Electronic Supplementary Material (ESI) for ChemComm.

Carbon nano-dots induced gelation of a histidine based amphiphile: Application as a fluorescent Ink and modulation of Gel stiffness

Subir Paul, Kousik Gayen, Nibedita Nandi and Arindam Banerjee*

[a]Department of Biological Chemistry, Indian Association for the Cultivation of Science,

Jadavpur, Kolkata, 700032 (India)

Fax: (+ 91) 33-2473-2805,

E-mail: bcab@iacs.res.in.

Experimental section:

NMR experiments

All NMR studies were carried out on Bruker DPX400 MHz and Bruker DPX500 MHz spectrometers at 300 K. Concentrations were in the range 5–10 mmol in $CDCl_3$ or DMSO-d_6.

Mass spectrometry

Mass spectra were recorded on a Q-Tofmicro™ (Waters Corporation) mass spectrometer by positive mode electrospray ionization process.

Field Emission Scanning Electron Microscopy (FE-SEM) study

FE-SEM experiments were performed by placing a small portion of gel sample on a microscope cover glass. Then, these samples were dried first in air and then in vacuum and coated with platinum for 90 seconds at 10 kV voltages and 10 μA current. The average thickness of the coating layer of platinum was 3 to 4 nm. After that micrographs were taken by using a JeolScanning Microscope model JSM-6700F.

Fourier Transform Infrared (FTIR) study

All FT-IR spectra were recorded by using the KBr pellet technique in a Nicolet 380 FT-IR spectrophotometer (Thermo Scientific).

MALDI-TOF:

MALDI-TOF MS analysis has been performed by using Applied Biosystems MALDI TOF/TOF Analyzer in dihydroxybenzoic acid as a matrix.

UV/Vis spectroscopy

UV/Vis absorption spectra were recorded on a Hewlett-Packard (model 8453) UV/Vis spectrophotometer (Varian Carry 50.bio).

Rheology study

The rheological measurements of the hydrogels were studied under dynamic and steady shear measurement at room temperature (25 °C) in a parallel-plate geometry (25 mm diameter, 1 mm gap). Rheological experiments were carried out using an Anton Paar modular compact rheometer (MCR 102). Measurements of the shear moduli of the hydrogels were performed at room temperature using parallel-plate geometry (PP-25 mm, gap 0.5 mm).

PL Spectroscopy

Fluorescence studies of the sample were carried out in a HORIBA Jobin Y von Fluorolog 3 Spectrometer instrument.

Absolute quantam yield study

The absolute quantam yield of xerogel was measured in a calibrated integrating sphere method in a PTIQM400 spectrometer.

Synthetic Procedure:

Synthesis of mono Boc protected ethylenediamine: The synthetic procedure has been described in*J. Mat. Chem. B.*, **2014**, *2*, 8528-8537.

Synthesis of 1 [CH_3-$(CH_2)_{12}$-CONH-$(CH_2)_2$-NHCO-O$(CH_3)_3$]: Myristic acid (2.28g, 10mmol) was dissolved in 10 ml DMF in a 250 ml round bottom flask in an ice water bath. 1.35g (10mmol) HOBt was added to it. Then mono Boc protected ethylenediamine (1.76g, 11 mmol dissolved in 25 ml ethyl acetate) followed by DCC (2.27g, 11mmol) was added to the reaction mixture. The reaction mixture was then allowed to come to room temperature and stirred for 48h. The reaction mixture was diluted with ethyl acetate and filtered to separate N, N- dicyclohexyl urea (DCU). The organic layer was washed with 1(N)HCl (3 × 30 ml), brine (1 × 30 ml), saturated sodium carbonate (3 × 30 ml) and brine (2 × 30 ml), dried over anhydrous Na_2SO_4 and evaporated in vacuum. A white material was obtained.

Yield: 2.96g (8 mmol, 80%)

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C):δ 6.27 (1H, br, NH), 4.99 (1H, br, NH), 3.35-3.38 (2H, m, α-CH_2 of ethylenediamine), 3.28-3.29(2H, m, β-CH_2ethylenediamine), 2.18(2H, t, J=8Hz), 1.46(9H, s, Boc-CH_3), 1.09-1.29 (24H, m, chain –CH_2), 0.90(3H, t, CH_3,J=6.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ174.20, 79.90, 50.98, 40.93, 40.43, 36.94, 34.02, 32.06, 29.82, 29.79, 29.77, 29.63, 29.49, 29.46, 28.50, 25.85, 25.04, 22.83, 14.24.HRMS (m/z): Calculated for $C_{21}H_{42}N_2O_3$: 370.57 Found: 393.20$(M+Na)^+$.

Synthesis of 2[CH_3-$(CH_2)_{12}$-CONH-$(CH_2)_2$-NH_2]: To 1.85 g (5 mmol) of **1**, 5 ml of 98% formic acid was added and the removal of the Boc group was monitored by TLC. After 6 h, formic acid was removed under vacuum. The residue was taken in water (8 ml) and pH of the aqueous solution was then adjusted to 8.0 with 30% aqueous NH_3. The aqueous portion was evaporated in a vacuum. A white material was obtained, purified using basic alumina in chloroform and methanol (9:1) as eluent.

Yield: 1.08g (4 mmol, 80%)

^{1}H NMR (400 MHz DMSO-d_6, 25 °C):δ 7.69 (1H, br, NH), 2.98-3.04(2H, m, β-CH_2 of ethylenediamine), 2.49-2.50(2H, m, NH_2), 2.02(2H, t, J=7.6Hz), 1.22-1.47(22H, m, chain CH_2), 0.84(3H, t, J=6Hz). ^{13}C NMR (100 MHz, DMSO-d_6):δ172.07, 42.21, 41.39, 35.40, 31.23, 28.95,28.88, 28.72, 28.63, 25.24, 22.02, 13.88.HRMS (m/z): Calculated for $C_{16}H_{34}N_2O$: 270.27 Found: 271$(M+H)^+$.

Synthesis of di-Boc Histidine: The synthetic procedure has been described inMirzahosseini et. al.ARKIVOC, 2014(VI), 1-9.

Synthesis of3[CH_3-$(CH_2)_{12}$-CONH-$(CH_2)_2$-NHCO-$CH_2(CH_2$-$C_3H_2N_2)$-NHCO-$O(CH_3)_3)_2$]:2(2.7 g, 10mmol) was dissolved in 15 ml DMF in a 250 ml round bottom flask in an ice water bath. 1.35g (10mmol) HOBt was added to it. The diboc protected histidine(3.91g, 11 mmol) was dissolved in 25 ml ethyl acetateand then DCC (2.27g, 11mmol) was added to the reaction mixture. The reaction mixture was allowed to come to room temperature and stirred for 48h. The reaction mixture was diluted with ethyl acetate and filtered to separate N, N-dicyclohexyl urea (DCU). The organic layer was washed with 1(N)HCl (3 × 30 ml), brine (1 × 30 ml), saturated sodium carbonate (3 × 30 ml) and brine (2 × 30 ml), dried over anhydrous Na_2SO_4 and evaporated in vacuum. A white material was obtained, purified using basic alumina in chloroform and methanol (9:1) as eluent.

Yield: 3.94g (6.5mmol, 65%)

^{1}H NMR (400MHz, $CDCl_3$, 25 °C):δ 7.98(1H, s, imidazole CH), 7.18(1H, s, imidazole proton, 6.82(1H, br, NH), 6.60(1H, br, NH), 5.96(1H, br, NH), 4.33-4.35(1H, m, CH of His), 3.32-3.38(4H, m, CH_2 of ethylenediamine), 2.94-2.99 (2H, m,CH_2 of His), 2.14(2H, t, J=7.6Hz), 1.32-1.64(22H, m, chain CH_2), 1.24(18H, s, Boc-CH_3), 0.87(3H, t, J=6.4Hz).^{13}C NMR (100 MHz, $CDCl_3$): δ173.96, 172.35, 146.92, 138.95, 136.88, 115.11, 86.01, 80.33, 55.90, 54.54, 39.64,

36.85, 35.07, 34.10, 32.05, 30.51, 29.78, 29.65, 29.51, 28.48, 28.11, 28.02, 25.86, 25.61, 25.08, 24.83, 22.82, 14.24. HRMS (m/z): Calculated for $C_{32}H_{57}N_5O_2$: 607.43 Found: 630.16$(M+Na)^+$, .

Synthesis of P1[CH_3-$(CH_2)_{12}$-CONH-$(CH_2)_2$-NHCO-$CH_2(CH_2$-$C_3H_2N_2)$-NH_2]: To 3.03 g (5 mmol) of **3**,8ml of 98% formic acid was added and the removal of the Boc group was monitored by TLC. After 6 h, formic acid was removed under vacuum. The residue was taken in water (8 ml) and pH of the aqueous solution was then adjusted to 8.0 with 30% aqueous NH_3. The aqueous portion was evaporated in a vacuum. A white material was obtained, purified using basic alumina in chloroform and methanol (9:1) as eluent.
Yield: 1.62 g (4 mmol, 80%)

^{1}H NMR (400MHz, DMSO-d_6, 25 °C):δ 11.82 (1H, br, NH of imidazole), 7.91(1H, s, imidazole CH), 7.85(1H, br, NH), 7.50 (1H, s, imidazole CH), 6.79(1H, br, NH), 4.43-4.48(1H, α-CH of His), 3.05-3.10(4H, m, CH_2 of ethylenediamine), 2.81-22.84(2H, d, β-CH_2 of His), 2.02(2H, t, J=7.2 Hz), 1.45-1.47(2H, br, NH_2), 1.21-1.25(22H, m, chain CH_2), 0.84(3H, t, J=6.4 Hz).^{13}C NMR (100 MHz, DMSO-d_6): δ174.75, 172.67, 161.13, 134.81, 55.24, 38.88, 38.41, 38.39, 35.56, 31.36, 29.11, 29.09, 28.99, 28.86, 28.76, 25.30, 22.16, 14.01. HRMS (m/z): Calculated for $C_{22}H_{41}N_5O_2$: 407.33 Found: 408.14$(M+H)^+$, 430.15$(M+Na)^+$.

Synthesis of Boc-Phe-OH: The synthetic procedure has been described in *Chem. Commun*,2016,52, 5045-5048.

Synthesis of 4[CH_3-$(CH_2)_{12}$-CONH-$(CH_2)_2$-NHCO-$CH(CH_2$-$C_6H_5)$-NHCO-$O(CH_3)_3)_2$]:2 (2.7 g, 10mmol) was dissolved in 15 ml DMF in a 250 ml round bottom flask in an ice water bath. 1.35g (10mmol) HOBt was added to it. Boc protected phenyl alanine(2.38g, 9 mmol) was dissolved in 25 ml ethyl acetate andthen DCC (2.27g, 11mmol) was added to the reaction mixture. The reaction mixture was then allowed to come to room temperature and stirred for 48h. The reaction mixture was diluted with ethyl acetate and it was filtered to separate N, N-dicyclohexyl urea (DCU). The organic layer was washed with 1(N)HCl (3 × 30 ml), brine (1 × 30 ml), saturated sodium carbonate (3 × 30 ml) and brine (2 × 30 ml), dried over anhydrous Na_2SO_4 and evaporated in vacuum. A white material was obtained, purified using basic alumina in ethylacetate and petroleum ether (2:8) as eluent.

Yield: 3.62g (6.3mmol, 70%)

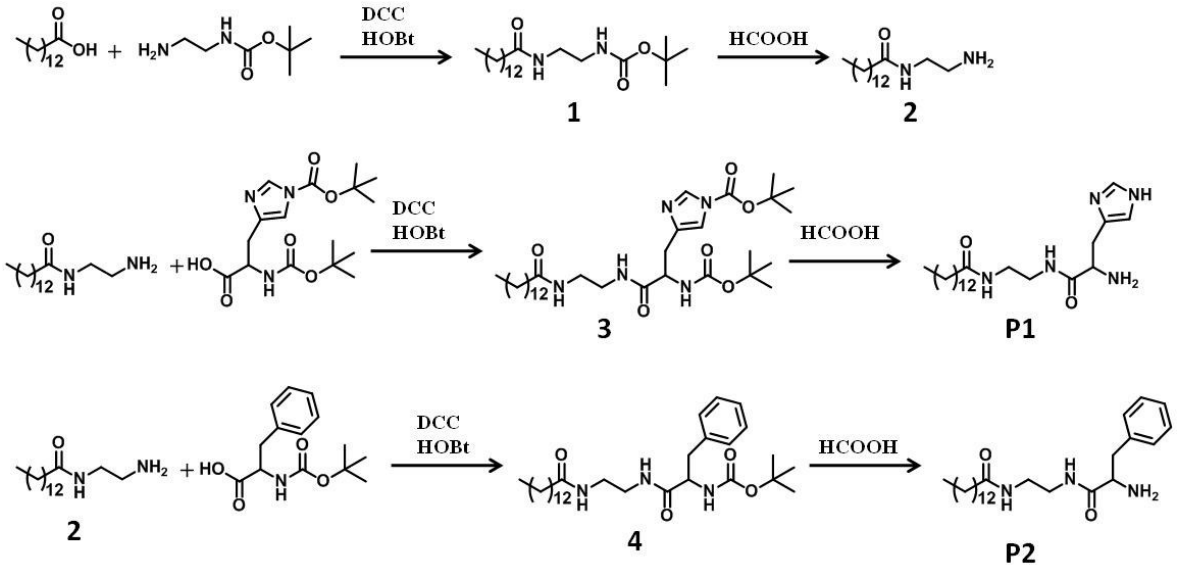
^{1}H NMR (500MHz, $CDCl_3$, 25 °C):δ 7.18-7.30(5H, m, CH of Phe), 6.56(1H, br, NH), 6.27(1H, br, NH), 5.17(1H, br, NH), 4.29-4.31(1H, m, α-CH of Phe), 3.01-3.29(6H, m, CH_2 of ethylenediamine and β-CH_2 of Phe), 2.12(3H, t, J=8Hz), 1.24-1.57(22H, m, chain CH_2), 0.88(3H, t, J=7.5Hz). ^{13}C NMR (125 MHz, $CDCl_3$):δ 174.72, 172.76, 155.70, 136.75, 129.57, 129.43, 128.83, 128.67, 128.58, 127.17, 127.11, 126.79, 126.20, 117.61, 111.24, 39.93, 39.79,

38.73, 36.77, 32.06, 29.82, 29.78, 29.64, 29.47, 28.41, 25.81, 22.82, 14.22. HRMS (m/z): Calculated for $C_{30}H_{51}N_3O_4$: 517.74 Found: 540.39$(M+Na)^+$.

Synthesis of P2[CH_3-$(CH_2)_{12}$-CONH-$(CH_2)_2$-NHCO-CH(CH_2-C_6H_5)-NH_2]: To 2.58 g (5 mmol) of **4**, 8ml of 98% formic acid was added and the removal of the Boc group was monitored by TLC. After 6 h, formic acid was removed under vacuum. The residue was taken in water (8 ml) and pH of the aqueous solution was then adjusted to 8.0 with 30% aqueous NH_3. The aqueous portion was evaporated in a vacuum. A white material was obtained, purified using basic alumina in chloroform and methanol (9:1) as eluent.

Yield: 1.67g (4 mmol, 80%)

^{1}H NMR (500MHz, DMSO-d_6, 25 °C):δ 7.88(1H, br, NH), 7.74(1H, br, NH), 7.18-7.28(5H, m, CH of Phe), 4.50-4.51(1H, m, α-CH of Phe), 2.89-3.17(6H, m, CH_2 of ethylenediamine and β-CH_2 of Phe), 2.02(3H, t, J=7.5 Hz), 1.33(2H, br, NH_2), 1.22-1.33(22H, m, chain CH_2), 0.83-0.85(3H, m, J=6Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.43, 172.27, 170.64, 160.70, 138.68, 129.15, 129.04, 127.99, 126.21, 125.98, 56.28, 52.51, 41.02, 40.09, 38.45, 38.27, 38.23, 38.00, 37.79, 35.40, 31.18, 28.94, 28.91, 28.81, 28.70, 28.58, 25.11, 21.98, 13.83. HRMS (m/z): Calculated for $C_{25}H_{43}N_3O_2$: 417.63 Found: 418.62$(M+H)^+$,440.62$(M+Na)^+$.



Scheme 1: Synthetic scheme for molecules **P1** and **P2.**

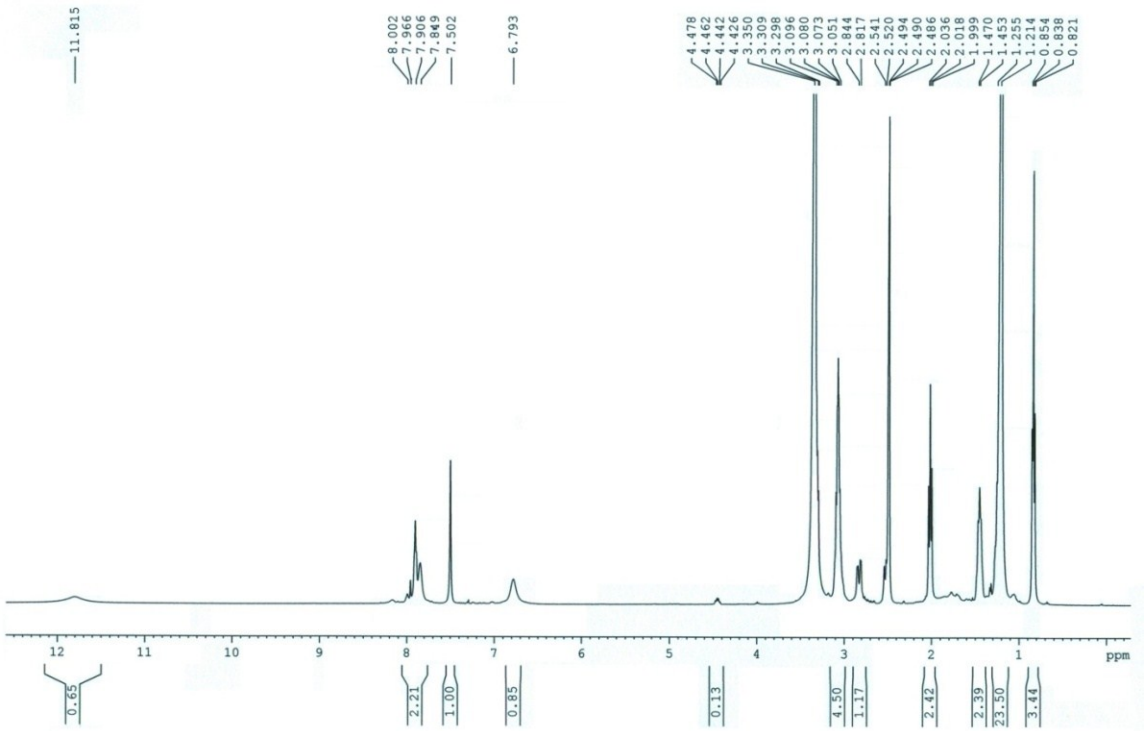


Fig. S1 400 MHz ^{1}H NMR spectrum of **P1** in DMSO-d_6.

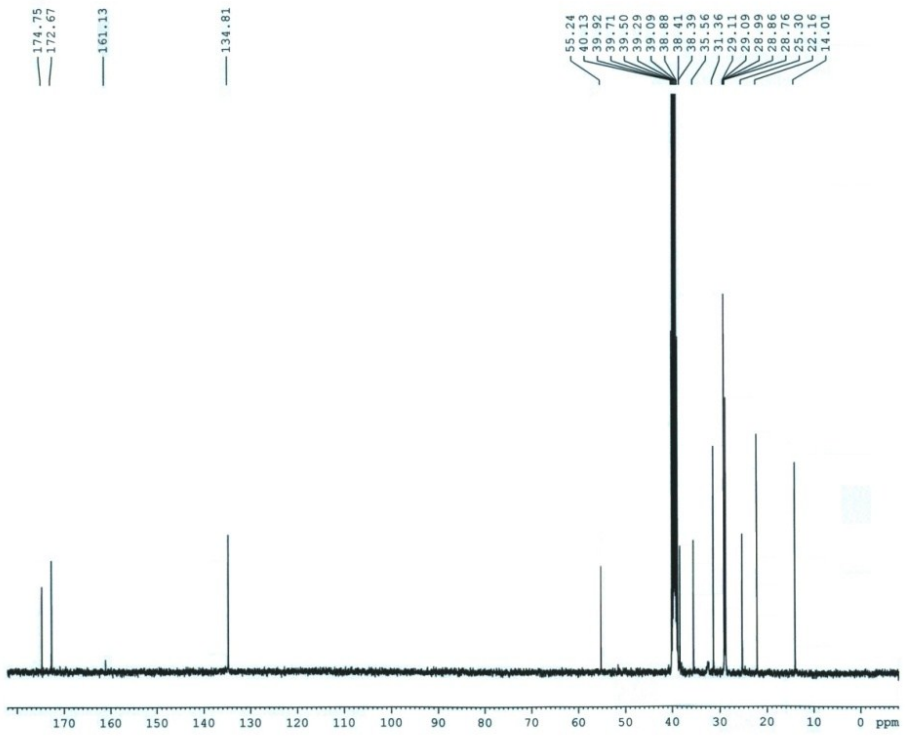
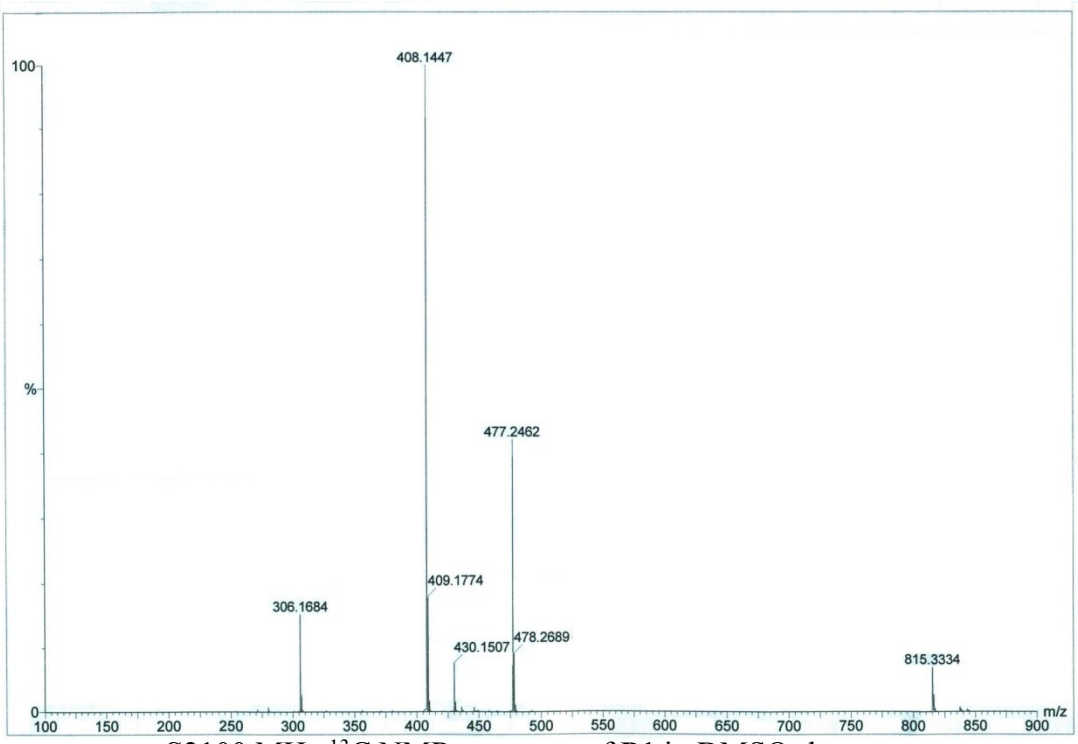


Fig.



S2100 MHz ^{13}C NMR spectrum of **P1** in DMSO-d_6.

Fig. S3 HRMS spectrum of **P1.**

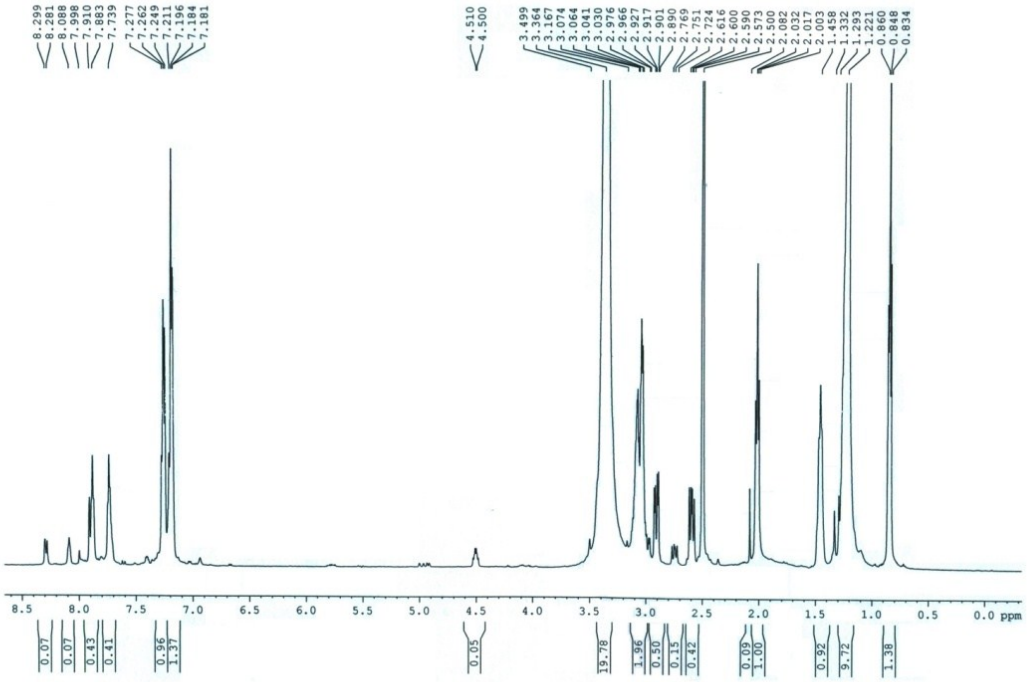


Fig.S4500 MHz ^{1}H NMR spectrum of **P2** in DMSO-d_6.

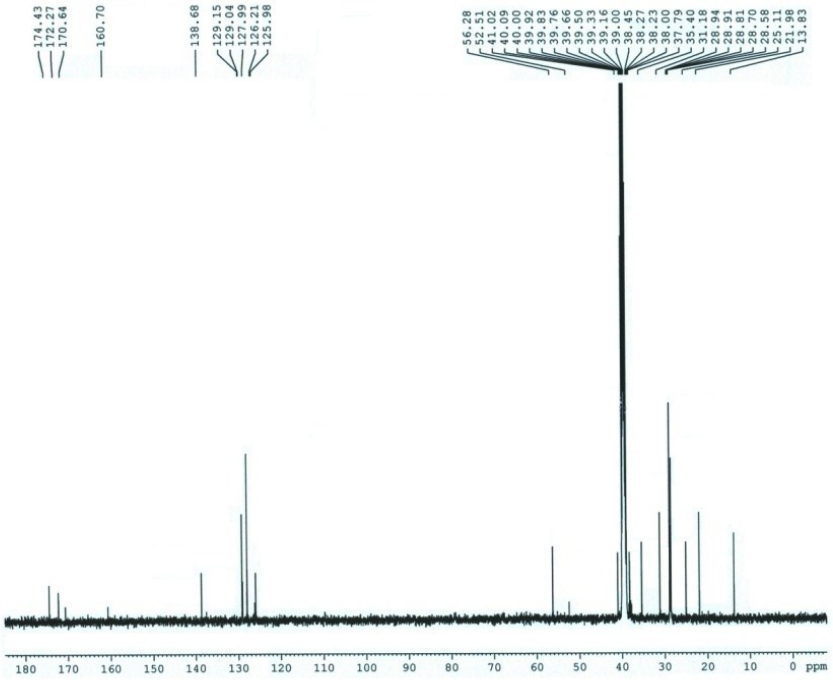


Fig. S5125 MHz ^{13}C NMR spectrum of **P2** in DMSO-d_6.

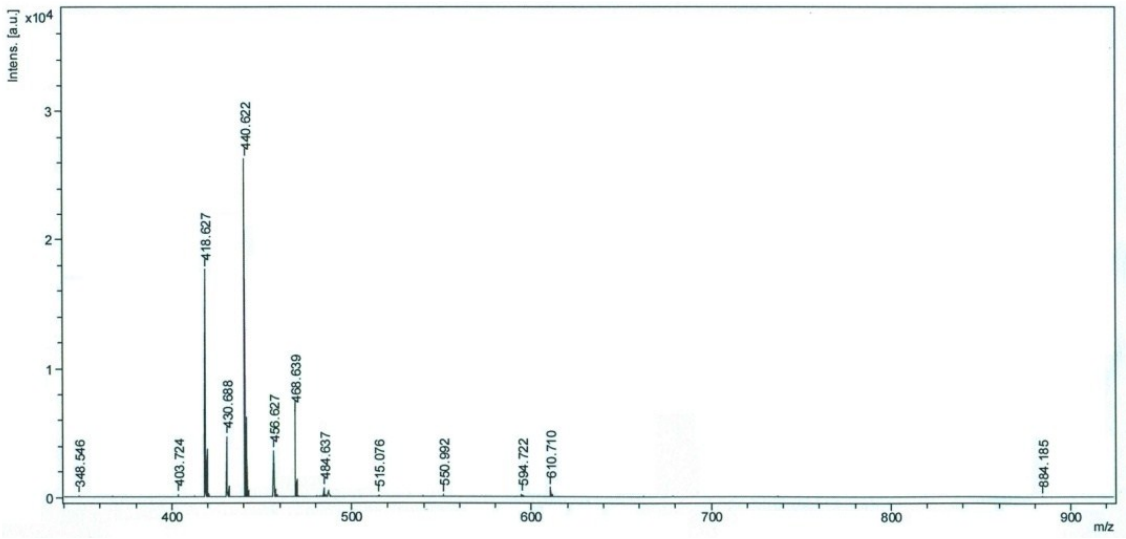


Fig. S6 MALDI-TOF spectrum of **P2.**

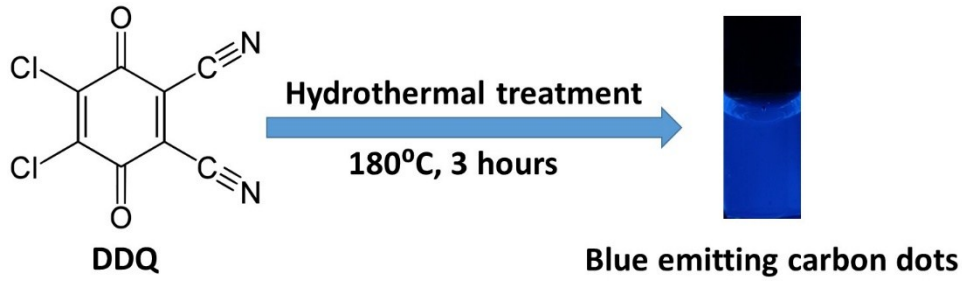
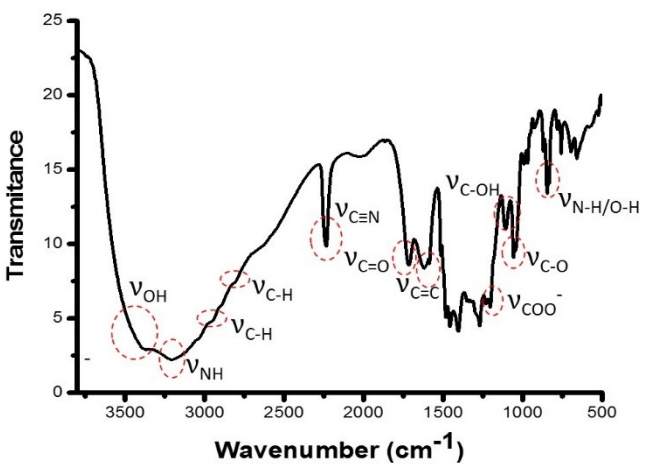


Fig. S7 Synthetic scheme for the preparation of blue emitting carbon dots (at 365 nm) upon hydrothermal treatment of 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).



Peak Position	Functional group
843 cm^{-1}	N-H and O-H bending
1057 cm^{-1}	C-O Stretching
1204 cm^{-1}	COO^{-} stretching
1404 cm^{-1}	O-H deformation peak
1622 cm^{-1}	C=C Stretching
1715 cm^{-1}	C=O Stretching
2234 cm^{-1}	C≡N Stretching
2931 cm^{-1}	-C-H Stretching
3033 cm^{-1}	=C-H Stretching
3198 cm^{-1}	N-H Stretching
3382 cm^{-1}	O-H Stretching

Fig. S8 FTIR spectra of C-dots and their functional group assignment shown in a tabulated form.

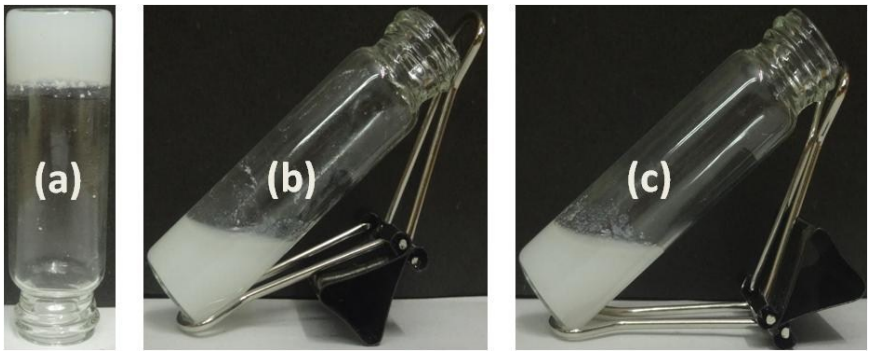


Fig. S9 Photographs of **(a) P1** and benzenetricarboxylic acid triggered the hydrogelation in mili-Q water, **(b)** Insoluble aggregate of **P1** and TREN **(c)** Insoluble aggregate of **P1** and catichol.

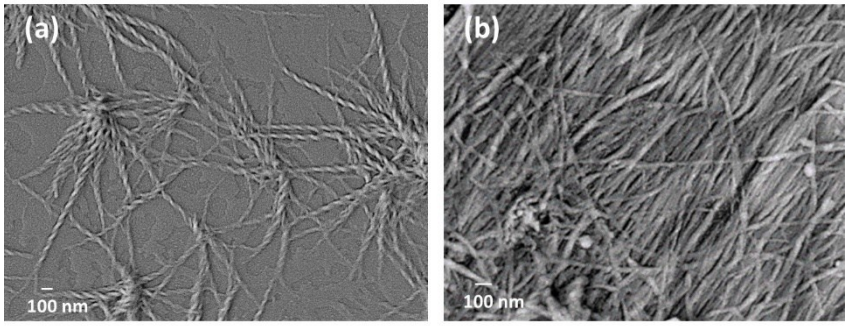


Fig. S10FE-SEM images of (a) **P1** aggregate (in water) and (b) **P1**-C dots composite hydrogel.

Fig. S11 Macroscopic illustration of the thixotropic nature of the C-dots hydrogel.

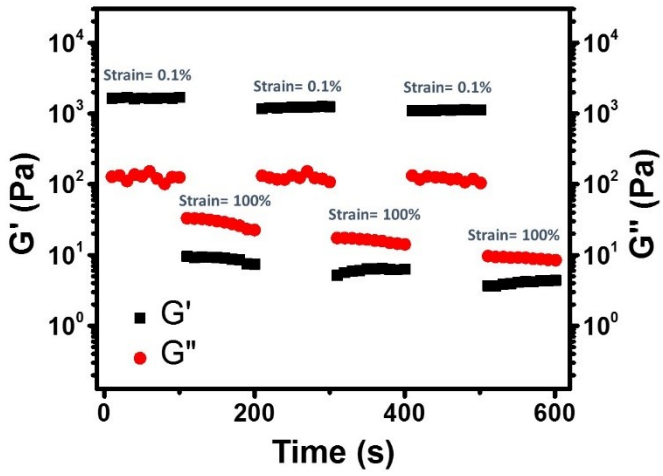


Fig. S12 Time dependent step−strain rheological analysis of the C-dotshydrogel (amphiphile P1:C dots = 2:1) at a fixed angular frequency of 1 rad/s (25 ^{0}C).

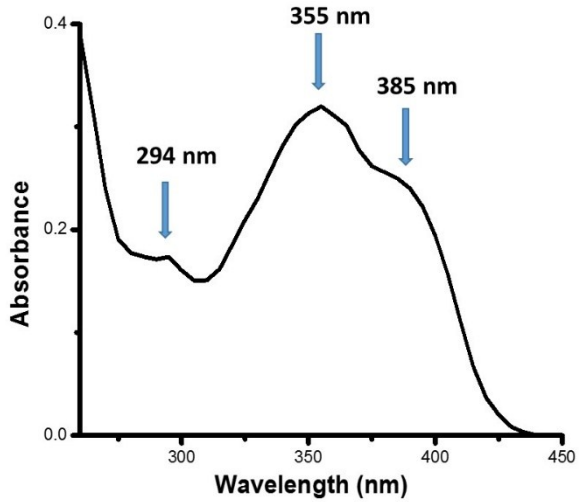


Fig. S13 UV-Visible absorption spectrum of C-dots in water.

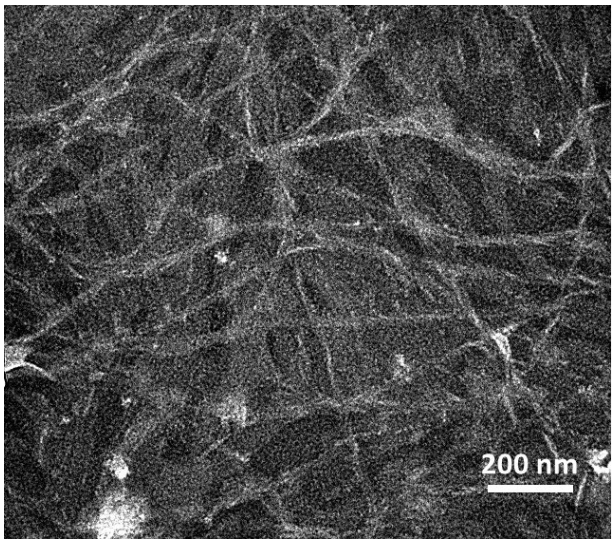


Fig. S14 FEG-TEM image of the amphiphile P1

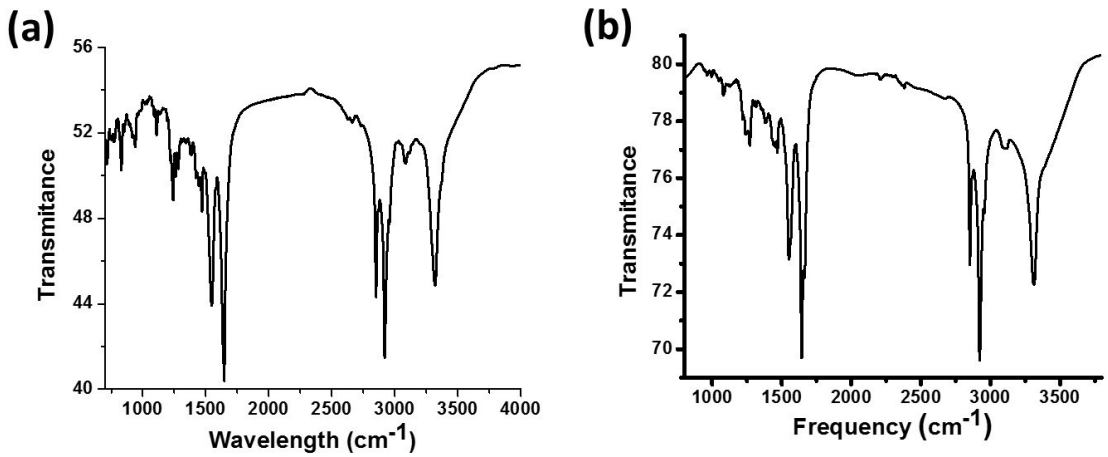


Fig. S14 FT-IR spectra of (a) P1 Aggregate and (b) xerogel