Comparing the release rates of ciprofloxacin from different polymer structures with different linker chemistries

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Abstract

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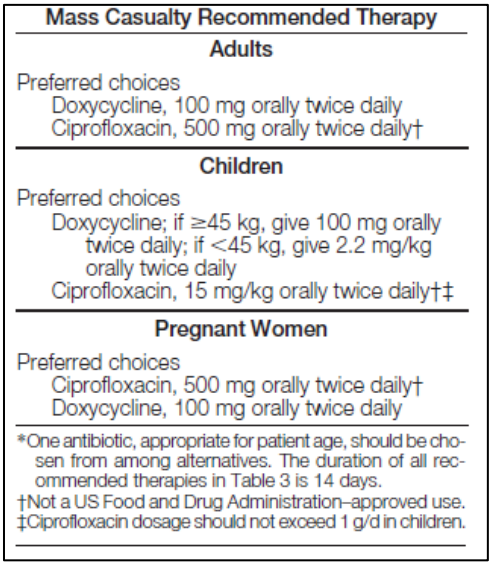
Polymeric prodrugs of ciprofloxacin for inhalation are attractive alternatives to orally-administered free ciprofloxacin and inhalable liposomal formulations. This study investigated the release kinetics of ciprofloxacin from four different polymers synthesized from two types of monomeric ciprofloxacin prodrug, HBC containing the alkyl ester linker and CTM containing the phenyl ester linker. The four polymers synthesized and characterized were poly(O950-*b*-HBC), poly[O950-*b*-(HBC-*co*-MAA)], poly(O950-*co*-CTM) and poly[(O950-*co*-CTM)-*b*-(O950-*co*-HBC)]. The release rates were quantified using HPLC. The inclusion of the hydrophilic methacrylic acid (MAA) in the diblock copolymer of HBC increased the release rates. Unexpected levels of ciprofloxacin release were observed for the CTM polymers using H_2O/ACN/acetic acid (94:5:1, v/v/v) as the mobile phase, while the contrary was obtained for poly(O950-*co*-CTM) with 2% aqueous acetic acid and acetonitrile (84:16, v/v) as the mobile phase. Hence, further analyses using the latter mobile phase will need to be performed before conclusions can be drawn regarding the CTM polymers.

Introduction

Francisella tularensis is one of the most infectious pathogenic bacteria known, requiring inoculation or inhalation of as few as 10 organisms to cause tularemia[1]. The bacteria are aerobic and gram-negative[1]. *F. tularensis* can be divided into two major subspecies: type A and type B[2]. Type A is more virulent than type B, and is the most common biovar isolated in North America[3]. *F. tularensis* disseminates easily, and has a huge potential to cause illness and death[4-6]. As a result, *F. tularensis* has long been considered a potential biological weapon[1]. The Working Group on Civilian Biodefense believes that an aerosol release of *F. tularensis* would have the greatest adverse medical and public health consequences[1], in comparison to other ways of exposure to the bacteria. The Center for Disease Control and Prevention (CDC) has estimated a total base cost to society of $5.4 billion for every 100,000 persons exposed in an *F. tularensis* aerosol attack[5].

In an event of *F. tularensis* biological attack, the Working Group on Civilian Biodefense suggests that orally-administered ciprofloxacin and doxycycline should be the preferred choices for treatment for both adults and children[1], as shown in **Figure 1**. Although fluoroquinolones (e.g. ciprofloxacin) have been reported to cause cartilage damage in immature animals and are not FDA-approved for use in children, the Group believes the benefits to children from short courses of fluoroquinolones outweigh the risks of their use[1]. However, ciprofloxacin administered orally tends to suffer from poor biodistribution, because the free drug does not preferentially accumulate in lungs and thus has low sustain therapeutic levels[7]. In addition, such formulation tends to demonstrate poor pharmacokinetics with a short half-life of approximately 3.5 hrs[8]. As with most systematically delivered free drugs, systemic toxicity is also often an issue. Hence, to help overcome these problems, a liposomal formulation of ciprofloxacin for inhalation has been developed and marketed by Aradigm®. Conventional liposomes are readily taken up by phagocytic cells of the RES system, including



Mass Casualty Recommended Therapy	
Adults	
Preferred choices	
Doxycycline, 100 mg orally twice daily	
Ciprofloxacin, 500 mg orally twice daily†	
Children	
Preferred choices	
Doxycycline; if ≥45 kg, give 100 mg orally twice daily; if <45 kg, give 2.2 mg/kg orally twice daily	
Ciprofloxacin, 15 mg/kg orally twice daily†‡	
Pregnant Women	
Preferred choices	
Ciprofloxacin, 500 mg orally twice daily†	
Doxycycline, 100 mg orally twice daily	

*One antibiotic, appropriate for patient age, should be chosen from among alternatives. The duration of all recommended therapies in Table 3 is 14 days.
†Not a US Food and Drug Administration–approved use.
‡Ciprofloxacin dosage should not exceed 1 g/d in children.

Figure 1. Working Group consensus recommendations for treatment of patients with tularemia in a mass casualty setting[1].

macrophages, and therefore they target intracellular sites where *F. tularensis* resides[7]. The targeted delivery *via* inhalation helps lower the risks of systemic toxicity as well as increase the possibility of preferential deposit in lungs. Additionally, the sustained release of ciprofloxacin from the liposomes may prolong its half-life in the body[7]. However, the liposomal formulation is still limited by low drug loading efficiencies, rapid release of drug contents, and more complex formulation procedures that challenges large-scale manufacturing[9].

Polymeric prodrugs provide an alternative to liposomes for targeted and controlled drug delivery, with one advantage of the polymeric prodrugs over liposomes being the relative ease of large-scale synthesis of polymers. Woo et al.[10] demonstrated a novel biodegradable polymeric prodrug that released ciprofloxacin in response to inflammatory related enzymes, to be potentially used in the control of medical device associated infections. Das et al.[9] reported the release kinetics of ciprofloxacin from four different polymeric prodrugs. The four prodrugs included two distinct polymer structures (statistical copolymer and diblock copolymer) and two different ester linkers that connected ciprofloxacin to the polymer backbones (phenyl ester and alkyl ester). Das et al.[9] concluded that the statistical copolymer structure and the phenyl ester linker resulted in faster ciprofloxacin release rates, and thus a combination of the two gave the fastest releasing polymer among the four different combinations.

As a continuation of Das et al.'s work[9], to further analyze how polymer structures and linker chemistries affect the release kinetics of ciprofloxacin from polymeric prodrugs, four different polymers were synthesized and characterized, and their release kinetics in 1xPBS and human serum were determined using high-performance liquid chromatography (HPLC). The four polymers were a micelle-forming diblock copolymer of the slower-releasing linker connected ciprofloxacin, a similar micelle-forming diblock copolymer with the core of the micelles diluted with a hydrophilic moiety, a statistical copolymer of the faster-releasing linker connected ciprofloxacin, and a polymer that combined the faster-releasing and the slower-releasing linkers. This study aimed to illustrate how the dilution with a hydrophilic moiety would affect the release kinetics, and how the release kinetics would alter with a polymer that contained both the phenyl ester linker and the alkyl ester linker.

Methods

Synthesis of PEGMA 950 (O950) macroCTA *via* RAFT polymerization

The RAFT polymerization of O950 macroCTA was performed in dimethyl sulfoxide (DMSO), with 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CTP) and 4, 4'-azobis(4-cyanovaleric acid) (ABCVA) as the chain transfer agent (CTA) and initiator (I), respectively. The $[M]_0$:$[CTA]_0$ and $[CTA]_0$:$[I]_0$ were 25:1 and 10:1, respectively. In a 50 mL round-bottom flask was added 4 g O950 (4.21 mmol), 47.1 mg CTA (0.168 mmol), 4.72 mg ABCVA (0.0168 mmol), and 16 mL DMSO. The solution was then sealed with a septum, and purged with nitrogen for 30 min. After nitrogen purge, the reaction was allowed to proceed at 70°C for 18 hrs. The polymerization solution was precipitated into diethyl ether, and the resulting polymer was dried under vacuum before its ^{1}H-NMR was taken on Bruker Avance 500 in $CDCl_3$.

Synthesis of poly(O950-*b*-HBC) and poly[O950-*b*-(HBC-*co*-*t*BMA)] *via* RAFT polymerization

The RAFT polymerization of poly(O950-*b*-HBC) from the O950 macroCTA was performed in glacial acetic acid, with ABCVA as the initiator. The $[M]_0$:$[CTA]_0$ and $[CTA]_0$:$[I]_0$ were 25:1 and 10:1, respectively. In a 5 mL pear-shaped flask was added 255 mg HBC (0.469 mmol), 300 mg O950 macroCTA (0.0188 mmol), 0.525 mg ABCVA (0.00188 mmol), and 1.17 mL acetic acid. The solution was then sealed with a septum, and purged with nitrogen for 30 min. After nitrogen purge, the reaction was allowed to proceed at 70°C for 18 hrs. The polymerization solution was precipitated into diethyl ether, and the resulting polymer was dried under vacuum.

The RAFT polymerization of poly[O950-*b*-(HBC-*co*-*t*BMA)] from the same O950 macroCTA was performed in glacial acetic acid, with ABCVA as the initiator. The $[M]_0$:$[CTA]_0$ and $[CTA]_0$:$[I]_0$ were 25:1 and 10:1, respectively. In a 5 mL pear-shaped flask was added 99.1 mg HBC (0.182 mmol), 51.8 mg *t*BMA (0.365 mmol), 350 mg O950 macroCTA (0.0219 mmol), 0.613 mg ABCVA (0.00219 mmol), and 0.779 mL acetic acid. The solution was then sealed with a septum, and purged with nitrogen for 30 min. After nitrogen purge, the reaction was allowed to proceed at 70°C for 18 hrs. The polymerization solution was precipitated into diethyl ether, and the resulting polymer was dried under vacuum.

Synthesis of poly(O950-*co*-CTM) *via* RAFT polymerization

The RAFT polymerization of poly(O950-*co*-CTM) was performed in glacial acetic acid, with CTP and ABCVA as CTA and initiator, respectively. The $[M]_0$:$[CTA]_0$ and $[CTA]_0$:$[I]_0$ were 25:1 and 10:1, respectively. In a 10 mL round-bottom flask was added 250 mg CTM (0.328 mmol), 886 mg O950 (0.933 mmol), 14.1 mg CTA (0.0504 mmol), 1.41 mg ABCVA (0.00504 mmol), and 4.54 mL acetic acid. The

solution was then sealed with a septum, and purged with nitrogen for 30 min. After nitrogen purge, the reaction was allowed to proceed at 70°C for 18 hrs. The polymerization solution was precipitated into diethyl ether, and the resulting polymer was dried under vacuum.

Synthesis of poly[(O950-*co*-CTM)-*b*-(O950-*co*-HBC)] *via* RAFT polymerization

The RAFT polymerization of poly[(O950-*co*-CTM)-*b*-(O950-*co*-HBC)] from the poly(O950-*co*-CTM) macroCTA was performed in glacial acetic acid, with ABCVA as the initiator. The $[M]_0$:$[CTA]_0$ and $[CTA]_0$:$[I]_0$ were 25:1 and 10:1, respectively. In a 5 mL pear-shaped flask was added 82.4 mg HBC (0.152 mmol), 410.1 mg O950 (0.432 mmol), 350 mg poly(O950-*co*-CTM) macroCTA (0.0233 mmol), 0.654 mg ABCVA (0.00233 mmol), and 2.15 mL acetic acid. The solution was then sealed with a septum, and purged with nitrogen for 30 min. After nitrogen purge, the reaction was allowed to proceed at 70°C for 18 hrs. The polymerization solution was precipitated into diethyl ether, and the resulting polymer was dried under vacuum.

Gel permeation chromatography (GPC)

The polymers were dissolved in dimethylformamide with 0.1 wt% LiBr at 10 mg/mL. GPC was performed using an Agilent 1200 Series Liquid Chromatography System (Santa Clara, CA) and Wyatt Technology miniDAWN TREOS, 3 angle MALS light scattering instrument and Optilab TrEX, and refractive index detector (Santa Barbara, CA). Tosoh SEC TSK-GEL α-3000 and α-e4000 columns (Tosoh Bioscience, Montgomeryville, PA) were used, with HPLC-grade dimethylformamide containing 0.1 wt% LiBr at 60°C as the mobile phase at a flow rate of 1 mL/min.

Deprotection and purification of the polymeric prodrugs

The polymeric prodrugs were dissolved in neat trifluoroacetic acid (TFA) at 50 mg/mL for 24 hrs at room temperature, to remove the Boc protecting group on the ciprofloxacin residues. The *tert*-butyl residues on poly[O950-*b*-(HBC-*co*-*t*BMA)] were also removed by neat TFA to generate carboxylic acid residues, and thus poly[O950-*b*-(HBC-*co*-MAA)] was generated. The TFA solutions were precipitated into diethyl ether, and the resulting polymers were dried under vacuum. The polymers were then dissolved in 2.5 mL of 0.5 M Na_2HPO_4 buffer (pH=7), eluted through PD-10 columns (GE Healthcare, Little Chalfont, UK) with DI H_2O following manufacturer's protocol, and lyophilized. All dried polymers, except poly(O950-*b*-HBC), were dissolved in $CDCl_3$ and their NMR taken on Bruker Avance 500. Poly(O950-*b*-HBC) was dissolved

in DMSO-d_6/TFA (50:50, v/v) and its NMR taken on Bruker Avance 500. The mass of poly(O950-*co*-CTM), poly(O950-*b*-HBC), poly[O950-*b*-(HBC-*co*-MAA)] and poly[(O950-*co*-CTM)-*b*-(O950-*co*-HBC)] right before PD-10 column purification or dialysis was 218.8 mg, 335.2 mg, 213.8 mg and 330.8 mg in that order.

Ciprofloxacin analysis by high-performance liquid chromatography (HPLC)

The HPLC analysis of ciprofloxacin was performed with H_2O/ACN/acetic acid (94:5:1, v/v/v) as the aqueous mobile phase and ACN as the organic mobile phase. The flow rate was set at 1.0 mL/min, and the sample injection volume was 10 µL. The UV detector was operated at 277 nm. The Agilent Zorbax Rx-C_{18} (4.6×150 mm, 5 µm) analytical column (Agilent Technologies, CA) was used, with the column temperature set at 26°C.

A stock solution of ciprofloxacin was prepared from ciprofloxacin hydrochloride, used as received, in the aqueous mobile phase at 5 mg/mL. A secondary series of stock solutions was diluted from the 5 mg/mL stock solution using the aqueous mobile phase to concentrations of 2.5 mg/mL, 250 µg/mL and 25 µg/mL. Working solutions of ciprofloxacin for standard curves were then made by dilution using the aqueous mobile phase to concentrations of 1 µg/mL, 2 µg/mL, 4 µg/mL and 8 µg/mL from the 25 µg/mL stock solution, to concentrations of 20 µg/mL, 40 µg/mL and 100 µg/mL from the 250 µg/mL stock solution, and to concentrations of 200 µg/mL and 400 µg/mL from the 2.5 mg/mL stock solution. Each working solution was subsequently diluted with a 1:1 (v/v) ratio of either mobile phase:1xPBS or mobile phase:human serum for pharmaceutical and biological analysis, respectively. Another 1:1 (v/v) addition of ACN into both the non-serum (mobile phase:1xPBS) and serum standards created final ciprofloxacin standards of 0.25 µg/mL, 0.5 µg/mL, 1 µg/mL, 2 µg/mL, 5 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL and 100 µg/mL. The ACN was added to promote protein precipitation. The standards were vortexed for 30 seconds and centrifuged at 12,000×g for 10 minutes. The non-serum standards and the supernatant of the serum standards were filtered through 0.20 µm PVDF syringe filters (4mm; Thermo Scientific, TN) before HPLC analysis.

All standards were processed through HPLC using a gradient elution profile. The aqueous mobile phase started at 100%, decreased to 70% over 3 minutes, and further transitioned to 100% ACN over another 35 minutes, followed by 10 minutes of column washing with ACN and 5 minutes of column equilibrium with the aqueous mobile phase. All HPLC analyses were performed with an Agilent 1260 Quaternary

HPLC Pump, Agilent 1260 Infinity Programmable Absorbance Detector, and Agilent ChemStation software for LC system (Palo Alto, CA).

Drug release from the polymeric prodrugs

The drug release studies were performed in 1xPBS and human serum at 37°C at a polymer concentration of 6 mg/mL. Sample aliquots were collected at designated time points and stored at -80°C until the samples were ready for analysis. Right before HPLC analysis, the samples were treated with a 1:1 (v/v) dilution using the aqueous mobile phase, followed by another 1:1 (v/v) dilution using ACN. The resulting samples were vortexed for 30 seconds and centrifuged at 12,000×g for 10 minutes. The 1xPBS samples and the supernatant of the serum samples were filtered through the 0.20 µm PVDF syringe filters before HPLC analysis.

The total weight percent of ciprofloxacin in each polymeric prodrug was measured by dissolving the polymer in 10% aqueous H_2SO_4 for 48 hrs at room temperature. The 100% release samples were processed differently from the drug release samples collected at designated time points. The 100% release samples were diluted with a 1:9 (v/v) ratio of 10% aqueous H_2SO_4:aqueous mobile phase, followed by a 1:1 (v/v) dilution with 1xPBS and another 1:1 (v/v) dilution with ACN. After these processing, the ciprofloxacin concentration was diluted 40x. The processed samples were then vortexed and filtered through the 0.20 µm PVDF syringe filters before HPLC analysis.

Quality controls were included in each HPLC sequence run to make sure that the standard curves could accurately predict the amount of released ciprofloxacin and that no drift in the elution profile occurred. The quality controls were prepared separately from the standards. A stock solution of ciprofloxacin was prepared in the aqueous mobile phase at 6 mg/mL. A secondary series of stock solutions was diluted from the 6 mg/mL stock solution using the aqueous mobile phase to concentrations of 1.5 mg/mL and 150 µg/mL. Working solutions of ciprofloxacin for quality controls were then made by dilution using the aqueous mobile phase to a concentration of 3 µg/mL from the 150 µg/mL stock solution, and to concentrations of 15 µg/mL and 8 µg/mL from the 1.5 mg/mL stock solution. Each working solution was subsequently diluted with a 1:1 (v/v) ratio of either mobile phase:1xPBS or mobile phase:human serum. Another 1:1 (v/v) addition of ACN into both the non-serum and serum quality controls created final ciprofloxacin quality controls of 0.75 µg/mL, 3.75 µg/mL and 37.5 µg/mL. The quality controls were

vortexed for 30 seconds and centrifuged at 12,000×g for 10 minutes. The non-serum quality controls and the supernatant of the serum quality controls were filtered through the 0.20 µm PVDF syringe filters before HPLC analysis.

The same HPLC settings as with the ciprofloxacin standards were used for all drug release, 100% release and quality control samples. The percent release of ciprofloxacin from the polymeric prodrugs was quantified by the following equation, where the amount of the drug was calculated from the standard curves based on the area under the curve (AUC) of the ciprofloxacin peak.

$$\text{Percent release} = \frac{\text{drug released at a certain time point} - \text{drug present at time zero}}{\text{total amount of drug on the polymer}}$$

Results and Discussion

Characterization of the polymeric prodrugs

Poly(O950) (poly. # 1), poly(O950-*b*-HBC) (poly. # 2), poly[O950-*b*-(HBC-*co*-MAA)] (poly. # 3), poly(O950-*co*-CTM) (poly. # 4) and poly[(O950-*co*-CTM)-*b*-(O950-*co*-HBC)] (poly. # 5) were synthesized *via* RAFT polymerization. The gel permeation chromatography (GPC) traces of the polymeric prodrugs before removal of the protecting Boc groups were shown in **Figure 2**, and the number-averaged

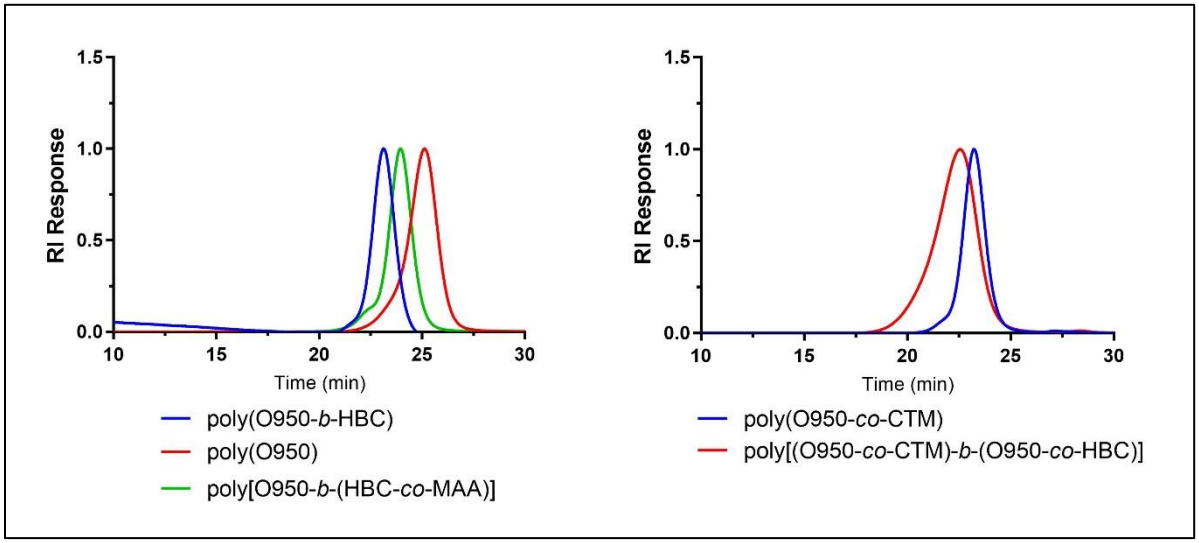


Figure 2. The gel permeation chromatography (GPC) traces of the polymeric prodrugs before removal of the Boc protecting groups on the ciprofloxacin residues.

molecular weight (M_n) and polydispersity ($Đ$) obtained from GPC were shown in **Table 1**. With the addition of a second block, the elution time shifted to earlier time points, confirming the successful

synthesis of the diblock copolymer. All the polymers were relatively narrowly-dispersed based on the *Đ* values.

Table 1. The number-averaged molecular weight (M_n) and polydispersity (*Đ*) obtained from GPC analysis of the polymeric prodrugs.

Poly. #	M_n (kDa)	*Đ*
1	13.3	1.10
2	51.8	1.18
3	26.7	1.10
4	22.5	1.12
5	48.2	1.28

Proton nuclear magnetic resonance (^{1}H-NMR) was also measured, and the successful synthesis of each polymeric prodrug was confirmed. Example ^{1}H-NMR spectra of poly[O950-*b*-(HBC-*co*-MMA)] and poly[(O950-*co*-CTM)-*b*-(O950-*co*-HBC)] were shown in **Figure 3 and Figure 4**. Key resonances included the 3H O950 methoxy residues at δ = 3.4 ppm and the 1H in the 1-cyclopropane-4-pyridone residues on the ciprofloxacin moiety at δ = 8.3 ppm. The absence of the sharp singlet at δ = 1.5 ppm confirmed successful removal of the Boc protecting group (9H) on the ciprofloxacin moiety. The ^{1}H-NMR spectra of poly(O950), poly(O950-*co*-CTM) and poly(O950-*b*-HBC) and were shown in **Figure S1**, **Figure S2** and **Figure S3**, respectively.

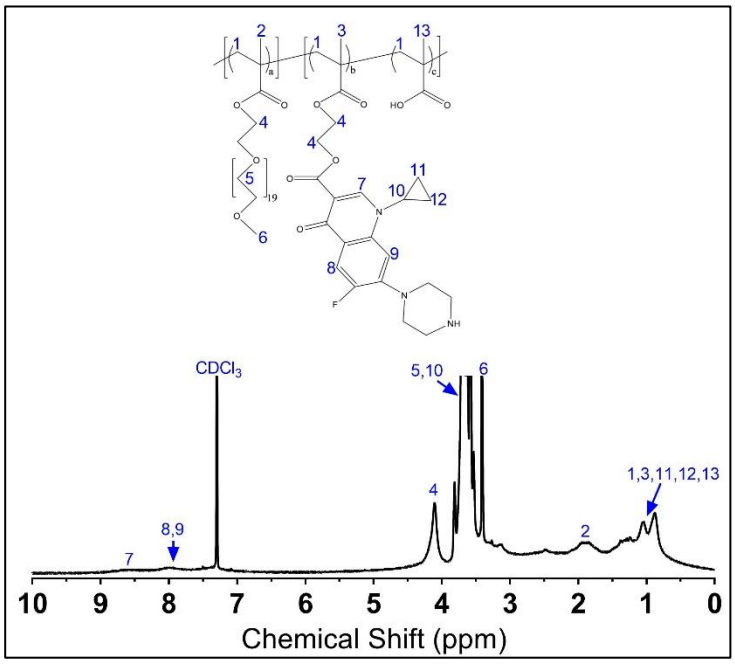


Figure 3. The ^{1}H-NMR spectrum of poly[O950-*b*-(HBC-*co*-MAA)] in $CDCl_3$.

Release kinetics of ciprofloxacin from the polymeric prodrugs quantified by HPLC

In HPLC analysis, the free ciprofloxacin eluted at approximately 5.6 min. The free ciprofloxacin standard curves in 1xPBS and in human serum were shown in **Figure 5**, with the linear regression equations as follows.

$$\text{AUC (1xPBS)} = 10.15 \times [\text{Cipro}]$$

$$\text{AUC (Serum)} = 10.23 \times [\text{Cipro}]$$

The total amount of ciprofloxacin in each polymeric prodrug was quantified by 100% release in 10% H_2SO_4 over 48 hrs, and the results were shown in **Table 3**.

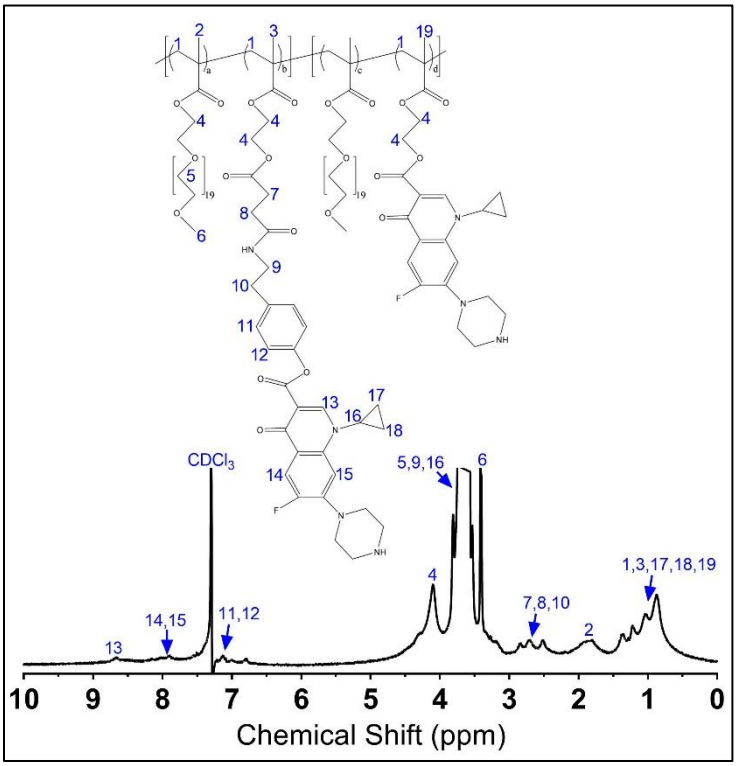


Figure 4. The ^{1}H-NMR spectrum of poly[(O950-*co*-CTM)-*b*-(O950-*co*-HBC)] in $CDCl_3$.

Table 3. The weight percent of ciprofloxacin in each polymeric prodrug as determined by the 100% release study.

Poly. #	2	3	4	5
Cipro wt%	38.0	16.5	15.3	13.7

The release kinetics of ciprofloxacin from poly(O950-*b*-HBC) in comparison with poly[O950-*b*-(HBC-*co*-MAA)] was shown in **Figure 6**. The release profiles for both polymers in 1xPBS and in human serum conformed to near-zero order, suggesting that the diffusion of water molecules was the rate-determining step in alkyl ester hydrolysis of the polymers due to the low dielectric environments within the polymer backbones[9,11]. Comparing the ciprofloxacin release rate in 1xPBS to that in human serum for either polymer, it can

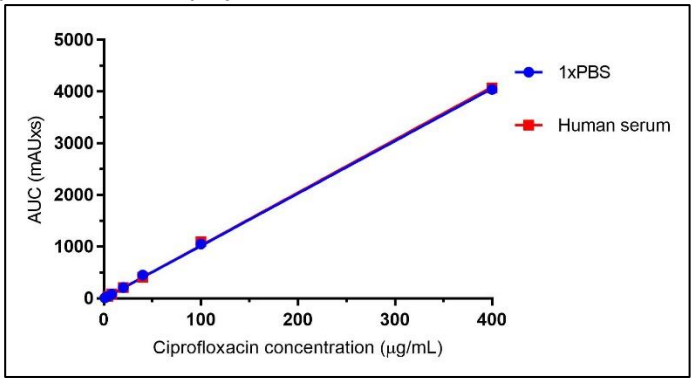


Figure 5. The standard curves of AUC at 277 nm versus the ciprofloxacin concentration in 1xPBS and human serum.

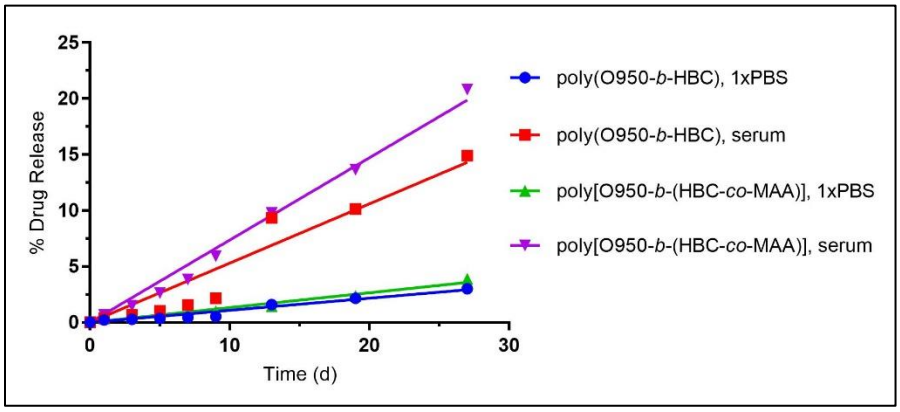


Figure 6. The release kinetics in 1xPBS and human serum over time for poly(O950-*b*-HBC) and poly[O950-*b*-(HBC-*co*-MAA)].

be concluded that the hydrolysis of alkyl esters was faster in human serum than in 1xPBS, most likely due to the presence in serum of enzymes such as esterase, which lowered the activation energy of the hydrolysis and sped up the reaction. In 1xPBS and in human serum, the ciprofloxacin was released more slowly from poly(O950-*b*-HBC) than from poly[O950-*b*-(HBC-*co*-MAA)]. One possible reason of such observation was that the presence of the carboxylic acid functional group in the polymerized methacrylic acid residue afforded more hydrophilicity to the hydrophobic core of the block copolymer compared to poly(O950-*b*-HBC). As a result, water molecules could access the alkyl ester bonds more easily, and the bonds were cleaved more readily.

Das et al.[9] demonstrated that phenyl esters hydrolyzed more rapidly than alkyl esters for both CPM and HBC monomers and O950 copolymers and diblock polymers synthesized from these monomers, because the phenoxide leaving group in CPM (and similarly in CTM) was resonance-stabilized[12] (2016). They also showed that diblock polymers underwent ester hydrolysis more slowly than copolymers, because diblock polymers formed micelles, with O950 in the corona and ciprofloxacin in the core, which reduced water access in comparison to unimerically soluble copolymer chains[9]. In this study, however, the ciprofloxacin release rate in human serum from poly(O950-*co*-CTM) was higher than that from poly(O950-*b*-HBC) in the first six days and was lower afterwards, as shown in **Figure 7**. Specifically, the percent ciprofloxacin release in serum at Day 27 was 6.9% for poly(O950-*co*-CTM) and 14.9% for poly(O950-*b*-HBC). Similar unexpected results were observed for poly[(O950-*co*-CTM)-*b*-(O950-*co*-HBC)]. For this polymer, a two-stage release of ciprofloxacin was expected, a fast release attributed to the CTM block followed by a slow release from the HBC block. Nonetheless, not only were the two-stage release not observed, but its percent release in serum at Day 27 was only 8.8%, lower than that of poly(O950-*b*-HBC).

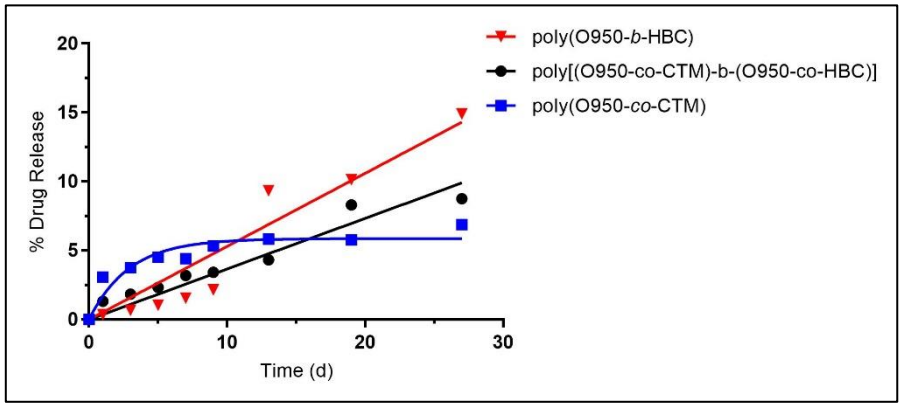


Figure 7. The release kinetics in human serum over time for poly(O950-b-HBC), poly(O950-*co*-CTM) and poly[(O950-*co*-CTM)-*b*-(O950-*co*-HBC)].

Due to the unexpected release trends observed for poly(O950-*co*-CTM) and poly[(O950-*co*-CTM)-*b*-(O950-*co*-HBC)], another preliminary study was performed on poly(O950-*co*-CTM) using a different aqueous mobile phase. In this preliminary study, as described elsewhere[9,13], 2% aqueous acetic acid and acetonitrile (84:16, v/v) was used as the aqueous mobile phase, and the gradient elution profile was altered to transitioning from 100% aqueous mobile phase to 100% acetonitrile over 15 minutes, followed by 10 minutes of column washing with acetonitrile and water and 5 minutes of column equilibrium with the aqueous mobile phase. Three time points—time zero, Day 1 and Day 2—were collected for the ciprofloxacin release from poly(O950-*co*-CTM) in human serum, and the 100% release was again quantified for the polymer. The percent release for Day 1 and Day 2 was determined to be 7.3% and 15.5%, respectively, as expected from previous observations[9].

Given those results, one factor that might have contributed to the differences in quantification might be that the higher percentage of ACN in the new mobile phase increased the hydrophobicity of the mobile phase. This might have changed the charge states of ciprofloxacin and other molecules present in the release samples, and thus the intermolecular interactions among them, which in turn resulted in quantification of ciprofloxacin release by HPLC that deviated from expected levels.

Conclusions

Based on the comparisons of the release kinetics of poly(O950-*b*-HBC) and poly[O950-*b*-(HBC-*co*-MAA)], it could be concluded that the dilution of the core of the micelles formed by the diblock copolymers with a hydrophilic moiety increased the release rates of ciprofloxacin from the polymeric prodrugs, possibly because of easier water access to the sites of hydrolysis. However, the effects of including both the faster-releasing and the slower-releasing linkers in the same polymer could not be determined yet, due to

the unexpected levels of ciprofloxacin release from CTM polymers as quantified using H_2O/ACN/acetic acid (94:5:1, v/v/v) as the mobile phase in HPLC. With the preliminary study on poly(O950-*co*-CTM) using 2% aqueous acetic acid and acetonitrile (84:16, v/v) as the new mobile phase giving more reasonable levels of ciprofloxacin release, the drug release from the CTM polymers will need to more accurately quantified using the new HPLC method before any conclusions can be drawn.

References

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Supplementary Information

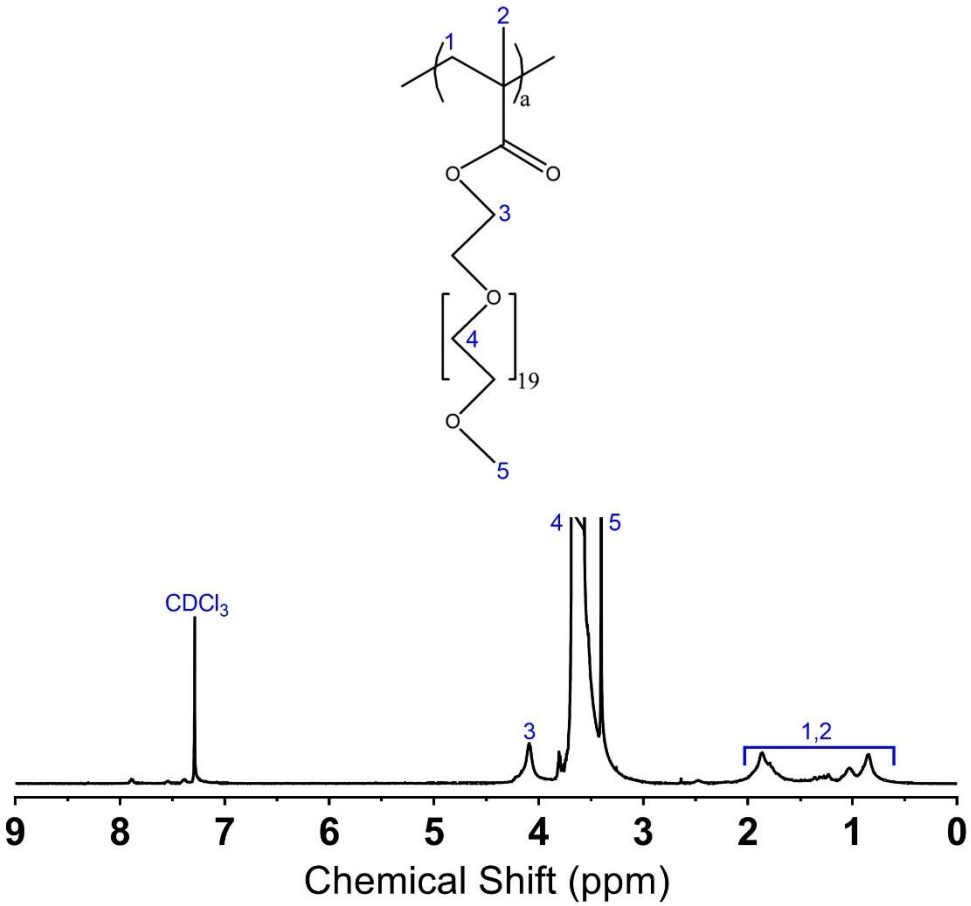

Figure S1. The ^{1}H-NMR spectrum of poly(O950) in $CDCl_3$.

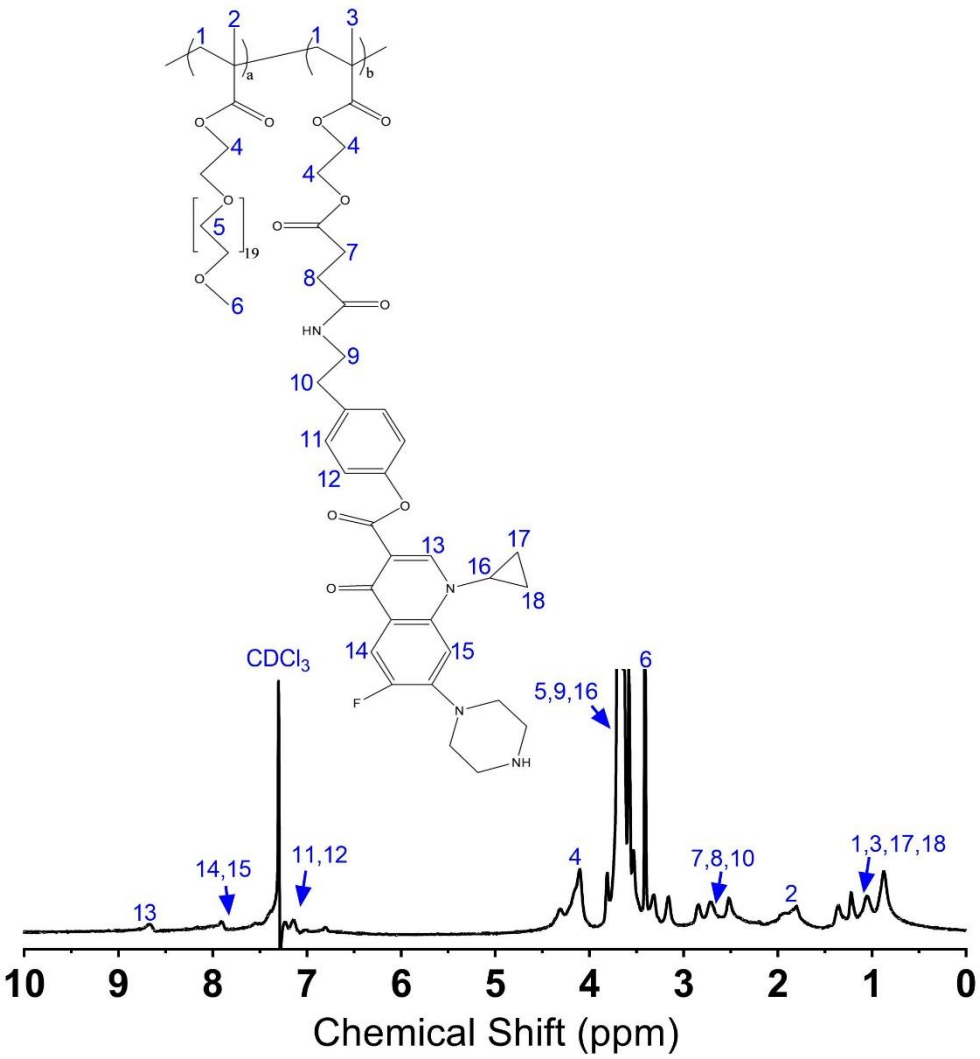

Figure S2. The ^{1}H-NMR spectrum of poly(O950-*co*-CTM) in $CDCl_3$.

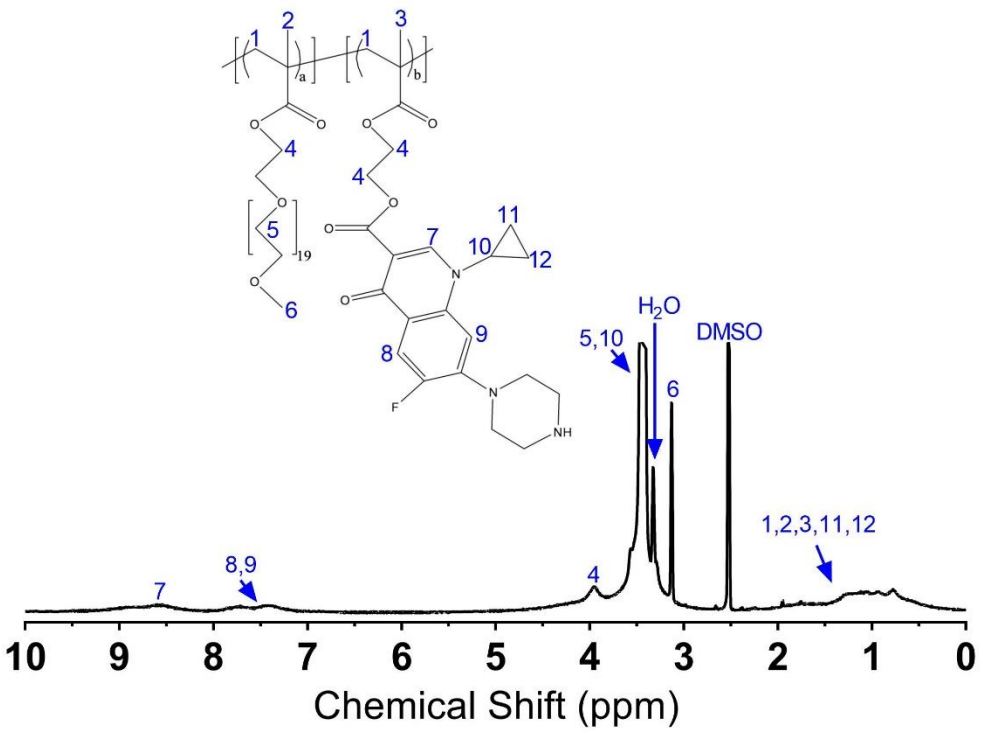

Figure S3. The ^{1}H-NMR spectrum of poly(O950-*b*-HBC) in DMSO-d_6/TFA (50:50, v/v).