Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Can the Stereogenicity in Aromatic/Non-Aromatic Residues influence the Mechanical Integrity, Antimicrobial and Anti-inflammatory Preferences of the Auxin derivatized Hydrogels?

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Table of Contents

S.No.	Category	Page No.
1.	T_{gel} Graphs of Hydrogelators **III, IV & V**	2
2.	Enlarged view of the FT-IR Spectra of Hydrogelators **III, IV & V** for the xerogels	3
3.	Enlarged CD data of the Hydrogelators **III, IV & V**	3-4
4.	Strength of Hydrogelators graph	4
5.	Torsional angles of Hydrogelators **III, IV & V**	5
6.	Energy Minimized Structure of Hydrogelators **III, IV & V**	5
7.	Overlayed structures of Hydrogelators **III, IV & V**	6
8.	Enlarged view of the MTT assay of Hydrogelators **III, IV & V** with data in tabular format	6
9.	IC_{50} values of Hydrogelators **III, IV & V**	6
10.	Data of Antibacterial assay determined by turbidity experiments.	7-8
11.	Thixotropic behaviour of Hydrogelators **III, IV & V**	9
12.	Zymographic study of the anti-inflammatory activities of Hydrogelators **III, IV & V**	10
13.	Diagram of Zymographic study of the anti-inflammatory activities of Hydrogelators **III, IV & V**	10
14.	Experimental Section	11-12
15.	Chemical structures of Hydrogelators **III, IV & V**	13
16.	^{1}H NMR of Hydrogelator- **III**	14
17.	^{1}H NMR of Hydrogelator- **IV**	14
18.	^{1}H NMR of Hydrogelator- **V**	15
19.	Mass Spectra of Hydrogelator- **III**	15
20.	Mass Spectra of Hydrogelator- **IV**	16
21.	Mass Spectra of Hydrogelator- **V**	16

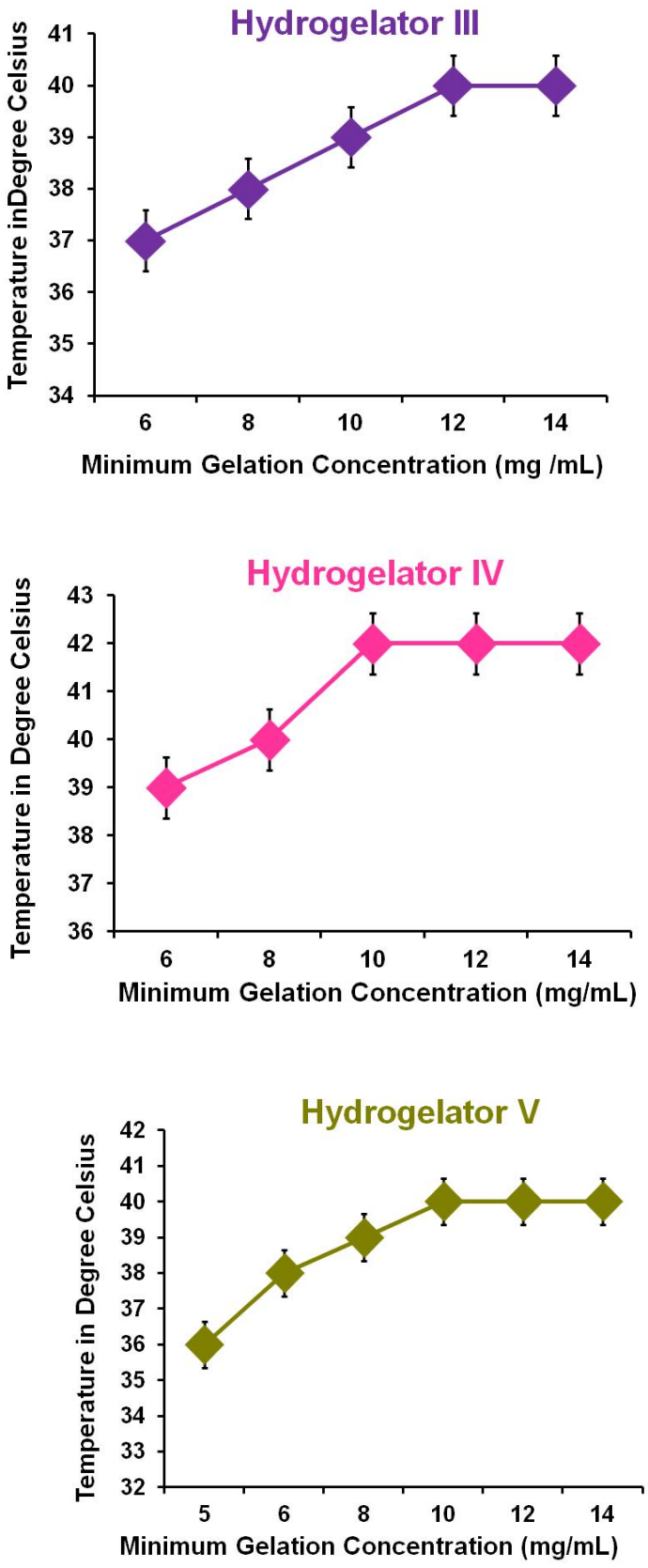


Figure S1: - T_{gel} Graphs of Hydrogelator **III-V**

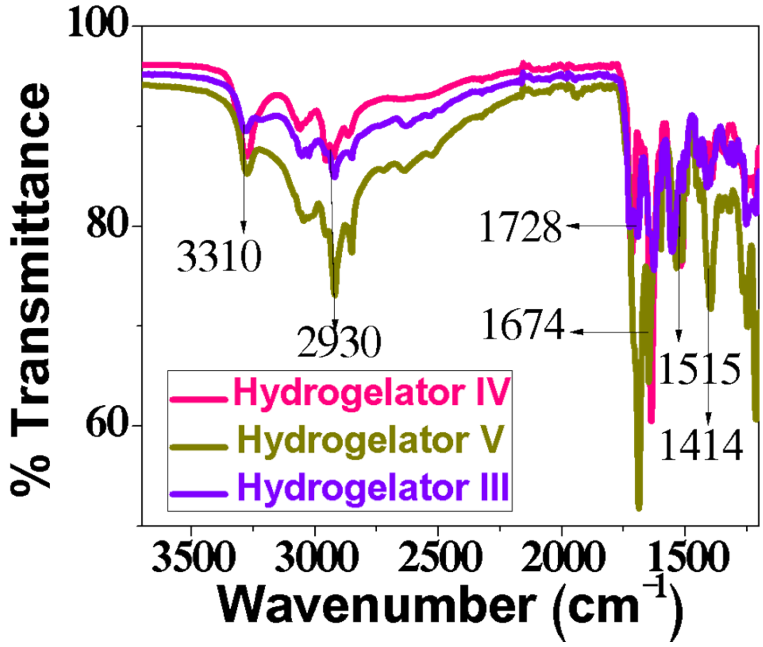
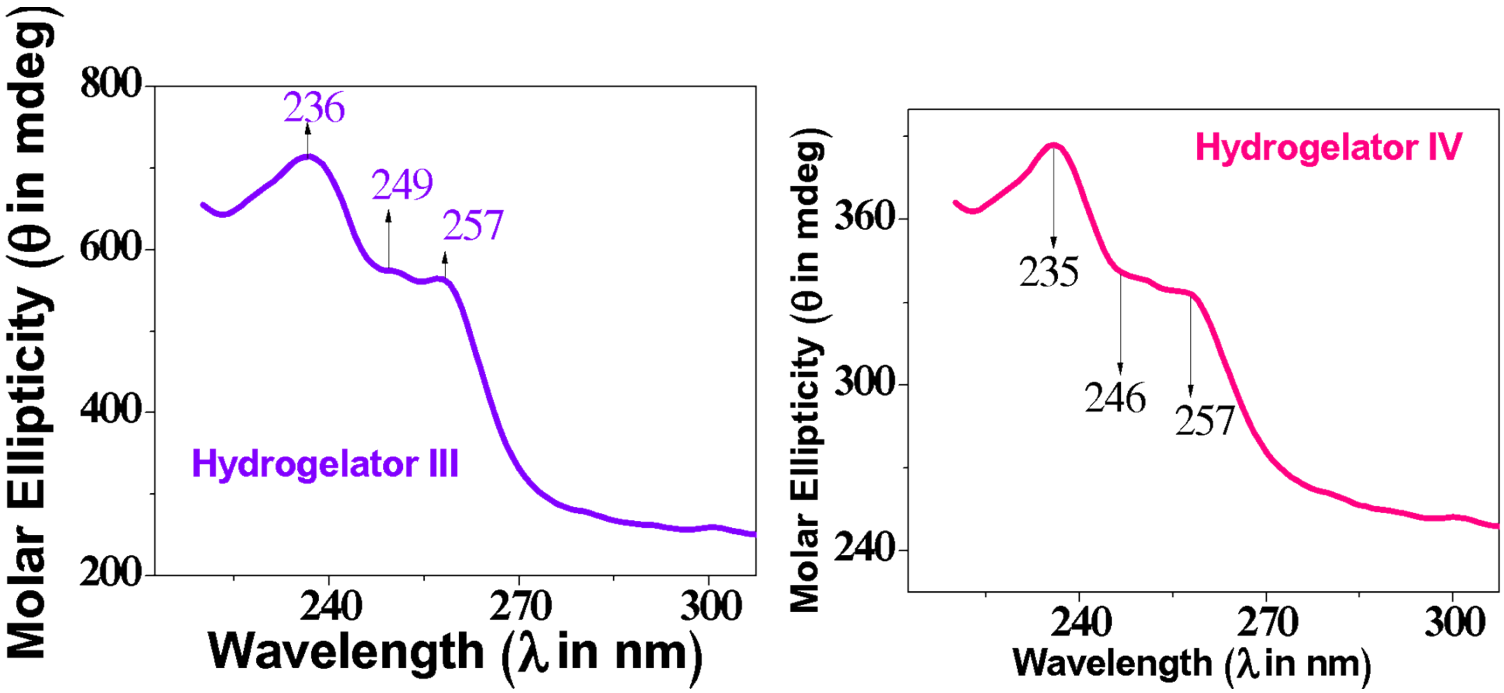


Figure S2: - Enlarged view of the FT-IR Spectra of Hydrogelators **III-V**



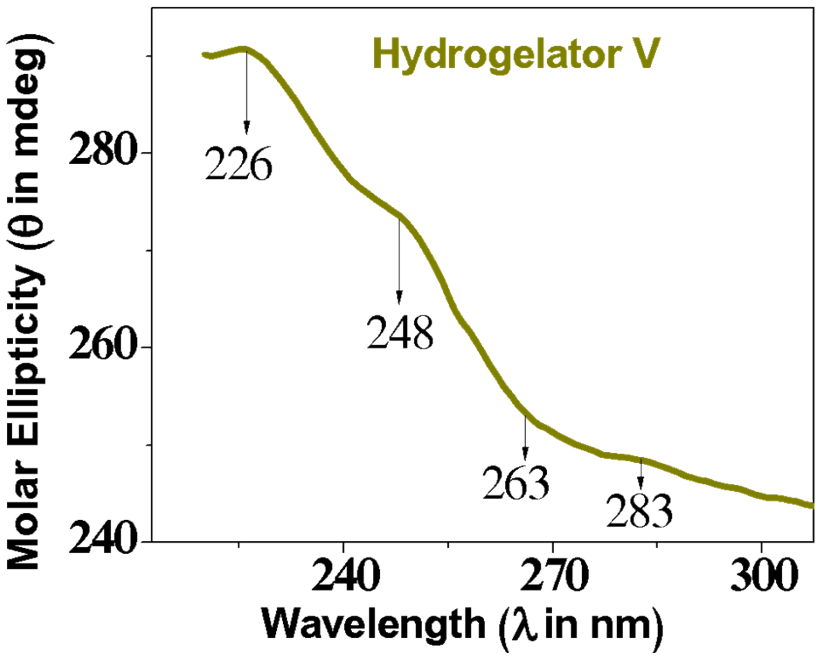


Figure S3: - Enlarged CD data of the Hydrogelators **III-V**

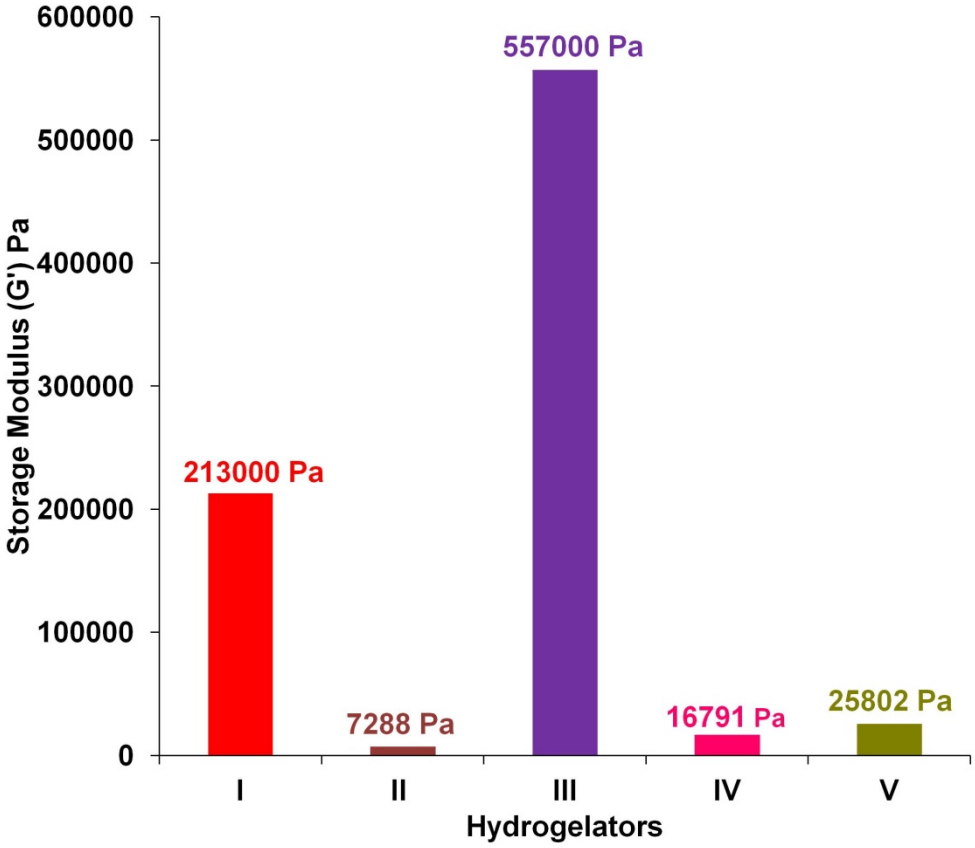
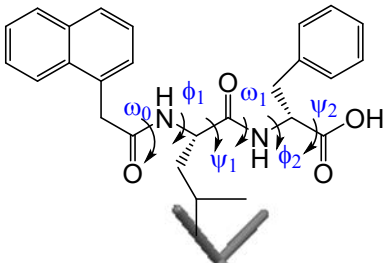
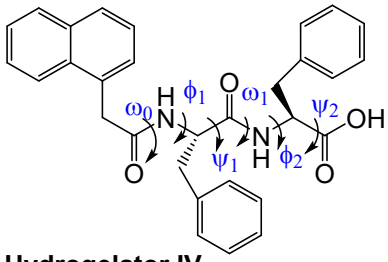


Figure S4: - Graph of Strength of Hydrogelators **III-V**





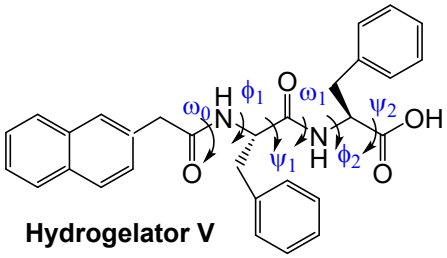
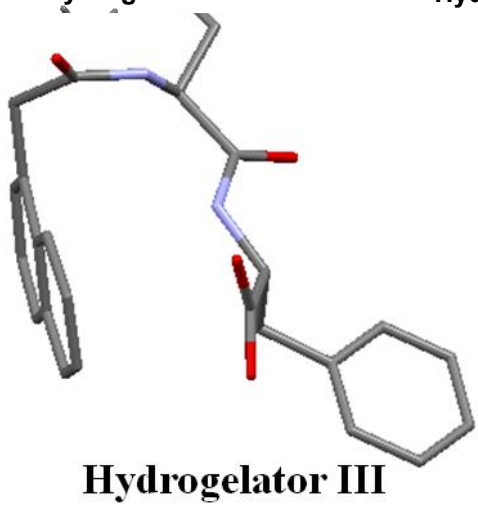
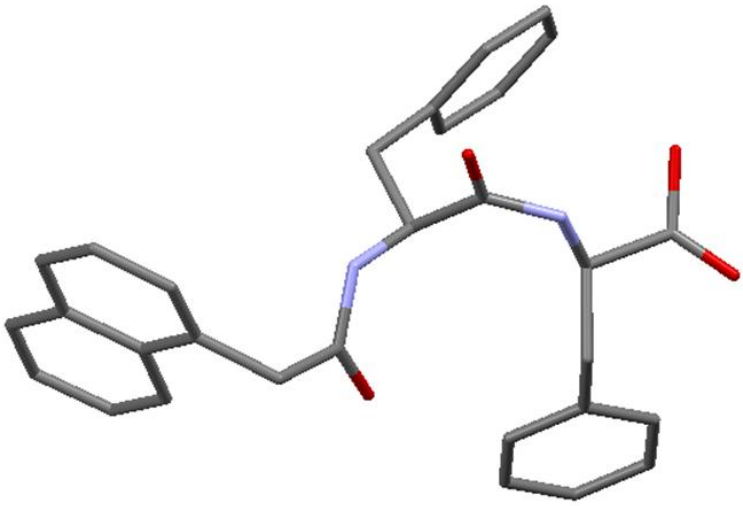


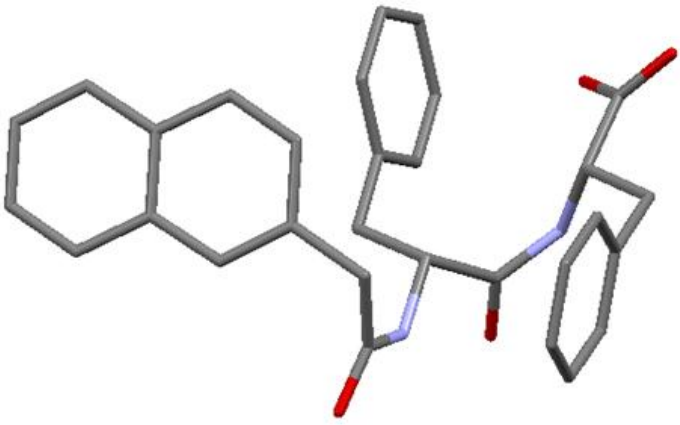
Figure S5: - Torsional angles of Hydrogelators- **III, IV& V**



Hydrogelator III



Hydrogelator IV



Hydrogelator V

Figure S6: - Energy Minimized Structures of Hydrogelators- **III, IV& V**

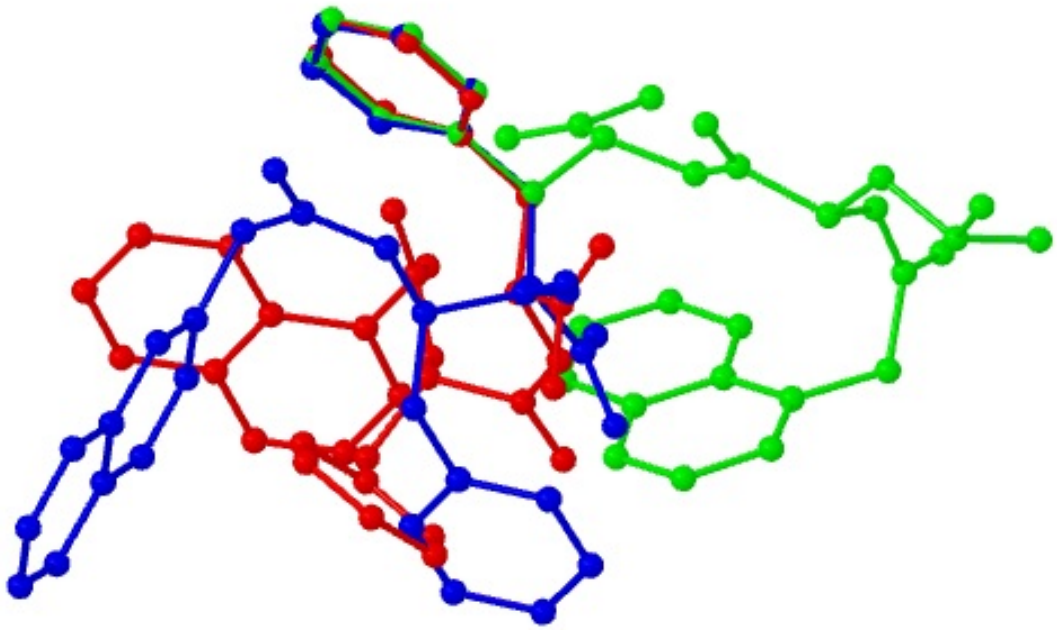


Figure S7: - Overlayed Structures of Hydrogelators- **III (green) , IV (red) & V(blue)**

Table S1:- MTT assay data of Hydrogelators **III-V** in tabular format.

	200 µg/ mL	**100 µg/ mL**	**50 µg/ mL**	**25 µg/ mL**	**12.5 µg/ mL**	**6.25 µg/ mL**	**NC**
Hydrogelator-III	**77.60**	**81.55**	**94.47**	**94.73**	**96.18**	**98.94**	**100**
Hydrogelator-IV	**55.20**	**57.31**	**59.94**	**64.69**	**80.89**	**85.77**	**100**
Hydrogelator-V	**70.88**	**72.59**	**74.17**	**75.75**	**80.63**	**94.86**	**100**

Table S2: - IC_{50} Value of the Hydrogelator compounds (µg/mL)

Compounds	**HEk293**	
	Mean	**SD**
Hydrogelator-III	606.53	31.57
Hydrogelator-IV	110.47	4.49
Hydrogelator-V	268.70	6.71

Table S3: - Data of Antibacterial assay as determined by turbidity experiments

S. No.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
E.coli											
01	**Hydrogelator III**	R	R	R	R	R	R	R	R	R	R
02	**Hydrogelator IV**	S	R	R	R	R	R	R	R	R	R
03	**Hydrogelator V**	S	R	R	R	R	R	R	R	R	R
Klebsiella											
01	**Hydrogelator III**	R	R	R	R	R	R	R	R	R	R
02	**Hydrogelator IV**	S	R	R	R	R	R	R	R	R	R
03	**Hydrogelator V**	R	R	R	R	R	R	R	R	R	R
S.aureus											
01	**Hydrogelator III**	S	S	R	R	R	R	R	R	R	R
02	**Hydrogelator IV**	S	S	S	R	R	R	R	R	R	R
03	**Hydrogelator V**	S	S	S	S	R	R	R	R	R	R
S.mutans											
01	**Hydrogelator III**	S	S	S	S	**S**	R	R	R	R	R
02	**Hydrogelator IV**	S	R	R	R	R	R	R	R	R	R
03	**Hydrogelator V**	S	R	R	R	R	R	R	R	R	R
Candida											
01	**Hydrogelator III**	S	S	S	S	S	S	R	R	R	R
02	**Hydrogelator IV**	S	S	S	S	S	R	R	R	R	R
03	**Hydrogelator V**	S	S	S	S	S	S	R	R	R	R
A.niger											
01	**Hydrogelator III**	S	S	S	S	S	S	S	S	R	R
02	**Hydrogelator IV**	S	S	S	S	R	R	R	R	R	R
03	**Hydrogelator V**	S	S	S	S	S	R	R	R	R	R

S.No.	Samples	100 µg / mL	50 µg / mL	25 µg / mL	12.5 µg / mL	6.25 µg / mL	3.12 µg / mL	1.6 µg / mL	0.8 µg / mL	0.4 µg / mL	0.2 µg / mL
E.coli											
01	**Hydrogelator III**	1.288	1.568	1.878	1.879	1.902	1.959	1.976	1.978	1.991	1.994
02	**Hydrogelator IV**	0.894	1.317	1.522	1.527	1.596	1.605	1.634	1.635	1.638	1.681
03	**Hydrogelator V**	0.867	1.308	1.508	1.568	1.582	1.601	1.653	1.665	1.668	1.753
Klebsiella											
01	**Hydrogelator III**	1.686	1.971	1.986	2.007	2.022	2.032	2.051	2.128	2.169	2.325
02	**Hydrogelator IV**	1.030	1.706	1.747	1.772	1.826	1.958	1.969	1.976	1.988	2.002
03	**Hydrogelator V**	1.159	1.651	1.747	1.748	1.753	1.774	1.789	1.790	1.851	1.896
S.aureus											
01	**Hydrogelator III**	0.861	1.083	1.105	1.147	1.151	1.310	1.468	1.549	1.589	1.716
02	**Hydrogelator IV**	0.423	0.535	0.567	1.086	1.104	1.152	1.220	1.242	1.284	1.345
03	**Hydrogelator V**	0.419	0.511	0.528	0.568	0.841	0.885	1.121	1.177	1.178	1.300
S.mutans											
01	**Hydrogelator III**	0.441	0.506	0.592	0.820	0.985	1.053	1.239	1.333	1.479	1.663
02	**Hydrogelator IV**	0.660	1.189	1.468	1.686	1.749	1.761	1.781	1.799	1.945	2.070
03	**Hydrogelator V**	0.582	1.189	1.715	1.726	1.732	1.749	1.766	1.827	1.856	1.894
Candida											
01	**Hydrogelator III**	0.710	0.785	0.922	0.944	0.946	0.978	1.043	1.051	1.126	1.195
02	**Hydrogelator IV**	0.696	0.701	0.730	0.733	0.757	0.772	0.785	0.808	0.868	0.887
03	**Hydrogelator V**	0.453	0.605	0.660	0.690	0.714	0.746	0.757	0.759	0.801	0.871
A.niger											
01	**Hydrogelator III**	0.352	0.358	0.371	0.393	0.400	0.409	0.426	0.441	0.462	0.492
02	**Hydrogelator IV**	0.298	0.309	0.319	0.324	0.332	0.341	0.356	0.357	0.3838	0.400
03	**Hydrogelator V**	0.303	0.308	0.311	0.322	0.331	0.332	0.339	0.354	0.358	0.376

Hydrogelator III: Non - Thixotropic **Hydrogelato**

Hydrogelator V: Thixotropic

Figure S8: - Thixotropic behaviour of Hydrogelators **III, IV & V**

Table S4: - Zymographic study of the Anti-Inflammatory activities of Hydrogelator **III-V.**

S.No.	NAME OF THE COMPOUND	% BANDS OF MMP		% INHIBITION OF MMP	
		MMP 2	MMP 9	MMP 2	MMP 9
1	Hydrogelator III	45	49	55	51
2	Hydrogelator IV	32	33	68	67
3	Hydrogelator V	49	46	51	54
4	POSITIVE CONTROL	00	00	100	100
5	NEGATIVE CONTROL	100	100	00	00

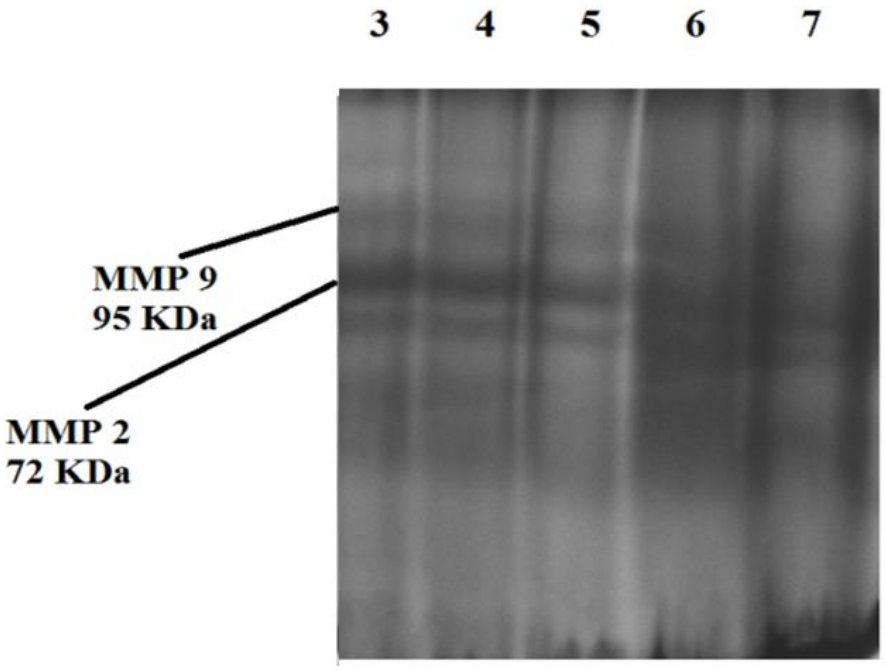


Figure S9: - Diagram of Zymographic study of the anti-inflammatory activities of Hydrogelator **III, IV & V**

Experimental Procedure

Preparation of the Hydrogels

The requisite amounts of hydrogelators were separately dissolved in 7.5 pH phosphate buffer by slightly warming until a transparent solution was produced. It was kept undisturbed for some time when hydrogel formation took place, confirmed by inverted vial method.

Conformational analysis of the Hydrogels.

The temperature dependent ^{1}H, ^{13}C NMR, COSY and ROESY experiments were performed using Bruker Advance instrument operating at 500MHz NMR, with d_6-DMSO as solvent.

Fourier-Transform Infrared (FTIR) Spectroscopy

FTIR spectra for both the xerogels were recorded using a KBr pellet on an Agilent CARY 620 FTIR spectrophotometer. The background was collected using a blank KBr pellet.

Circular Dichroism

Far-UV CD measurements of the Hydrogelators were recorded in methanol at 25^{o}C with a 0.5 s averaging time, a scan speed of 50nm/min, using a JASCO spectropolarimeter (J 720 model) equipped with a 0.1 cm path length cuvette. The measurements were taken at 0.2 nm wavelength intervals, 2.0 nm spectral bandwidth, and five sequential scans were recorded for each sample.

Morphological Study of the Hydrogels.

Field-emission scanning electron microscopy (FE-SEM) experiment was performed on a JEOL scanning electron microscope (model no. JSM-7600F) with xerogels, obtained from the hydrogels of same concentration 8 mg/ml.

Rheological Properties of the Hydrogels. Rheological experiments were performed at 25 °C on an Anton Paar Physica MCR 301 rheometer. The viscoelastic properties of hydrogels were measured by measuring the storage modulus (G′) and loss modulus (G″). Hydrogel (1 mL) was transferred on a rheometer plate by using a microspatula and kept hydrated by using a solvent trap. A stainless steel parallel plate (diameter: 25 mm) was used to sandwich the hydrogels with TruGap (0.5 mm). The dynamic strain sweep experiment was performed to determine the region of deformation of hydrogels in which linear viscoelasticity is valid. The exact strains for hydrogel materials were determined by linear viscoelastic regime at a constant frequency of 10 rad s^{-1}. The mechanical strengths of the hydrogels were determined by frequency sweep experiment. In the frequency sweep measurement, the graph was plotted as a function of frequency in the range of 0.05−100 rad s^{-1}. The thixotropic properties were investigated by step-strain experiments at the constant frequency of 10 rad s^{-1}, and applied strains were varied from 0.1 to 40%. The concentrations of hydrogelators used were 8 mg/mL.

DFT Calculations

The molecules (reactants and products) were modelled using Spartan08 software and energy minimization was done within the software. Calculations were performed on a single molecule as described in the reference 1.[1]

The PROTEOLYTIC STABILITY: The hydrogelators were incubated with the proteolytic enzyme proteinase K and recording the degradation rate by Mass Spectrometry at regular intervals of time as described in References 2 and 3. [2, 3]

MTT Assay: This is a colorimetric assay that quantifies the reduction of *yellow* 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinated dehydrogenase. Since the reduction of MTT occurs in metabolically active cells, the level of

activity is a measure of the viability of the cells. Eventually the IC_{50} values were determined which would give a measure of the biocompatibility $IC_{50} > 100\mu M$).

Antibacterial Experiment.
Bacterial Culture. *S. Aureus* ATCC – 25923, *S. Mutans* ATCC – 25175, *E. Coli* ATCC – 25922, *Klebsiella Pneumonia* ATCC - 1705 were obtained as a lyophilized powder. Before beginning the experiments, fresh inoculums of the organisms were prepared.

Antimicrobial Properties: *The* antimicrobial activities were done through MIC (minimum inhibitory concentration) approach using Turbidity Experiment. Before beginning the experiments, fresh inoculums of the organisms were prepared from their lyophilized powder.
For this study various dilutions of the hydrogelators were used and the experiments were done in triplicates. In each experiment, tentatively ten microliter of peptide hydrogels of a particular concentration were added to each well which were further diluted for rest of the experiments. The bacterial solution devoid of hydrogels in nutrient broth was considered as the control and only nutrient broth as blank. These plates containing test organisms and hydrogels were incubated at 37 °C for 24 h. Finally the antibacterial properties of peptide hydrogels were confirmed with a microplate reader using 96-well microplates at 25°C by comparing the absorbance of the test solution with the control experiment.[1] *The Antifungal activity was done using the same protocol.*

In vitro Anti-inflammatory Activity
The Matrix Metalloproteinase enzymes are mainly known to regulate this activity and they were extracted in tris buffer as described in Reference 1.[1] Again in the same Tris buffer an equal amount of hydrogel was incubated for an hour. Only the ENZYME Solution was used as negative control (NC) and ENZYME Solution with an equal amount of standard anti-inflammatory drug were used as positive control (PC). The final solutions were mixed with a non reducing buffer in equal volume and from this a certain volume of sample was loaded in each well, connected with the electrodes and POWER was started until the bromophenol blue reached the bottom of the plates. After electrophoresis, the apparatus were dissembled; the gel was removed and washed with zymogram renaturing buffer i.e. 2.5%Triton x-100 for one hour to remove SDS completely allowing the gels to renature. It was further incubated at 37ºC overnight, followed by staining with Coomassie blue R-250 for one hour and then destained with appropriate solution. After staining, the background was stained blue with Coomassie stain where the gelatin was degraded, while white bands appeared indicating the presence of gelatinases.
The percentage inhibition of protein denaturation was calculated by using the following formula
% Inhibition = 100 x (Abs of control -Abs of sample) /Abs of control.[1]

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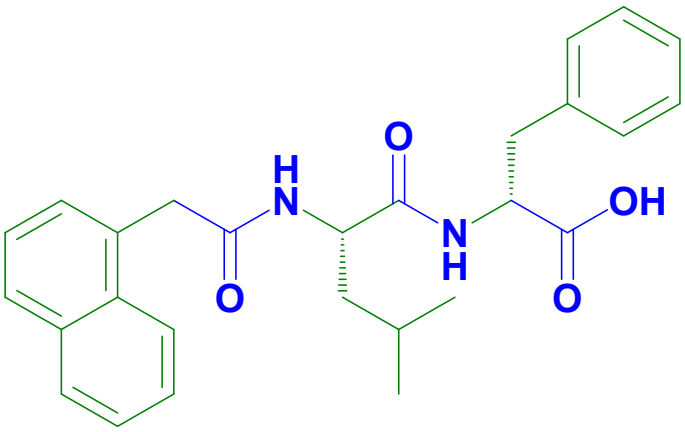
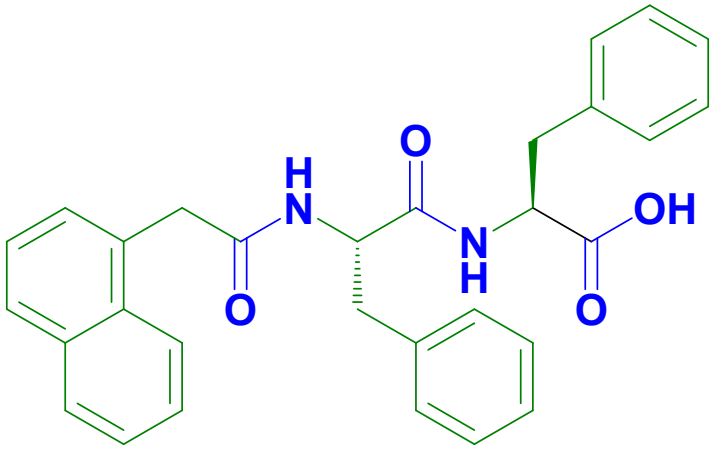
2 P. Tiwari, A. Basu, A. Vij, S. Bera, A. K. Tiwari and A. D. Konar, *Chemistry Select*, 2019, **4**, 6896–6905.

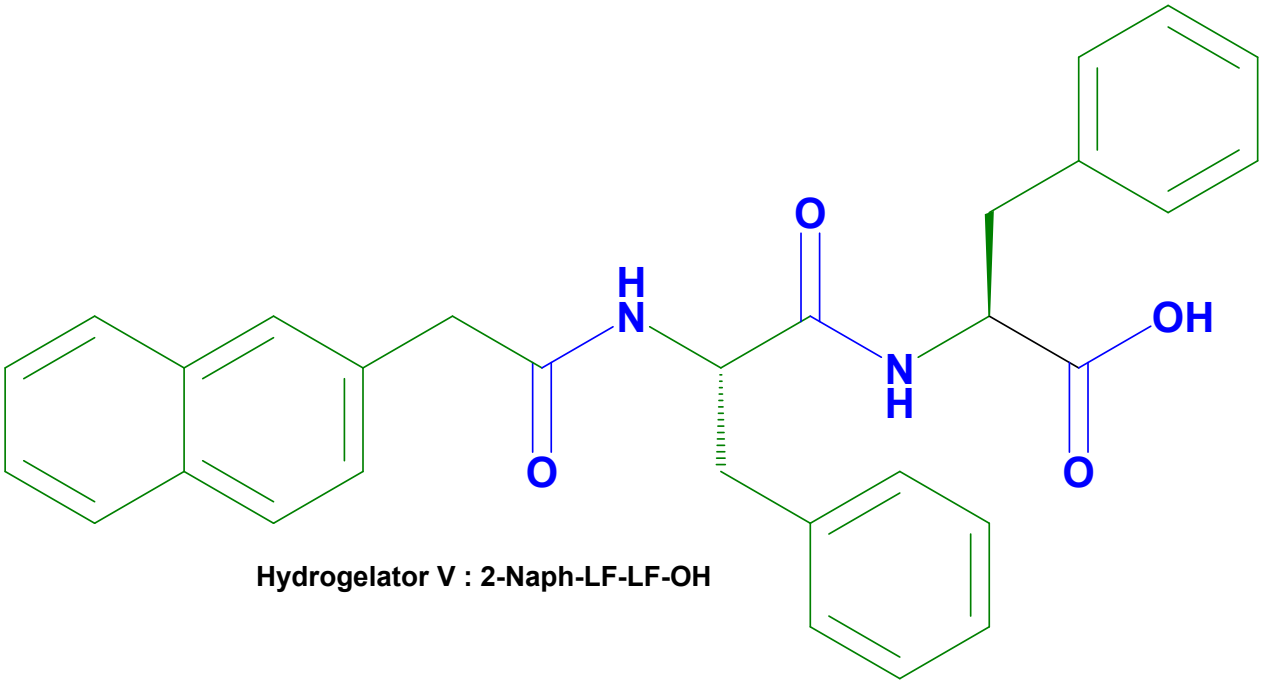
3 R. R. Mehra, P. Tiwari, A. Basu and A. Duttkonar, *New J. Chem.*, 2019, **43**, 11666–11678.



Hydrogelator III : 1-Naph-Leu-DF-OH



Hydrogelator IV : 1-Naph-LF-LF-OH



Hydrogelator V : 2-Naph-LF-LF-OH

Figure S10: - Lipophobic and Lipophillic parts of the Hydrogelators **III-V** are labelled in Green and Blue respectively.

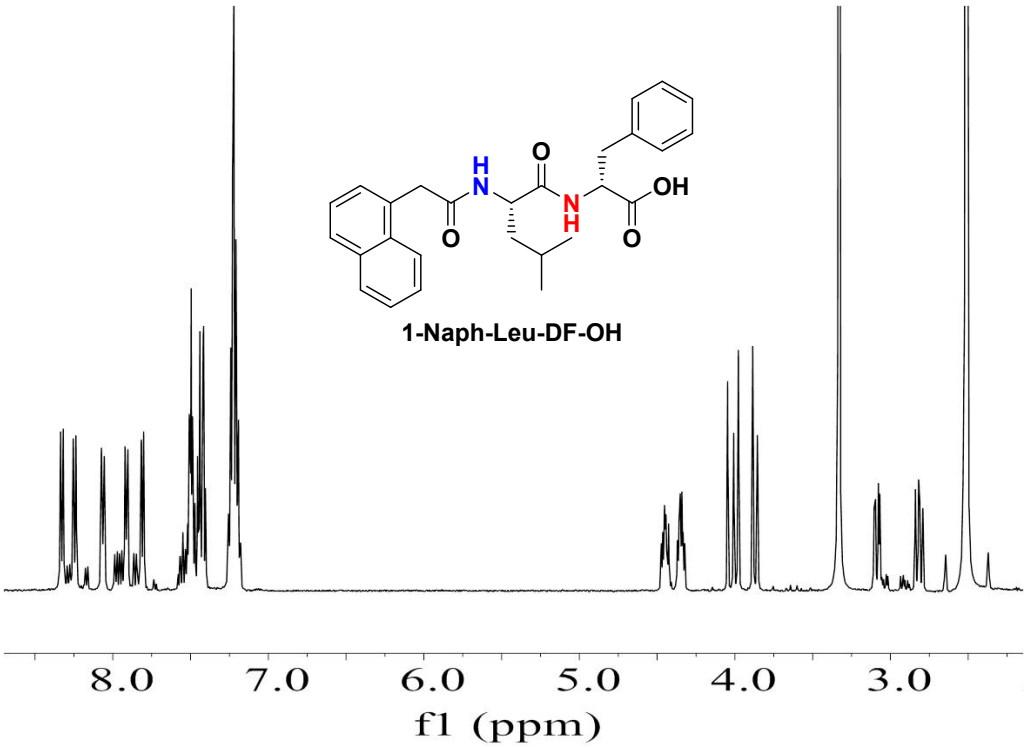


Figure S11: - ^{1}H NMR of Hydrogelator- **III**

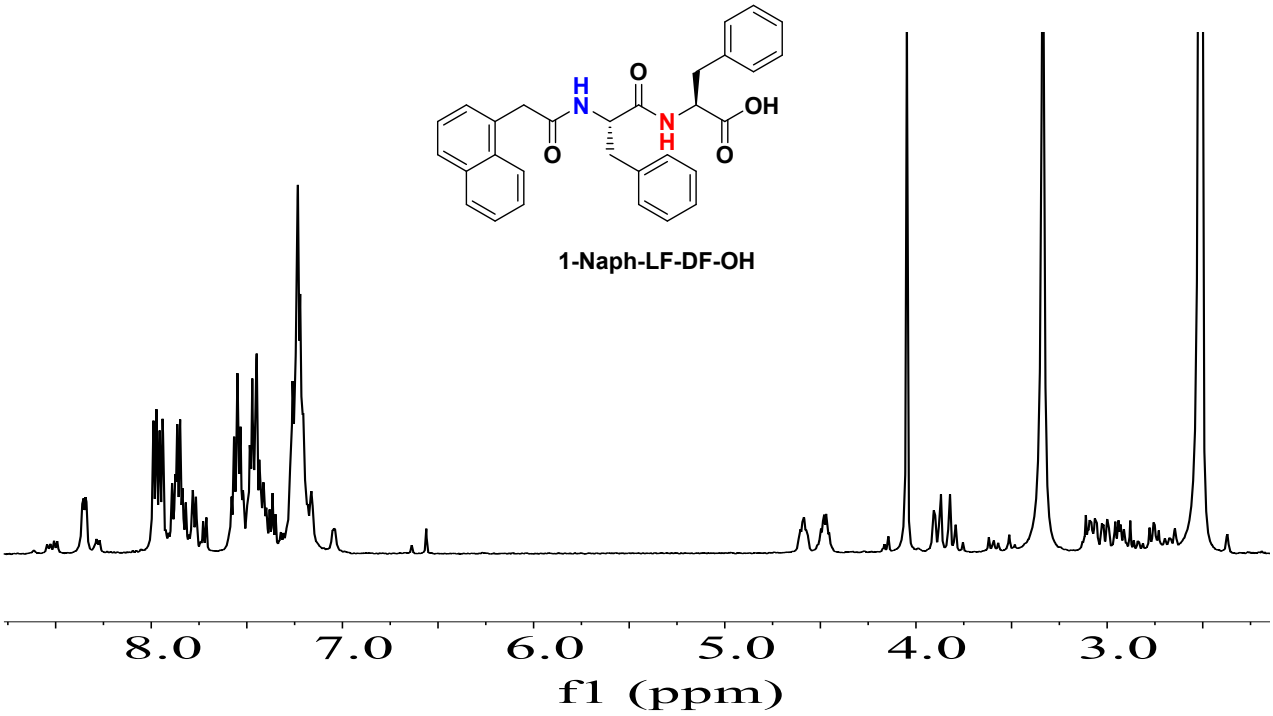


Figure S12: - ^{1}H NMR of Hydrogelator- **IV**

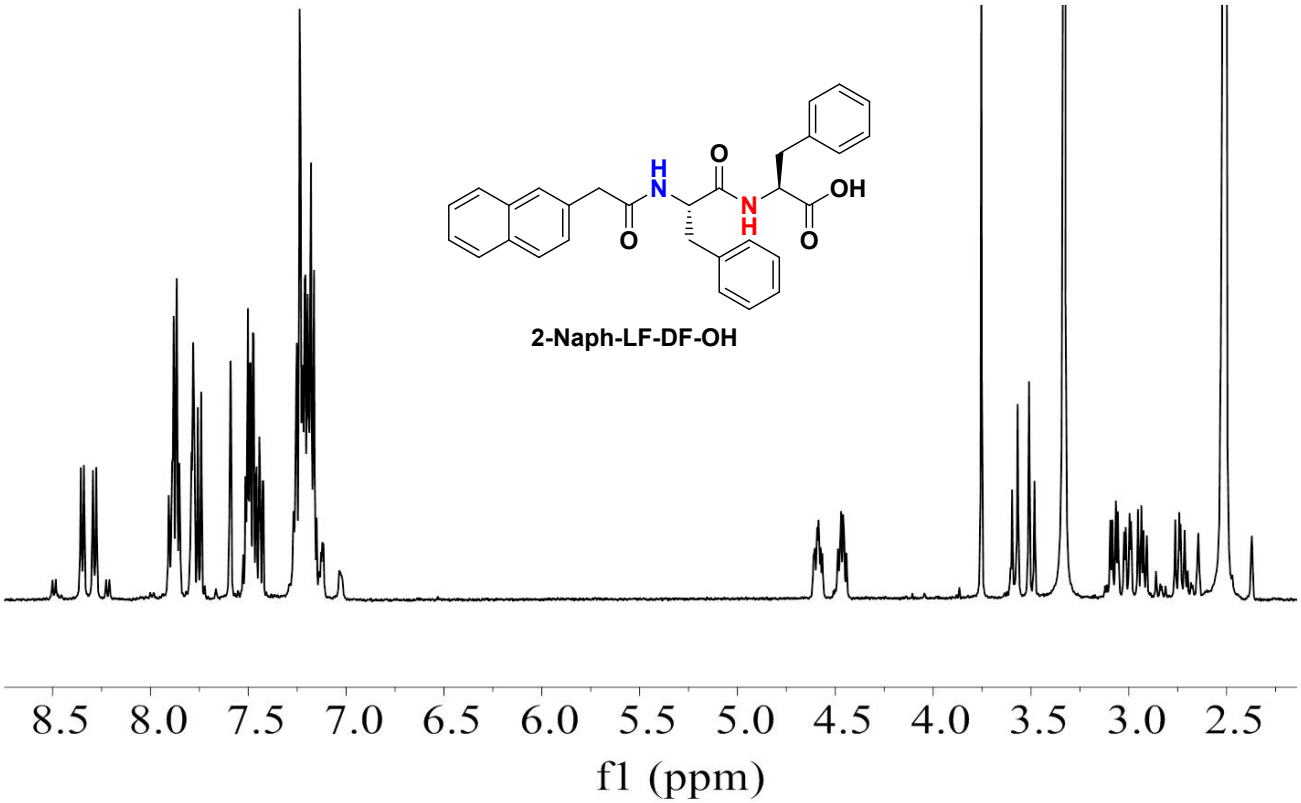


Figure S13: - ^{1}H NMR of Hydrogelator- **V**

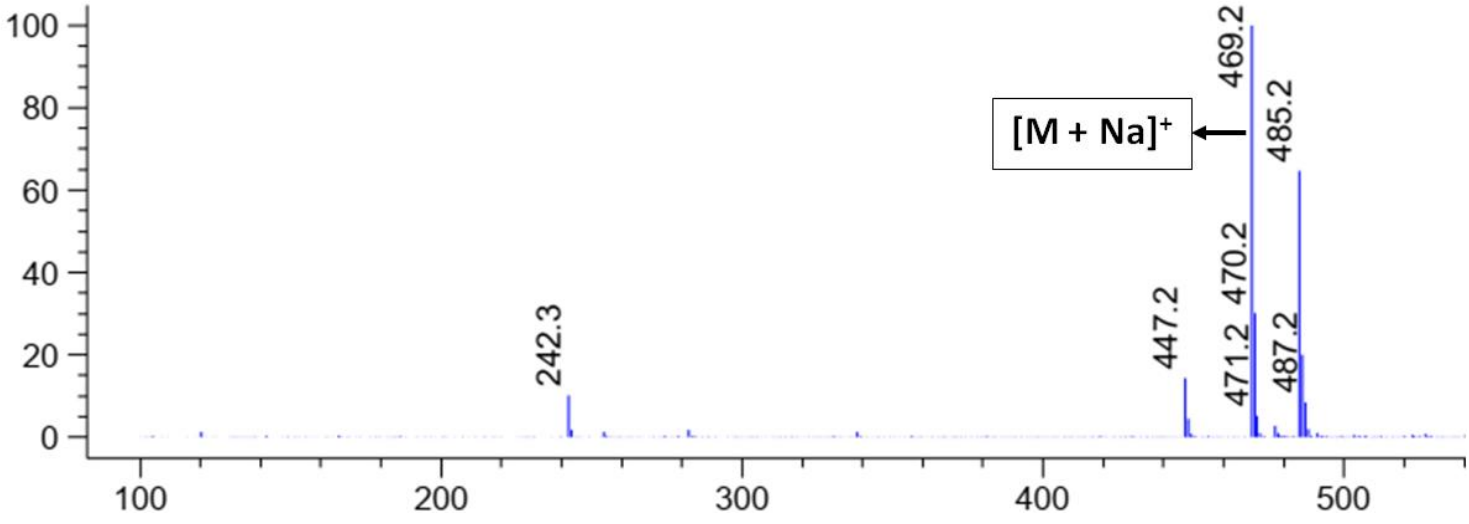


Figure S14: - Mass Spectra of Hydrogelator- **III**

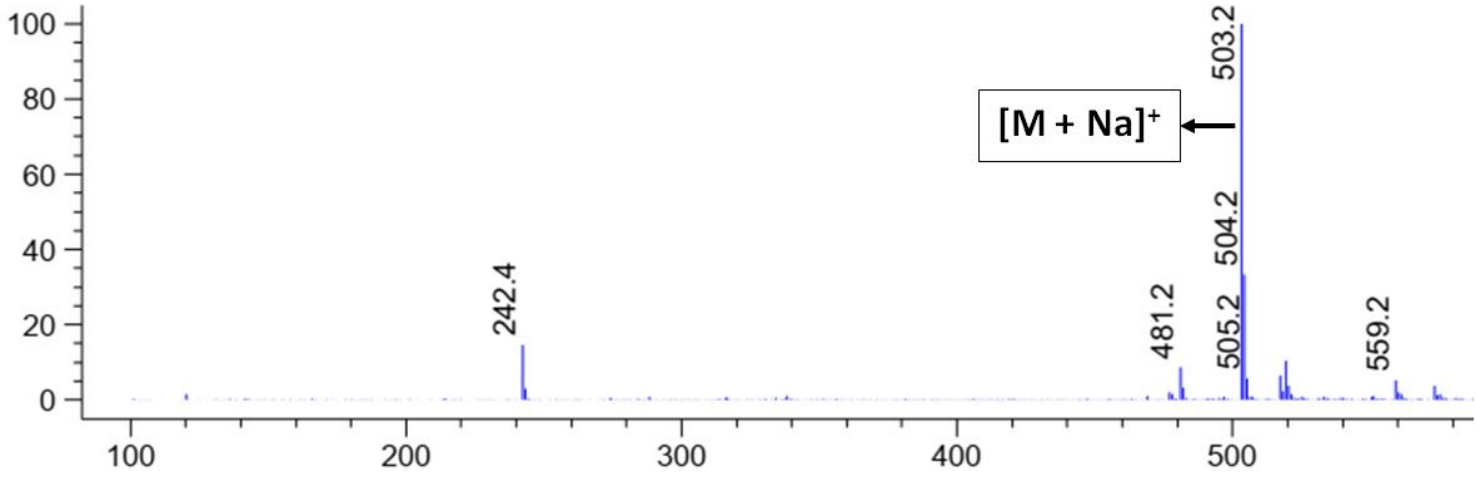


Figure S15: - Mass Spectra of Hydrogelator- **IV**

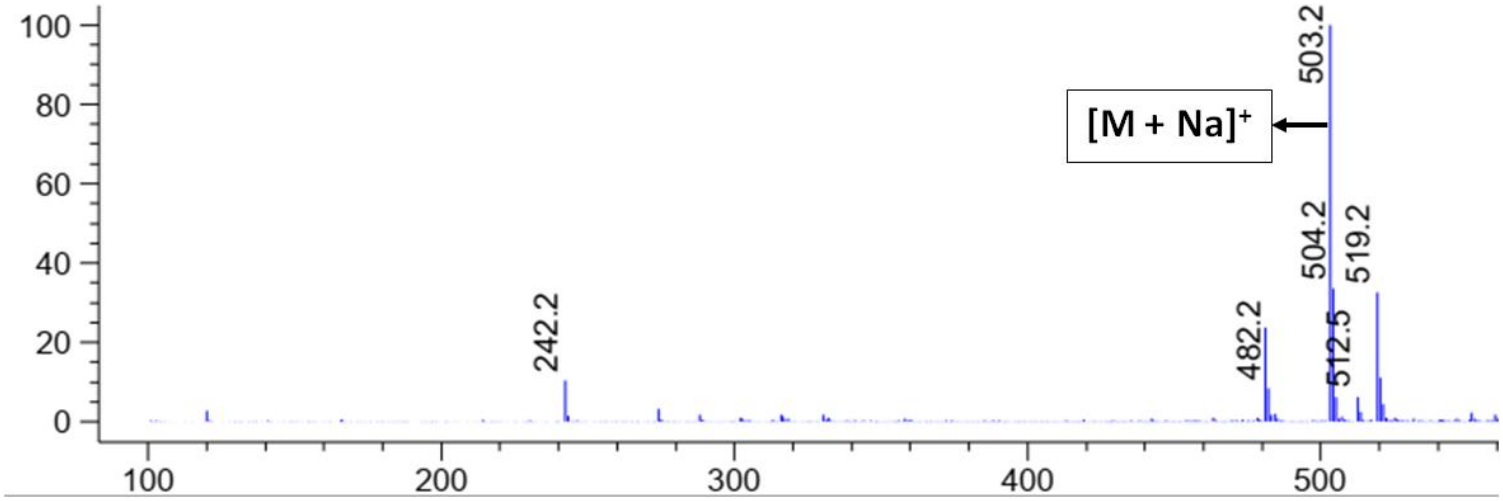


Figure S16: - Mass Spectra of Hydrogelator- **V**