Supporting Information

PEG-based Core-Degradable Nanoparticles via RAFT-Mediated Reverse-Sequence PISA.

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1. Materials and Instruments

All chemicals were used as obtained without any further purification unless otherwise stated. Chemical reagents were purchased as follows: 4-(*N*,*N*-dimethylamino)pyridine (DMAP, ≥ 97%, Aldrich), *N*,*N*-dicyclohexylcarbodiimide (DCC, 99%, Aldrich), acetone (≥ 99,8%, Carlo Erba), 4,4′-azobis(4-cyanovaleric acid) (ACVA, ≥ 98%, Aldrich), diethyl ether (Et_2O, ≥ 99.7%, Aldrich), ethyl acetate (EtOAc, ≥ 99.5%, Aldrich), dichloromethane (CH_2Cl_2, ≥ 99.8%, Carlo Erba), cyclohexane (≥ 99.8%, Carlo Erba), dimethylformamide (DMF, ≥ 99.9%, Aldrich), *N,N*-dimethylacrylamide (DMAA, 99%, Aldrich), dimethylsulfoxyde-d_6 (DMSO-d_6, 99.8 atom% D, Aldrich) Chloroform D ($CDCl_3$, 99.8 atom% D, Aldrich).

4-cyano-4-(((ethylthio)carbonothioyl)thio)pentanoic acid (CEPA) chain transfer agent (CTA) was synthesized according to an experimental procedure previously reported[1]. The synthesis of poly[(ethylene oxide)$_{43}$-*co*-(ethylene carbonate)$_{51}$] (PECEO) batch used in this study was published elsewhere[2].

^{1}H Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR). The ^{1}H NMR spectra were acquired using a Bruker 400 MHz Fourier Transform NMR spectrometer. The samples were formulated in deuterated solvents, namely DMSO-d_6 (δ = 2.50 ppm), and chloroform-d (δ = 7.26 ppm), with signals referenced to the residual non-deuterated signals of the respective solvents.

Size Exclusion Chromatography (SEC). The determination of molecular characteristics of the precursor polymers was accomplished through size exclusion chromatography (SEC) using *N*, *N*-dimethylformamide (DMF) as the eluent at a flowrate of 1 mL.min^{-1}. The SEC analyses were conducted employing a Shimadzu instrument equipped with mixed-C columns and refractive index (RI) detection. Molecular weight distributions ($Đ$) were computed utilizing poly(ethylene glycol) (PEG) standards.

Dynamic Light Scattering (DLS). The intensity-average sphere equivalent diameter of polymer nano-objects was determined at 25 °C. Dynamic Light Scattering (DLS) was employed for these measurements using a Malvern Zetasizer Nano S instrument, applying the Stokes−Einstein equation, which assumes perfectly monodisperse, non-interacting spheres. Since all samples were stored directly in water after polymerization without undergoing any further purification steps, they experienced appropriate dilution in water for the measurements. The Z-average hydrodynamic diameters (D_h) reported in this study were calculated based on the average of three repeated measurements. All the analyzed PECEO-*b*-PDMAA NPs samples were diluted to proper concentrations prior to measurement.

Transmission Electron Microscopy (TEM). Dry-state transmission electron microscopy (TEM) imaging was conducted using an FEI TECNAI F20 microscope with an acceleration voltage of 200 kV. Aqueous suspension samples were initially diluted with deionized water and subsequently applied onto formvar-graphene oxide coated copper grids. The samples were directly diluted 100 times with ultrapure water before being deposited onto the grid. After an incubation period of approximately 90 sec, excess sample was removed by blotting from the grid. Following this, the grid underwent staining with an aqueous 1 wt.% uranyl acetate (UA) solution for 90 sec before being subjected to blotting, drying, and subsequent microscopic analysis. ImageJ was employed for image processing.

2.1. Synthesis of Hydrophilic Poly[(ethylene carbonate)$_{51}$-*co*-(ethylene oxide)$_{43}$] Macromolecular Chain Transfer Agent (PECEO macro-CTA)

The synthesis of the water soluble PECEO macro-CTA was adapted from a previously reported literature procedure[3]. Poly [(ethylene carbonate)-*co*-(ethylene oxide)] (PECEO, 2 g, 0.4 mmol, 1 equiv.), was dissolved in 70 mL dry CH_2Cl_2. The resulting solution was then purged with N_2 stream for 30 min. After complete dissolution of PECEO, CEPA CTA (0.422 g, 1.6 mmol, 4 equiv.), DCC (165 mg, 0.8 mmol, 2 equiv.), and DMAP (10 mg, 0.08 mmol, 0.2 equiv.) were added to the reaction mixture. The esterification reaction proceeded with stirring at room temperature for 18 h under N_2 atmosphere. After this period, further DCC (165 mg, 0.8 mmol, 2 equiv.) and DMAP (10 mg, 0.08 mmol, 0.2 equiv.) were added to the reaction mixture that was stirred at room temperature for an additional period of 6 h under N_2 atmosphere. The reaction mixture was filtered off to remove unreacted DCC and DMAP following evaporation of CH_2Cl_2 via air blowing. The obtained PECEO macro-CTA copolymer was purified by precipitating the CH_2Cl_2 solution of the PECEO macro-CTA against 500 mL cold Et_2O. Upon filtration, the PECEO macro-CTA collected after re-dissolved in a minimum volume of CH_2Cl_2, reprecipitated another time in cold Et_2O and harvested by filtration. After dissolution in deionized water, it was dialyzed against deionized water for 2 days (dialysis membrane MWCO = 1000 Da). The obtained PECEO macro-CTA solution was finally freeze-dried to afford the final product as an orange oil. The degree of functionalization of PECEO by CEPA was estimated by comparing the integration of the CEPA end-group signal (proton a) with that of a characteristic PECEO backbone signal (proton f) and was found to be approximately 95%. ^{1}H NMR (400 MHz, $CDCl_3$) as shown in **Figure S1**: δ (ppm) 4.44 – 4.17 (m, 147H), 3.69 (m, 59H), 3.61 (m, 172H), 3.31 (dd, 2H), 2.63 (t, 2H), 2.05 (s, 3H), 1.84 (s, 3H), 1.32 (t, 3H).

2.2. Reverse sequence RAFT polymerization of DMAA in concentrated aqueous solution using hydrophobic PECEO macro-CTA followed by subsequent dilution with water.

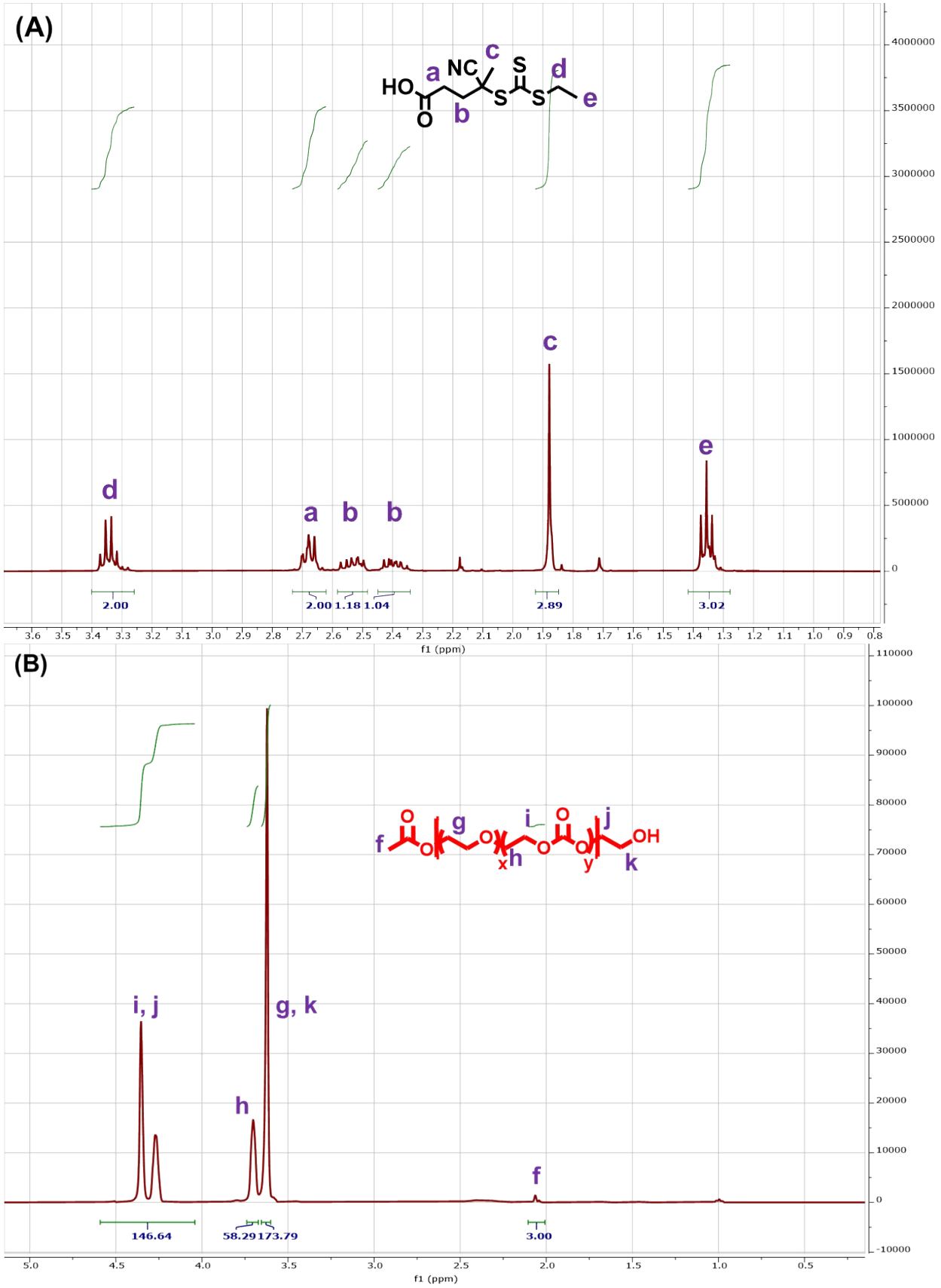
A series of PECEO-*b*-PDMAA diblock copolymers were synthesized by varying the degree of polymerization (DP) values (60, 80, 100, 120) of DMAA and the solids content (10, 15, 20 wt.%) through reverse sequence PISA. The quantities of reagents are summarized in **Table S2**. All

polymerization proceeded with an initiator/CTA molar ratio = 0.2. A representative synthetic procedure, employing a DP of 100 and a solids content of 15 wt.%, is described in detail as follows: Initially, 70 mg of PECEO macro-CTA (1.75×10^{-5} mol, 1 equiv.) was weighted in a 2-mL high-performance liquid chromatography (HPLC) vial. Subsequently, 173 µL of DMAA (1.75×10^{-3} mol, 100 equiv.) was added to dissolve the PECEO macro-CTA followed by addition of 98.1 µL (3.5×10^{-6} mol, 0.2 equiv.) of a 10mg/mL ACVA stock solution in water. The vial was then sealed with a rubber septum and subjected to degassing under a continuous flow of nitrogen (N_2) while stirring for 30 min to ensure complete oxygen removal. The vial was then placed into a 80 °C reactor under stirring. The reaction mixture became highly viscous after 7 min, owing to polymerization of DMAA. At this point, 1.38 mL degassed water was added to the polymerization mixture using a purged syringe to diluted the suspension down to 15 wt.% solids content. The reaction mixture was finally exposed to ambient air and subsequently cooled down in a water bath after 19h to stop the polymerization. In order to investigate the reaction kinetics during the PISA process, aliquots of the polymerization mixture were withdrawn at predetermined time intervals. These samples were used for subsequent ^{1}H NMR analysis in deuterated DMSO-d_6 and SEC analysis using DMF as the mobile phase at a flow rate of 1 mL.min^{-1} and PEG standards.

3. Hydrolytic degradation of block copolymers in aqueous solution

PECEO-*b*-PDMAA with a degree of polymerization (DP) of 120 at 10 wt.% was selected as a model to investigate its degradation behavior. Full degradation was first conducted under strong basic conditions (pH = 13) as follows: 100 µL of a PECEO-*b*-PDMAA$_{120}$ (10 wt.%) polymer solution was immersed in 900 µL of 0.1 N NaOH aqueous solution (pH = 13) at 37 °C under stirring for 67 h. Subsequently, the incubated block copolymer sample was freeze-dried and purified via precipitation against Et_2O for ^{1}H NMR analysis.

Additionally, hydrolytic behavior was evaluated under milder conditions, specifically using incubation media at pH = 10.9 and 2.7 (in PBS buffer), following the same abovementioned procedure. Samples were withdrawn at predetermined time intervals and analyzed using SEC to monitor the degradation process.


(A)
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1.18
1.04
2.89
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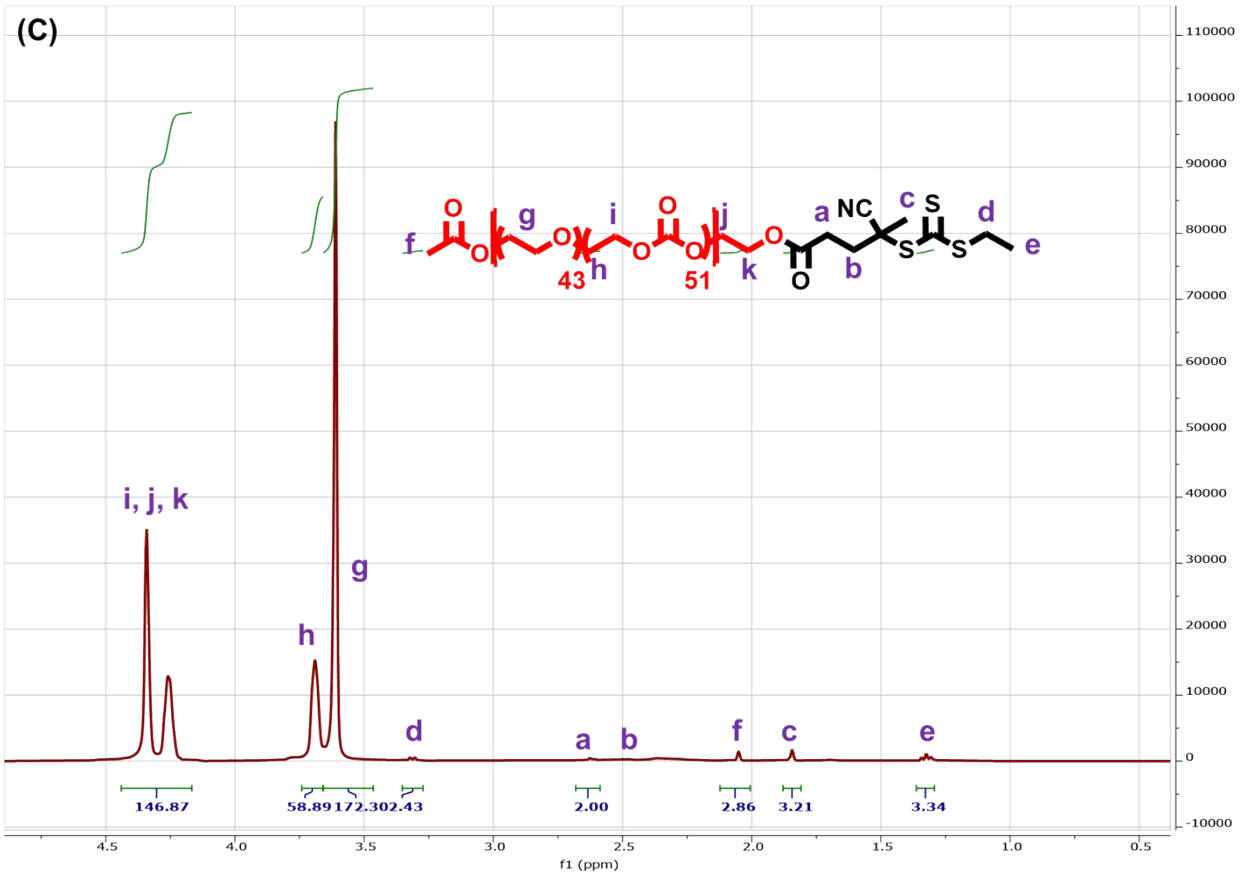

Figure S1. Assigned ^{1}H NMR spectrum of (A) CEPA RAFT agent, (B) hydrophobic poly[(ethylene oxide)$_{43}$-*co*-(ethylene carbonate)$_{51}$] (PECEO) copolymer precursor, and (C) poly[(ethylene oxide)$_{43}$-co-(ethylene carbonate)$_{51}$] macro-CTA (PECEO-mCTA) in $CDCl_3$.

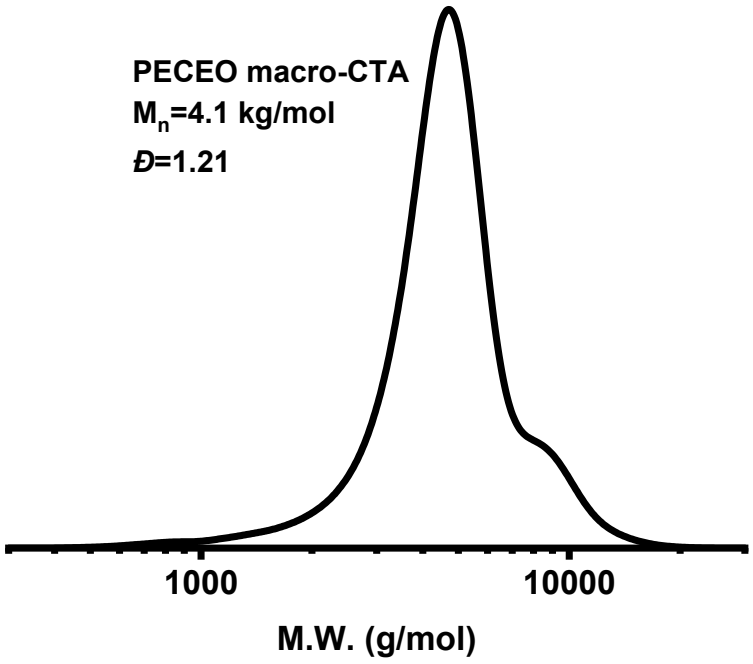

Figure S2. Normalized SEC-RI trace of hydrophobic PECEO macro-CTA and its corresponding M_n and *Đ* values.

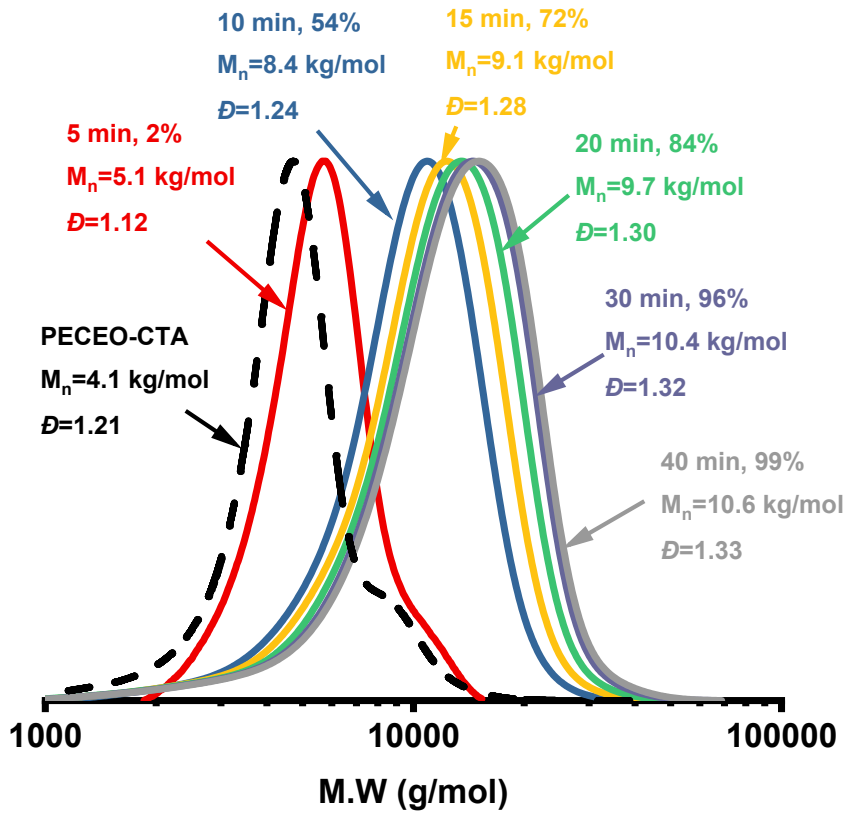

Figure S3. Evolution of normalized SEC-RI traces of PECEO-*b*-PDMAA diblock copolymers against time (DP = 100, solids content = 15 wt.%) during PISA from PECEO-based macro-CTA at 80 °C.

Reaction time (min)	Monomer conversion (%)	M_n	M_w	$Đ$	$M_{n,\ theory}$
5	2	5.1	5.8	1.12	4.2
10	54	8.4	10.4	1.24	9.4
15	72	9.1	11.6	1.28	11.1
20	84	9.7	12.7	1.3	12.3
30	96	10.4	13.6	1.32	13.5
40	99	10.6	14.1	1.33	13.8

Table S1. Molecular features of PECEO-*b*-PDAAM diblock copolymers (DP = 100, solid content = 15 wt.%) obtained as a function of polymerization time. M_n theoretical values $M_{n,\ theory}$) were calculated from ^{1}H NMR (DMSO-d_6): $M_{n,\ theory} = MW_{monomer} \times DP \times conversion / 100 + MW_{CTA}$, Value of M_n and M_w were determined by SEC (PEG standards, DMF as the mobile phase at a flow rate of 1 mL.min^{-1})

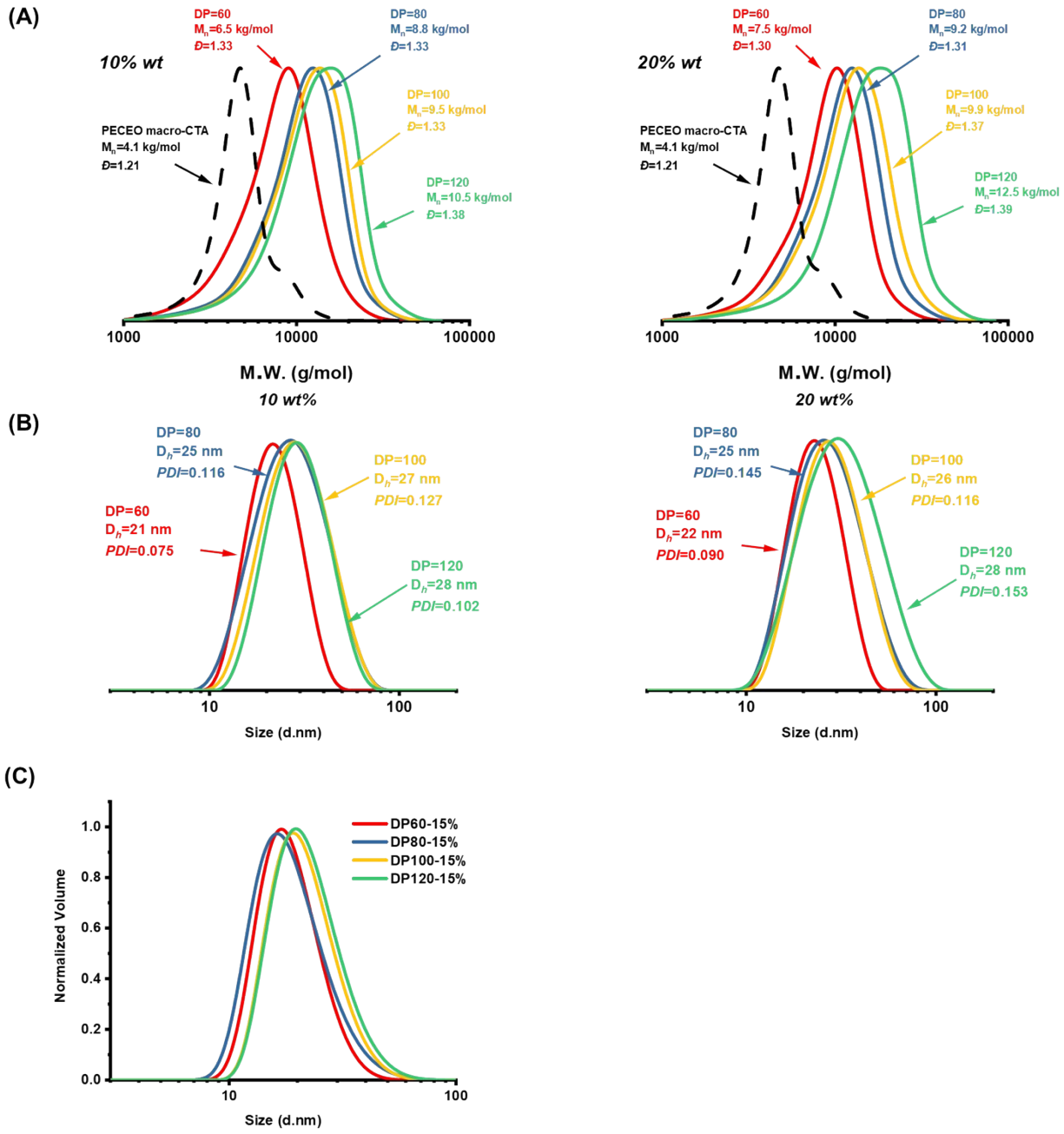

Figure S4. (A) Normalized SEC-RI traces of PECEO macro-CTA and corresponding PECEO-*b*-PDMAA diblock copolymers at 10 wt.% and 20 wt.% solids content with varying DP values (60, 80, 100, 120), and their associated M_n and $Đ$ values. (B) Normalized intensity DLS size distribution curves obtained for PECEO-*b*-PDMAA copolymers at 10 wt.% and 20 wt.% solids content with varying DP values (60, 80, 100, 120). (C) Normalized volume DLS size distribution curves obtained for PECEO-*b*-PDMAA copolymers at 15 wt.% solids content with varying DP values (60, 80, 100, 120)

Solids content	DP	PECEO-CTA (mg)	DMAA (mg)	Amount of added water (mL)	Dilution time (min)
10%wt	60	30	44.6	0.67	13
	80	30	59.5	0.8	7
	100	30	74.3	0.93	7
	120	30	89.2	1.07	5
15%wt	60	40	59.5	0.56	20
	80	40	79.3	0.67	7
	100	30	74.3	0.59	9
	120	30	89.2	0.67	7
20%wt	60	50	74.3	0.5	9
	80	40	79.3	0.48	9
	100	40	99.1	0.56	7
	120	40	119	0.64	8

Table S2. Summary of reagent quantities used in initially concentrated polymerization mixtures for reverse sequence PISA of DMAA, of deoxygenated water volume added for subsequent dilution, and related dilution time.

Solids content	DP	Reaction time (h)	Monomer conversion	$M_{n,SEC}$ (kg/mol)	$M_{w,SEC}$ (kg/mol)	*Đ*	D_h	PDI
10%wt	60	19	≥99%	6.5	8.6	1.33	21	0.075
	80	19	≥99%	8.8	11.7	1.33	25	0.116
	100	19	≥99%	9.5	12.6	1.33	27	0.127
	120	19	≥99%	10.5	14.5	1.38	28	0.102
15%wt	60	19	≥99%	8.1	10.3	1.26	22	0.062
	80	19	≥99%	9.4	12.5	1.33	26	0.159
	100	19	≥99%	10.9	14.8	1.35	26	0.096
	120	19	≥99%	12.1	16.5	1.36	28	0.101
20%wt	60	19	≥99%	7.5	9.8	1.3	22	0.09
	80	19	≥99%	9.2	12.0	1.31	25	0.145
	100	19	≥99%	9.9	13.5	1.37	26	0.116
	120	19	≥99%	12.5	17.5	1.39	28	0.153

Note：The $M_{n,SEC}$ and $M_{w,SEC}$ values correspond to apparent molecular weights determined by SEC using PEG standards.

Table S3. Table gathering SEC and DLS results obtained from a series of PECEO-*b*-PDMAA diblock copolymers with varying DP (60, 80, 100, 120) and solids content (10, 15, 20 wt%) after 19 hours polymerization.

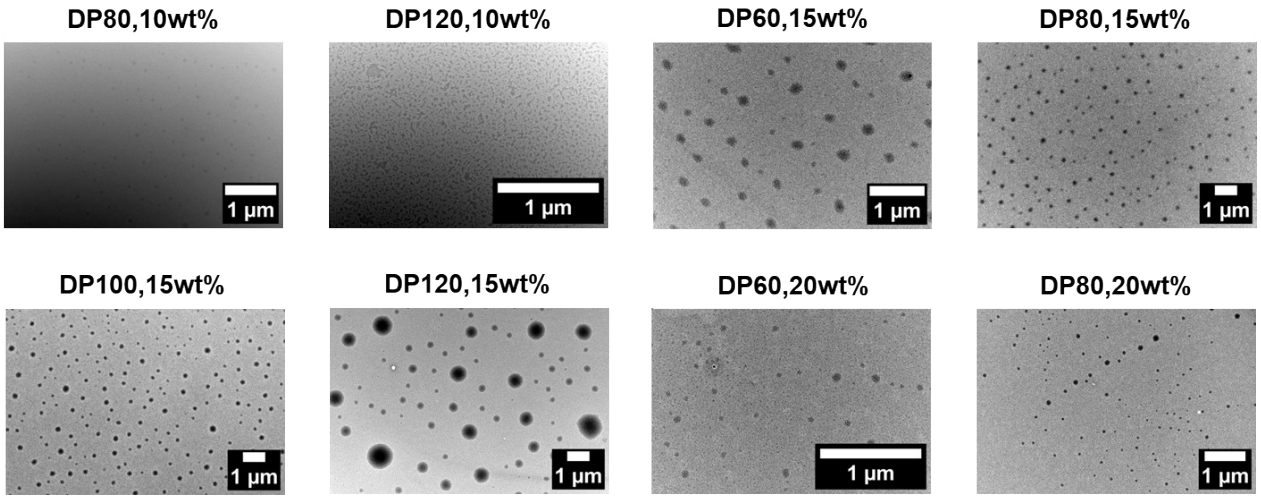

Figure S5. Representative micrographs for PECEO-*b*-PDMAA-based nanoparticles obtained from dry-state TEM.

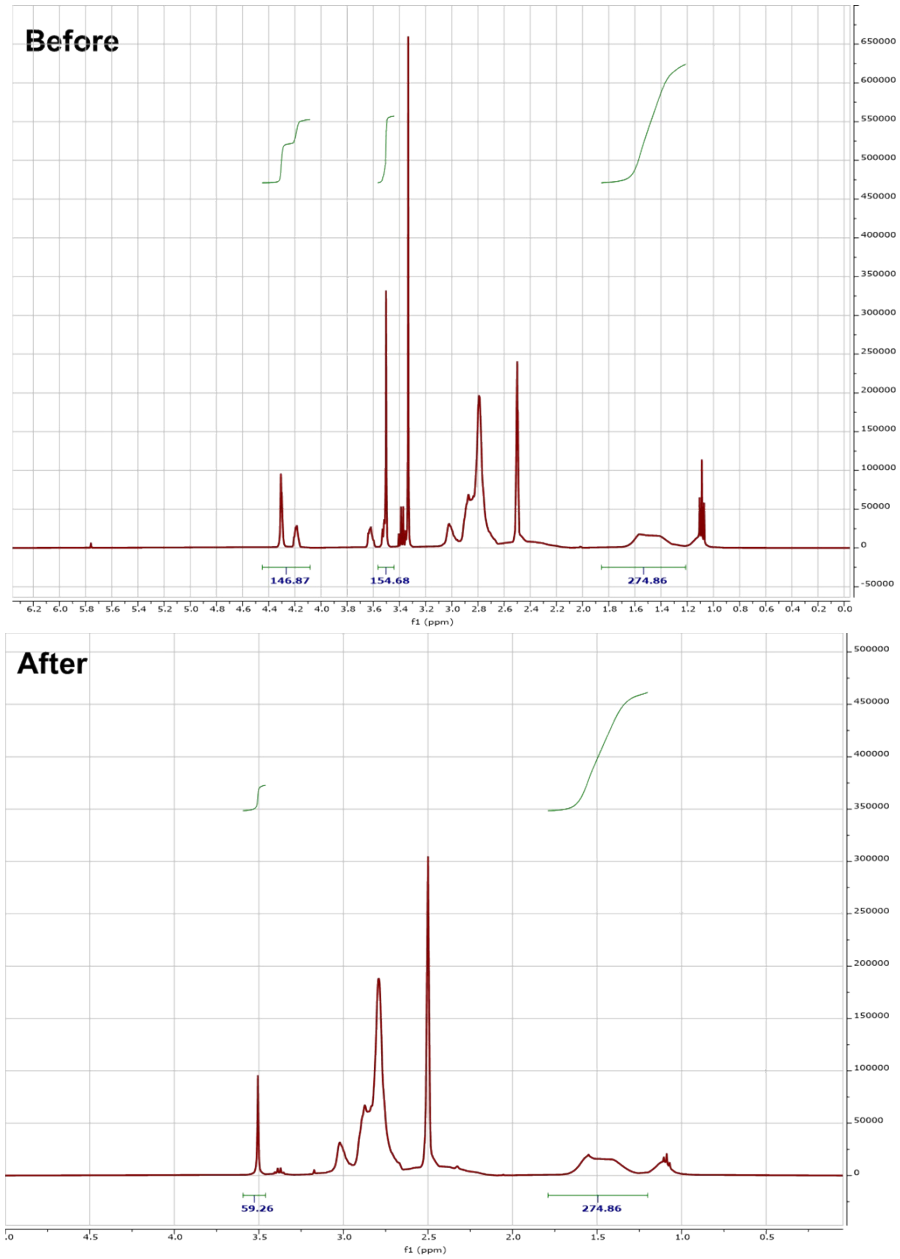

Figure S6. The integrated ^{1}H-NMR of PECEO-*b*-PDMAA before and after degradation.

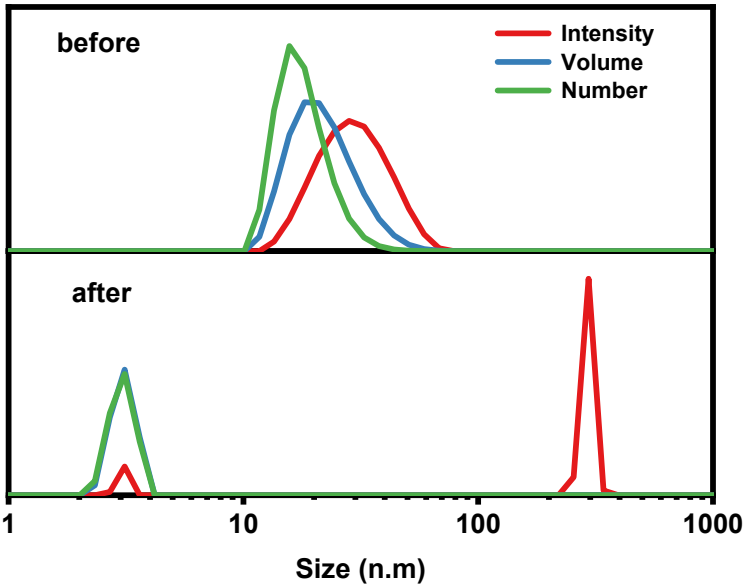

Figure S7. Evolution of PECEO-*b*-PDMAA-based (DP of 120, solids content of 10 wt%) self-assembly monitored by DLS before and after 138 hours incubation period in basic medium (pH = 10.9) at 37 °C.

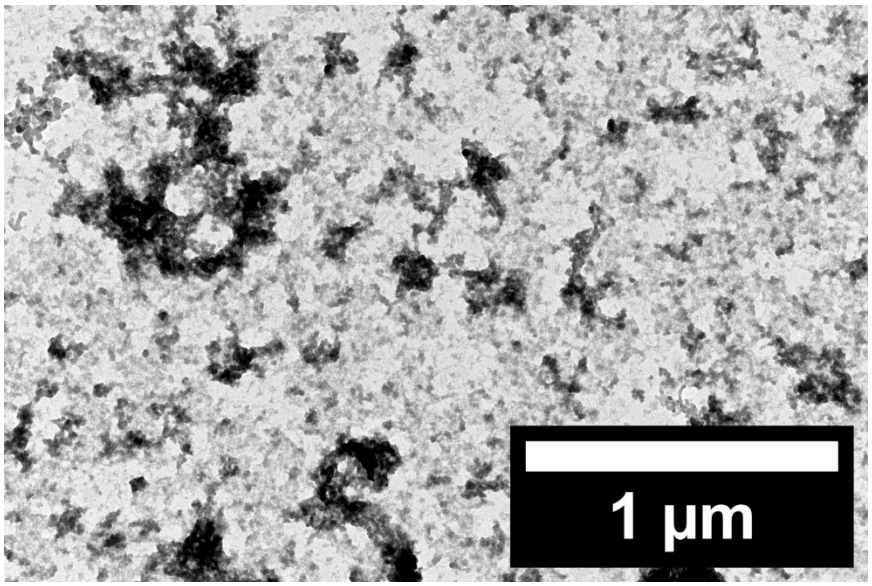

Figure S8. Representative micrograph for PECEO-*b*-PDMAA-based nanoparticles (DP of 120, solids content of 10 wt.%) after degradation obtained from dry-state TEM.

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RAFT-mediated emulsion polymerization. *Polymer Chemistry* **2015,** *6* (20), 3865-3874. doi.10.1039/C5PY00166H
2. Jia, M.; Zhang, D.; Gnanou, Y.; Feng, X., Surfactant-Emulating Amphiphilic Polycarbonates and Other Functional Polycarbonates through Metal-Free Copolymerization of CO2 with Ethylene Oxide. *ACS Sustainable Chemistry & Engineering* **2021,** *9* (30), 10370-10380. doi.10.1021/acssuschemeng.1c03751
3. Lei, L.; Patil, N.; Arnoux, A.; Le Cœur, C.; de Rancourt de Mimérand, Y.; Grande, D.; Le Droumaguet, B.; Feng, X.; Gnanou, Y.; Couturaud, B., Transition from Steric to Electrostatic Stabilization in Shell-Degradable Waterborne Particles Obtained by Photopolymerization-Induced Self-Assembly. *Macromolecules* **2024,** *57* (22), 10513-10521. doi.10.1021/acs.macromol.4c01863