

## Supporting Information

# Orotic acid as a useful supramolecular synthon for the fabrication of an OPV based hydrogel: Stoichiometry dependent injectable behavior

Subham Bhattacharjee<sup>a</sup> and Santanu Bhattacharya<sup>\*,a,b</sup>

<sup>a</sup>Department of Organic Chemistry, Indian Institute of Science, Bangalore, India. Fax: +91-80-23600529; Tel: +91-80-22932664; E-mail: sb@orgchem.iisc.ernet.in

<sup>b</sup>Chemical Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India

### 1. Physical Measurements and Instrumentation.

**Materials and Methods.** All chemicals, solvents and silica gel for TLC and column chromatography were obtained from well-known commercial sources and were used without further purification, as appropriate. Solvents were distilled and dried by standard procedure before use. <sup>1</sup>H-NMR (400 MHz) and <sup>13</sup>C-NMR (100 MHz) spectra were recorded in Bruker-400 Avance NMR spectrometer. Chemical shifts were reported in ppm downfield from the internal standard tetramethylsilane (TMS). Elemental analysis was recorded in Thermo Finnigan EA FLASH 1112 SERIES.

**Gelation Studies.** A weighed amount of PV and OA was added into a sealed glass vial containing absolute water and heated and sonicated to get a clear solution. The solution was then cooled to room temperature and left for 0.5 h to check the stability of the gel using inverse flow method and each experiment was performed in duplicate. If a gel was formed, it was evaluated quantitatively by determining the critical gelator concentration (CGC) which is the minimum amount of gelator required to immobilize 1 mL of water.

**FT-IR Spectroscopy.** FT-IR spectra of the compounds were recorded in Perkin Elmer Spectrum BX FT-IR system.

**UV-Vis and Fluorescence Spectroscopy.** The UV-Vis and fluorescence spectra of solutions and gels of various gelators were recorded on Shimadzu model 2100 spectrophotometer and Hitachi F-4500 spectrofluorimeter respectively, both equipped with a temperature controller bath.

**Atomic Force Microscopy (AFM).** Diluted solutions of each of these two-component gelators at different molar ratios were drop casted on mica and then carefully air dried. Each of the samples were analyzed using Bruker diInnova SPM instrument: Tapping mode, 10 nm tip radius, silicon tip, 292 KHz resonant frequency, 0.7-1 Hz scan speed, 256x256 and 512x512 – pixels placed at JNCASR, Bangalore 560012.

**Transmission Electron Microscopy (TEM).** The aqueous aggregates of the gelators were examined under transmission electron microscopy. A 10 µL sample was loaded onto 400 mesh copper grids and allowed to remain for 5 min. Excess fluid was removed from the grids by touching their edges with filter paper, and 10 µL of 0.1% uranyl acetate was applied on the same grid after which the excess stain was similarly removed from the grids. First, the grids were air-dried and then the last traces of water were removed under vacuum. The samples were observed under TEM (TECNAI T20) operating at an acceleration voltage (dc voltage) of 100 kV.

**Dynamic Light Scattering (DLS).** DLS measurements were performed at room temperature using a Malvern Zetasizer Nano ZS particle sizer (Malvern Instruments Inc., Westborough, MA). Samples were prepared and examined under dust-free conditions. Mean hydrodynamic diameters reported were obtained from the Gaussian analysis of the intensity-weighted particle size distributions.

**Rheological Studies.** For rheology experiment of the gels, an Anton Paar 100 rheometer with a cone and plate geometry (CP 25-2) having an adjustable peltier temperature controlling system was used. All the measurements were done fixing the gap distance between the cone and the plate at 0.05 mm. The gels were scooped on the plate of the rheometer. An oscillatory strain amplitude sweep experiment was performed at a constant oscillation frequency of 1 Hz for the applied strain range 0.001-100 % at 20 °C. The software US-200 converted the torque measurements into either  $G'$  (the storage modulus) and  $G''$  (the loss modulus) and represent  $G'$  and  $G''$  with either strain or shear stress. Oscillatory frequency sweep experiments were performed in the linear viscoelastic region (strain 0.001 %) to ensure that calculated parameters correspond to an intact network structures. Hysteresis loop test was carried out by recording the shear stress at different shear rate in ascending and descending order simultaneously and then measuring the thixotropic area.

**Polarized Optical Microscopy (POM).** A diluted solution of the PV:OA in water was drop casted on a pre-cleaned glass slide and it was left overnight for drying in a dust free environment and finally evacuated. Birefringence was followed under polarized optical microscope (Olympus BX51) equipped with a heating stage (Mettler FP82HT) and a central processor (Mettler FP90).

**Fluorescence Microscopy.** A diluted solution of the gelators was drop-coated on a pre-cleaned glass slide and it was left overnight for drying in a dust free environment and finally evacuated. The fluorescence microscopic image was taken on an Olympus IX-71 microscope with the excitation of 340-400 nm UV-light.

**Stoichiometry determination by Job's method.** The Job plot is a method of continuous variation for determining the stoichiometry of interaction between the two species. The total molar concentration of the two binding species was kept constant and the mole fraction was varied. Further the change in the absorbance was plotted against the mole fraction. The maxima or minima thus obtained gave the stoichiometry of interaction.

**Differential Scanning Calorimetry.** Thermotropic behavior of the hydrogels was investigated using a high-sensitivity differential scanning calorimetry using a CSC-4100 model multicell differential scanning calorimeter (Calorimetric Sciences Corp., Utah, USA).

**Powder X-ray Diffraction (PXRD).** Gels were scooped onto glass slide and dried under vacuum for the corresponding PXRD measurement. These samples were analyzed using a Bruker D8 Advance instrument ( $\theta$ ,  $2\theta$  geometry with Scintillation Detectors). The X-ray beam generated with rotating Cu anode at the wavelength of KR beam at  $1.5418\text{\AA}$  was directed toward the film edge and scanning was done up to a  $2\theta$  value of  $30^\circ$ . Data were analyzed and interpreted using the Bragg equation.

**2. Synthesis and Characterization:** The syntheses of the bis-alcohol<sup>(S1)</sup> (**2**), 4-[(diethylphosphono)methyl]pyridine<sup>(S2)</sup> (**5**) and biphosphonates<sup>(S3)</sup> (**6**) were carried out based on reported procedures. Synthetic scheme of PV<sup>(S4)</sup> (**1**) and BPV<sup>(S5)</sup> (**2**) was shown below.

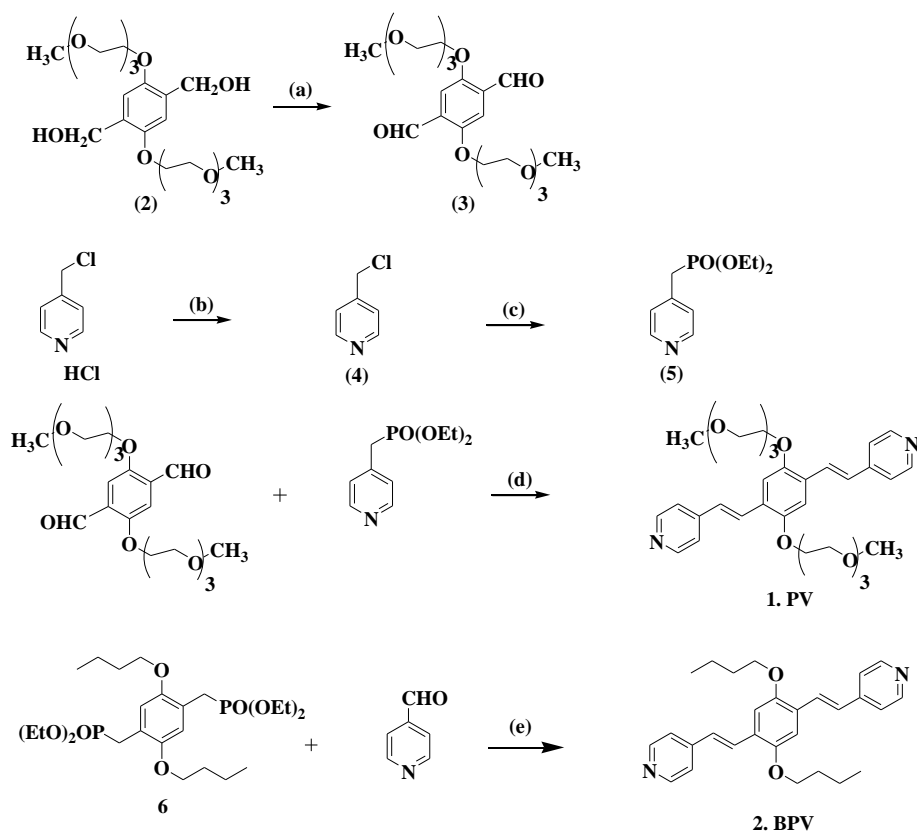
**Bis-aldehyde (3):** To a solution of **2** (2 g, 4.3 mmol) in 50 ml of dry DCM, PCC (3.73 g, 17.3 mmol) was added at a time. The mixture was stirred for 2 hours at room temperature. After completion of the reaction small amount of silica gel was added to the Rb and adsorbed by low pressure vacuum pump. It was then packed in a column ( $\text{SiO}_2$ ) and the product was eluted with 3 % MeOH/ $\text{CHCl}_3$  solution. 1.8 g of **5** was isolated as a light yellow oil: yield 90.8%;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.37 (s, 6H); 3.54-3.56 (m, 4H), 3.64-3.68 (m, 8H), 3.71-3.74 (m, 4H), 3.88-3.91 (m, 4H), 4.27 (t,  $J = 4.8$  Hz, 4H), 7.46 (s, 2H), 10.52 (s, 2H);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  58.9, 68.8, 69.4, 70.5, 70.6, 70.8, 71.8, 112.1, 129.4, 155.1, 189.2; HRMS  $m/z$  calcd for  $\text{C}_{22}\text{H}_{34}\text{O}_{10}$  ( $\text{M} + \text{Na}$ )<sup>+</sup> 481.22, found 481.2050.

**PV (1):** To a solution of bis-aldehyde (**3**) (1.23 g, 2.68 mmol) and 4-[(Diethylphosphono)methyl]-pyridine (**5**) (1.23 g, 5.37 mmol) in dry THF (20 ml),  $^t\text{BuOK}$  (1.2 g, 10.71 mmol) was added and the solution was stirred at room temperature for 6 minute. After completion of the reaction, few drops of water were added to the reaction mixture. Solvent was evaporated using high vacuum and the product was purified by flash column

chromatography (SiO<sub>2</sub>) using 3.5% MeOH/CHCl<sub>3</sub> solution. 1.6 g of 1 was isolated as a wax like solid: yield 98.3 %; IR (Neat, cm<sup>-1</sup>) 2874, 1597, 1491, 1416, 1205, 1107; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.35 (s, 6H), 3.51-3.54 (m, 4H), 3.64-3.66 (m, 4H), 3.69-3.72 (m, 4H), 3.78-3.80 (m, 4H), 3.93-3.95 (m, 4H), 4.24-4.26 (m, 4H), 7.06 (d, *J* = 16.4 Hz, 2H), 7.18 (s, 2H), 7.39 (d, *J* = 5.6 Hz, 4H), 7.65 (d, *J* = 16.4 Hz, 2H), 8.57 (d, *J* = 5.6 Hz, 4H); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>) δ 58.98, 69.16, 69.80, 70.55, 70.66, 70.83, 71.86, 111.62, 120.89, 126.87, 127.1, 127.64, 145.03, 150.08, 151.41; HRMS *m/z* calcd for C<sub>34</sub>H<sub>44</sub>N<sub>2</sub>O<sub>8</sub> (M + H)<sup>+</sup> 609.31, found 609.3204; Elemental analysis: calcd for C<sub>34</sub>H<sub>44</sub>N<sub>2</sub>O<sub>8</sub> ·H<sub>2</sub>O: C, 65.16; H, 7.4; N, 4.47; Found: C, 65.33; H, 7.31; N, 4.59.

**BPV (2):** To a mixture of biphosphonate (6) (1 mmol) and 4-Pyridinecarboxaldehyde (2 mmol) in dry THF, K<sup>t</sup>BuO (4 mmol) was added under Ar atmosphere and the mixture was stirred at room temperature for 5 to 6 minutes. The completion of the reaction was confirmed by monitoring TLC. Excess <sup>t</sup>BuOK was quenched by adding a few drops of water and the solvent was removed under high vacuum pump. The mixture was partitioned between CHCl<sub>3</sub> and water. Rotary evaporation of the organic part gave a crude product which was purified by repetitive leaching of the solid residue in *n*-hexane: yield = 75%; yellow-orange crystal; m.p. 202-203 °C; IR (Neat, cm<sup>-1</sup>): 3368, 3053, 2953, 2933, 2872, 1630, 1593, 1492, 1422, 1265, 1207, 1066, 975, 860, 808, 740; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 1.04 (t, *J* = 7.6 Hz, 6H), 1.541 (m, 4H), 1.85 (m, 4H), 4.09 (t, *J* = 6.4 Hz, 4H), 7.06 (d, *J* = 16.8 Hz, 2H), 7.13 (s, 2H), 7.37 (d, *J* = 6 Hz, 4H), 7.64 (d, *J* = 16.4 Hz, 2H), 8.58 (d, *J* = 5.6 Hz, 4H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>) δ 13.85, 19.36, 31.38, 68.99, 110.77, 120.80, 126.53, 127.72, 144.97, 150.06, 151.29; HRMS: *m/z* calcd. for C<sub>28</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>: 429.25, found: 429.2544; Elemental analysis: calcd. for C<sub>28</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub>: C, 78.47; H, 7.53; N, 6.54; found: C, 78.72; H, 7.77; N, 6.92.

## Synthetic Scheme.

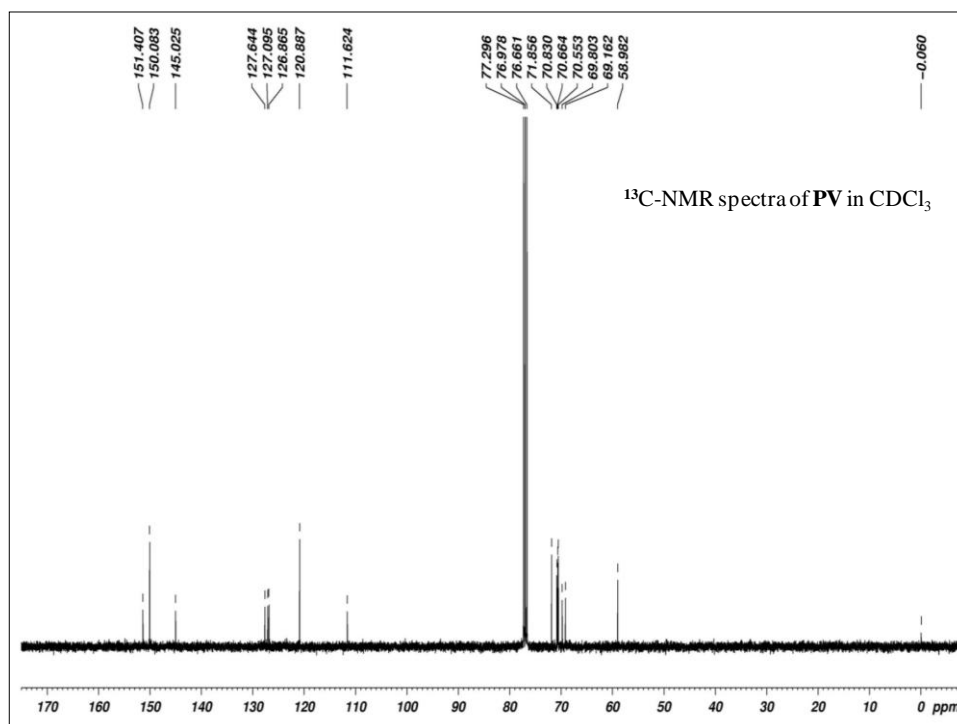
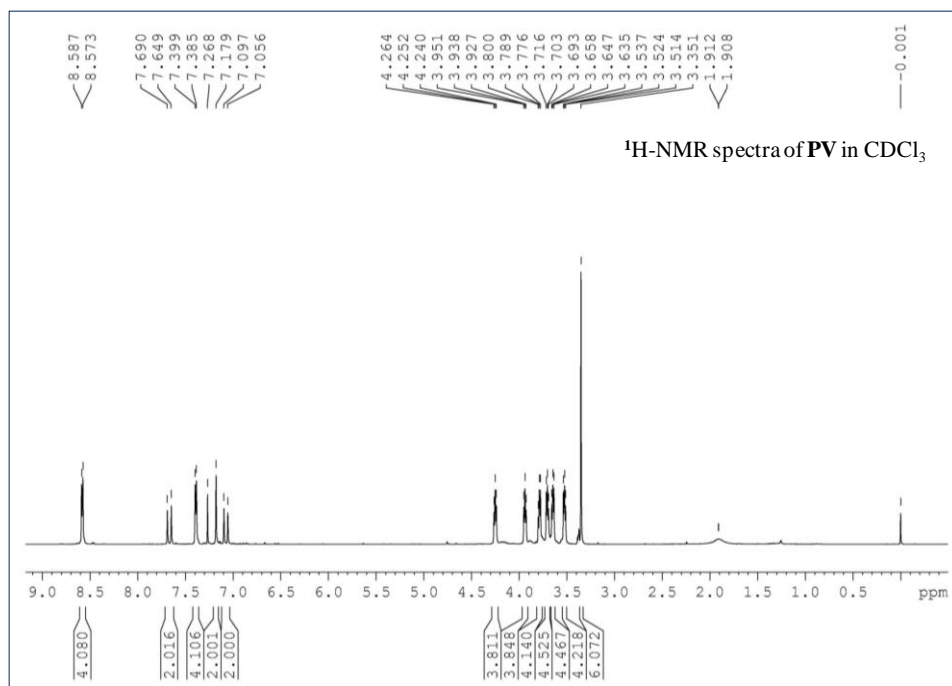


**Reagents, conditions and yields:** (a) PCC, dry DCM, rt, 2h, 90.8%; (b) Na<sub>2</sub>CO<sub>3</sub>, H<sub>2</sub>O, rt, 2 min; (c) NaH, diethyl phosphite, dry toluene, 80 °C, 30 min, 80%; (d) 3, 5, <sup>t</sup>BuOK, dry THF, rt, 6 minute, 98.3%; (e) 6, 4-Pyridinecarboxaldehyde, <sup>t</sup>BuOK, dry THF, 5-6 min, 75%.

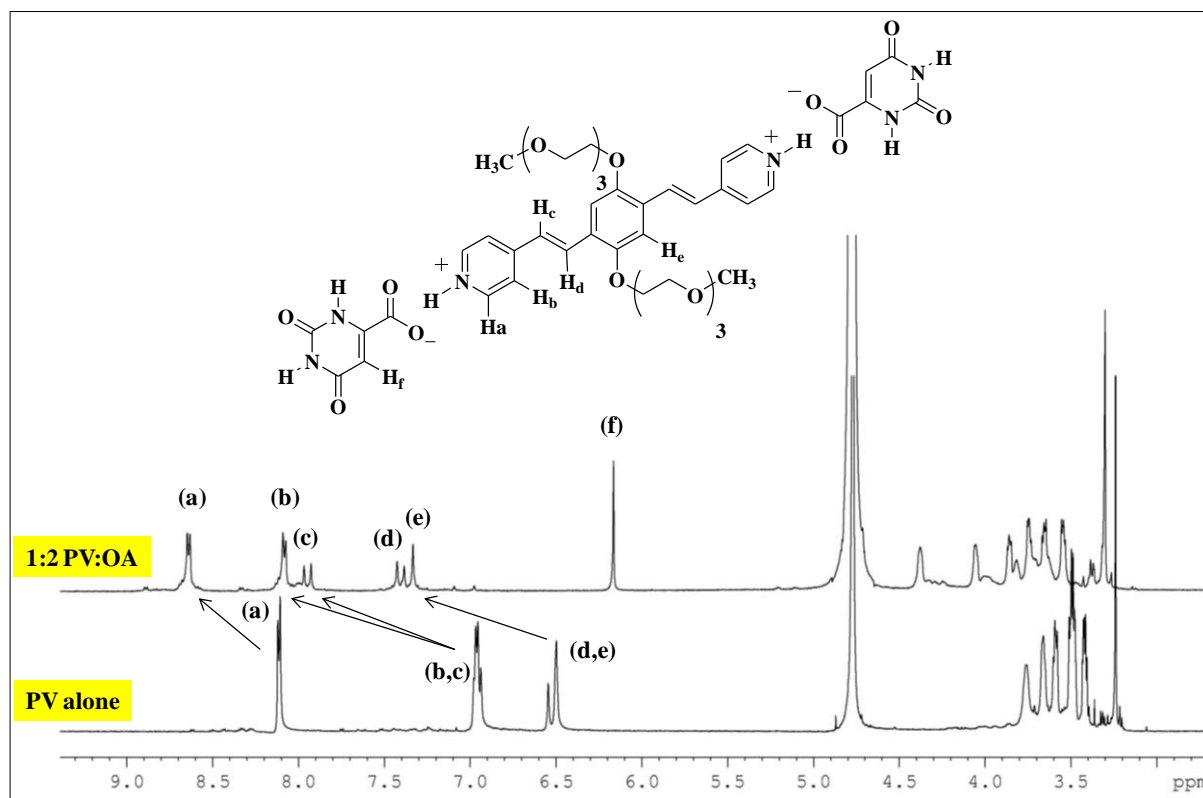
## References.

- (S1) G. Fabbrini, R. Ricc3, E. Menna, M. Maggini, V. Amendola, M. Garbin, M. Villano and M. Meneghetti, *Phys. Chem. Chem. Phys.* 2007, **9**, 616.
- (S2) A. J. Hutchison, M. Williams, C. Angst, R. de Jesus, L. Blanchard, R. H. Jackson, E. J. Wilusz, D. E. Murphy, P. S. Bernard, J. Schneider, T. Campbell, W. Guida and M. A. Sills, *J. Med. Chem.* 1989, **32**, 2171.
- (S3) a) H. Shao, X. Chen, Z. Wang, P. Lu, *J. Phys. Chem. B* 2007, **111**, 10386; b) J. -Y. Jin, Z. -Z. Zin, Y. Xia, Z. -Y. Zhou, X. Wu, D. -X. Zhu, Z. -M. Su, *Polymer* 2007, **48**, 4028.
- (S4) S. Bhattacharjee and S. Bhattacharya, *Chem. Commun.* 2014, **50**, 11690.
- (S5) S. Bhattacharjee, S. Datta and S. Bhattacharya, *Chem. Eur. J.* 2013, **19**, 16672.

### 3. Characterization of PV.



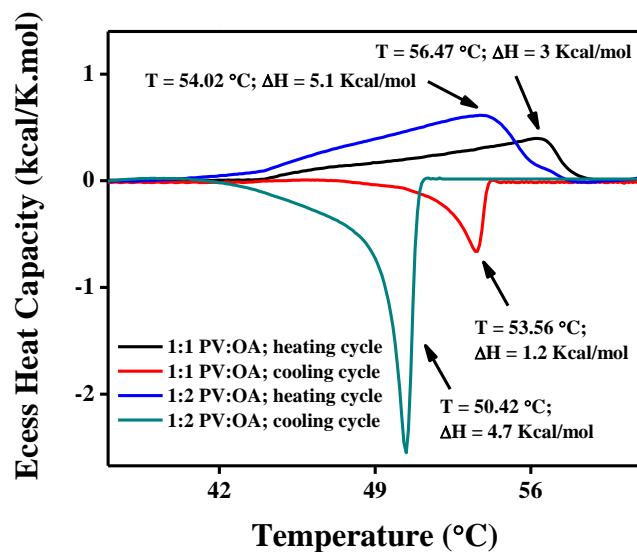
#### 4. Supporting Figures.



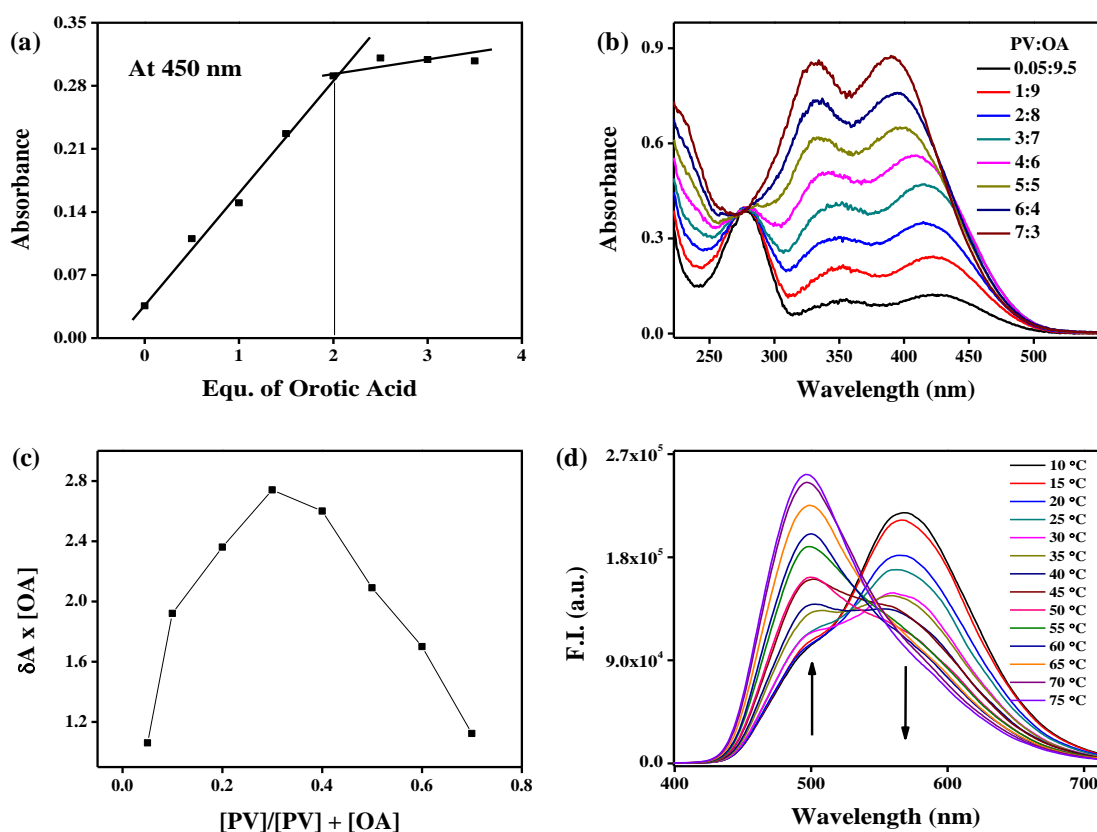
**Figure S1.**  $^1\text{H}$  NMR spectra of PV and 1:2 PV:OA in  $\text{D}_2\text{O}$  showing the chemical shifts (downfield) of different protons of 1:2 PV:OA compared to PV as a result of protonation.

**Table S1.** Summary of gelation of PV:OA at four different molar ratios in water.

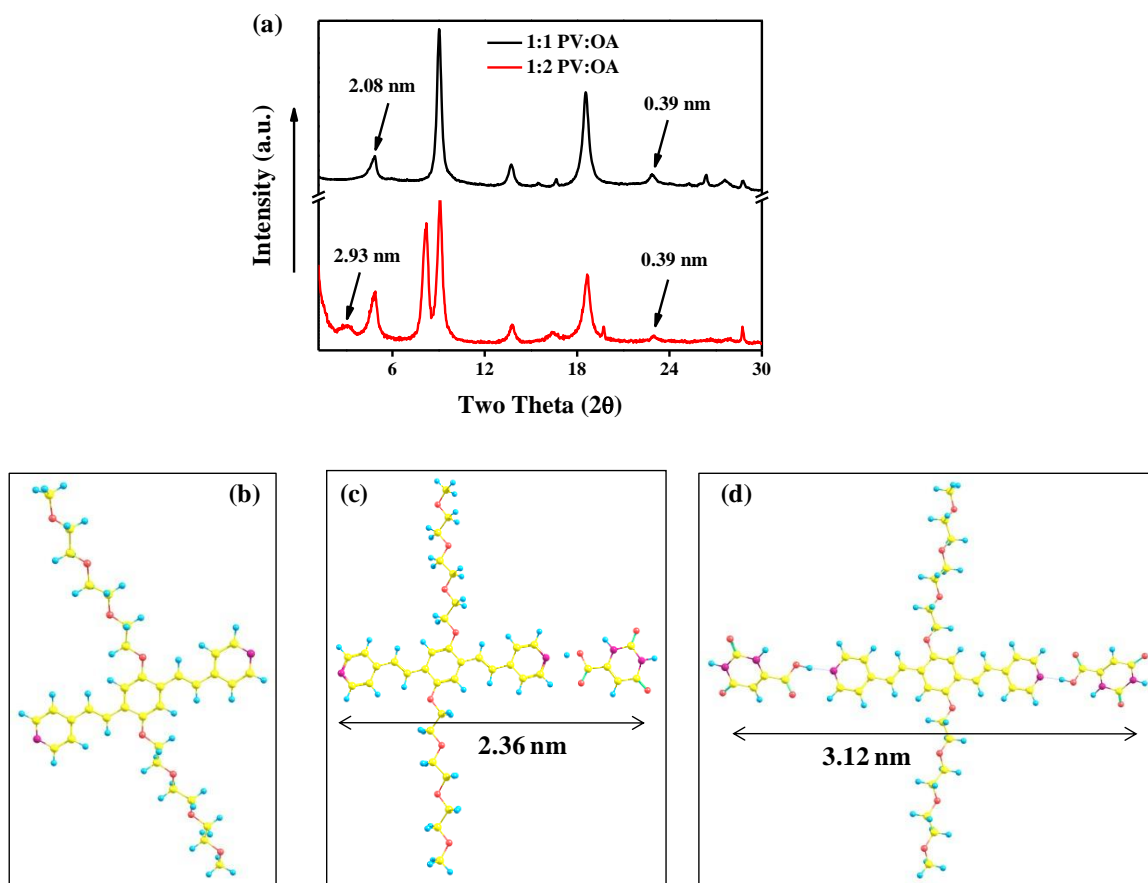
System	CGC (mg/ml)	CGC (mM)
<b>PV:OA = 1:0.5</b>	15:2.15	24.64:12.32
<b>PV:OA = 1:1</b>	10:2.86	16.43:16.43
<b>PV:OA = 1:1.5</b>	6.67:2.86	10.96:16.44
<b>PV:OA = 1:2</b>	6.31:3.61	10.36:20.72



**Figure S2.** DSC thermograms of the hydrogels derived from 1:1 and 1:2 PV:OA complexes respectively; [PV] = 30 mM.

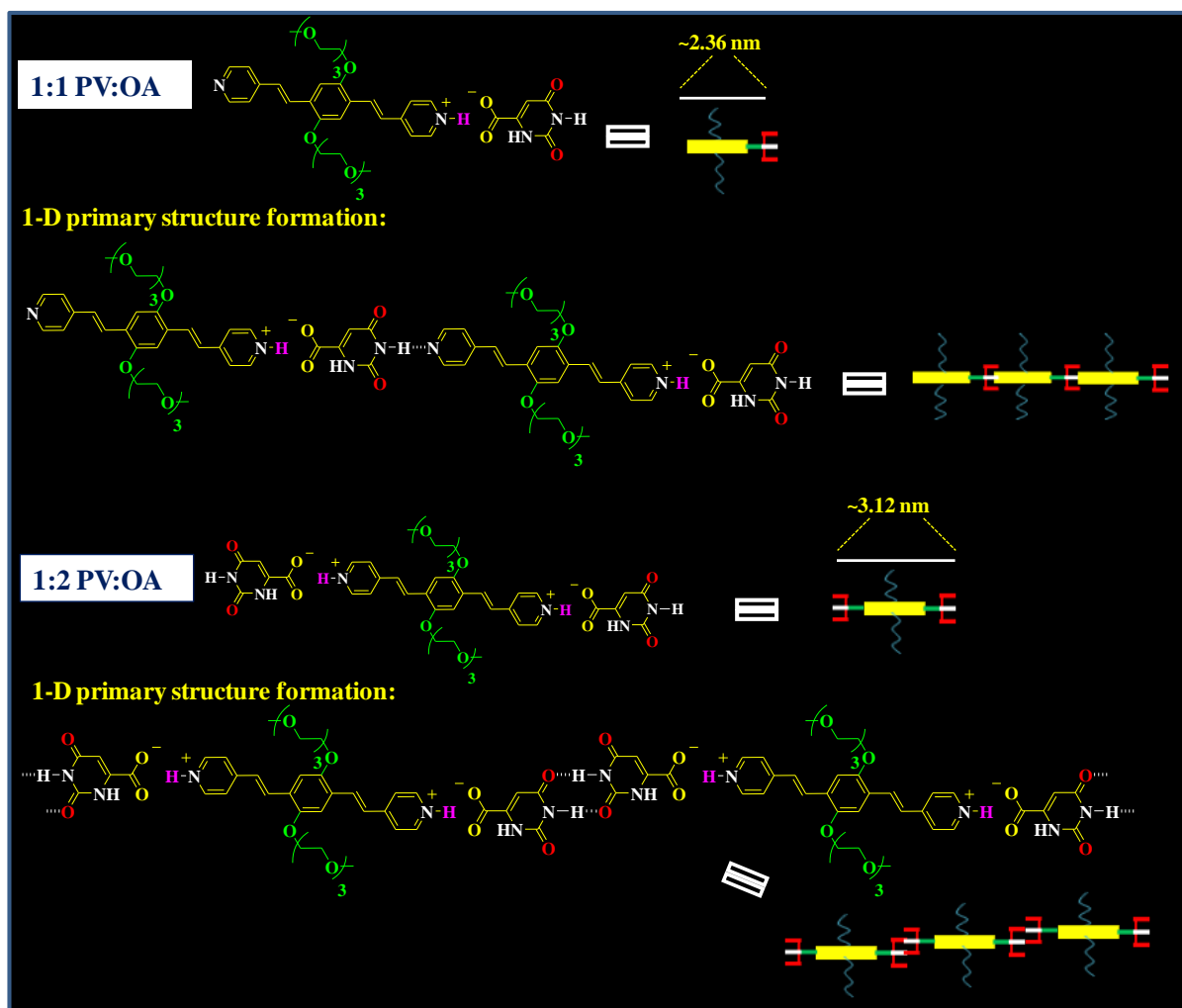


**Figure S3.** (a) Plot of absorbance vs. equiv. of OA at 450 nm; ([PV] = 0.04 mM). (b,c) UV/Vis absorption spectra of PV:OA at different molar ratios by keeping the total concentration 50  $\mu$ M and the corresponding Job plot showed 1:2 stoichiometry of the PV:OA complex respectively (absorbance values at 425 nm were taken for Job plot analysis). (d) Temperature-dependent fluorescence spectra of 1:2 PV:OA at a concentration of [PV] = 0.04 mM; ( $\lambda_{\text{ex}}$  = 370 nm).

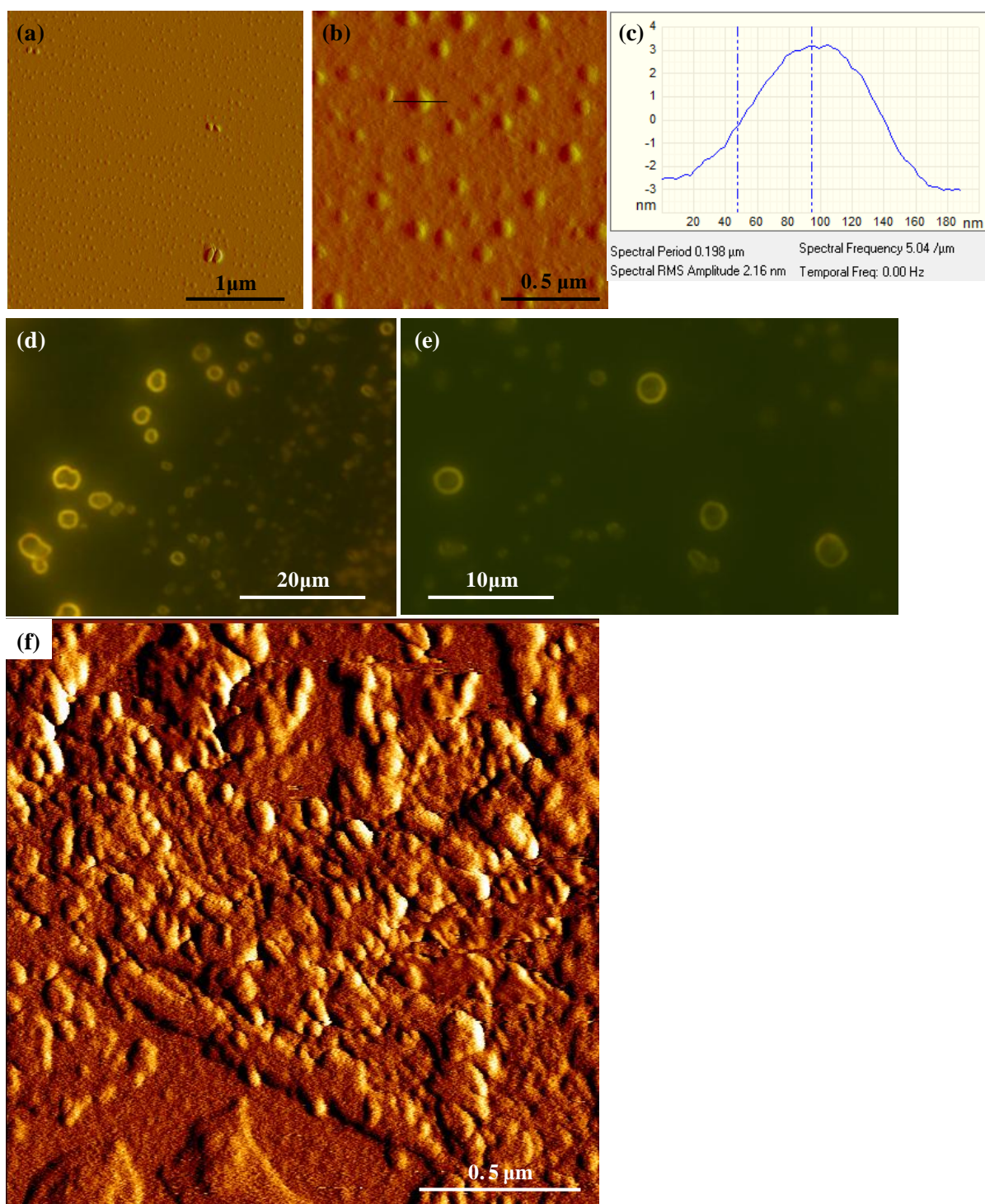


**Figure S4.** (a) XRD patterns of the freeze-dried gel of 1:1 PV:OA and 1:2 PV:OA. (b) Energy minimized structure of (b) PV, (c) 1:1 PV:OA and (d) 1:2 PV:OA system respectively performed using B3LYP/6-31 G\* level of theory.

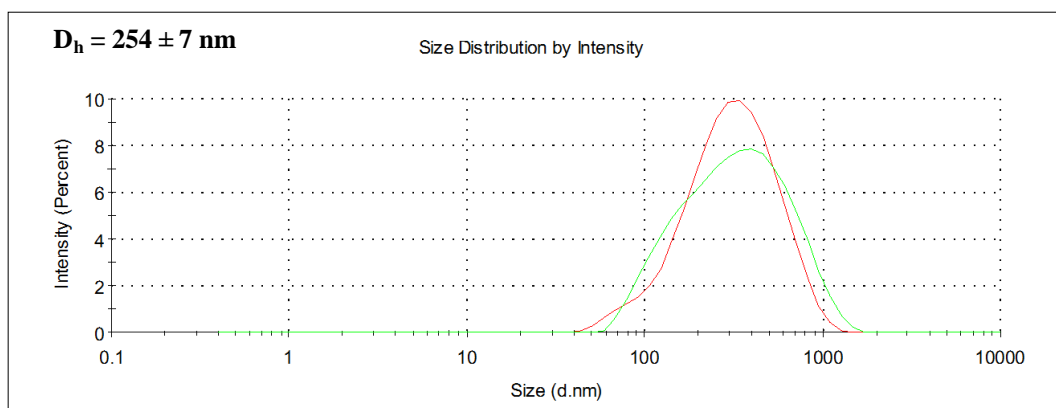




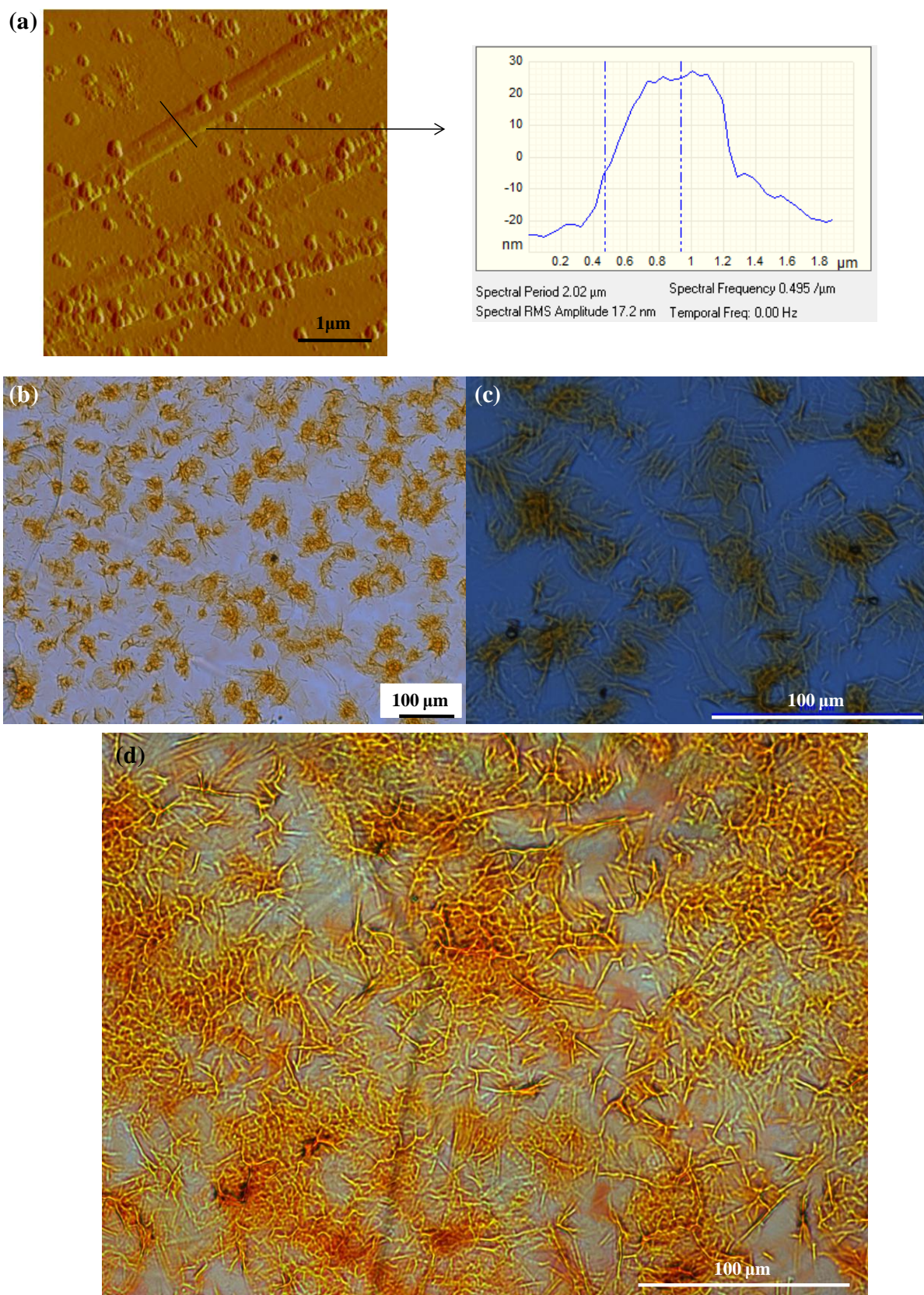
**Figure S5.** A schematic of the possible aggregation mode of 1:1 and 1:2 PV:OA gelators yielding the 1-D primary structure in water through the linear growth of the gelator units via complementary H-bonding interactions. These 1-D primary structures of the respective gelator complexes further self assemble in 3-D via various non-covalent interactions.



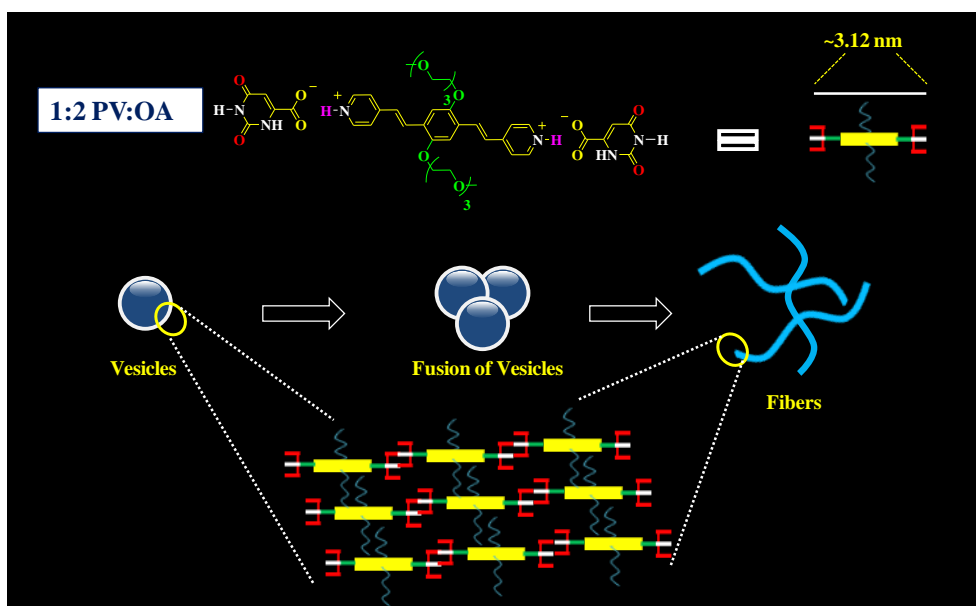
**Figure S6.** (a,b) AFM images of the vesicles of 1:1 PV:OA at different magnifications and (c) height image of a single vesicle respectively; ([PV] = 0.1 mM). (d,e) fluorescence microscopic images of the vesicular aggregates of 1:1 PV:OA at different positions and magnifications respectively; ([PV] = 0.1 μM). (f) AFM image of the cluster-type aggregate of 1:1 PV:OA complex at [PV] = 0.3 mM.



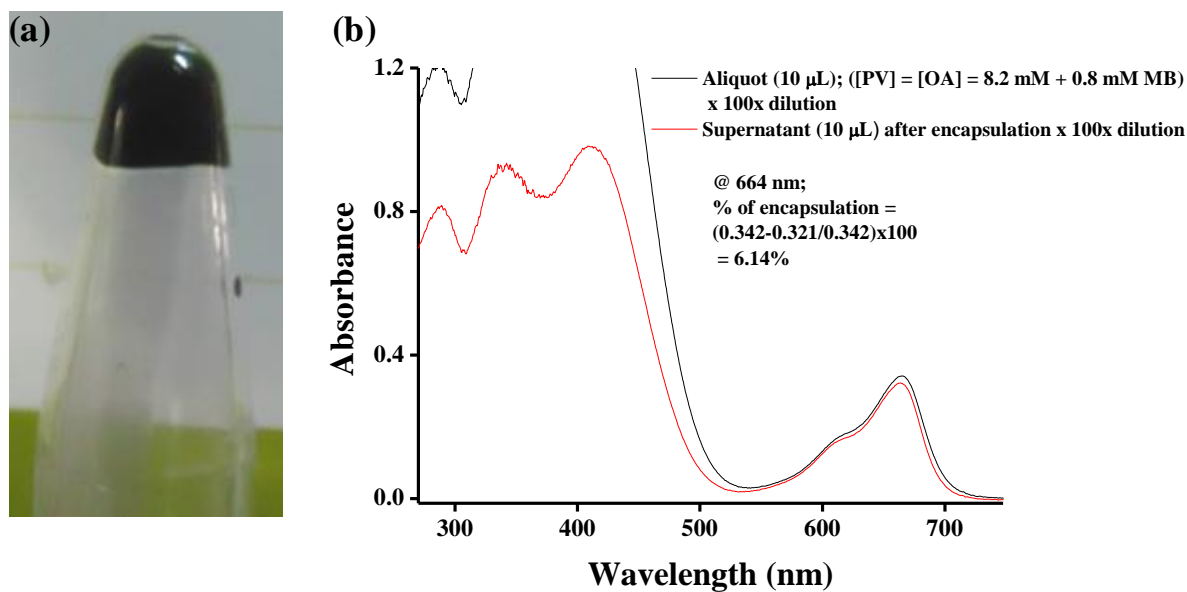
**Figure S7.** DLS studies of 1:1 PV:OA system in water; ([PV] = 0.1 mM).



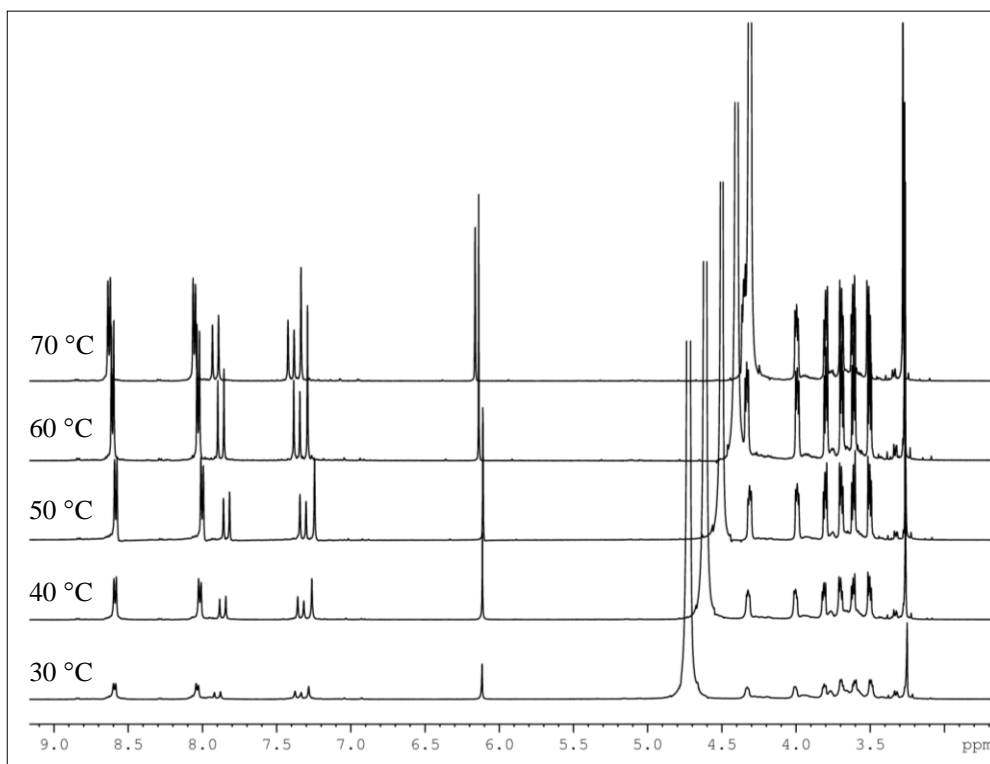
**Figure S8.** (a) AFM image shows the presence of the fiber-like aggregates along with vesicles from the 1:2 PV:OA system and the height image of the fibrous aggregate respectively; ([PV] = 0.2 mM). (b,c) Bright-field and (d) POM images showing the presence of dense fiber networks of 1:2 PV:OA gel; ([PV] = 11 mM).



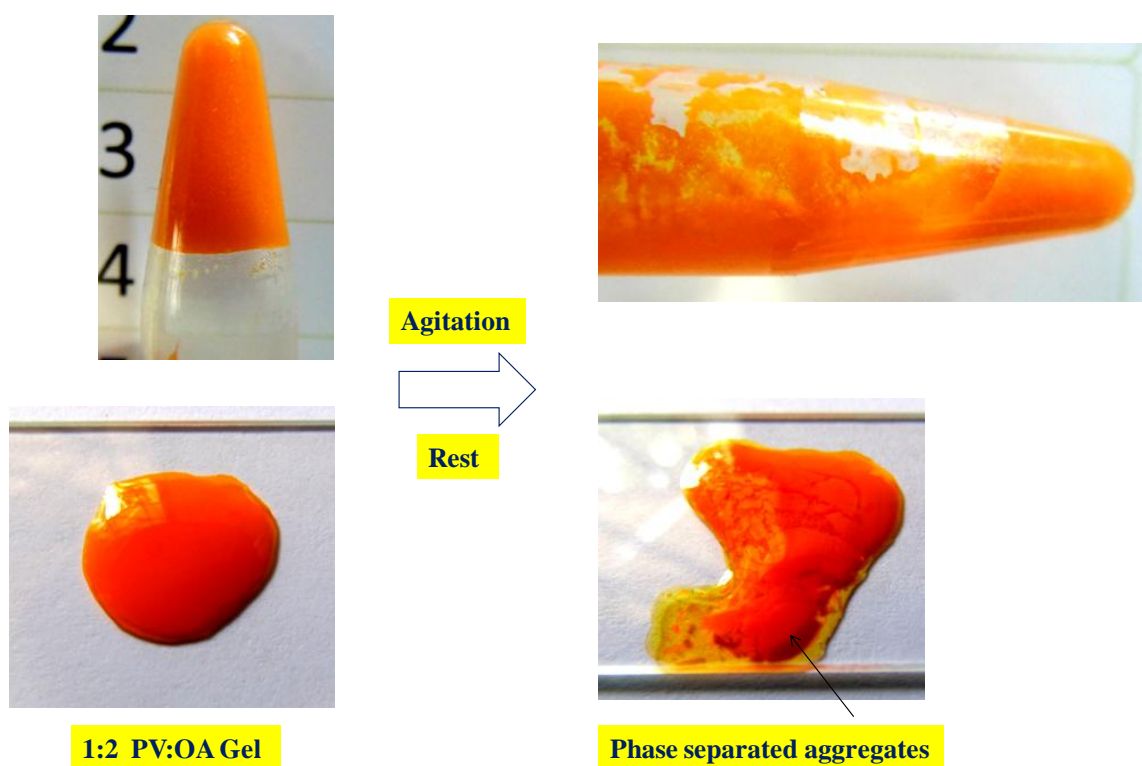
**Figure S9.** A schematic of the vesicle-to-fiber transformation of the 1:2 PV:OA gelator upon increasing concentration of the gelators.



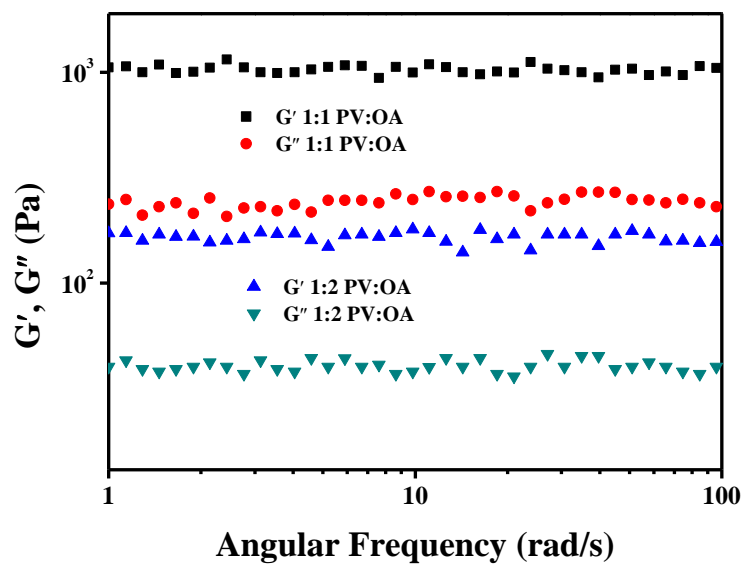
**Figure S10.** (a) Photographs of the precipitates of the vesicular aggregates along with encapsulated dye. (b) Analysis of the % of encapsulation of the dye, MB in the inner vesicular compartment of the gelator.



**Figure S11.** Variable temperature  $^1\text{H}$  NMR spectra of 1:2 PV:OA hydrogel in water; ( $[\text{PV}] = 21 \text{ mM}$ ).



**Figure S12.** Demonstration of the brittle or non thixotropic nature of the 1:2 PV:OA hydrogel. The hydrogel (either in the vial or in the glass plate) transformed into phase separated aggregates upon agitation which does not revert back to the native gel state on resting.



**Figure S13.** Oscillatory frequency sweep test of the PV:OA gelator at two different molar ratios; ( $[PV] = 25$  mM for both the gels).