quantity of copolymer was dissolved in methanol, water was added slowly to obtain a methanol/water ratio of 1:10 (v/v) and the solutions were stirred in open air for 24 h. Prior to measurement, all the solutions were filtered through a 0.45 μm hydrophilic polypropylene filter. The weight-average molecular weight of the micelles ($M_{w,mic}$) was determined by multiangle static light scattering (MASLS) using a Malvern Autosizer 4800 (Malvern, Worcestershire, UK) as previously described [24]. The measurement was conducted at 25 °C at nine angles ranging from 50 to 130°. Each experiment was performed in duplicate. The aggregation number (N_{agg}) was calculated by dividing $M_{w,mic}$ by the M_w of the polymer.

2.5. Incorporation of CDN in micelles

2.5.1. Dialysis method

Accurately weighed quantities of copolymer (5 mg) and CDN (1.0 or 1.25 mg) (copolymer:CDN, 5:1 or 4:1 w/w) were dissolved in 2.5 mL of methanol and the same volume of water was added slowly under constant stirring. The solutions were dialyzed against water (molecular weight cut-off 3500) for 24 h. After dialysis, they were filtered (1.2-μm glass microfiber) to remove any precipitated drug and the filtrate was freeze-dried. Each experiment was performed in quadruplicate.

2.5.2. Solvent evaporation method

Accurately weighed quantities of copolymer (5 mg) and CDN (1 or 1.25 mg) (copolymer:CDN, 5:1 or 4:1 w/w) were dissolved in 0.5 mL of methanol, and 4.5 mL of water was added slowly under constant stirring. Stirring was continued for 24 h in open air to complete the self-assembling process. Then, the solutions were filtered (1.2-μm glass microfiber) and freeze dried. Each experiment was performed in quadruplicate.

2.6. Characterization of CDN-loaded micelles

2.6.1. Measurement of drug loading

To measure CDN loading, an accurately weighed amount of lyophilized micelles prepared by either the dialysis or the solvent evaporation method was dissolved in methanol and the solution was assayed by spectrophotometry (CARY 100 Bio UV–Visible spectrophotometer, Varian, Walnut Creek, CA) at 255 nm, after appropriate polymer blank correction.

2.6.2. Atomic force microscopy (AFM)

The CDN-loaded PEG_{115}-*b*-P(*n*BA_{38}-*co*-MAA_{43}) micelles were analyzed by AFM using a Nanoscope III Dimension 3100 instrument (Digital Instruments, Santa Barbara, CA). A drop of micelle solution (polymer concentration 6 mg/mL) was allowed to stabilize on freshly cleaved mica surface for 20–25 min for imaging in the liquid state. The imaging

was performed in ambient conditions under tapping mode with a gold-coated tip operating at 8 kHz with 0.1 N/m force constant.

2.6.3. Differential scanning calorimetry (DSC)

Calorimetric measurements were carried out using a DSC 2910 calorimeter (TA Instruments Inc. New Castle, DE) calibrated with indium. Briefly, lyophilized micelles with and without drug, physical mixtures of drug and polymer, polymer and drug alone were placed separately in aluminum pans and sealed with lids. Samples were scanned from −100 to 250 °C at a rate of 10 °C/min under helium.

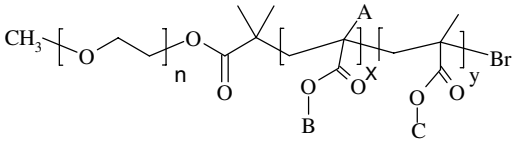
2.7. In vitro release of CDN from micelles

Micelles containing ~20% (w/w) of unlabelled CDN (on polymer weight basis) and 0.4 μCi of ^{3}H-CDN were prepared by the solvent evaporation method. The solution was passed through a 1.2-μm filter and transferred to the dialysis bag (molecular weight cut-off 15,000). The bag (containing 0.14 mg of CDN loaded in PM) was immersed in a flask containing 400 mL of SGF maintained at 37 °C under sink conditions. After 2 h, the pH of the release medium was adjusted to 7.2 by the addition of NaOH (100 mM) and potassium dihydrogen phosphate (22.8 mM). One-milliliter samples were withdrawn periodically with replacement and drug content was assessed by radioactivity counting. The release of the free drug from the dialysis bag was studied at pH 1.2 and 7.2 using a dilute solution (300-fold less concentrated compared to CDN-loaded PM solution) in order to avoid precipitation. Each release experiment was performed in triplicate.

3. Results and discussion

3.1. Copolymer synthesis and characterization

PEG-*b*-P(Al(M)A-*co*-MAA)s were prepared from two different molecular weight PEG macroinitiators and several structurally-related (meth)acrylates. *n*BA, *iso*BA and *Pr*MA have all 7 atoms of carbon, but the latter are positioned differently on the polymeric backbone. These monomers were copolymerized in a 1:1 molar ratio with *t*BMA. The pH-sensitivity of the copolymer was conferred by cleaving *t*BMA into MAA [23]. Control pH-insensitive polymers comprised of the non-hydrolyzed polymers as well as PEG-*b*-P(*n*BA-*co*-MMA) (Scheme 1). As reported in Table 1, polymers with well-controlled M_n and relatively low PI (*i.e.* M_w/M_n) (<1.5) were obtained. The SEC chromatograms displayed a single M_w distribution supporting the block copolymer structure (data not shown). ^{1}H NMR analysis showed a reasonable correlation with the initial monomer feed ratios and the polymerization yield was close to 90%. The MAA copolymers had similar pK_a values in the range of 6.2 to 6.6 (Table 2), which is higher than that of MAA (4.83) [25].



A	B	C	Copolymer
H	$CH_2CH_2CH_2CH_3$	t-C_4H_9	PEG-*b*-P(*n*BA-*co*-*t*BMA)
H	$CH_2CH(CH_3)CH_3$	t-C_4H_9	PEG-*b*-P(*iso*BA-*co*-*t*BMA)
CH_3	$CH_2CH_2CH_3$	t-C_4H_9	PEG-*b*-P(*Pr*MA-*co*-*t*BMA)
H	$CH_2CH_2CH_2CH_3$	CH_3	PEG-*b*-P(*n*BA-*co*-MMA)

Scheme 1. General structure of the PEG-*b*-P(Al(M)A-*co*-*t*BMA) and PEG-*b*-P(Al(M)A-*co*-MAA) copolymers.

When dissolved in water at concentrations above their CAC, all polymers formed supramolecular assemblies. As shown in Table 2, the CACs of the MAA copolymers were significantly higher (26–67 mg/L) than those of their *t*BMA counterparts (⩽5 mg/L). This suggests that the hydrolysis of *t*BMA decreased the hydrophobicity of the micelle core-forming segment. For a given composition, a slight reduction in the CAC was observed with an increase in the total molecular weight. The simultaneous increase in both PEG and hydrophobic chain lengths prevented a more drastic reduction in CAC, which would have been otherwise expected upon doubling the hydrophobic block length. The position of the carbons in Al(M)A also appeared to influence the overall hydrophobicity of the core-forming block. Indeed, copolymers containing *n*BA exhibited the highest CAC, followed by those of *iso*BA and *Pr*MA. For instance, in the case of PEG_{44}-*b*-P(nBA_{21}-*co*-MAA_{19}), the substitution of *n*BA by *iso*BA in the hydrophobic chain decreased the CAC from 67 to 54 mg/L. Likewise, the replacement of *iso*BA by *Pr*MA further reduced the CAC to 32 mg/L. Surprisingly, substituting *t*BMA by the smaller non-ionizable MMA unit had no effect on the CAC (compare #1 with 13 and #3 with 14). The CAC of MAA copolymers was also determined in SGF to investigate the effect of acidic conditions on their self-assembling properties. At pH 1.2, the CAC could be determined only for the high molecular weight MAA copolymers (PEG_{115} series) (entries 4, 8, and 12). Indeed, the polymers containing PEG_{44} precipitated at pH 1.2 possibly because of extensive hydrogen bonding under acidic conditions (see Section 3.2), which may have interfered with the assembly of the polymer chains into well-defined core-shell type structures. As expected, the CACs were significantly reduced (*i.e.* 2-fold) in SGF *vs.* water due to the complete protonation of the MAA units at pH 1.2 (Table 2). In SFG, substitution of *n*BA by *iso*BA had no significant effect on the CAC. However, as also observed in water the introduction of *Pr*MA significantly reduced the CAC of the copolymer.

Table 1
Characteristics of the diblock copolymers

Copolymer no.	Copolymers	M_n[a] (theory)	M_n[b] (NMR)	M_n (SEC)	M_w (SEC)	M_w/M_n (SEC)	Al(M)A/*t*BMA[c] (mol%)
1	PEG_{44}-*b*-P(*n*BA_{21}-*co*-*t*BMA_{19})	6700	7300	6200	7000	1.12	52.5:47.5
2	PEG_{44}-*b*-P(*n*BA_{21}-*co*-MAA_{19})	5700	6300	–	–	–	–
3	PEG_{115}-*b*-P(*n*BA_{38}-*co*-*t*BMA_{43})	14,500	16,000	10,200	13,400	1.31	46.9:53.1
4	PEG_{115}-*b*-P(*n*BA_{38}-*co*-MAA_{43})	12,600	13,600	–	–	–	–
5	PEG_{44}-*b*-P(*iso*BA_{20}-*co*-*t*BMA_{20})	6700	7300	5800	6900	1.19	50:50
6	PEG_{44}-*b*-P(*iso*BA_{20}-*co*-MAA_{20})	5700	6200	–	–	–	–
7	PEG_{115}-*b*-P(*iso*BA_{35}-*co*-*t*BMA_{38})	14,500	15,000	11,900	17,900	1.49	48:52
8	PEG_{115}-*b*-P(*iso*BA_{35}-*co*-MAA_{38})	12,600	12,800	–	–	–	–
9	PEG_{44}-*b*-P(*Pr*MA_{17}-*co*-*t*BMA_{16})	6700	6400	6100	6800	1.11	51.5:48.5
10	PEG_{44}-*b*-P(*Pr*MA_{17}-*co*-MAA_{16})	5700	5500	–	–	–	–
11	PEG_{115}-*b*-P(*Pr*MA_{36}-*co*-*t*BMA_{36})	14,500	14,800	10,400	12,900	1.24	50:50
12	PEG_{115}-*b*-P(*Pr*MA_{36}-*co*-MAA_{36})	12,600	12,800	–	–	–	–
13	PEG_{44}-*b*-P(*n*BA_{20}-*co*-MMA_{17})	6000	6200	7600	10,400	1.37	54:46
14	PEG_{115}-*b*-P(*n*BA_{40}-*co*-MMA_{32})	13,100	13,400	9300	11,400	1.23	55.5:44.5

[a] From the initial feed composition.
[b] Determined from ^{1}H NMR by comparing characteristic peak intensities of Al(M)A (CH_2COO $\delta = 4.09$) and *t*BMA (($CH_3)_3$ $\delta = 1.43$) with that of PEG (OCH_2CH_2 $\delta = 3.66$).
[c] Evaluated by ^{1}H NMR, theoretical Al(M)A/*t*BMA molar ratio is 50:50.

Table 2
Characteristics of PM

Copolymer no.	Copolymers	CAC ± SD[a] (mg/L) in water	CAC ± SD[a] (mg/L) in SGF	d_h (nm) [PI][b] in water	d_h (nm) [PI][b] in SGF	pK_a ± SD[a]	N_{agg}	ρ_p in water (10^{-2} g/cm^3)
1	PEG_{44}-*b*-P(*n*BA_{21}-*co*-*t*BMA_{19})	5 ± 0.4	–	25 [0.04]	–	–	108	15.5
2	PEG_{44}-*b*-P(*n*BA_{21}-*co*-MAA_{19})	67 ± 9.7	–	183 [0.13]	*	6.29 ± 0.08	–	–
3	PEG_{115}-*b*-P(*n*BA_{38}-*co*-*t*BMA_{43})	4 ± 0.9	–	35 [0.06]	–	–	83	8.2
4	PEG_{115}-*b*-P(*n*BA_{38}-*co*-MAA_{43})	63 ± 0.8	28	60 [0.20]	64 [0.12]	6.53 ± 0.05	157	2.5
5	PEG_{44}-*b*-P(*iso*BA_{20}-*co*-*t*BMA_{20})	4 ± 0.8	–	27 [0.08]	–	–	102	11.4
6	PEG_{44}-*b*-P(*iso*BA_{20}-*co*-MAA_{20})	54 ± 7.8	–	185 [0.06]	*	6.33 ± 0.01	–	–
7	PEG_{115}-*b*-P(*iso*BA_{35}-*co*-*t*BMA_{38})	3 ± 1.5	–	38 [0.19]	–	–	66	6.8
8	PEG_{115}-*b*-P(*iso*BA_{35}-*co*-MAA_{38})	39 ± 10.8	24	56 [0.25]	54 [0.17]	6.36 ± 0.13	135	3.8
9	PEG_{44}-*b*-P(*Pr*MA_{17}-*co*-*t*BMA_{16})	0.9 ± 0.1	–	37 [0.14]	–	–	85	3.6
10	PEG_{44}-*b*-P(*Pr*MA_{17}-*co*-MAA_{16})	32 ± 11.4	–	283 [0.17]	*	6.31 ± 0.07	–	–
11	PEG_{115}-*b*-P(*Pr*MA_{36}-*co*-*t*BMA_{36})	0.8 ± 0.2	–	37 [0.06]	–	–	62	5.0
12	PEG_{115}-*b*-P(*Pr*MA_{36}-*co*-MAA_{36})	26 ± 7.1	12	122 [0.15]	87 [0.12]	6.26 ± 0.07	129	0.2
13	PEG_{44}-*b*-P(*n*BA_{20}-*co*-MMA_{17})	5 ± 1.2	–	26 [0.11]	–	–	–	–
14	PEG_{115}-*b*-P(*n*BA_{40}-*co*-MMA_{32})	3 ± 1.1	–	35 [0.28]	–	–	–	–

[a] Mean ± SD of three independent measurements.
[b] PI = polydispersity index.
* Large aggregates >500 nm.

3.2. Micelle characterization

The mean hydrodynamic diameters and PI of the PM are reported in Table 2. Regarding *t*BMA copolymers, the substitution of *n*BA with *iso*BA or *Pr*MA had no major effect on the size. However, size increased with the molecular weight of the copolymer. For instance, the diameters of PEG_{44}-*b*-P(nBA_{21}-*co*-$tBMA_{19}$) and PEG_{115}-*b*-(nBA_{38}-*co*-$tBMA_{43}$) were 25 nm and 35 nm, respectively. Hydrolysis of *t*BMA also led to a significant size increase and in some cases, to the formation of relatively large particles (>180 nm). This could be attributed to swelling of the micelle core due to the partial ionization of MAA in water and to secondary aggregation resulting from hydrogen bonding between the surface exposed carboxylic acid groups of MAA and the oxygen of the ethylene oxide units of PEG [26]. The implication of COOH groups on size increase is further corroborated by the fact that substitution of *t*BMA by MMA, a non-ionizable unit, barely affected the mean diameter (25–35 nm). Secondary aggregation could be reduced by using higher molecular weight polymers (see PEG_{115} series). In the case of MAA copolymers, substitution of *n*BA by *iso*BA had no significant effect on PM size, whereas introduction of *Pr*MA units yielded larger aggregates. It is possible the lateral methyl group of the *Pr*MA units has a negative impact on the packing density of the core forming segment. In SGF a trend similar to that observed in water was obtained. The PEG_{44} series yielded very large aggregates, whereas the MAA copolymers prepared with PEG_{115} allowed the formation of narrowly distributed micelles with PEG_{115}-*b*-P($PrMA_{36}$-*co*-MAA_{36}) giving the largest PM.

The N_{agg} were calculated for all PM except low molecular weight MAA copolymers and MMA copolymers. At the lowest concentrations used in MASLS analysis, these polymers showed a small population (<15%) of large aggregates, which could distort the results (data not shown). The micelles exhibited N_{agg} values ranging from 60 to 160. No clear trend could be identified upon the substitution of *n*BA by *iso*BA or *Pr*MA. Generally, irrespective of the Al(M)A unit, the N_{agg} was higher for polymers having lower molecular weights. For example, the N_{agg} of PEG_{44}-*b*-P(nBA_{21}-*co*-$tBMA_{19}$) ($M_w = 7000$) micelles dropped from 108 to 83 upon increasing the copolymer M_w to 14,400. The most noticeable feature was the 2-fold rise in N_{agg} following the hydrolysis of *t*BMA into MAA, which paralleled the increase in micelle size measured by DLS. To gain further insight on the micelle structure, the apparent micelle density (ρ_p) was calculated according to the following equation. This equation does not take into account the fact that the PM are hydrated in water.

$$\rho_p = 3M_{w,mic}/4\pi N_A R_h^3$$

where N_A is the Avogadro's number and R_h, the micelle hydrodynamic radius.

As reported in Table 2, the unhydrolyzed PM exhibited higher ρ_p than MAA copolymer micelles, which is indicative of the formation of less hydrated and more tightly packed aggregates. Among the systems for which ρ_p could be calculated, PM containing *Pr*MA units were those presenting the lowest density (compare entries 1 and 5–9, and entries 4 and 8–12). As stated above, the *Pr*MA moiety may impair the tight packing of the core unit segments.

3.3. Incorporation of CDN in self-assemblies

The effects of loading method (*i.e.* dialysis and solvent evaporation), polymer composition and molecular weight on the incorporation of CDN into the micelles were assessed. Irrespective of the drug loading method, entrapment efficiency (EE) was found to be above 60% for all investigated copolymers and was often superior to 90%. Indeed, when the initial feed ratio was set at 25% (w/w), drug loadings as high as 23.8% (w/w) could be achieved. While comparing the two loading methods, it was observed that the solvent evaporation method generally yielded smaller micelles (data not shown). AFM analysis revealed that CDN-loaded PM were spherical in shape and of uniform size (Fig. 1).

Table 3 reports the characteristics of the drug-loaded micelles prepared by the solvent evaporation method using a 20% (w/w) initial drug loading. For all copolymers, the hydrolysis of the *t*BMA units into MAA barely affected the EE. However, as observed with the unloaded micelles, it substantially increased the mean micelle size from about 20–30 nm to 40–100 nm. The EE was not affected by either the nature of the AL(M)A units (*n*BA, *iso*BA or *Pr*MA) or the molecular weight of the copolymer. The most distinct feature was the substitution of *t*BMA by MMA (compare copolymers PEG_{44}-*b*-P(nBA_{21}-*co*-$tBMA_{19}$) with PEG_{44}-*b*-P(nBA_{20}-*co*-MMA_{17}) and PEG_{115}-*b*-P(nBA_{38}-*co*-$tBMA_{43}$)

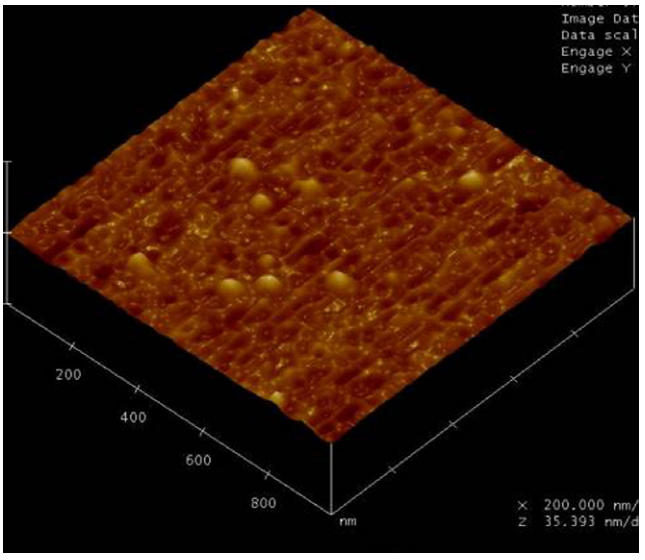


Fig. 1. AFM image of CDN-loaded PEG_{115}-*b*-P(nBA_{38}-*co*-MAA_{43}) micelles.

Table 3
Characteristics of CDN-loaded PM prepared by the solvent evaporation method with 20% (w/w) of initial feed ratio

Copolymer no.	Copolymers	d_h (nm) [PI][a]	Final drug loading (% w/w)	EE (%)
1	PEG_{44}-*b*-P(*n*BA_{21}-*co*-*t*BMA_{19})	21 [0.35]	18.0	90 ± 6.4
2	PEG_{44}-*b*-P(*n*BA_{21}-*co*-MAA_{19})	86 [0.14]	17.2	86 ± 3.2
3	PEG_{115}-*b*-P(*n*BA_{38}-*co*-*t*BMA_{43})	28 [0.33]	19.2	96 ± 7.3
4	PEG_{115}-*b*-P(*n*BA_{38}-*co*-MAA_{43})	43 [0.21]	19.0	95 ± 8.4
5	PEG_{44}-*b*-P(*iso*BA_{20}-*co*-*t*BMA_{20})	30 [0.37]	18.0	90 ± 7.6
6	PEG_{44}-*b*-P(*iso*BA_{20}-*co*-MAA_{20})	106 [0.21]	17.8	89 ± 5.7
7	PEG_{115}-*b*-P(*iso*BA_{35}-*co*-*t*BMA_{38})	31 [0.29]	18.4	92 ± 4.5
8	PEG_{115}-*b*-P(*iso*BA_{35}-*co*-MAA_{38})	45 [0.27]	17.8	89 ± 8.0
9	PEG_{44}-*b*-P(*Pr*MA_{17}-*co*-*t*BMA_{16})	30 [0.33]	18.0	90 ± 7.0
10	PEG_{44}-*b*-P(*Pr*MA_{17}-*co*-MAA_{16})	73 [0.20]	17.2	86 ± 9.0
11	PEG_{115}-*b*-P(*Pr*MA_{36}-*co*-*t*BMA_{36})	26 [0.29]	18.4	92 ± 3.3
12	PEG_{115}-*b*-P(*Pr*MA_{36}-*co*-MAA_{36})	52 [0.29]	17.4	87 ± 12
13	PEG_{44}-*b*-P(*n*BA_{20}-*co*-MMA_{17})	21 [0.37]	12.4	62 ± 8.4
14	PEG_{115}-*b*-P(*n*BA_{40}-*co*-MMA_{32})	27 [0.40]	14.2	71 ± 5.7

[a] PI, polydispersity index.

with PEG_{115}-*b*-P(*n*BA_{40}-*co*-MMA_{32}) which brought about a considerable drop in the EE.

3.4. Thermal analysis of micelles

Thermal analysis of PEG_{115}-*b*-P(*n*BA_{38}-*co*-*t*BMA_{43}) revealed an endothermic peak at 54 °C, which corresponded to the melting temperature (T_m) of the PEG block (Fig. 2A) [27]. CDN exhibited a T_m at 163 °C, followed by an exothermic peak attributed to its decomposition. The endothermic peak reflected the crystalline nature of CDN in bulk form. This peak was also present in the physical mixture, but was smaller due to the lower amount of drug in the aluminum pan. On the contrary, the lyophilized drug-loaded PM did not exhibit the T_m of CDN, suggesting its amorphous nature in the micellar system. The same analyses were carried out with the hydrolyzed polymer (Fig. 2B). Bulk PEG_{115}-*b*-P(*n*BA_{38}-*co*-MAA_{43}) showed two endothermic peaks at 47 and 55 °C which were assigned to the T_m of the PEG block [28]. The presence of multiple melting peaks possibly corresponds to a different crystal folding (fully extended and folded once) in PEG chains [29]. These two peaks were preserved in the physical mixture. However, after micellization, they fused to produce one broad peak. This may be due to reorientation of once-folded PEG chains to an extended conformation. As described for the unhydrolyzed polymers, the melting peak of CDN was not present in the drug-loaded micelles confirming the amorphous nature of the entrapped drug.

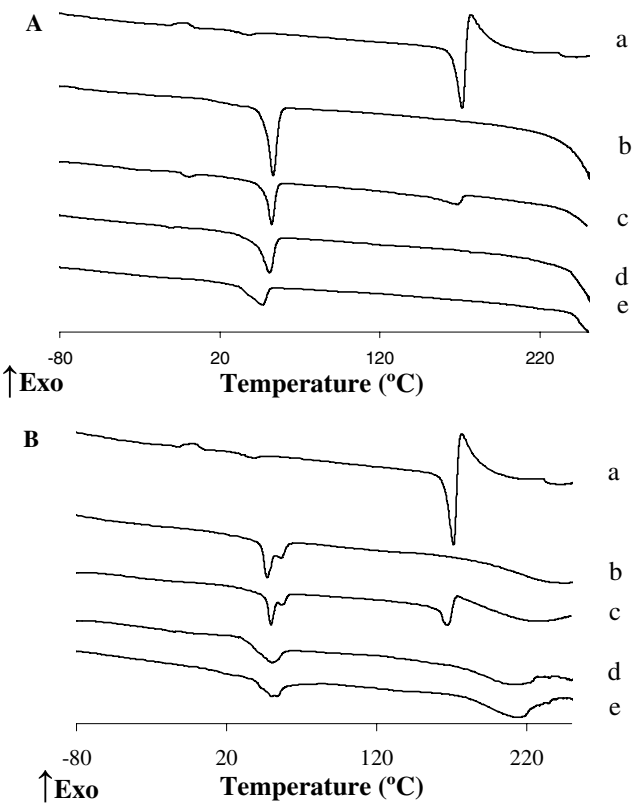


Fig. 2. (A) Thermograms of CDN (a), PEG_{115}-*b*-P(*n*BA_{38}-*co*-*t*BMA_{43}) (b), physical mixture of CDN and copolymer (1:5, w/w) (c), PM (d) and CDN-loaded PM (e). (B) Thermograms of CDN (a), PEG_{115}-*b*-P(*n*BA_{38}-*co*-MAA_{43}) (b), physical mixture of CDN and copolymer (1:5, w/w) (c), PM (d) and CDN-loaded PM (e).

3.5. In vitro release of CDN from micelles

Release of a drug from PM can be affected by the level of drug encapsulation, its physical state, the nature of the polymer and the level of polymer-drug compatibility [30–33]. Fig. 3 shows the *in vitro* cumulative release profiles of CDN from different micelle formulations. Prior to conducting these release assays, it was verified that CDN could freely diffuse through the dialysis membrane (Fig. 3A), and that sink conditions were respected. As illustrated in Fig. 3A, the micelles prepared with PEG-*b*-P(*iso*BA-*co*-*t*BMA) or PEG-*b*-P(*iso*BA-*co*-MAA) released <10% of CDN after 2 h in simulated gastric fluids. This suggests that the hydrophobicity of the micelle core was sufficiently high to minimize the burst release at acidic pH. When the pH was shifted from 1.2 to 7.2 (arrows), the release rate of CDN from pH-insensitive PEG-*b*-P(*iso*BA-*co*-*t*BMA) micelles slightly increased from ~4 to 6%/h. Indeed, this

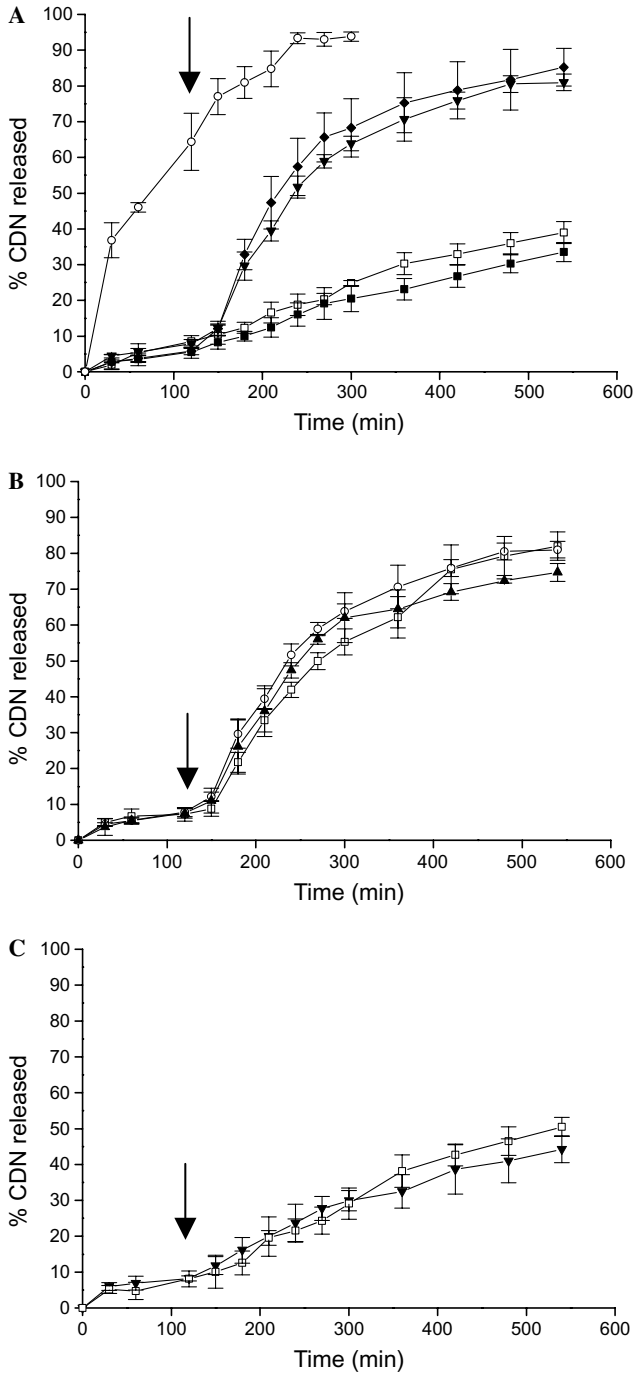


Fig. 3. (A) *In vitro* release of CDN from different *iso*BA containing PM prepared by solvent evaporation method at pH 1.2 for 2 h followed by 7 h at pH 7.2: (□) PEG_{44}-*b*-P(*iso*BA_{20}-*co*-*t*BMA_{20}), (■) PEG_{115}-*b*-P(*iso*BA_{35}-*co*-*t*BMA_{38}), (♦) PEG_{44}-*b*-P(*iso*BA_{20}-*co*-MAA_{20}), (▼) PEG_{115}-*b*-P(*iso*BA_{35}-*co*-MAA_{38}) and (○) CDN release in the absence of copolymer. (B) Effect of Al(M)A monomer on *in vitro* release of CDN from PM: (▼) PEG_{115}-*b*-P(*n*BA_{38}-*co*-MAA_{43}), (○) PEG_{115}-*b*-P(*iso*BA_{35}-*co*-MAA_{38}) and (□) PEG_{115}-*b*-P(*Pr*MA_{36}-*co*-MAA_{36}). (C) Effect of *t*BMA substitution by MMA on *in vitro* release of CDN from PM: (□) PEG_{44}-*b*-P(*n*BA_{20}-*co*-MMA_{17}) and (▼) PEG_{44}-*b*-P(*n*BA_{21}-*co*-*t*BMA_{19}). Mean ± SD ($n = 3$). Arrow corresponds to the change in pH. *Note*: For all investigated copolymers, a similar pattern of CDN release was observed for micelles prepared by the dialysis method (data not shown).

minimal change in the slope can be attributed to a difference in the solubility of CDN in simulated gastric (0.6 μg/mL) *vs.* intestinal fluids (8.6 μg/mL). Yet, the release pattern was essentially pH-independent. In contrast, in the case of PEG-*b*-P(*iso*BA-*co*-MAA) micelles, an abrupt rise in the release rate (30%/h) occurred upon an increase in pH to 7.2, with approximately 85% of the drug released after 9 h, irrespective of the polymer molecular weight. This is attributed to the ionization of the carboxyl group of the MAA core units leading to partial or complete destabilization of the micelles [19,34]. Fig. 3B shows that the substitution of *n*BA by *iso*BA or *Pr*MA in the pH-sensitive copolymer had no significant effect on the release pattern. As for PEG-*b*-P(*n*BA-*co*-*t*BMA), micelles prepared from non-ionizable PEG-*b*-P(*n*BA-*co*-MMA) exhibited release kinetics which were essentially pH-independent (Fig. 3C).

When developing oral colloidal delivery systems for highly hydrophobic drugs such as CDN, it is important to adequately control the release rate in order to avoid precipitation upon dilution in the stomach and maximize the absorption in the small bowel. The proposed pH-sensitive micelles exhibit minimal leakage at acidic pH and almost complete drug release at pH 7.2 within a time interval compatible with the transit time in the intestine. Other micellar systems may not achieve these objectives and might lead to sequestration of the drug in the GI tract especially when used at high concentrations. Indeed, Pierri et al. [35] reported that the *in vitro* release of griseofulvin in simulated intestinal fluid from pH-insensitive PEG-*b*-P(D,L-lactide) micelles was less than 45% in 6 days. Similarly, slow release (<50% in 48 h) of risperidone from conventional micelles prepared from self-assembling biodegradable PEG-*b*-(caprolactone/trimethylene carbonate) was reported [9]. In these cases, the slow drug release observed *in vitro* reflects the fact that the micelles remained intact during the assay.

4. Conclusions

The present study revealed that PEG-*b*-P(Al(M)A-*co*-MAA)s can self-assemble in aqueous media to form pH-sensitive micelles of narrow size distribution. The CAC and PM hydrodynamic diameter were found to depend on the nature of the Al(M)A units and the polymer molecular weight. The poorly water-soluble compound CDN was efficiently incorporated at high concentrations into the micelles. The release rate of CDN from pH-sensitive PM increased substantially when the pH was shifted from 1.2 to 7.2, while that from micelles prepared from unhydrolyzed copolymers was mostly unaffected. Such pH-sensitive PM could potentially enhance the oral absorption of CDN by releasing it near its site of preferential absorption. Future work will focus on studying the permeability across gastrointestinal mucosa and the bioavailability of CDN-loaded micelles *in vivo*.

Acknowledgements

Financial support from the Natural Sciences and Engineering Research Council of Canada and the Canada Research Chair program is acknowledged. Mrs G. Gaucher is acknowledged for her critical reading of the manuscript.

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