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Phenylboronic Acid-Installed Polycarbonates for the pH-Dependent Release of Diol-Containing Molecules

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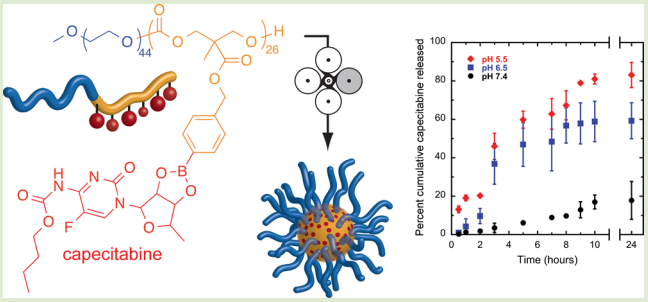
Supporting Information

ABSTRACT: Environmental responsiveness is an appealing trait of emerging polymeric materials, as shown for a variety of pH-responsive drug delivery systems. The chemical versatility of the conjugation site and conjugate lability to physiologically relevant changes in pH will largely determine their applicability. Herein, we report on the use of a drug−polymer complex based on boronic acid-functionalized polycarbonates (PPBC) as the substrate for the pH-sensitive delivery of a diol-containing drug, capecitabine (CAPE). Complexation of CAPE with a PEGylated-PPBC block copolymer, via boronic ester formation, resulted in amphiphiles capable of self-assembling into spherical nanoparticles. We examined nanoparticle stability and release kinetics in neutral and acidic media and relate differences in release profiles and particle stability with changes to polymer chemistry. Comparison of complexed nanoparticles with their noncomplex analogues revealed striking differences in release rate and particle stability. Illustrated herein for capecitabine, the pH-sensitive dissociation of boronate esters from PPBCs can be applied in a general manner to diol- or catechol-containing solutes, demonstrating the utility of these polymers for biomedical applications.



Environmental responsiveness is an appealing trait of emerging polymeric materials. The chemical versatility of synthetic polymers can be harnessed to trigger physicochemical changes in response to stimuli such as temperature, light, enzymatic activity, redox potential, and pH.[1,2] Among these, pH is a common chemical triggering mechanism for biomaterials applications due to microenvironmental differences in pH at the organ, tissue, or intracellular levels. For example, differences between extracellular pH and those existing in the endosomal (5.5−6.0) and lysosomal (4.5−5.0) compartments have been used to activate the occurrence of events resulting in the delivery of a therapeutic agent.[3−6] For polymer-based drug carriers, the susceptibility to effect release under acidic conditions can be programmed to occur by matrix degradation or, as in the case of polymer−drug conjugates, through hydrolysis of the linker or conjugation site.[7−11] The most prevalent examples of functional groups exhibiting high sensitivity to acidic conditions include acetals, esters, hydrazones, and orthoesters.[2,12]

Boronic ester derivatives comprise a class of reversible complexes exhibiting high pH sensitivity.[4,5] The growing interest for organoboron polymers in biomaterials applications stems from their capacity to reversibly bind to 1,2- or 1,3-diols and catechol-containing molecules, their reactivity toward H_2O_2, and the delicate effect of molecular structure on boronic acid pK_a.[13−19] We have recently reported on the synthesis of polycarbonates installed with boronic acids (PPBCs).[20] Functional polycarbonates have been studied for their use as drug delivery agents in the form of micelles or nanoparticles of well-controlled size and core properties.[21−23] Although a large variety of functional polycarbonates are known,[21,24−27] only a few of these have been involved in the formation of polymeric prodrugs.[28−30]

Herein, we discuss the use of amphiphilic PPBC copolymers as carriers for a 1,2-diol-containing molecule and its triggered release under physiologically relevant acidic conditions. Illustrated for capecitabine (CAPE), the pH-sensitive dissociation of boronate esters from PPBCs can be applied in a general manner to other diol- or catechol-containing solutes, demonstrating the utility of these polymers for biomaterials applications.

When catalyzed by the superbase DBU, derivatives of trimethylene carbonate containing pendant boronic esters are polymerized to high monomer conversion (∼70%) to yield polymers with controlled molecular weight and narrow polydispersity (<1.1).[20] The hydrophobic nature of the carbonate backbone enabled block copolymers of PPBCs and poly(ethylene glycol) (PEG) to self-assemble by hydrophobic association into, for the particular monomer ratios examined, spherical aggregates. In this work, we focus on the pinacol-protected PEG_{44}-*b*-$PPBC_{26}$ (Figure 1, **1**) and its deprotected analogue (**2**); subscripts represent repeat units. Capecitabine (CAPE, 5′-deoxy-5-fluoro-*N*-[(pentyloxy)carbonyl]cytidine), the model 1,2-diol-containing molecule and an orally administered fluoropyrimidine carbamate precursor to 5-

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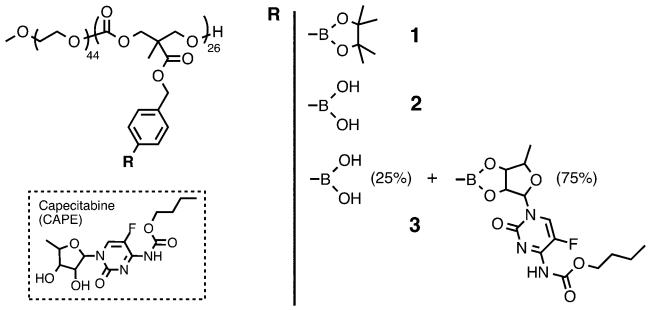

Figure 1. Boronic acid-installed polycarbonate amphiphiles used in this study: pinacol-protected (**1**), deprotected (**2**), and complexed with capecitabine to 75% efficiency (**3**). The structure of CAPE is provided for reference (inset).

fluorouracil, was complexed onto **2** via CAPE *cis*-diols to yield **3**. Boronate ester formation was performed under anhydrous conditions, and the extent of esterification could be modulated according to the amount of CAPE added. Following purification by dialysis against methanol to remove unreacted CAPE, conjugation efficiency was calculated by a peak integration ratio of −C*H*−O− protons of CAPE at δ = 4.79 ppm and C*H*-benzylidene protons of the pendant boronic acid groups at 7.7 ppm (Figure S1, Supporting Information). For instance, addition of 1.5 equiv of CAPE/boronic acid resulted in a grafting efficiency of 75% (Figure 1, **3**), whereas 3 equiv led to 100% grafting. All results discussed herein, except GPC, were carried out for the polymer with 75% conjugation efficiency.

GPC traces of the polymer with 100% complexation were monomodal with a molecular weight distribution identical to that of the parent (protected) copolymer **1** but with a small shift to shorter elution times (Figure S2, Supporting Information). This difference is attributed to the higher molecular weight of CAPE compared to the pinacol protecting group. At 75% grafting, there is an average of 20 CAPE units per chain, which corresponds to approximately 44% of the total mass of the copolymer. The appearance of the characteristic signal of CAPE at 305 nm was observed for **3** by UV/vis spectrometry (Figure S3, Supporting Information).

Nanoparticles of block copolymer amphiphiles **1** and **3** (NP-**1** and NP-**3**, respectively) were formed by a rapid change in solvent quality inside a multi-inlet vortex mixer. In this process, aggregate size and size dispersity vary according to solvent:nonsolvent ratio and mixing velocity.[31] Nanoparticles of free CAPE stabilized by **1** were also produced, to contrast the effect of complexation on particle stability and solute loading; these will be denoted as (CAPE)NP-**1**. To ensure that formation of NP-**3** occurred under conditions of high supersaturation of the polymer, we first determined the critical micelle concentration (C_{CMC}) of **3** by pyrene fluorescence (Figure S4, Supporting Information). According to the data, grafting CAPE onto the backbone of **2** had no appreciable impact on the C_{CMC} of the copolymer (3.69 vs 3.64 μg/mL),[20] which we attribute to the high solubility of CAPE in water (S_w = 26 mg/mL), despite the fact that the polar *cis*-diol is now part of the boronate ester. The C_{CMC} of **1** (1.45 μg/mL) was nearly half that of **3**. Nevertheless, the relatively low C_{CMC} of **3** is important in that its aggregates are expected to remain stable despite dilution, for example, upon systemic circulation.

As measured by DLS, replacing pinacol with CAPE results in a decrease in particle size of ∼13 nm, with no major differences in particle size dispersity (Table 1). We attribute this to CAPE

Table 1. Properties of the Nanoparticles Used in This Study

nanoparticles[a]	D_h (nm)[b]	PDI[b]	DLC[c,d] (%)	DLE[c,d] (%)
NP-**1**	34.7	0.19		
NP-**3**	21.2	0.15	25.0	64.3
(CAPE)NP-**1**	34.1	0.09	21.5	27.5

[a]NP-**1** and **3** refer to nanoparticles formed by the self-assembly of **1** and **3**, respectively. [b]Determined by dynamic light scattering. [c]Estimated by UV/vis spectroscopy. [d]Drug loading capacity (DLC) and efficiency (DLE) are defined as DLC (%) = (mass of drug in nanoparticles)/(total mass of drug used) × 100 and DLE (%) = (mass of drug in nanoparticles)/(total mass of drug used) × 100.

complexation, which lessens the hydrophobic character of the polycarbonate block. This results in lower interfacial core tension and hydrophobic/hydrophilic ratio, both of which lead to smaller nanoparticles. While no considerable effect was observed for average particle size upon addition of CAPE to the precipitating solution of **1** (34.1 vs 34.7 nm), the presence of the solute does appear to effect narrowing of the particle size distribution.

Drug loading capacity (DLC) and efficiency (DLE) were measured for NP-**3** and (CAPE)NP-**1** (Table 1). The difference between the observed and theoretical values for DLC and DLE of the former (DLC_{theo} = 44% and DLE_{theo} = 100%) is likely due to dissociation of the boronic ester during extended dialysis (12 h) used to remove the organic solvent from the mixture since the pH of the water used was not controlled and fluctuated slightly between 6.7 and 7.4. Formation of the boronate ester did, nevertheless, greatly improve both DLC and DLE compared to particles prepared using the free solute, (CAPE)NP-**1**. In this case, the values obtained differed greatly from the theoretical ones (DLC_{theo} = DLE_{theo} = 100%). The rapid precipitation method used in this work generally results in nanoparticles with high solute loading, the premise being that the solute possesses a strong hydrophobic character and is present under conditions of high supersaturation. Therefore, it is not surprising that both the DLC and DLE of (CAPE)NP-**1** were far below their theoretical values since the solubility of CAPE in water is high (26 mg/mL) and the solute was present at a concentration below its supersaturation limit during precipitation. Even if nanoprecipitation of free CAPE had occurred under conditions of high supersaturation, it is expected that this solute would not form stable nanoparticles as the clogP of CAPE is low (1.38, as calculated by molinspiration, www.molinspiration.com). As shown by Macosko and Prud'homme, stable nanoparticles are formed by solutes with clogP values greater than 7.[32]

Boronic acid-diol/catechol complexes are formed through dynamic covalent chemistry with high pH-dependent reversibility.[4,5] However, under acidic conditions, polymer **1** (or **3**) may also exhibit reactivity at the carbonate and/or ester groups. To examine the changes in polymer structure under such an environment, NP-**1** was incubated in phosphate buffer saline (10 mM) at pH 5.5, the lowest pH used in this study. Nanoparticles were then dialyzed against water to remove salts and lyophilized; the resulting polymers were dissolved in $CDCl_3$ for characterization by ^{1}H NMR (Figure 2). After 6 h of incubation, of the three susceptible groups, only the boronate ester underwent dissociation, observed by a decrease of pinacol methyls at 1.25 ppm. Signals from the carbonate (4.25 ppm) and the ester (5.1 ppm) remained unchanged during this time. The decrease of the pinacol signal is attributed to dissociation,

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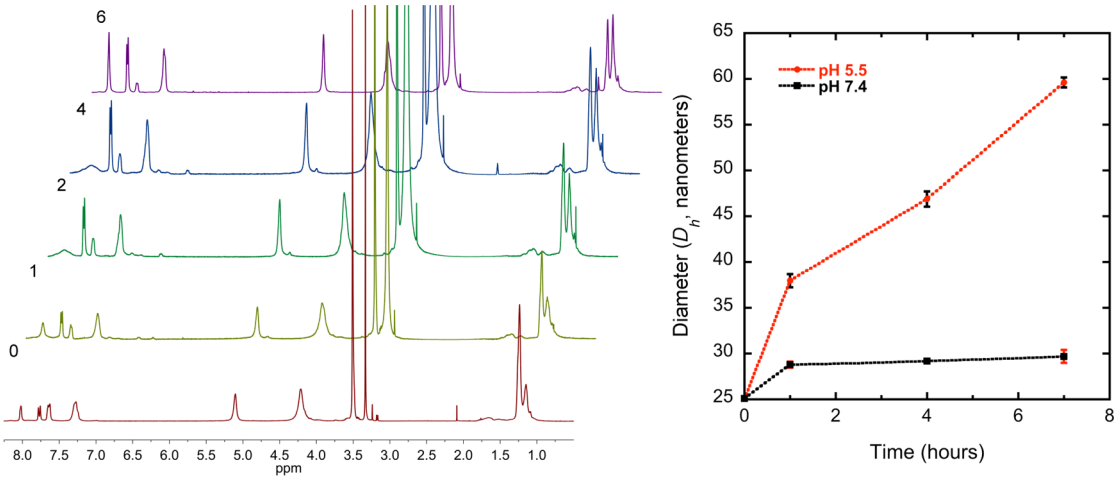

Figure 2. (**A**) ^{1}H NMR of NP-**1** incubated in PBS 10 mM at 37 °C and pH 5.5; incubation times shown. Signals from the ester, carbonate, and pinacol appear at 5.1, 4.25, and 1.25 ppm, respectively. (B) Stability of NP-**1** in neutral and acidic media.

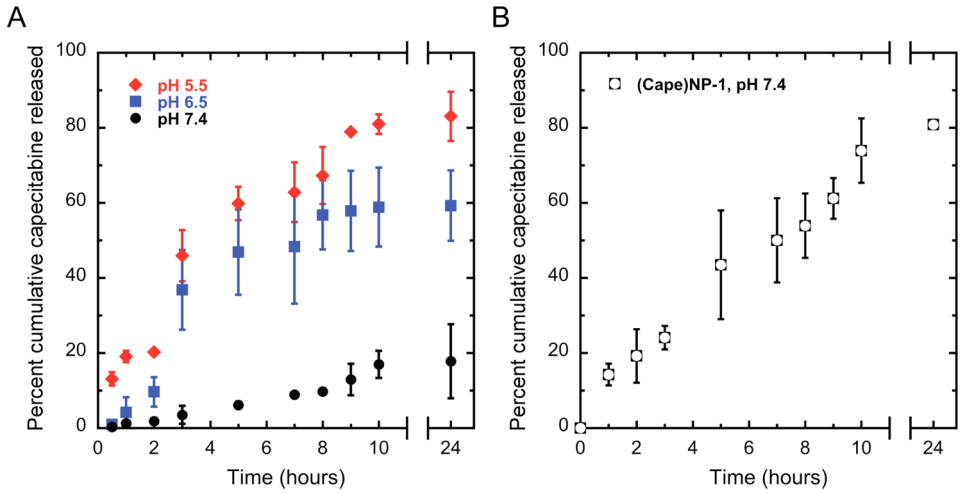

Figure 3. (A) pH-dependent release of CAPE from NP-**3**, showing the susceptibility of boronic ester dissociation under biologically relevant acidic conditions. (B) CAPE release at pH 7.4 for free drug stabilized by **1** ((CAPE)NP-**1**).

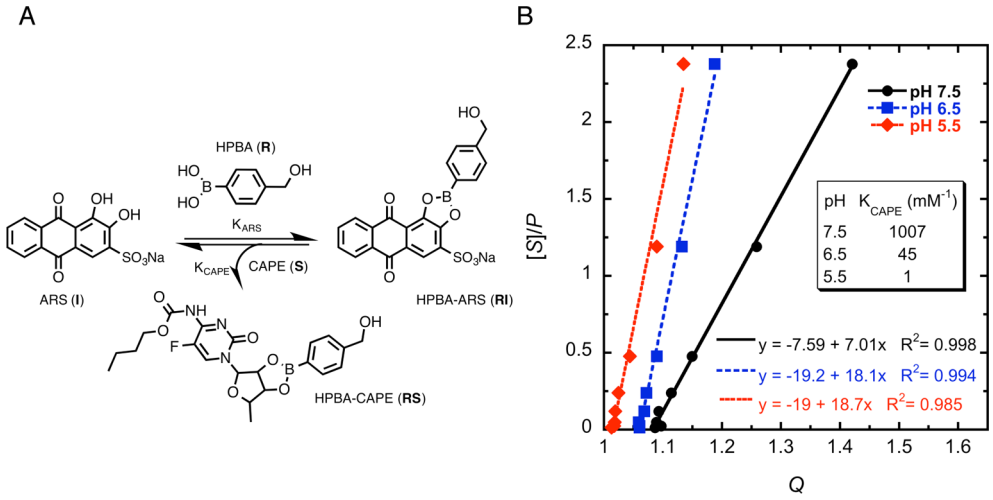

Figure 4. (**A**) Binding studies of the ternary system consisting of alizarin red (ARS, **I**), 4-(hydroxymethyl)phenylboronic acid (HPBA, **R**), and capecitabine (CAPE, **S**). (B) Binding constants for the HPBA−CAPE complex (K_{CAPE}) were calculated by dividing the value of the K_{ARS} by the slope of the $[S]/P$ vs Q plot (B), according to the Benesi−Hildebrand method, for the corresponding pH.[35,36] A summary table of K_{CAPE} is provided.

followed by partitioning from the particle core to the aqueous phase, as the concentration of pinacol used for this experiment is below its solubility limit. Dissociation of **1**, therefore, results in the boronic acid-functionalized polycarbonate (**2**) and free

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pinacol. This is important in the case of drug−polymer conjugates, as it demonstrates that the diol/catechol-containing drug would be released in its free form.

Boronate ester dissociation also impacted nanoparticle properties, as confirmed by DLS. The average size of NP-**1** increased slightly (∼14%) after ∼1 h of incubation in PBS at pH 7.4 and remained constant thereafter (Figure 2). However, in an acidic medium, average particle diameter progressively increased from 25 to 59 nm over 7 h. Given that under these conditions the boronate ester is the only reactive functional group, the increase in size is attributed to displacement of the pinacol protecting group, rendering the core more hydrophilic and susceptible to swelling.

Release profiles of the pH-dependent dissociation of CAPE−polymer conjugates (**3**) were measured at pH 7.4, 6.5, and 5.5 (Figure 3). CAPE solubility was also measured in PBS for the three conditions examined; the solubility dropped from 22.4 to 19.6 mg/mL as the pH decreased from 7.4 to 5.5 (Table S1, Supporting Information). These values, however, are considerably higher than the conditions used to measure release (∼0.4 mg/mL), so CAPE solubility should not be considered a limiting parameter. We report the sum of released CAPE at a specific time divided by the amount of loaded drug or its cumulative release. For a 24 h incubation period, 17%, 58%, and 85% of CAPE was released at pH 7.4, 6.5, and 5.5, respectively. As shown by the kinetic profiles, boronate ester dissociation is highly pH sensitive within physiologically relevant conditions. Similar profiles had been observed for the opposite combination of reagents, i.e., boronic-acid-containing drugs bound to catechol-containing polymers.[5] Release kinetics from (CAPE)NP-**1** and NP-**3** were characterized by a linear profile over the first 10 h of incubation at pH 7.4 (Figure S5, Supporting Information). The difference in passive release rates between them (∼4×) is partially attributed to solute complexation; diffusion through the polycarbonate core may also influence the observed difference in release rates; however, we ignore the location of the solute within (CAPE)NP-**1**.

The sensitivity of the boronic acid−CAPE conjugate dissociation to pH was further assessed by competitive binding experiments (Figure 4). For this, we determined the equilibrium constant of the complex formed between 4-(hydroxymethyl)phenylboronic acid (HPBA, **R** in Figure 4) and CAPE (**S**) by competitive binding with alizarin red (ARS, **I**), a catechol dye whose fluorescence intensity increases upon binding to boronic acid (Figure S6, Supporting Information). We chose HPBA for these measurements as both **1** and **2** had limited solubility in water and because it is a precursor to the PBC monomer. Binding experiments for HPBA−CAPE (K_{CAPE}) were conducted at the three pH values used for prodrug release studies. Measured binding constants for HPBA−ARS (K_{ARS}, Figure S6, Supporting Information) were considerably lower than those reported for the complex formed between phenylboronic acid (PBA) and ARS. For instance, the binding constant for HPBA−ARS was K_{ARS} = 7054 mM^{-1}, a value considerably lower than that reported for PBA−ARS, with a K_{ARS} = 1300 M^{-1}. The striking difference can be explained by the presence of additional hydroxyl groups in HPBA and the buffer strength, both of which have been shown to affect binding constants.[33] It is expected, then, that binding between CAPE−PPBC would be stronger than that measured here for CAPE−HPBA. Nevertheless, as previously observed for other diol−boronic acid systems and as the release studies indicate, CAPE exhibits increasing binding affinity toward HPBA with pH, as shown in Figure 4. The exponential decrease of K_{CAPE} with pH (Figure S6, Supporting Information), previously observed for other model diol−boronic acid combinations (e.g., fructose, glucose, and galactose),[34] along with the high solubility of the drug throughout the pH range examined serve to explain the stability of NP-**3** at physiological pH and rapid release under acidic conditions.

Suspensions of NP-**3** also showed differences during incubation under neutral and acidic conditions. Particle suspensions appeared to be stable at pH 7.4, whereas incubation at pH 5.5 resulted in a gradual increase of sample turbidity over time; TEM images reveal the formation of large nanoparticle aggregates (Figure S7, Supporting Information). These differences are likely caused by dissociation of the boronic ester and diffusion of CAPE from the core of the particles, increasing core hydrophilicity and possibly destabilizing nanoparticle suspensions. While hydrolysis of the boronate ester occurs on exposure of either NP-**1** or NP-**3** to acidic media, the latter appears to result in greater particle destabilization, possibly a consequence of the fact that CAPE represents a higher fraction of the polymer conjugate compared to pinacol.

Organoboron polymers offer ample possibilities for the design of responsive biomaterials because of their capacity to reversibly bind to diols and catechol-containing molecules and the sensitivity of boronate esters to subtle changes in pH. We have examined the use of a boronic acid functionalized polycarbonate (PPBC) for the pH-triggered release of a diol-containing molecule, capecitabine. Facile complexation between CAPE and PEG-*b*-PPBC copolymer resulted in the formation of an amphiphilic conjugate, which readily self-assembled into spherical nanoparticles. NMR studies revealed a selective reactivity of the boronate ester in PPBCs, while capecitabine release studies and competitive binding experiments confirmed the pH-dependent sensitivity of capecitabine conjugates under physiologically relevant acidic conditions. The facile loading and release sensitivity provided by complexes of diols and boronic acids via PPBCs can be generalized for the pH-triggered delivery of solutes containing diols or catechols, demonstrating the utility of these polymers for biomaterials applications.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures including GPC, UV/vis spectra, C_{CMC} measurement, release kinetics, TEM, and binding study plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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