Electronic Supplementary Information

Effect of molar ratio and concentration on rheological properties of two-component supramolecular hydrogels: Tuning of morphological and drug releasing behaviour

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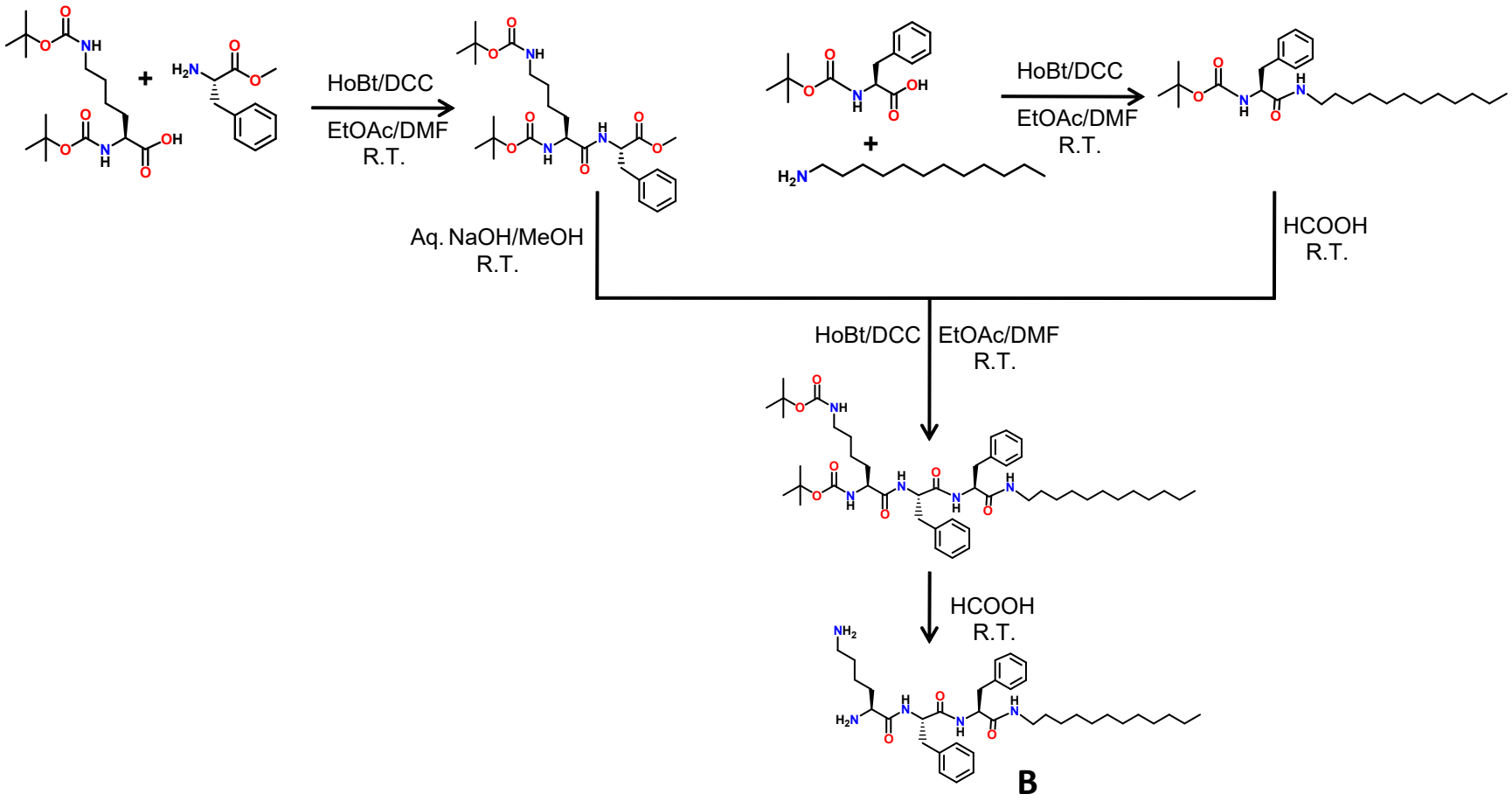
Synthesis


HoBt/DCC
EtOAc/DMF
R.T.
HoBt/DCC
EtOAc/DMF
R.T.
Aq. NaOH/MeOH
R.T.
HCOOH
R.T.
HoBt/DCC
EtOAc/DMF
R.T.
HCOOH
R.T.
B

Synthesis of Boc-Phe-C_{12} (BFC_{12}):6.63 g (25mmol) Boc-Phe-OHwas taken in 250mL RB and dissolved in minimum amount of DMF (10mL).100mL of ethyl acetate was added to it followed by the subsequent addition of 4.63 g (25mmol) of dodecylamine (NH_2-C_{12}). 3.38g HOBt.H_2O (25mmol) was added to the reaction mixture in ice-bath condition. After 15 minutes, 6.44g (31.25 mmol) DCC was added to the mixture. The reaction was allowed to stirred 36 h at 0º to 25º C. The DCU produced during the course of the reaction was filtered out and the residue was washed with 1(N) HCl (3 x 50 mL), saturated sodium carbonate solution (3 x 50mL) followed by saturated brine solution (2 x 50 mL). Then it was extracted in ethyl acetate and dried over anhydrous sodium sulfate (Na_2SO_4)which later on is evaporated in high vacuum in a rotary evaporator. The crude product was purified by silica gel column(100–200 mesh) in petroleum ether and ethyl acetate mixture and white dry powder as pure product was obtained.

Yield: 9.50 g (22mmol, 88.00%). ESI-MS (m/z): Calculated: - 432.64, Found: - 455.17 $[M+Na]^+$

^{1}H NMR (500 MHz, $CDCl_3$, TMS, 25°C): δ7.30-7.19 (5H, m), 5.70 (1H, br,), 5.12 (1H, br), 4.26-4.25 (1H, d, j= 5), 3.14-3.12 (2H, t, j=5), 3.10-3.05 (2H, m), 3.02-2.98 (2H, m), 1.41 (9H, s), 1.25-1.23 (18H, m), 0.89-0.86 (3H, t, j= 5)

^{13}C NMR (400 MHz, $CDCl_3$, TMS, 25°C):δ 171.07, 155.52, 137.06, 129.46, 128.77, 127.03, 80.27, 56.30, 39.62, 38.97, 32.05, 29.82, 29.77, 29.75, 29.71, 29.63, 29.47, 29.37, 28.43, 26.91, 22.81, 14.22.

Synthesis of $(Boc)_2$-Lys-Phe-OMe(B_2KFO):8.65g (25mmol)diBoc-Lys- OH was dissolved in 150mL ethyl acetate in 250 mL RB. Methyl ester protected phenylalanine 4.48g (25mmol) was dissolved in minimum amount of DMF (10mL). Two solutions were mixed together and place in ice-bath condition. 3.38g HOBt.H_2O (25mmol) was added to the reaction mixture. After 15

minutes, 6.44g (31.25 mmol) DCC was added. The reaction was allowed to stirred 36 h at 0º to 25º C. The DCU produced during the course of the reaction was filtered out and the residue was washed with 1(N) HCl (3 x 50 mL), saturated sodium carbonate solution (3 x 50mL) followed by saturated brine solution (2 x 50 mL). Then it was extracted in ethyl acetate and dried over anhydrous sodium sulfate (Na_2SO_4) which later on is evaporated in high vacuum in a rotary evaporator. The crude product was purified by silica gel column(100–200 mesh) in petroleum ether and ethyl acetate mixture and white dry powder as pure product was obtained.

Yield: 10.15g (20mmol, 80.00%).ESI-MS (m/z): Calculated: - 507.62, Found: - 530.14 $[M+Na]^+$

^{1}H NMR (400 MHz, $CDCl_3$, TMS, 25°C): δ 7.30-7.24 (5H, m), 7.12-7.10 (2H, d, j= 8), 5.04 (1H, br)4.61 (1H, br), 4.03 (1H, br), 3.71(3H, s), 3.14-3.07 (2H, m), 1.59 (8H, s), 1.43(18H, s,).

^{13}C NMR (400 MHz, $CDCl_3$, TMS, 25°C):δ 171.88, 171.81, 156.28, 135.91, 129.41, 128.76, 127.31, 53.28, 52.47, 38.09, 34.11, 32.10, 29.78, 28.60, 28.46, 25.09, 22.59.

Synthesis of $(Boc)_2$-Lys-Phe-Phe-C_{12}(B_2KFFC_{12}):6.48 g(15mmol)Boc-Phe-C_{12} was taken in 250mL RB and treated with 50 mL concentrated formic acid for deprotection of N-terminal which was allowed to stirred for 8h. The formic acid was evaporated in high vacuum. The reaction mixture was dissolved in water (30 mL) and 100 mL ethyl acetate was added to it in a separating funnel. The mixture was washed with 1(N) HCl (3 x 50 mL) followed by saturated brine solution (2 x 50 mL). The organic layer was separated and dried over anhydrous sodium sulfate (Na_2SO_4).On the other hand 7.61 g (15mmol) $(Boc)_2$-Lys-Phe-OMe was taken in another 250 mL RB and dissolved in 80mL methanol. Then it was treated with 60.88 mL 1(N) NaOH aqueous solution allowed to stir for 8h. Then the reaction was monitored by thin layer chromatography (TLC) time to time to check the hydrolysis process. After complete hydrolysis

the methanol was evaporated in high vacuum and obtained aqueous part was neutralized by 1(N) HCl. Then it was extracted in ethyl acetate and dried overanhydrous sodium sulfate (Na_2SO_4). Now both the ethyl acetate solutions were mixed together and 10mL DMF was added to the mixture for well dissolution of the deprotected species. 2.03 g HOBt.H_2O (15mmol) was added to the reaction mixture in ice-bath condition. To the reaction mixture 1.25 equivalent (18.75mmol 3.86 g)ofDCC was added and stirred for 72h. Produced DCU was separated by filtration. Filtrate was washed with 1(N) HCl (3 x 50 mL) and saturated sodium carbonate solution (3 x 50mL) followed by saturated brine solution (2 x 50 mL).The crude product was extracted in ethyl acetate, and dried over anhydrous sodium sulfate (Na_2SO_4).Then solvent was evaporated in rotary evaporator. The purification process of the crude product was carried out in column chromatography using silica gel. 3.5% methanol in chloroform was used as eluent. White powder was obtained as pure product.

Yield: 8.08 g (10mmol, 66.66%)ESI-MS (m/z): Calculated: - 808.10, Found: - 808.3381 $[M]^+$, 830.311 $[M+Na-H]^+$, 831.3143 $[M+Na]^+$, 708.3166 [M+H-mono Boc fragmented]$^+$

^{1}H NMR (400 MHz, DMSO-d_6, TMS, 25°C): δ 7.24-7.08 (10H, m), 6.53 (1H, br), 4.69 (2H, br), 4.52-4.50 (1H, d, j= 8), 3.16- 3.10 (2H, m), 3.06-3.01 (2H, m) 2.95-2.90 (2H, m),1.95-1.91 (2H, m), 1.71-1.56(2H, m) 1.46-1.36 (18H, s), 1.30-1.06 (24H, m),0.89-0.86 (3H, t, j=12)

^{13}C NMR (400 MHz, $CDCl_3$, TMS, 25°C): δ 170.64, 170.50, 156.89, 156.80, 136.14, 129.22, 129.09, 129.04, 128.59, 127.36, 126.75, 80.84, 76.50, 55.84, 54.09, 49.34, 39.88, 39.15, 37.76, 37.24, 34.11, 32.07, 30.05, 29.83, 29.73, 29.40, 28.64, 28.46, 27.00, 25.79, 25.09, 22.83, 21.70, 14.24

Synthesis of $(NH)_2$-Lys-Phe-Phe-C_{12} ($KFFC_{12}$):4.86g (8mmol) $(Boc)_2$-Lys-Phe-Phe-C_{12} was taken in 100mL RB and to it 30mL concentrated formic acid was added. The reaction for the deprotection of N-terminal was allowed to stir for 8h. After complete deprotection (monitored by TLC) the formic acid was evaporated in high vacuum. Then the reaction mixture was dissolved in 50mL double distilled water and transferred in a separating funnel. It was neutralized by 1(N) NaOH solution and extracted in ethyl acetate. Then it was dried over anhydrous sodium sulfate (Na_2SO_4). The solvent was evaporated in rotary evaporator. The crude product was purified by by column chromatography using chloroform and methanol as eluents. Pure white product was obtained in 5% methanol.

Yield: 3.04 g, (5mmol, 62.5%),ESI-MS (m/z): Calculated:607.87, Found: 608.2939 $[M+H]^+$, 609.2988 $[M+2H]^+$

^{1}H NMR (400 MHz, DMSO-d_6, TMS, 25°C): δ8.46-8.24 (1H, m), 7.99-7.98(1H, br), 7.87-7.86(1H, br), 7.25-7.15 (10H, m), 4.52-4.46 (2H, br), 3.06-2.86 (4H, m), 2.84-2.74 (4H, m), 1.35-1.27 (4H, m), 1.23-1.18(22H, m), 0.87-0.84 (3H, t, j=8)

^{13}C NMR (400 MHz, DMSO-d_6, TMS, 25°C):δ174.92, 174.74, 170.73, 170.57, 170.32, 137.85, 137.64, 137.42, 129.24, 129.19, 129.11, 127.94, 127.79, 126.15, 126.07, 54.65, 54.03, 53.02, 37.72, 34.47, 31.23, 29.00, 28.95, 28.93, 28.64, 26.23.

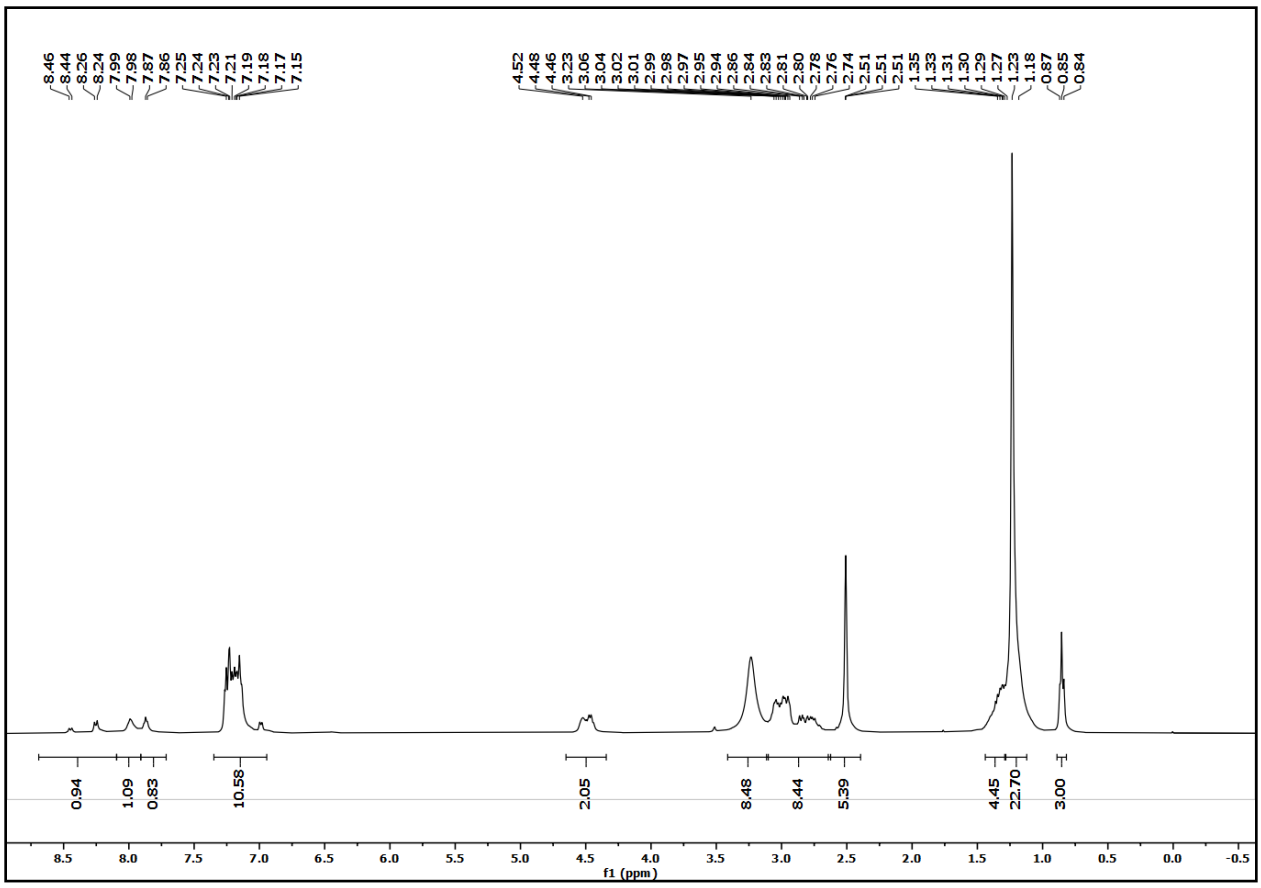


Fig. S1. ^{1}H-NMR spectrum of peptide amphiphile **B** in DMSO-d_6.

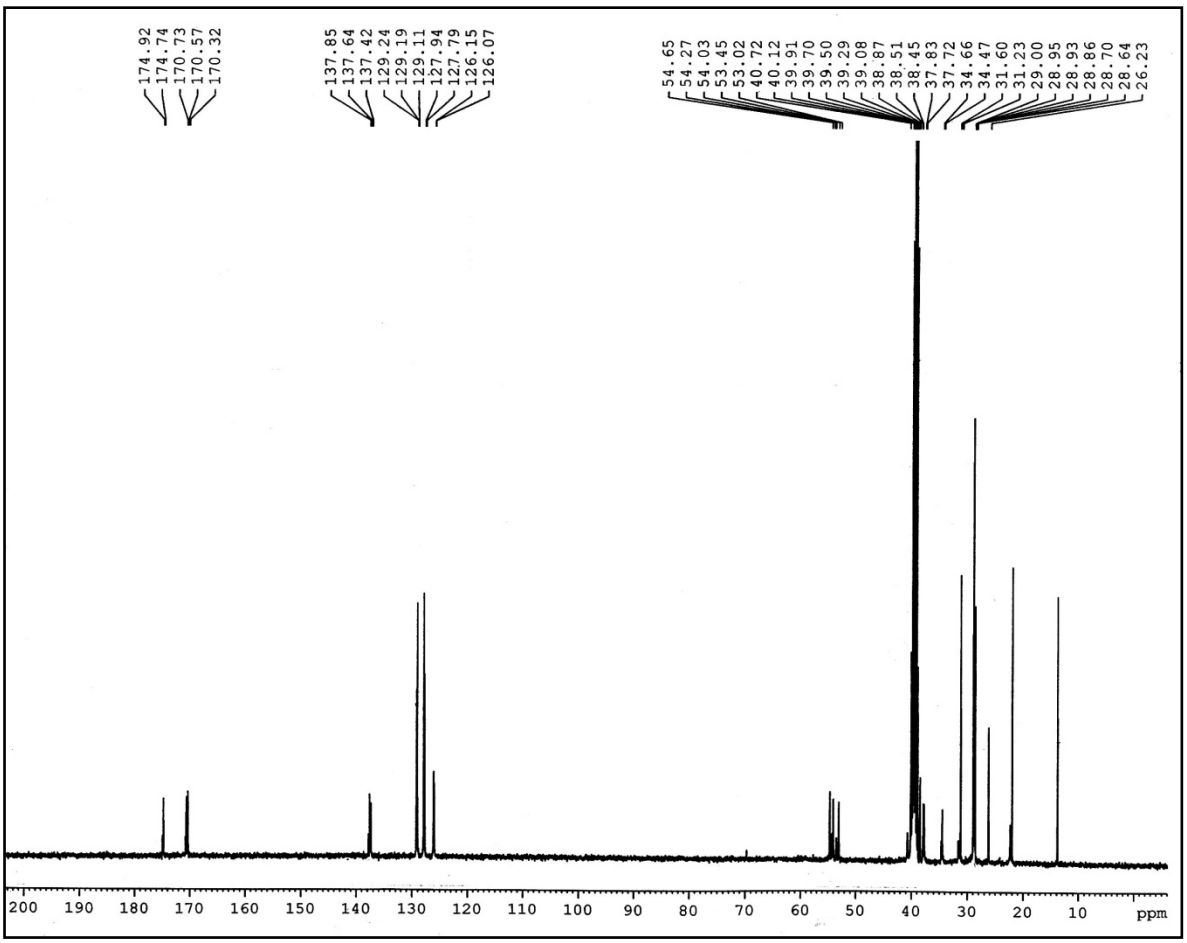


Fig. S2. ^{13}C-NMR spectrum of peptide amphiphile **B** in DMSO-d_6.

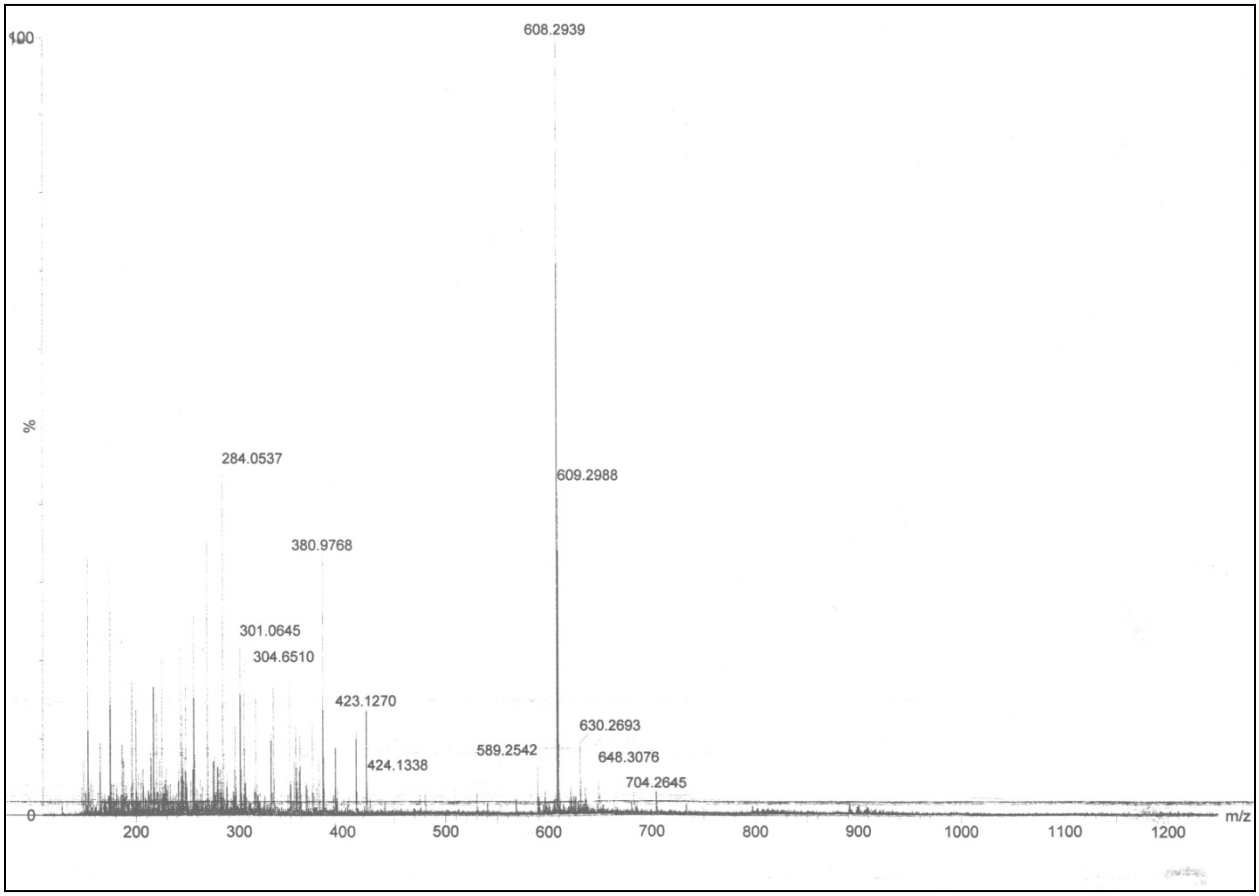


Fig. S3. Mass spectrum of peptide amphiphile **B** .

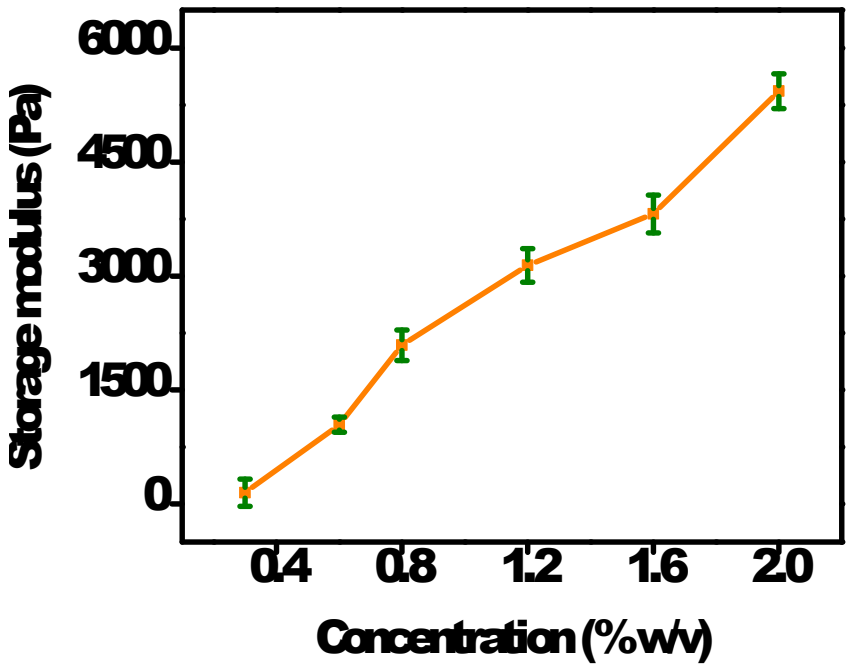


Fig. S4. Variation of storage modulus with increasing concentration of the gelators A and B for 1:1 hydrogel.

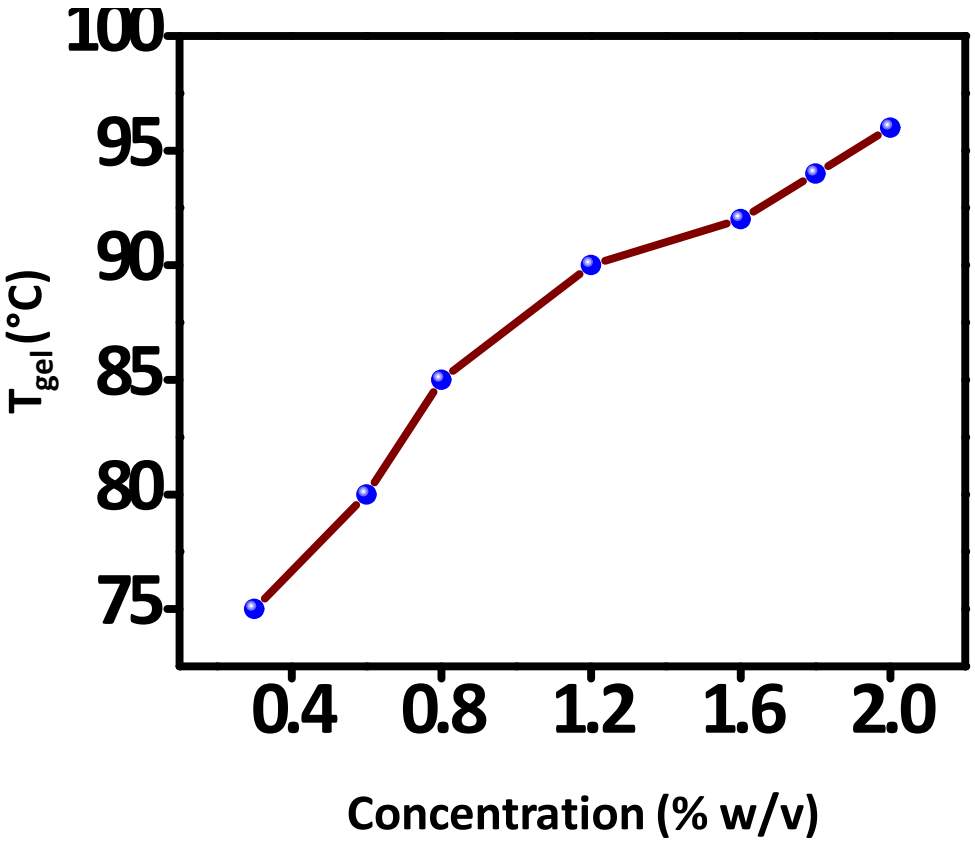


Fig. S5. Variation of T_{gel} with increasing concentration of the gelators **A** and **B** for 1:1 hydrogel**.**

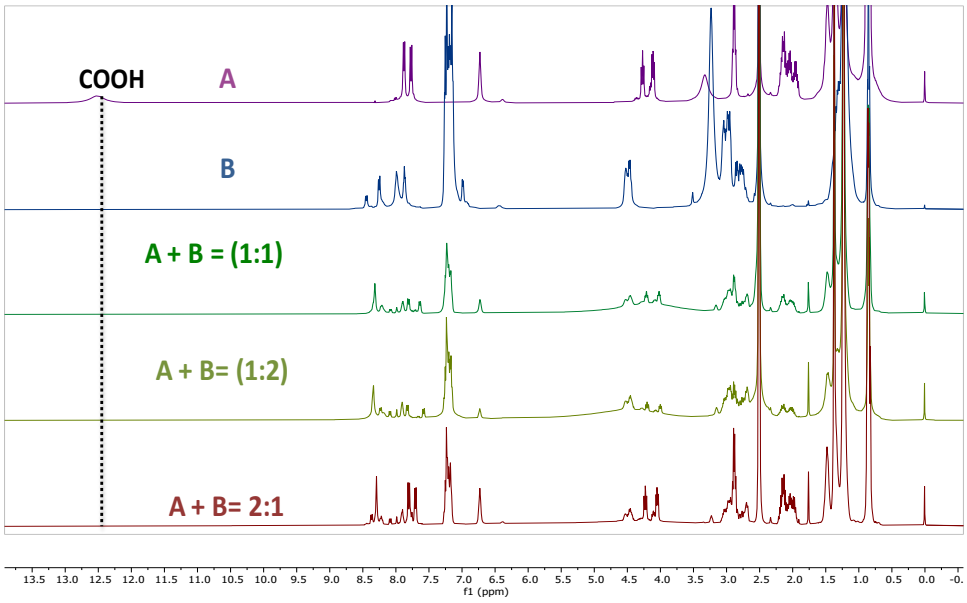


Fig. S6 ^{1}H NMR spectra showing the electrostatic interaction between the two components.

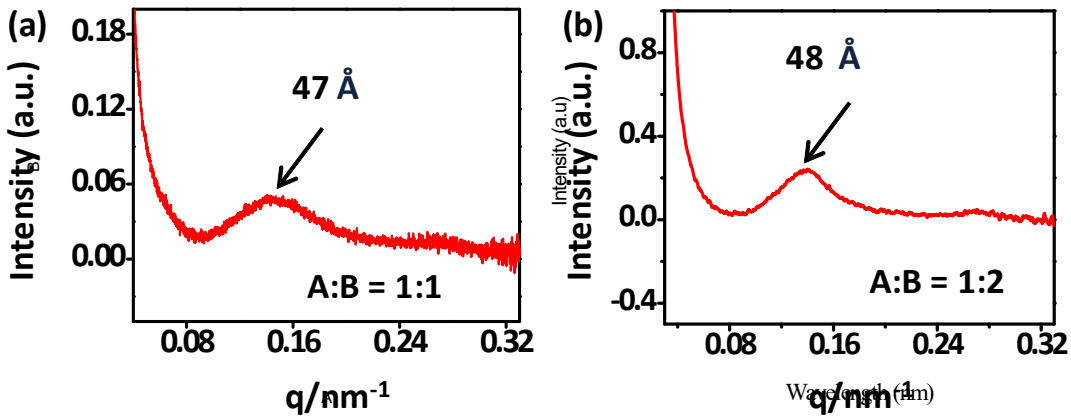


Fig. S7. SAXS data obtained from the (a)1:1 and (b)1:2 hydrogels.

Fig. S8. First order kinetics (red line fit) for each of the hydrogels loaded with drugs (a) amoxicillin, (b) rifampicin, and (c) doxocubicin.