

## Supplementary Materials

# Co-loading of Temozolomide and Curcumin into a Calix[4]arene-Based Nanocontainer for Potential Combined Chemotherapy: Binding Features, Enhanced Drug Solubility and Stability in Aqueous Medium

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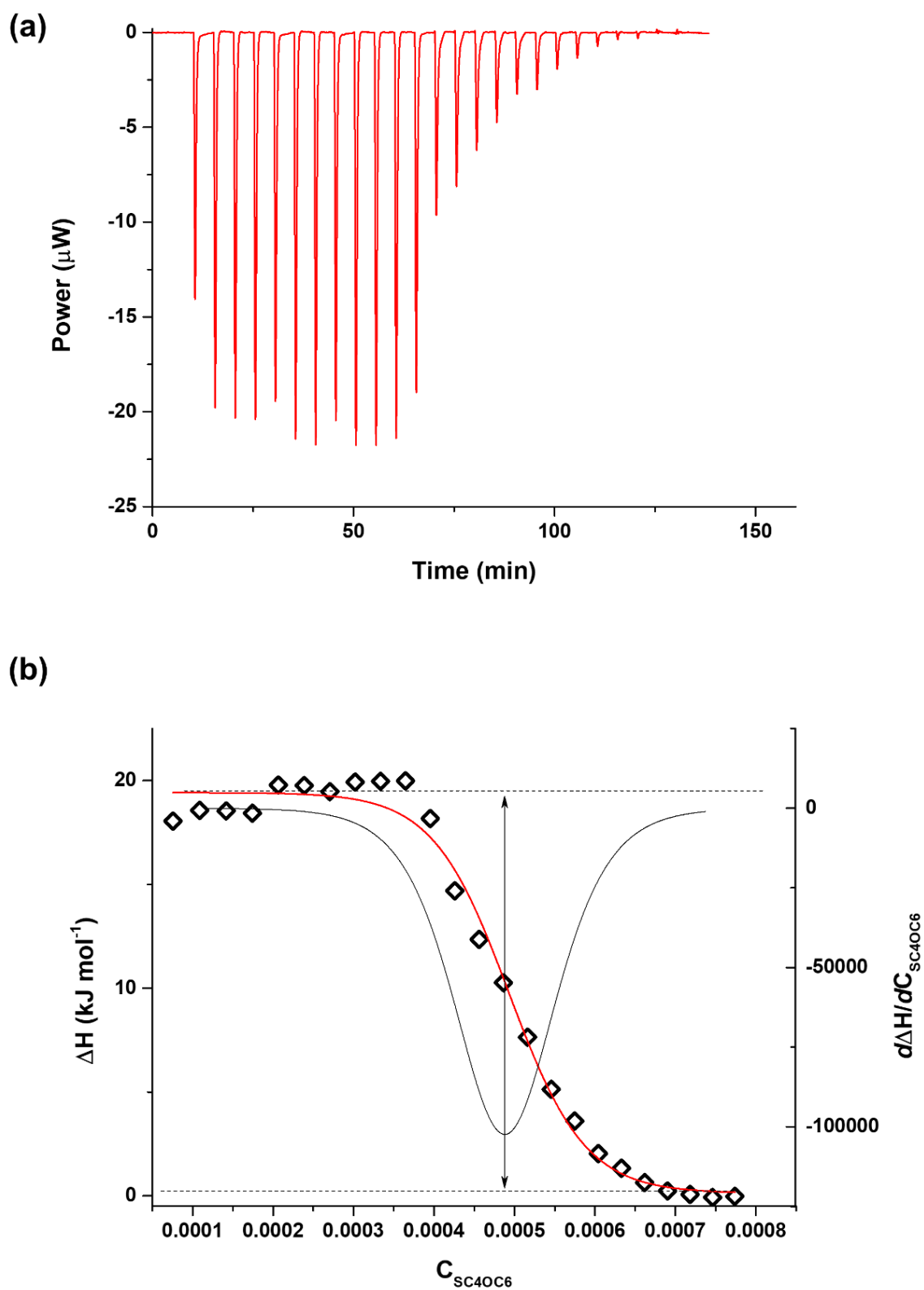
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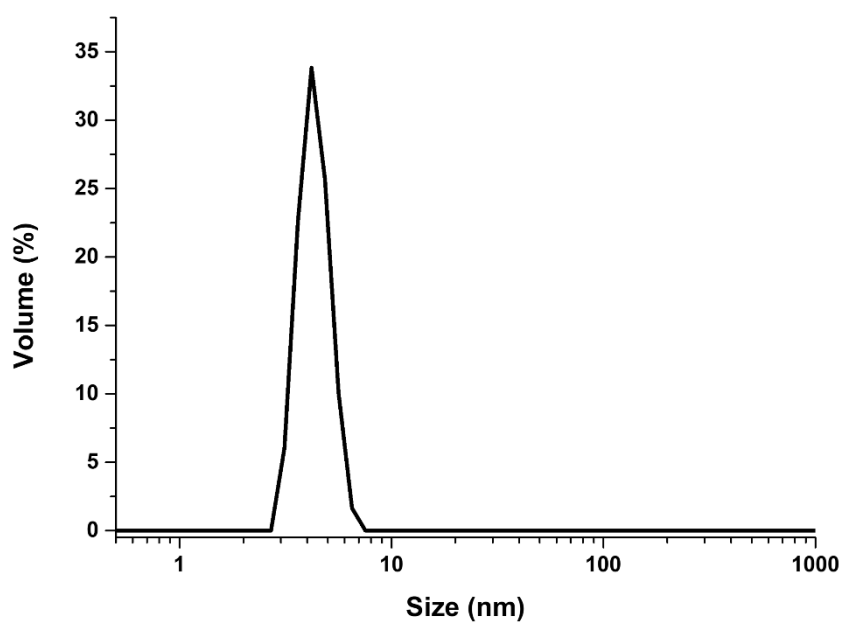
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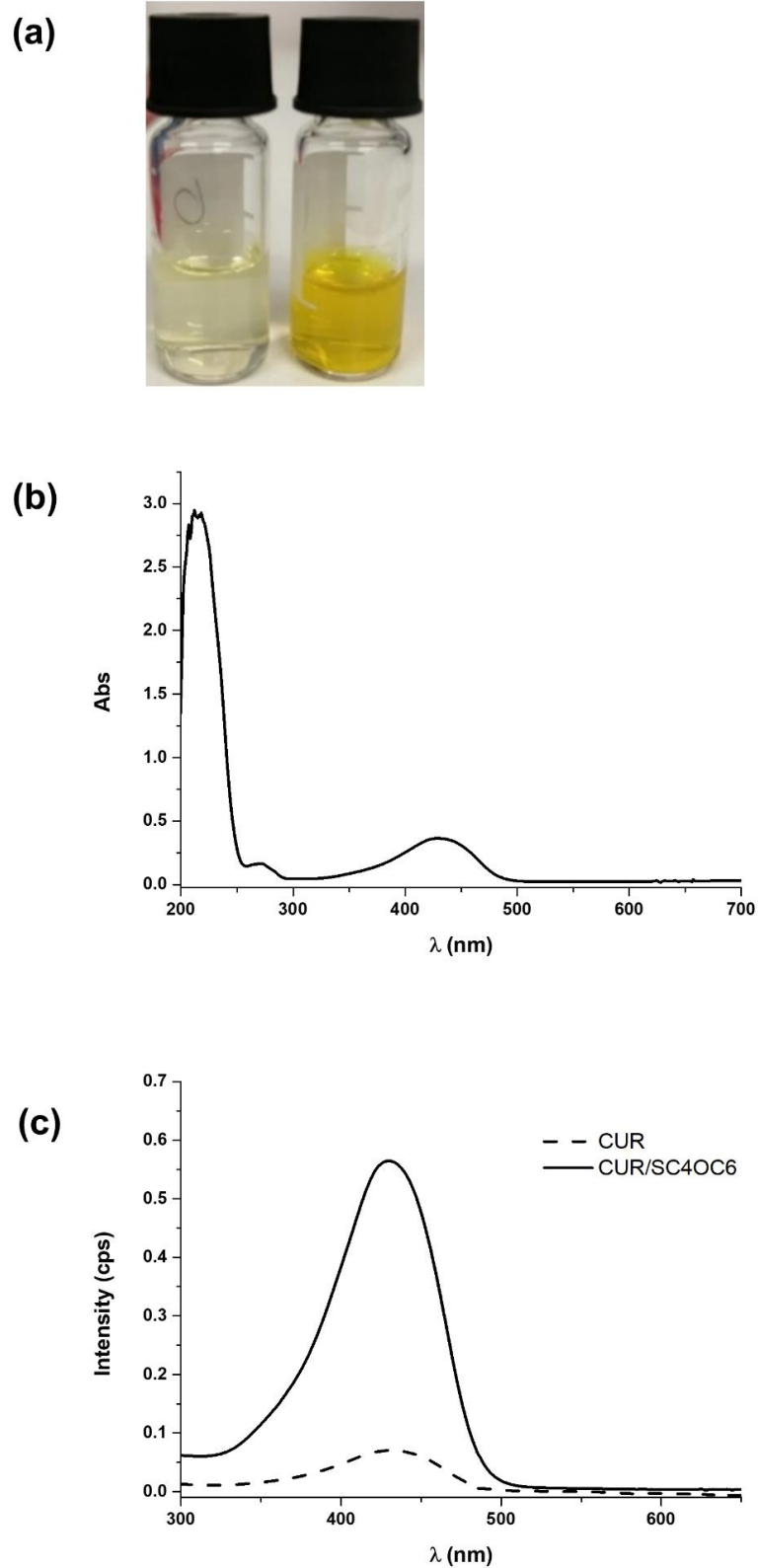
**Figure S1.** (a) ITC titration of SC4OC6 4 mM into plain water at 25 °C; (b) reaction enthalpy as a function of the total surfactant concentration ( $C_{\text{SC4OC6}}$ ) in the calorimetric cell.

**Table S1.** CMC and enthalpy values for SC4OC6 self-aggregation process at 25 °C.

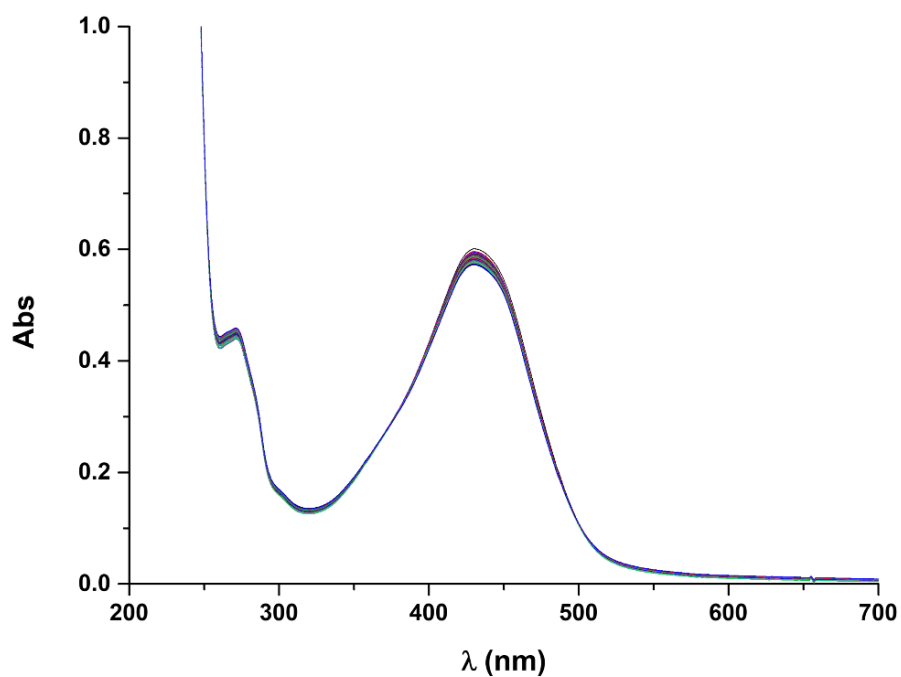
	CMC	$\Delta H$
	(mM)	(kJ mol <sup>-1</sup> )
Plain water	0.492 (5)	-21.7 (2)
Phosphate buffer (I = 20 mM)	0.05 (1)	-4.8 (2)



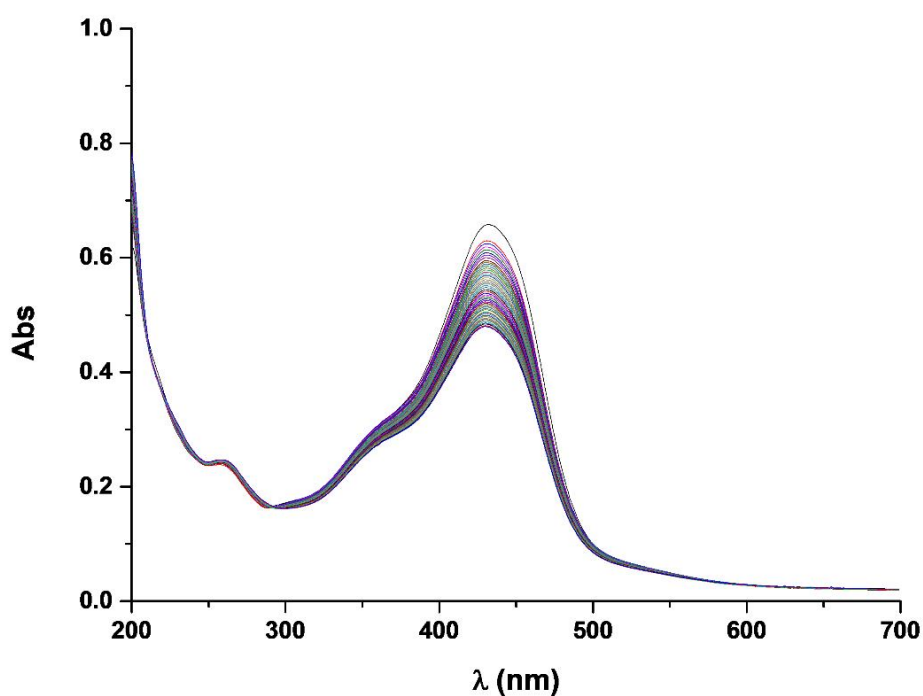
**Figure S2.** Volume weighted hydrodynamic diameter distribution of SC4OC6 (1.7 mM) in 10 mM phosphate buffer.



**Figure S3.** (a) Picture of the colloidal solution of CUR alone (right) and with SC4OC6 (left) in 10 mM phosphate buffer; (b) UV-vis spectrum of SC4OC6/CUR (0.091 mM/0.012 mM); (c) fluorescence spectra of CUR alone (0.0016 mM, dashed line) and CUR (0.012 mM, solid line) in the presence of SC4OC6 in 10 mM phosphate buffer.



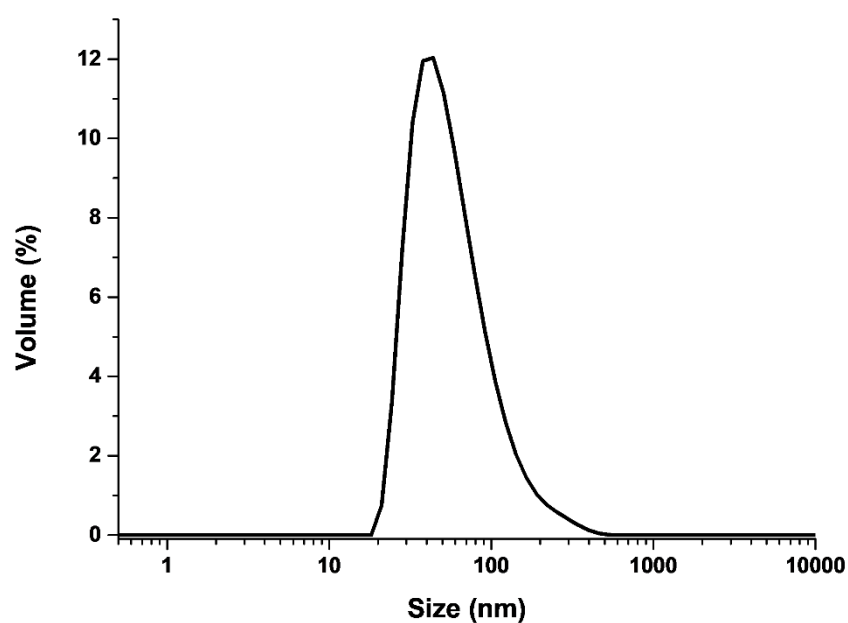
**Figure S4.** UV-vis spectra of SC4OC6/CUR (0.11 and 0.014 mM respectively) in 10 mM phosphate buffer recorded every 15 minutes at 37 °C.



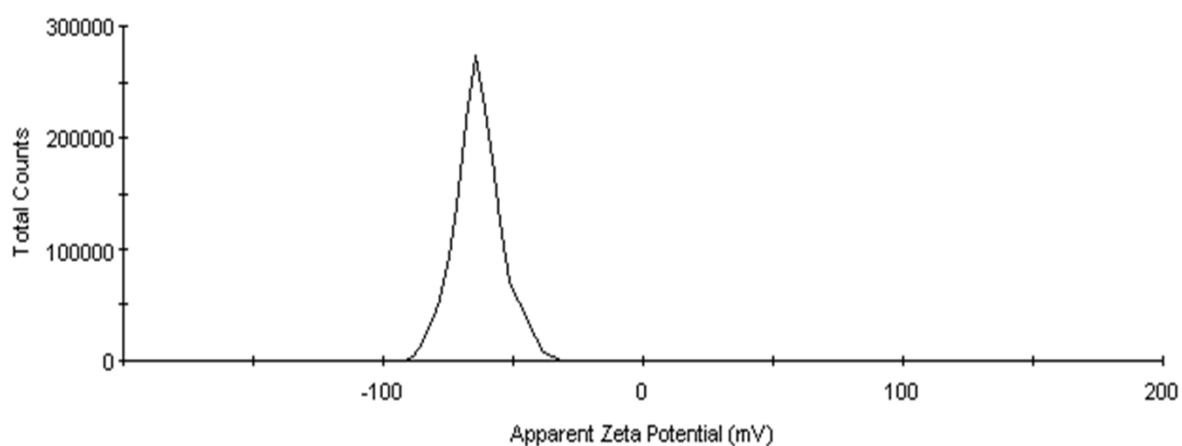
**Figure S5.** UV-vis spectra of curcumin (0.014 mM) in 30% ethanol/phosphate buffer recorded every 15 minutes at 37 °C.

**Table S2.** First order degradation rate constant and half-life values for curcumin in 30% ethanol/phosphate buffer and for SC4OC6/CUR in 10 mM phosphate buffer at 37 °C.

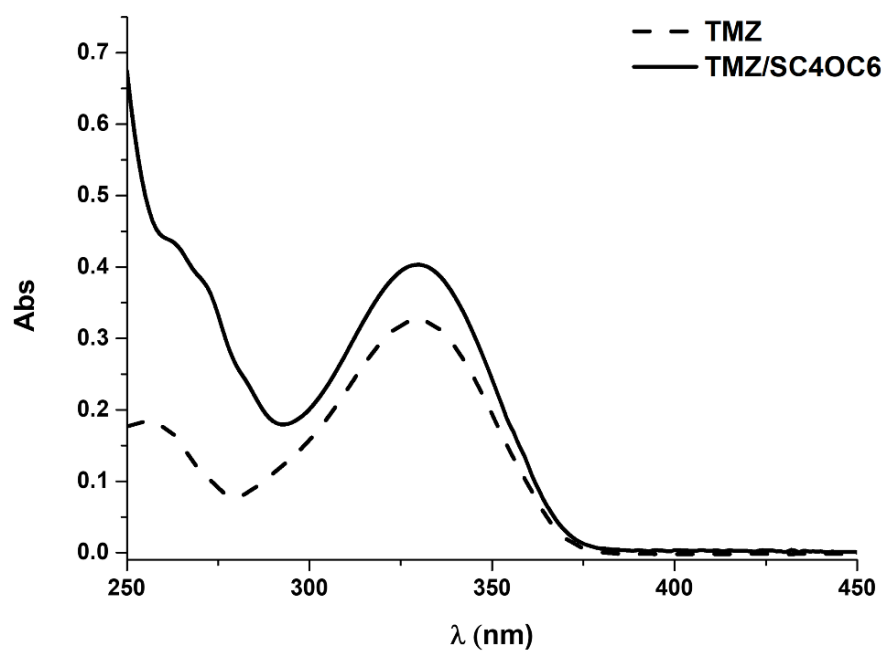
	<b>k</b> <b>(min<sup>-1</sup>)</b>	<b>t<sub>1/2</sub></b> <b>(h)</b>
Free CUR	0.00049 (1)	23.5
CUR in SC4OC6 micelles	0.000075 (2)	154



**Figure S6.** Volume weighted hydrodynamic diameter distribution of SC4OC6/CUR (0.85 mM/0.11 mM) in 10 mM phosphate buffer.

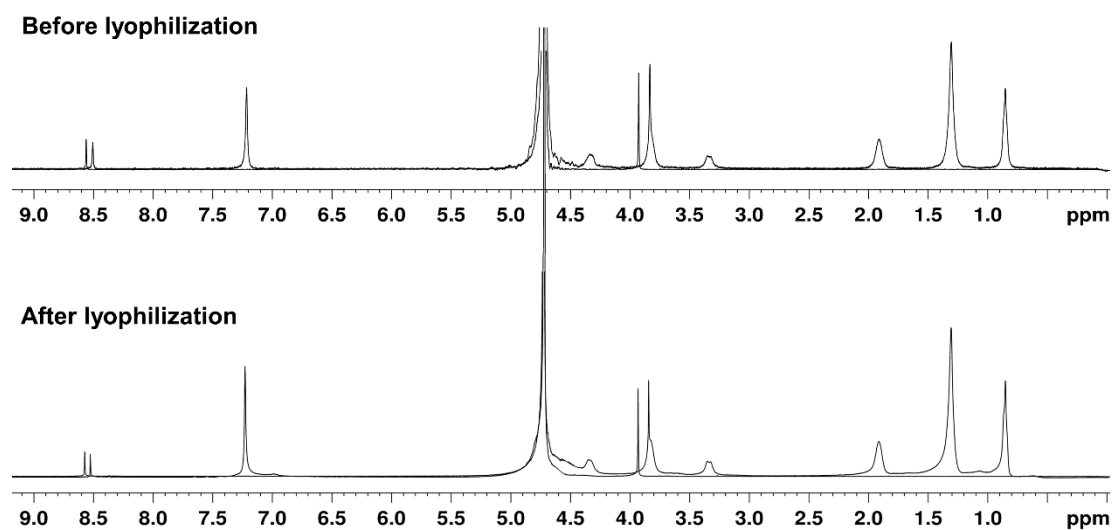


**Figure S7.** Zeta potential distribution of SC4OC6/CUR (0.85 mM/0.11 mM) in 10 mM phosphate buffer.

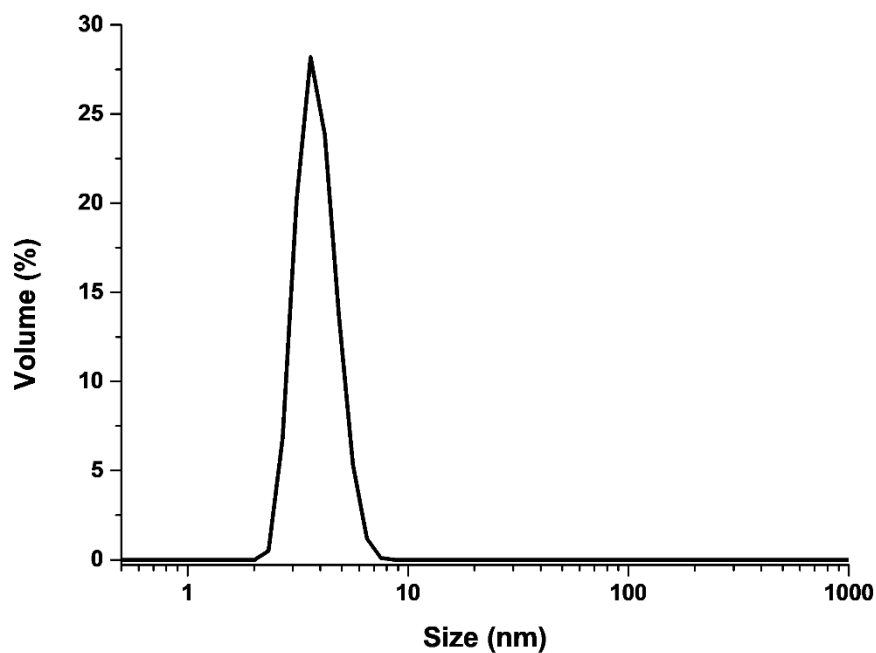


**Figure S8.** UV-vis spectra of TMZ alone (0.03 mM, dashed line) and TMZ (0.0365 mM) in the presence of SC4OC6 (solid line) in 10 mM phosphate buffer.

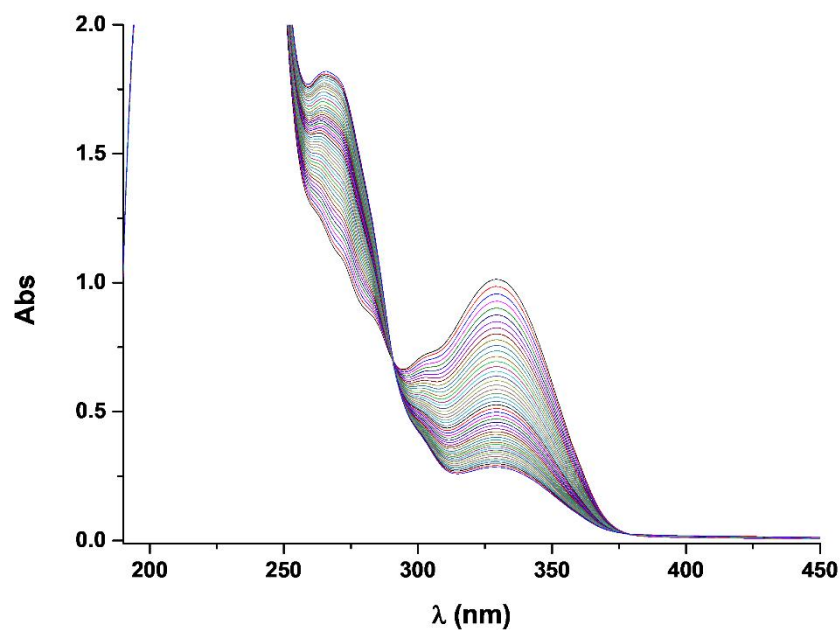




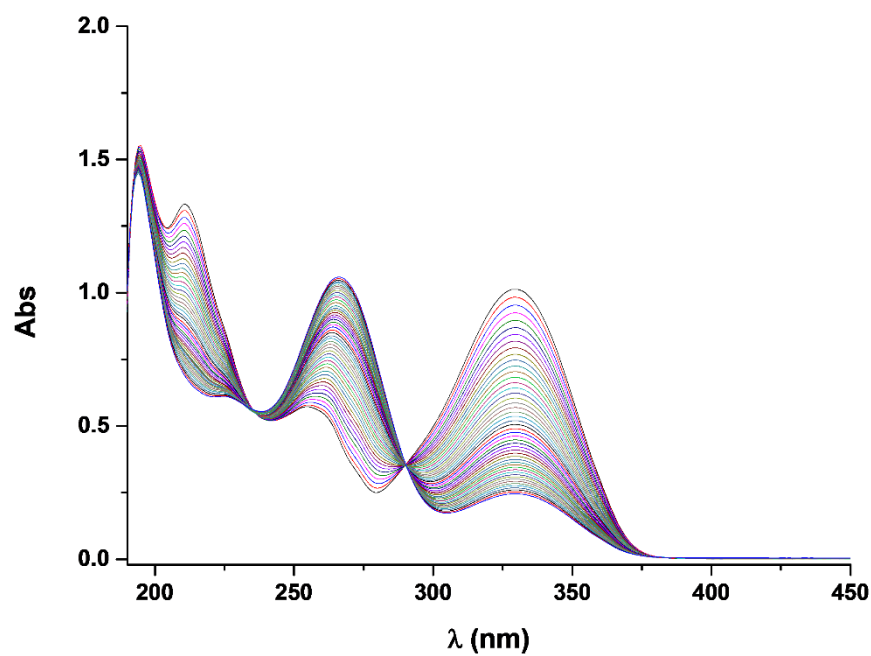
**Figure S9.** Overlapped  $^1\text{H}$  NMR spectra ( $\text{D}_2\text{O}$ , phosphate buffer, 297 K) of TMZ (4.5 mM) and SC4OC6/TMZ (7.4 mM/4.5 mM) complex before (up) and after (bottom) freeze-drying.



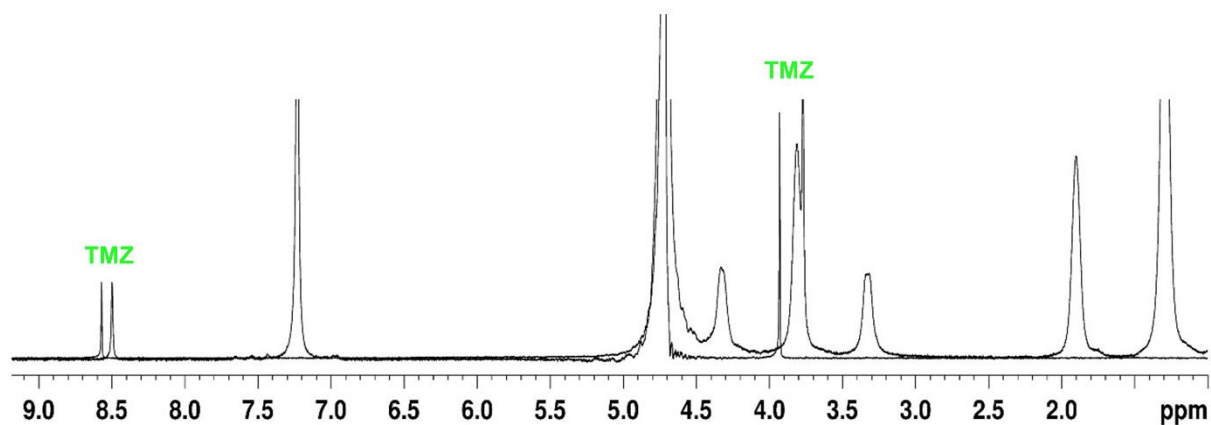
**Figure S10.** Volume weighted hydrodynamic diameter distribution of SC4OC6/TMZ (1.7 mM/1.03 mM) complex in 10 mM phosphate buffer.



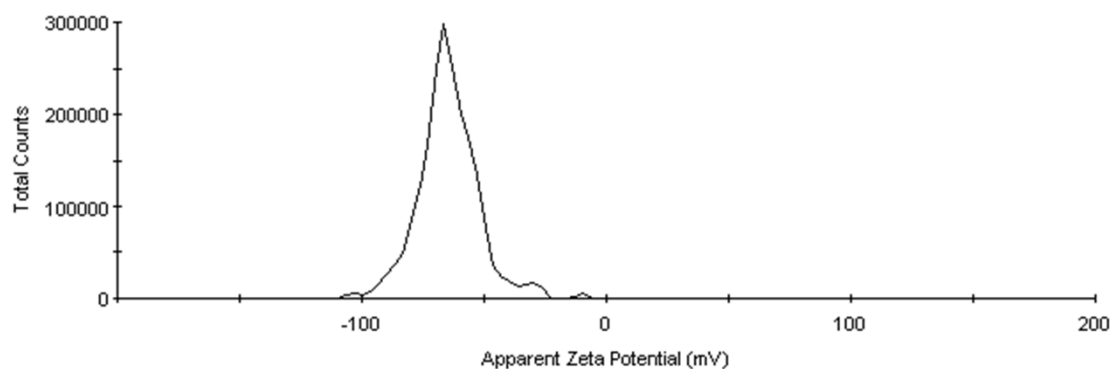
**Figure S11.** UV-vis spectra of SC4OC6/TMZ (0.2 and 0.1 mM respectively) in 10 mM phosphate buffer recorded every 15 minutes at 37 °C.



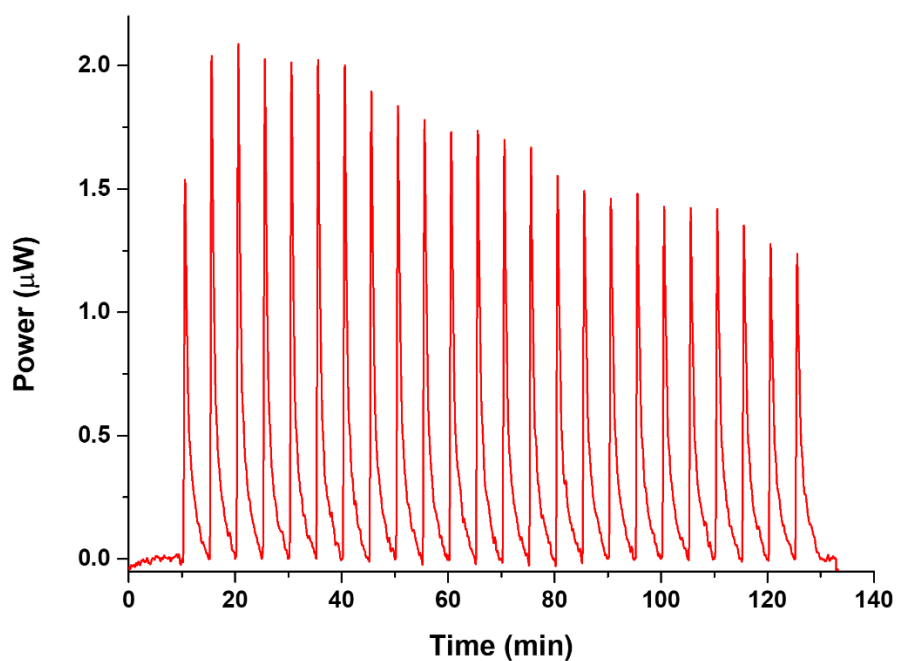
**Figure S12.** UV-vis spectra of TMZ (0.1 mM) in 10 mM phosphate buffer recorded every 15 minutes at 37 °C.



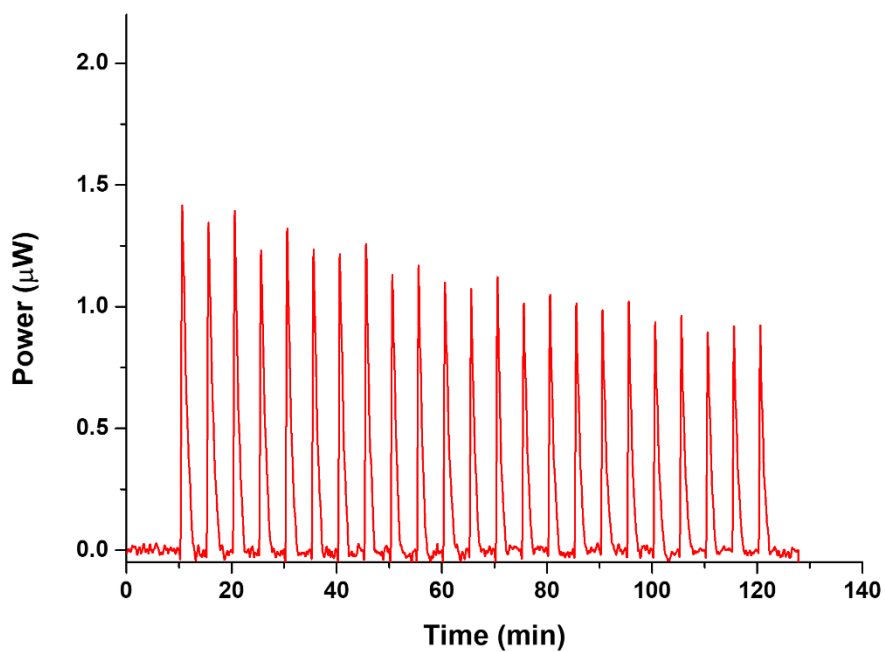
**Figure S13.** Overlapped  $^1\text{H}$  NMR spectra ( $\text{D}_2\text{O}$ , phosphate buffer, 297 K) of TMZ (4.5 mM) and lyophilized SC4OC6/CUR/TMZ (7.4 mM/0.95 mM/4.6 mM).



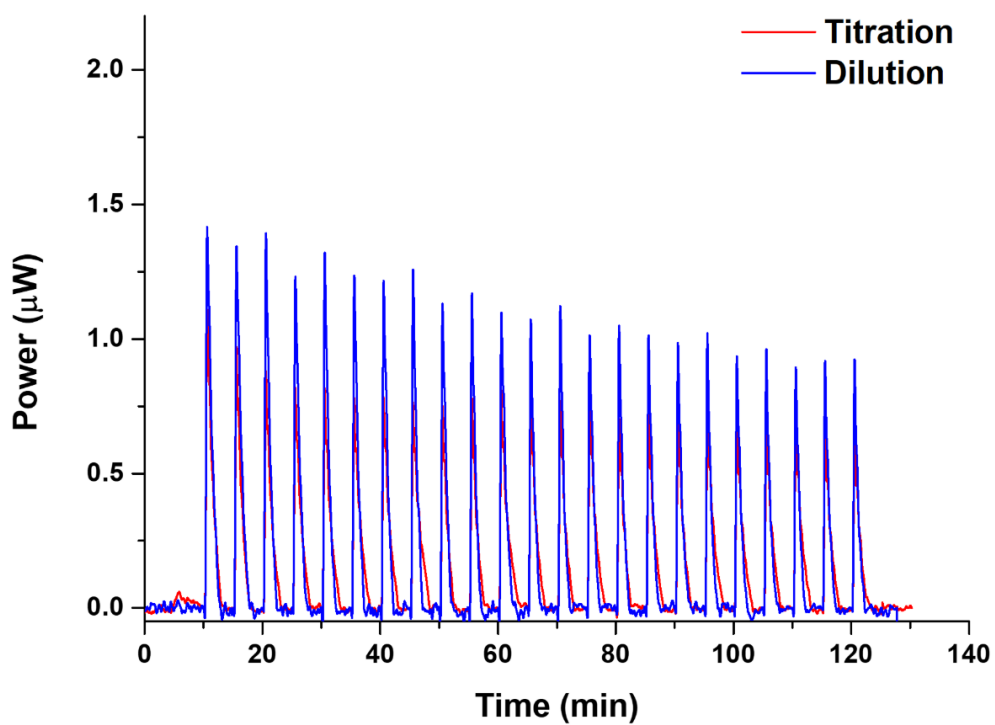
**Figure S14.** Zeta potential distribution of SC4OC6/CUR/TMZ (0.85 mM/0.11 mM/0.5 mM) in 10 mM phosphate buffer.



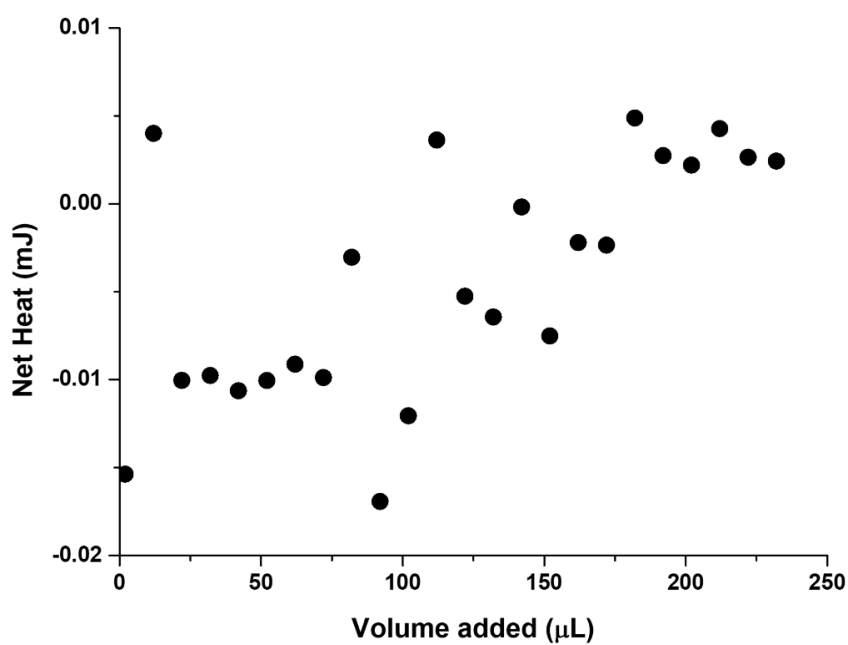
**Figure S15.** ITC titration curve of TMZ 5 mM into SC4OC6 0.3 mM (above CMC) at 25 °C in 10 mM phosphate buffer.



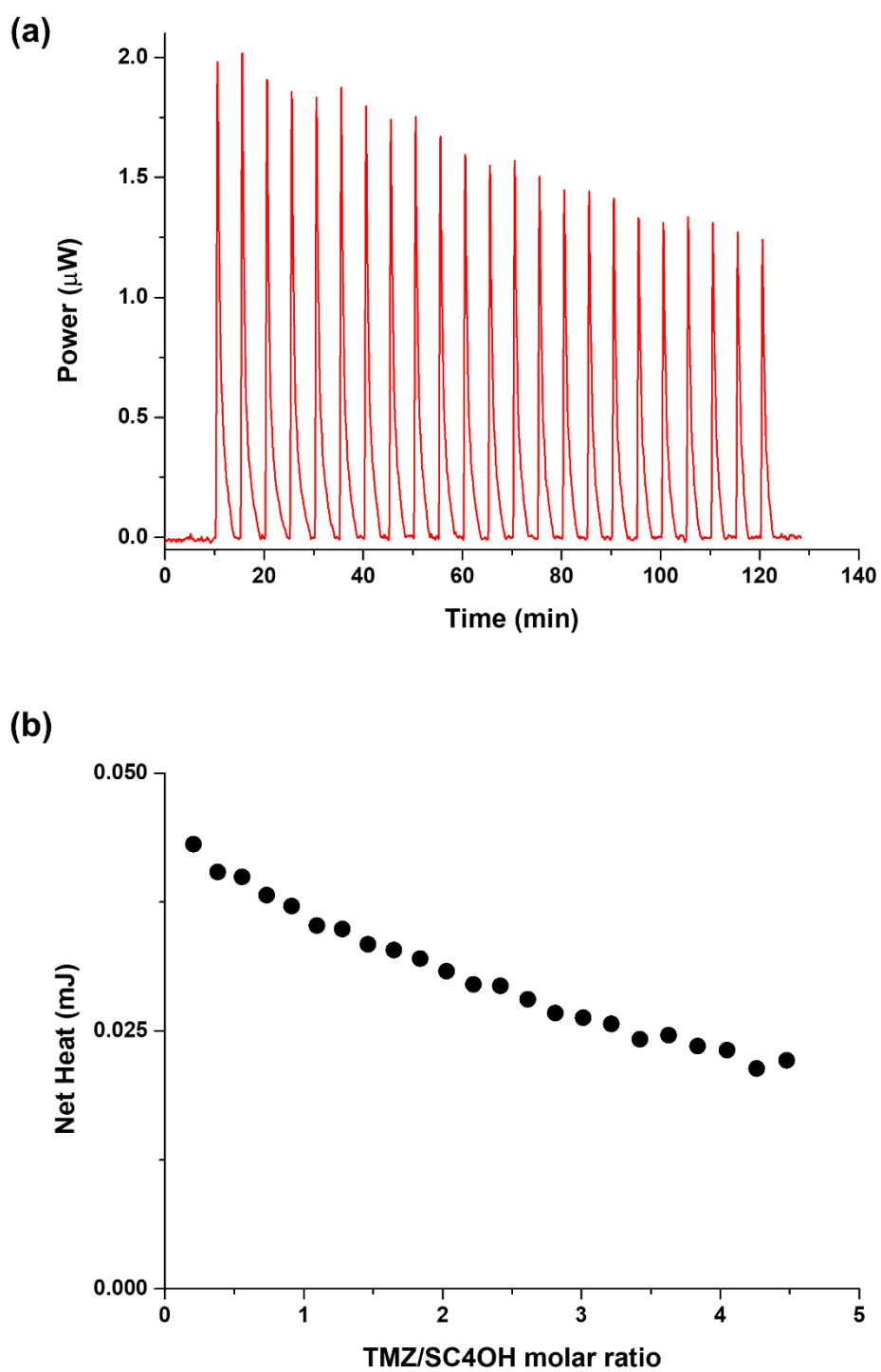
**Figure S16.** Typical "blank" experiment. ITC titration curve of TMZ 5 mM into phosphate buffer 10 mM at 25 °C.



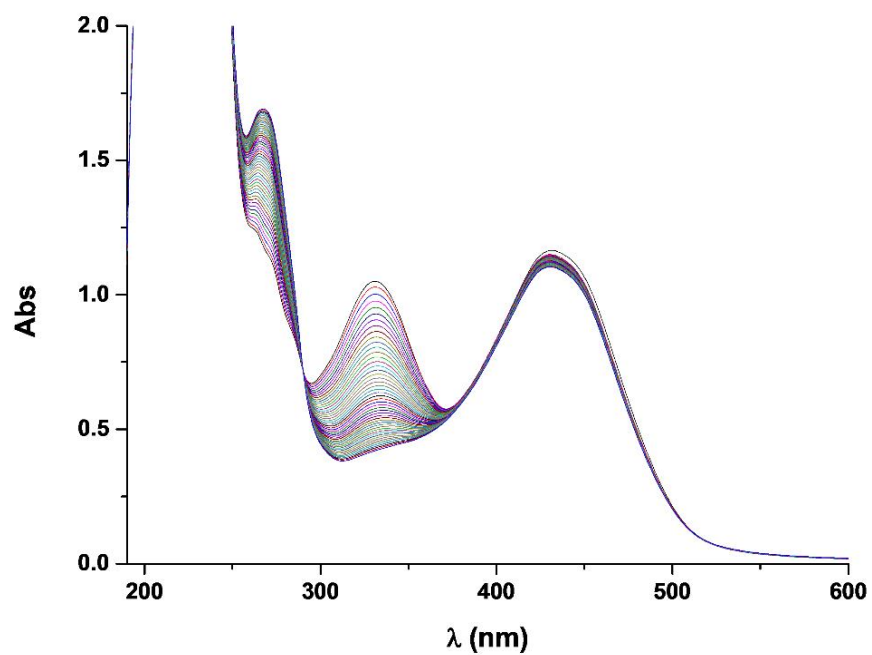
**Figure S17.** Overlap between TMZ (5 mM) into SC4OC6 (0.02 mM, below CMC) calorimetric titration (red) and the corresponding blank experiment (blue) at 25 °C in 10 mM phosphate buffer.



**Figure S18.** Net heat curve for the TMZ (5 mM) / SC4OC6 (0.02 mM, below CMC) system.



**Figure S19.** (a) ITC titration curve of TMZ 5 mM into SC4OH 0.3 mM at 25 °C in 10 mM phosphate buffer; (b) integrated heat data.



**Figure S20.** UV-vis spectra of SC4OC6/CUR/TMZ (0.2, 0.025 and 0.1 mM respectively) in 10 mM phosphate buffer recorded every 15 minutes at 37 °C.

**Table S3.** First order degradation rate constant and half-life values for free TMZ, TMZ-SC4OC6 micelles and TMZ-SC4OC6 micelles loading curcumin in 10 mM phosphate buffer at 37 °C.

	<b>k</b> <b>(min<sup>-1</sup>)</b>	<b>t<sub>1/2</sub></b> <b>(h)</b>
Free TMZ	0.00199 (1)	5.8
TMZ-SC4OC6 micelles	0.00183 (1)	6.3
TMZ-SC4OC6 micelles loading curcumin	0.00139 (1)	8.3