Electronic Supplementary Information

A Tripeptide-based Self-Shrinking Hydrogel for Waste-Water Treatment: Removal of Toxic Organic Dyes and Lead (Pb^{2+}) Ions

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Instrumentation:

NMR experiments: All NMR studies were carried out on a Brüker DPX 300 MHz and Brüker DPX 500 MHz spectrometer at 300 K. Compounds concentrations were in the range 1–10 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.

Field emission scanning electron microscopic (FESEM) study: FE-SEM experiments were performed by placing a small portion of gel 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 Jeol Scanning Electron Microscope JSM-6700F.

FT-IR Spectroscopy: FT-IR spectroscopy was performed using Nicolate 380 FT-IR spectrophotometer (Thermo Scientific). A reported FT-IR spectrum was taken using the spectroscopic cell with CaF_2 window.

Small Angle X-Ray Scattering (SAXS): SAXS Measurements were performed using aBrukerNanostar instrument using CuKα radiation and a Vantec 2000 detector. The sample-to-detectordistance was 1.07 m. The $q = 4\pi\sin\theta/\lambda$ (scattering angle 2θ) scale was calibrated using silver behenate. Samples were mounted in quartz capillaries.

Powder X-ray diffraction study: X-ray diffraction measurements on the xerogel were carried out by placing the sample on a glass plate. Experiments were carried out by using an X-ray diffractometer (Bruker D8 Advance) with a parallel beam optics attachment. The instrument was operated at a 35 kV voltage and 30 mA current using Ni-filtered CuKα radiation and the instrument was calibrated with a standard silicon sample before use. Samples were scanned from 2º to 30º (2θ) in the step scan mode (step size 0.03°, preset time 2s) and diffraction patterns were recorded using a scintillation scan detector.

Atomic Absorption Spectroscopy (AAS): Atomic absorption spectroscopic measurements were carriedout using a Shimadzu AA-6300AAS spectrometer fitted with a double beam monochromator. AMetrohm 861 Advanced Concept IC ion chromatograph wasused for the determination of the concentrations of Pb^{2+} ions in expelled solution and in shrinkage gel.

UV/Vis spectroscopy: UV/Vis absorption spectra were recorded on a hewlett-packard (model 8453) UV/Vis spectrophotometer (Varian carry 50.bio).

ANS Fluorimetric analysis: Fluorescence studies of fresh and aged hydrogels in a sealed cuvette were carried out in a Horiba Fluoromax 4 Fluorescence Spectrometer instrument. All the experiments were carried out with the excitation slit width 2 nm and emission slit width 2 nm. The excitation wavelength was fixed at 365 nm.

Elemental Mapping: Elemental mapping of Pb(element)before and after the shrinkage of the hydrogel were carried out in Jeol JSM-6700F Scanning Electron Microscope (SEM) and in JEOL JEM-2100 Transmission Electron Microscope (operated at a maximum accelerating voltage of 200 kV).

X-ray Photoelectron Spectroscopic (XPS) Study:XPS analysis of Pb^{2+} ion containing shrunken hydrogelwas carried out by using an X–ray photoelectron spectroscopic (XPS, Omicron, model: 1712–62–11) method. The measurement was done by using an Al-Kα radiation source under 15 kV voltages and 5 mA current.

Materials:

L-Phenylalanine (Phe) and Myristic acid (C_{14}) were purchased from Aldrich. HOBt (1-hydroxybenzotriazole) and DCC (N, N'-Dicyclohexylcarbodiimide) were purchased from SRL, India.

Methods:

The peptide amphiphile **MF** was synthesized by conventional solution phase methods using racemization free fragment condensation strategy. The C-terminus was protected as a methyl ester. Couplings were mediated by DCC/HOBt. All compounds were purified by column chromatography using silica gel (100-200 mesh size) as stationary phase and chloroform and ethyl acetate as eluent. Finally, compounds were characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometry.

Peptide Synthesis.

1) C_{14}-Phe-COOMe: Myristic acid (4.56 g, 20 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Phe-OMe was isolated from the corresponding methyl ester hydrochloride (8.62 g, 40 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 10 mL. Then it was added to the reaction mixture, followed immediately by DCC (4.12 g, 20 mmol) and HOBt (3.06 g, 20 mmol). The reaction mixture was stirred for three days. The reaction mixture was taken in ethyl acetate (60 mL) and

the DCU (dicyclohexylurea) was filtered off. The organic layer was washed with 1M HCl (3×50 mL), brine (2×50 mL), 1M sodium carbonate (3×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulphate and evaporated in vacuum to yield peptide as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform and ethyl acetate as eluent.

Yield: 6.4 g, (16.5 mmol, 82.5 %).

^{1}H NMR (300 MHz, $CDCl_3$, TMS, 25 °C): δ 7.27-7.04 (Aromatic Hs, 5H, m), 5.90-5.87 (NH, 1H, d, *J* = 7.5 Hz), 4.90-4.84 ($C^{\alpha}H$, 1H, q), 3.70 (OCH_3, 3H, s), 3.16-3.02 ($C^{\beta}H$, 2H, m), 2.16-2.11 ($^{\alpha}CH_2$, 2H, t, *J* = 7.5Hz), 1.56-1.52 ($^{\beta}CH_2$, 2H, m), 1.22 ($10CH_2$, 20H, m), 0.87-0.82 (CH_3, 3H, t, *J* = 6.4Hz).^{13}C NMR (75 MHz, $CDCl_3$, TMS, 25 °C): δ 177.31, 172.79, 172.20, 135.86, 134.30, 129.25, 128.54, 127.10, 52.90, 52.32, 37.90, 36.54, 31.92, 29.64, 29.46, 29.35, 29.32, 29.20, 25.56, 24.80, 22.68, 14.12. HRMS (m/z): 390.2827 $(M+H)^+$, 412.2621$(M+Na)^+$.

2) C_{14}-Phe-COOH:C_{14}-Phe-COOMe (6.4 g, 16.5 mmol) was dissolved in MeOH (20 mL) and then 1M NaOH (10 mL) was added. The reaction mixture was stirred and the progress of saponification was monitored by thin layer chromatography (TLC). After 10 h methanol was removed under vacuum, the residue was taken in 50 mL of water, washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were dried over anhydrous sodium sulphate, and evaporated in vacuum to yield as a white solid sample.

Yield: 5.62 g, (15 mmol, 90.9 %).

^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ 12.58 (COOH, 1H, s), 8.09-8.06 (NH, 1H, d, *J* = 8.2Hz), 7.28-7.15 (Aromatic Hs, 5H, m), 4.46-4.38 ($C^{\alpha}H$, 1H, m), 3.08-2.78 ($C^{\beta}H$, 2H, m), 2.04-2.00 ($^{\alpha}CH_2$, 2H, t, *J* = 7.26 Hz), 1.39-1.34 ($^{\beta}CH_2$, 2H, t, *J* = 7.07 Hz), 1.24-1.10 (10 CH_2, 20H, m), 0.87-0.82 (CH_3, 3H, t, *J* = 6.57 Hz). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 173.79, 172.68, 138.34, 129.60, 128.60, 126.82, 53.78, 37.30, 35.62, 31.88, 29.64, 29.60, 29.50, 29.38, 29.30, 29.02, 25.74, 22.67, 14.50. HRMS: (m/z) 376.4160 $(M+H)^+$, 398.4019 $(M+Na)^+$.

3) C_{14}-Phe-Phe-COOMe:C_{14}-Phe-COOH(5.62 g, 15 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Phe-OMe was isolated from the corresponding methyl ester hydrochloride (6.46 g, 30 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 10 mL. Then it was added to the reaction mixture, followed immediately by DCC (3.09 g, 15 mmol) and HOBt (2.29 g, 15 mmol). The reaction mixture was stirred for three days. The reaction mixture was taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 1M HCl (3×50 mL), brine (2×50 mL), 1M

sodium carbonate (3×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulphate and evaporated in vacuum to yield peptide as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform and ethyl acetate as eluent.

Yield: 4.82 g, (9 mmol, 60 %).

^{1}H NMR (300 MHz, $CDCl_3$, TMS, 25 °C): δ 7.28-6.96 (Aromatic Hs, 10H, m), 6.32 (NH, 1H, s), 6.06-6.05 (NH, 1H, d, *J* = 6.0 Hz), 4.78-4.72 ($C^{\alpha}H$, 1H, q), 4.70-4.65 ($C^{\alpha}H$, 1H, q), 3.70 (OCH_3, 3H, s), 3.14-2.98 ($C^{\beta}H$, 4H, m), 2.20-2.10 ($^{\alpha}CH_2$, 2H, m), 1.55-1.54 ($^{\beta}CH_2$, 2H, m), 1.34-1.26 ($10CH_2$, 20H, m), 0.92-0.89 (CH_3, 3H, t, *J* = 6.75 Hz).^{13}C NMR (75 MHz, $CDCl_3$, TMS, 25 °C): δ 173.23, 171.40, 170.68, 136.57, 135.75, 129.46, 129.31, 128.77, 128.70, 127.26, 127.15, 54.21, 53.56, 52.43, 38.19, 38.01, 36.68, 32.06, 29.79, 29.59, 29.49, 29.47, 29.31, 25.68, 22.82, 14.24. HRMS: (m/z) 537.6808 $(M+H)^+$, 559.6556 $(M+Na)^+$, 575.6509 $(M+K)^+$.

4) C_{14}-Phe-Phe-COOH:C_{14}-Phe-Phe-COOMe(4.82 g, 9 mmol) was dissolved in MeOH (20 mL) and then 1M NaOH (10 mL) was added. The reaction mixture was stirred and the progress of saponification was monitored by thin layer chromatography (TLC). After 10 h methanol was removed under vacuum, the residue was taken in 50 mL of water, washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were dried over anhydrous sodium sulphate, and evaporated in vacuum to yield as a white solid sample.

Yield: 3.39 g, (6.5 mmol, 72.2 %).

^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ 12.74 (COOH, 1H, s), 8.20-8.17 (NH, 1H, d, *J* = 7.5 Hz), 7.93-7.90 (NH, 1H, d, *J* = 8.4 Hz), 7.29-7.10 (Aromatic Hs, 10H, m), 4.55-4.44 ($C^{\alpha}H$, 2H, m), 3.06-2.67 ($C^{\beta}H$,4H, m), 1.99-1.94 ($^{\alpha}CH_2$, 2H, t, *J* = 7.23 Hz), 1.33-1.03 ($11CH_2$, 22H, m), 0.88-0.83 (CH_3, 3H, t, *J* = 6.57 Hz).^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 173.41, 173.26, 172.49, 172.38, 172.06, 171.79, 138.59, 138.52, 137.94, 129.83, 129.70, 128.72, 128.42, 126.98, 126.62, 53.94, 37.98, 35.74, 31.87, 29.63, 29.60, 29.46, 29.38, 29.29, 29.00, 25.72, 22.67, 14.51. HRMS: (m/z) 523.4360 $(M+H)^+$, 545.3513 $(M+Na)^+$,561.3912 $(M+K)^+$.

5) C_{14}-Phe-Phe-Phe-COOMe:C_{14}-Phe-Phe-COOH (5.62 g, 15 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Phe-OMe was isolated from the corresponding methyl ester hydrochloride (6.46 g, 30 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 10 mL. Then it was added to the reaction mixture, followed immediately by DCC (3.09 g, 15 mmol) and HOBt (2.29 g,

15 mmol). The reaction mixture was stirred for three days. The reaction mixture was taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 1M HCl (3×50 mL), brine (2×50 mL), 1M sodium carbonate (3×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulphate and evaporated in vacuum to yield peptide as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform and ethyl acetate as eluent.

Yield: 4.82 g, (7.05 mmol, 47 %).

^{1}H NMR (500 MHz, $CDCl_3$, TMS, 25 °C): δ 7.28-7.00 (Aromatic Hs, 15H, m), 6.42-6.40 (NH, 1H, d, *J* = 7.6 Hz), 6.26-6.24 (NH, 1H, d, *J* = 7.6 Hz), 5.84-5.82 (NH, 1H, d, *J* = 7.6 Hz), 4.74-4.70 ($C^{\alpha}H$, 1H, q), 4.59-4.52 ($C^{\alpha}H$, 2H, m), 3.68 (OCH_3, 3H, s), 3.10-2.95 ($C^{\beta}Hs$, 6H, m), 2.07-2.02 ($^{\alpha}CH_2$, 2H, m), 1.49-1.44 ($^{\beta}CH_2$, 2H, m), 1.32-1.25 ($10CH_2$, 20H, m), 0.89-0.86 (CH_3, 3H, t, *J* = 6.4 Hz).^{13}C NMR (125 MHz, $CDCl_3$, TMS, 25 °C): δ173.47, 171.49, 170.95, 170.04, 136.53, 136.36, 135.93, 129.41, 129.34, 128.93, 128.77, 128.73, 127.28, 127.16, 54.55, 54.20, 53.57, 52.45, 37.97, 37.74, 36.62, 32.07, 29.83, 29.80, 29.78, 29.61, 29.50, 29.47, 29.32, 25.60, 22.83, 14.25. HRMS: (m/z) 684.6069 $(M+H)^+$, 706.5923 $(M+Na)^+$, 722.5646 $(M+K)^+$.

6) C_{14}-Phe-Phe-Phe-COOH: C_{14}-Phe-Phe-Phe-COOMe(4.82 g, 7.05 mmol) was dissolved in MeOH (20 mL) and then 1M NaOH (10 mL) was added. The reaction mixture was stirred and the progress of saponification was monitored by thin layer chromatography (TLC). After 10 h methanol was removed under vacuum, the residue was taken in 50 mL of water, washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were dried over anhydrous sodium sulphate, and evaporated in vacuum to yield as a white solid sample.

Yield: 3.39 g, (5.06 mmol, 71.7 %).

^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ 12.70 (COOH, 1H, s), 8.29-8.27 (NH, 1H, m), 7.96-7.81 (NH, 1H, m), 7.28-7.02 (Aromatic Hs, 15H, m), 4.54-4.43 ($C^{\alpha}H$, 3H, m), 3.09-2.37 ($C^{\beta}H$, 6H, m), 1.95-1.93 ($^{\alpha}CH_2$, 2H, t, *J* = 7 Hz), 1.30-1.02 ($12CH_2$, 24H, m), 0.86-0.83 (CH_3, 3H, t, *J* = 6.75 Hz): ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C): δ 172.59, 172.56, 171.97, 171.94, 171.10, 171.03, 170.76, 138.00, 137.85, 137.70, 137.48, 137.38, 137.32, 129.31, 129.21, 129.06, 128.15, 127.92, 127.88, 127.82, 127.74, 126.38, 126.15, 126.00, 53.50, 37.67, 37.53, 37.23, 36.73, 35.16, 35.09, 31.24, 28.98, 28.96, 28.80, 28.73, 28.64, 28.40, 28.38, 25.07, 25.02, 22.03, 13.89. HRMS: (m/z) 692.1702 $(M+Na)^+$.

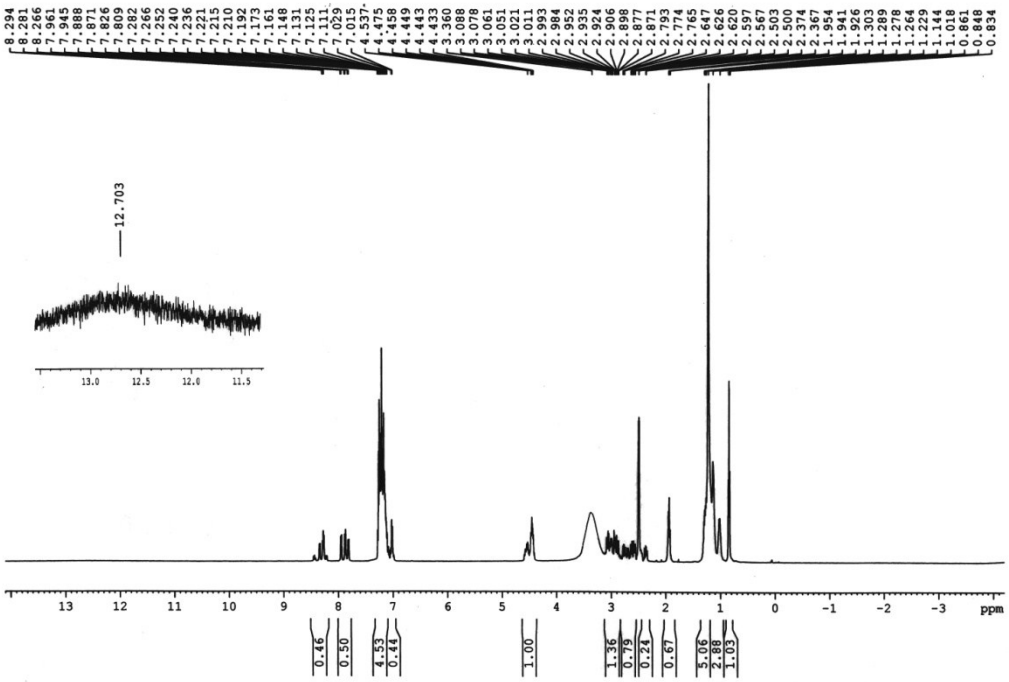

Fig. S1: ^{1}H-NMR spectrum of gelator peptide **MF**.

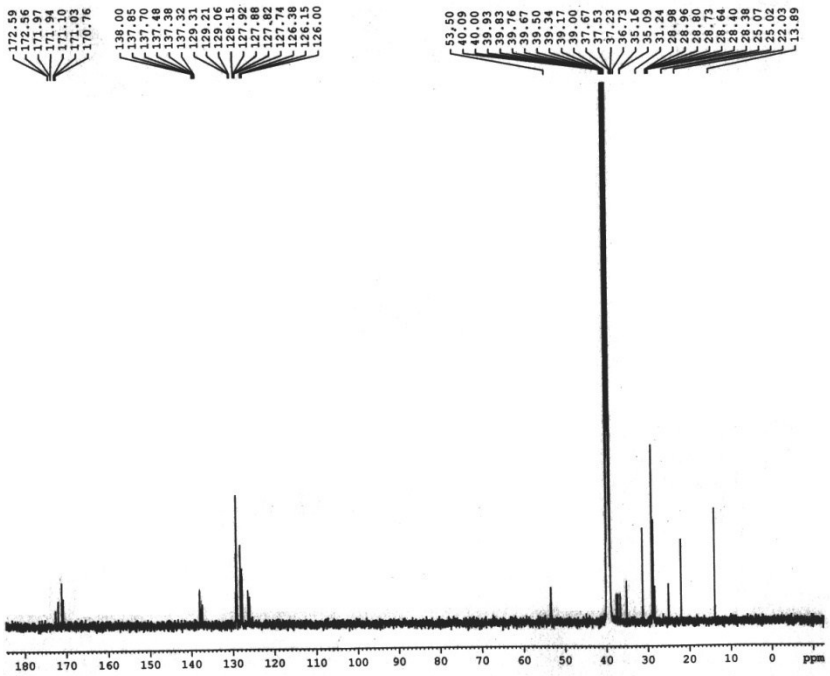

Fig. S2: ^{13}C-NMR spectrum of gelator peptide **MF**.

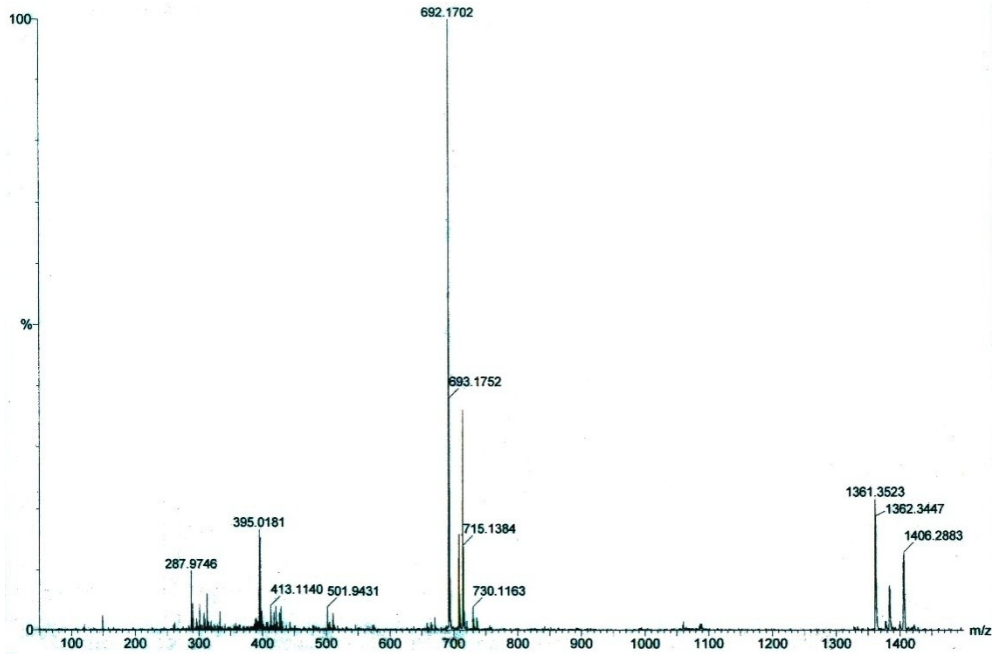

Fig. S3: Mass spectrum of gelator peptide **MF**.

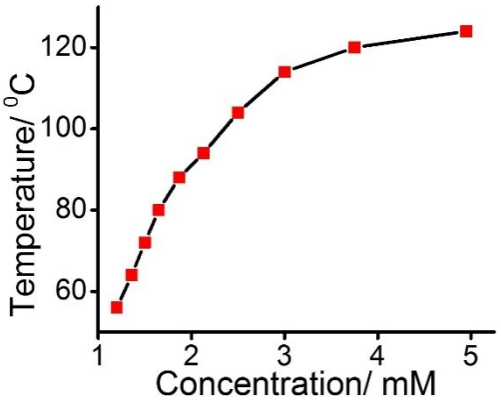

Fig. S4: T_{gel} plot obtained from the hydrogel showing high melting temperature at higher concentration.

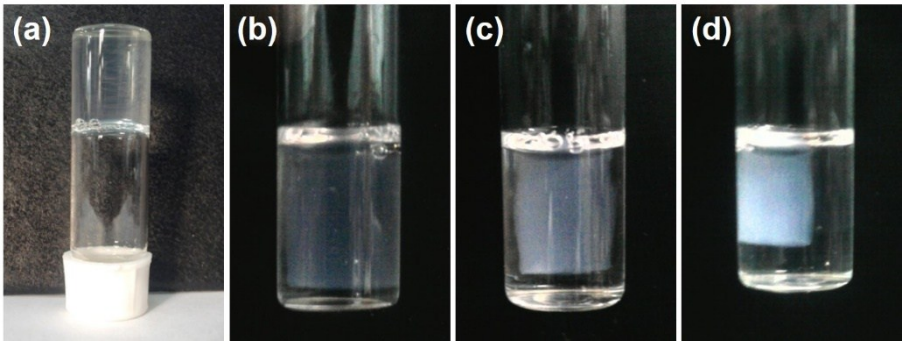

Fig. S5: Image showing thevial pictures of the hydrogel obtained from **MF**at different time intervals[(a) 0 hour, (b) 1 day, (c) 4 days, (d) 7 days]during syneresis process.

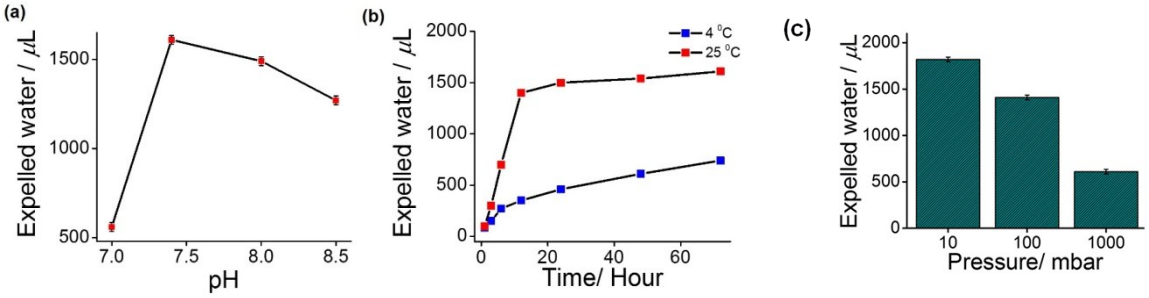

Fig. S6: (a) Amount of expelled water with pH shows the dependency of syneresis on pH of the solution. (b) Amount of expelled water with time at low temperature (4 ^{0}C) and room temperature (25^{0}C) by the hydrogel obtained from gelator **MF**. (c) Amount of expelled water at different pressure. (10, 100 and 1000 mbar in a vacuum apparatus with a continuous flow of Argon)

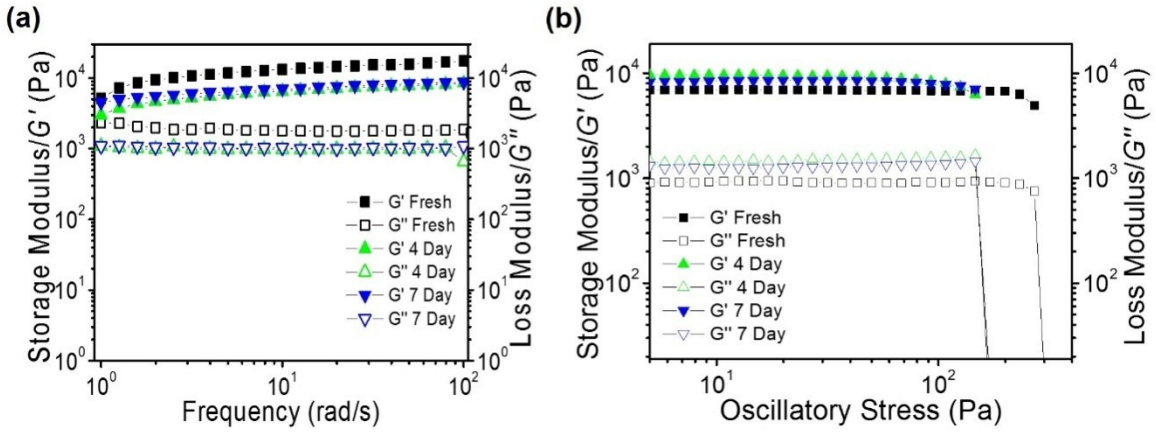

Fig. S7: (a) Modulus frequency sweep experiment of hydrogels (fresh, 4-day and 7-day aged gels) at 2.25 mM concentration (at 25 ^{0}C). (b)Dynamicshear rheology of fresh, 4-day and 7-day aged hydrogels illustrates oscillatory stress sweep experiment at 25 °C. Concentration in the hydrogel was initially 2.25 mM.

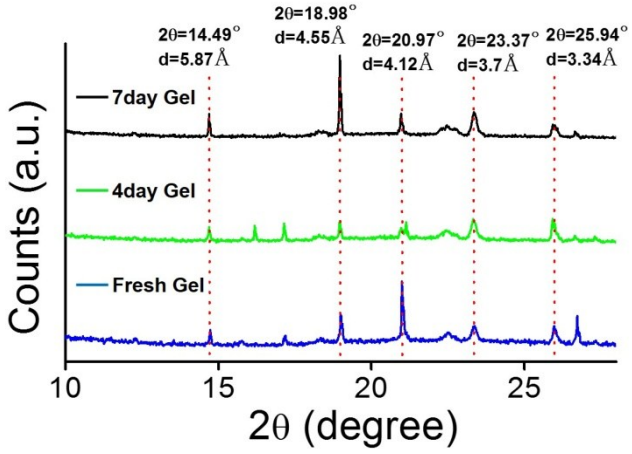

Fig. S8: Wide angle powder XRD of xerogels obtained from gels at different time intervals during syneresis.

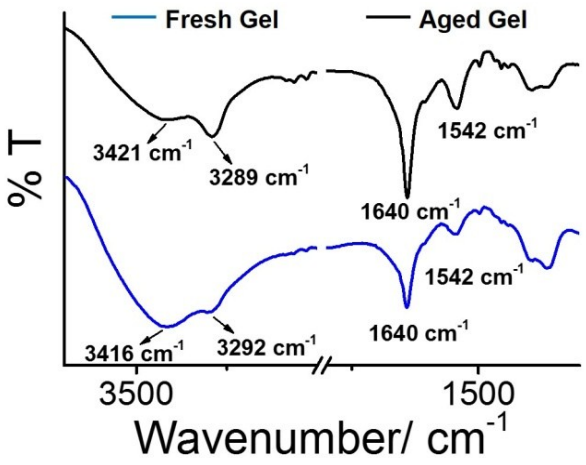

Fig. S9: FTIR spectra from xerogels obtained from freshly prepared and 7-day aged gel.

Fig. S10: Image showing removal of Pb^{2+} ions (0.33 mM) by the hydrogel during syneresis process, where $Pb(CLO_4)_2$used as a Pb^{2+} ion source.

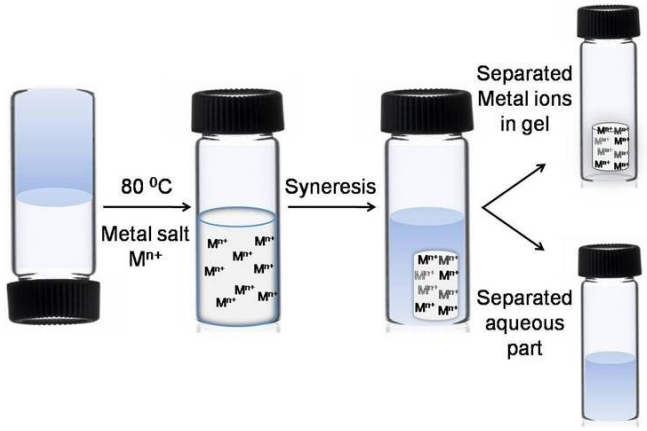

Fig. S11: A schematic diagram showing the removal of toxic heavy metal ion Pb^{2+} by the hydrogel. M^{n+} refers to the Pb^{2+} ions.

Table S1: The maximum capacity of removal toxic dyes and Pb^{2+} ions by the hydrogel.

Pollutant	**λ_{max} value of the dye solution (only)**	**Maximum load Capacity per gm gelator (1.5 mM hydrogel)**	**Maximum % of dye/ metal ion absorption by 1.5 mM hydrogel**
Methylene blue	650 nm	420 mg (1.31 mmol)	99.9
Brilliant blue	595 nm	120 mg (0.14 mmol)	99.8
Pb^{2+}	-	1995 mg (9.63 mmol)	98.4

Fig. S12: Image showing removal of toxic substance mixtureaided by syneresis process: Pb^{2+} ions (0.33 mM), along with Brilliant blue (0.005mM) and Methylene blue (0.005 mM) dyes. $Pb(NO_3)_2$ is used as a Pb^{2+} ion source.

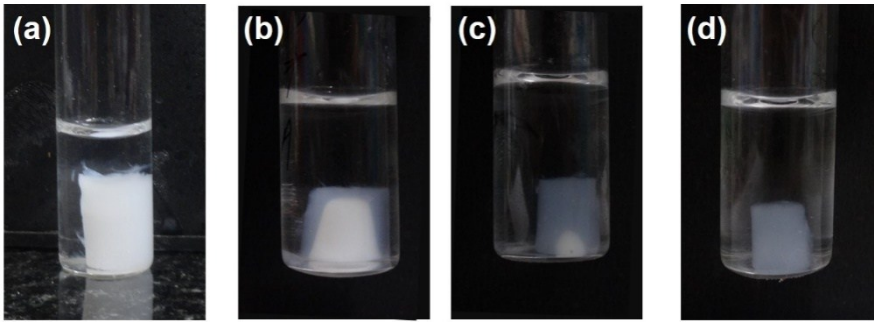

Fig. S13: Image showing Pb^{2+} containing shrunken hydrogel (1.5 mM concentration) at different time interval [(a) 0 hour, (b) 8 hours, (c) 20 hours, (d) 24 hours] duringtreatment with saturated EDTA-Na_2 solution.

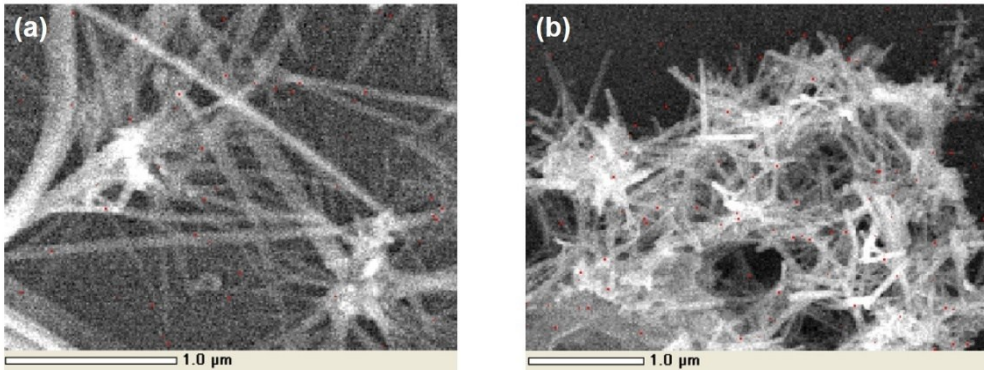

Fig. S14: FE-SEM images show the elemental mapping of Pb(element) (Red mark) within gel network (c) before and (d) after shrinkage of lead containing hydrogel.

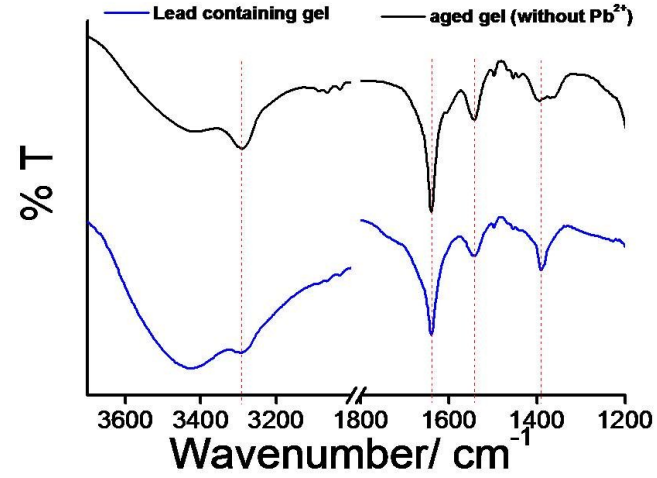

Fig. S15: FTIR spectra from xerogels obtained from 7-day aged gel with (blue line) and without Pb^{2+} ions (black line).

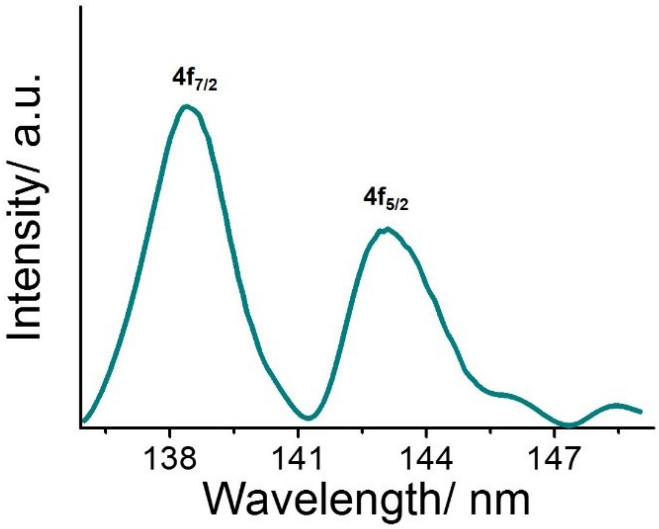

Fig. S16: XPS analysis of Pb^{2+} ion containing hydrogel. The binding energy peaks at 138.43 and 143.07 eV corresponds to $4f_{7/2}$ and $4f_{5/2}$ state respectively confirming the presence of hydroxide of Pb^{2+}.

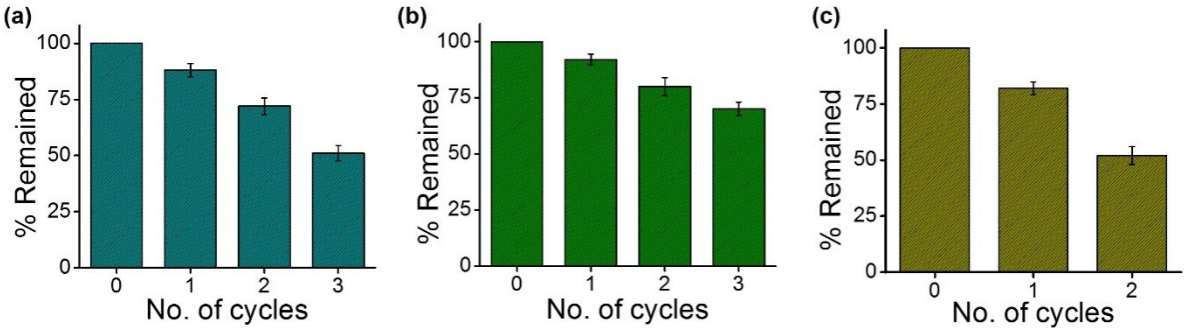

Fig. S17: Graphs showing recyclability. Amount of recoveredgelator from the hydrogels containing (a) Brilliant blue, (b) Methylene blue and (c) Pb^{2+} ions.