Diblock terpolymers are tunable and pH responsive vehicles to increase hydrophobic drug solubility for oral administration

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Supporting Information

1. Materials and Methods:

Materials. All chemicals were used as received (reagent grade) unless otherwise noted. All solvents utilized were HPLC or analytical grade. Anhydrous D-trehalose (99%, Acros Organics), iodine (Aldrich, >99.8%), triphenylphosphine (Aldrich, 99%), acetic anhydride (99.6%, Fisher), dry pyridine (99.8%, Sigma Aldrich), sodium azide (Aldrich, >99.5%), Pd/C (Aldrich), sodium methoxide (Aldrich, 95%), sodium chloride (Fisher), silica gel (Sorbent technologies, porosity 60Å size 40-60μm), chlorotrimethylsilane TMSCl (Fisher, 98%), triethylamine (TEA) (Acros Organics, 99.7%), and HCl 1.25M in methanol (Fluka), 2,2'-Azobis(2-methylpropionitrile) (AIBN, Aldrich, 98%) were used as received. Freshly distilled methacryloyl chloride (Acros, 95%) was used for synthesis. *N*,*N*-Dimethylacrylamide (DMA) (Aldrich, 99+%) was purified by passage through activated basic alumina columns to remove

trace amounts of inhibitors. The monomers N-isopropylacrylamide (NIPAAm) (Aldrich, >99%), 2-(diethylamino)ethylmethacrylate (DEAEMA) (Aldrich, 99%), and 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (Aldrich, 98%) were used as received. *Sec*-butyllithium (1.4 M in cyclohexane, Aldrich), 1,3-isoprene (Aldrich, 99%), and ethylene oxide (Aldrich, 99.5+%) were degassed with three freeze-pump-thaw cycles followed by removing trace amounts of acidic impurities by multiple treatments with *n*-butyllithium (2.5 M in hexanes, Aldrich) for 1 hour each and *n*-butylmagnesium chloride (2.0 M in diethyl ether, Aldrich) for 4 hours each, respectively. Toluene (Sigma-Aldrich, HPLC grade, 99.9+%), and dichloromethane (Sigma-Aldrich, anhydrous, 99.8+%), and tetrahydrofuran (THF, Sigma-Aldrich, HPLC grade, 99.9+%, inhibitor free) were purified via an MBRAUN solvent purification system. Probucol (PBC) was purchased from Sigma-Aldrich (Milwaukee, WI) and used without further purification. HPMCAS (AFFINISOL™ 912G, The Dow Chemical Company) was used as received with degree of substitution (DS) as follows: DS of succinate 0.28, DS of acetate 0.57, DS of methoxy, and 1.94 DS hydroxypropyl 0.25. Fasted simulated intestinal fluid powder (FaSSIF) was purchased from Biorelevant (Surrey, UK). Phosphate buffered saline (PBS) was prepared that consisted of 82 mM sodium chloride (Fisher, ≥99.0%), 20 mM sodium phosphate dibasic heptahydrate (Fisher, 98%), and 47 mM potassium phosphate monobasic (J.T. Baker, ≥99.0%).

The synthesis of trimethylsilyl protected 2-methacrylamidotrehalose (TMS-MAT) was synthesized as previously described by our group.[1,2] The ^{1}H NMR was in agreement with previously published spectral data.[1,2] The diblock terpolymer PEP-P(DMA-*grad*-MAT) was synthesized using a combination of anionic and reversible addition fragmentation chain transfer (RAFT) copolymerizations as previously published.[2] The PEP-b-P(DMA-*grad*-MAT) was

characterized by ^{1}H NMR and size exclusion chromatography (SEC). The analysis and characterization data were also in agreement with the previously published data.[2] The synthesis of the diblock terpolymers PNIPAAm-b-P(DMA-*grad*-MAT) (18, 32, and 48 kDa) (Figure S5, ^{1}H NMR) and PDEAEMA-b-P(DMA-*grad*-MAT) (Figure S6, ^{1}H NMR) was achieved via polymerization from of the macromolecular chain transfer agents (CTA), PNIPAAm-CTA and PDEAEMA-CTA, respectively. For example, a 25 mL round conical flask was charged with PNIPAAm-CTA (0.6 g, 0.1 mmol), DMA (1.8 g, 18 mmol), TMS-MAT (1.8 g, 2 mmol), AIBN (0.8 mg, 0.0005 mmol), and 1,4-dioxane (10 mL). The reaction mixture was degassed for 1 h by bubbling nitrogen through the solution and adding the flask to a preheated oil bath at 70 °C for 12 h. Both polymers were purified by precipitation in diethyl ether twice and dried in a vacuum oven for 24 h at 45 °C. Detailed characterization information is reported in Table 1 and Figure S7, which shows SEC chromatograms for PNIPAAm-b-P(DMA-*grad*-MAT) and PDEAEMA-b-P(DMA-*grad*-MAT). PNIPAAm-CTA (Figure S1, ^{1}H NMR) and PDEAEMA-CTA (Figure S2, ^{1}H NMR) were obtained by RAFT polymerization using a small molecule trithiocarbonate-based CTA, 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid in 1,4-dioxane using AIBN as an initiator at 70 °C. For example, a 50 mL round conical flask was charged with NIPAAm (5 g, 45 mmol), 357 mg of CTA (357 mg, 0.88 mmol), Azobisisobutyronitrile (AIBN) (7.2 mg, 0.044 mmol), and 1, 4-dioxane (22 mL, 1.69 M). The reaction mixture was degassed for 45 min by bubbling nitrogen through the solution. The reaction flask was then added to a preheated oil bath at 70 °C for 6 h. The PNIPAAm-CTA was isolated by precipitation into pentane and purified by dialysis against water to remove trace amounts of impurities such as monomers and solvent. PDEAEMA-CTA was purified by precipitation into diethyl ether twice. Detailed characterization information is reported in Table S1. The yields for PNIPAAm-CTA and PDEAEMA-CTA were

87% and 72%, respectively. The macromolecular-CTAs were dried in a vacuum oven for 48 h at 40 °C before utilization to synthesize diblock terpolymers. For PNIPAAm-containing diblock terpolymers, three reactions were setup where feed ratio of monomers to macro-CTA was varied along with the time of the reaction. For instance, for PNIPAAm-b-P(DMA-*grad*-MAT) – 18 kDa synthesis, 250 mg of PNIPAAm macro-CTA (0.0409 mmol), 527 mg of N,N-dimethylacrylamide (5.33 mmol) and 357 mg of TMS-MAT (0.409 mmol) were mixed together in 4 mL of toluene, degassed with nitrogen for 30 minutes and placed in an oil bath at 70 °C for 18 hours. After, polymer was precipitated in 200 mL of diethyl ether, redissolved in methanol and treated with 5 mL of HCl/MeOH solution overnight. Solution was dialyzed in water to obtain pure product (yield was 88%). To obtain two other PNIPAAm-based diblock terpolymers (32 and 48 kDa), a ratio of CTA to monomers was changed to 1 : 180 : 20 (PNIPAAm : DMA : TMS-MAT) and 1: 420 : 35 (PNIPAAm : DMA : TMS-MAT) respectively and reaction time was increased to 32 or 48 hours (yields were 78% and 85% respectively).

Poly(N,N-dimethyl acrylamide) (PDMA) was synthesized using 4-Cyano-4-(dodecylsulfanylthiocarbonyl) sulfanylpentanoic acid as a chain transfer agent. For this synthesis, N,N-dimethylacrylamide (DMA, 2.00 g, 20.2 mmol) was added to 12 mL of 1,4-dioxane in a 25 nL flask with 4-Cyano-4-(dodecylsulfanylthiocarbonyl)sulfanyl pentanoic acid (32.6 mg, 0.0808 mmol) and AIBN (0.663 mg, 0.00404 mmol). The mixture was capped with a septum and degassed with nitrogen flow for 30 minutes while being submerged in water/ice bath. After, the reaction was placed in a preheated block at 70 °C for 6 hours. When the reaction was completed, the flask was opened to air and cooled using loquid nitrogen. The mixture was precipitated in pentane and polymer was dried in a vacuum overnight (yield was 92%).

Methods.

Size Exclusion Chromatography (SEC) Method: SEC measurements were carried out on an Agilent 1260 Infinity liquid chromatograph equipped with a Waters Styragel guard column and three Waters Styragel columns (HR6, HR4, and HR1; 100-10,000,000 g/mol) to provide effective separation for molecular weight determination. The detectors used were an Agilent 1260 VWD UV-vis detector, a Wyatt Dawn Heleos II light-scattering detector, and a Wyatt Optilab T-rEX refractive-index detector. Tetrahydrofuran was used as the mobile phase at 1.0 mL/min at 25 °C.

Spray drying: Spray drying was performed on a Bend Research Mini Spray Drier under the following conditions: inlet temperature of 68 °C, nitrogen flow rate of 12.8 SLPM, and a 0.65 mL/min syringe flow rate. The SDDs were collected on a 4" Whatman filter. Unless otherwise noted, the total solute content spray dried was always one weight percent. Solutions were sprayed from a THF:MeOH mixture (15:2, v/v). All diblock terpolymers were completely soluble in a THF:MeOH mixture prior to spray drying. The SDD composition is reported as the weight percent (wt %) drug in the dispersion. For example, 30 mg of probucol and 270 mg of polymer were dissolved in 29.7 g of THF:MeOH mixture to make 10 wt% probucol with (PEP-b-P(DMA-*grad*-MAT)). Three different compositions were used for the polymer/drug dispersions: 10, 25, and 50 wt % probucol relative to polymer.

Dynamic Light Scattering (DLS): A Brookhaven Instrument system was used for DLS measurements. It includes a Mini L-30 laser source (λ = 637 nm), a BI-APD avalanche photodiode detector, and a BI-9000AT digital correlator, all aligned on a BI-200SM goniometer with a decaline thermo regulating bath. All samples were filtered using 0.45 μm Teflon syringe

filters. For measurements, polymers were dissolved in THF: MeOH (15:2, v/v) mixture until a 1 wt % concentration was reached. For each sample, the second-order scattering intensity correlation function ($g2(t)$) was measured at a 90 degree angle and converted using the regularized positive exponential sum[4] (REPES) algorithm. The size distribution of aggregates is shown in the Figure S1. All polymer samples show some degree of aggregation, which changes depending on the hydrophobicity of the PNIPAm, PDEAEMA, or PEP blocks.

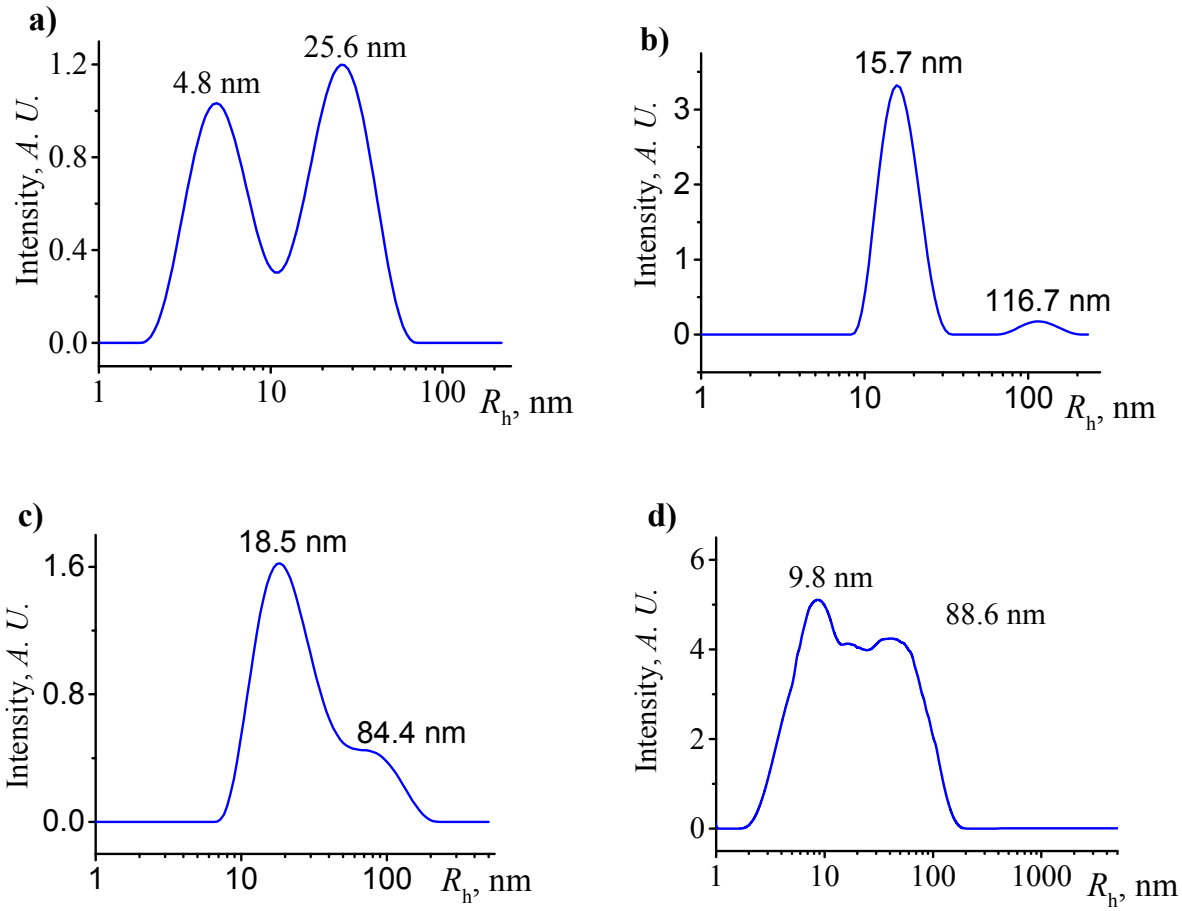

Figure S1. REPES analysis of data obtained from intensity correlation function at a 90° angle for the solutions in THF:MeOH (15:2, v/v) of a) PNIPAm-b-P(MAT-*grad*-DMA) – 32 kDa, b) PDEAEMA-b-P(MAT-*grad*-DMA), c) PEP-b-P(MAT-co-DMA), and d) PNIPAm-b-P(MAT-*grad*-DMA) – 18 kDa. Polymer concentration is at 1 wt %.

Powder X-ray Diffraction (Powder XRD). PXRD experiments were carried out on a Bruker- AXS (Siemens) D5005 diffractometer. Samples (50 mg) were packed into standard 0.5 mm deep glass holders with zero background. The x-ray source (KCuα, λ = 1.54 A) was operated at a voltage of 45 kV and a current of 40 mA. Data for each sample was collected from 5° to 40° on the 2θ scale over approximately 30 minutes at a scan step of 1 seconds and a step size of 0.02°/s.

Differential Scanning Calorimetry (DSC). Modulated differential scanning calorimetry (MDSC) was used to determine the thermal features of the SDDs and was conducted on a TA-Instruments Discovery DSC equipped with an autosampler. Samples from 5−10 mg were placed in T-zero aluminum pans and sealed with a hermetic lid. MDSC analysis was performed with a nitrogen flow rate of 50.00 mL/min and a heating rate of 1 °C/min from 0 to 180 °C. The temperature was modulated at ±2 °C with a period of 40 s. The first heating scans are reported. For polymer only samples (not spray dried), the temperature was not modulated, but was ramped between -50 °C and 180 °C at a rate of 10 °C/min. The second heating scans are reported for those samples. For all samples, TA TRIOS software version 2.2 was used to analyze *T*g values and enthalpic components.

Scanning Electron Microscopy (SEM). A Hitachi S-900 microscope was used, and samples were sputtered with gold/palladium for 30 s at 40 kV on a Denton DV-502A high vacuum deposition system to provide a conductive coating for analysis. SEM was used to obtain particle size and information about morphology data from the SDDs.

All spray dried dispersions with probucol revealed wrinkled, collapsed sphere morphologies according to SEM, which is indicative of the large surface area of the SDD powders that encapsulate the amorphous drug to aid dissolution and supersaturation maintenance.

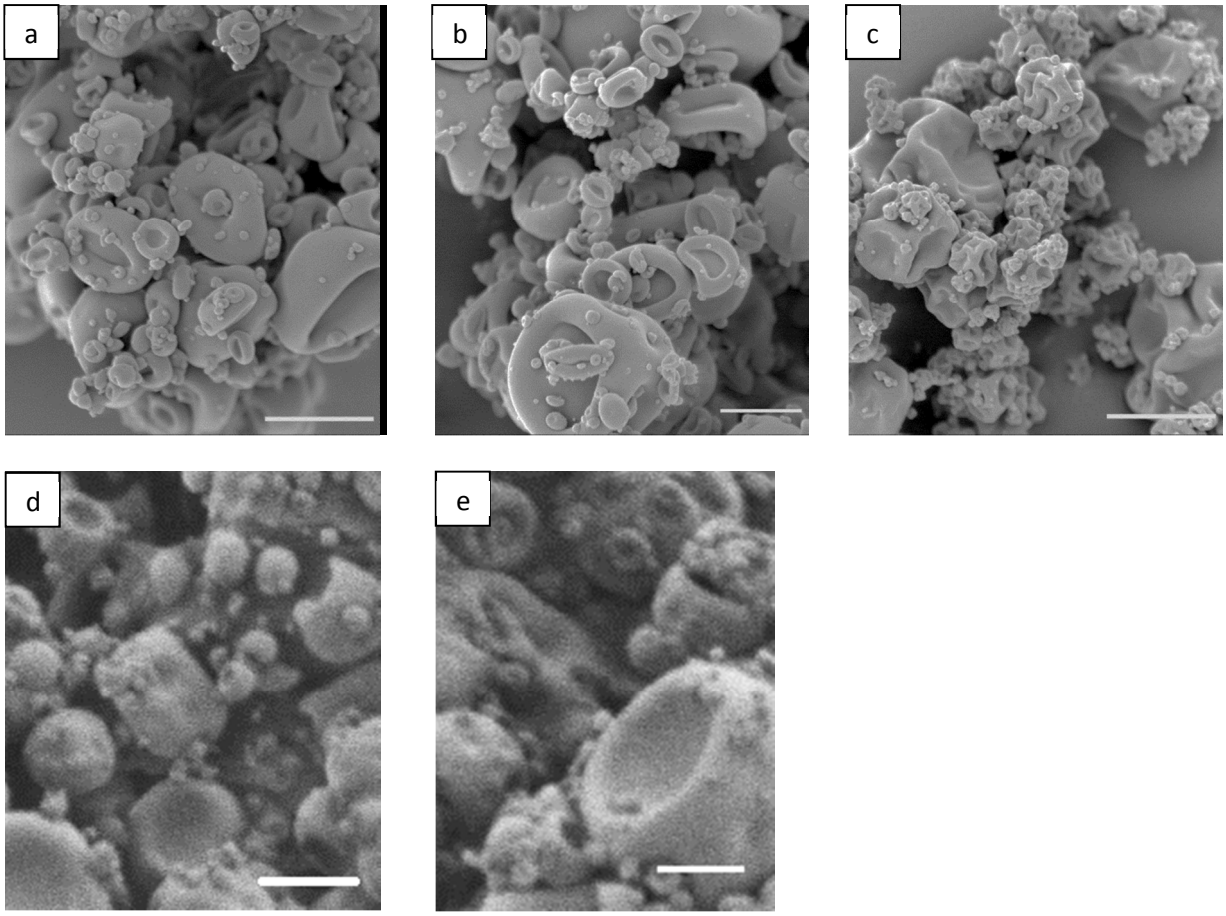

Figure S2. SEM images of the SDDs created by spraying a THF:MeOH (15:2) solution of probucol with (a) PEP-b-P(DMA-*grad*-MAT), (b) PNIPAAm-b-P(DMA-*grad*-MAT) – 32 kDa, (c) PDEAEMA-b-P(DMA-*grad*-MAT), (d) PNIPAAm-b-P(DMA-*grad*-MAT) – 18 kDa, and (e) PNIPAAm-b-P(DMA-*grad*-MAT) – 48 kDa as the polymer excipients at 25 weight percent of drug loading. The scale bars indicate 1 μm.

In Vitro Dissolution Test. Dissolution testing was performed on each SDD formulation and the crystalline drug to determine the concentration of drug in the dissolution media and maintenance of supersaturation. The dissolution medium consisted of phosphate buffer saline (82 mM sodium chloride, 20 mM sodium phosphate dibasic, 47 mM potassium phosphate monobasic) supplemented with 0.5 wt% FaSSIF. The medium was adjusted to pH 6.5 with NaOH. An appropriate amount of SDD or crystalline drug was weighed and added into 2.0 mL microcentrifuge tubes to yield a final total drug concentration of 1000 mg/mL (if all material is fully dissolved). For example: At 10 wt % of drug loading, we took 18.0 mg of SDD consisting of 1.8 mg of drug and 16.2 mg of polymer and diluted the SDD with 1.8 mL of PBS buffer (containing FaSSIF) solution for dissolution testing. All samples were analyzed in duplicate (n = 2). The first step in dissolution testing involved vortexing the samples for 1 min in 1.8 mL of PBS+FaSSIF medium and then placing the sample into an isothermal aluminum heating block held at 37°C. At each time point (4, 10, 20, 40, 90, 180, and 360 min), tubes were removed from the heating blocks and centrifuged at 13,000 rpm, 37 °C for 1 min to remove undissolved drug from dissolved drug, and then a 50 μL aliquot of the supernatant was transferred to an HPLC vial. The samples were again vortexed for 30 s and held at 37 °C until the next time point. The supernatant in the HPLC vials was then diluted with 250 μL of methanol and analyzed for drug via HPLC.

Reverse Phase HPLC. Drug concentration in each aliquot was determined by reverse phase HPLC. The HPLC consisted of a reversed-phase EC-C18 column (Poroshell 120, 4.6 × 50 mm, 2.7 μm, Agilent, USA). A mobile phase of 96:4 (v/v) acetonitrile:water was used for probucol detection with a flow rate of 1.0 mL/min at 30 °C. A 10 μL aliquot of sample was injected, and the column effluent was detected at 241 nm with a UV detector (1260 Infinity

Multiple Wavelength Detector, Agilent). The probucol concentration in the samples was determined using a calibration curve of 0.1−500 μg/mL concentrations.

Table S1. Molecular characterization of the homopolymers

Sample	[AIBN] : [CTA] : [Monomer]	$[M_0]$	Time (h)	Conv. of monomer	M_n, kg/mole[a]	$Đ$[b]
PNIPAm-CTA	0.05 : 1 : 50	1.69	6	99%	5.6	1.07
PDEAEMA-CTA	0.05 : 1 : 50	1.69	8	87%	8.0	1.15
PDMA	0.05 : 1 : 250	1.80	6	93%	23	1.17

[a]The number average molecular weight of the poly (CTA) as determined by ^{1}H NMR spectroscopy. [b]The dispersities of poly (CTA) as determined by SEC using THF as a mobile phase.

Note: CTA used: 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid.

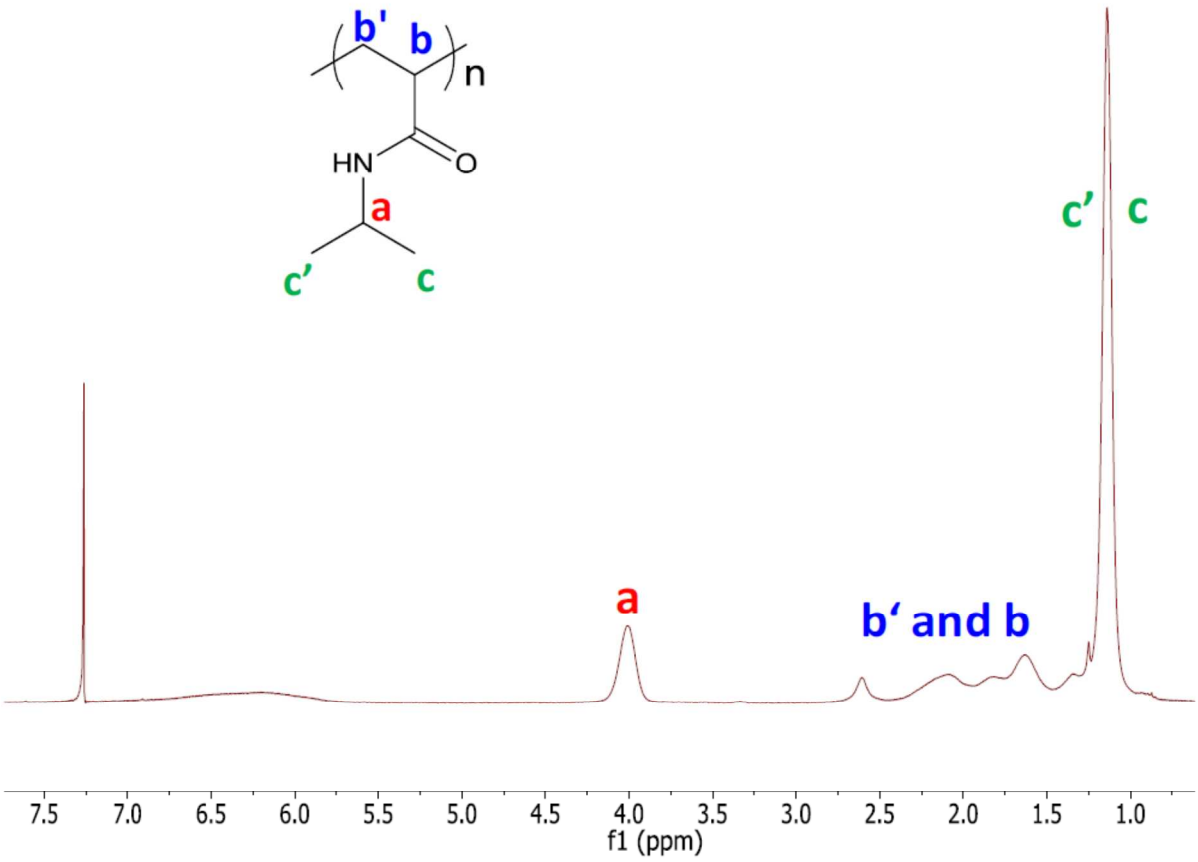

Figure S3: ^{1}H NMR spectrum recorded for PNIPAAm-CTA in $CDCl_3$

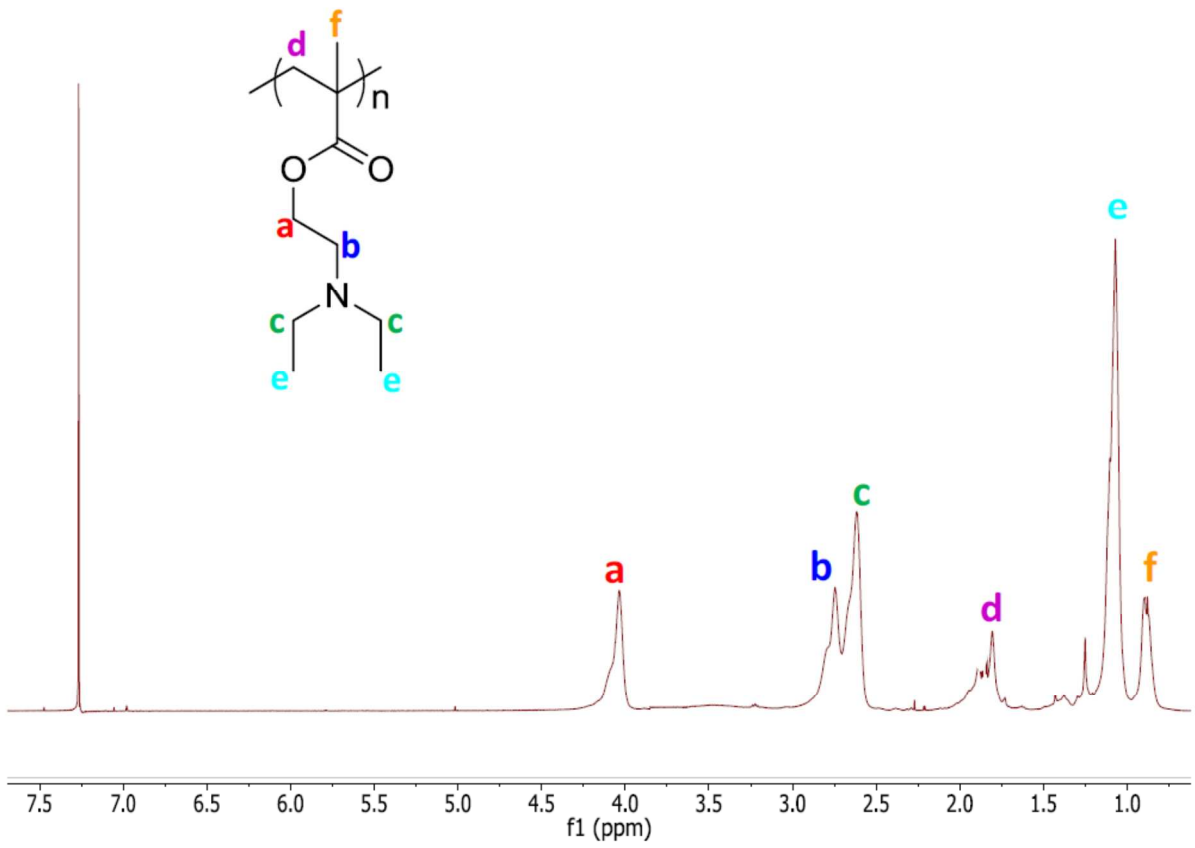

Figure S4: ^{1}H NMR spectrum recorded for PDEAEMA-CTA in $CDCl_3$

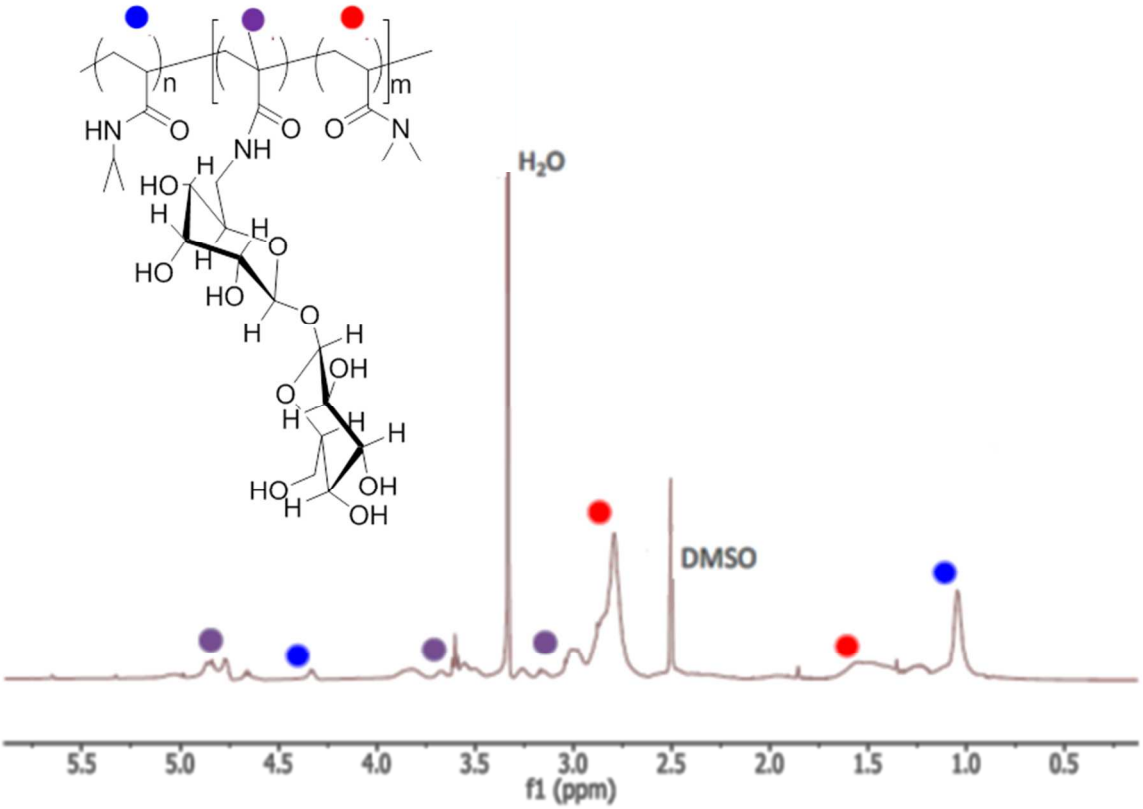

Figure S5: ^{1}H NMR spectrum recorded for PNIPAm-b-P(DMA-*grad*-MAT) in d6-DMSO

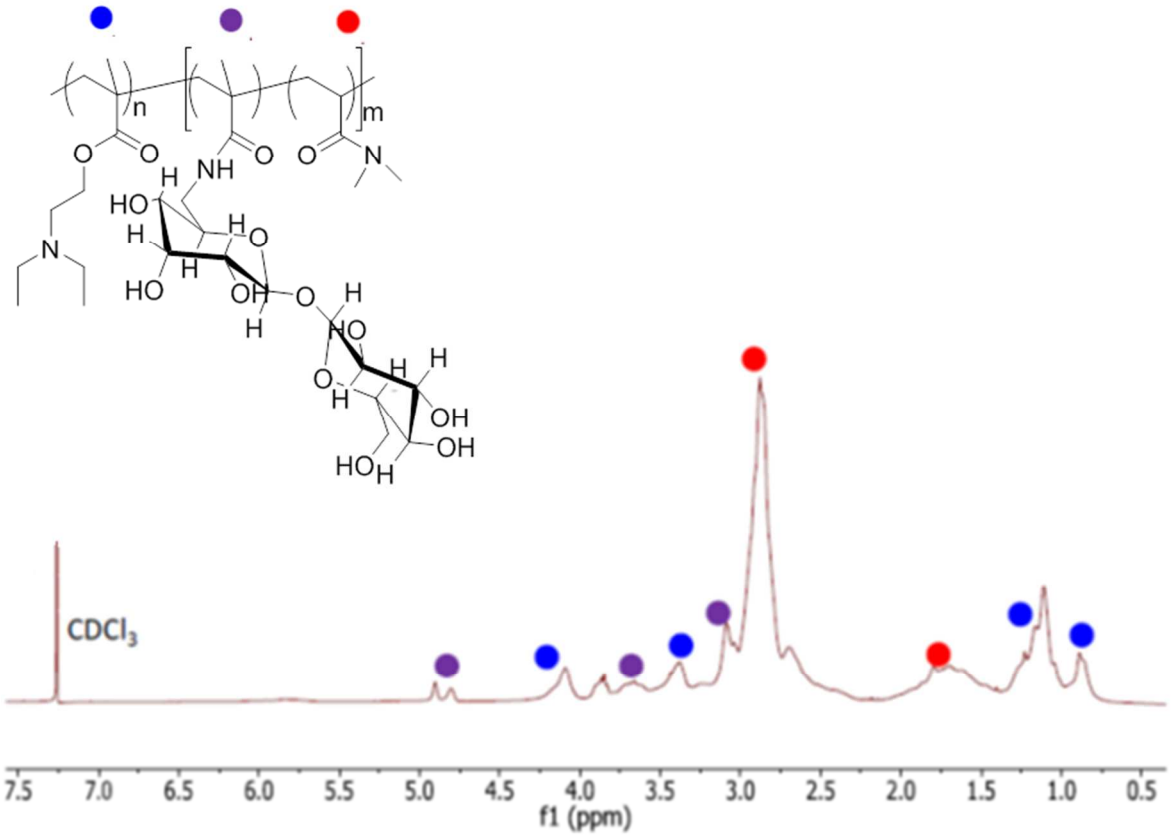

Figure S6: ^{1}H NMR spectrum recorded for PDEAEMA-b-P(DMA-*grad*-MAT) in $CDCl_3$

Table S2. Comparison of the weight fraction of monomers and drugs in the SDDs and the percent of probucol crystallinity.

Sample	SDD weight fraction					Probucol loading	% PBC Crystallinity
	PEP	PNIPAm	PDEAEMA	PDMA	PMAT		
PEP-b-P(DMA-*grad*-MAT)	0.06	0	0	0.31	0.13	0.50	42.1
PNIPAm-b-P(DMA-*grad*-MAT) – 18 kDa	0	0.16	0	0.24	0.10	0.50	25.7
PNIPAm-b-P(DMA-*grad*-MAT) – 32 kDa	0	0.09	0	0.29	0.12	0.50	35.8
PNIPAm-b-P(DMA-*grad*-MAT) – 48 kDa	0	0.07	0	0.31	0.12	0.50	34.0
PDEAEMA-b-P(DMA-*grad*-MAT)	0	0	0.13	0.26	0.11	0.50	0

Table S3. MDSC analysis of SDDs with probucol.

Probucol Loading	SDD	T_g (°C)	T_{c1} (°C)	Enthalpy (J/g)	T_{c2} (°C)	Enthalpy (J/g)	T_{m1} (°C)	Enthalpy (J/g)	T_{m2} (°C)	Enthalpy (J/g)	% PBC Crystallinity
10 wt %	PEP-b-P(DMA-*grad*-MAT)	106.4	-	-	-	-	-	-	-	-	-
	PNIPAm-b-P(DMA-*grad*-MAT) – 18 kDa	104.2	-	-	-	-	-	-	-	-	-
	PNIPAm-b-P(DMA-*grad*-MAT) – 32 kDa	127.6	-	-	-	-	-	-	-	-	-
	PNIPAm-b-P(DMA-*grad*-MAT) – 48 kDa	141.1	-	-	-	-	-	-	-	-	-
	PDEAEMA-b-P(DMA-*grad*-MAT)	150.2	-	-	-	-	-	-	-	-	-
25 wt %	PEP-b-P(DMA-*grad*-MAT)	105.1	-	-	-	-	107.1	5.2	-	-	14.1
	PNIPAm-b-P(DMA-*grad*-MAT) – 18 kDa	102.8	74.3	3.8	101.1	2.3	106.3	5.3	-	-	9.3
	PNIPAm-b-P(DMA-*grad*-MAT) – 32 kDa	116.6	80.2	2.6	-	-	107.6	4.8	-	-	12.9
	PNIPAm-b-P(DMA-*grad*-MAT) – 48 kDa	118.8	80.4	3.2	-	-	108.1	5.9	-	-	14.2
	PDEAEMA-b-P(DMA-*grad*-MAT)	126.9	-	-	-	-	-	-	-	-	-
50 wt %	PEP-b-P(DMA-*grad*-MAT)	104.3	56.8	15.9	-	-	107.9	28.9	120.3	1.46	42.1
	PNIPAm-b-P(DMA-*grad*-MAT) – 18 kDa	103.7	78.2	3.4	101.3	1.3	114.2	15.3	123.4	2.1	28.4
	PNIPAm-b-P(DMA-*grad*-MAT) – 32 kDa	105.9	74.9	5.3	98.2	2.1	112.4	18.4	121.1	1.2	35.8
	PNIPAm-b-P(DMA-*grad*-MAT) – 48 kDa	112.2	73.1	5.7	98.3	2.4	113.2	24.3	-	-	40.2
	PDEAEMA-b-P(DMA-*grad*-MAT)	111.1	-	-	-	-	-	-	-	-	-

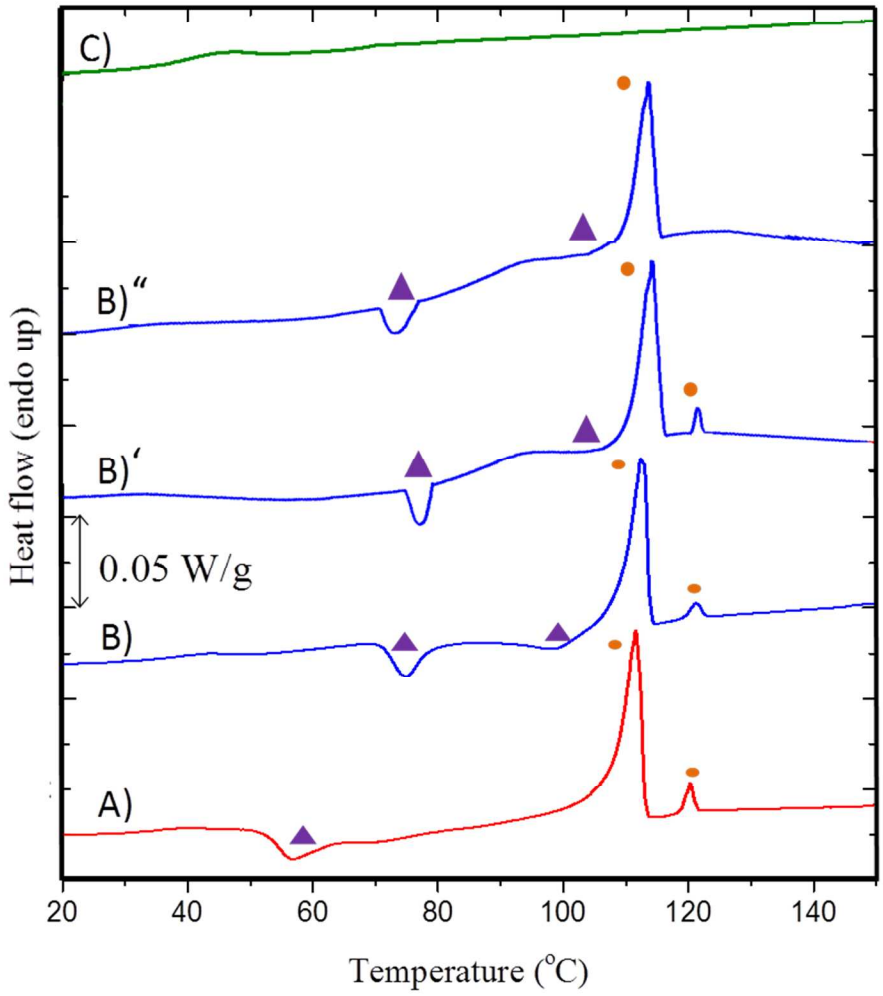

Figure S7. MDSC thermograms of total heat flow from SDDs containing 50 weight percent of probucol A) PEP-b-P(DMA-*grad*-MAT), B) PNIPAm-b-P(DMA-*grad*-MAT) – 32 kDa, B)' – PNIPAm-b-P(DMA-*grad*-MAT) – 18 kDa, B)'' – PNIPAm-b-P(DMA-*grad*-MAT) – 48 kDa, and C) PDEAEMA–b-P(DMA-*grad*-MAT).

Table S4. Calculated area under the curve (AUC) for solubilization of probucol and all polymer excipient SDD formulations at 10, 25 and 50 weight percent drug loading.

Polymer	10 wt %		25 wt %		50 wt %	
	AUC_{360min} (µg/mL)	AUC_{360min}/ AUC_{360max} (µg/mL)	AUC_{360min} (µg/mL)	AUC_{360min}/ AUC_{360max} (µg/mL)	AUC_{360min} (µg/mL)	AUC_{360min}/ AUC_{360max} (µg/mL)
HPMCAS – 6.5	3.4×10^5	0.97	3.3×10^5	0.92	2.3×10^5	0.64
PEP-b-P(DMA-*grad*-MAT) – 6.5	4.8×10^4	0.13	6.7×10^4	0.19	4.2×10^4	0.12
PNIPAm-b-P(DMA-*grad*-MAT) – 6.5 (18 kDa)	3.1×10^5	0.84	1.6×10^5	0.43	9.8×10^4	0.26
PNIPAm-b-P(DMA-*grad*-MAT) – 6.5 (32 kDa)	3.7×10^5	~1.0	3.7×10^5	~1.0	6.6×10^4	0.18
PNIPAm-b-P(DMA-*grad*-MAT) – 6.5 (48 kDa)	3.7×10^5	~1.0	3.6×10^5	0.97	7.2×10^4	0.19
PDEAEMA-b-P(DMA-*grad*-MAT) – 6.5	3.9×10^2	0	5.1×10^3	0.01	2.3×10^4	0.06
PDEAEMA-b-P(DMA-*grad*-MAT) – 5.1	4.2×10^2	0	1.2×10^4	0.03	2.4×10^4	0.07
PDEAEMA-b-P(DMA-*grad*-MAT) – 3.1	2.9×10^5	0.81	3.1×10^5	0.86	3.2×10^5	0.89
PDMA – 6.5	3.0×10^6	0.83	2.9×10^4	0.08	1.1×10^4	0.03

The AUC_{360min} values for PDMA at pH 6.5 were obtained from previously published work.[3]

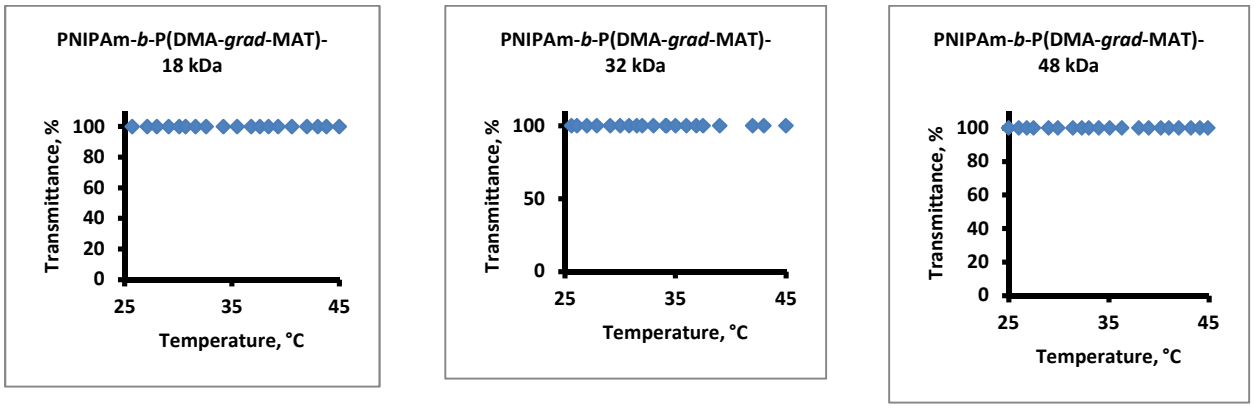

Figure S8. Cloud point transition measurement for (a) PNIPAm-*b*-P(DMA-*grad*-MAT) – 18 kDa, (b) PNIPAm-*b*-P(DMA-*grad*-MAT) – 32 kDa, and (c) PNIPAm-*b*-P(DMA-*grad*-MAT) – 48 kDa. Solvent – water, polymer concentration – 1 mg/ml.

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