

One-pot Synthesis of Biopolymeric Hollow Nanospheres by Photocrosslinking

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Supplementary Information

Experimental section

Chemicals

Chitosan (CS, MW=5.6×10⁵ Da) with a deacetylation degree of 91.13% was purchased from the Zhejiang Yuhuan Biotechnology Company. 4-Aminobenzaldehyde was provided by 3B Scientific Corporation, USA. Sodium azide (analytical grade) was purchased from Sinopharm Group Chemical Reagent Co., Ltd.

Preparation of 4-azidobenzaldehyde.

4-Aminobenzaldehyde (3.5 g, 0.03 mol) was added to glacial acetic acid (13 ml) containing concentrated sulfuric acid (3.2 ml), which yielded a tarry black solid. Maintaining the reaction mixture below 5 °C, a solution of sodium nitrite (2.07 g, 0.03 mol) in a minimal amount of cold water was then added dropwise with stirring, giving a yellow solution. After 15 min, a solution of sodium azide (1.95 g, 0.03 mol) in a minimal amount of cold water was added dropwise, during which copious frothing occurred. After 1 h, the reaction product was extracted with diethyl ether, and the combined ether layers were washed with saturated aqueous NaHCO₃. The organic layer was dried over anhydrous sodium sulfate and concentrated under minimal laboratory light using a rotary evaporator at less than 40 °C to yield the product as a dark orange oil.^{ref S1}

Synthesis of carboxymethyl chitosan (CMCS).

CMCS was synthesized according to the literature.^{ref S2} In brief, 3 g of chitosan (MW=5.6×10⁵Da, deacetylation degree = 91.1%) was stirred with isopropanol (35 ml) for 6 h and then filtered. NaOH solution (50%, wt %) was added to the beaker with the filter residue, and this solution was fully stirred to mix evenly and then put in the refrigerator, where it froze overnight. Chloroacetic acid (4.5 g) dissolved in isopropanol (10 ml) was then dropped onto the frozen chitosan mixture, and the mixture was stirred at a temperature below 15 °C for 20 h. This solution was then filtered and the filter residue was washed with absolute ethanol. The crude product was then removed by centrifugation and subsequent dialysis against deionized water, and then freeze-dried to obtain CMCS. The elemental analyses results indicate that the N,O-substitution degree of CMCS is about 0.70, and the degree of N-substitution is about 0.17. The viscosity-average molecular weight of CMCS measured by an Ubbelohde viscometer is 5.1×10⁵.

Preparation of photocrosslinkable carboxymethyl chitosan (Az-CMCS).

CMCS was dissolved in deionized water. Azidobenzaldehyde dissolved in ethanol was dropped into the CMCS solution, and this mixture was stirred overnight at room temperature. A photocrosslinkable carboxymethyl chitosan solution was then obtained. The solution was divided into two parts. One part was directly used for the preparation of nanocapsules before photocrosslinking, the other part was added with cold alcohol. The resulting precipitate was collected by filtration, then washed thrice with 95% alcohol (v/v), redissolved in deionized water, dialyzed against deionized water, and then freeze-dried to obtain Az-CMCS.

The FT-IR spectra of photocrosslinkable carboxymethyl chitosan (see curve b), and carboxymethyl chitosan (see curve a) are shown in Figure S1. Upon comparing the IR spectrum of curve b with that of curve a, the Schiff bases have strong peaks at 1630–1660 cm⁻¹, which are assigned to the characteristic absorbance of the imino groups [C=N],^{ref S3} a characteristic absorption band of –N₃ at 2130 cm⁻¹^{ref S4} is observed to be present in curve b but not in curve a. These results indicate that azide substitution occurred.

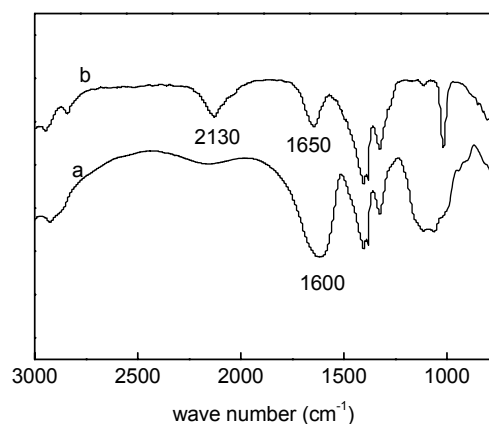


Figure S1. FT-IR spectra of (a) CMCS and (b) Az-CMCS.

The structure of photocrosslinkable carboxymethyl chitosan was also characterized by NMR spectroscopy (see Figure S2). The assignments and chemical shifts of the ¹H NMR signals of photocrosslinkable carboxymethyl chitosan are given as follows. Photocrosslinkable carboxymethyl chitosan: ¹H NMR (D₂O) δ=2.05 (COCH₃), δ=3.13 (H-2), δ=3.30 (H'-2), δ=3.7-3.9 (H-3, H-4, H-6), δ=4.17 (CH₂COOH), δ=4.22 (H-5), δ=4.79 (H-1), δ=7.09(Hb), 7.79(Ha).

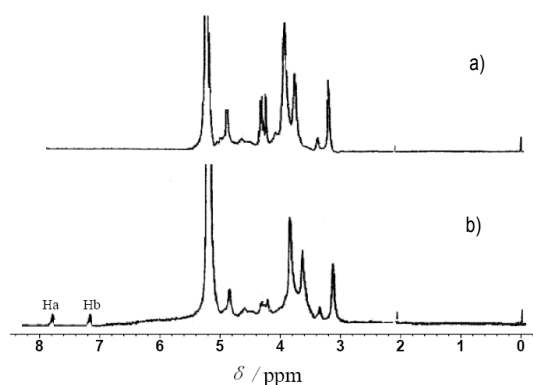


Figure S2. ¹H NMR spectra of a) CMCS and b) Az-CMCS.

Preparation of nanocapsules before photocrosslinking (Az-CMCS nanocapsules).

The nanocapsules before photocrosslinking were prepared by a sonication method. The photocrosslinkable carboxymethyl chitosan solution was sonicated for 3 min using

a probe type sonifier (JY92-2D, made by Ningbo Xinzhi Bio-tech Co. LTD.) at 100 W using a pulse function (pulse on 2.0 s, pulse off 2.0 s). Then, the solution was divided into two parts. One part was directly used for the preparation of photocrosslinked hollow nanospheres, the other was dialyzed against buffer solution, and used for analysis and testing. All operations were carried out at the dark environment.

Preparation of photocrosslinked hollow nanospheres.

The nanocapsule samples before photocrosslinking were exposed to UV light for 15 min using an ultraviolet lamp (20 W, 253.7 nm), and the photocrosslinked hollow nanospheres were obtained. The dispersions then were dialyzed against buffer for analysis and testing.

The FT-IR spectrum of the photocrosslinked carboxymethyl chitosan in the hollow nanospheres is shown in Figure S3 (see curve b). Compared with FT-IR spectrum of Az-CMCS in the nanocapsules (see curve a), the characteristic absorption band at 2130cm^{-1} is disappeared in curve b, which indicates the formation of cross-linking between azide groups after UV irradiation.

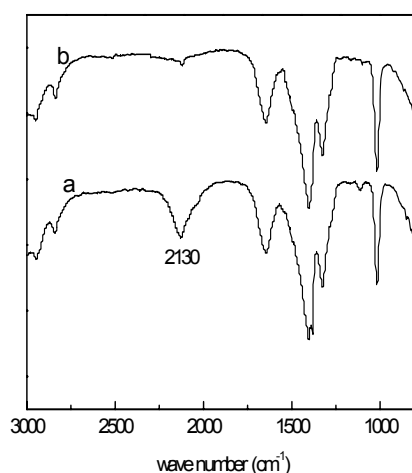
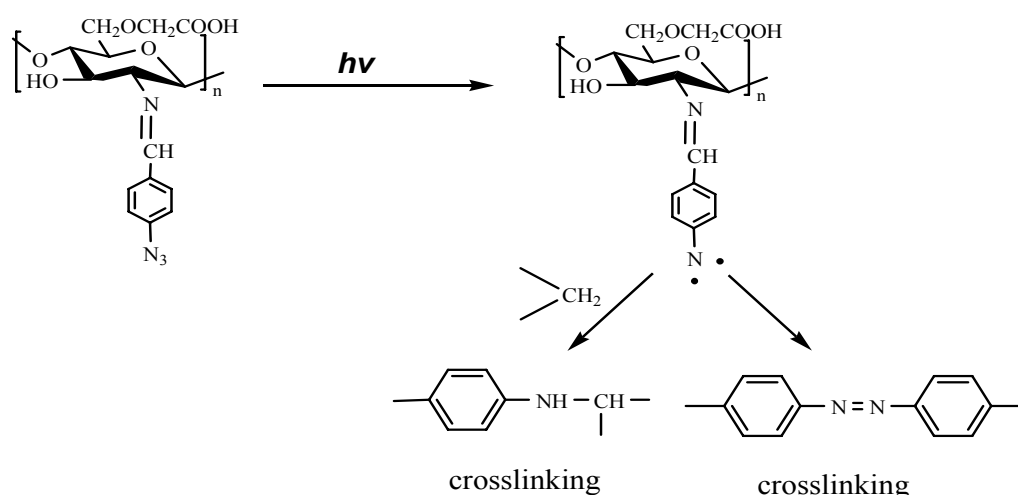


Figure S3. FT-IR spectra of (a) Az-CMCS in the nanocapsules before photocrosslinking and (b) the photocrosslinked carboxymethyl chitosan in the photocrosslinked hollow nanospheres.

According to the literature,^{ref S5} an azidobenzoate group can produce a nitrene and N₂ when irradiated with light of an appropriate wavelength. The nitrene can be converted to primary, and secondary aminobenzoate and azobenzoate groups by hydrogen abstraction and coupling. Based on the structure of Az-CMCS, we propose that the secondary aminobenzoate and azobenzoate cross-linking structures are formed between Az-CMCS chains under UV irradiation, as shown in the following scheme.



Scheme S1. Mechanism of photocross-linking for Az-CMCS.

The diameters and the size distributions of the nanocapsules before photocrosslinking and the photocrosslinked hollow nanospheres characterized by DLS are shown in Table S1. The diameters of the two sets of nanoparticles decrease with an increasing of the substitution degree of Az (the molar number of benzaldehyde azide per mole of chitosan monomeric unit), this result is apparently due to a propensity of the photocrosslinkable chitosan with the higher degree of hydrophobic substitution to coil a more shrinking structure. Compared with that of the nanocapsules, the size distribution index of the photocrosslinked hollow nanospheres is obviously smaller. This shows that the nanocapsules had some degree of aggregation before DLS testing.

Table S1. The particle sizes and distribution and Zeta potentials of the nanocapsules before photocrosslinking and the photocrosslinked nanocages at neutral pH.

samples	nanocapsules			nanocages		
	particle size (nm)	distribution index	Zeta potential (mV)	particle size (nm)	distribution index	Zeta potential (mV)
Az-CMCS-1	254±0.21	0.352±0.023	-27.8±3.56	210±0.18	0.165±0.018	-25.3±2.86
Az-CMCS-2	203±0.05	0.234±0.014	-24.0±1.45	159±0.02	0.102±0.006	-19.6±0.65
Az-CMCS-3	153±0.14	0.205±0.030	-19.3±2.64	123±0.12	0.153±0.014	-17.5±1.54

References

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