Electronic Supplementary Information

Amino Acid-based Amphiphilic Hydrogels: Metal Ion Induced Tuning of Mechanical and Thermal Stability

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Experimental Section

All NMR studies were carried out on a Bruker spectrometer 400 MHz 300 K using DMSO-d_6 solvent. Mass spectroscopy was performed on Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS spectrometer. MALDI-TOF MS analysis for metal complex solutions has been performed by using Bruker microFlex MALDI-TOF analyzer and α-Cyano-4-hydroxycinnamic acid as a matrix. FTIR spectra of freeze dried hydrogels were recorded using a Bruker ALPHA FTIR spectrometer equipped with a diamond ATR. Transmission Electron Microscopy (TEM) of hydrogels were studied using Hitachi 7500 transmission EM. Field emission scanning electron microscopy (FESEM) experiment was performed using LEO (Zeiss) 1540XB SEM. Rheological measurements were carried out using a TA Instruments DHR-1 rheometer. Gel melting temperature determination was performed by heating gels in an oil bath fitted with a thermometer at a heating rate of 2 °C/ 5 minute until the gel melted. The calculated error range in T_{gel} determination was found to be ±1 °C. Circular Dichroism (CD) spectra were recorded with a JASCO J-810 spectrometer using a 1 mm path-length cell. All CD experiments were carried by diluting 16 mM hydrogels at 25 °C.

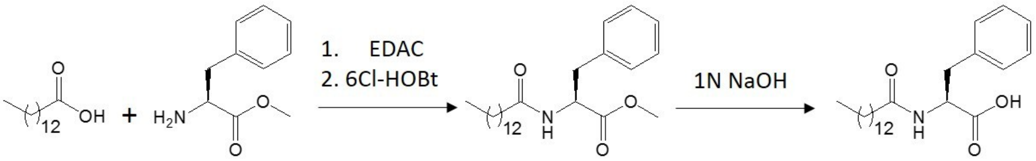
Synthesis Procedure: H-Phe-OMe.HCl, EDAC and 6Cl-HOBt were purchased from Matrix Innnovation. Whereas, Myristic acid (C_{14}) and Triethyl amine (TEA) were purchased from Sigma Aldrich. The compound was synthesized (Scheme S1) by conventional solution-phase methodology using racemisation-free fragment condensation strategy.

C_{14}-Phe-OMe: In a round bottom flask 10 mmol (2.28 g) of Myristic acid (C_{14}) was dissolved in 100 mL of DCM in an ice bath. To the cooled mixture, 20 mmol of EDAC.HCl was added following the addition of 20 mmol of 6Cl-HOBt. Excess of TEA was added to neutralize the HCl. In another round bottom flask, 15 mmol of H-Phe-OMe.HCl was dissolved in 50 mL DCM, excess of TEA was added and stirred for 30 mins. Excess solvent was evaporated under reduced pressure until a slurry was obtained. This slurry was added to above mentioned solution and stirred at room temperature for 24 hrs. After 24 hrs, the solution was washed with saturated 10 % citric acid, saturated sodium bicarbonate and brine solution respectively. The resulting solution was dried over anhydrous Na_2SO_4 and evaporated in vacuum. A white material was obtained. Compound was further purified using flash column chromatography with hexane-ethylacetate as an eluent. Yield: 2.8 g (7.19 mmol, 71 %)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27-7.04 (5H, Aromatic Hs, m), 5.90-5.87 (1H, NH, d, *J* = 7.5), 4.91-4.84 (1H, $^{\alpha}$H, m), 3.70 (3H, OCH_3, s), 3.16-3.02 (2H, $^{\beta}$H, m), 2.16-2.11 (2H, $^{\alpha}CH_2$, t, *J* = 7.5), 1.57-1.52 (2H, $^{\beta}CH_2$, m), 1.22 (20H, $10CH_2$, m), 0.87-0.82 (3H, CH_3, t, *J* = 6.4). ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.31, 172.79, 172.20, 135.87, 134.31, 129.25, 128.55, 127.11, 52.91, 52.32, 37.90, 36.54, 31.92, 29.65, 29.46, 29.35, 29.32, 29.20, 25.56, 24.80, 22.69, 14.12. Anal. Calcd. for $C_{24}H_{39}NO_3$ (389.2929): MS (ESI) m/z 390.2827 $[M+H]^+$.

C_{14}-Phe-COOH (1): To 2.8 g (7.1 mmol) of C_{14}-Phe-OMe, 50 mL MeOH and 25 mL 1N NaOH were added a nd the progress of saponification was monitored by thin layer chromatography (TLC). After 10 hr, methanol was removed under vacuum, the residue was taken in 50 ml of water, washed with diethylether (2 × 50 ml). Then the pH of the aqueous layer was adjusted to 2 using 1N HCl and it was extracted with ethyl acetate (3 × 50 ml). The extracts were pooled, dried over anhydrous Na_2SO_4 and evaporated in vacuum. A white solid material was obtained. Yield: 2.4 g (6.5 mmol, 91 %)

^1H NMR (400 MHz, DMSO-d6) δ 12.65 (1H, s, COOH), 8.12-8.10 (1H, d, *J* = 8.2 Hz, NH), 7.29-7.17 (m, 5H, Aromatic), 4.46-4.40 (1H, m, $^{\alpha}H$), 3.08-2.81 (2H, m, $^{\beta}H$), 2.05-2.01 (2H, t, *J* = 7.3, $^{\alpha}CH_2$), 1.40-1.34 (2H, t, J = 7.3, $^{\beta}CH_2$), 1.30-1.08 (20H, m, $10CH_2$), 0.88-0.85 (3H, m, CH_3). ^{13}C NMR (101 MHz, DMSO-d6) δ 173.21, 172.09, 137.75, 129.02, 128.03, 126.25, 53.20, 40.11, 39.90, 39.69, 39.48, 39.27, 39.06, 38.85, 36.72, 35.04, 31.29, 29.05, 29.02, 28.92, 28.79, 28.72, 28.43, 25.16, 22.09, 13.93. Anal. Calcd. for $C_{23}H_{37}NO_3$ (375.2773): MS (ESI) m/z 376.4160 $[M+H]^+$. Anal. Calcd. C, 73.56; H, 9.93; N, 3.73; Obtained, C, 73.46; H, 9.93; N, 3.71.



Scheme. S1 The general synthesis of the gelator **1**.

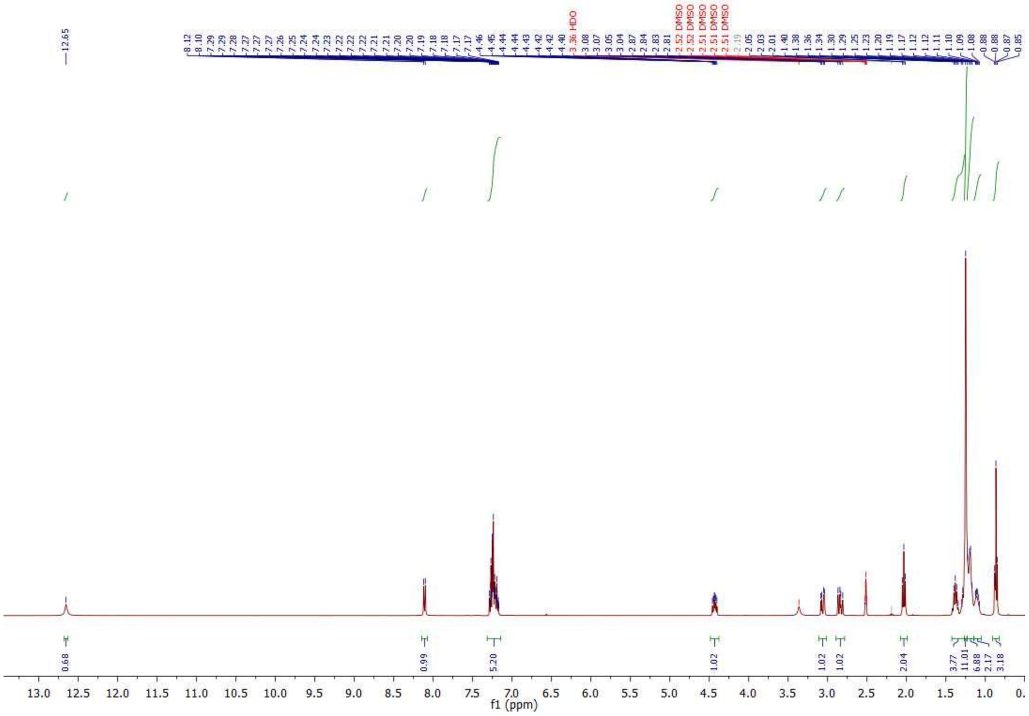


Fig. S1 ^{1}H NMR spectrum of the compound **1** in DMSO-d6.

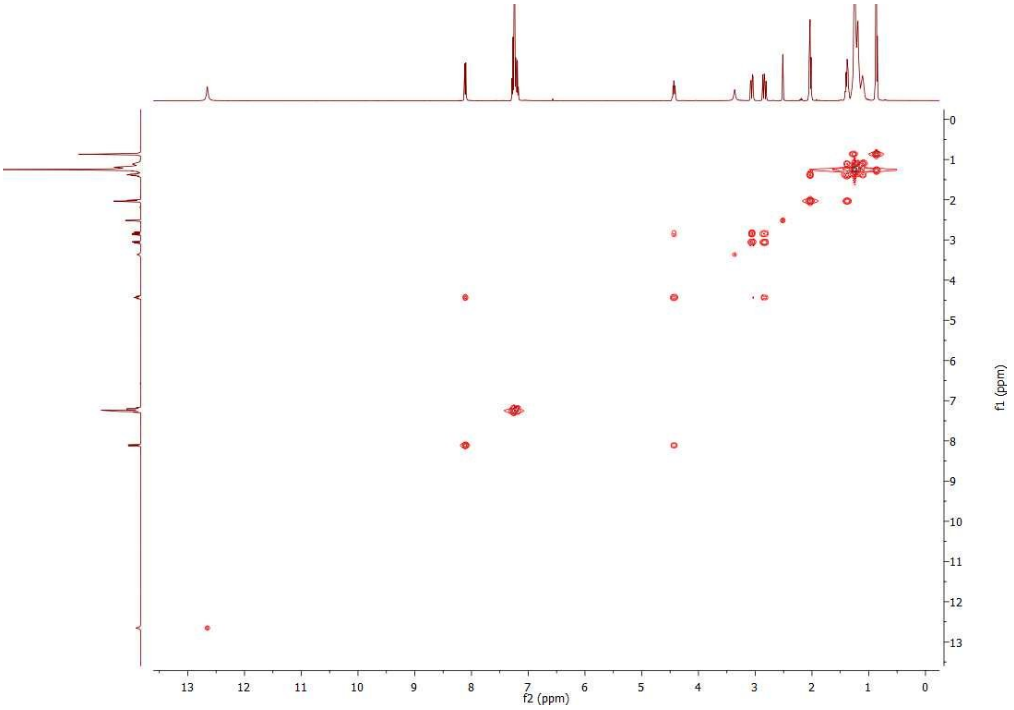


Fig. S2 ^{1}H-^{1}H COSY of spectra of the compound **1** in DMSO-d6.

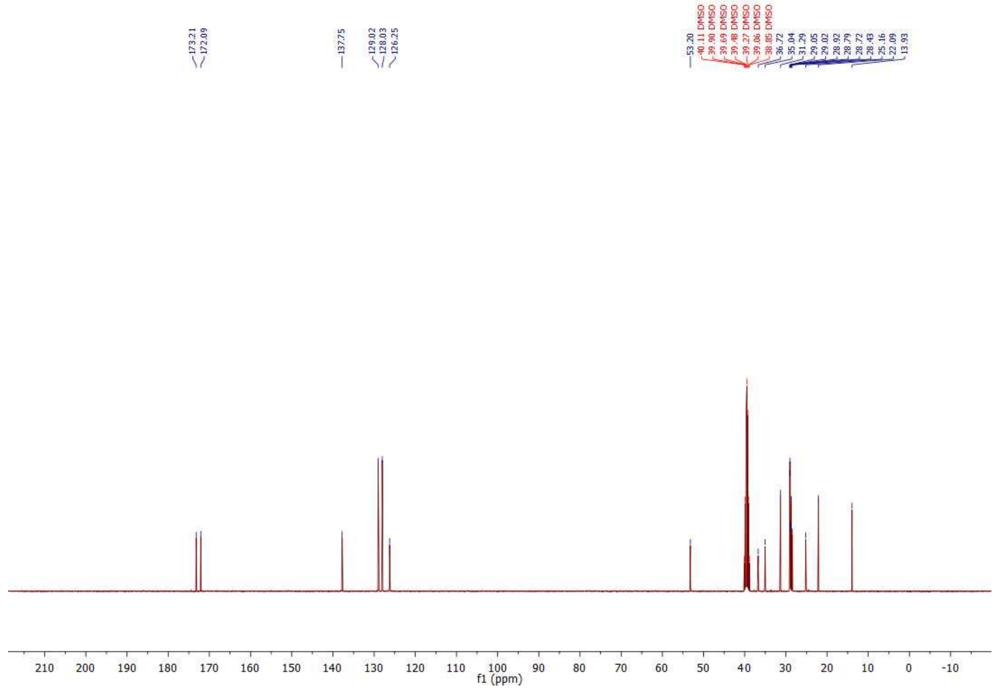


Fig. S3 ^{13}C spectrum of the compound **1** in DMSO-d6.

Table. S1 The hydrogel forming ability, T_{gel} and mechanical strength of native and metallo-hydrogels.

Hydrogels	**Gelation Time / hour**	**MGC / mM**	**T_{gel} / °C**	**G' / Pa***
1	≈ 24	16	41	1.30553
1+**Ca^{2+}**	0.5	6	50	1971.94
1+**Co^{2+}**	≈ 4-5	8	99	11115.1
1+**Ni^{2+}**	≈ 4-5	8	98	9986.86

* avarage value of three consicutive run for one sample at 16 mM.

Table. S2 The hydrogel forming ability, T_{gel} and mechanical strength of metallo-hydrogels at varying metal ion concentration.

Gelator Concentration / mM	**Metal Ion Concentration / mM**	**Ratio**	**T_{gel} / °C**	**G′ / Pa***
8	6 (Ca^{2+})	1: 0.75	50	1.08
8	4 (Ca^{2+})	1: 0.50	50	7.43
8	2 (Ca^{2+})	1: 0.25	40	137
8	1 (Ca^{2+})	1: 0.125	38	127
8	6 (Co^{2+})	1: 0.75	98	1660
8	4 (Co^{2+})	1: 0.50	99	2630
8	6 (Ni^{2+})	1: 0.75	101	4340
8	4 (Ni^{2+})	1: 0.50	98	1590

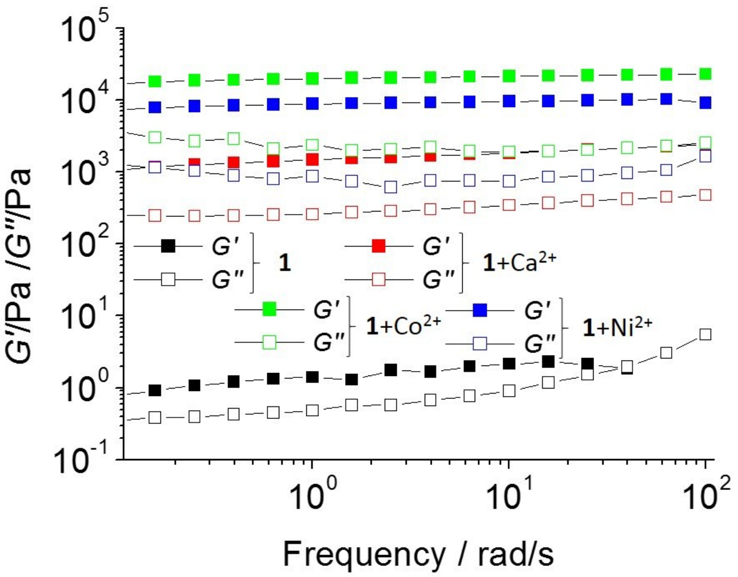


Fig. S4 Frequency sweep (from 0.1 to 100 rad/s) rheology study of native hydrogel and metallo-hydrogels (concentration was 16 mM).

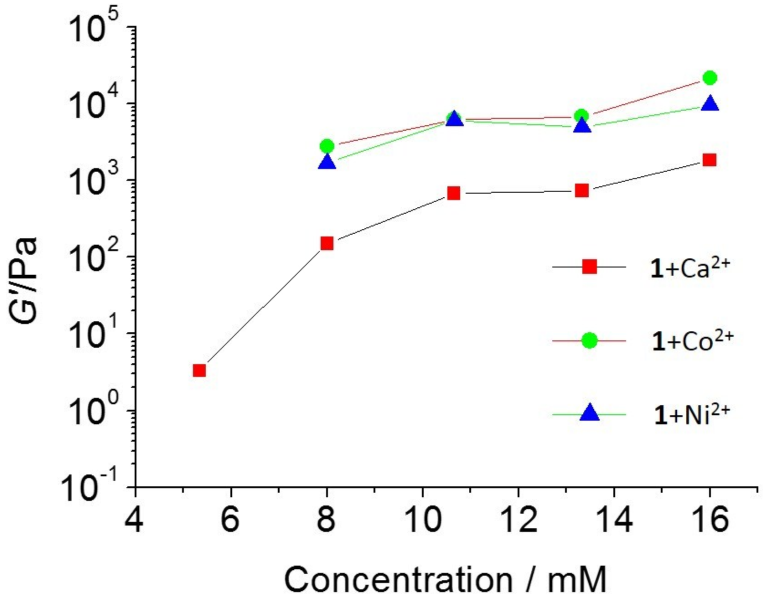


Fig. S5 G′ values (at 10 rad/s) of metallo-hydrogels obtained from concentration dependent frequency sweep rheology study.

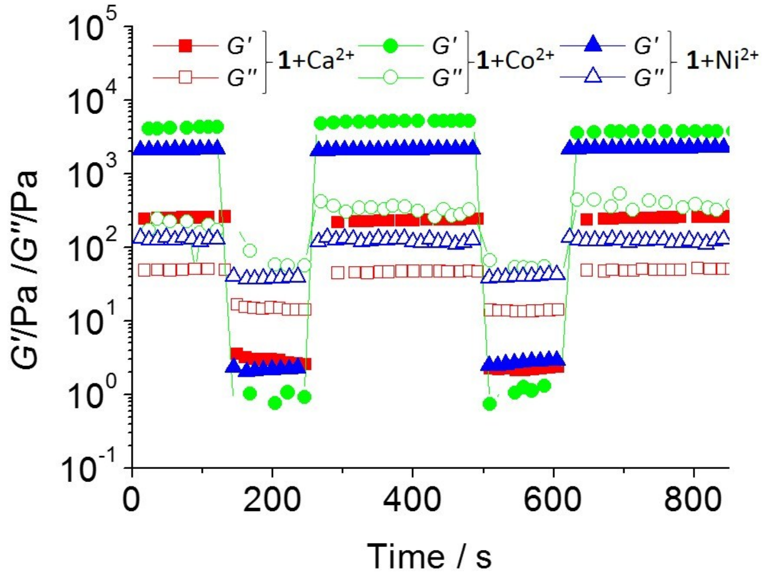


Fig. S6 Alternating strain rheology tests performed for **1**+Ca^{2+}, **1**+Co^{2+} and **1**+Ni^{2+} show metallo-hydrogels undergo shear-induced breaking and reformation at constant angular frequency of 10 rad/s. Applied strain: 0.1% (120 s) → 100% (120 s) → 0.1% (240 s) →100% (120 s) → 0.1% (240 s).

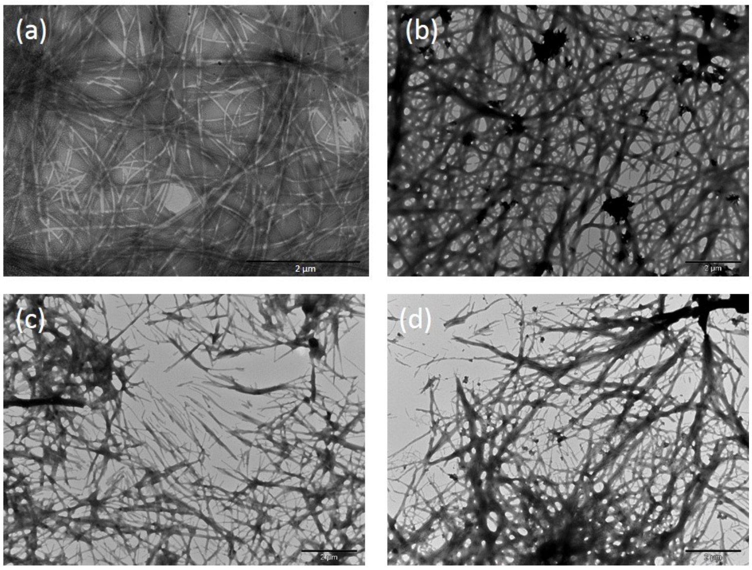


Fig. S7 TEM images of (a) native hydrogel and metallo-hydrogels obtained from (b) **1**+Ca^{2+}, (c) **1**+Co^{2+} and (d) **1**+Ni^{2+}.

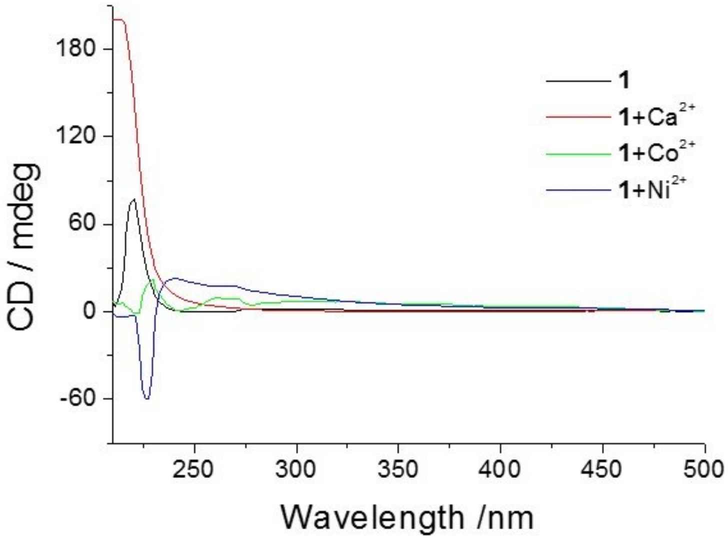


Fig. S8 CD spectra of **1**, **1**+Ca^{2+}, **1**+Co^{2+} and **1**+Ni^{2+} show supramolecular chirality. The **1**+Ca^{2+} metallo-hydrogel shows a considerable increase in peak intensity that reflects in FE-SEM image.

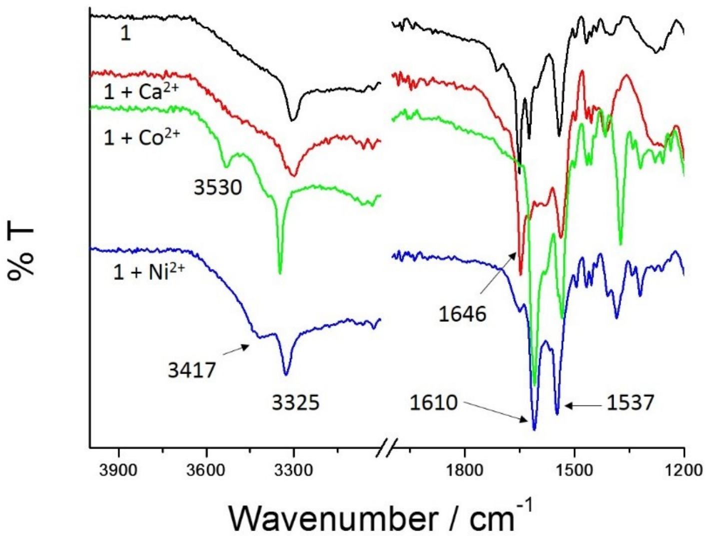


Fig. S9 FT-IR studies of freeze dried native hydrogel and metallo-hydrogels. Peaks indicate hydrogen bonding is involved in the hydrogel network. Temperature and concentration maintained at 25 °C and 16 mM respectively.

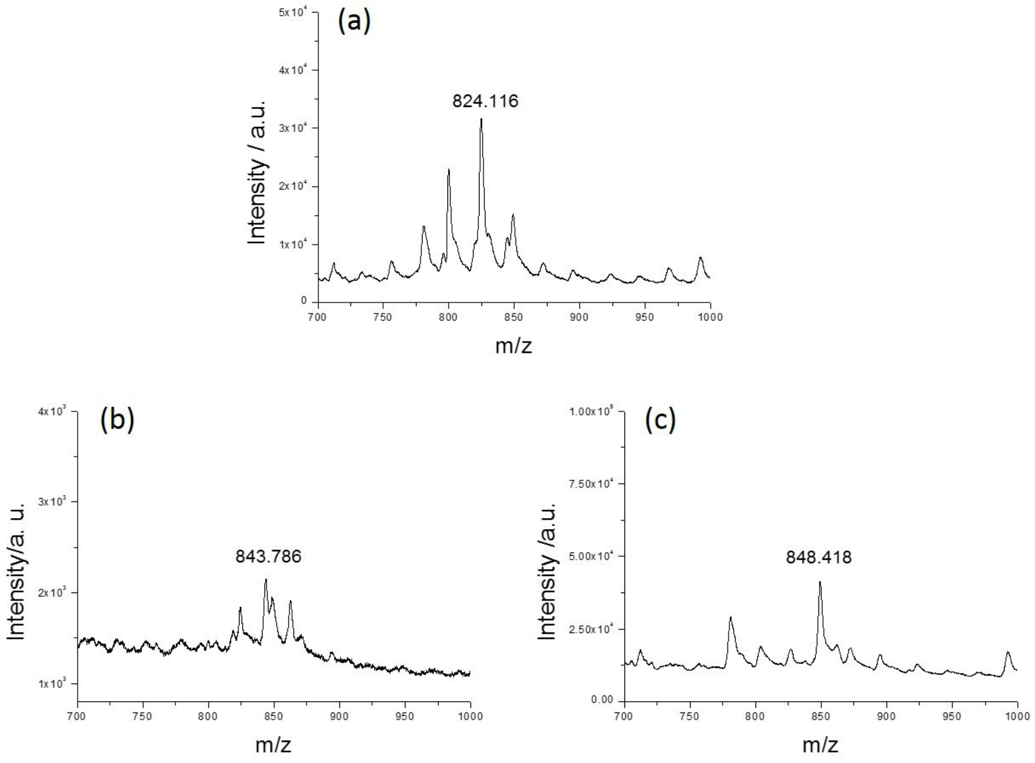


Fig. S10 MALDI-ToF MS obtained for (a) **1**+Ca^{2+} hydrogel, (b) **1**+Co^{2+} hydrogel and (c) **1**+Ni^{2+} hydrogel. For **1**+Ca^{2+} and **1**+Co^{2+} hydrogels MALDI TOF matches well with calculated mass (823.166 and

842.021 respectively) of metal complexes. However, for **1**+Ni^{2+} it is surprising and 6 unit more (calculated 841.781 Ni^{2+}).

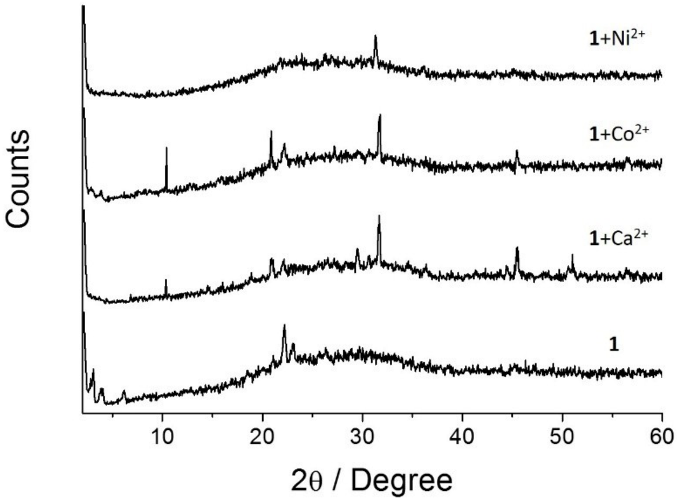


Fig. S11 Powder XRD of freeze dried samples obtained from native hydrogel and metallo-hydrogels. Metallo-hydrogels show sharp peaks in the wide angle region indicate the presence of metal hydroxides.