SUPPORTING INFORMATION

Π- Π Stacking Increases the Stability and Loading Capacity of Thermosensitive Polymeric Micelles for Chemotherapeutic Drugs

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1. Characterizations of mPEG-*b*-poly(HPMAm-Bz/Nt-*co*-HPMAm-Lac) block copolymers by gel permeation chromatograph (GPC) and nuclear magnetic resonance (NMR).

Table S1. Characteristics of mPEG-*b*-poly(HPMAm-Bz/Nt-*co*-HPMAm-Lac)

feed ratio of HPMAm-Bz to HPMAm-Lac (mol/mol)	mol % of HPMAm-Bz in copolymers (^{1}H NMR)	yield (%)	M_n (NMR)	M_n (GPC)	M_w (GPC)	PDI (M_w/M_n)
10/90	11.8	85	17	16	28	1.8
15/85	16.1	87	19	16	29	1.7
20/80	20.2	90	20	18	31	1.7
25/75	24.1	72	15	17	29	1.7
30/70	26.9	86	17	14	23	1.7
35/65	30.4	87	21	17	29	1.7
40/60	33.1	82	20	18	32	1.8
50/50	48.5	84	18	22	59	2.7
75/25	74.2	83	21	17	25	1.7
feed ratio of HPMAm-Nt to HPMAm-Lac (mol/mol)	mol % of HPMAm-Nt in copolymer (^{1}H NMR)	yield (%)	M_n (NMR)	M_n (GPC)	M_w (GPC)	PDI (M_w/M_n)
5/95	5.7	84	18	17	30	1.7
10/90	15.8	83	16	15	27	1.8
15/85	17.5	85	16	16	27	1.7
20/80	23.6	84	16	16	27	1.7
25/75	28.4	77	16	16	29	1.8
30/70	34.2	67	12	17	29	1.7
40/60	37.4	75	16	16	27	1.7
50/50	48.9	75	18	22	44	1.9
75/25	72.6	74	16	16	28	1.7

Table S2. Compositions of mPEG-*b*-poly(HPMAm-Bz/Nt-*co*-HPMAm-Lac) at low conversion

feed ratio of HPMAm-Bz to HPMAm-Lac	mol % of HPMAm-Bz in copolymers (^{1}H NMR)	Conversion of HPMAm-Bz (%)	Conversion of HPMAm-monolactate (%)

(mol/mol)			
25/75	26.1	6.9	6.5
75/25	72.6	8.1	9.2
feed ratio of HPMAm-Nt to HPMAm-Lac (mol/mol)	mol % of HPMAm-Nt in copolymer (^{1}H NMR)	Conversion of HPMAm-Nt (%)	Conversion of HPMAm-monolactate (%)
25/75	24.9	7.7	7.8
75/25	74.0	5.7	6.0

Polymerization mixtures with 25 or 75% HPMAm-Bz or HPMAm-Nt were quenched in liquid N_2 after around 50-70 minutes under the polymerization condition described in the main text, section 2.2. The compositions of the obtained polymers were examined by ^{1}H NMR spectroscopy (Table S2). The results show that the compositions of the polymers formed at early stage with relatively low monomers conversions are close to that of feed, which means that HPMAm-Bz/Nt and HPMAm-Lac have similar relativities yielding random copolymers.

2. Thermal decomposition kinetics of the mPEG$_2$-ABCPA macroinitiator

In a dried flask, 100 mg of mPEG$_2$-ABCPA macroinitiator was charged and 2 mL of dried ACN was added. 31.2 mg of TEMPO (20 molar equivalents of the macroinitiator) was added to the mixture to quench free radicals formed by thermal cleavage of the macroinitiator. The flask was immersed in an oil bath at 70 ^{o}C and samples were taken at predetermined time points and then diluted 10 time with DMF containing 10 mM LiCl. The samples were analyzed by GPC with the method described in the main text, section 2.3. The mPEG$_2$-ABCPA macroinitiator has a M_n of 10 kDa. The formed mPEG free radicals with a M_n of 5 kDa were quenched by TEMPO and therefore mPEG of 5 kDa was generated. By comparing the percentage of area under curve (% AUC) of PEG 5 kDa and 10 kDa traces, the amount of formed mPEG 5 kDa was calculated.

Figure S1 shows that the concentration of mPEG 5 kDa increases in time at 70 ^{o}C. The decomposition half-life of the macroinitiator ($t_{1/2}$) is 5.1 h in ACN, which is close to that of 4,4'-azobis(4-cyanopentanoic acid) (from which the macroinitiator was synthesized) which is 4.2 h in acetone. From this figure it is concluded that 18 h of heating at 70 ^{o}C is sufficient for full decomposition of the macroinitiator. The lower amount of PEG 5 kDa formed in the absence of TEMPO points to the combination of two PEG 5000 radicals to yield PEG 10 kDa.

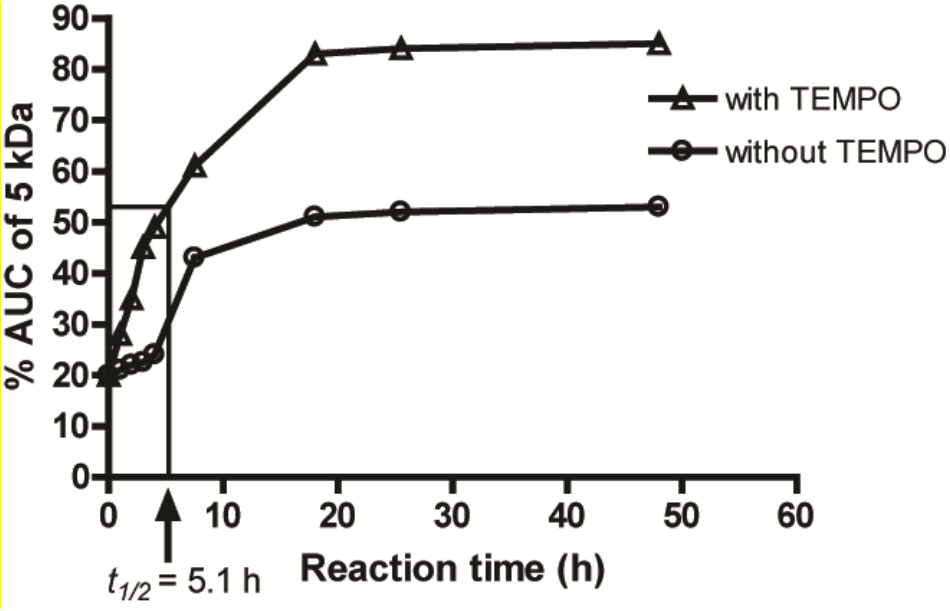

Figure S1. % AUC of mPEG 5 kDa at as a function of time.

3. Fluorescence excitation spectra of pyrene in aqueous solutions with different copolymers.

The excitation spectra of mPEG-*b*-poly(HPMAm-Bz$_{20}$-*co*-HPMAm-Lac$_{80}$), mPEG-*b*-poly(HPMAm-Nt$_{24}$-*co*-HPMAm-Lac$_{76}$) and pyrene were recorded as described in Section 2.5 in main text. The excitation spectra of the three samples were shown in Figure S2.

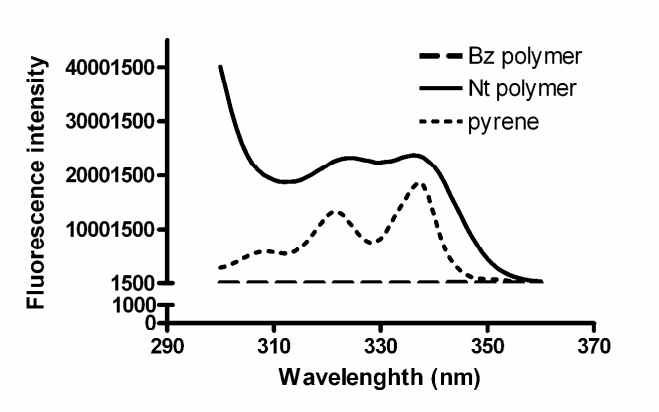

Figure S2. Excitation spectra of pyrene and copolymers in aqueous solutions at 37 ^{o}C.

Emission wavelength was 390 nm, Bz polymer: mPEG-*b*-poly(HPMAm-Bz_{20}-*co*-HPMAm-Lac_{80}), Nt polymer: mPEG-*b*-poly(HPMAm-Nt_{24}-*co*-HPMAm-Lac_{76}). The concentration of the polymer and pyrene were 1 mg/ml and 1.8×10^{-4} M, respectively.

4. Monomer degradation.

Stock solutions (1 M) of HPMAm-Bz/Nt in DMSO were prepared and then diluted 100 times with DMSO. Na_2CO_3 and $NaHCO_3$ (7/3, w/w) were dissolved in RO water at a concentration of 0.1 M and the pH was adjusted to 10.0. AMPSO, NaH_2PO_4 and Na_2HPO_4 were dissolved in RO water at a concentration of 0.1 M and the pHs were adjusted to 9.0 and 7.4, respectively. Next, 0.5 ml of the solutions of HPMAm-Bz/Nt in DMSO (10 mM) were added to 4.5 mL of buffers of different pHs in glass vials. The solutions were incubated at 37 ^{o}C and at different time points, samples were drawn and analyzed using HPLC. HPLC analysis was done using a SunFire C18 (5μm, 4.6×150mm) column and detection was done at 254 nm. A gradient system was run using eluent A of ACN/H_2O (95/5, v/v, with 1% perchloric acid) and B of ACN containing 1% perchloric acid. The gradient was run from 100% A to 100% B in 15 min. The injection volume was 20 μL and the running time was 20 min.

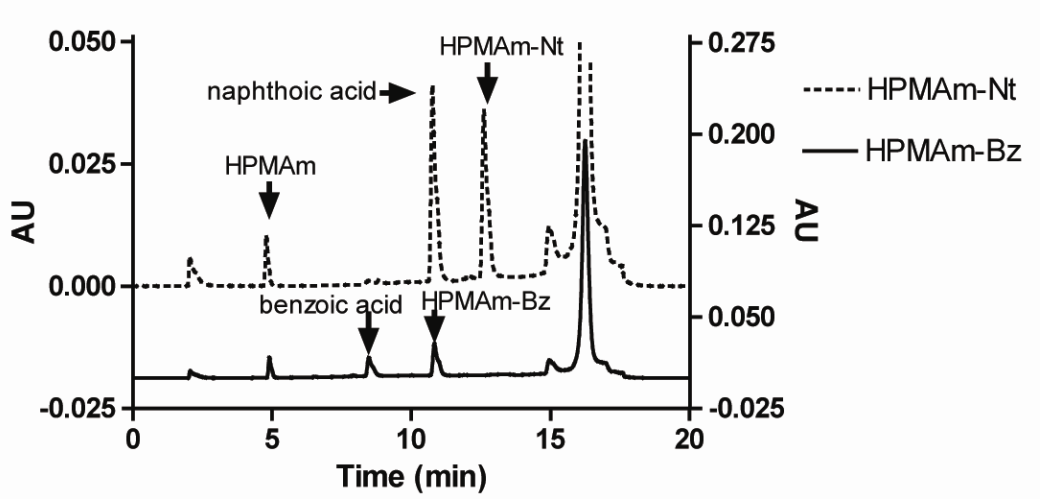

Figure S3. Chromatograms of the monomers (HPMAm-Bz and HPMAm-Nt) and their degradation products (HPMAm, benzoic acid and naphthoic acid).

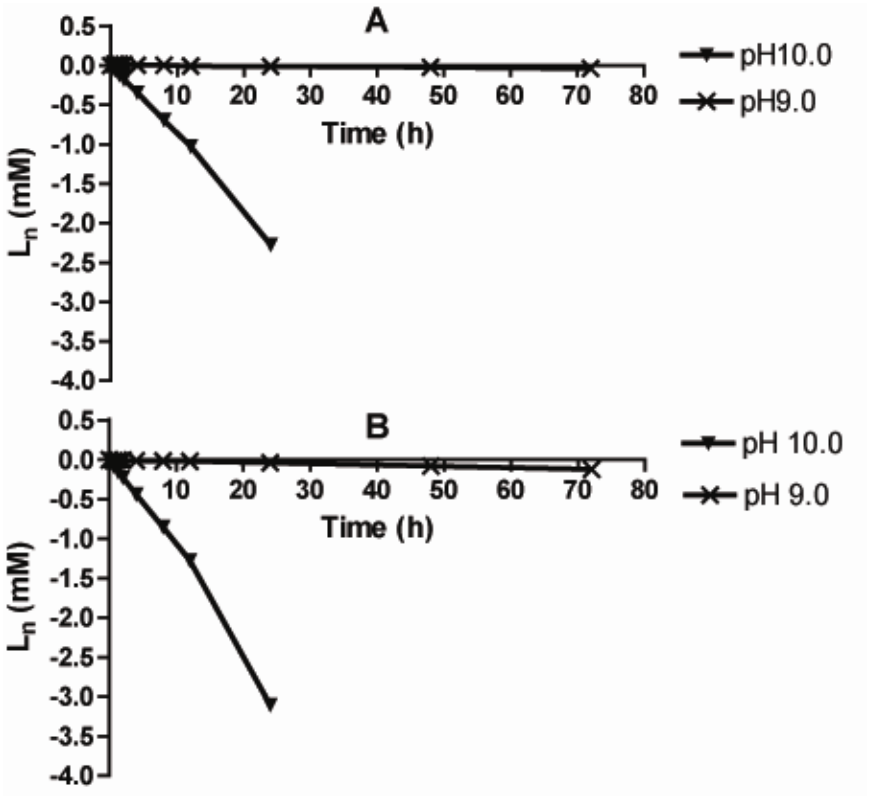

Figure S4. Logarithmic concentration of HPMAm-Bz (A) and HPMAm-Nt (B) against hydrolysis time at pH 9 and 10, and at 37 ^{o}C.

The detected hydrolysis products of HPMAm-Bz/Nt were HPMAm and benzoic acid/naphthoic acid (Figure S3). This demonstrates that as expected the ester bond is hydrolytically sensitive and the amide bond is stable under the selected reaction conditions (no formation of methacrylic acid was observed) . The degradation rate of HPMAm-Bz was slower than that of HPMAm-Nt under the same conditions. The hydrolysis products of HPMAm-Bz/Nt at pH 7.4 and 37 ^{o}C could hardly be detected after 48 h of incubation (results not shown). At pH 9.0, however, both monomers underwent hydrolysis, with 12 % of HPMAm-Nt and 4 % of HPMAm-Bz hydrolyzed at 37 ^{o}C in 48 h. When the pH was increased to 10.0, 95 % of HPMAm-Nt and 90 % of HPMAm-Bz were hydrolyzed in 24 h (at 37 ^{o}C). Both monomers were hydrolyzed according to first-order kinetics. The observed first-order reaction rate constants (k) at 37 °C and pH 9 were (70.7 ± 1.5) × 10^{-5} h^{-1} ($t_{1/2}$ = 980 ± 21 h) and (127 ± 21) × 10^{-5} h^{-1} ($t_{1/2}$ = 557 ± 85 h) for HPMAm-Bz and HPMAM-Nt, respectively. Assuming first order kinetics in hydroxyl ion concentration, the k of the hydrolysis of HPMAm-Bz/Nt at pH 7.4 were calculated to be (1.78 ± 0.04) × 10^{-5} h^{-1} ($t_{1/2}$ = 39000 ± 850 h) and (3.20 ± 0.05) × 10^{-5} h^{-1} ($t_{1/2}$ = 2200 ± 3400 h).

5. GPC analysis of mPEG-*b*-p(HPMAm-Bz$_{24}$-*co*-HPMAm-Lac$_{76}$), mPEG 5 kDa, 10 kDa and macroinitiator.

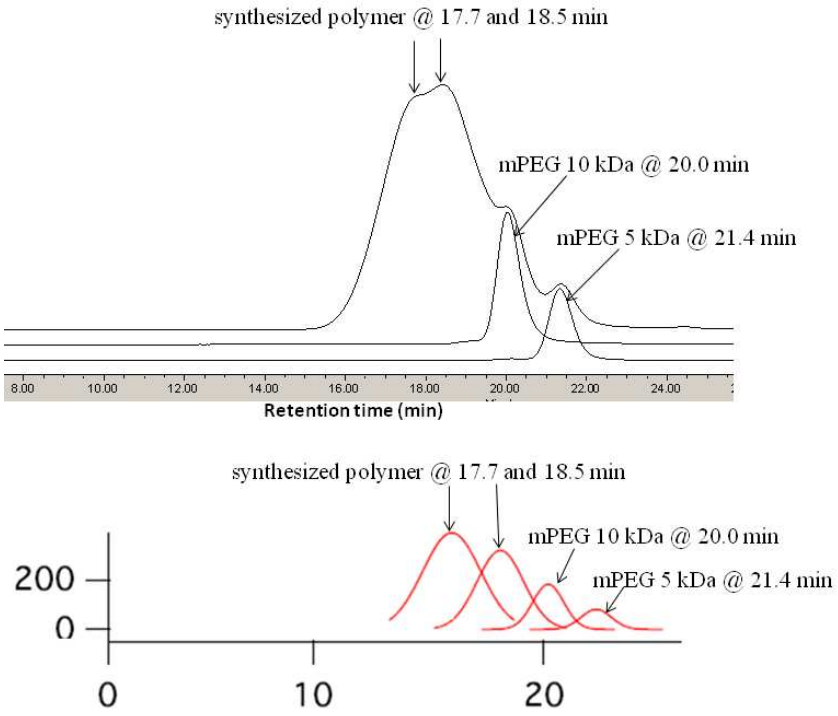

Figure S5. GPC traces of mPEG-*b*-p(HPMAm-Bz$_{24}$-*co*-HPMAm-Lac$_{76}$), mPEG 5 kDa, 10 kDa (top) and deconvoluted trace of mPEG-*b*-p(HPMAm-Bz$_{24}$-*co*-HPMAm-Lac$_{76}$) (bottom).

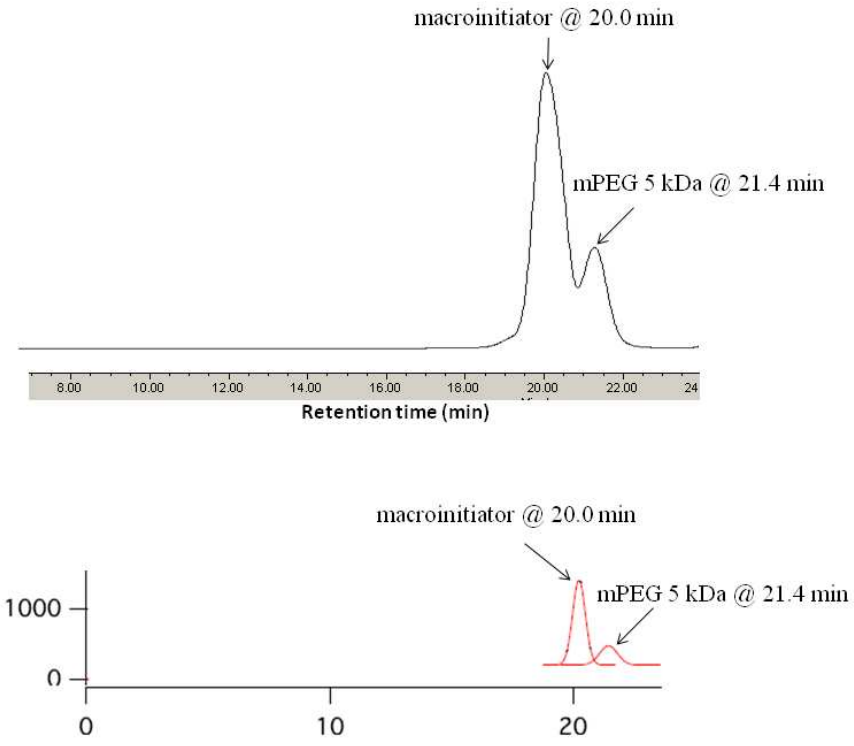

Figure S6. GPC trace of macroinitiator (top) and deconvoluted trace (bottom).

6. Light scattering intensity of mPEG 5 kDa at different temperature

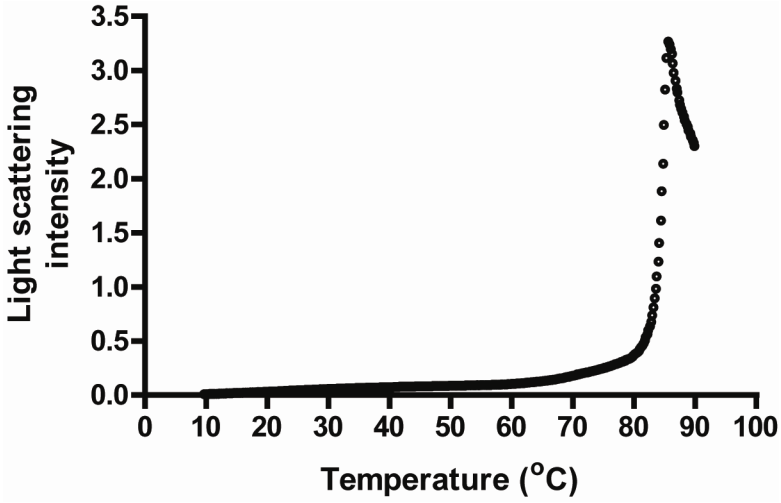

Figure S7. Light scattering intensity change of mPEG 5 kDa (10 mg/mL in 120 mM pH 5.0 ammonium acetate buffer) at different temperatures.

7. EE and LC of the micelles with different feed DTX concentrations

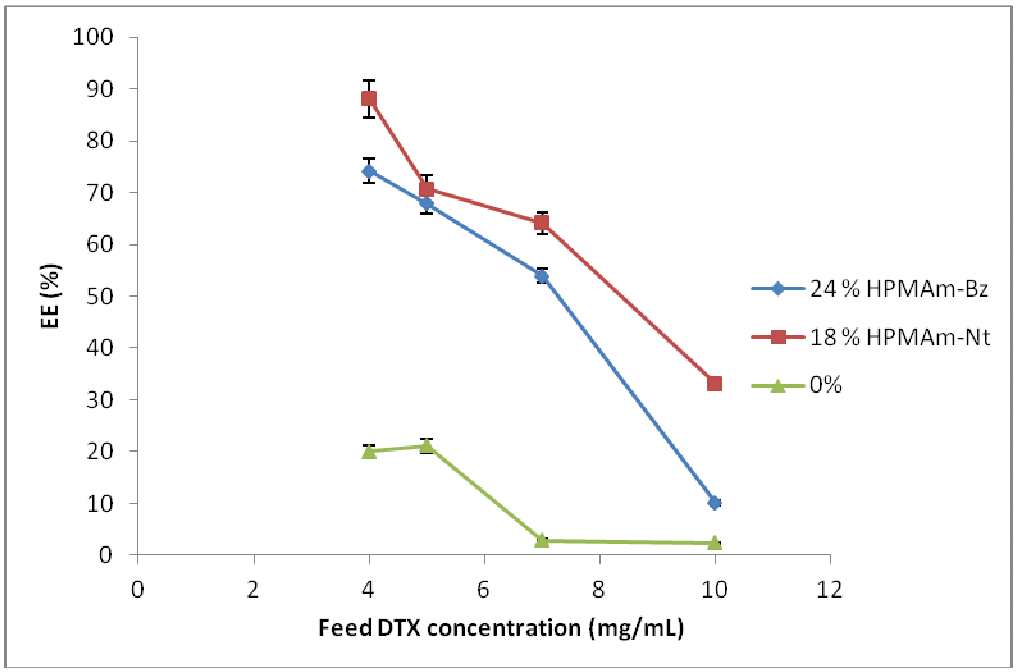

Figure S8. Encapsulation efficiency of the micelles with different feed DTX concentration; 0% refers to the polymer without aromatic groups (mPEG-*b*-p(HPMAm-dilactate).

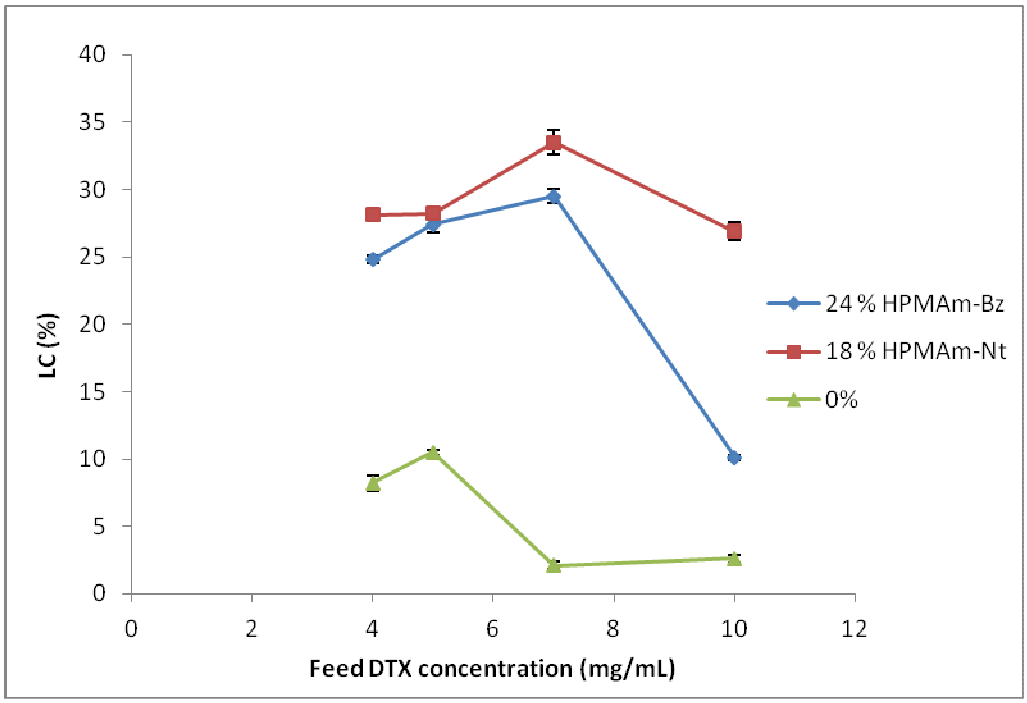

Figure S9. Loading capacity of the micelles with different feed DTX concentration. 0 % refers to mPEG-*b*-p(HPMAm-dilactate); the other polymers are mPEG-*b*-p(HPMAm-Bz/Nt-*co*-HPMAm-Lac) with different amounts of HPMAm-Bz/Nt, respectively (n = 3).

References:

(1) Polymer Handbook, Eds. Brandrup, J; Immergut, E.H.; Grulke, E.A., 4th Edition, John Wiley, New York, 1999, II/2-69;