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Stimuli Responsive hydrogel derived from Renewable Resource: Synthesis, Self-Assembly in water and application in drug delivery

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We designed and synthesised coumarin-tris derivatives from renewable resource and well characterized using different spectral techniques. Self-assembly of coumarin-tris amphiphile into hydrogel were examined relative to the molecular structure. The reversible morphological transition from nanofibers to vesicles and nanotubes has been observed, upon pH variation. Reversible processes and self-assembled structures such as gel, vesicle and nanotube formation have been investigated by optical microscopy and High Resolution Transmission Electron Microscopy (HRTEM). ^{1}H NMR and XRD studies clearly suggest the π-π stacking interaction and hydrogen bonding were the driving force for the process of gelation. The flow behaviour of hydrogel has been identified using rheological measurements. More importantly, chemopreventive drug, curcumin has been encapsulated into the gel and further release has been achieved by gel-to-sol transition induced by pH and metal ion, Fe^{3+} stimuli. Reported hydrogel could play a substantial role in the development of new generation stimuli responsive drug delivery systems for *in vivo* formulations

Introduction

Natural systems are having the ability to change its physiological functions in direct response with environmental conditions such as temperature, light, pH, electro/magnetic field, mechanical stress and chemical stimuli has always been an interesting source of inspiration for scientists to develop new technologies for advanced "smart" materials.[1,2] Remarkable changes in micro/nanoscopic (molecular level) and macroscopic (surface level) are responsible for stimuli-responsive behaviour of natural systems. Among the different types of stimuli-responsive materials, gels are considered as assuring materials for the bottom-up nanofabrication tools in various fields such as biology, biomedicine, tissue engineering, drug delivery, sensors, catalysis, cosmetics, and as therapeutics.[2] Gels are classified in to two categories: (1) chemical gel and (2) physical gel. Usually chemical gels are covalently cross-linked irreversible structures (gels derived from polymers), whereas physical gels, are self-assembled structures based on reversible intermolecular interactions.[3] Depending up on the molecular structure, gels can acquire certain prescribed conformations and self-assemble into preciously defined biomimetic structures (helices, sheets, nanotubes, vesicles, micelles, etc.) through non-covalent interactions such as hydrogen bonding, π-π interaction, electrostatic and van der Waals interactions, hydrophilic-lipophilic balance (HLB) and other supramolecular weak forces.[4] Such self-assembled soft materials are continuous in structure and own solid-like in rheological behaviour, which is due to the entrapment of solvent in self-assembled 3D solid matrix through surface tension and capillary forces.[4] In particular, supramolecular hydrogels derived from small molecules are more interesting because of its inherent and excellent biocompatibility and they were considered as best alternative for chemical gel (polymeric gel). Due to the dynamic reversible nature of hydrogel derived from small molecules, the phase transition either sol-to-gel or gel-to-sol can be triggered by the external stimuli.[5] This phenomenon could be useful for understanding the gelation mechanism and advanced applications like drug delivery and switchable devices with memory function. We report herein the ability of small amphiphilic system bearing a biologically interesting coumarin moiety to form stable supramolecular hydrogel that are responsive to external stimuli, pH and biologically important Fe^{3+} ion. In particular, we have synthesised and studied the self-assembly (hydrogelation) and stimuli responsive behaviour of coumarin coupled tris derivatives, which in turn derived from renewable plant-derived resource, cashew nut shell liquid. Renewable resources has been substantial focus on establishing and optimizing efficient materials and production of fine chemicals and fuels that address the needs of the 21st century.[6] Cardanol, a bio based non-isoprene lipid directly incurred by distillation of cashew

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nutshell liquid (CNSL), an important by-product obtained from the cashew nut industry. Cardanol comprising of rich mixture of phenolic lipids: 5% of 3-*n*-pentadecylphenol (3-PDP), 50% of 3-(8*Z*-pentadecenyl)phenol, 16% of 3-(8*Z*,11*Z*-pentadecadienyl)phenol and 29% of 3-(8*Z*,11*Z*,14-pentadecatrienyl)phenol. The uniqueness of cardanol stem from its structural lineament and easily accessible saturated and unsaturated hydrocarbon chains.[7]

Experimental

General

All reagents and solvents were purchased from Sigma Aldrich, Merck, Alfa Aesar and Avra chemicals and were used as such for the synthesis without further purification. The progress of the reactions were monitored by thin-layer chromatography (TLC) using pre-coated silica gel plates purchased from Merck and visualized under UV cabinet and/or p-anisaldehyde stain and/or molecular iodine. We have used LR grade solvents for the compounds purification and AR grade solvents for gelation studies. If not indicated particularly, all other reagents were purchased from commercial source and used without further purification. Silica Gel (100-200 mesh) purchased from Avra synthesis, INDIA was used for column chromatography. ^{1}H- and ^{13}C-NMR spectra were recorded in either $CDCl_3$ or $CDCl_3$ with few drops of DMSO-d_6 at room temperature on a Bruker DRX 300 MHz instrument. Chemical shifts (δ) are reported in parts per million (ppm) with respect to internal standard TMS and coupling constants (J) are given in Hz. Proton multiplicity is assigned using the following abbreviations: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). MM2 energy minimised diagram was performed using ChemBio 3D Ultra 13. Red colour dotted line shows the possible hydrogen bond formation.

Distillation of Cardanol

The key constituent of CNSL is cardanol **1b**, a bio based non isoprene lipid, consisting of phenolic lipid mixture: 5% of 3-*n*-pentadecylphenol (3-PDP), 50% of 3-(8*Z*-pentadecenyl) phenol, 16% of 3-(8*Z*, 11*Z*-pentadecadienyl) phenol and 29% of 3-(8*Z*,11*Z*,14-pentadecatrienyl) phenol. CNSL was distilled at a temperature between 210 and 280°C, under a pressure from 2 to 8 mm Hg to get cardanol. Cardanol was obtained as pale yellow liquid which darkens during storage. After a second distillation, mixture of cardanol mono-, di- and tri-ene was obtained.

Synthesis

General procedure for synthesis of compounds 2b and 2c.

Dry paraformaldehyde (35 mmol) was added to a mixture of 3-alkyl phenol (4 mmol), anhydrous magnesium chloride (6 mmol) and triethylamine (15 mmol) in acetonitrile (25 mL) and the mixture was heated under reflux for about 12-15h. After the completion of the reaction as monitored by TLC, the reaction mixture was cooled to room temperature and 5% aq. HCl was added. The crude product was extracted with ethylacetate, dried under Na_2SO_4 and pure product was isolated by column chromatography using 95:5 v/v hexane-ethyl acetate as eluent.

Compound 2b

Isolated as yellow liquid; yield =88%. ^{1}H NMR (300MHz, $CDCl_3$) δ = 0.88 (t, *J* = 7.2 Hz, 3H), 1.25-1.30 (m, 16H), 1.59-1.64 (m, 4H), 1.95-2.05 (m, 2H), 2.61 (t, *J* = 7.5 Hz, 2H), 5.33-5.39 (m,2H), 6.80 (s, 1H), 6.83 (d, *J* = 7.8 Hz, 1H), 7.44 (d, *J* =7.8 Hz, 1H), 9.83 (s, 1H), 11.05 (s, 1H); ^{13}C NMR (75MHz, $CDCl_3$) δ = 195.8, 161.8, 153.8, 133.6, 130.0, 129.7, 120.5, 118.9, 117.1, 36.4, 32.6, 31.8, 30.7, 29.7, 29.7, 29.7, 29.6, 29.4, 29.3, 29.3, 29.2, 29.2, 29.0, 27.2, 27.2, 22. 7, 14.1.

Compound 2c

Isolated as white solid; Yield = 92%. ^{1}H NMR (300MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.29-1.34 (m, 24H), 1.57-1.66 (m,2H), 2.61 (t, *J* = 7.8 Hz, 2H), 6.80 (s, 1H), 6.83 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 9.83 (s, 1H), 11.05 (s, 1H); ^{13}C NMR (75MHz, $CDCl_3$) δ= 195.8, 161.8, 153.8, 133.6, 120.5, 118.8, 117.1, 36.5, 31.9, 30.7, 29.7, 29.7, 29.5, 29.4, 29.4, 29.3, 22.7, 14.1.

General procedure for synthesis of compounds 3b and 3c

To a solution of 2-hydroxyarylaldehyde (**2a-c**) (3 mmol) and diethyl malonate (3.2 mmol) in ethanol (15mL), piperidine (0.1 mL) and glacial acetic acid (1 or 2 drop) were added and the reaction mixture was refluxed for 6h. After completion of reaction as identified using TLC, 20 mL of water was then added to the reaction mixture. Crude product was extracted using ethyl acetate and dried over anhydrous Na_2SO_4. Pure product was isolated by column chromatography using 75:25 v/v hexane-ethylacetate as eluent.

Compound 3b

Isolated as yellow liquid; Yield = 87%. ^{1}H NMR ($CDCl_3$, 300MHz): δ 0.81 (t, *J* = 6.9 Hz, 3H), 1.19-1.24 (m, 18H), 1.34 (t, *J* = 6.9 Hz, 3H), 1.5-1.62 (m, 2H), 1.90-1.95 (m, 2H), 2.65 (t, *J* = 7.5 Hz, 2H), 4.34 (q, *J* = 7.2 Hz, 2H), 5.27-5.31 (m, 2H), 7.07 (s, 1H), 7.08 (d, *J* = 6.9 Hz, 1H), 7.43 (dd, *J* = 6.9, 1.5 Hz, 1H), 8.45 (s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ = 166.6, 163.3, 157.0, 155.4, 151.3, 148.7, 129.9, 129.6, 129.2, 125.5, 116.9, 116.2, 115.7, 61.8, 61.5, 41.7, 36.7, 30.8, 29.7, 29.7, 29.3, 29.2, 29.1, 28.9, 27.2, 27.1, 22.6, 14.2.

Compound 3c

Isolated as white solid; Yield = 89%. ^{1}H NMR ($CDCl_3$, 300MHz): δ 0.83 (t, *J* = 6.9 Hz, 3H), 1.18-1.23 (m, 26H), 1.34 (t, *J* = 6.9 Hz, 3H), 2.65 (t, *J* = 7.8 Hz, 2H), 4.30, (t, *J* = 7.2 Hz, 2H), 7.09 (d, *J* = 6.9 Hz, 1H), 7.20 (s, 1H), 7.44 (dd, J = 6.9, 1.5 Hz, 1H), 8.45 (s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ = 163.3, 1571, 155.4, 151.3, 148.7, 129.2, 125.5, 116.2, 115.7, 61.8, 36.3, 31.9, 30.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.4, 29.2, 22.7, 14.2, 14.1.

General procedure for synthesis of compound 4a-c

Ethyl 7-alkylcoumarin-3-carboxlate (**3a-3c**) (1 mmol) and tris (0.12 g, 1 mmol) (1:1 ratio) in methanol were refluxed with

stirring for about 24h. After completion of reaction as identified by TLC, the reaction mixture was then cooled to room temperature. The precipitate thus formed was filtered and washed several times with cold methanol. Filtrate obtained was kept under refrigeration for further crop of product. Pure product was obtained by recrystallization in ethanol.

Compound 4a

Isolated as white solid; Yield = 92%. ^{1}H NMR (DMSO-d_6, 300MHz): δ 3.68 (d, *J* = 5.4 Hz, 6H), 4.85 (t, *J* = 5.4 Hz, 3H), 7.46 (t, *J* = 7.5 Hz, 1H), 7.53 (d, *J* = 8.1 Hz, 1H), 7.77 (ddd, J = 8.7, 7.2, 1.5 Hz, 1H), 8.0 (dd, *J* = 7.8, 1.5 Hz, 1H), 8.93 (s, 1H), 8.98 (s, 1H).

Compound 4b

Isolated as pale yellow semi solid; Yield = 79%. ^{1}H NMR ($CDCl_3$, 300MHz): δ = 0.89 (t, 3H), 1.25-1.32 (m, 14H), 1.66-1.90 (m,8H), 2.75 (m, 2H), 3.90 (s, 6H), 5.31-5.32 (m, 2H), 7.20-7.28 (m, 2H), 7.59 (d, *J* = 8.4 Hz, 1H), 8.86 (s, 1H), 9.74 (s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ = 162.1, 160.4, 153.7, 153.7, 150.4, 147.1, 128.7, 115.9, 115.4, 115.1, 61.7, 61.3, 35.3, 30.7, 29.8, 28.7, 28.4, 28.2, 26.2, 21.6, 13.1. HRMS (ESI): calcd for $C_{29}H_{43}NO_6$, *m/z* 502.3169 $[M+H]^+$; found *m/z* 502.3184.

Compound 4c

Isolated as white precipitate; Yield = 78%; mp 106-110 ^{0}C. ^{1}H NMR ($CDCl_3$, 300MHz): δ 0.87 (t, *J* = 6.3 Hz, 3H), 1.25-1.32 (m, 24H), 1.64-1.67 (m, 2H), 2.74 (t, *J* = 7.8 Hz, 2H), 3.74 (d, *J* = 5.7 Hz, 6H), 4.83-4.85 (m, 3H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.22 (s, 1H), 7.64-7.66 (m, 1H), 8.88 (s, 1H), 9.53 (s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz) δ = 162.0, 160.7, 154.2, 150.6, 147.8, 129.2, 125.5, 116.9, 115., 115.3, 62.3, 61.7, 38.7, 35.7, 31.3, 30.3, 29.1, 29.0, 28.9, 28.8, 28.7, 28.6, 22.1, 13.7. HRMS (ESI): calcd for $C_{29}H_{45}NO_6$, *m/z* 504.3325 $[M+H]^+$; found m/z 504.3326.

Gelation studies

A known quantity of gelator was mixed with appropriate amount of solvent in a sealed test tube, and the system was heated until the solid get dissolved. By this procedure the solvent boiling point becomes higher than that under standard atmospheric pressure. The resulting solution was slowly allowed to cool to room temperature, and gelation was visually observed by inverting the test tube. A gel sample thus obtained which exhibited no gravitational flow in inverted tube is denoted as "G". Different solvents and vegetable oils such as hazelnut oil, sesame oil, jojoba oil, olive oil, soya bean oil, linseed oil, heavy paraffin oil, light paraffin oil, dodecanol, ethanol, n-butanol, octanol, toluene, cyclohexane, n-heptane, ethylene glycol, and mixture of solvents such as DMSO, DMF, DMSO-water, DMF-water were used for gelation studies and results were given in ESI.

Gel-sol melting temperature (T_g)

Gel melting temperature was determined by flow of gel by test tube inversion method. Above the gelation temperature, the gel phase becomes solution, but could be returned to their original gel state upon cooling. Gel was prepared in a 5 mL glass vial as described above, the vial was immersed in the oil-bath 'upside down' and the vial is slowly heated. The temperature at which the gel melted down to solution was recorded as T_g.

Self-assembly studies

In a round bottom flask, 2 mg of compound **4c** was dissolved in 200 µL of ethanol and then 5 mL of double distilled water was added until to get a bluish white tinge. Entire content was refluxed for 1h to get a clear solution and then cooled at room temperature. Formation of self-assembled fibers has been observed within 24h. This experiment has also been carried out in both acidic and basic pH levels.

Morphological analysis

Morphological analysis of hydrogel formed by gelator was studied using Carl Zeiss AXIO ScopeA1 fluorescent/phase contrast microscope. A glass slide containing a small portion of gel was mounted on Phase Contrast Microscope and the morphology of gel was identified. The reversible morphological transition from nanofibers to vesicles and nanotubes have been investigated by using JEOL JEM 2100F FETEM.

X-Ray diffraction studies

A small portion of a wet hydrogel sample was placed on a glass slide and allowed to dry. X-ray diffraction pattern of xerogel thus formed was analyzed on XPert-PRO Diffractometer system.

Fourier Transform Infrared Spectrometer (FTIR)

Infrared spectrum was recorded using FTIR Shimadzu 8000 Spectrometer in the spectral range of 4000 cm^{-1} to 400 cm^{-1}. Approximately 1.5 mg of sample was ground with 15-25 mg of potassium bromide using pestle and mortar. The fine powder was then transferred to the 'dye' and 10 tonne of pressure was applied for 2 minutes. The resulting pellet was put in the sample holder and the FTIR spectrum was recorded.

Rheological measurements

The mechanical properties of hydrogel and curcumin encapsulated composite gel were investigated with a stress controlled rheometer (Anton Paar 302 rheometer) equipped with a steel-coated parallel-plate geometry (25 mm diameter). The gap between two plates was fixed as 1 mm. The measurements were carried out at 23°C. Firstly, amplitude sweep measurement was conducted, which provides the information about linear viscoelastic range which is directly proportional to the mechanical strength of the gel sample. Secondly, the storage modulus, G' and the loss modulus, G" were monitored as functions of frequency from 0.1 to 100 rad s-1.

Preparation of curcumin encapsulated hydrogel (composite gel)

We have used curcumin, one of the anticancer drug occurring naturally, for drug loading and release studies. The encapsulation of chemopreventive drug curcumin was done by

adding 5 mg of curcumin into the hydrogel (5mg of **4c** in 5mL of DMSO-water mixture i.e. 1 % wt/v) followed by heating to get a clear yellow solution and cooled to room temperature. The formation of composite gel was confirmed by the observation of no gravitational flow upon test tube inversion.

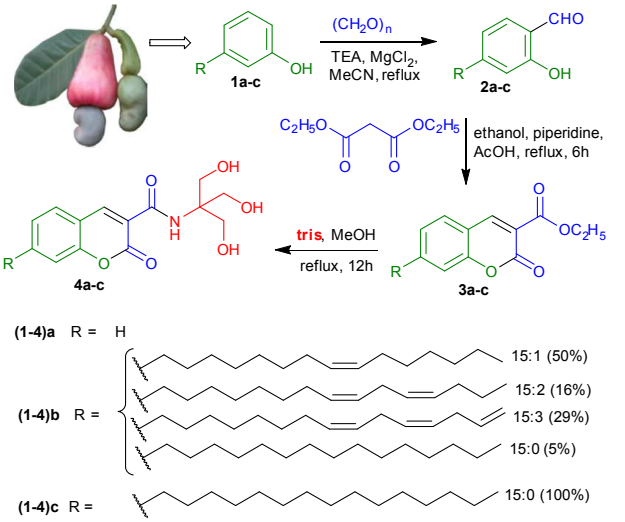
Stimuli responsive drug release studies

pH as stimuli responsive drug release studies: To the composite gel 10 mL of acidic buffer (pH = 4) was added and 1mL of aliquots was retrieved at different time intervals. On adding acidic buffer, the simultaneous degradation of composite gel and release of curcumin drug was visually observed. The drug release profile was monitored by UV-vis spectra by diluting 1.0 mL of the retrieved aliquot to 2.0 mL using double distilled water.

Metal ion, Fe^{3+} as stimuli responsive drug release studies: To the composite gel 10 mL of $FeCl_3$ solution of concentration $1X10^{-3}$ M was added and 1mL of aliquots was retrieved at different time intervals. On adding $FeCl_3$ solution, the slow diffusion of Fe^{3+} ions with simultaneous degradation of composite gel and release of curcumin drug was observed. The drug release profile was monitored by UV-vis spectra by diluting 1.0 mL of the retrieved aliquot to 2.0 mL using double distilled water.

Results and discussion

The stimuli responsive coumarin-tris derivatives were synthesised as follows: Electrophilic aromatic substitution reaction of cardanol, **1b** and n-pentadecylphenol (PDP), **1c** with paraformaldehyde in presence of $MgCl_2$ and TEA lead to the formation of cardanol-aldehyde **2b** and PDP-aldehyde **2c** respectively. The Knoevenagel reaction of compounds **2a-c** with diethylmalonate led to the desired ethyl coumarin-3-carboxylate **3a-c** in good yields. Ethylesters **3a-c** were converted into corresponding tris amide (**4a-c**) under mild conditions in good yield (Scheme 1). In this report we have synthesised and completely characterized (NMR and Mass spectral analysis) three different coumarin-tris compounds with varying hydrophobicity. Synthetic protocol used for the construction of amphiphiles are simple, green and economical for practical applications. Biocompatible tris were introduced as the hydrophilic part to empower the amphiphile for excellent biological functions. The gelation ability of compound **4a-c** was consistently studied for both hydrophilic and hydrophobic solvents by "stable to inversion" method.[2] Compound **4c** exhibit excellent hydrogelation in DMSO-water by heating, followed by sonication for few seconds. Compound **4c** shows critical gelation concentrations (CGCs) of 0.8 % (wt/v) in DMSO-water (1:2 ratio). Gel thus formed are thermo reversible, and stable at room temperature even for more than 5 months. In case of **4a** and **4b** remarkable stifling of gelation ability was observed, probable due to the lack of hydrophobic part in **4a** and the existence of a kink in hydrophobic part of **4b**. This result highlight the importance of maintenance of optimum hydrophilic and hydrophobic interactions, which driven to assemble into macromolecular structures.[8] In fact hydrogel formed by **4c** display a gel to sol transition upon heating-cooling cycles (T_g = 45°C) and T_g increases with increase in concentration of gelator. To acquire perceptivity into the protons involved in the self-assembly process, we have recorded ^{1}H NMR spectra of gelator **4c** in both solution and gel states.



Scheme 1. Synthesis of coumarin coupled tris amphiphile **4a-c**

In general a gradual shift or broadening of signals under aggregated/self-assembled state were observed in 1D ^{1}H NMR spectrum and folding/unfolding of self-assembled structure could be identified by 2D NMR spectra.[9] In 1D ^{1}H NMR spectra of **4c** dissolved in DMSO-d_6, protons in coumarin moiety H_a-H_d resonates at δ 8.87, 7.65, 7.22, 7.21 ppm respectively, whereas in gel form H_a-H_d protons experience downfield shift and resonate at δ 8.91, 7.92, 7.38, 7.42 ppm respectively. Note that for NMR studies, hydrogel was prepared by dissolving **4c** in DMSO-d_6 - D_2O solvent mixture and in gel form, all –OH and –NH peaks disappear because of D_2O exchange. This result clearly depicts the existence of π-π stacking in gel state (Figure 1), which was further confirmed by XRD analysis. The stacked ^{1}H NMR spectra of gelator **4c** and its gel is given in ESI.

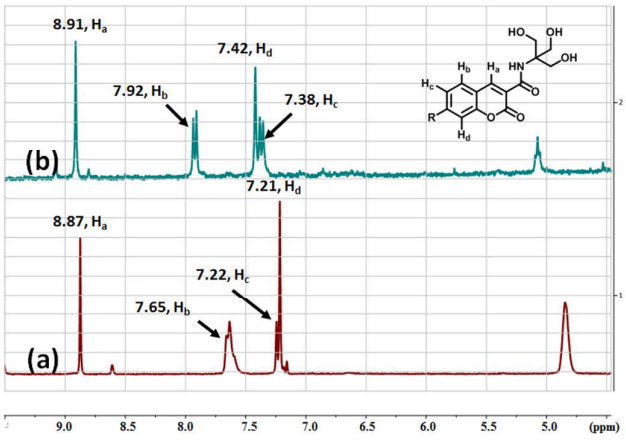


Figure 1. ^{1}H NMR spectra (aromatic region alone) of (a) gelator, **4c** in DMSO-d_6 and (b) gel formed by **4c** in DMSO-d_6-D_2O (1:2 ratio).

In order to obtain further insight into the aggregation mode at nanoscale we performed optical microscopy and FE-TEM. A slice of the gel was carefully taken and subjected for morphological analysis. The existence of well dispersed nanofibers in gel was also identified by optical microscopy (Figure 2a). From HRTEM analysis one can consistently observe a well-developed network structure composed of 3D entangled fibrous network with diameter range of 50-200 nm (Figure 2b). The existence of nanofiber with greater void volumes can trap more solvent in it as it is documented from HRTEM analysis.

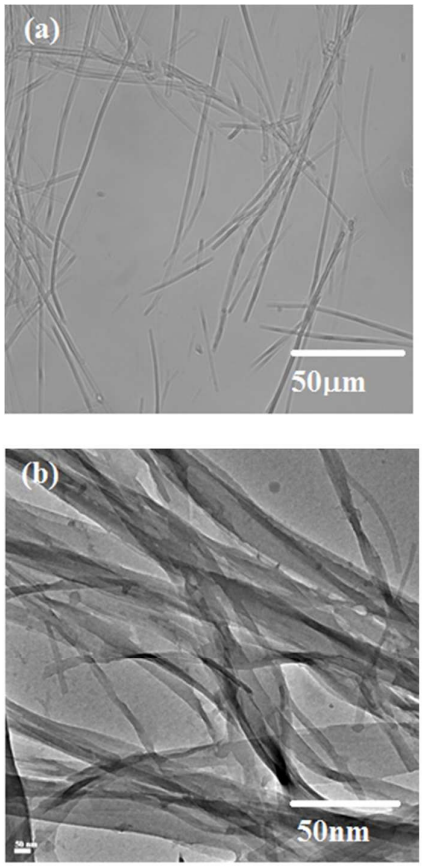


Figure 2. Morphological analysis of gel, **4c**. (a) Optical microscopy image of hydrogel formed by **4c** and (b) HRTEM image of xerogel of **4c**.

Small angle X-ray diffraction (SAXD) was employed to gain the additional perceptiveness into the structure that comprise the gel formed from **4c**. XRD of wet gel gave a Bragg's reflection at 4.02, 1.35, 1.00 and 0.45 nm. The energy minimized structure of compound **4c** indicate the molecular length of 2.74 nm, which is different from the observed d-spacing value (4.02 nm) from XRD studies (Figure 3a). The d-spacing value observed is in between the mono and double layer thickness formed by the end to end length of **4c**, which clearly validate the molecular self-assembly in an interdigitated fashion of hydrophobic moiety. The broad peak at 20º (0.45 nm) is assignable to the (001) aspect of π-π stacking of coumarin moiety (see ESI).[10] A schematic representation of gelator arrangement in gel phase is given in Figure 3b.

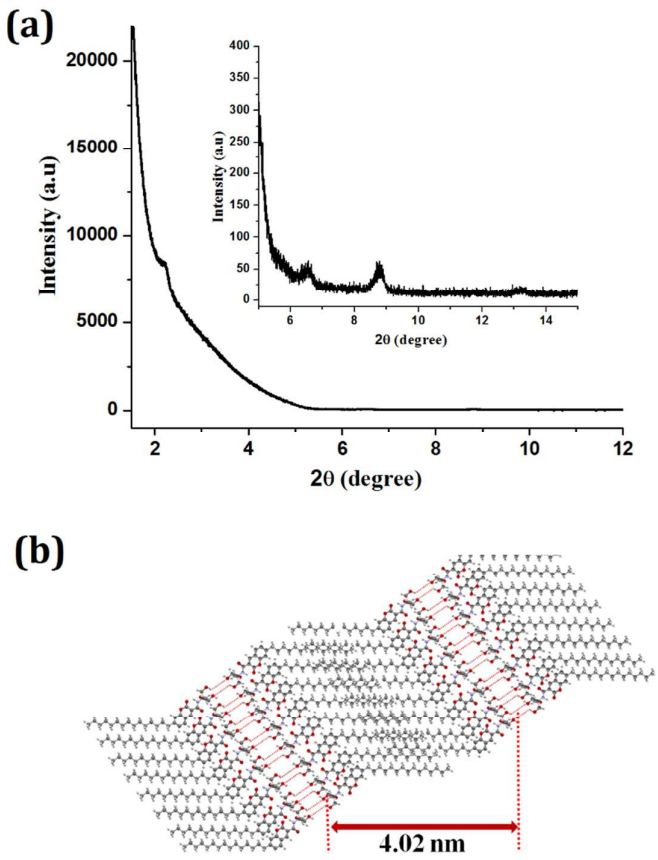


Figure 3. (a) SAXD pattern of gel formed by **4c** and (b) schematic representation of possible molecular packing models for the gel.

This gel is highly stable in neutral and basic pH for a long time, while change in pH from neutral to acidic condition by adding buffer solution (pH = 4.0) drive the slow conversion of gel to sol state. This behavior could be attributed due to the disturbance of H-bonding exerted by amide group. The gel-to-sol conversion in response with the external stimuli, pH could be directly visualized by naked eye. Molecular level commutes[11] occurred during this process was identified by HRTEM analysis. On addition of acidic buffer, fibrillar morphology of gel are changed to vesicular and nanotubular structures with average diameter of 300 nm and 50 nm respectively (Figure 4a & 4b). The average size of the vesicles was further confirmed using particle size analyzer. The average size of the vesicles formed by adding acidic buffer (pH = 4) into the gel, formed by **4c** was 243 nm (see ESI). It should be noted that the vesicles and nanotubes formed by **4c** were stable for even more than 3 months. The observed results indicate that the molecule self-assemble into fibers in both neutral and basic medium (as gel form) and vesicles and nanotubes in acidic medium. This property could be attributed to the H-bonding triggered self-assembly[12] behavior of **4c** under

different pH solution. Solubilization of hydrophobic drugs formulating a suitable delivery system is an ambitious chore in biotechnology.[13] By getting clue from the self-assembly behavior of **4c** in different pH levels, we gear-up to encapsulate hydrophobic drug, curcumin into the hydrophobic part of the hydrogel (Figure 4c). Subsequently encapsulated drug was released by breaking the gel by bringing the pH to acidic level (Figure 4d). Curcumin encapsulated gel (composite gel) was found to be reversible with respect to pH and temperature. Drug release by means of gel degradation is due to the dis-assembly of composite gel in acidic pH and this phenomenon could be visually monitored (Figure 4d). The pH triggered release of curcumin has been identified by adding acidic solution (pH=4.0) to composite gel and recorded the UV-vis spectra of the supernatant solution periodically. Aliquot collected at 0 min did not display any absorbance peak corresponding to curcumin. As time increases, degradation of composite gel started and supernatant solution becomes yellow in color. Aliquot collected at different time intervals such as 10 min, 20 min and 30 min displays absorbance peak of curcumin and intensity increases with increase in time (Figure 4e). Metal ion, Fe^{3+} plays a significant role in biological processes as metalloenzyme and hemeprotein, to name a few.[15] It has been reported in literature that coumarin coupled tris can form co-ordination complex with Fe^{3+} ions.[16] By getting clue from the literature,[16] we step-up further to release the encapsulated drug by adding Fe^{3+} solution, as an external stimuli. In addition to pH, degradation of composite gel was also triggered by adding a solution containing Fe^{3+} ion. Metal ion actuated release of curcumin was achieved by adding Fe^{3+} solution of (1 X 10^{-3} M) on preformed composite gel and recorded UV absorption of aliquots collected at different intervals of time. Gel degradation occurred via slow diffusion of Fe^{3+} ion into the composite gel. Aliquots collected at various interval of time (1 min, 10 min, 20 min, 1 h, 2 h, 3h and 12h) showed absorption maxima at 445 nm, which corresponds to the absorption peak of curcumin. From UV-vis measurements, we could clearly observe the steady release of curcumin from the composite gel with respect to time (Figure 4f).

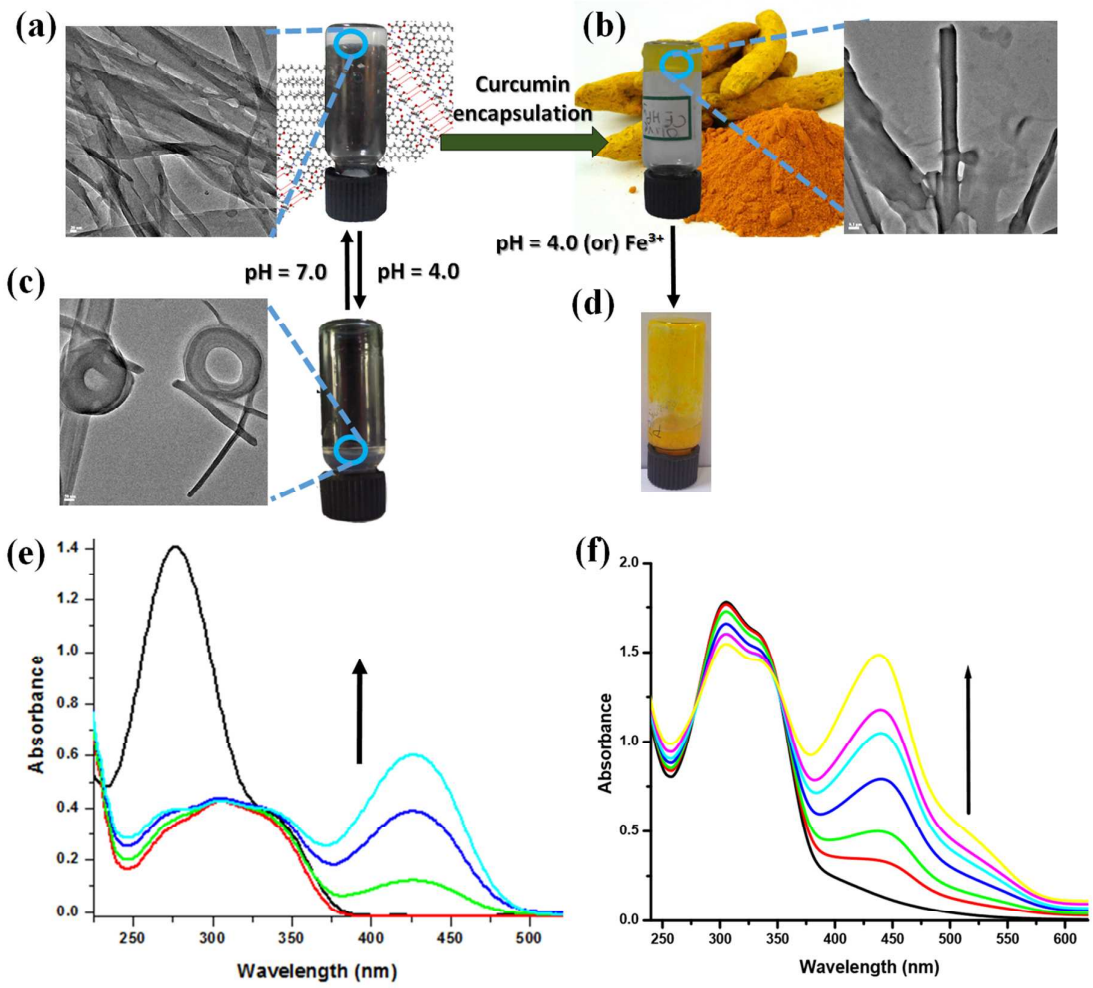


Figure 4. (a & b) Photographs of hydrogel and composite gel formed by **4c** and its corresponding HRTEM images respectively; (c) photograph of reversible conversion of gel-to-sol under various pH level and its corresponding HRTEM image; (d) photograph showing the degradation of composite gel under acidic pH and Fe^{3+} ion and (e) UV-vis spectra of gelator, **4c** dissolved in ethanol, 1X10^{-5}M (black line) and profile of pH as stimuli responsive curcumin release from composite gel at regular

interval of time such as 0 min, 10 min, 20 min and 30 min (red to cyan blue) respectively; (f) UV-vis profile of Fe3+ as stimuli responsive curcumin release from composite gel at different time intervals such as 1 min, 10 min, 20 min, 1 h, 2 h, 3h and 12h (red to yellow) respectively. For UV-vis measurements, 1 mL of aliquot was retrieved at different intervals of time and diluted to 2 mL using distilled water. Direction of arrow shows the increase in intensity of curcumin absorption peak with respect to time.

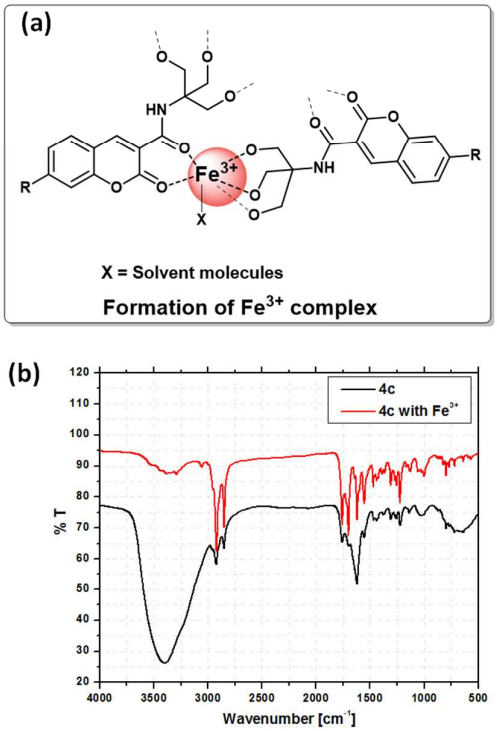


Figure 5. (a) Proposed binding mode of compound **4c** with Fe^{3+} ions and (b) FT-IR spectra of xerogel of **4c** and its corresponding Fe^{3+} complex.

Subsequent coordination of Fe^{3+} with carbonyl and –OH groups of **4c,** as identified using FT-IR spectra, lead to the dis-assembly of composite gel which directly trigger the drug release (Figure 5a & 5b). The possible mode of binding of Fe^{3+} with **4c** is given in figure 5a.There are few stimuli responsive molecular gels were reported.[17] However, only a little is known regarding the multi stimuli responsive biocompatible hydrogel. This report represents the first example of a pH and metal ion as stimuli responsive coumarin-tris based hydrogel derived from a renewable resource.

The rigidity and flow behavior of gel formed by **4c** were investigated. The elastic behavior of a material could be directly identified by measuring the difference between G′ and G′′.[18] Storage moduli (G′) and loss moduli (G′′) of gel formed from **4c** and composite gel as the function of angular frequency (elastic response) is shown in Figure 6. In both the cases throughout the entire range of frequency sweep, the values of G′ are found to be more than that of G′′. The difference in these two moduli ΔG (G′-G′′) of gel formed by **4c** is calculated as 27300 pa, which is comparatively higher than that of corresponding composite gel (ΔG = 23440 pa). Thus incorporation of drug in the gel network have only little effect in the rheological behavior (Figure 6a). Visco-elastic behavior of these gels were independent of frequency sweep, and this result suggesting that it possess good tolerance to external forces.

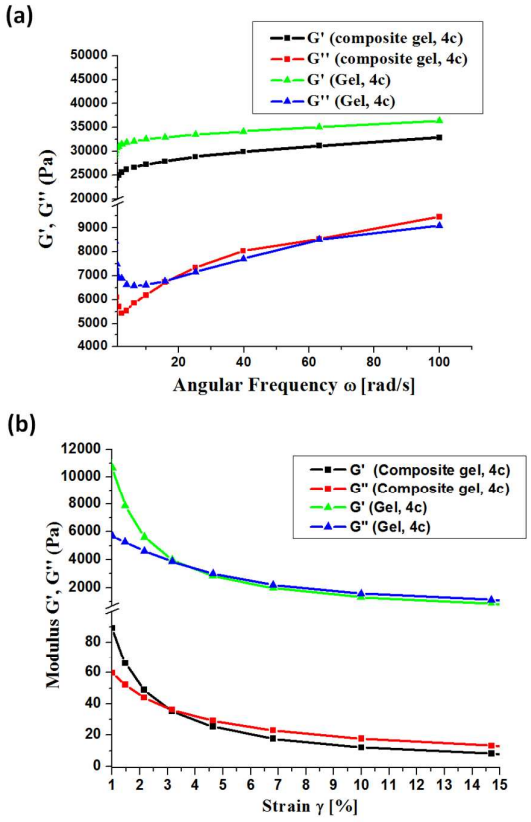


Figure 6. (a) Angular frequency dependence of G′ and G′′ of gel, **4c** and composite gel and (b) strain amplitude dependence of G′ and G′′ of gel, **4c** and composite gel.

The rheological property of a viscoelastic material are independent of strain up to a critical strain level (γ_c). Beyond γ_c, the storage modulus start decay and the material behaves in a non-linear fashion. It is worth to measure G′ and G′′ of gel formed from **4c** and its corresponding composite gel in dependence of strain amplitude. Figure 6b shows a strain sweep of gel formed from **4c** and its composite gel. With gradual increase in strain, G′ and G′′ remains constant and at a certain point gradual decrease was observed in a linear fashion and cross over occurs between G′ and G′′, the point at which the cross over occurs is considered as critical strain (γ_c) of a gel. The γ_c for gel formed by **4c** and its composite gel is 3.88 and 3.31% respectively. Below γ_c (G′>G′′) the self-assembled structure is intact and have solid like behavior, and this result clearly depicts the formation of highly structured material. By increasing the strain above γ_c, disturbs the self-assembled network and eventually become fluid-like. The crossover of G′ and G′′ demonstrate the reversible nature of gel.

Conclusions

In conclusion, we have designed and synthesised a new class of coumarin-tris based amphiphiles from renewable resource, that self-assemble into gels at neutral and basic pH levels, and vesicles and nanotubes at acidic pH level. Self-assembled structures were stabilized through the extensive hydrogen bonding and π-π stacking interactions that are existing in the gelator. We have demonstrated the reversible sol-gel switching of coumarin-tris based gel in the presence of pH and metal ion stimuli. We also depicted the encapsulation of chemopreventive drug curcumin into the hydrogel, and pH and Fe^{3+} triggered encapsulated drug release into the solution also performed. Rheological studies clearly depicts the stability and mechanical strength of gel and composite gel. We envision that this hydrogel could play a significant role in pharmaceutical science, in particular for the development of new generation of stimuli responsive drug delivery systems for in vivo formulations.

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