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pH-Responsive, Bolaamphiphile-Based Smart Metallo-Hydrogels as Potential Dye-Adsorbing Agents, Water Purifier, and Vitamin B$_{12}$ Carrier

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A protein amino acid, phenylalanine-based bolaamphiphile **1** containing a centrally located oligomethylene group has been found to be a hydrogelator at nearly physiological pH (pH 6.5−7.2) in the presence of divalent metal salts (such as MnCl$_2$, CoCl$_2$, CuSO$_4$, and NiCl$_2$) on sonication. Other structurally related synthetic bolaamphiphiles **2**−**4** do not form hydrogels under similar conditions. The metallo-hydrogels have been characterized using transmission electron microscopic (TEM) studies and FT-IR studies. TEM study of these metallo-hydrogels indicates a network of nanofibrilar morphology that might be responsible for gelation. The pH sensitivity of these metallo-hydrogels can be potentially utilized for entrapment and slow release of biologically important molecules. It has been demonstrated that one of these gels entraps and slowly releases vitamin B$_{12}$ molecules depending upon the pH of the medium. Moreover, these metallo-hydrogels can efficiently adsorb various toxic dyes including Crystal Violet and Naphthol Blue Black from water and this property can be utilized for the removal of different types of toxic dye molecules from wastewater.

Introduction

Discovery of low molecular weight hydrogels is an emerging field of current research.[1] Hydrogels formed by self-assembly of small organic molecules are exquisitely interesting due to numerous applications of hydrogels. They can be used for tissue engineering,[2] pollutant capture and removal,[3] drug delivery vehicles,[4] and other applications.[5] Low molecular weight hydrogelators are structurally diverse and over the past few years different types of molecules, such as bis-urea,[6] bis[7]- and tris[8]-amides, azo-benzene-based sugar derivatives,[9] de novo designed β-hairpin-forming oligopeptides,[10] short peptide derivatives,[11] 2′-deoxyuridine derivatives,[12] tripodal cholic acid-based compounds,[13] bile acid-based compounds,[14] peptide-based amphiphiles,[15] derivatives of vancomycin,[4] and others[16] have been discovered to be low molecular weight (LMW) hydrogelators. Low molecular weight pH-responsive hydrogelator[17] belongs to a unique class of hydrogelators in which the change of pH

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plays a vital role in modulating sol−gel phase transition and this property can be nicely exploited to develop new soft materials for encapsulation and slow release of biologically important molecules like vitamins.[17b] Pollutants including water-soluble toxic dye molecules can also be removed from wastewater using the organogel scaffold.[18] Though metal ion-sensitive organogels have been studied extensively,[19] examples of metal-ion-induced hydrogels are quite few in number.[20] Here, we present metal-ion-induced, pH-responsive hydrogel formation using amino acid-based bolaamphiphilic molecule containing phenylalanine residues and the application of these gels to remove efficiently different types of water-soluble dyes to purify contaminated water. One of these metallo-hydrogels can also trap vitamin B_{12} molecules and release slowly the vitamin molecules by changing the pH of the system and this property may be utilized to carry vitamin B_{12} molecules at the target site.

Experimental Section

General Methods and Materials. Sebacic acid, HOBt (1-hydroxy benzotriazole), and DCC (dicyclohexyl carbodiimide) were purchased from Sigma. Metal salts ($MnCl_2$, $CoCl_2$, $NiCl_2$, and $CuSO_4$) and amino acids were purchased from Merck.

Bolaamphiphile Synthesis. Bolaamphiphiles (**1**−**4**) were synthesized by coupling corresponding methyl esters of the respective L-amino acids with a long chain dicarboxylic acid (sebacic/azelaic acid) at −5 to 10 °C in dimethylformamide using dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC−HOBt) and subsequent saponification of the bisamedic diesters using 1 M NaOH. See Supporting Information for NMR and MS data for pure bolaamphiphiles.

Methyl Ester of Bis(N-α-amido-phenylalanine)-1,10-decane Dicarboxylic Acid ***5***. A solution of 1,10-decane dicarboxylic acid (sebacic acid) (1.0 g, 5 mmol) and HOBt (1.35 g, 10 mmol) in DMF was cooled in an ice-water bath. H-Phe-OMe was isolated from (4.31 g, 20 mmol) the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and concentration to 10 mL. Then this was added to the precooled solution, followed immediately by the addition of DCC (2.06 g, 10 mmol). The reaction mixture was stirred for 2 h at 0 °C and brought to room temperature gradually. The reaction mixture was taken in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3 × 50 mL), brine (2 × 50 mL), 1 M sodium carbonate (3 × 50 mL), and brine (2 × 50 mL) respectively. This washed solution was dried over anhydrous sodium sulfate and evaporated in vacuo to yield **5** as a white solid.

Yield = 2.35 g (4.5 mmol, 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.12−7.07 (m, 10H); 5.91−5.88 (d, J = 7.5 Hz, 2H); 4.93−4.86 (m, 2H); 3.73 (s, 6H); 3.19−3.04 (m, 4H); 2.18−2.13 (m, 4H); 1.64 (m, 4H); 1.59−1.52 (m, 8H). ESI-MS m/z 525.5 $[M + H]^+$, 547.3 $[M + Na]^+$, M_{calcd} = 524. Anal. Calcd for $C_{30}H_{40}N_2O_6$ (524): C, 68.70; H, 7.63; N, 5.34. Found: C, 68.7; H, 7.41; N, 5.4%.

Bis(N-α-amido-phenylalanine)-1,10-decane Dicarboxylic Acid ***1***. To a solution of **5** (2.09 g, 4 mmol) in 20 mL of MeOH was added 10 mL of 2 M NaOH at room temperature and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10 h methanol was removed under vacuum, and the residue was taken in 50 mL of water and washed with diethyl ether (2 × 50 mL). Then the pH of the aqueous layer was adjusted to 2 by adding 1 M HCl dropwise and it was extracted with ethyl acetate (3 × 50 mL). These extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuo to yield **1** as a white solid.

Yield = 1.5 g (3 mmol, 75%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.5 (b, 2H); 8.03−8.00 (d, J = 9 Hz, 2H); 7.21−7.09 (m, 10H); 4.39−4.31 (m, 2H); 3.01−2.95, 2.79−2.71 (m, 4H); 1.97−1.92 (m, 4H); 1.32−1.27 (m, 4H); 1.16−1.04 (m, 8H). $[\alpha]^{20}_D + 19.0$ (c 0.66, CH_3OH). HRMS m/z 497.3029 $[M + H]^+$, 519.2999 $[M + Na]^+$, M_{calcd} = 496. FT-IR (KBr): 3286, 2927, 1722, 1650, 1542 cm^{-1}. Anal. Calcd for $C_{28}H_{36}N_2O_6$ (496): C, 67.74; H, 7.25; N, 5.64. Found: C, 67.7; H, 7.2; N, 5.4%.

Methyl Ester of Bis(N-α-amido-tyrosine)-1,10-decane Dicarboxylic Acid ***6***. A solution of 1,10-decane dicarboxylic acid (seabcic acid) (1.0 g, 5 mmol) and 1-hydroxy benzotriazole (HOBT) (1.35 g, 10 mmol) in DMF was cooled in an ice-water bath. H-Tyr-OMe was isolated from (4.63 g, 20 mmol) the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and concentration to 10 mL. Then this was added to the precooled solution, followed immediately by the addition of dicyclohexylcarbodiimide (DCC) (2.06 g, 10 mmol). The reaction mixture was stirred for 2 h at 0 °C and brought to room temperature gradually. The reaction mixture was taken in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3 × 50 mL), brine (2 × 50 mL), 1 M sodium carbonate (3 × 50 mL), and brine (2 × 50 mL) respectively. This washed solution was dried over anhydrous sodium sulfate and evaporated in vacuo to yield **6** as a white solid.

Yield = 2.5 g (4.5 mmol, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.96−6.93 (m, 8H); 6.75−6.72 (d, J = 8.4 Hz, 2H); 4.74−4.69 (m, 2H); 3.71 (s, 6H); 3.06−2.85 (m, 4H); 2.19−2.14 (m, 4H); 1.55−1.54 (m, 4H); 1.35−1.14 (m, 8H). ESI-MS m/z 557.3 $[M + H]^+$, M_{calcd} = 556. FT-IR (KBr): 3422, 3329, 2930, 2851, 1720, 1645, 1544, 1517 cm^{-1}. Anal. Calcd for $C_{30}H_{40}N_2O_8$ (556): C, 64.74; H, 7.19; N, 5.03. Found: C, 64.5; H, 7.4; N, 5.0%.

Bis(N-α-amido-tyrosine)-1,10-decane Dicarboxylic Acid ***2***. To a solution of **6** (2.22 g, 4 mmol, 80%) in 20 mL of MeOH was added 10 mL of 2 M NaOH at room temperature and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10 h methanol was removed under vacuum, and the residue was taken in 50 mL of water and washed with diethyl ether (2 × 50 mL). Then the pH of the aqueous layer was adjusted to 2 by adding 1 M HCl dropwise and it was extracted with ethyl acetate (3 × 50 mL). These extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuo to yield **2** as a white solid.

Yield = 1.7 g (3.2 mmol, 81%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.12 (s, 2H); 7.95−7.92 (d, J = 8.1 Hz, 2H); 6.94−6.91 and 6.58−6.55 (d, J = 8.4 Hz, 8H); 4.28−4.21, 3.99−3.92 (m, 2H); 2.87−2.81, 2.68−2.60 (m, 4H); 2.01−1.92 (m, 4H); 1.32 (m, 4H); 1.16−1.08 (m, 8H). $[\alpha]^{20}_D + 18.8$ (c 0.73, CH_3OH). HRMS m/z 529.3052 $[M + H]^+$, 551.2463 $[M + Na]^+$, M_{calcd} = 528. FT-IR (KBr): 3370, 3300, 2926, 1720, 1647, 1614, 1541 cm^{-1}. Anal.

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Calcd for $C_{28}H_{36}N_2O_8$ (528): C, 63.39; H, 6.79; N, 5.28. Found: C, 63.0; H, 6.4; N, 5.0%.

Methyl Ester of Bis(N-α-amido-norphenylalanine)-1,10-decane Dicarboxylic Acid ***7***. A solution of 1,10-decanedicarboxylic acid (sebacic acid) (1.0 g, 5 mmol) and 1-hydroxy benzotriazole (HOBT) (1.35 g, 10 mmol) in DMF was cooled in an ice-water bath. H-nor-Phe-OMe was isolated from (4.03 g, 20 mmol) the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and concentration to 10 mL. Then this was added to the precooled solution, followed immediately by the addition of dicyclohexylcarbodiimide (DCC) (2.06 g, 10 mmol). The reaction mixture was stirred for 2 h at 0 °C and brought to room temperature gradually. Reaction mixture was taken in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3 × 50 mL), brine (2 × 50 mL), 1 M sodium carbonate (3 × 50 mL), and brine (2 × 50 mL), respectively. This washed solution was dried over anhydrous sodium sulfate and evaporated in vacuo to yield **7** as a white solid.

Yield = 2.2 g (4.4 mmol, 88.7%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35−7.32 (m, 10H); 6.45−6.42 (d, J = 7.2 Hz, 2H); 5.60−5.57 (d, J = 7.2 Hz, 2H); 3.72 (s, 6H); 2.25−2.20 (m, 4H); 1.86−1.81 (m, 4H); 1.27 (m, 8H). Anal. Calcd for $C_{28}H_{36}N_2O_6$ (496): C, 67.74; H, 7.25; N, 5.64. Found: C, 67.3; H, 7.02; N, 5.6%.

Bis(N-α-amido-norphenylalanine)-1,10-decane Dicarboxylic Acid ***3***. To a solution of **7** (2.09 g, 4 mmol) in 20 mL of MeOH was added 10 mL of 2 M NaOH at room temperature and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10 h methanol was removed under vacuum, and the residue was taken in 50 mL of water and washed with diethyl ether (2 × 50 mL). Then the pH of the aqueous layer was adjusted to 2 by adding 1 M HCl dropwise and it was extracted with ethyl acetate (3 × 50 mL). These extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuo to yield **3** as a white solid.

Yield = 1.6 g (3.4 mmol, 87.9%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.74 (b, 2H); 8.46−8.43 (d, J = 7.5 Hz, 2H); 7.31−7.24 (m, 10H); 5.27−5.25 (d, J = 7.5 Hz, 2H); 2.12−2.07 (m, 4H); 1.40 (m, 4H); 1.14−0.96 (m, 8H). $[\alpha]^{20}_D$ + 34.6 (c 0.73, CH_3OH). HRMS m/z 469.4901 $[M + H]^+$, 491.4526 $[M + Na]^+$, M_{calcd} = 468. Anal. Calcd for $C_{26}H_{32}N_2O_6$ (468): C, 66.66; H, 6.83; N, 5.98. Found: C, 66.3; H, 6.7; N, 5.6%.

Methyl Ester of Bis(N-α-amido-phenylalanine)-1,9-nonane Dicarboxylic Acid ***8***. A solution of 1,9-nonane dicarboxylic acid (Azelaic acid) (1.0 g, 5 mmol) and 1-hydroxy benzotriazole (HOBT) (1.35 g, 10 mmol) in DMF was cooled in an ice-water bath. H-Phe-OMe was isolated from (4.31 g, 20 mmol) the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and concentration to 10 mL. Then this was added to the precooled solution, followed immediately by the addition of dicyclohexylcarbodiimide (DCC) (2.06 g, 10 mmol). The reaction mixture was stirred for 2 h at 0 °C and brought to room temperature gradually. The reaction mixture was taken in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3 × 50 mL), brine (2 × 50 mL), 1 M sodium carbonate (3 × 50 mL), and brine (2 × 50 mL), respectively. This washed solution was dried over anhydrous sodium sulfate and evaporated in vacuo to yield **8** as a white solid.

Yield = 2.2 g (4.3 mmol, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.09−7.06 (m, 10H); 5.90−5.87 (d, J = 7.2 Hz, 2H); 4.92−4.85 (m, 2H); 3.71 (s, 6H); 3.17−3.08 (m, 4H); 2.17−2.12 (m, 4H); 1.27−1.22 (m, 4H); 1.0−0.09 (m, 6H). Anal. Calcd for $C_{29}H_{38}N_2O_6$ (510): C, 68.23; H, 7.45; N, 5.49. Found: C, 68.0; H, 7.2; N, 5.62%.

Bis(N-α-amido-phenylalanine)-1,9-nonane Dicarboxylic Acid ***4***. To a solution of **8** (2.04 g, 4 mmol) in 20 mL of MeOH was added 10 mL of 2 M NaOH at room temperature and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10 h methanol was removed under vacuum, and the residue was taken in 50 mL of water and washed with diethyl ether (2 × 50 mL). Then the pH of the aqueous layer was adjusted to 2 by the dropwise addition of 1 M HCl and it was extracted with ethyl acetate (3 × 50 mL). These extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuo to yield **4** as a white solid.

Yield = 1.7 g (3.52 mmol, 88.5%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.58 (b, 2H); 8.02−7.99 (d, J = 8.1 Hz, 2H); 7.21−7.03 (m, 10H); 4.39−4.31 (m, 2H); 3.01−2.95, 2.79−2.66 (m, 4H); 1.97−1.92 (m, 4H); 1.30−1.28 (m, 4H); 1.01−0.08 (m, 6H); $[\alpha]^{20}_D$ + 20.9 (c 0.85, CH_3OH); HRMS m/z 483 $[M + H]^+$, 505 $[M + Na]^+$, M_{calcd} = 482. Anal. Calcd for $C_{27}H_{34}N_2O_6$ (482): C, 67.21; H, 7.05; N, 5.80. Found: C, 67.0; H, 6.9; N, 5.6%.

Gel Melting Temperature. The melting temperatures of the resultant metallo-hydrogel (T_{gel}) were determined by the inverted test tube method. The temperature was increased at the rate of 2 °C/min.

Transmission Electron Microscopic Study. Morphologies of the metallo-hydrogels, dye-adsorbed metallo-hydrogels, and vitamin B_{12} entrapped metallo-hydrogels were investigated by using transmission electron microscopy (TEM). A piece of gel of the corresponding compounds (at its minimum gelation concentration) or a piece of dye-adsorbed/vitamin B_{12}-entrapped gel was placed on a carbon-coated copper grid (200 mesh) and allowed to dry in vacuo at room temperature for 2 days. Images were taken at an accelerating voltage of 200 kV. TEM was done by a JEM-2010 electron microscope.

NMR Experiments. All NMR studies were carried out on a Brüker DPX 300 MHz spectrometer at 300 K. Compounds concentrations were in the range 1−10 mmol in $CDCl_3$ and $(CD_3)_2SO$.

Mass Spectroscopy. Mass spectra were recorded on a Hewlett Packard Series 1100MSD and Micromass Qtof Micro YA263 mass spectrometer by positive mode electrospray ionization.

UV−Vis Spectroscopy. UV−vis absorption spectra were recorded on a Hewlett-Packard (Model 8453) UV−vis spectrophotometer (Varian Carry 50.Bio).

FT-IR Spectroscopy. All reported FT-IR spectra were taken using a Shimadzu (Japan) model FT-IR spectrophotometer. A Nicolet FT-IR instrument [Magna IR-750] spectrometer (series II) was used to obtain solid state and dried gel state FT-IR spectra. For the solid-state measurements the KBr disk technique was used.

Adsorption of Dye. Measured amounts of dyes (0.11 mM for Crystal Violet, 0.1 mM for Naphthol Blue Black, and 0.05 mM for pyrene) were added to each set of 7 mL stock solution of buffer at pH 6.5. Then, 25 mg of dry metallo-hydrogel containing $CuSO_4$ or $NiCl_2$ was added to these solutions and they were left for 24 h at room temperature without any disturbance. Then the kinetic study of the dye adsorption by the metallo-hydrogel was demonstrated using UV/vis spectroscopy at pH 6.5.

Controlled Release of the Entraped Vitamin B_{12} Molecule. At first 7 mL stock solution of vitamin B_{12} (0.05 mM) in buffer having pH 6.5 was prepared. Then 25 mg (0.05 mM) of bolaamphiphile 1 and $CoCl_2$ salt (0.025 mM) were added to it. The total solution was then sonicated for a few minutes to form the vitamin B_{12} containing metallo-hydrogel. The pH of the metallo-hydrogel was then changed to be in the acidic region (pH ≥ 2) by adding 1 M HCl solution dropwise. The metallo-hydrogel containing vitamin B_{12} slowly precipitated from solution at that acidic pH. Gradually,

Scheme 1. Schematic Representation of Bolaamphiphiles 1−4

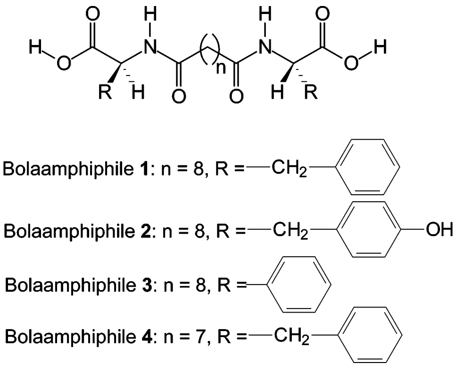


Table 1. Hydrogel-Forming Behavior of Bolaamphiphiles 1−4 (0.35% w/v) in Water at pH 6.5 Containing Divalent Transition Metal Salts on Sonication[a]

metal salts	1	2	3	4
$MnCl_2$	G (65 °C)	S	P	P
$CoCl_2$	G (52 °C)	S	P	P
$NiCl_2$	G (48 °C)	S	P	P
$CuSO_4$	G (63 °C)	S	P	P

[a] G: stable gel formed at room temperature; S: soluble; P: precipitate. In parentheses T_{gel} values are given. In all cases bolaamphiphiles:metal salts = 2:1.

the volume of buffer solution was separated from the gel and it was then taken out of the system as aliquot to test the concentration of the released vitamin B_{12}. The released amount of the vitamin was measured by analyzing time-dependent UV/vis spectra of the released vitamin B_{12} in solution.

Results and Discussion

Gel Formation. The bolaamphiphile **1** has been found to form hydrogels in the presence of various metal salts ($CuSO_4$, $CoCl_2$, $MnCl_2$, and $NiCl_2$). Other amino acid-based bolapmphiphiles (**2**, **3**, and **4**) that are structurally related to the gelator molecule **1** (Scheme 1) have been synthesized, purified, and characterized and their gel-forming tendencies have been studied to address the question of whether they are able to form hydrogels by varying the pH of the solution and/or in the presence of a similar type of transition metal ions. All reported amino acid-based bolaamphiphiles **1**−**4** are sparingly soluble in water. However, their solubilities in water are very sensitive to pH. A stock solution of the bolaamphiphile **1** was prepared by adding 25 mg (0.05 mmol) of the bolaamphiphile to 6 mL of 30 mmol NaOH solution. The pH was then adjusted to 6.5 by the dropwise addition of 50 mmol citric acid solution. Then, either $MnCl_2$ or $CoCl_2$ or $NiCl_2$ or $CuSO_4$ (0.025 mmol) was added to the each set of stock solution. These samples were sonicated and kept at room temperature to form gels. The gelation test data and the respective T_{gel} values of the metallo-hydrogels are listed in Table 1.

Only the bolaamphiphile **1** is able to gelify water up to 99.65% w/v in the presence of selective divalent metal ions (Mn^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+}), indicating that each gelator molecule can entrap ~6620 water molecules in the gel state. These gels are stable at room temperature and maintain its gel state for several months. As bolaamphilies **2**, **3**, and **4** do not gelate water in the presence of the above-mentioned metal salt at this particular pH or in the vicinity of this pH, it is expected that gelation significantly depends upon the chain length of the centrally located oligomethylene units as well as the aromatic side chain residue. A subtle change in the structure of the gelator molecule abolishes the hydrogelation property. Previous results also demonstrate that a fine balance between hydrophilicity and hydrophobicity dictates the effective hydrogel formation using a series of bis urea dicarboxylic acids[6] or bis (amino acid) oxalyl amides.[7]

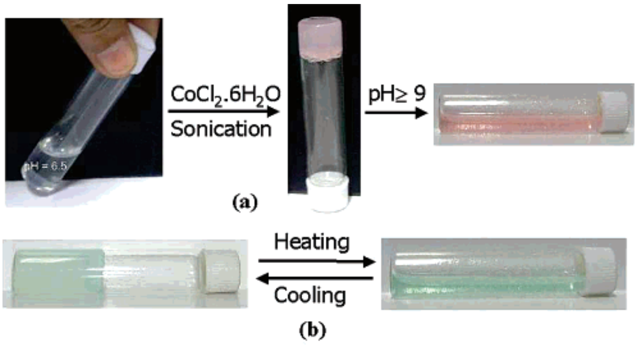


Figure 1. (a) Photograph showing the gel formation of bolaamphiphile 1 with $CoCl_2$ salt and its pH responsiveness. (b) Photograph of metallo-hydrogel showing the thermoresponsive nature of the gel (in the presence of $NiCl_2$).

Anthranilic acid (6.8 mg, 0.05 mmol) was added to the Ni-metal hydrogel of bolaamphiphile **1**, and it was then sonicated for a few minutes. Within 1 h a light yellow colored precipitation gradually occurred. The addition of anthranilic acid to the metallo-gel of compound **1** containing Ni^{2+} resulted in the disruption of gel formation as anthranilic acid is a much stronger complexing agent for Ni salt than bolaamphiphile **1** itself; this leads to the prevention of gel formation and this indicates that the presence of bolaamphiphile **1**−Ni complexation is required for gel formation.

pH Dependence and Thermoresponsiveness. The gel is highly stable around the pH range 6.5−7.2. When the pH is lowered below 6.5, the gel starts breaking slowly and completely breaks at pH 2 and at that pH the bolaamphiphile **1** is entirely precipitated out, separating into two phases, the solid phase and the solution phase. However, at higher pH (>7.2) the gel also starts breaking slowly with the concomitant appearance of the solution and at pH 9 the gel to sol transition occurs completely (Figure 1). The reason for the gel to sol transition at higher pH is not clearly understood. At the higher pH both the carboxylic acid moieties are ionized and this might help the gel to sol transition. However, at the lower pH (<6.5) one or both of the carboxylic acid groups are protonated and this decreases the solubility and the gel-forming tendency of the bolaamphiphile **1** to a great extent. Thermal stability of the metallo-hydrogel was analyzed by inverted test tube method. The bolaamphiphile **1** forms thermoreversible gels in the presence of various divalent metal ions. Gel to sol transition temperatures for various metallo-gels are listed in Table 1 at a fixed pH 6.5 and at a constant metal salt concentration (0.025 mmol). The gel melting temperature of the $NiCl_2$−metallo-hydrogel has been found to increase with increasing the gelator concentration within the range of 0.04−0.05 mmol and then the gel melting temperature decereases with further increse of the

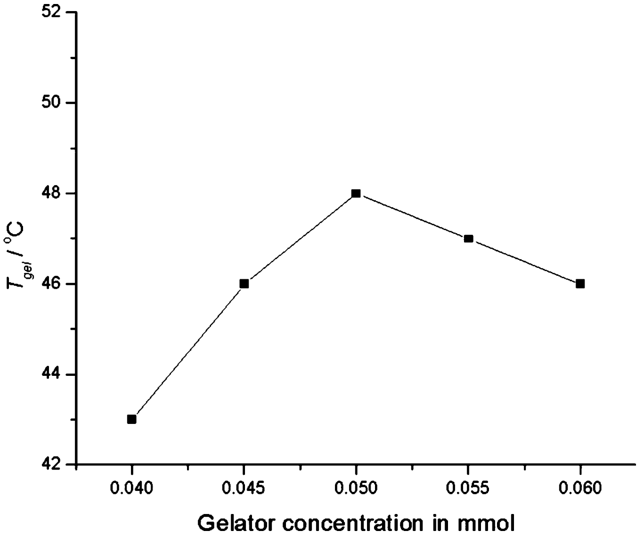


Figure 2. Plot of T_{gel} vs concentration of bolaamphiphile **1** in the presence of constant $NiCl_2$ salt concentration (0.025 mmol) at pH 6.5.

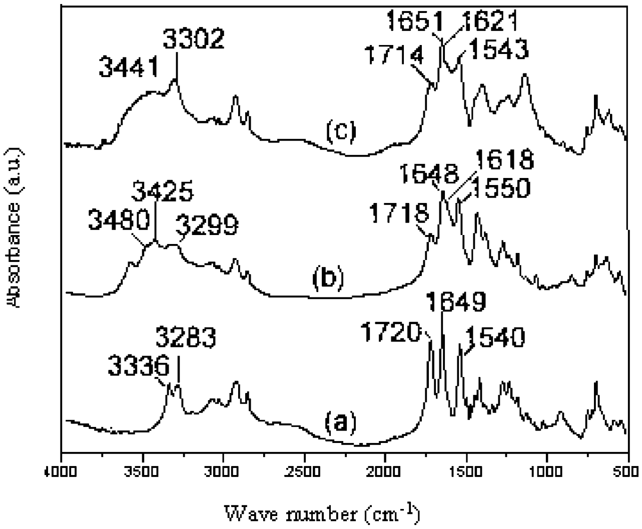


Figure 3. FT-IR spectra of (a) solid bolaamphiphile **1** and its metallo-hydrogel having metal salts (b) $NiCl_2$ and (c) $CoCl_2$.

gelator concentration, keeping the pH and the metal ion concentration constant (pH = 6.5 and metal ion concentration = 0.025 mmol) (Figure 2).

Infrared Spectroscopy. Preliminary information for the structural preference of the gelator bolaamphiphile **1** and the hydrogel in the presence of $NiCl_2$ and $CoCl_2$ are obtained from the FT-IR studies (Figure 3). The amide CO stretching frequency of the gelator bolaamphiphile alone appears at 1649 cm^{-1}. However, in the gel state in the presence of Ni salt or Co salt, the above-mentioned peak splits into two peaks; the new peaks are at 1648 and 1618 cm^{-1} for Ni^{2+}-containing gel and 1651 and 1621 cm^{-1} for Co^{2+}-containing gel. The appearance of a new peak at 1618 or 1621 cm^{-1} in Ni^{2+} or Co^{2+}-containing metallo-hydrogels indicates that a lone pair of the amide CO might be involved in metal ion coordination in the gel state. There is no significant change in the stretching frequency of the CO group of COOH for metallogels and the solid gelator compound. The amide NH is hydrogen-bonded in all studied states, i.e., in the bulk solid of the gelator compound **1** and in metallo-hydrogels.

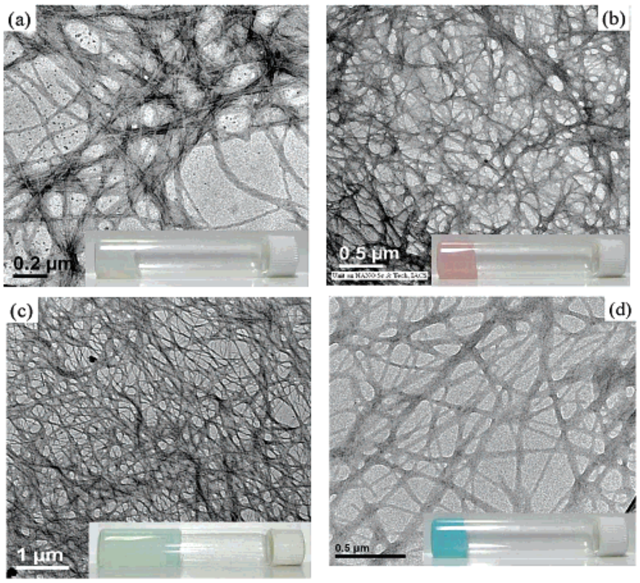


Figure 4. TEM images of metallo-hydrogels prepared from bolaamphiphile **1** at pH 6.5 with metal salt concentrations required for minimum gel concentration (MGC) (a) in the presence of $MnCl_2$, (b) in the presence of $CoCl_2$, (c) in the presence of $NiCl_2$, and (d) in the presence of $CuSO_4$.

TEM Microscopy. Morphological features of the dried metallogels have been studied using a high-resolution transmission electron microscope (HR-TEM). Figure 4 shows the TEM image of the metallo-hydrogel in the presence of (a) $MnCl_2$, (b) $CoCl_2$, (c) $NiCl_2$, and (d) $CuSO_4$. It is clear from the TEM study that all metallo-hydrogels form nanofibrillar network structures, which might be responsible for encapsulating a large volume of water molecules to form gels in the presence of different divalent transition metal ions. The average widths of the nanofibrils are in the range of 20−30 nm (Figure 4).

Bolaamphiphile **1** self-assembles in the presence of selective divalent metal ions (Mn^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+}) in a particular pH range (6.5−7.2) to form a nanofibrillar network structure (20−30 nm) that enables the entrapment of a large volume of water molecules (99.65% w/v) to form metallo-hydrogels. It may be suggested that metal ion chelation, intermolecular hydrogen bonding, and $\pi-\pi$ stacking play a significant role in promoting the self-organization and self-association of the bolaamphiphile **1** in the gel state.

Dye Adsorption Study. The property of pH responsiveness of these gels can be utilized for the entrapment and slow release of dye molecules and/or biologically important molecules. Many of these gels (Mn^{2+}-, Cu^{2+}-, or Ni^{2+}-induced hydrogels) have been tested for adsorbing different types of toxic dyes from water with an aim to purify dye-contaminated wastewater. When these dry gels have been submerged in aqueous solutions of various dyes (cationic, anionic, and electrically neutral dyes (ESI Figure S9)), they efficiently adsorb the dye molecules from water and within a few hours they are capable of removing all dye content, leaving only clear water. Hence, these pH-sensitive gels can efficiently be used as water-purifying agents. It has been observed from Figure 5 that the dried gel containing Cu^{2+} adsorbs cationic dye (Crystal Violet) from solution and the gel breaks into pieces after adsorbing all dye molecules. The capacity of a given amount of this gel for adsorbing various

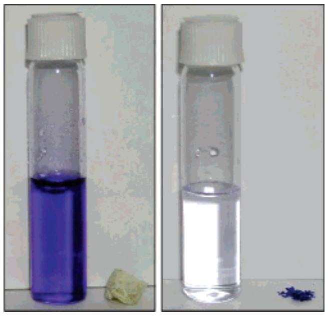


Figure 5. Photograph showing adsorption of the cationic dye (crystal violet) by the dried metallo-hydrogel containing $CuSO_4$.

Table 2. Adsorption Data for Bolaamphiphile 1-Cu-salt Hydrogel for Various Dyes

dye	λ_{max}[a] (nm)	C[b] (mg/L) [mM]	Q_{max}[c] (mg/g)
Crystal Violet (cationic dye)	590	45 [0.11]	63
Naphthol Blue Black (anionic dye)	616	61 [0.1]	84
Pyrene (nonionic dye)	336	1 [0.005]	2.8

[a] Wavelength of light absorption maximum of dye solution only. [b] Initial aqueous concentrations of the corresponding dyes (corresponding molarity in brackets). [c] Quantity of the adsorbed dye per gram of the adsorbent.

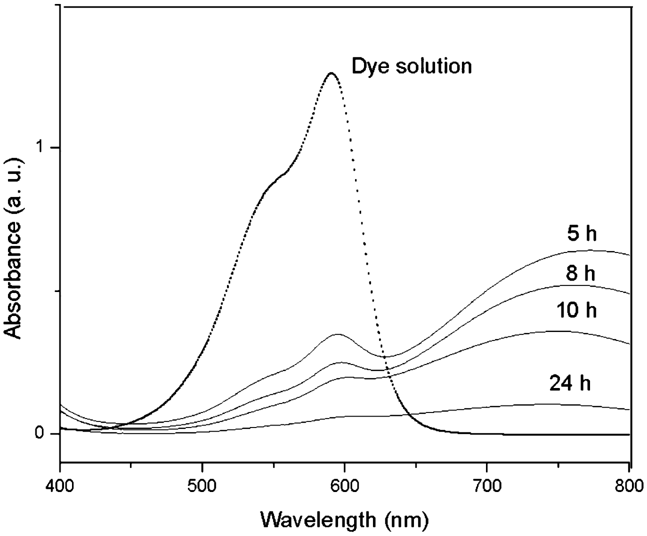


Figure 6. Dotted line indicates the UV−vis spectrum for only dye solution (at pH 6.5) and solid lines indicate the UV−vis spectra of dye-adsorbed gel indicating the time-dependent adsorption of cationic dye (crystal violet) from water solution by the metallo-hydrogel containing $CuSO_4$.

dyes have been quantitatively estimated and it has been listed in Table 2. The gel adsorbs ionic dyes such as Crystal Violet and Naphthol Blue Black more efficiently than pyrene (a nonionic dye).

Adsorption of dyes by the dried gels has been studied by using UV−visible spectra. From the time-dependent UV−vis spectra of the dye solution after submerging the dried gel into the solution at pH 6.5 (Figures 6 and 7), it has been observed that absorption maxima have been shifted toward longer wavelength and the intensity of the peak also decreases with respect to time and after 24 h it becomes almost flat. The shift in the peak position of UV/vis spectra which has been observed for the adsorbed dye by the metallo-hydrogel is possibly due to the binding of dye molecules with the metallo-hydrogel. This indicates the adsorption of dye molecules by the porous framework of the gel.

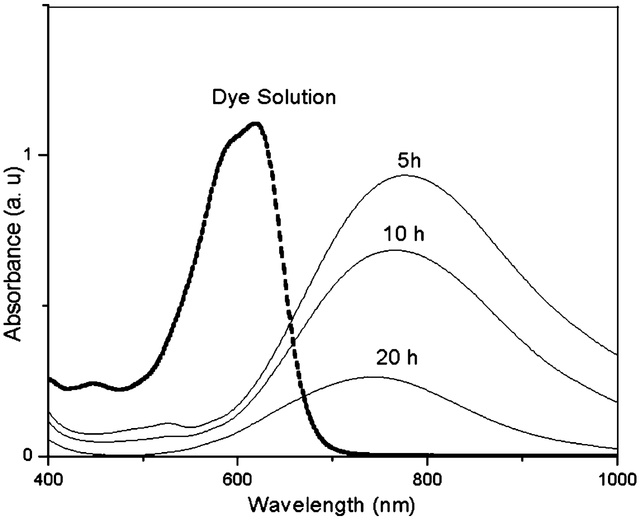
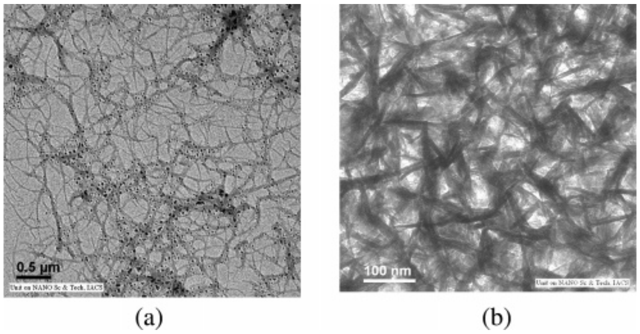


Figure 7. UV data for time-dependent adsorption of anionic dye (Napthol Blue Black) from water solution by the application of bolaamphiphile **1**−Cu salt hydrogel. Dotted line indicates the UV−vis absorption spectrum of the dye solution only. Solid lines indicate time-dependent UV−vis absorption spectra of the dye-entrapped gel.



(a) (b)

Figure 8. TEM images of metallo-hydrogel in the presence of Cu salt after adsorbing various dye molecules (a) in the presence of anionic dye Napthol Blue Black and (b) in the presence of cationic dye Crystal Violet.

TEM Study of Dye-Adsorbed Metallo-hydrogel. A transmission electron microscopic study has been performed to visualize the trapped dye molecule within the gel and it clearly indicates that dye particles are adsorbed on the nanofibrillar network of the gel (Figure 8). It has been observed from the EDX profile (ESI Figure S10) of the black dots present in the TEM image that there is no peak corresponding to the relevant metal atom present in the metallo-hydrogel. This study clearly suggests that the black dots observed in the TEM image are not the complex of hydrogelator and metal. So these black dots most probably correspond to the adsorbed dyes.

Controlled Release of Vitamin B_{12}. We also performed an experiment to elucidate the vitamin encapsulation ability of one of these metallo-hydrogels (the hydrogel containing $CoCl_2$). Water-soluble vitamin, such as vitamin B_{12}, has been encapsulated by the hydrogel during the gel formation process. Twenty-five milligrams (0.05 mM) of bolaamphiphile **1** and $CoCl_2$ salt (0.025 mM) were added into 7 mL buffer solution having pH 6.5 with vitamin B_{12} (0.05 mM). Then the mixture was sonicated for a few minutes and was kept at 37 °C for 1 day. Pink colored metallo-hydrogel containing vitamin B_{12} was formed. This gel is sensitive to

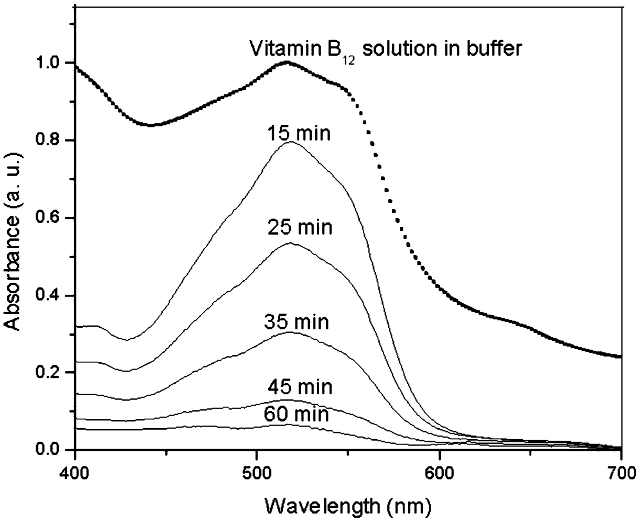


Figure 9. Time-dependent UV−vis absorption spectra showing absorption of vitamin B_{12} by Co^{2+}-metallo-hydrogel. Dotted line indicates UV−vis absorption spectrum of only vitamin B_{12} solution whereas solid lines indicate UV−vis absorption spectra of vitamin B_{12} containing metallo-hydrogel.

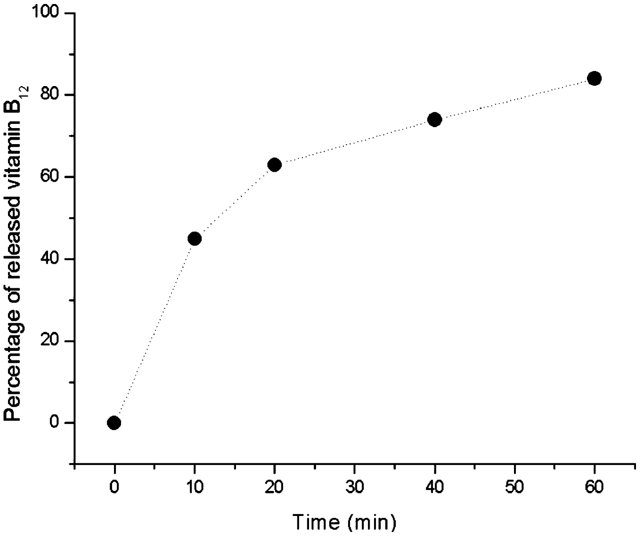


Figure 10. Quantitative estimate of the release of vitamin B_{12} from the metallo-hydrogel with respect to time at acidic pH (pH = 2.0).

pH and it slowly breaks in response to the change of pH of the medium (on lowering the pH).

It has been observed from the time-dependent UV−visible spectra that vitamin B_{12} has been gradually entrapped into the metallo-hydrogel (Figure 9). It has been found that 1 g of metallo-hydrogel can entrap ∼17 mg of vitamin B_{12}.

It has been observed that changing (lowering) the pH (≥2) facilitates slow release of vitamin B_{12} molecules from the gel. Figure 10 shows the time course of slow release of vitamin B_{12} from the gel at acidic pH (pH = 2).

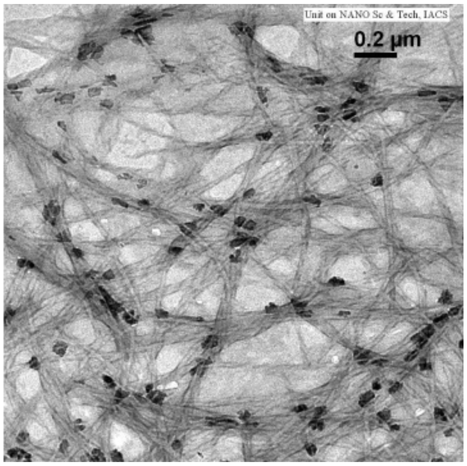


Figure 11. TEM image of the metallo-hydrogel prepared from bolaamphiphile **1**−Co salt at pH 6.5 in the presence of vitamin B_{12} showing the trapped vitamin molecules within the gel nanofiber network.

TEM Study of Vitamin B_{12}-Entrapped Metallo-hydrogel. From the TEM study it has also been observed that the vitamin B_{12} molecules are trapped within the gel nanofiber network structure (Figure 11).

Conclusions

Our study clearly demonstrates the formation of the pH-responsive transition metal-ion-induced thermoreversible hydrogels using a bolaamphiphile containing phenylalanine residues. The pH-responsive metallo-hydrogel formation occurs at nearly physiological pH (6.5−7.2). This study also demonstrates that these pH-responsive metallo-hydrogels can be used for adsorbing different types of dyes from water, indicating their possible applications in wastewater treatment. One of these metallo-hydrogels also entrap vitamin B_{12} molecules and the vitamin can be released by changing the pH of the medium, suggesting a possible use of this gel as a carrier of biologically important compounds.

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Supporting Information Available: NMR spectra and ESI-mass spectra of the reported bolaamphiphiles **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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