Supplementary Information for:

Hemiaminal ether Linkages Provide a Selective Release of Payloads from Polymer Conjugates

Farzad Seidi, Victor Druet, Nguyen Huynh, Treethip Phakkeeree, Daniel Crespy*

Department of Material Science and Engineering, School of Molecular Science and Engineering, Vidyasirimedhi Institute of Science and Technology (VISTEC), Rayong 21210, Thailand

**E-mail: daniel.crespy@vistec.ac.th*

Experimental section

Materials

n-Butyl methacrylate (BMA, 99%, TCI) and 2-hydroxyethyl methacrylate (HEMA, >95%, TCI) were purified by vacuum distillation before using. Methacryloyl chloride was synthesized following a reported method for the synthesis of acryloyl chloride.[S1] A mixture of methacrylic acid and benzoyl chloride (1:2 molar ratio) was distilled at 100 mbar at a temperature range of 70-95 °C and further purified by two times by re-distillation at 200 mbar at 50 °C. Methyl methacrylate (MMA, 99%, Merck) was purified by vacuum distillation before use. 1,4-Butanediol vinyl ether (99%, Aldrich), 1,1'-azobis(cyclohexanecarbonitrile) (ABCN, 98%, Sigma-Aldrich), triethyl amine (99.5%, Carlo Erba), benzotriazole (BTA, 99%, Acros), trifluoroacetic acid (TFA, 99%, Acros), 1,2-dimethoxyethane (99%, Acros), 1,4-dioxane (99.8%, Carlo Erba), dichloromethane (DCM, 99.9%, Carlo Erba), diisopropyl ether (99%, Carlo Erba), chloroform-D (99.8%, Cambridge Isotope Laboratories), and sodium dodecyl sulfate (SDS, 99%, Acros) were used as received without further purification.

Synthesis of 4-(vinyloxy)butyl methacrylate (VBMA)

In a 250 mL one neck round-bottom flask, 1,4 butanediolvinyl ether (10 g, 86.1 mmol, 1 eq) and triethylamine (10.1 g, 99 mmol, 1.15 eq) were dissolved in 150 mL dichloromethane. Then, the reaction mixture was cooled in an ice-bath. Methacryloyl chloride (9.455 g, 90.4 mmol, 1.05 eq) was added dropwise to the reaction mixture in an ice-bath. Afterwards, it was stirred for

another 2 h in an ice-bath for 3 h. The mixture was transferred to a separatory funnel and washed sequentially with distilled water (100 mL), saturated $NaHCO_3$ solution (100 mL), saturated NaCl (100 mL), and then dried over $NaSO_4$ and filtered. The solvent was removed with a rotary evaporator to afford a crude light yellow product that was purified by column chromatography on silica gel with a mixture hexane:dichloromethane (3:1) to provide 14.27 g (90% yield) of pure product as colorless liquid. ^{1}H-NMR (600 MHz, $CDCl_3$): δ 6.43 (m, 1H, -OC**H**=CH_2), 6.07 (d, 1H, C**H$_2$**=C), 5.52 (d, 1H, C**H$_2$**=C), 4.13-4.16 (m, 2H of -COOC**H$_2$**- & 1H of -OCH=C**H$_2$**), 3.95 (d, 1H, -OCH=C**H$_2$**), 3.69 (t, 2H, -C**H$_2$**OCH=CH_2), 1.91 (s, -C**H$_3$**), 1.71-1.77 (m, 4H, -C**H$_2$**C**H$_2$**-). ^{13}C NMR (600 MHz, $CDCl_3$): δ 18.2, 25.3, 25.7, 64.2, 67.2, 86.3, 125.1, 136.4, 151.7, 167.2.

Synthesis of 4-(1-(benzotriazole-N-yl) ethoxy)butyl methacrylate (BEBMA)

The preparation of BEBMA monomer was performed similar to a previously reported procedure with little modifications.[S2] In a two-neck round bottom flask, VBMA (10 g, 54.28 mmol, 1 eq) and BTA (6.72 g, 56.45 mmol, 1.04 eq) were dissolved in 22 mL 1,2-dimethoxyethane. Then, 0.1 mL of TFA was added to the reaction mixture and the reaction was continued at 50 °C for 3 h under nitrogen atmosphere. Then, 1-2 mL triethylamine was added to the reaction mixture to neutralize TFA and stop the reaction. The reaction mixture was transferred to the column chromatography and washed firstly with around 150 mL hexane (containing 1 mL TEA) and then with a mixture of hexane:dichloromethane (1:1) to provide 14 g (85% yield) of pure product as viscous colorless liquid. ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.03 (d, 1H, Ar), 7.74 (d, 1H, Ar), 7.45 (t, 1H, Ar), 7.35 (t, 1H, Ar), 6.21 (m, 1H, -C**H**(CH_3)-), 6.00 (d, 1H, C**H$_2$**=C), 5.49 (d, 1H, C**H$_2$**=C), 4.04 (t, 2H, -COOC**H$_2$**-), 3.48 (d, 1H, enantiotopic -C**H$_2$**-CH), 3.16 (d, 1H, enantiotopic -C**H$_2$**-CH), 1.83-1.87 (m, 6H, two -C**H$_3$**), 1.55-1.65 (m, 4H, -C**H$_2$**C**H$_2$**-). ^{13}C-NMR (600 MHz, $CDCl_3$): δ 167.2, 146.7, 136.2, 131.0, 127.4, 125.2, 124.1, 120.0, 110.9, 87.1, 68.2, 64.1, 25.8, 25.2, 20.9, 18.1. HRMS for $C_{16}H_{22}N_3O_3$: calculated 304.16 $[M+H]^+$, found 304.12.

Copolymerization of BEBMA with BMA and HEMA

BEBMA was copolymerized with BMA or HEMA with various ratios by free-radical polymerization. Generally, certain amounts of BEBMA, BMA, HEMA, and ABCN were dissolved in dioxane at room temperature (Table S1). The mixture was bubbled with nitrogen for 15 min and then placed in an oil bath at 90 ^{o}C under nitrogen atmosphere and the polymerization was continued for 24 h. The reaction mixture was cooled to room temperature and the product was precipitated in 100 mL hexane containing 4-5 drops of triethylamine. The obtained polymer was purified by redissolution in dichloromethane and reprecipitation in hexane containing few drops of triethylamine. Redissolution/reprecipitation cycles were repeated four times more to remove all traces of unreacted monomers. Finally, the product was dried under high vacuum to provide the related copolymers as white solids. Compositions of the polymers were determined from their ^{1}H-NMR spectra in $CDCl_3$.

Table S1. Conditions for radical copolymerization of BEBMA with BMA and HEMA.

Entry	Monomer [g]			ABCN [mg]	Dioxane [mL]	Feed ratio			Conv. [%]	Yield [%]
	BEBMA	BMA	HEMA			BEBMA	BMA	HEMA		
P(BMA$_{0.80}$-*co*-BEBMA$_{0.20}$)	1.1	2.06	0	180	16.5	1	4	0	95	88
P(BMA$_{0.86}$-*co*-BEBMA$_{0.14}$)	1.1	3.1	0	248	21	1	6	0	95	87
P(BMA$_{0.89}$-*co*-BEBMA$_{0.11}$)	1.1	4.1	0	319	26	1	8	0	93	80
P(BMA$_{0.57}$-*co*-HEMA$_{0.29}$-*co*-BEBMA$_{0.14}$)	1.1	2.06	0.95	248	21	1	4	2	92	90

Copolymerization of MMA with BMA

In a two-neck round bottom flask, 5 g (35.2 mmol) BMA, 0.88 g (8.8 mmol) MMA, and 0.05 g (0.2 mmol) ABCN were dissolved in 21 mL dioxane at room temperature and then the solution was bubbled with nitrogen for 15 min. Afterwards, reaction flask was placed in an oil bath at 90 ^{o}C under nitrogen atmosphere and the polymerization was continued for 24 h. Finally, two-third of dioxane was removed using rotary evaporator and the viscous solution was added to 100 mL of hexane to obtain a white gummy solid. The solid was separated and re-dissolved in 3 mL of

diethyl ether and then precipitated again in hexane. Finally, product was separated and washed two more times with pure hexane and then dried under high vacuum before corrosion tests.

Preparation of nanoparticles from polymer conjugates

Nanoparticles (NPs) were prepared with different polymer conjugates by the miniemulsion-solvent evaporation process.[S3] Copolymers (200 mg) were dissolved in 4 g DCM and then mixed with a solution of SDS (38 mg) in 12 g of a phosphate buffer (0.05 M, pH ~ 7.0). The mixture were stirred for 45 min at room temperature and exposed to ultrasonication for 300 s (10 s pulse, 5 s pause) at 50% amplitude using a Branson the sonifier SFX550 with a micro-tip (3 mm) in an ice-bath to produce miniemulsion. Finally, dichloromethane was removed with a rotary evaporator. Size and polydispersity in size of the NPs were determined by dynamic light scattering (DLS).

Table S2. Hydrodynamic diameters of the nanoparticles before and after release of BTA.

Copolymer precursors	D_h[a]		D_h[b]		D_h[c]		D_h[d]	
	[nm]	PDI	[nm]	PDI	[nm]	PDI	[nm]	PDI
P(BMA$_{0.80}$-*co*-BEBMA$_{0.20}$)	131	0.13	149	0.11	155	0.12	138	0.13
P(BMA$_{0.86}$-*co*-BEBMA$_{0.14}$)	130	0.11	138	0.12	142	0.12	138	0.14
P(BMA$_{0.57}$-*co*-HEMA$_{0.29}$-*co*-BEBMA$_{0.14}$)	188	0.13	185	0.11	188	0.11	189	0.08

[a] Before release; [b] after release at pH ~ 2.0; [c] after release at pH ~ 3.5; [d] after release at pH ~ 5.0.

Hydrolysis of the hemiaminal ether group of BEBMA at various pH values

To evaluate the hydrolytic stability of the hemiaminal ether bonds in the BEBMA monomer, 5 mg of BEBMA was mixed with 1 mL D_2O phosphate buffer (0.1 M) of various pH values (2.0, 3.5, 7.0) in an NMR tube. The degree of hydrolysis was calculated from ^{1}H-NMR spectra of aliquots taken at various time intervals by comparing the integrals of the alkene protons of BEBMA with the ones of 4-hydroxybutyl methacrylate (HBMA), which are produced upon hydrolysis of hemiaminal ether linkage.

Release of benzotriazole from the nanoparticles

λ_{max} of BTA in phosphate buffer solutions at pH 2.0, 3.5, and 7.0 was determined to be 252, 255, and 260 nm, respectively. Calibration curves at different pH values were prepared in the concentration range of 5-80 ppm (see Figure S7). The amount of the released BTA from polymer NPs was determined at different time intervals using these calibration curves.

For studying the release behavior from polymer NPs, 800 µL of the dispersions of polymer conjugates (16.5 $mg.mL^{-1}$) was placed in a dialysis tube (MWCO = 6,000 $g.mol^{-1}$) that was immersed in 8 mL phosphate buffer solution (0.05 $mol.L^{-1}$) at various pH values (2.0, 3.5, and 7.0). At various time intervals, 1 mL release medium was taken for UV measurements and 1 mL of fresh buffer solution containing SDS (~ 2 mM) was added to the release media to keep the total volume constant. UV absorbance of the solutions was measured at their determined λ_{max}.

Comparison of the hydrolysis rate of 1H- and 2H-isomers

A dispersion of nanoparticles (~ 12 mL) of P($BMA_{0.86}$-*co*-$BEBMA_{0.14}$) copolymer was prepared starting from 200 mg of the copolymer using the same procedure as we mentioned above in the "Preparation of nanoparticles from polymer conjugates" section. This nanoparticle dispersion was then mixed with phosphate buffer (80 mL, 0.05 M, pH ~ 2.5) and stirred at room temperature. At various interval times, 15 mL of the nanoparticle dispersion was taken and centrifuged at 9500 rpm for 30 min. The centrifuged nanoparticles were freeze-dried and their NMR were recorded after dissolution in $CDCl_3$.

Table S3. Release rate of 1H- and 2H-isomers of BTA at various time intervals at pH ~ 2.5.

Time	Total released BTA [%]	Released 1H isomer [%]	Released 2H isomer [%]
6 h	6.7	4.7	15.7
20 h	11.4	8.5	24.5
28 h	13.3	10.2	27.4
4 days	33.0	23.7	75.4
7 days	49.5	40.9	88.6

Analytical tools

Molar mass of VBMA and BEBMA were obtained from measurements with a LC-quadrupole-time-of-flight tandem mass spectrometry (MS, model Compact QTOF, Bruker). UV-Vis spectra were determined with an UV-Vis spectrometer (Lambda 1050, PerkinElmer). A 600 MHz NMR spectrometer from Bruker was used for recording ^{1}H and ^{13}C NMR spectra of samples in $CDCl_3$ at room temperature. Thermogravimetric analysis (TGA) measurements were carried out in nitrogen atmosphere with a heating rate of 10 °C min^{-1} from 25 to 700 °C on a LINSEIS STA PT 1600 thermogravimetric analyzer. The apparent average molecular weights and molar weight dispersity (Đ) of the copolymers were determined with a gel permeation chromatography (GPC, model Viscotek TDAmax, Malvern). Copolymer solutions in THF at a concentration of 4 mg/mL were used for GPC measurements with a three single-pore GPC/SEC columns (6, 7, and 10 µm particle size, linear M) at 35 °C with 1 $mL.min^{-1}$ flow rate and a RI-detector. Linear polystyrene (from PSS, Mainz) with different molecular weights and narrow Đ were used for the calibration curves. The hydrodynamic diameters of the polymer nanoparticles were determined with a dynamic light scattering (DLS, model NanoPlus-3, Micromeritics) operating with a laser at a wavelength of 660 nm and a detection angle at 165 °. To calculate the released amount of BTA from polymer nanoparticles at various time intervals, calibration curves were drawn for each pH values (see Fig. S8).

Investigation of the anticorrosion properties of the polymers

Surface of cold-rolled steels (SPFC 780Y grade) substrates were coated with P($BMA_{0.80}$-*co*-$BEBMA_{0.20}$) or P($BMA_{0.80}$-*co*-$MMA_{0.20}$) by dip-coating the substrates 10 times in 2% w/v solution of polymer in chloroform. After each coating, polymer films were left to dry under nitrogen flow for 10 min. After dip-coating the substrates 10 times, the samples were allowed to dry for 1 h under nitrogen flow at room temperature and then 1 h in vacuum (30 mmHg). Thickness of the coatings were measured by optical microscopy (OM, Zeiss SteREO Discovery V12) to be ~ 5-7 µm. Solutions of polymers were coated on cold-rolled steel (SPFC 780Y grade). The corrosion potential E_{corr}, polarization resistance R_p, corrosion current density I_{corr}, and corrosion rate CR of coated and uncoated cold-rolled steels were investigated with electrochemical measurements

(Autolab PGSTAT302N, Metrohm Siam Ltd.). Platinum electrode and Ag/AgCl electrode were used as counter electrode and reference electrodes, respectively. The corrosive medium was 0.1 M HCl. Potentiodynamic polarization was conducted in the potential range ± 500 mV. Before potential sweep, the electrode was left under open-circuit for 30 min in order to reach a steady state open-circuit potential. CR was evaluated from the intersection of Tafel lines extrapolation. Results revealed that the nanoparticles with the benzotriazole payloads are useful as anticorrosive agents (Table R1). We synthesize also a new copolymer without benzotriazole by copolymerizing butyl methacrylate and methyl methacrylate and prepared nanoparticles with this polymer. Coatings prepared with the polymer-benzotriazole conjugate displayed the best anticorrosion properties with the lowest corrosion rate, I_{corr} and E_{corr}, and the largest R_p.

Table S4. Corrosion parameters for coated and uncoated cold-rolled steel in 0.1 M HCl.

Entry	E_{corr} [mV]	I_{corr} [μA cm^{-2}]	R_p [Ω]	Corrosion rate [mm/year]
Coated with P(BMA$_{0.80}$-*co*-BEBMA$_{0.20}$)	-529	978.3	89.2	11.32
Coated with P(BMA$_{0.80}$-*co*-MMA$_{0.20}$)	-508	1199.7	74.1	13.88
Uncoated	-476	6025.8	14.9	69.70

Supplementary Figures

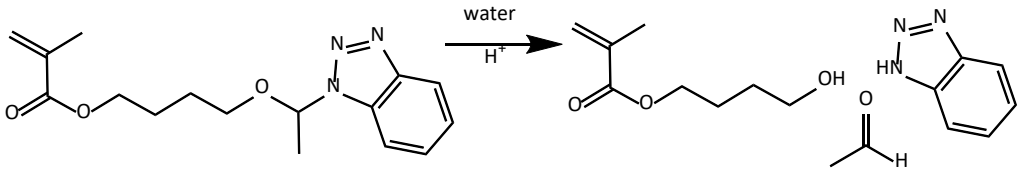


Figure S1. Scheme of the hydrolysis of the hemiaminal ether group in the BEBMA monomer.

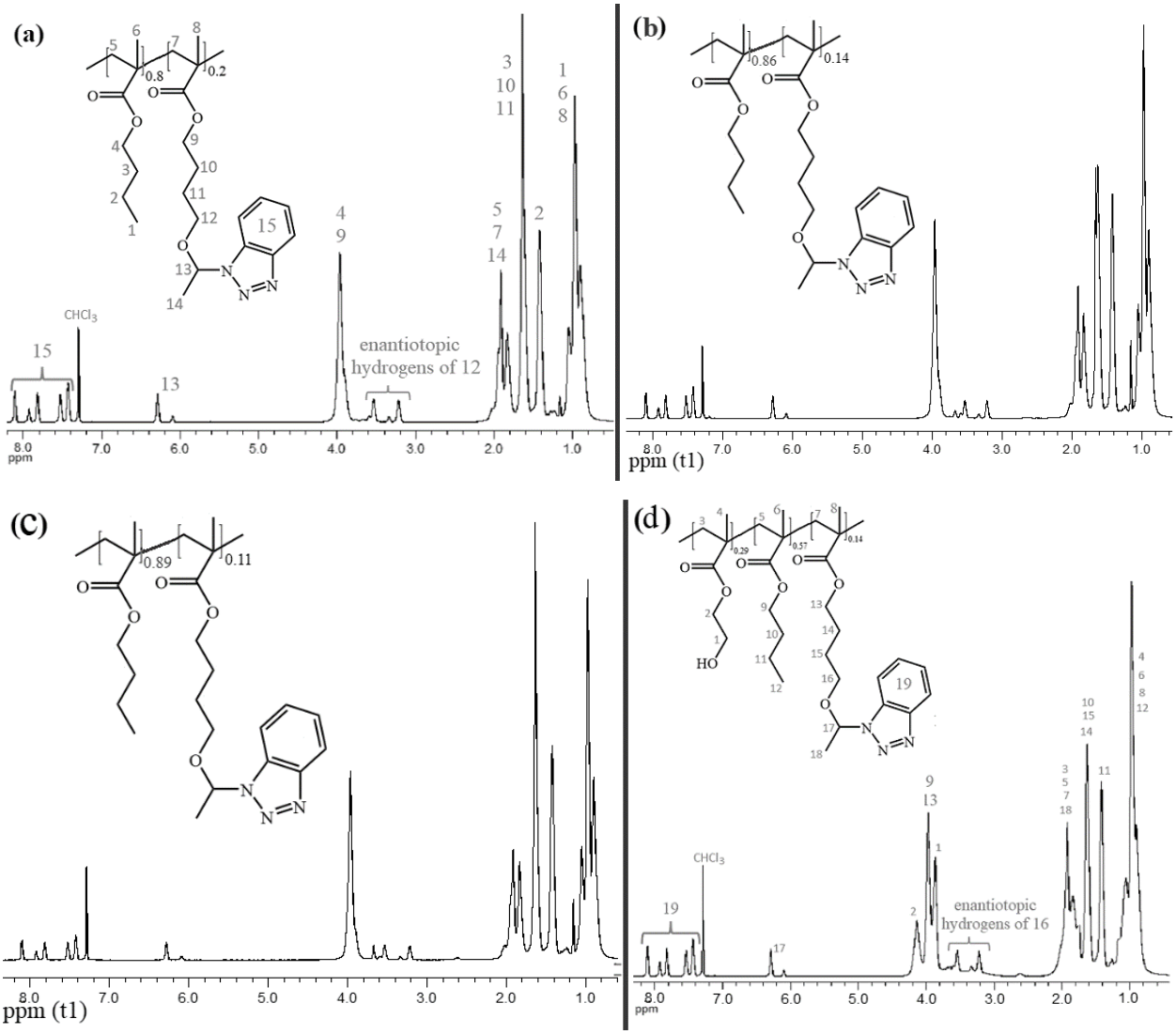


Figure S2. ^{1}H-NMR spectra of (a) P(BMA$_{0.80}$-*co*-BEBMA$_{0.20}$), (b) P(BMA$_{0.86}$-*co*-BEBMA$_{0.14}$), (c) P(BMA$_{0.89}$-*co*-BEBMA$_{0.11}$), and (d) P(BMA$_{0.57}$-*co*-HEMA$_{0.29}$-*co*-BEBMA$_{0.14}$) in $CDCl_3$.

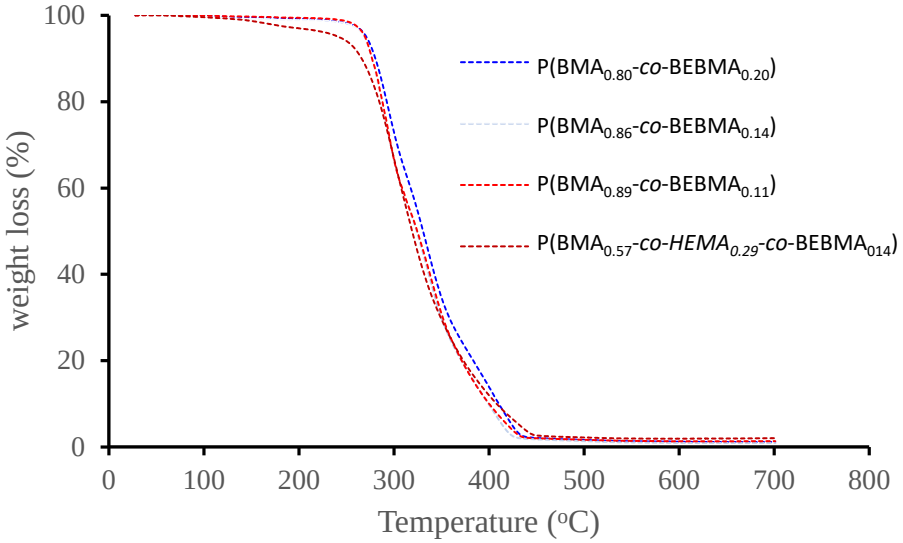


Figure S3. TGA thermograms of different polymer conjugates.

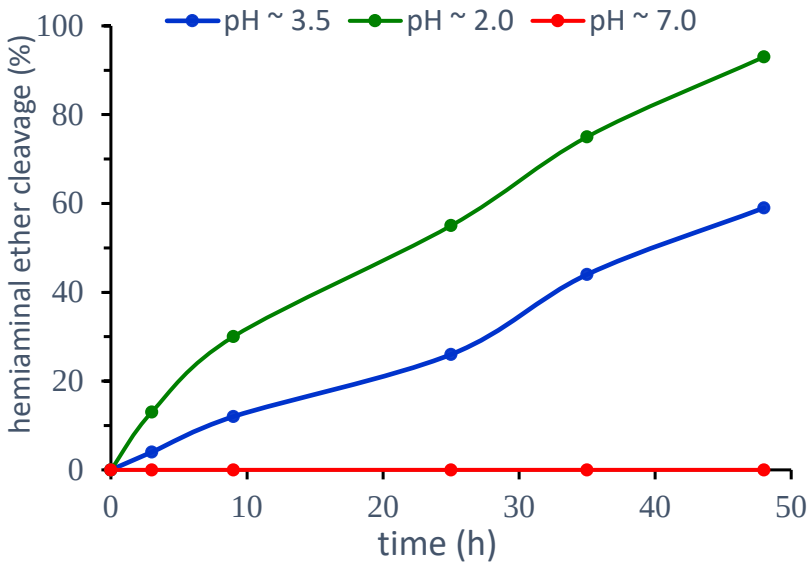


Figure S4. Kinetics of hydrolydic degradation of 4-(1-(benzotriazole-*N*-yl) ethoxy)butyl methacrylate (BEBMA) monomer in aqueous media at three different pH values.

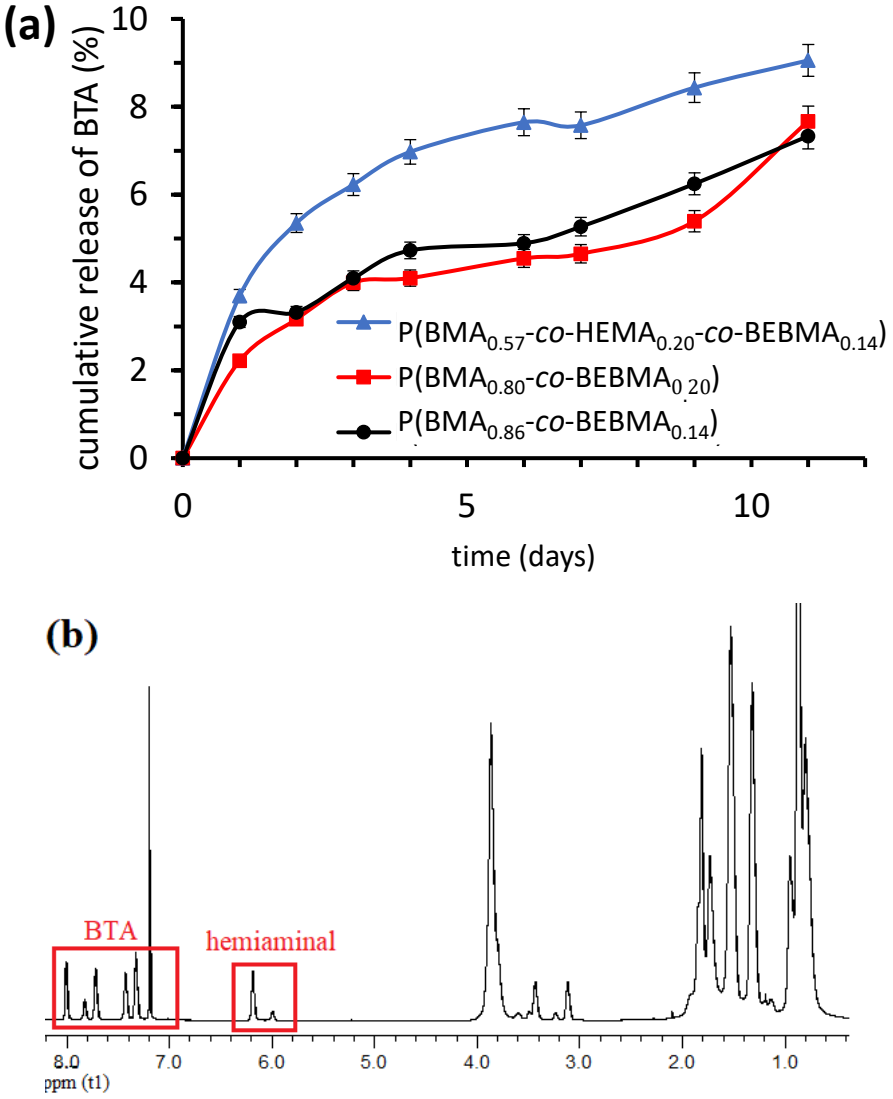


Figure S5. (a) Release profile of BTA from nanoparticles of polymer conjugates at pH ~ 5.0 and (b) ^{1}H NMR spectrum of P(BMA$_{0.80}$-*co*-BEBMA$_{0.20}$) nanoparticles after release.

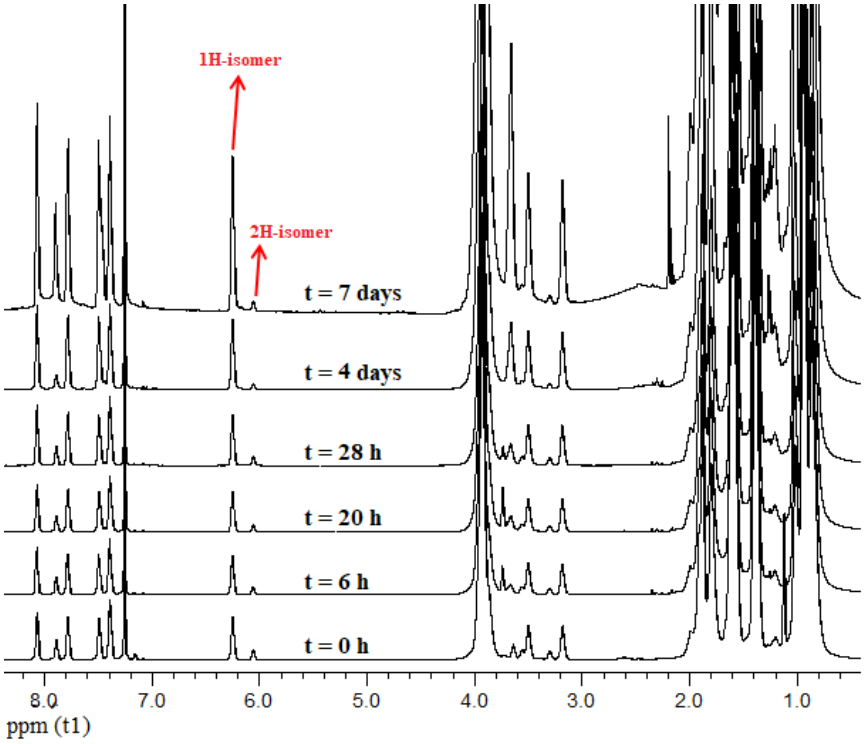
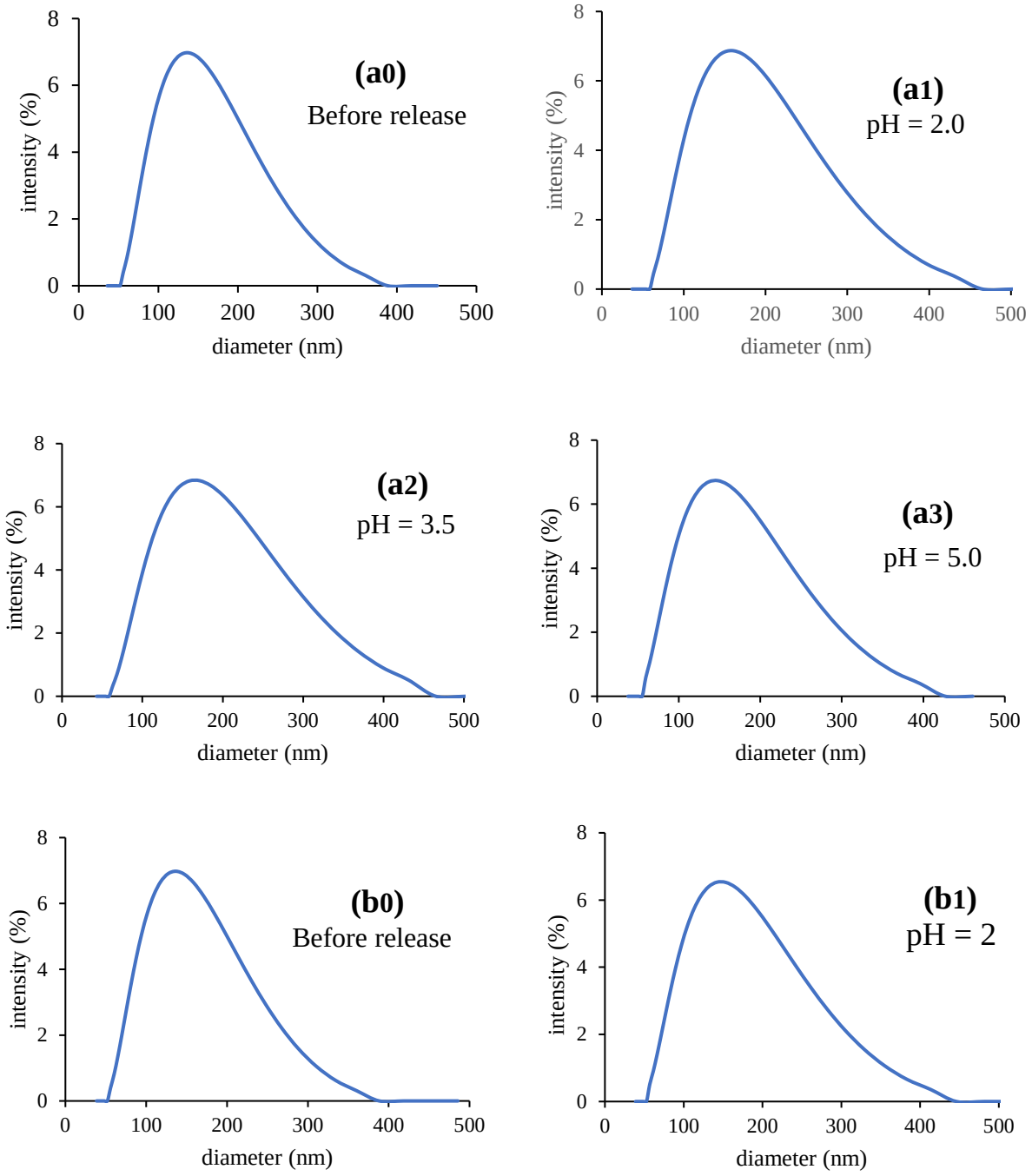


Figure S6. ^{1}H NMR spectra of P(BMA$_{0.86}$-*co*-BEBMA$_{0.14}$) nanoparticles at various time intervals at pH ~ 2.5 for evaluation of the release rate of 1H- and 2H-isomers of BTA.


(a0)
Before release
(a1)
pH = 2.0
(a2)
pH = 3.5
(a3)
pH = 5.0
(b0)
Before release
(b1)
pH = 2
intensity (%)
diameter (nm)
0
2
4
6
8
0
100
200
300
400
500

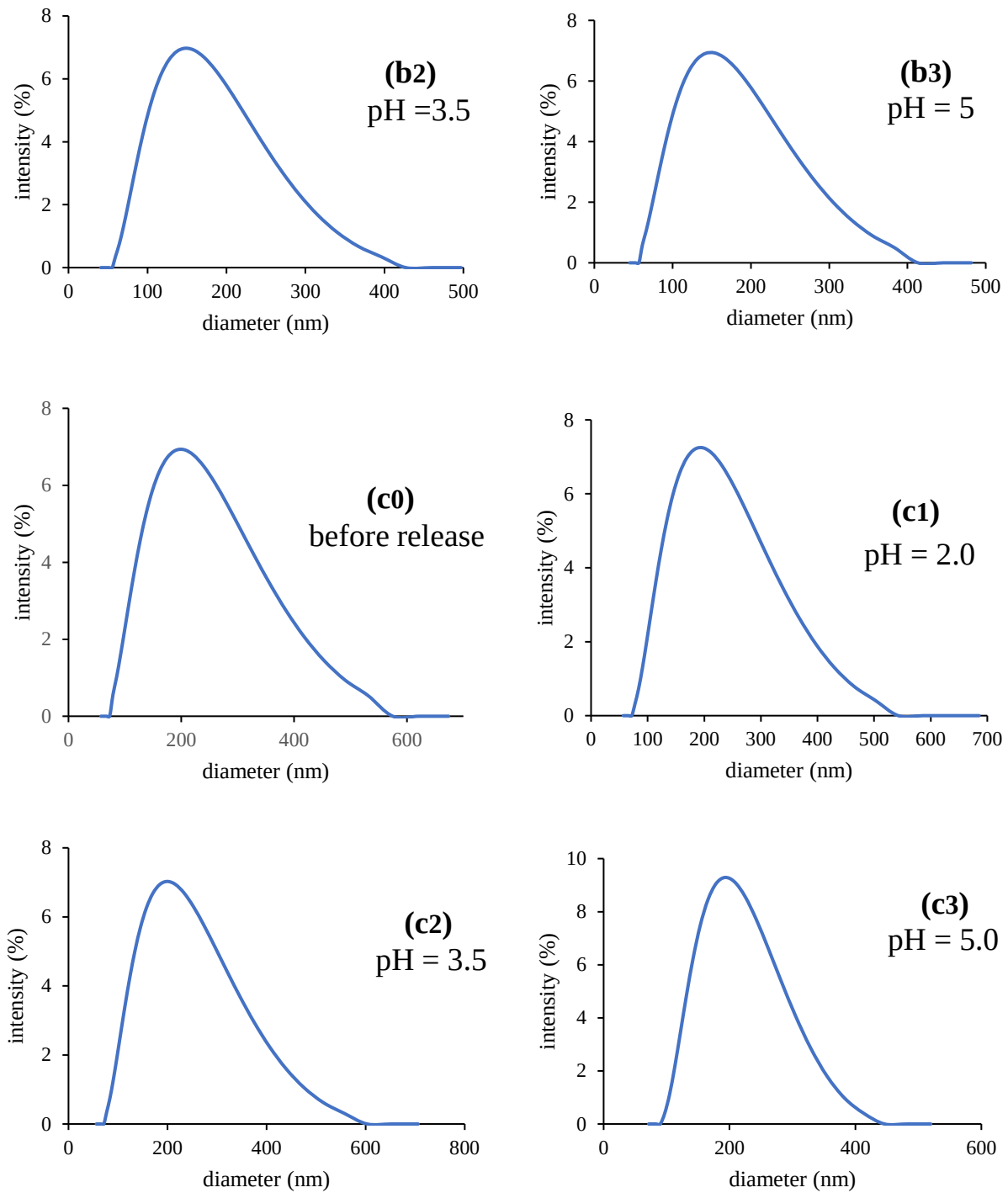


Figure S7. Particle size distribution of nanoparticles made from (a0) P(BMA$_{0.80}$-*co*-BEBMA$_{0.20}$) copolymer before release, (a1) after release at pH = 2.0, (a2) after release at pH = 3.5, (a3) after release at pH = 5.0, (b0) P(BMA$_{0.86}$-*co*-BEBMA$_{0.14}$) copolymer before release, (b1) after release at pH = 2.0, (b2) after release at pH = 3.5, (b3) after release at pH = 5.0, (c0) P(BMA$_{0.57}$-*co*-

HEMA$_{0.29}$-*co*-BEBMA$_{0.14}$) copolymer before release, (c1) after release at pH = 2.0, (c2) after release at pH = 3.5, (c3) after release at pH = 5.0.

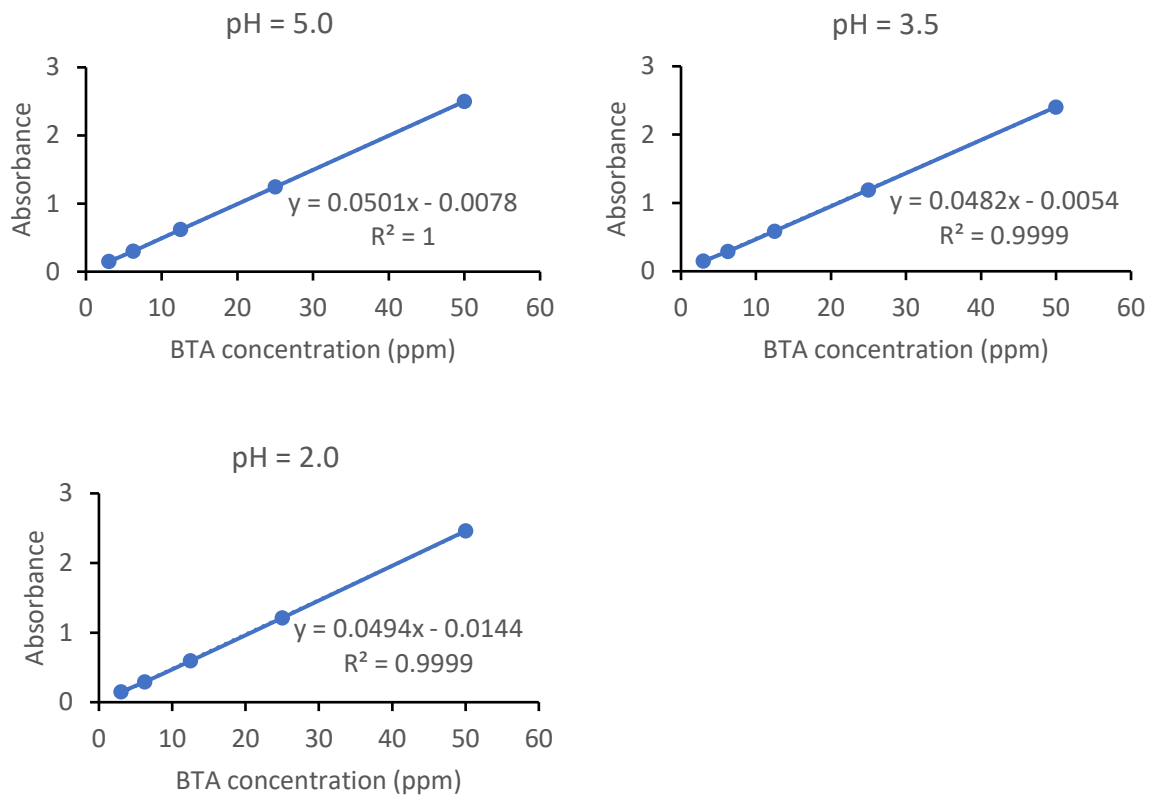


Figure S8. Calibration curves of UV absorbance of BTA versus concentration of BTA at pH~ 2.0, 3.5, and 5.0.

References

S1 V. Dhanapal and K. Subramanian, *Carbohydr. Polym.*, 2015, **117**, 123.

S2 O. Vysotskaya, L. Oparina, L. Parshina, B. Timokhin, N. Gusarova and B. Trofimov, *Russ. J. Org. Chem.*, 2003, **39**, 585.

S3 R. H. Staff, K. Landfester and D. Crespy, in *Hierarchical Macromolecular Structures: 60 Years after the Staudinger Nobel Prize II*, Springer, 2013, pp. 329.