Supporting information

Efficient Synthesis of Nitric Oxide-Releasing Hollow Double-Layered Polymer Microspheres with Multiple Stimuli-Responsive Properties

Tuanwei Liu, Wei Zhang, Tao Song, Xinlin Yang*, and Chenxi Li

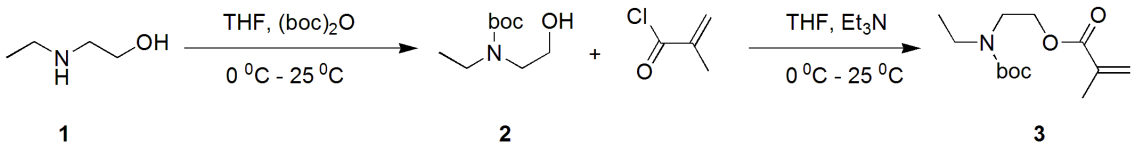
Key Laboratory of Functional Polymer Materials, Ministry of Education, Institute of Polymer Chemistry, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin 300071, P. R. China

* Corresponding author: Tel: +86-22-23502023, Fax: +86-22-23503510, E-mail: xlyang88@nankai.edu.cn

1. Materials

Tetraethyl orthosilicate ($Si(OEt)_4$, TEOS)) was purchased from Aldrich and used without any further purification. 3-(Methacryloxy) propyltrimethoxysilane (MPS) was available from Aldrich and distilled under vacuum. Ethylene glycol dimethacrylate (EGDMA, Alfa Aesar, 98 %) was purified by distillation under vacuum. Benzoyl peroxide (BPO) (analytical grade from Chemical Factory of Nankai University) was recrystallized from methanol. Acetonitrile (analytical grade, Tianjin Chemical Reagents II Co.) was dried over 4 Å molecular sieves and purified by distillation. Tetrahydrofuran (THF, Tianjin Jiangtian Chemicals, 99%) was refluxed over sodium and then distilled. 2-(Ethylamino)ethanol (Tianjin Heowns Biochemical Technology Co., Ltd., China, 98 %). Di-*tert*-butyl dicarbonate and methacryloyl chloride were purchased from Tianjin Heowns Biochemical Technology Co., Ltd., China). N-Isopropylacrylamide (NIPAAm) was purchased from Acros Co. and recrystallized from hexane. 2-(dimethylamino) ethyl methacrylate (DMAEMA, Tianjin heowns Biochemical Technology Co., Ltd., China, 97 %). Nitric oxide (NO) with high purity was bought from Tianjin Best Gas Co. Ltd, China. Griess reagent (Shanghai Beyotime Institute of Biotechnology, S0021). Trifluoroacetic acid (TFA, Tianjin Heowns Biochemical Technology Co., Ltd., China, 98 %). Hydrofluoric acid (Tianjin Guangfu chemical Co., Ltd., China).

2. Synthesis of Monomer Compound



Scheme S1. Synthesis of AmEMA precursor **2** and bocAmEMA monomer **3**.

2.1 Synthesis of 2-(Ethyl(boc)amino)ethanol (bocAmE)

To a vigorously stirred solution of 0.1 mol of amine alcohol in 60 mL of dry THF, 0.11 mol of di-*tert*-butyl dicarbonate was added dropwise at 0 °C. After stirred overnight at 25 °C, the reaction mixture was washed with ethyl acetate and water. The organic phase was dried with sodium sulfate and evaporated in vacuo. The residue was used without any further purification for the next step of the reaction.

2.2 Synthesis of monomer 2-(Ethyl(boc)amino)ethyl methacrylate (bocAmEMA).

A solution containing the boc-protected 2-(ethyl(boc)amino)ethanol and 0.1 mol of triethylamine was prepared in dry THF at 0 °C. Then, 0.1 mol of methacryloyl chloride was added dropwise maintaining the temperature below 5 °C. After stirred overnight at 25 °C, the reaction mixture was filtered, and the filtrate was washed with ethyl acetate and water. After removal of solvent, the crude product was purified by column chromatography on silica gel using a mixture of ethyl acetate and petroleum ether (1:5) as eluant to afford the monomer compound **bocAmEMA** as a white oil. ^{1}H NMR ($CDCl_3$, 400 MHz): 6.13 (s, 1H), 5.59 (s, 1H), 4.25 (s, 2H), 3.50 (t, 2H), 3.24 (q, 2H), 1.95 (s, 3H), 1.46 (s, 9H), 1.12 (s, 3H).

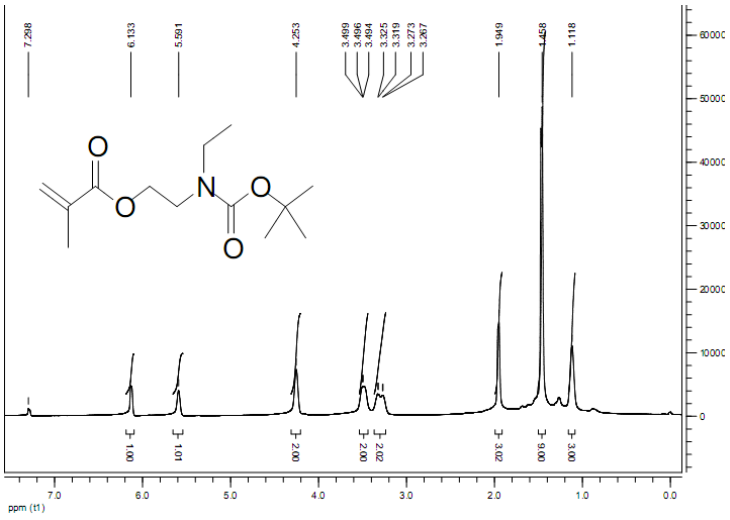


Figure S1. The ^{1}H spectrum of the monomer compound **bocAmEMA.**

3. Synthesis of MPS-modified silica microspheres

Silica microspheres were prepared according to the classical Stober method: 12 mL of TEOS was added to the mixture of 200 mL ethanol, 20 mL de-ionized water and 15 mL aqueous solution of 25% ammonium with vigorous stirring at room temperature and the reaction was continued further for 24 h with stirring. The MPS-modified silica microspheres were afforded by coupling silica alcosol particles according to the literature: excess MPS (1.0 g, 4.0 mmol) was introduced into 20 mL of the silica mixture under stirring in a 50 mL round-bottom flask. Coating of silica particles with MPS was achieved by stirring the mixture of alcosol silica particles and MPS for 48 h at room temperature. The resultant MPS-modified silica particles were purified by three cycles of centrifugation, decantation, and resuspension in ethanol with ultrasonic-bathing. The MPS-modified silica particles were dried in a vacuum oven at 50 °C till constant weight.

4. Preparation of the control double-layered core-shell microspheres

The preparation of SiO_2/P(bocAmEMA-*co*-EGDMA)/PEGDMA, SiO_2/P(bocAmEMA-*co*-EGDMA)/P(NIPAAm-*co*-EGDMA), and SiO_2/P(bocAmEMA-*co*-EGDMA)/P(DMAEMA-*co*-EGDMA)double-layered core-shell composite microspheres were performed via distillation precipitation polymerization in acetonitrile with BPO as initiator in presence of SiO_2/P(BMA-*co*-EGDMA) core-shell microspheres as templates.

A typical procedure for SiO_2/P(bocAmEMA-*co*-EGDMA)/PEGDMA polymerization is as follows: 100 mg of SiO_2/P(BMA-*co*-EGDMA) core-shell microspheres were suspended in 40 mL of acetonitrile as seeds of the polymerization. Then EGDMA (900 ul, 4.5 mmol) and BOP (12 mg) were dissolved in the suspension. The flask was submerged in a heating mantle and the reaction mixture was heated from room temperature to the

boiling state. After 20 mL of acetonitrile was distilled out of the reaction system, the reaction was ended. The resultant SiO_2/P(bocAmEMA-*co*-EGDMA)/PEGDMA microspheres were purified by repeating centrifugation, decantation and resuspension in acetonitrile for three times.

A typical procedure for SiO_2/P(bocAmEMA-*co*-EGDMA)/P(NIPAAm-*co*-EGDMA) polymerization is as follows: 100 mg of SiO_2/P(BMA-*co*-EGDMA) core-shell microspheres were suspended in 40 mL of acetonitrile as seeds of the polymerization. Then NIPAAm (320 mg, 2.8 mmol), EGDMA (360 ul, 1.8 mmol) and BOP (12 mg) were dissolved in the suspension. The flask was submerged in a heating mantle and the reaction mixture was heated from room temperature to the boiling state. After 20 mL of acetonitrile was distilled out of the reaction system, the reaction was ended. The resultant SiO_2/P(bocAmEMA-*co*-EGDMA)/ P(NIPAAm-*co*-EGDMA)microspheres were purified by repeating centrifugation, decantation and resuspension in acetonitrile for three times.

A typical procedure for SiO_2/P(bocAmEMA-*co*-EGDMA)/P(DMAEMA-*co*-EGDMA) polymerization is as follows: 100 mg of SiO_2/P(BMA-*co*-EGDMA) core-shell microspheres were suspended in 40 mL of acetonitrile as seeds of the polymerization. Then DMAEMA (480 ul, 2.8 mmol), EGDMA (360 ul, 1.8 mmol) and BOP (12 mg) were dissolved in the suspension. The flask was submerged in a heating mantle and the reaction mixture was heated from room temperature to the boiling state. After 20 mL of acetonitrile was distilled out of the reaction system, the reaction was ended. The resultant SiO_2/P(bocAmEMA-*co*-EGDMA)/P(DMAEMA-*co*-EGDMA)microspheres were purified by repeating centrifugation, decantation and resuspension in acetonitrile for three times.

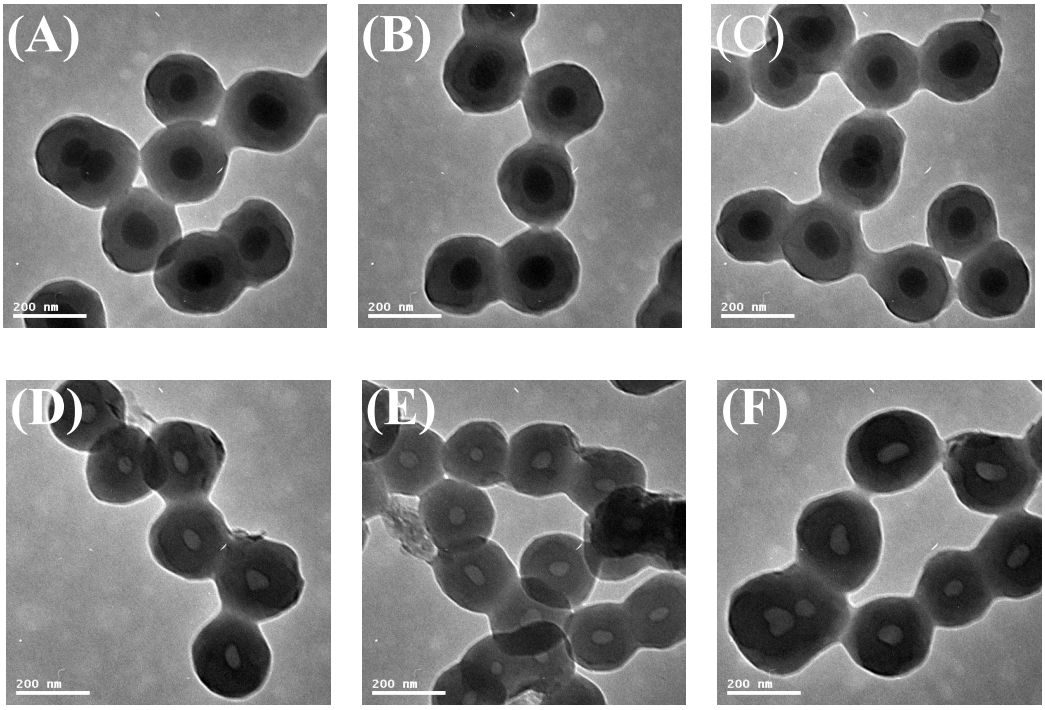


Figure S2 TEM micrographs: A) SiO_2/P(bocAmEMA-*co*-EGDMA)/PEGDMA double-layered core-shell microspheres **; B)** SiO_2/P(bocAmEMA-*co*-EGDMA)/P(PNIPAAm -*co*-EGDMA) double-layered core-shell microspheres**; C)** SiO_2/P(bocAmEMA-*co*-EGDMA)/P(DMAEMA-*co*-EGDMA) double-layered core-shell microspheres**;** D) Hollow P(AmEMA-*co*-EGDMA)/PEGDMA double-layered microspheres**;** E) Hollow P(AmEMA-*co*-EGDMA)/P(PNIPAAm-*co*-EGDMA) double-layered microspheres**;** and F) Hollow P(AmEMA-*co*-EGDMA)/P(DMAEMA-*co*-EGDMA) double-layered microspheres.

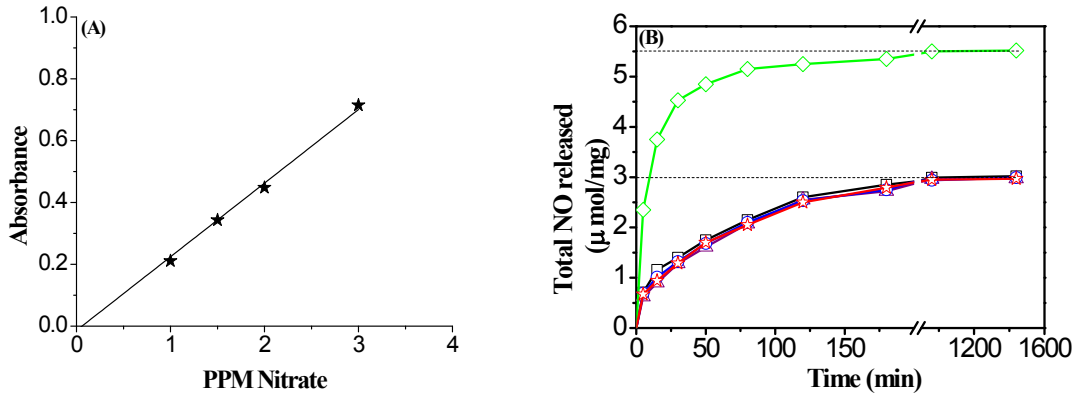


Figure S3 The standard working curve in 10^{-3} M KCl aqueous solution (A); And the total amount of the released NO *vs* time from 5 mg of *N*-diazeniumdiolated naked hollow P(AmEMA-*co*-EGDMA) (Green diamond), hollow double-layered P(AmEMA-*co*-EGDMA)/PEGDMA (Black square), hollow double-layered P(AmEMA-*co*-EGDMA)/P(NIPAAm-*co*-EGDMA) (Purple uptriangle), hollow double-layered P(AmEMA-*co*-EGDMA)/P(DMAEMA-*co*-EGDMA) (Blue circle), and hollow double-layered P(AmEMA-*co*-EGDMA)/P(NIPAAm-*co*-DMAEMA-*co*-EGDMA) (Red star) in 5 mL of aqueous with pH of 7.4 at 37 ^{o}C (**B**).

Table S1 The detail half life times of the NO release from *N*-diazeniumdiolated hollow double-shelled microspheres with different polymeric outer-layer

Entry	pH	Temp. (^{0}C)	-	Half-life ($t_{1/2}$, min)			
			-	Hollow P(AmEMA-*co*-EGDMA)/ PEGDMA microspheres	Hollow P(AmEMA-*co*-EGDMA)/ P(NIPAAm-*co*-EGDMA) microspheres	Hollow P(AmEMA-*co*-EGDMA)/ P(DMAEMA-*co*-EGDMA) microspheres	Hollow P(AmEMA-*co*-EGDMA)/ P(NIPAAm-*co*-DMAEMA-*co*-EGDMA) microspheres
1	7.4	20	Mean value	40	38	45	40
			S1-1	39	35	47	38
			S1-2	43	39	42	41
			S2-1	41	43	48	42
			S2-2	37	37	43	39
2	7.4	30	Mean value	36	36	40	36
			S1-1	35	34	41	34
			S1-2	33	35	37	39
			S2-1	39	38	39	38
			S2-2	37	37	43	33
3	7.4	37	Mean value	34	44	40	41
			S1-1	33	43	39	43
			S1-2	34	42	42	44
			S2-1	34	46	41	39
			S2-2	35	45	38	38
4	7.4	45	Mean value	17	43	35	42
			S1-1	16	41	33	43
			S1-2	19	44	37	45
			S2-1	15	42	36	39
			S2-2	18	45	34	41
5	7.4	55	Mean	11	28	15	38

			value				
			S1-1	9	26	16	39
			S1-2	12	31	15	41
			S2-1	13	30	14	35
			S2-2	10	25	15	37
6	7.4	60	Mean value	10	15	10	19
			S1-1	9	16	9	18
			S1-2	11	13	11	21
			S2-1	12	14	10	17
			S2-2	8	17	10	20
7	4.0	37	Mean value	9	8	9	10
			S1-1	11	9	10	11
			S1-2	9	7	7	10
			S2-1	9	8	11	9
			S2-2	7	8	8	10
8	5.0	37	Mean value	10	18	25	40
			S1-1	11	19	23	40
			S1-2	8	16	26	39
			S2-1	12	20	24	41
			S2-2	9	17	27	40
9	6.0	37	Mean value	15	30	45	45
			S1-1	16	32	46	46
			S1-2	13	30	44	45
			S2-1	17	30	43	45
			S2-2	14	28	47	44
10	7.0	37	Mean value	30	35	55	45
			S1-1	32	34	54	44
			S1-2	28	36	55	47
			S2-1	29	36	55	46
			S2-2	31	34	53	43
11	8.0	37	Mean	40	40	55	50

			value				
			S1-1	41	39	57	49
			S1-2	40	42	54	48
			S2-1	39	41	56	51
			S2-2	40	38	53	52
12	9.0	37	Mean value	110	60	67	60
			S1-1	106	58	69	58
			S1-2	113	63	66	61
			S2-1	107	57	68	59
			S2-2	114	62	65	62
13	10.0	37	Mean value	200	170	150	140
			S1-1	191	168	146	134
			S1-2	203	177	152	142
			S2-1	197	172	148	146
			S2-2	209	163	154	138
14	11.0	37	Mean value	300	400	450	430
			S1-1	309	403	441	422
			S1-2	294	389	456	439
			S2-1	306	397	444	438
			S2-2	291	411	459	421