Electronic Supplementary Material (ESI) for Inorganic Chemistry Frontiers.

Supporting Information

Engineered doxorubicin-calcium@silica nanospheres with tunable degradability for controlled drug delivery

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Experiment Sections

Chemicals. Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) and tetraethyl orthosilicate (TEOS) were purchased from Sigma-Aldrich. Doxorubicin hydrochloride (DOX) and ammonium bicarbonate (NH_4HCO_3) were purchased from Aladdin. Hoechst 33258 was purchased from Life Technologies. 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) was purchased from Biosharp. Other materials and chemicals were all commercially available.

Synthesis of ACC-DOX nanospheres. $CaCl_2 \cdot 2H_2O$ (12.5, 25, 50, 75, 120, or 200 mg) was ultrasonic dissolved in absolute ethanol (100 mL) in a glass bottle. Then, $NH_3 \cdot H_2O$ (3 μL) and DOX (1, 2, 4 or 8 mg) were dissolved in deionized water (500 μL) were added into the $CaCl_2$ ethanol solution. After stiring, the bottle was closely sealed by parafilm with some small pores and transferred into a desiccator. Two small bottles of ammonia bicarbonate powder were also placed in the desiccator. After the vapor diffusion reaction at 30 ºC after 48 h, the purple products were centrifuged at 8000rpm and 10 min and finally dispersed in absolute ethanol for storage. The synthesis of ACC-DOX nanospheres with 75 mg $CaCl_2 \cdot 2H_2O$ and 4 mg DOX were chosen for the next synthesis of ACC-DOX@silica nanospheres.

Synthesis of ACC-DOX@silica nanospheres. Firstly, ethylenediaminetetraacetic acid (EDTA) solution was produced by dissolving 0.6 g EDTA in solution that contained 12 mL ammonia solution and 28 mL deionized water. Briefly, ACC-DOX nanoparticles around 75 nm (2 mg), ammonia solution (350, 375, 400, 425 or 450 μL) and EDTA solution (25 μL) were dispersed in absolute ethanol (20 mL) and stirred for 0.5 hour. Then, TEOS (50 μL) was added and stirred for another 0.5 hour before deionized water (400 μL) was added. The mixture was continuously stirred for 24 hours. The final product was centrifuged (8,000 rpm and 10 min), washed with absolute ethanol for 2-3 times and storaged in ethanol. The synthesis of ACC-DOX@silica nanospheres with ammonia solution of 400 μL were chosen for the next synthesis of DOX-Ca@silica nanospheres.

Synthesis of DOX-Ca@silica nanoparticles. The centrifuged DOX-Ca@silica nanospheres (1 mg) were dispersed in deionized water (1 mL) for suspension. To fabricate DOX-Ca@silcia nanoparticles of A0, A1, A2, A3 and A4, different amount of HCl solution (0, 2, 5, 15, or 25 μL) was added respectively. The concentration of HCl was 12 M. In the acid-treatment solution, the pH value of A0 was 7. And the pH values of A1 to A4 were 0.5-2. After rotation at 4 ºC for 1 hour by a roller mixer, the mixture was centrifuged at 10000 rpm of 5 min and washed 3-5 times with deionized water. Finally, the product was redispersed in absolute ethanol for stroage**.**

Characterizations. Hydrodynamic diameters were characterized by Malvern Zetasizer Nano ZS90. Transmission electron microscopy (TEM) images were observed by Hitachi H-7650 at 100kv. TEE element mapping images were obtained with a JEOL-2010F analytical microscope. X-ray photoelectron spectroscopy (XPS) were performed using an ESCALAB-MKII X-ray photoelectron spectrometer. UV–vis measurements were collected on a UV-2600 spectrometer (Shimadzu Corporation). ICP-AES studies were acquired on an Atomscan Advantage Spectrometer (Thermo Ash Jarrell Corporation). FTIR spectra was investigated from 4000 to 400 cm^{-1} using a Bruker Vector-22 FTIR spectrometer at room temperature.

DOX release form ACC-DOX@silica nanoparticles. Centrifuged ACC-DOX@silica-A nanospheres (0.1 mg) were dispersed in deionized water (1 mL) and rotated at 37 ºC for predetermined time by a roller mixer. At the given time, the samples were centrifuged and the concentration of the released DOX was measured with a UV-vis spectrometer and TEM.

***Cell culture*:** HeLa cells and DOX-resistant MCF-7 cells were cultured in DMEM medium with 15% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 ºC in a 5% CO_2 incubator.

***MTS assay in vitro*:** Hela and drug-resistant MCF-7 cells were respectively seeded into 96-well plates and incubated for 24 hours to allow cell attachment. Then, free DOX and ACC-DOX@silica-A nanoparticles were respectively added each well at a certain DOX concentration range from 2.5 to 40 μg/mL. After different time, the culture media were replaced with MTT solution (100μL) and cultured for 2 h. Then dimethyl sulfoxide (DMSO, 100μL) was used to dissolve the purple formazan crystals. Finally, the absorbance was collected at 490 nm in a microplate reader (Thermo Scientific Multiskan FC, China).

***Internalization in vitro*:** HeLa cells and drug-resistant MCF-7 cells were seeded onto Thermo Scientific™ Nunc™ Lab-Tek™ II Chamber Slide™ System at a density of 5×10^4 cells per well. After 24 h of incubation, the culture media were removed, and DMEM media containing free DOX and ACC-DOX@silica-A nanospheres were then added into each well. For cell observation, after predetermined co-incubation time, the cells were washed by PBS and dyed with Hoechst 33258 (10 μg/mL) for 5 min, followed by being washed with PBS. At last, the samples were excited at 405 nm for nucleus and 488 nm for DOX using a confocal laser scanning microscopy (Zeiss 710), respectively.

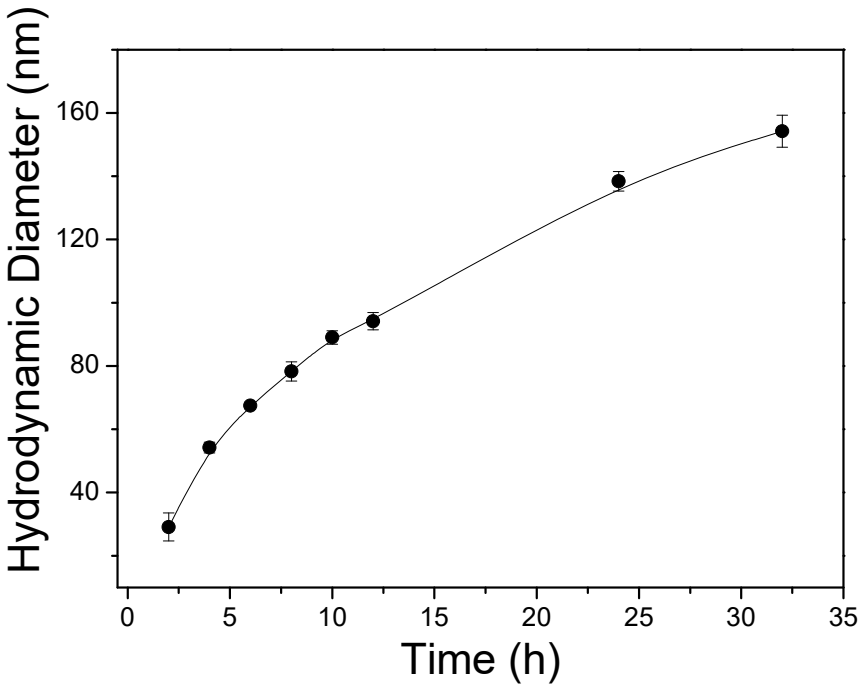

Figure S1. Time-dependent growth of ACC-DOX nanospheres during synthesis. The sizes of intermediates are characterized dynamic light scattering (DLS).

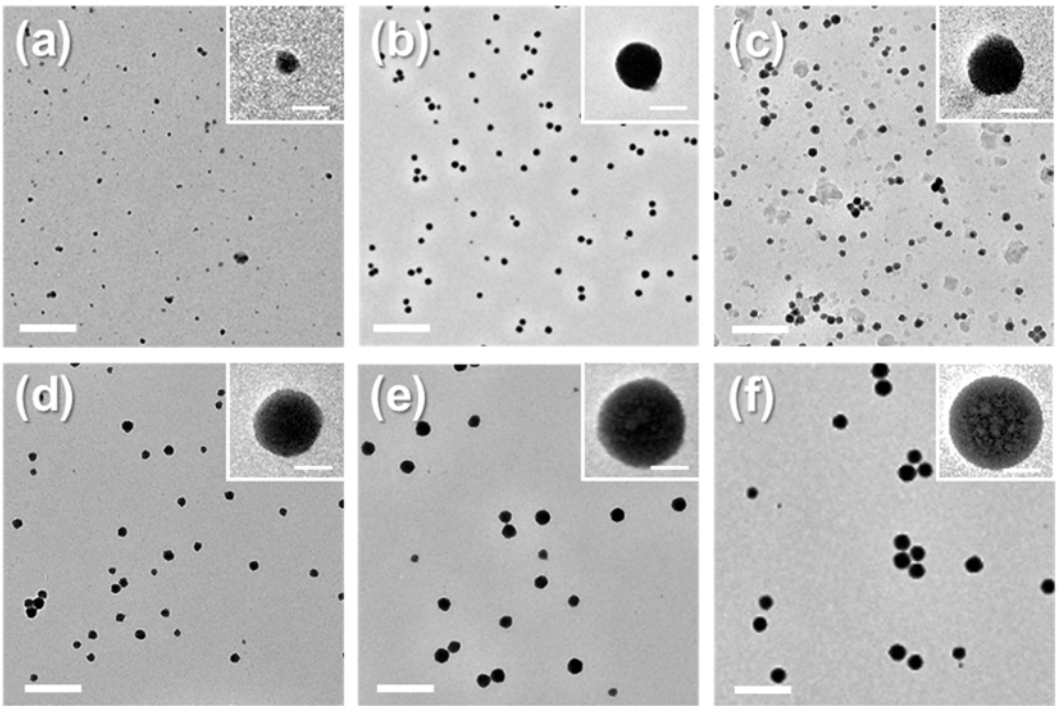

Figure S2. Representative TEM images of ACC-DOX nanospheres at predetermined time of (a) 2 h, (b) 4 h, (c) 8 h, (d) 12 h, (e) 24 h, and (f) 36 h with synthetic conditions of 2 mg/ml $CaCl_2 \cdot 2H_2O$ and 0.5% DOX solution (8 mg/ml in deionized water) at 30 ºC. Scale bars are 500 nm. Inset images are the enlarged view of the nanospheres and the scale bars are 50 nm.

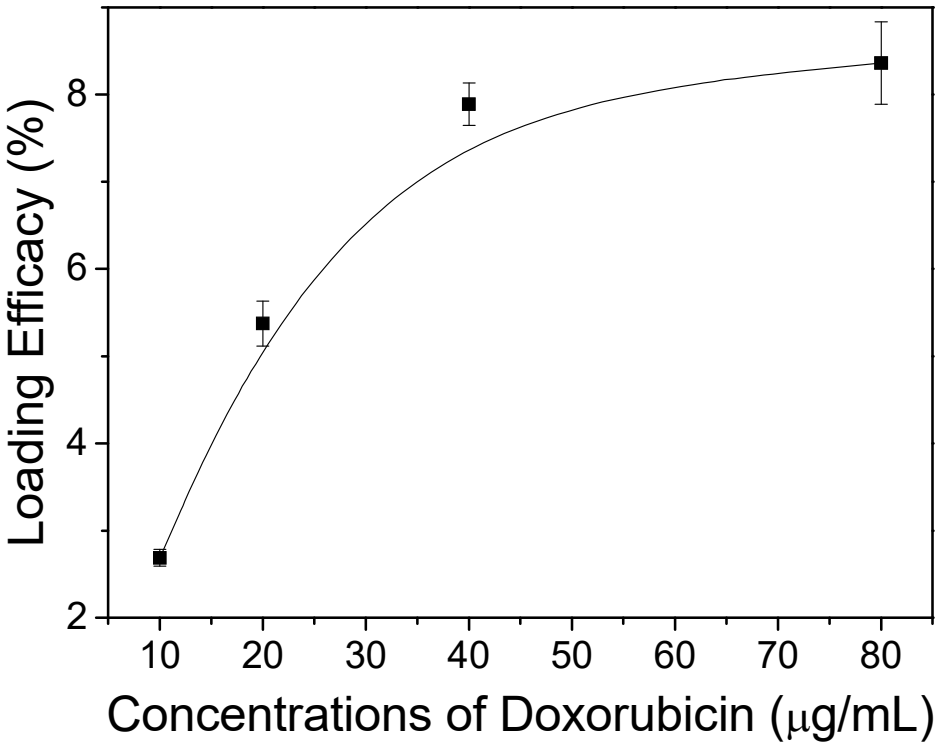

Figure S3. Loading efficacy of DOX in synthesis of ACC-DOX nanospheres with conditions of 2 mg/ml $CaCl_2 \cdot 2H_2O$ at 30 ºC and 0.5% DOX solution (deionized water) with series of final concentrations from 10-80 μg/ml in ethanol.

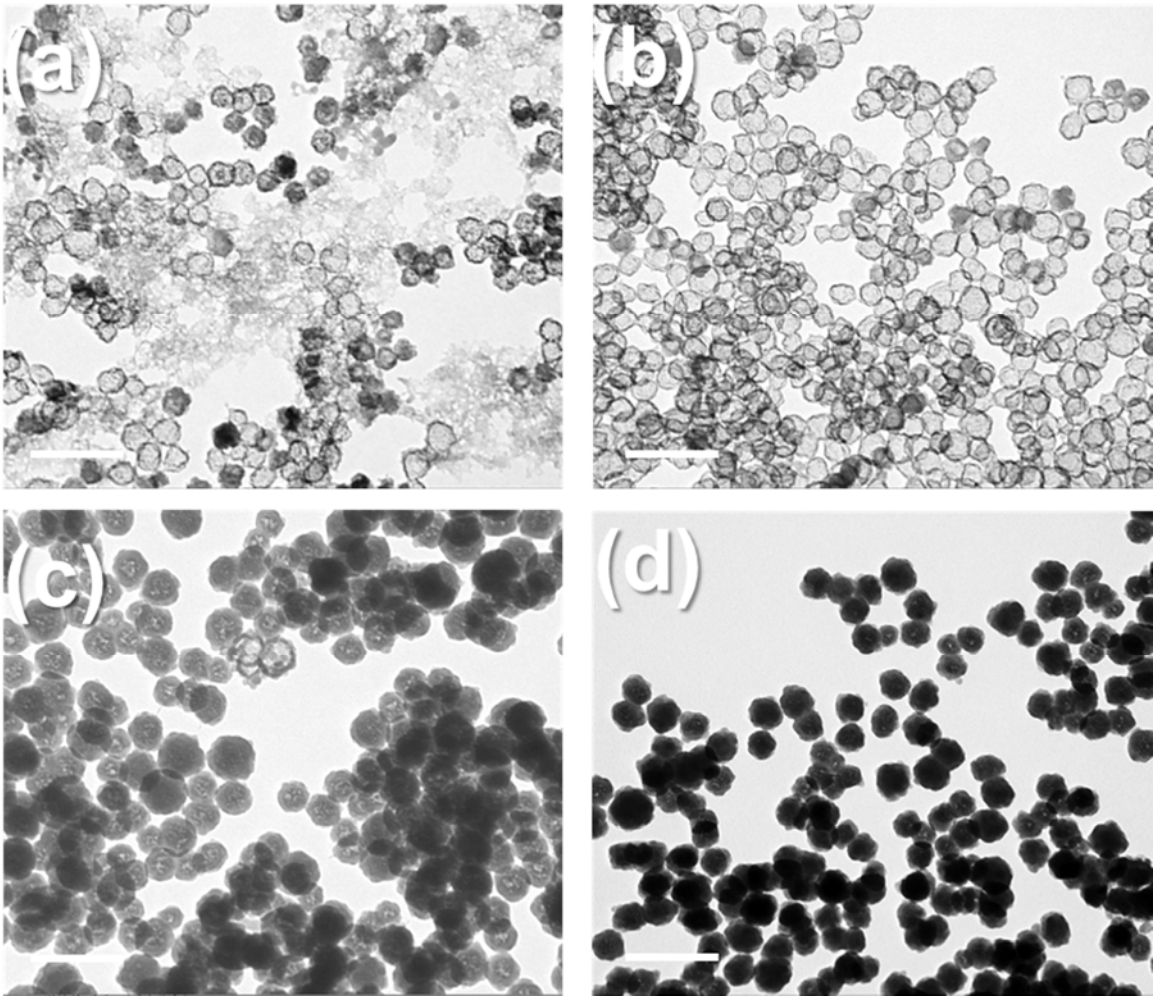

Figure S4. Representative TEM images of water treated ACC-DOX@silica nanoparticles with various synthetic ammonia concentration of (a) 1.75%, (b) 1.875%, (c) 2% and (d) 2.25%. Scale bars are 250 nm.

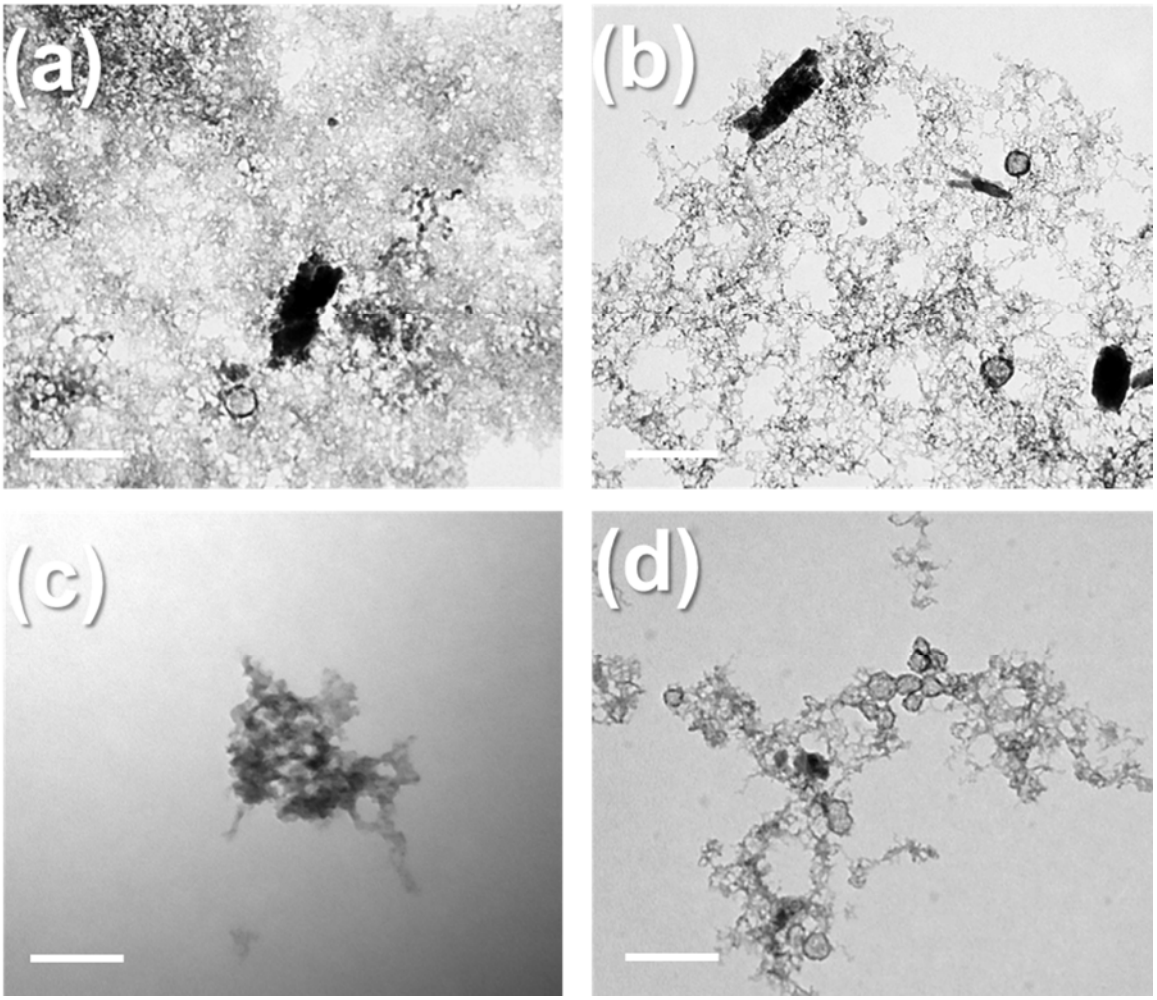

Figure S5. Representative TEM images of water treated ACC-DOX@silica nanoparticles with synthetic ammonia concentration of 1.75% at release procedure of (a) 1h, (b) 6h, (c) 12 h and (d) 24 h. Scale bars are 250 nm.

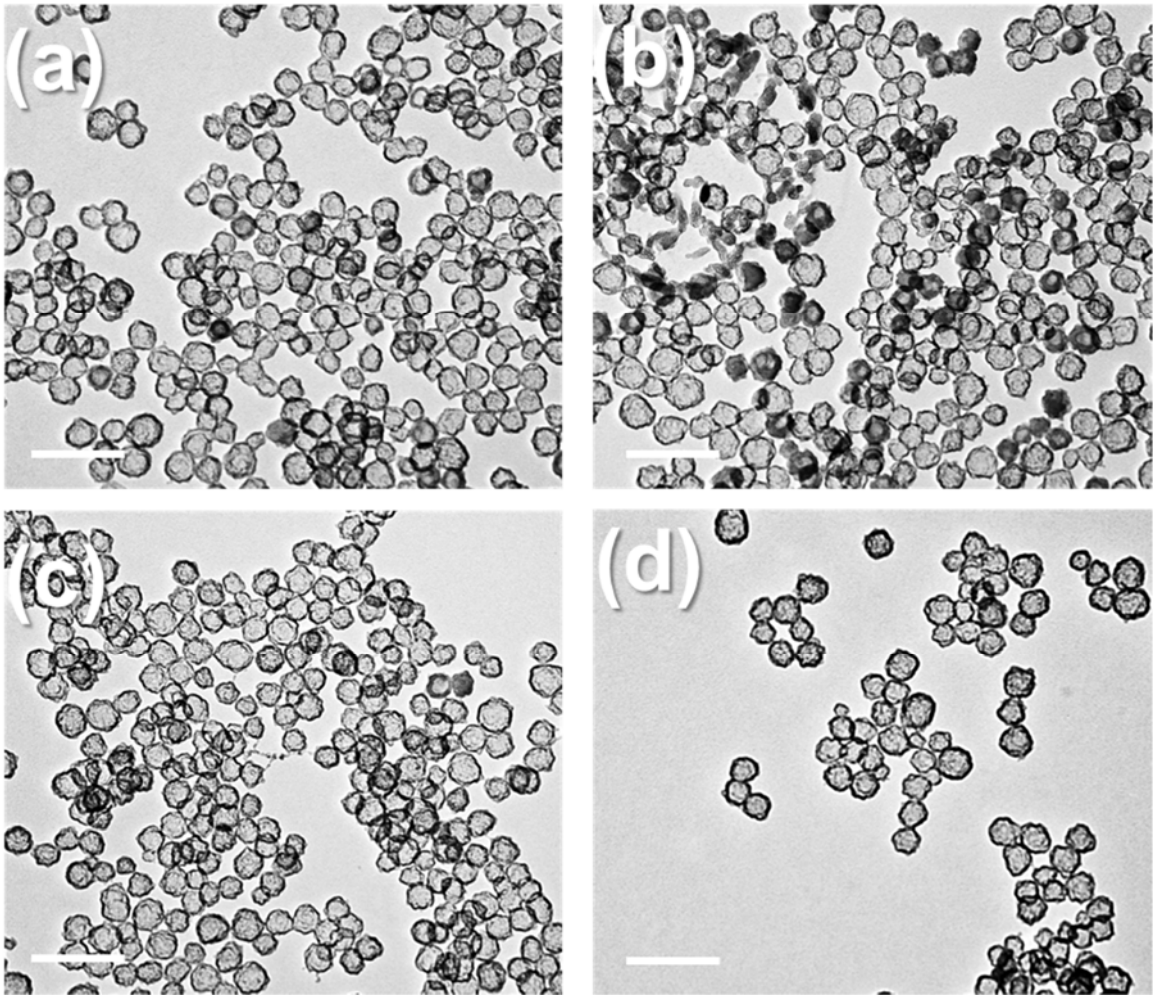

Figure S6. Representative TEM images of water treated ACC-DOX@silica nanoparticles with synthetic ammonia concentration of 1.875% at release procedure of (a) 1h, (b) 6h, (c) 12 h and (d) 24 h. Scale bars are 250 nm.

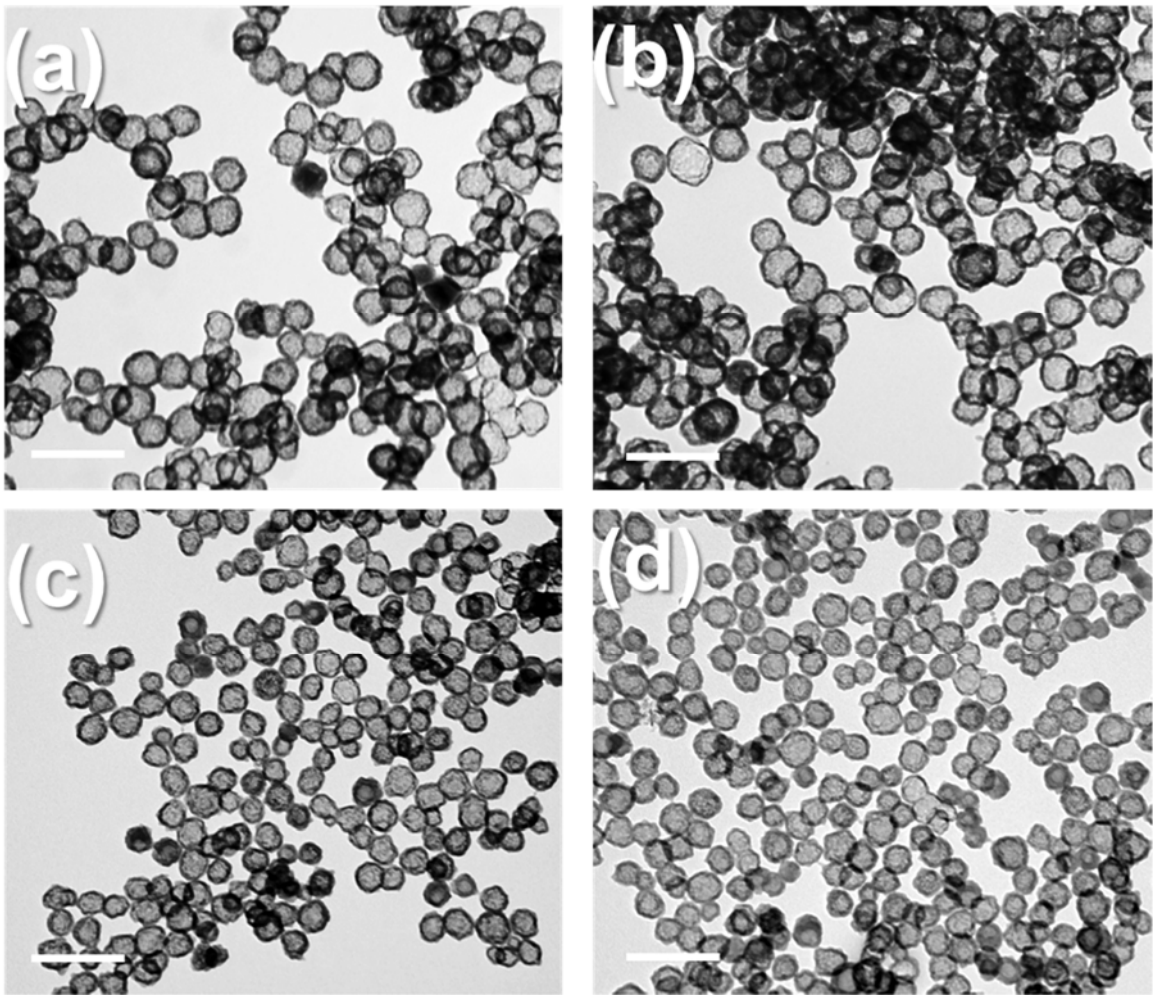

Figure S7. Representative TEM images of water treated ACC-DOX@silica nanoparticles with synthetic ammonia concentration of 2% at release procedure of (a) 1h, (b) 6h, (c) 12 h and (d) 24 h. Scale bars are 250 nm.

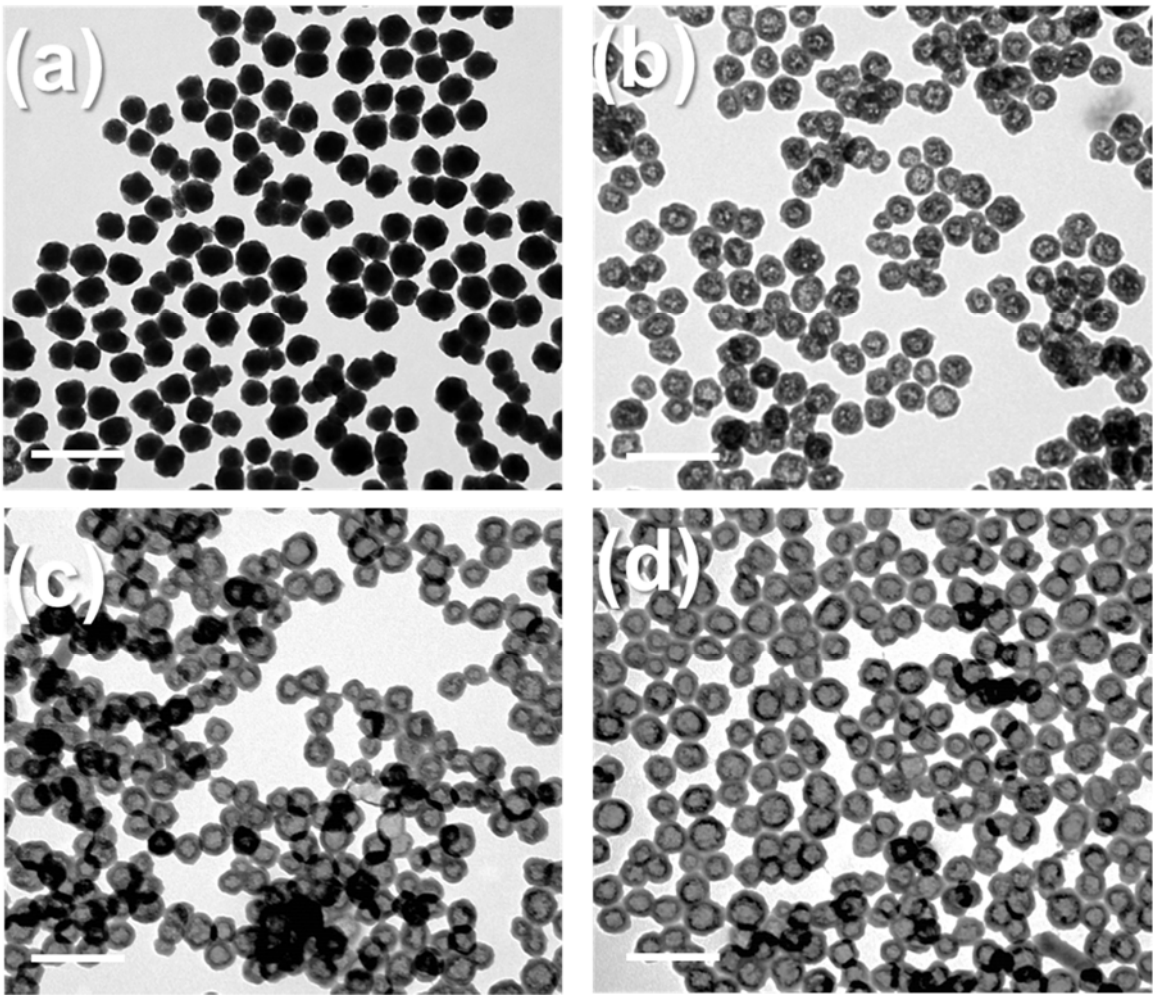

Figure S8. Representative TEM images of water treated ACC-DOX@silica nanoparticles with synthetic ammonia concentration of 2.25% at release procedure of (a) 1h, (b) 6h, (c) 12 h and (d) 24 h. Scale bars are 250 nm.

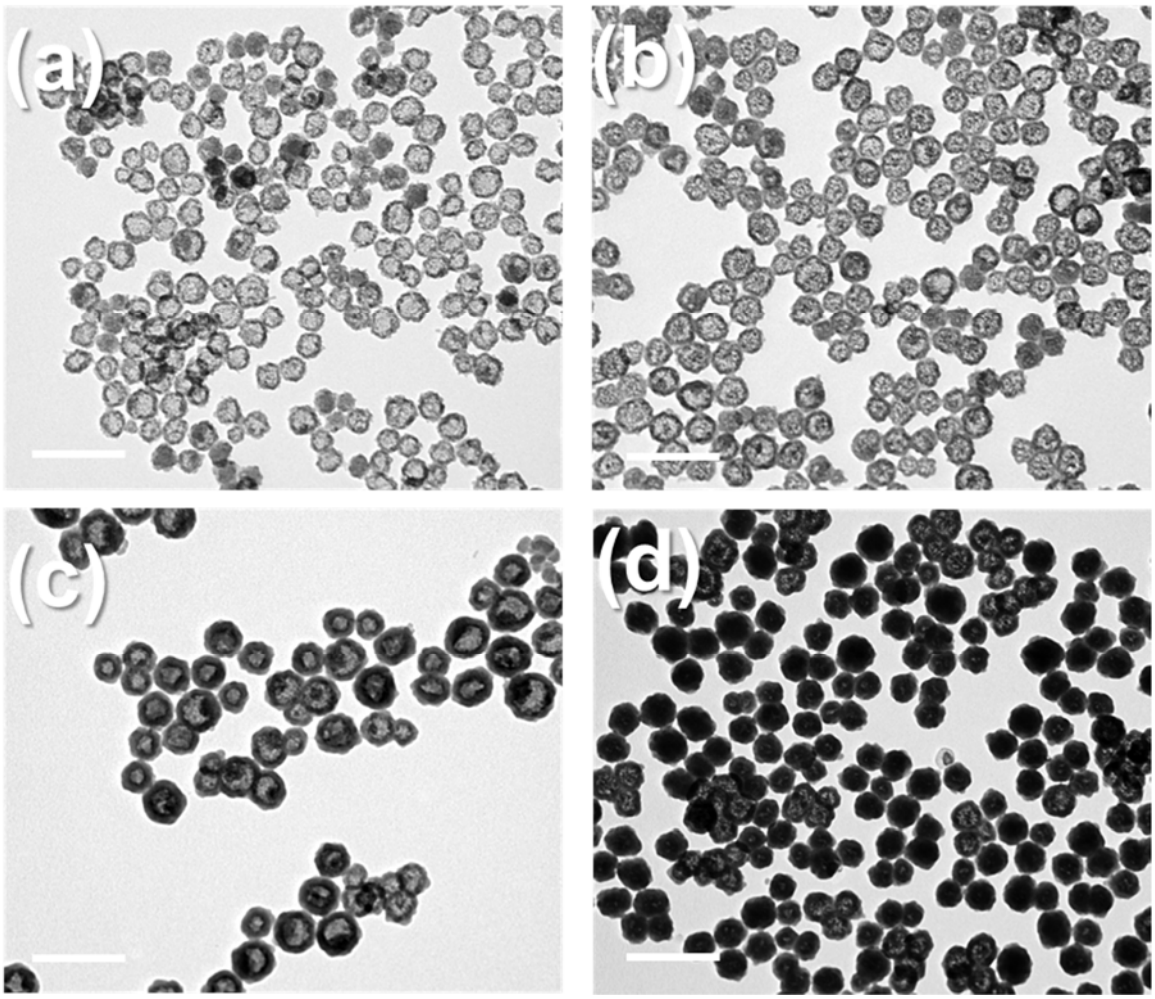

Figure S9. Representative TEM images of acid treated ACC-DOX@silica nanoparticles with various synthetic ammonia concentration of (a) 1.75%, (b) 1.875%, (c) 2% and (d) 2.25%. Scale bars are 250 nm.

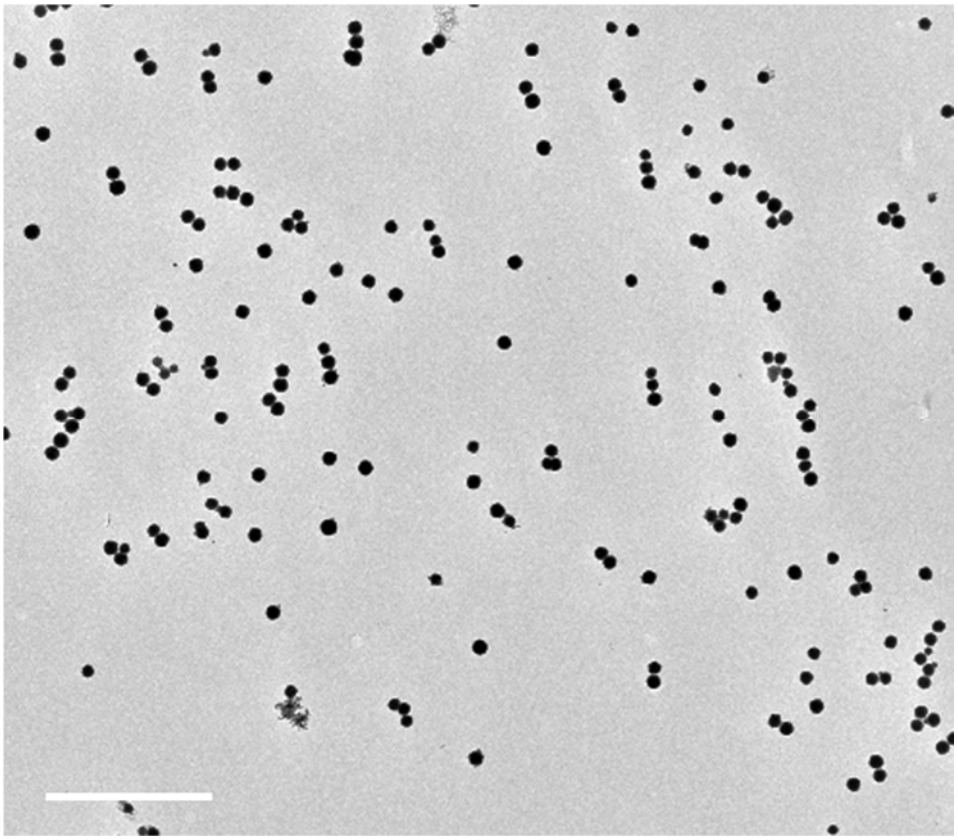

Figure S10. Representative TEM images of ACC-DOX@silica nanoparticles with synthetic ammonia concentration of 2%. Scale bar is 2 μm.

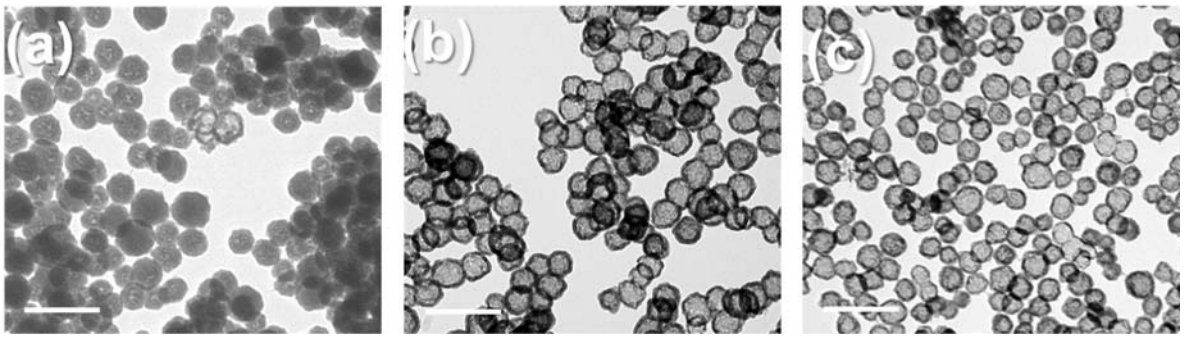

Figure S11. Representative TEM images of A0 at release procedure of (a) 0 h, (b) 1h and (c) 24 h. Scale bars are 250 nm.

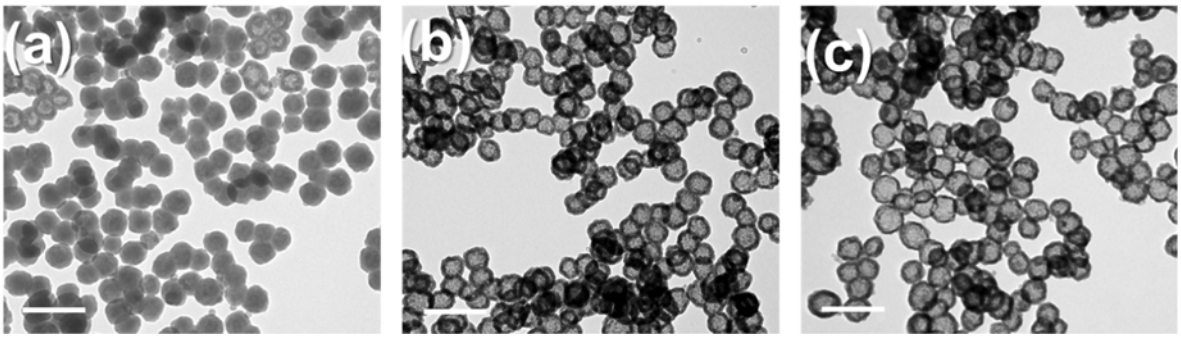

Figure S12. Representative TEM images of A2 at release procedure of (a) 0 h, (b) 1h and (c) 24 h. Scale bars are 250 nm.

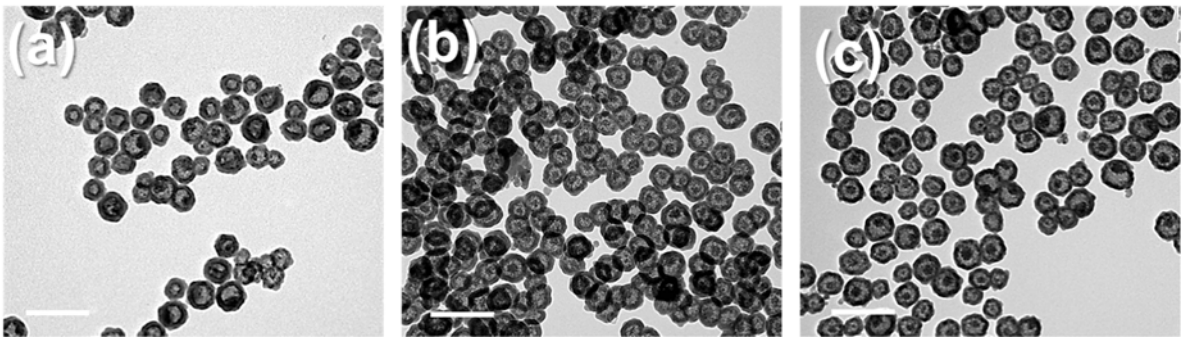

Figure S13. Representative TEM images of A4 at release procedure of (a) 0 h, (b) 1h and (c) 24 h. Scale bars are 250 nm.

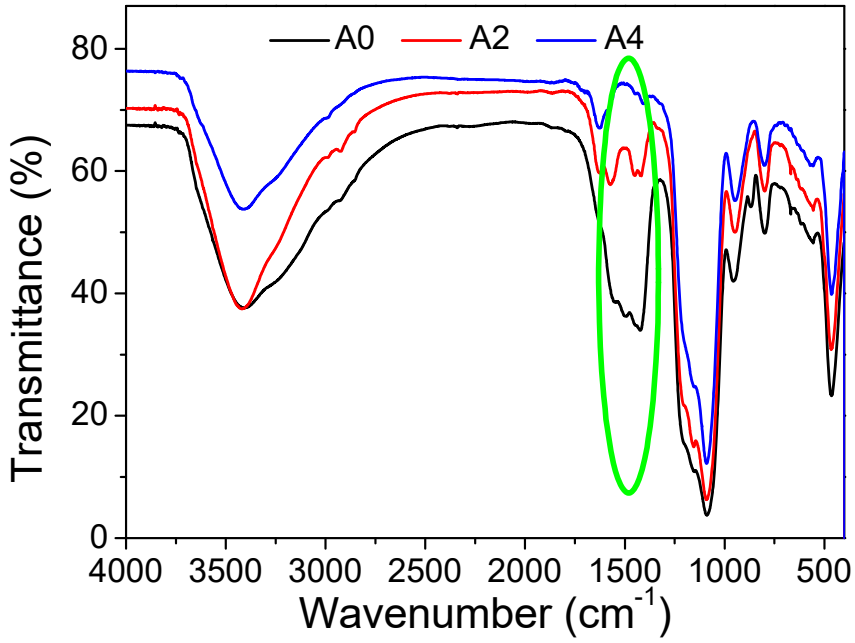

Figure S14. IR spectra of A0, A2 and A4. The gradually decreased peaks around 1430 cm^{-1} (in green circle) are attributed to the asymmetric stretch of CO_3^{2-}, which are typical characteristics of ACC vibrations. These results indicate that the gradual decrease of Ca from A0, A2 to A4.

Table 1 XPS and ICP characterizations of A0, A2 and A4.

XPS			
Sample	**Si (At. %)**	**Ca (At. %)**	**Si:Ca (molar ratio)**
A0	**26**	**1.28**	**20.3**
A2	**23.38**	**1.13**	**20.7**
A4	**26.9**	**0.25**	**107.0**
ICP			
Sample	**Si (mg/g)**	**Ca (mg/g)**	**Si:Ca (molar ratio)**
A0	**4.97**	**1.43**	**5.0**
A2	**5.07**	**1.12**	**6.5**
A4	**5.58**	**0.31**	**25.7**

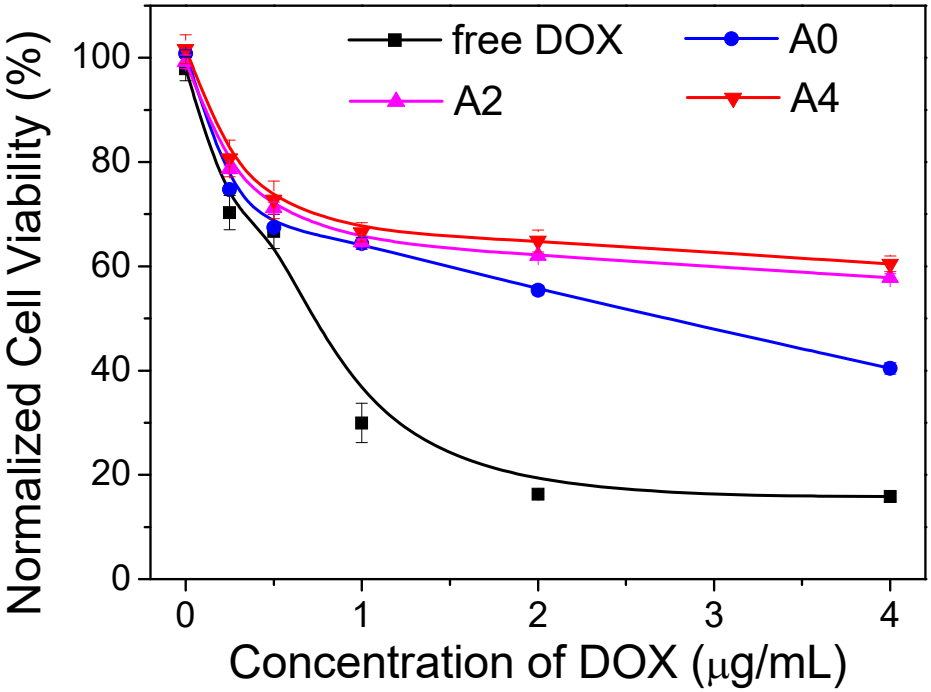

Figure S15. Cell Viability of free DOX, A0, A2 and A4 in HeLa cells for 24 h.

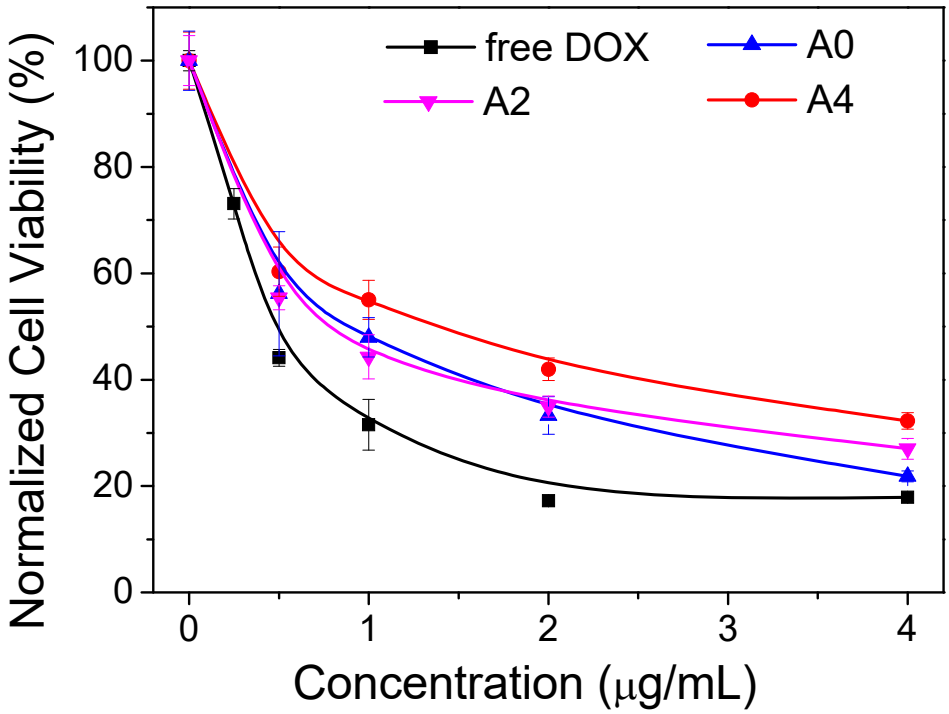

Figure S16. Cell Viability of free DOX, A0, A2 and A4 in HeLa cells for 48 h.

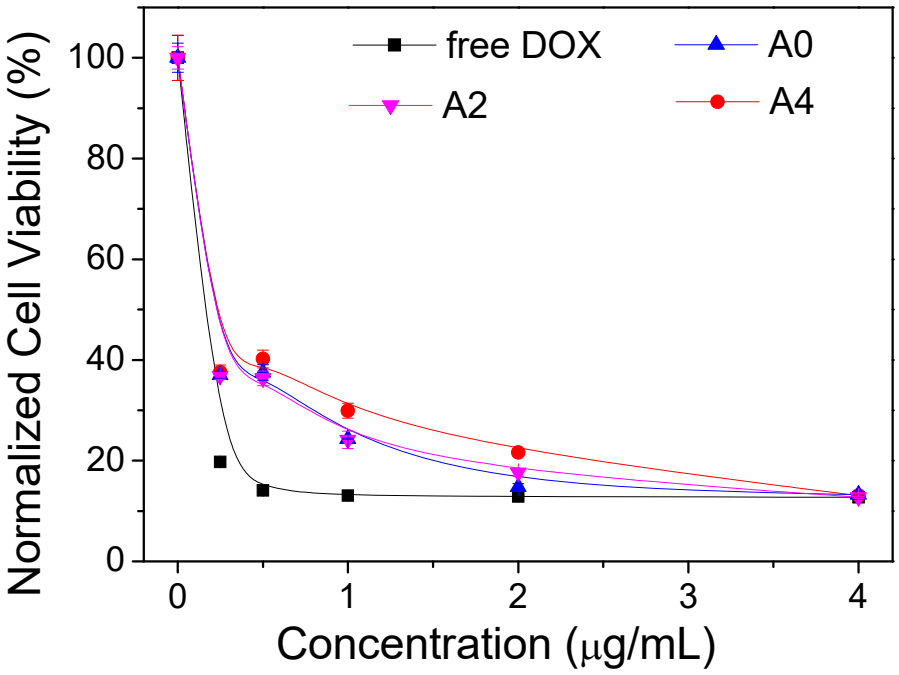

Figure S17. Cell Viability of free DOX, A0, A2 and A4 in HeLa cells for 72 h.

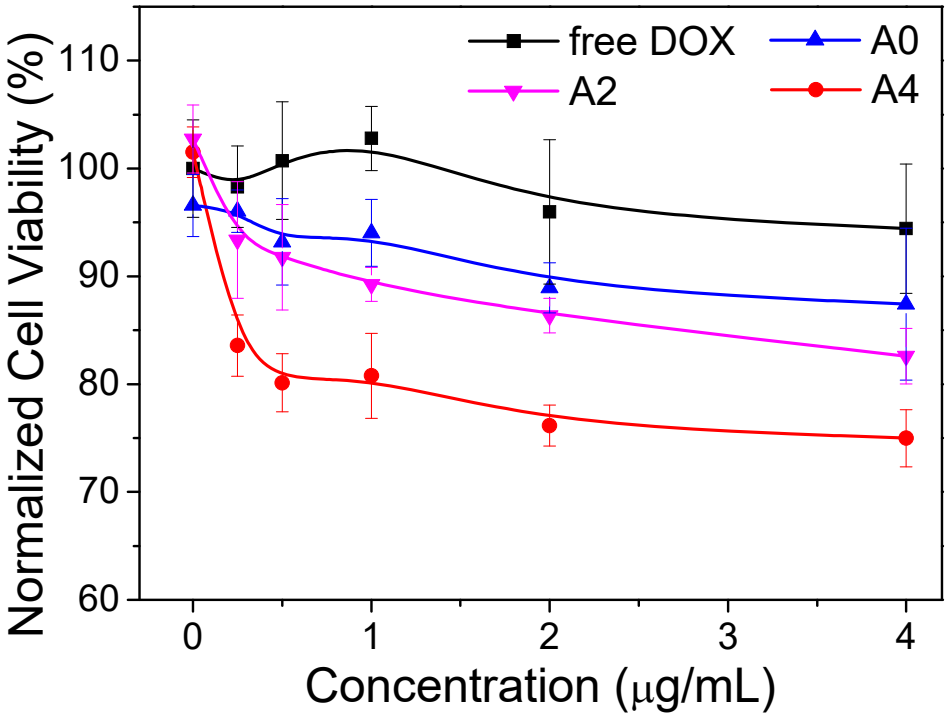

Figure S18. Cell Viability of free DOX, A0, A2 and A4 in DOX-resistant MCF cells for 24 h.

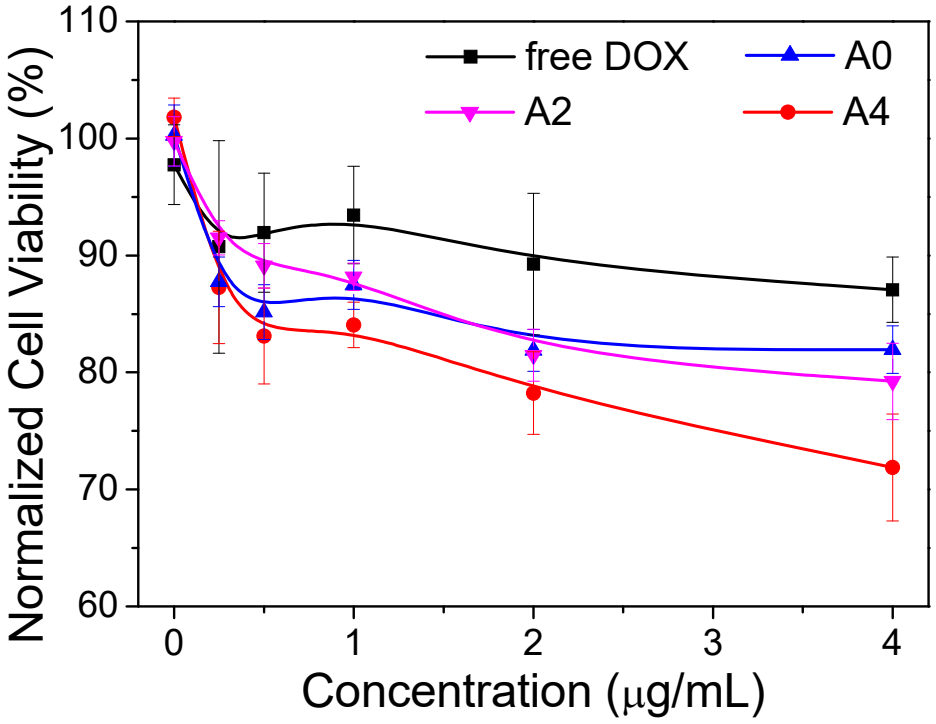

Figure S19. Cell Viability of free DOX, A0, A2 and A4 in DOX-resistant MCF cells for 48 h.

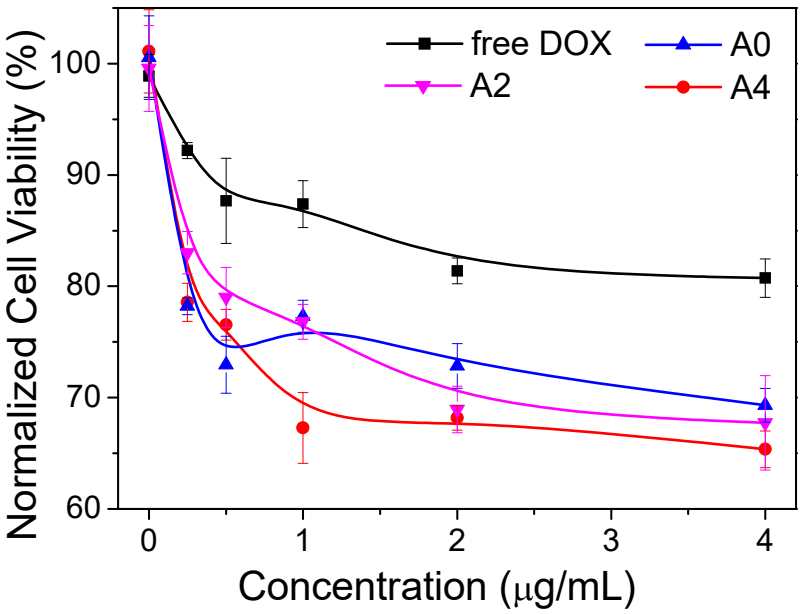

Figure S20. Cell Viability of free DOX, A0, A2 and A4 in DOX-resistant MCF cells for 72 h.

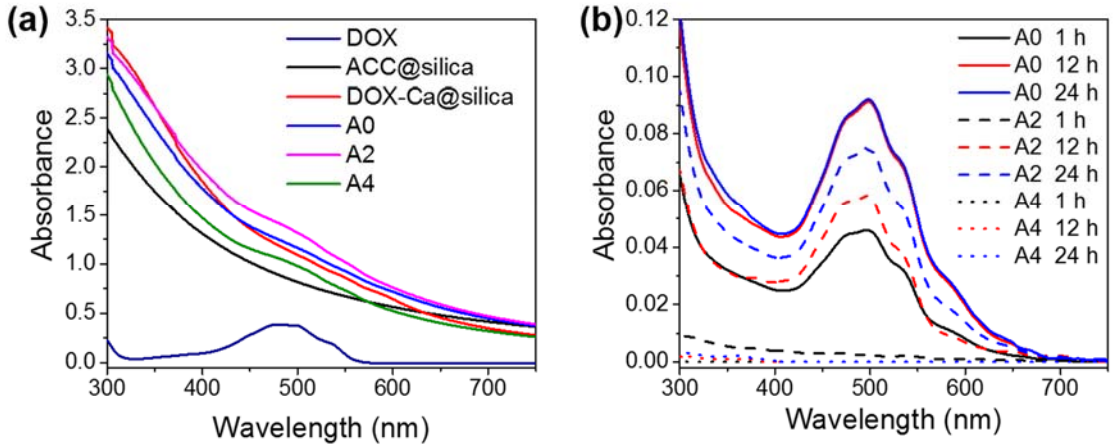

Figure S21. UV-vis adsorption spectra of (a) free DOX, ACC@silica, DOX-Ca@silica in ethanol, A0, A2 and A4; and (b) typical drug release supernates of A0, A2 and A4 at predetermined times of 1 h, 12 h and 24 h.

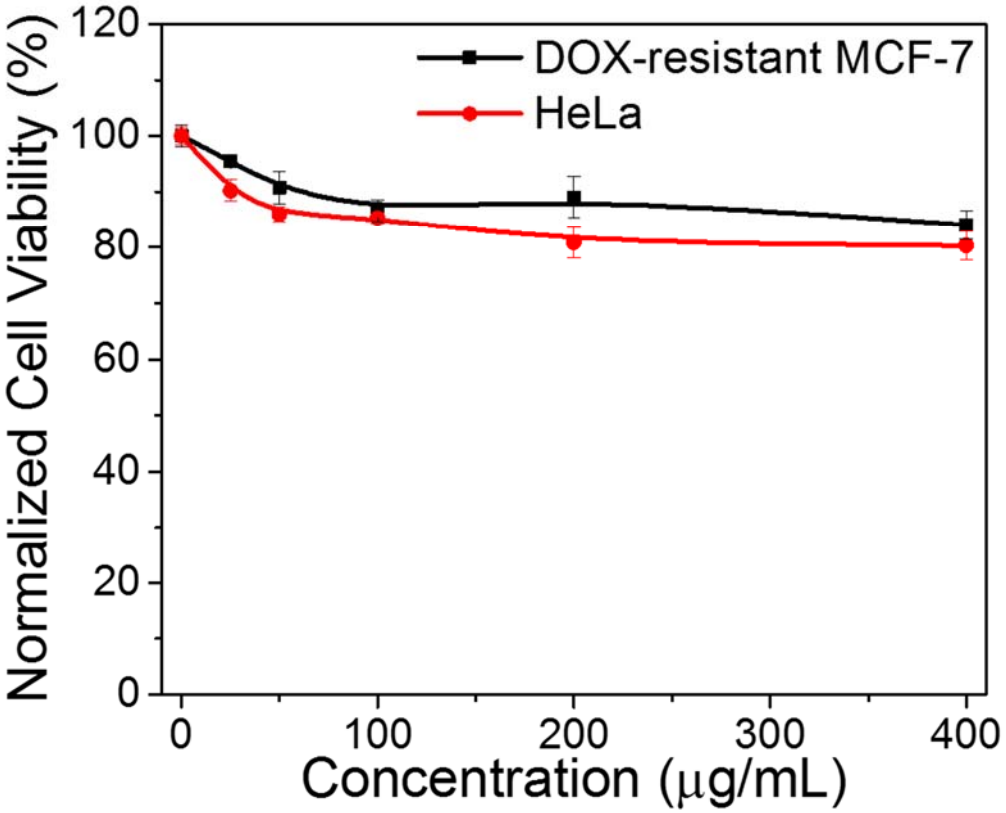

Figure S22. Cell viability of ACC@silica nanospheres in HeLa cell and DOX-resistant MCF-7 cell.

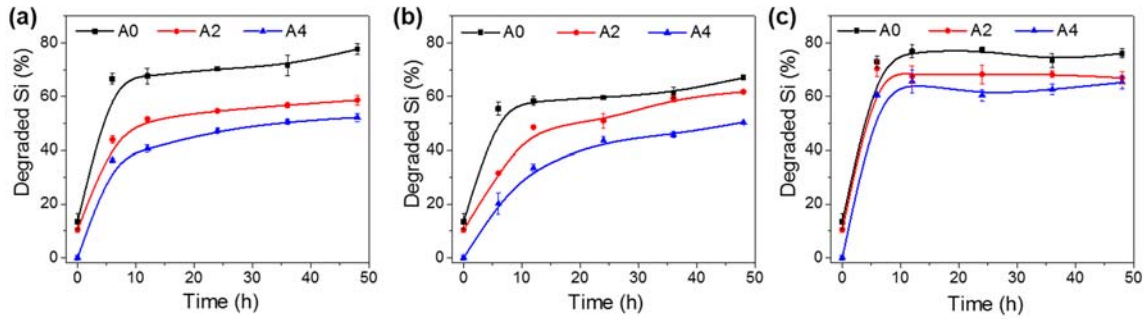

Figure S23. Time dependent silica degradation of DOX-Ca@silica nanospheres (A0, A2 and A4) at 37 ºC in (a) 0.2 M phosphate buffer at pH 6.5, (b) phosphate buffered saline (PBS, pH 7.4), and (c) Dulbecco's Modified Eagle's medium (DMEM, high glucose) with 10% Fetal bovine serum (FBS).