Supporting Information

Nanoscale Porous triazine-based Frameworks with Cyanate Ester Linkages for Efficient Drug Delivery

Junling Liu,‡[a] Yu Fu,‡[a] Xianbo Fu,[a] Yuxin Li,[a] Dongke Liang,[b] Yuan Song,[b] Chunyue Pan[a] Guipeng Yu,[a,b,c] and Xuxian Xiao[a] □

[a]College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, China

[b]Jiangsu Provincial Key Laboratory for Interventional Medical Devices, Huaiyin Institute of Technology, HuaiAn, Jiangsu Province 223003, China.

[c]State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012, China.

1. Materials

4-Cyanophenol (CP) and cyanogen bromide (BrCN) were purchased from Aldrich-Sigma Chemical Inc. and dried with CaH_2 before use. Triethylamine were purified by distillation under reduced pressure over calcium hydride. All reagents including trifluoromethanesulfonic acid (CF_3SO_3H), unless otherwise stated, were obtained from Sigma Aldrich and used without further purification. Ibuprofen and simulated body fluid (SBF) were purchased from Energy Chemical and used as received.

2. Instruments

^1H NMR spectra were recorded using a Bruker AV-400 spectrometer in $CDCl_3$ or DMSO-d_6 as a solution, with tetramethylsilane (TMS) as the internal reference, and chemical shifts were recorded in ppm. Solid state ^{13}C NMR spectrum was obtained on a Bruker AV-500 spectrometer operating at 75.5MHz. Infrared measurements were performed on a Thermo Nicolet Nexus 470 Fourier transform infrared spectroscopy (FT-IR), and spectra were recorded from in the range of 4000-400 cm^{-1} using the KBr pellet technique on a FTIR spectrum GX instrument. Thermoravimeric analysis (TGA) was performed on a Mettler TGA thermogravimetric analysis instrument in the nitrogen atmosphere or air atmosphere at a heating rate of 10^{o}C/min from 100 to 800^{o}C. The melt temperature (T_m), and curing temperature (T_c) of were determined with a Mettler DSC822 differential scanning calorimeter (DSC) under nitrogen flow at a heating rate of 10 ^{o}C/min from 100 to 400 ^{o}C. Scanning electron microscopy (SEM) was performed on a FEI Quanta-200 scanning electron microscope. Field-emission Transmission electron microscopes (FE-TEM) were performed on a JEM-2100F transmission electron microscope. Powder X-ray diffraction (PXRD) was obtained on a BD-86 X-ray diffractometer. UV−vis absorption of sample solutions of IBU in hexane was measured in a 1 cm quartz cell. UV−vis spectra were obtained using a Shimadzu UV-2550 UV−vis spectrophotometer. Elemental analysis of C, N and H was carried out in a Leco CNS-200 analyzer.

Surface areas and pore size distributions of NOP-14 were measured by nitrogen adsorption and desorption at 77 K using the ASAP 2020 volumetric adsorption

analyzer. The apparent surface areas (S_{BET}) for N_2 were calculated using the Brunauer−Emmett−Teller (BET) model in the relative pressure (P/P_0) range from 0.04 to 0.32. Porous volume and pore size distribution were calculated using the BJH method. Samples were degassed at 150°C for 10 h under vacuum (10^{-5} bar) before each measurement.

3. Synthesis procedures

3.1 Synthesis of 4,4',4''-(1,3,5-triazine-2,4,6-triyl)triphenol (THTZ)

To a vigorously stirred solution of trifluoromethanesulfonic acid (25.4 g, 0.34 mol) was dropwisely added 4-cyanophenol (HBN, 10.0g, 0.17mol) in dry $CHCl_3$ (135 mL) over a course of 1h at 0°C under the protection of nitrogen. After stirring for a further 1h at 0°C, the mixture was stirred for another 24h at ambient temperature and then poured into distilled-water containing a few drops of NH_4OH. The obtained mixture was filtered, and the residue was collected and purified by column chromatography, and then recrystallized in actone to afford a pale-yellow solid (7.2g, Yield: 72%) M.p. > 250°C. FTIR (KBr, cm^{-1}): 3431 (s, O-H), 1607(Ar-H), 1502(Ar-H), 1417 (C=N), 1352 (C-N). ^1H NMR (DMSO-d_6, 400 MHz): 8.56 (d, 6H, Ar-H), 6.99 (d, 6H, Ar-H).

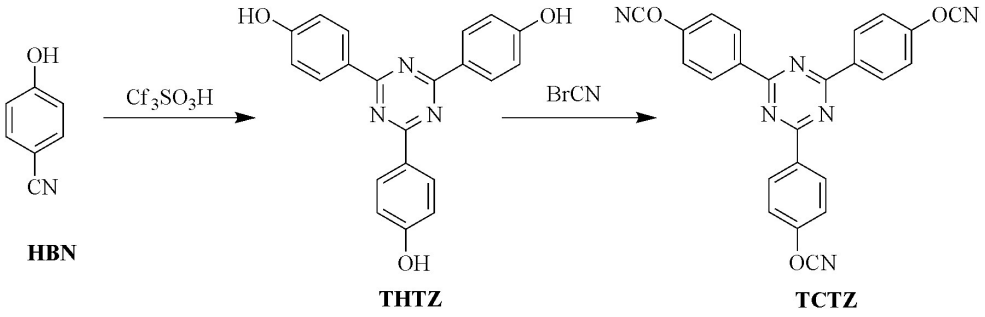

Fig. S1 Synthesis route of the intermediates THTZ and TCTZ

3.2 Synthesis of 2,4,6-tris(4-cyanatophenyl)-1,3,5-triazine (TCTZ)

A mixture of 4,4',4''-(1,3,5-triazine-2,4,6-triyl)triphenol *(*THTZ*,* 5.36 g, 15 mmol) and BrCN (4.87 g, 46.0 mmol) was rapidly charged into a 250mL round-bottom flask containing 50 mL of acetone at -30 °C under the protection of nitrogen. After rigorous stirring for 15 minutes, a solution of triethylamine (6.4 mL, 46.0 mmol) in 20 mL of actone was charged dropwisely. After another 2h of vigorously stirring at room

temperature (25°C), the formed salt was removed by filtration and the residue was collected and purified by distilled water. White solids were obtained by further recrystallization in toluene (Yield: 80%; M.p. >300 °C). ^{1}H NMR ($CDCl_3$, 400 MHz): 8.88(d, 6H), 7.54(d, 6H).

3.3 Synthesis of NOP-14

Preparation of NOP-14@1wt%: *TCTZ* (0.100g) was mixing evenly with a certain amount of diphenylsulfone (10.00 g) and dried under vacuum before the heating cycles. The whole reaction mass was sealed in a quartz ampule. For the polymerization of TCTZ, a cure schedule of 170 °C for 4 h, 190 °C for 4 h, 230 °C for 8 h, and lastly 270 °C for 20 h was employed under the protection of nitrogen. The solids obtained were crushed, and extracted with THF in a Soxhlet apparatus for 48h. The yellow or little black solid was then dried in vacuo at 180 °C. Yield: 89%. FTIR (KBr, cm^{-1}): 1518,1431,1363；

Selected date of NOP-14@5wt%: Yield: 92%. FTIR (KBr, cm^{-1}): 1537, 1411, 1362；Elemental analysis found for NOP-14@5wt%: C: 62.91%, H: 2.54%, N: 18.98. Calculated value: C: 66.67%, H: 2.80%, N: 19.44%.

Selected date of NOP-14@10wt%: Yield: 94%. FTIR (KBr, cm^{-1}): 1518, 1407, 1357；Elemental analysis found for NOP-14@10wt%: Calculated: C: 66.67%, H: 2.80%, N: 19.44%.

4. Drug loading and release experiments

4.1Drug loading experiments

A typical procedure was used to charge the NOP-14 materials with IBU. At various selected loading temperature (i.e. 298K, 308K or 318K, referring to Table S1), the model drug was dissolved in 10 mL of different solvent mediums (i.e. hexane, petroleum ether, acetone or ethanol, referring to Table S4), and the activated NOP-14@2wt% sample (degassed at 150°C for 10 h under vacuum of 10^{-5} bar) was added with constant stirring (1000 rpm) for 24 h, preventing the evaporation of hexane. Varying mass ratios of IBU against NOPs (i.e. 2:3, 1:1 or 2:1, referring to Table S3)

and repeated impregnations (i.e. once, twice, three times or four times, referring to Table S2) were also selected to probe their effects on the drug loading amount.

Table S1 The calculated IBU amount loaded with varying loading temperatures

Temperature	298K	308 K	318 K
IBU	15.00	15.78	15.85

where initial mass ratio of $M_{IBU}/M_{NOP\text{-}14@2wt\%}$ is set to be 2:3, utilizing hexane as solvent medium under 1st impregnation with a contact time of 24h in hexane.

Table S2 The calculated IBU amount loaded with different impregnations times

Times	1st	2nd	3rd	4th
IBU	15.00	15.31	15.57	15.68

where initial mass ratio of $M_{IBU}/M_{NOP\text{-}14@2\%}$ is set to be 2:3, utilizing hexane as solvent medium at 298K with a contact time of 24h in hexane.

Table S3 The calculated IBU amount loaded in various solvent mediums

Immerse solvent	hexane	petroleum ether	acetone	ethanol
NOP-14@1wt%	15.00	15.15	12.31	11.52

where initial mass ratio of $M_{IBU}/M_{NOP\text{-}14@2\%}$ is set to be 2:3, utilizing hexane as solvent medium at 298K under 1st impregnation with a contact time of 24h.

Table S4 The calculated IBU amount loaded with different contact time

Immerse solvent	4h	8h	16h	24h	36h	72h
NOP-14@1wt%	5.49	9.36	13.29	15.00	15.47	15.41

where initial mass ratio of $M_{IBU}/M_{NOP\text{-}14@2\%}$ is set to be 2:3, utilizing hexane as solvent medium at 298K under 1st impregnation in hexane.

The optimized drug loading experiments was listed as follows. The model drug IBU (330 mg) was dissolved in hexane (10 mL), and the activated NOP-14@2wt% sample (165 mg, degassed at 150°C for 10 h under vacuum of 10^{-5} bar) was added with constant stirring (1000 rpm) for 24 h at 298K, preventing the evaporation of hexane. UV-vis spectrophotometer was used to determine the amount of IBU absorbed by the polymer, reading at 273 nm.

Table S5 The calculated IBU amount loaded with varying ratios of IBU against NOPs

M_{IBU}/M_{NOP-14}	2:3	1:1	2:1
NOP-14@1wt%	15.00	39.58	37.06
NOP-14@2wt%	17.63	27.12	38.62
NOP-14@5wt%	17.94	48.64	50.75
NOP-14@10wt%	21.37	50.06	54.83

where the uploading is completed by utilizing hexane as solvent medium at 298K with only one impregnation with a contact time of 24h.

Table S6 Elemental analysis results for the NOP-14 series/IBU complexes

Sample	Calculated[a)]			Found		
(Estimated IBU content)	C (%)	H (%)	N (%)	C (%)	H (%)	N (%)
NOP-14@1wt% 18.0wt% IBU	68.08	3.73	16.46	65.87	3.81	15.78
NOP-14@2wt% 49.3wt% IBU	69.79	4.86	13.01	66.91	4.65	12.98
NOP-14@5wt% 50.1wt% IBU	69.82	4.88	12.94	66.86	4.51	12.77
NOP-14@2wt% 54.8wt% IBU	70.02	5.00	15.54	67.43	4.72	14.99

a) Elemental values based from the proposed NOP-14@IBU complexes

4.2 Drug release experiments

A release experiment of NOP-14@2wt%/IBU was given as an example. The release profile was obtained by soaking the NOP-14@2wt%/IBU sample (34 mg) in 250 mL of a simulated body fluid (SBF, 0.038 mg of ibuprofen of the sample per mL of fluid), and then monitoring the drug concentration in the fluid by means of a UV-vis spectrophotometer. SBF has a composition very similar to the human plasma (pmm: 142.0/5.0/2.5/1.5/147.8/4.2/1.0/0.5Na^+/K^+/Ca^{2+}/Mg^{2+}/Cl^-/HCO_3^-/HPO_4^{2-}/SO_4^{2-}).[20] These suspensions were kept under bidimensional agitation for different incubation time periods (from 30 min to 5 days). At each time point, an aliquot of 0.5 mL of supernatant was recovered by constant centrifugation (1000 rpm) and replaced with the same volume of fresh PBS. The released IBU amount was determined by UV-Vis spectrophotometer with the same procedure for the determination of the IBU loading amount.

5 . Characterization of monomers and polymers, heating cycles

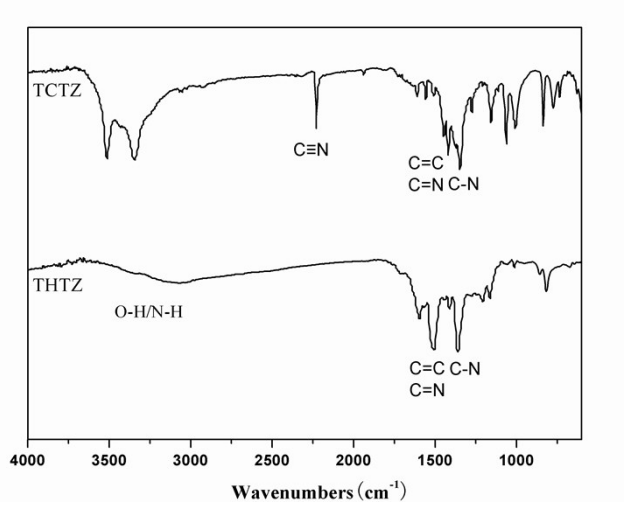

Fig. S2 FTIR spectra of THTZ and TCTZ

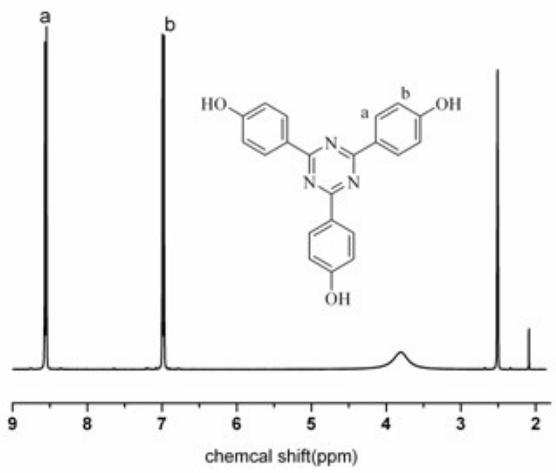

Fig. S3 ^{1}H NMR spectrum of THTZ compound

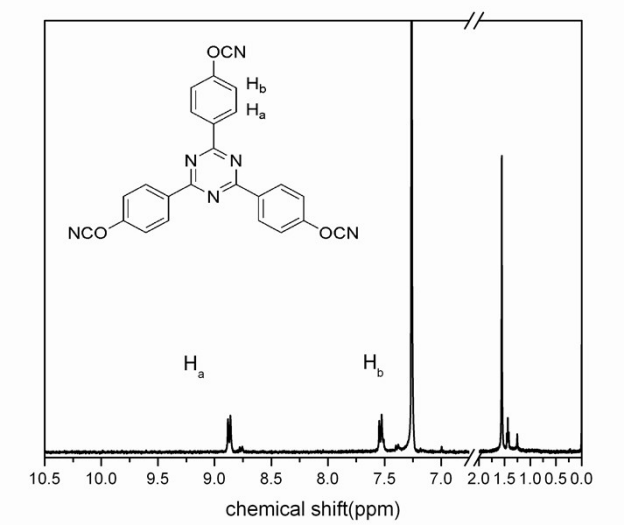

Fig. S4 1H NMR spectrum of TCTZ compound

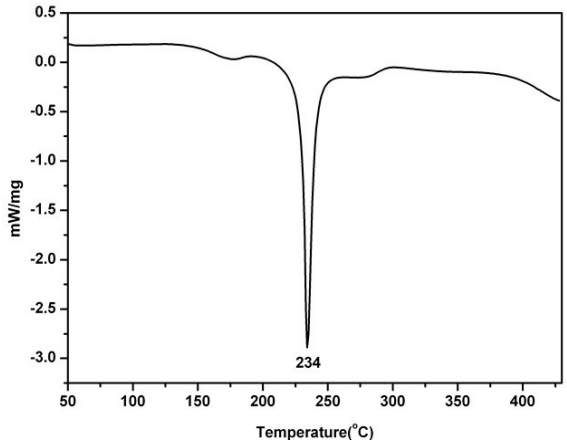

Fig. S5 DSC curve of cyanate monomer TCPT

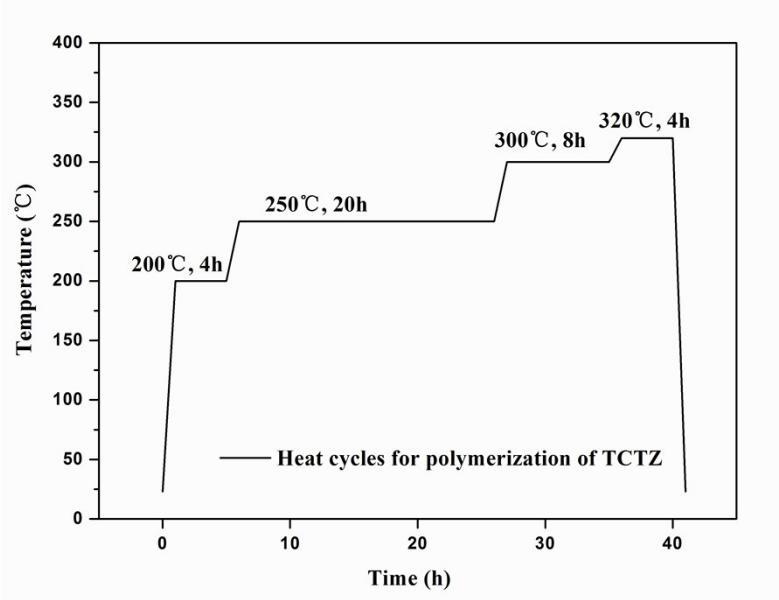

Fig. S6 Heating cycles for the polymerization of TCTZ

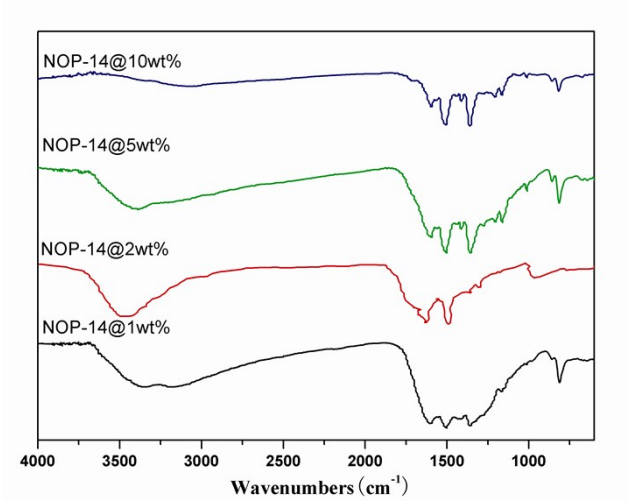

Fig. S7 FTIR spectra of NOP-14 series

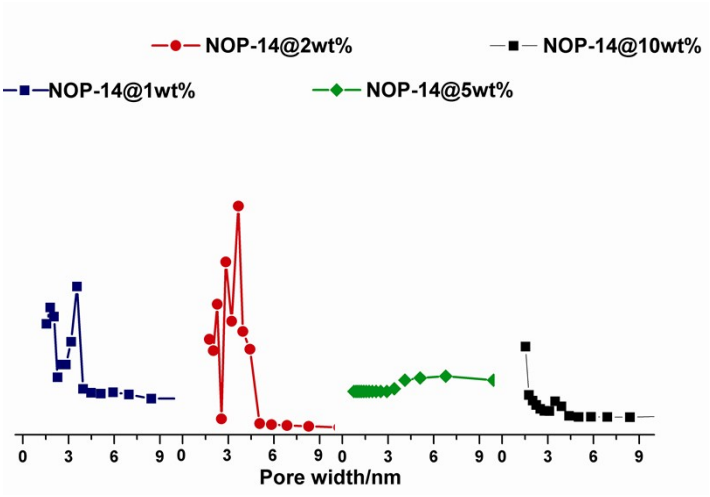

Fig. S8. Pore size distribution obtained by the BJH method (ranging from 0.1 to 9 nm).

6 . TGA traces of NOP-14@2wt% and NOP-14@2wt%-IBU

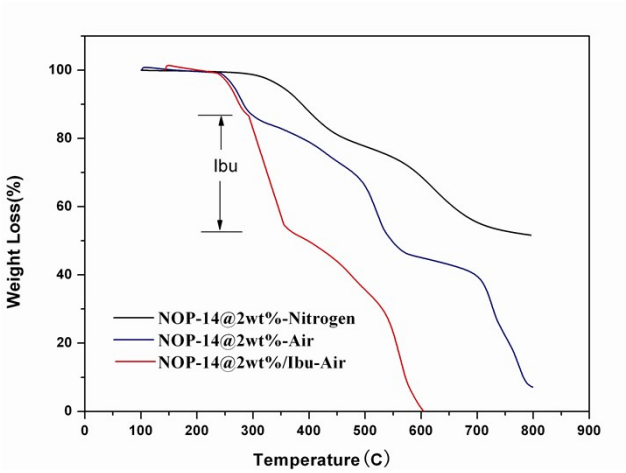

Fig. S9 TGA curves of NOP-14@2wt% and NOP-14@2wt%/IBU (nitrogen)

7. PXRD patterns of NOP-14@2wt% and NOP-14@2wt%-IBU

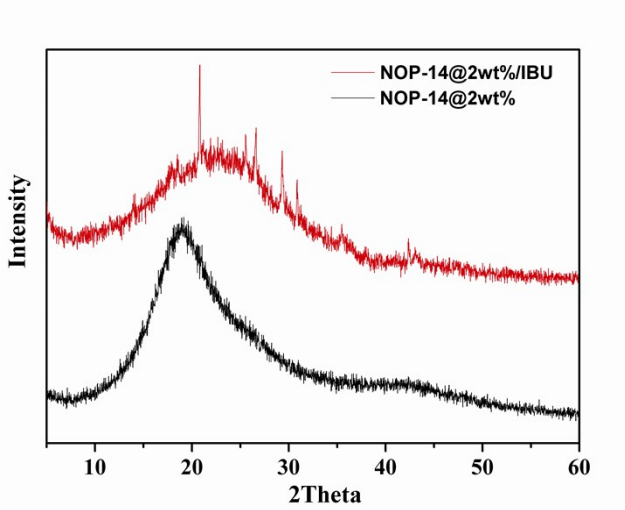

Fig S10 PXRD spectra of NOP-14@2wt% and NOP-14@2wt%/IBU

8. Morphology of NOP-14@1wt%, NOP-14@5wt% and NOP-14@10wt%

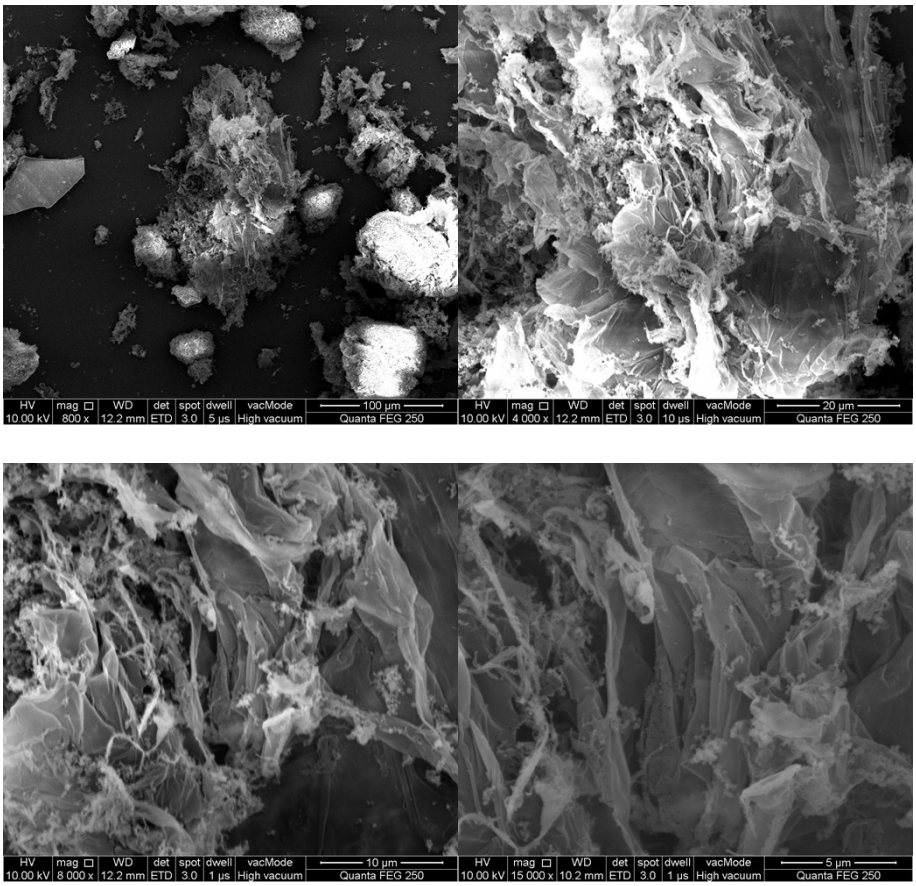

Fig. S11 SEM images for NOP-14@1wt%

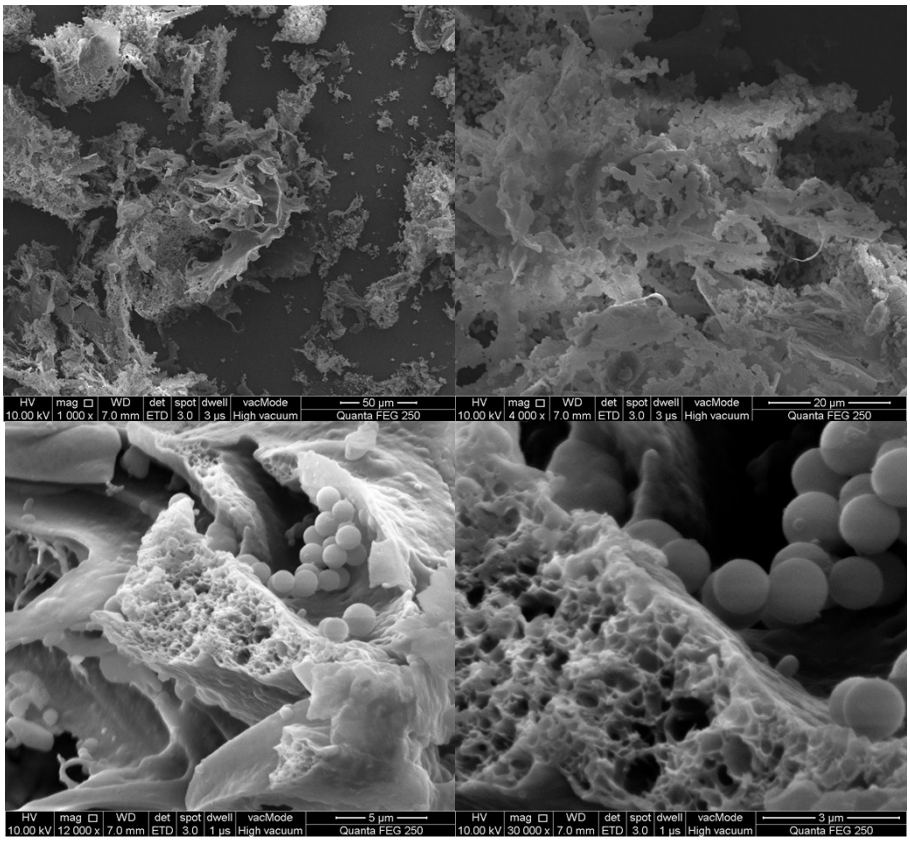

Fig. S12 SEM images for NOP-14@5wt%

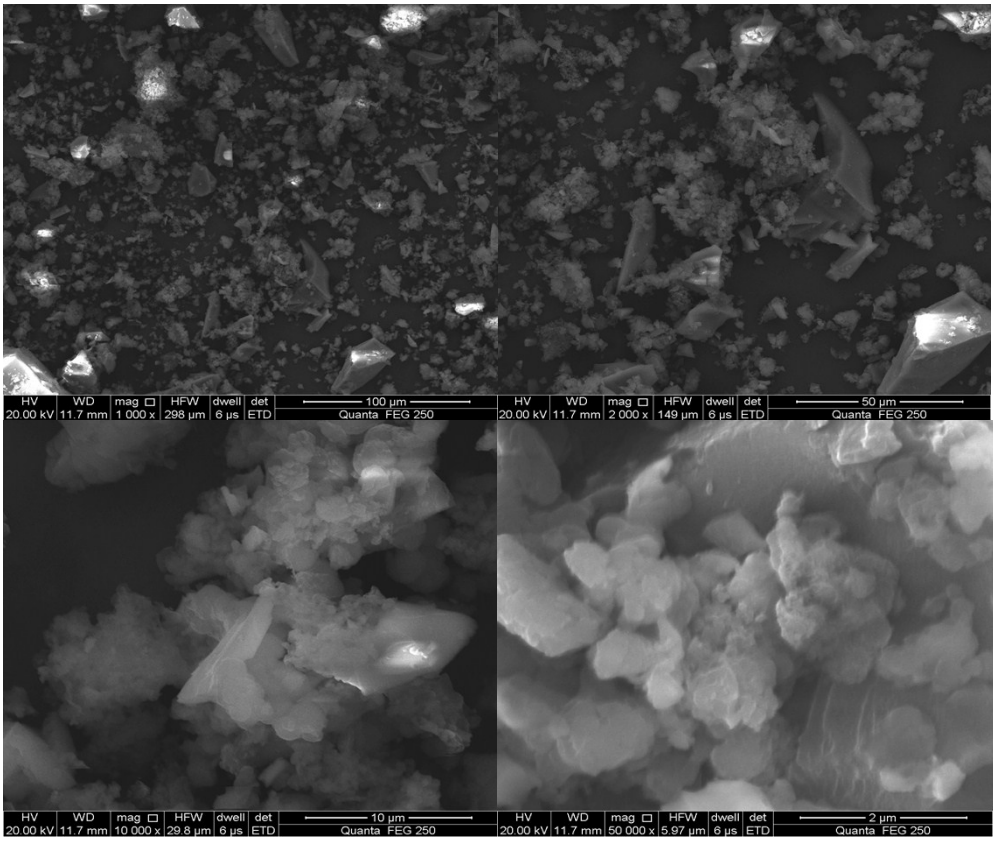

Fig. S13 SEM images for NOP-14@10wt%

9. Viability of cells in the presence of NOP-14@2wt% using MTT assay

The viability of cells in the presence of NOP-14@2wt% was investigated using 3-[4,5-dimethylthialzol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma) assay. For MTT assay, HeLa cells were seeded into 96-well plates at a density of 1×104 per well in 100 µL of media and grown overnight. The cells were then incubated with various concentrations of NOP-14 for 48 h. Following this incubation, cells were incubated in media containing 0.5 mg mL^{-1} of MTT for 4 h. The precipitated formazan violet crystals were dissolved in 100 µL of 10 % SDS in 10 mmol HCl solution at 37 °C overnight. The absorbance was measured at 570 nm by multi-detection microplate reader (SynergyTM HT, BioTek Instruments Inc, USA). The assay was carried out in triplicate in the above manner.

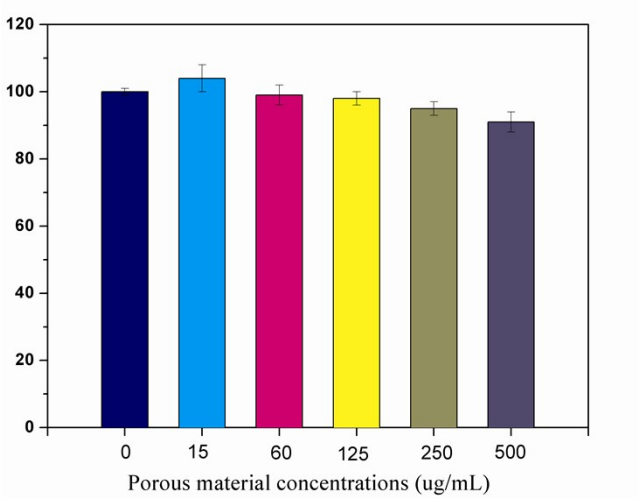

Fig. S14 Viability of cells in the presence of NOP-14@2wt% using MTT assay

10. Simulated structure of NOP-14

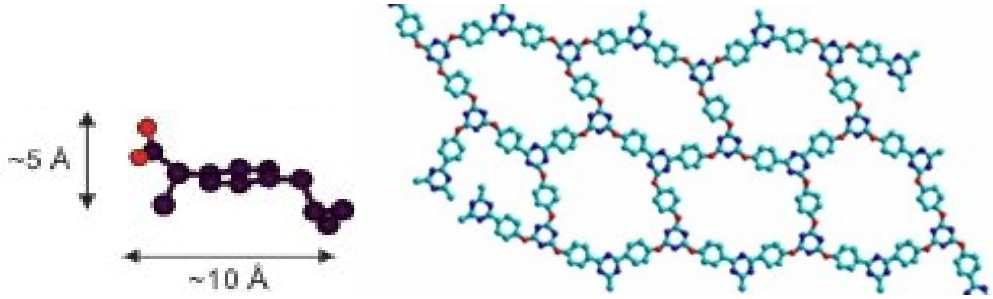

Fig S15 3D Schematic representation of IBU and NOP-14 series

11. **Reproducibility of NOP-14 as a reservoir for IBU delivery and release.**

Table S7 The calculated IBU uptake amount for the regenerative samples

Regenerative times	Original	1st	2nd	3rd
NOP-14@2wt%	38.62	37.14	35.70	32.81
	100%	96%	91%	85%

where initial mass ratio of $M_{IBU}/M_{NOP\text{-}14@2\%}$ is set to be 2:1, and the uploading is completed utilizing hexane as solvent medium at 298K with only one impregnation with a contact time of 24h.

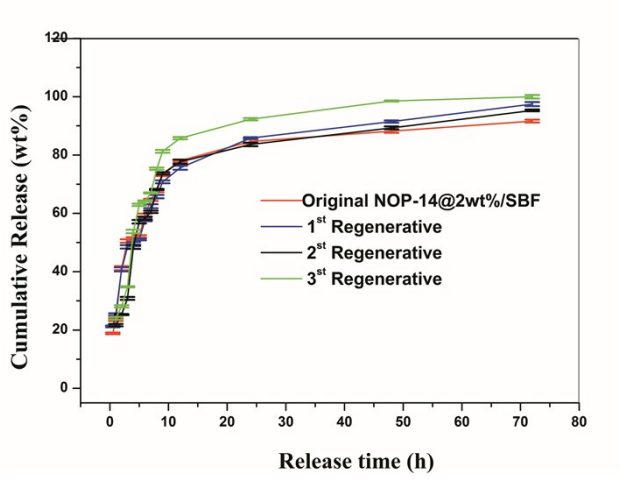

Fig S16 IBU release from the regenerative samples of NOP-14 in SBF.