Electronic Supplementary Material (ESI) for Polymer Chemistry.

Synthesis and Properties of CO_2-Switchable Dex-*g*-PAHMA Copolymers

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1. Optimization of polymerization conditions

The details of the selection of the optimal graft copolymerizing conditions are list in Table S1 and the influences of temperature, time, the concentration and ratio of the initiator to the graft ratio (G) and graft efficiency (G_e) are shown in Fig. S1. G and G_e were defined as $G = W_3/W_2 \times 100$ % and $G_e = W_3/W_1 \times 100$ %, where W_1, W_2, and W_3 are the mass of the feeding monomers, the graft copolymers, and the side chains in the copolymers, respectively. The resultant reaction mixture was dialyzed against pure water to remove the homopolymers and initiators, and purified graft copolymers were obtained. After explorations of the optimal conditions of the graft polymerization, the optimal parameters are at the initiator of $C(K_2S_2O_8)$ = 3 mmol L^{-1}, $C(NaHSO_3)$ = 2 mmol L^{-1} at 50 °C for 24 h.

Table S1. The details of the selection of the optimal graft copolymerizing conditions

Run	m_{Dex}/V_{H_2O} (mg/mL)	$C(K_2S_2O_8)$ (mmol L^{-1})	$C(NaHSO_3)$ (mmol L^{-1})	m_{PMA}/V_{H_2O} (mg/mL)	T (h)	T (°C)	G (%)	G_e (%)
1	100/2	1	1	100/1.6	9	**20**	4.76	5.00
2	100/2	1	1	100/1.6	9	**30**	5.93	6.30
3	100/2	1	1	100/1.6	9	**40**	12.84	14.74
4	100/2	1	1	100/1.6	9	**50**	16.44	19.68
5	100/2	1	1	100/1.6	9	**60**	16.18	19.30
6	100/2	1	1	100/1.6	**3**	30	4.40	4.60
7	100/2	1	1	100/1.6	**6**	30	5.42	5.74
8	100/2	1	1	100/1.6	**9**	30	6.75	7.24
9	100/2	1	1	100/1.6	**12**	30	9.69	10.73
10	100/2	1	1	100/1.6	**24**	30	12.54	14.34
11	100/2	1	1	100/1.6	**48**	30	12.52	14.31
12	100/2	**0.5**	**0.5**	100/1.6	9	30	2.44	2.50
13	100/2	**1**	**1**	100/1.6	9	30	5.78	6.13
14	100/2	**2**	**2**	100/1.6	9	30	10.34	11.53
15	100/2	**3**	**3**	100/1.6	9	30	7.58	8.21
16	100/2	**4**	**4**	100/1.6	9	30	7.07	7.61
17	100/2	**1**	1	100/1.6	9	30	7.54	8.15
18	100/2	**1.5**	1	100/1.6	9	30	9.02	9.91
19	100/2	**2**	1	100/1.6	9	30	8.70	9.88

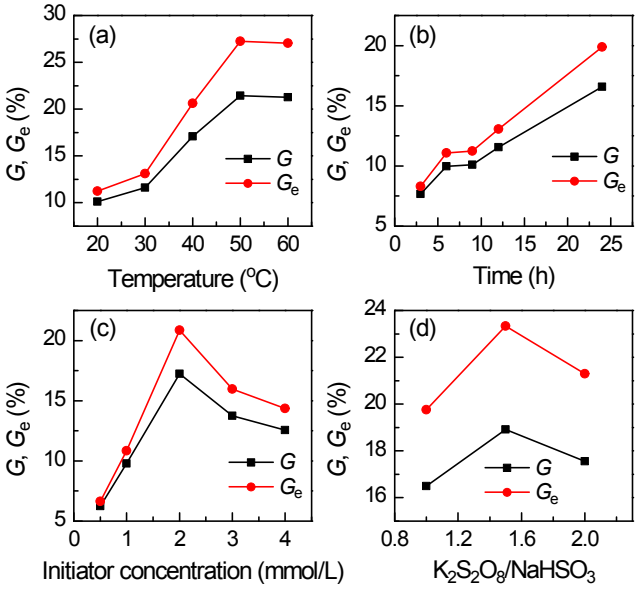

Fig. S1 The influences of temperature (a), time (b), the concentration (c) and ratio(d) of the initiator to the graft ratio (G) and graft efficiency (G_e).

2. Determination of the CMC (the critical micelle concentration) of Dex-*g*-PAHMA copolymers

CMC of dextran graft copolymer was estimated by the fluorescence probe technique. Pyrene was used as the probe and the concentration of pyrene was kept at 1.0×10^{-6} mol L^{-1} in all the solutions with different Dex-*g*-PAHMA concentrations (1.0×10^{-9} – 1.0 mg mL^{-1}). Then emission fluorescence spectra (excited at 334 nm) of the solutions were recorded. The state fluorescence spectrum has five characteristic peaks, where the variation in the intensity ratio of the first I_1 (373 nm) to the third vibrational peak I_3(384 nm) is quite sensitive to the polarity of microenvironment where pyrene is located[1,2]. When I_1/I_3 was plotted as a function of copolymer concentrations, the turn point was used as the CMC of the Dextran-*g*-PAHMA graft copolymers. The critical micelle concentration (CMC) of Dex-g-PAHMA copolymers of graft ratio of 16.5%, 32.3%, 59.4% are about 11.7, 8.5, and 10.8 μg mL^{-1}, respectively.

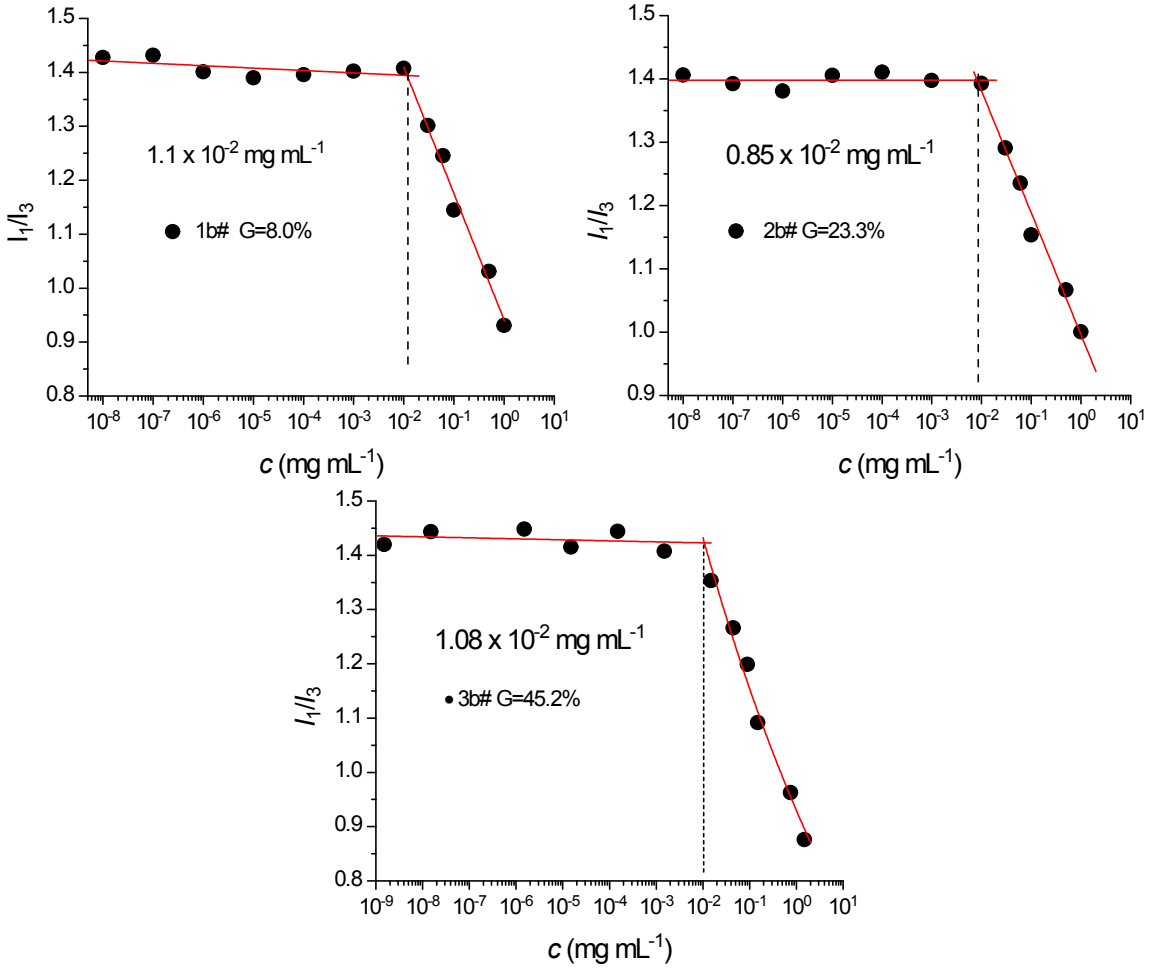

Fig. S2 The estimation of CMC of graft copolymers Dex-*g*-PAHMA with different graft ratios using the fluorescent method with pyrene as a probe.

3. Cytotoxicity of Dex-*g*-PHMA copolymers

The relative cell viability is gradually reduced with the increasing concentration of Dex-*g*-PHMA (Fig. S3), due to that the amino groups is toxic to cells.

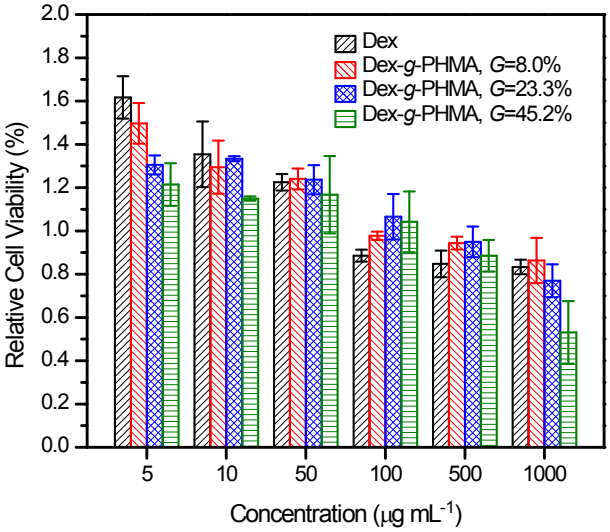

Fig. S3 Relative cell viability of MCF-7 cells after being cultured for 24 h in Dex-*g*-PHMA solutions with different concentration. MCF-7 cells incubated without copolymers were used as the control and the cell viability was determined by MTT assay. Each point is the mean of three parallel measurements.

4. Cellular uptake of DOX-loaded FITC-Dex-*g*-PAHMA micelles (DS_{Am} = 0.064 and DS_{Am} = 0.216)

As it can be seen from Fig. S4, The CLSM photos of DOX loaded copolymer micelles DS_{Am} = 0.064 and DS_{Am} = 0.216 showed similar phenomena as the copolymers DS_{Am} = 0.513. The DOX-loaded Dex-*g*-PAHMA copolymers micelles can be endocytosed by MCF-37 Cell efficiently and the DOX can release from micelles and diffuse partially into the cell nucleus.

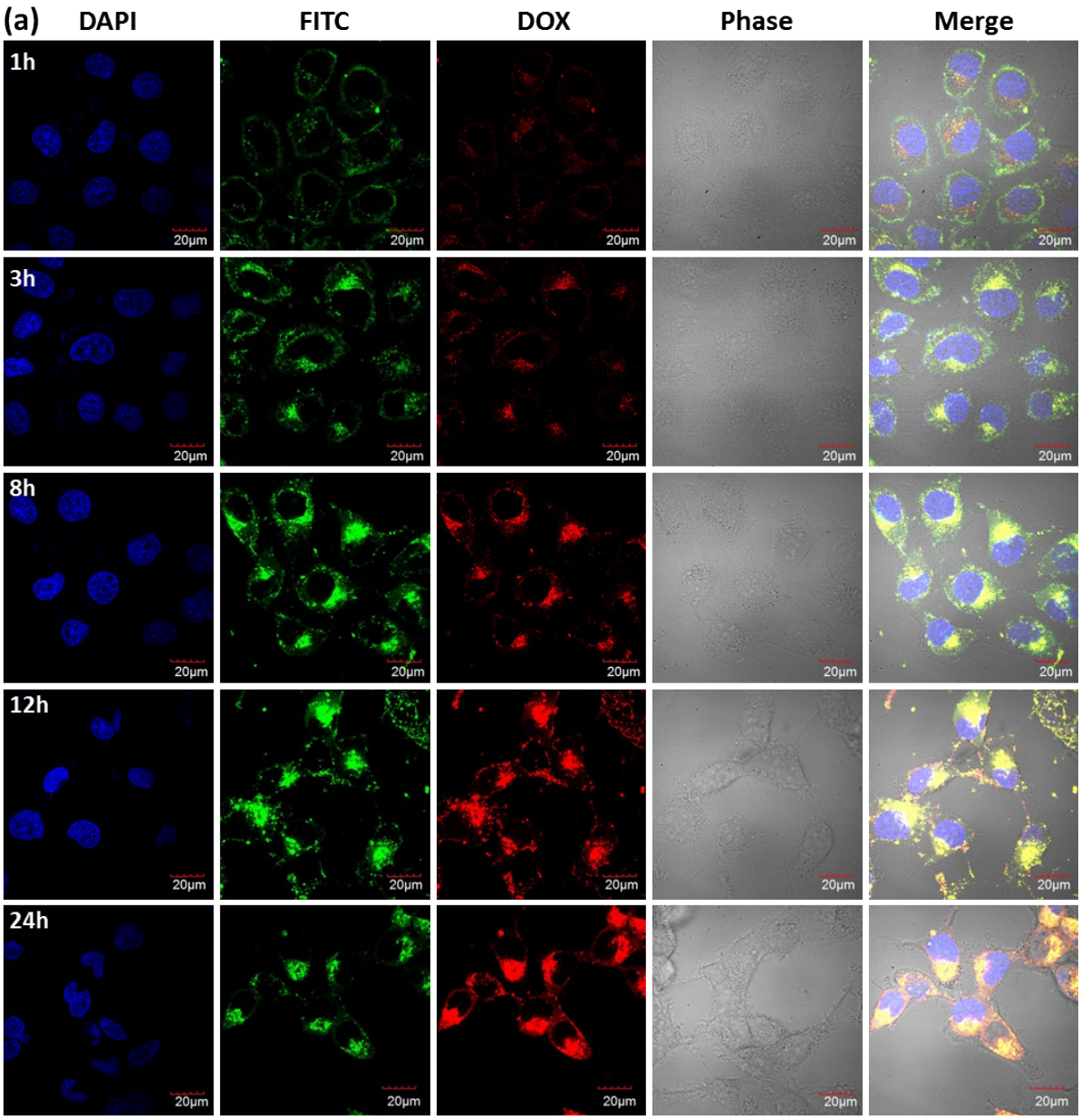

Fig. S4a Representative CLSM images of MCF-7 cells incubated with Dox-loaded FITC-Dex-*g*-PAHMA micelles with DS_{Am} of 0.064 for 1, 3, 8, 12, and 24 h, respectively. Cell nuclei were stained with DAPI.

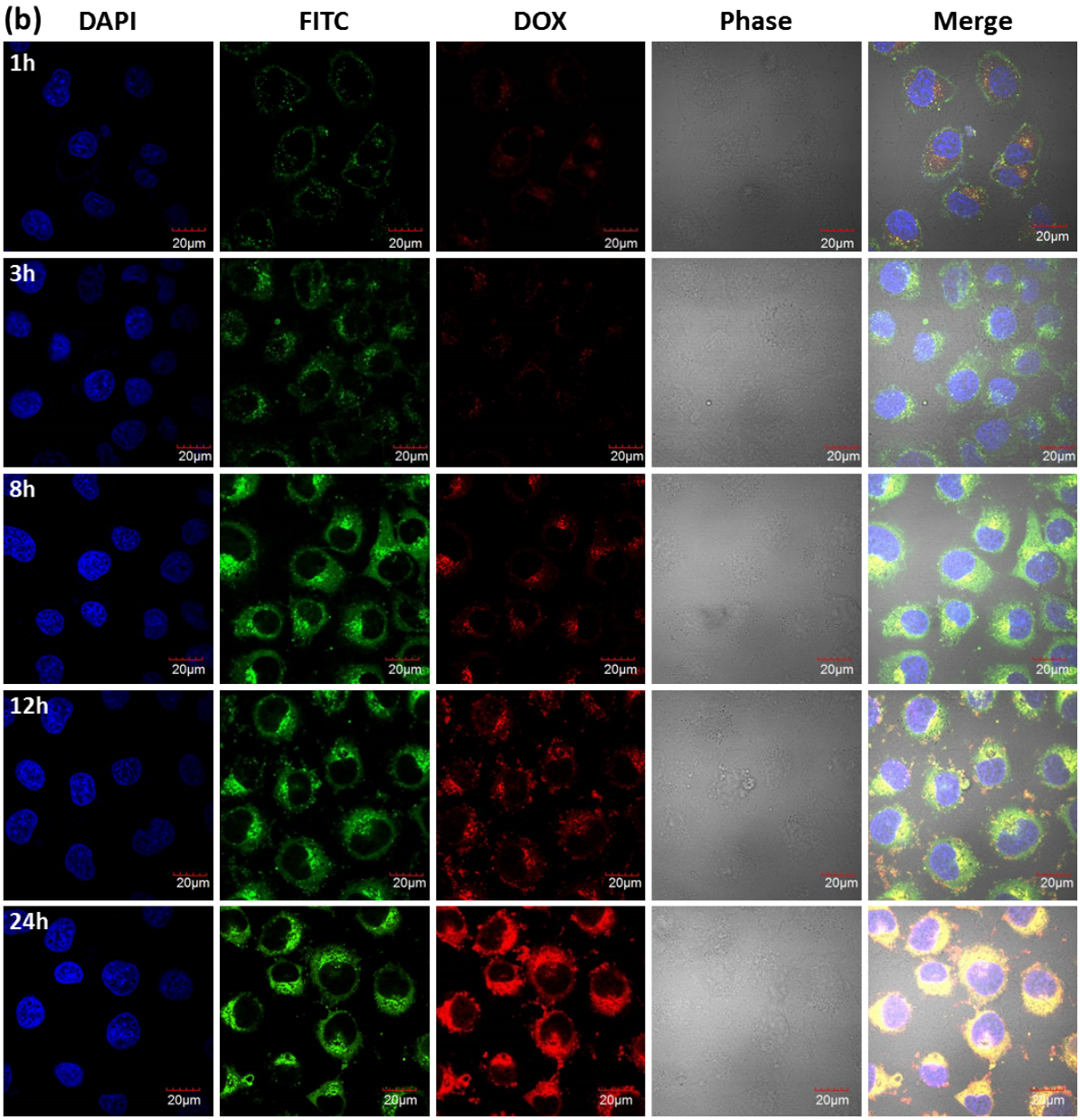

Fig. S4b Representative CLSM images of MCF-7 cells incubated with Dox-loaded FITC-Dex-*g*-PAHMA micelles with DS_{Am} = 0.216 for 1, 3, 8, 12, and 24 h, respectively. Cell nuclei were stained with DAPI.

5. In vitro DOX release from DOX-loaded Dex-*g*-PAHMA copolymers micelles

The release of DOX was carried out by a dialysis method. For the release stimulated by CO_2 and N_2 the DOX-loaded nanoparticles solutions (5 mL) were loaded in a dialysis bag (cut-off molecular weight of 14 kDa) to dialyze against 100 mL Milli-Q water at 37°C in a beaker with constant stirring. CO_2 (the flow rate is about 50 mL/min) was purged into the Milli-Q water to investigate the release of DOX conducted by CO_2. For DOX released in different pH aqueous solution, the DOX-loaded nanoparticles solutions dialyze against aqueous solution of pH=7.4 and 5.5 at 37°C in a beaker with constant stirring. At predetermined time intervals, 2 mL liquid was taken from the outer side of the dialysis bag and 2 mL Milli-Q water was added to ensure the constant volume of the release medium. The content of DOX in the solution was measured by UV-vis absorbance at 481 nm. Control experiments with N_2 were carried out for comparison. The cumulative percentage release was plotted against time based on the maximum amount of DOX released from the micelles. As shown in Fig. S3a, about 3.1% of loaded DOX could be released with N_2 bubbling in 60hr. While CO_2 was added, the accumulative release could reach to 11.5% at the same time intervals. The release rate and efficiency can be improved as CO_2 bubbling is due to the greatly expanded volume of the micelles decrease the density of the micelles as amidino groups being protonated, which makes it easier for DOX to travel through the core and shell of the micelles. In contrast，the DOX release rate from micelles can be delayed when N_2 bubbling throughout the whole process, which is caused by deprotonation of the core of the micelles. The enhanced hydrophobic packing of the shrunk micelle core hold DOX tightly, leading to a sluggish rate of release. The release of DOX in pH=7.4 and 5.5 aqueous solution (Fig. S3b) showed the same situation. The accumulative release of Dox in aqueous solution at pH 5.5 reached to 12.6% at 120 h while only 8.9% in pH=7.4 aqueous solution at the same time intervals. The result confirmed the pH sensitivity of the Dex-*g*-PAHMA copolymers.

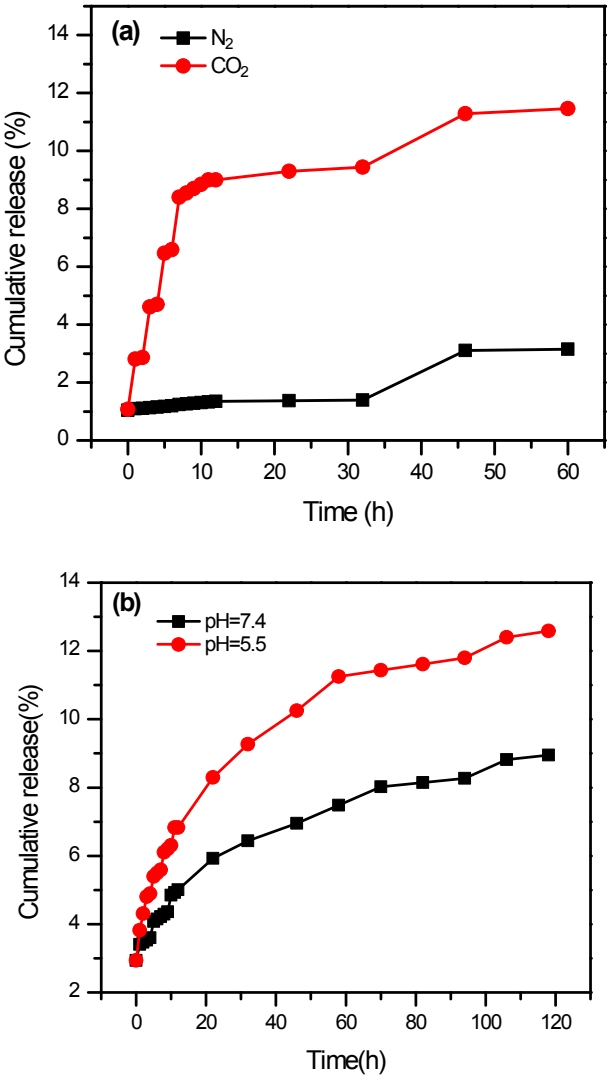

Fig. S5 DOX release from micelles with (a) CO_2 and N_2 bubbling throughout the whole process and (b) pH=7.4 and pH=5.5 aqueous solution .

References

(1) Astafieva, I.; Zhong, X. F.; Eisenberg, A. *Macromolecules* **1993**, *26*, 7339.

(2) Kalyanasundaram, K.; Thomas, J. K. *Journal of the American Chemical Society* **1977**, *99*, 2039.