

Dipeptide-SWCNTs-based Supramolecular Hybrid Hydrogel

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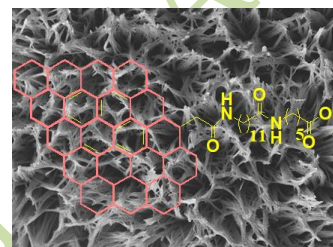
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Received: October 22, 2021 | Accepted: November 11, 2021 | Published online: December 23, 2021

Abstract

A dipeptide appended pyrenebutyric acid's amide forms hydrogel at basic pH (pH of the gelator dissolved solution ~ 13.7 at 25 °C temperature) with an estimated minimum gelation concentration (MGC) of 0.29 % w/v. The hydrogel was characterized meticulously by using various microscopic and spectroscopic techniques including Transmission Electron Microscopy (TEM), Field Emission Scanning Electron Microscopy (FE-SEM), X-ray diffraction, fluorescence spectroscopy and rheology. The hydrogel exhibits bluish green fluorescence emission colour. The gelator molecule itself can disperse pristine-single walled carbon nanotubes (SWCNTs) in basic aqueous medium and it can also form a hybrid hydrogel with SWCNTs under the hydrogelation condition. Fluorescence, morphological and rheological properties of the hybrid hydrogel are also different from the native hydrogel.



Keywords: Peptide. Amphiphile. Hydrogel. Fluorescence. SWCNTs. Rheology

1. Introduction

Low molecular weight hydrogels¹ are an emerging area of interest in the field of current research due to their numerous prospective applications². Hydrogelation arises from the fine-tuning of the self-assemble molecules in an aqueous medium based on the hydrogelator-hydrogelator, hydrogelator-solvent and indeed solvent-solvent interactions. Supramolecular hydrogels can be stimuli³ responsive including pH,⁴ temperature,⁵ light,⁶ ultrasounds⁷ and so on. Low molecular weight hydrogels can be considered as a new class of soft material offering emerging targeted applications.^{8,9,10,11} Hydrogel is an increasing field of research due to their application in biomaterials,¹² drug delivery¹³ and waste water treatment¹⁴. There are several examples in the literature of low molecular weight hydrogels.¹⁵ Among these reported hydrogelators, the peptide-based hydrogelators are attracted special research interest due to their biological relevance, functional modality, ease of synthesis, prone to self-assemble by using various non-covalent interactions and cost effectiveness. Peptide-based hydrogelators are also common in literature.¹⁶ However, peptide-based low molecular weight amphiphilic fluorescent bluish green emissive, large Stokes shift and narrow bandwidth hydrogelators are not yet well explored.¹⁷ Adams and coworkers have reported the self-assembling mechanism of naphthalene-alanylvaline which undergone hydrogelation in presence of glucono- δ -lactone.¹⁸ Xu and his coworkers have made a significant contribution in the field of fluorescent hydrogels.¹⁹ They have also demonstrated that the aromatic-aromatic interactions lead to the formation of penta-peptide-based fluorescent hydrogel.²⁰ Banerjee and co-workers have

reported recently a histidine-containing peptide-based amphiphile for three-dimensional cell culture using mouse fibroblast cell line and a tripeptide-based self-shrinking hydrogel for waste-water treatment and the removal of toxic organic dyes and lead ions.²¹ Hamley and co-workers have discovered RGD-based peptides for hydrogelation.²² Several research groups have contributed significantly in the field of peptide hydrogels.²³

However, synthesis and formation of amphiphilic fluorescent hydrogelator that can disperse nicely pristine single walled carbon nanotubes (SWCNTs) as well as form hydrogels with SWCNTs is a hot topic in the field of hybrid materials research. However, the main problem is the superior dispersion of pristine SWCNTs in aqueous solvents. Therefore dispersion and formation of pristine SWCNTs is a challenging issue. This is because of the fact that carbon nanotube and linked system have shown various interesting phenomenon like high aspect ratio, mechanical strength as well as electrical conductivity that can make them a suitable candidate ranging from optoelectronics to biomedical fields.²⁴ Carbon nanotube-based nanomaterials have attracted research interest due to their various applications.^{25, 26, 27} In carbon nanotube containing hydrogels the pristine SWCNTs were well dispersed into the gels that can lead to impact of π - π interactions of the fluorescent hydrogelators with the pristine carbon nanotube wall. Bhattacharya and his coworkers have demonstrated the incorporation of pristine as well as functionalized SWCNTs within the trans tri(p-phenylenevinylene) bis-aldoxime-based organogelators. They have also made L-alanine-based organogel that can able to form gels with SWCNTs, which show thermo responsive behavior triggered by near IR irradiation.²⁸

Ajayaghosh and coworkers have also demonstrated carbon nanotube triggered self-assembly of oligo(p-phenylene vinylene)s to make stable hybrid-organo- π -gels.²⁹ Aida and coworkers have synthesized imidazolium-ion-based ionic liquids for the dispersants of CNTs.³⁰ Harada and coworkers have demonstrated the β -cyclodextrins (β -CD) that can be used to make SWCNTs-based hybrid hydrogels using host-guest contacts between β -CDs of pyrene incorporated β -CD and SWCNTs. This SWCNTs containing hydrogel has also shown gel to sol transition with the addition of reasonable host as well as guest molecules.³¹ However, there is a real significant interest to make pristine SWCNTs disperse in aqueous media as well to form the fluorescent gelator.

In this study, the synthesis and characterization of low molecular weight pyrene-based dipeptide hydrogelators successfully in 1 (M) NaOH medium with a minimum gelation concentration of 0.29 % w/v has been demonstrated. The hydrogelator has shown excellent fluorescent properties including large Stokes shift, bluish green emissive and narrow bandwidth. The hydrogelator can disperse pristine SWCNTs in aqueous media up to a maximum level of 84 % as well make hydrogel with pristine SWCNTs. The morphology, fluorescence properties and rheological properties of the hydrogelators have also been changed upon the incorporation of pristine SWCNTs into the hydrogel.

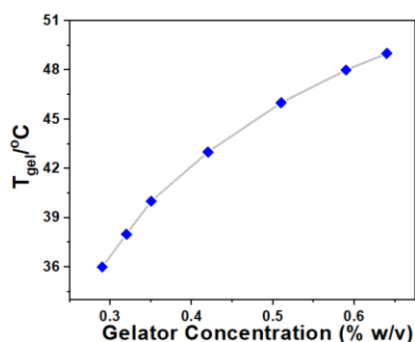


Figure 1. The change in T_{gel} values with respect to various concentrations of the hydrogelator.

2. Experimental

Synthesis of Dipeptide: The dipeptide was synthesized by conventional solution-phase methodology through racemization free fragment condensation approach. The Boc group was used for N-terminal protection and the C-terminus was protected as a methyl ester. Couplings were mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). Methyl ester deprotection was carried out by means of the saponification method, and the Boc group was deprotected by 98 % formic acid. The de-BOC compound has been coupled with 1-pyrenebutyric acid following solution phase methodology. All the intermediates were characterized by 300 MHz ^1H NMR and mass spectrometry. The final compound was fully characterized by 300 MHz ^1H NMR spectroscopy and mass spectrometry. The synthetic details,

instrumentation and supporting data have given in the supporting information.

3. Result and discussion

Preparation of hydrogel and characterization: Xu and coworkers have demonstrated antibiotic gelator-vancomycin-pyrene-based hydrogel via hydrophobic interactions and hydrogen bonding promoted self-assembly in water.³² Ossipov and coworkers have reported pyrene conjugated hyaluronic acid hydrogel.³³ Hahma and coworkers have reported pyrene-based low molecular weight organogels which have the ability to effectively bind with polar surface.³⁴ However, in this study, pyrenebutyric acid derivative of dipeptide has undergone hydrogelation in 1 (M) NaOH solution with a minimum gelation concentration of 0.29 % w/v. At first, the hydrogelator was dissolved in 1 (M) NaOH solution (2.9 mg/mL) under mild heating in a sealed tube. Then it was allowed to cool to room temperature for 1 h to obtain a transparent gel. Sol-gel transition temperatures (T_{gel}) for the gelator at different concentrations (% w/v) have been plotted against different gelator concentrations (Figure 1). This plot shows that the T_{gel} values of the dipeptide-based hydrogel increases with an increase in concentration (% w/v) of the hydrogelator until a typical concentration is attained. From the T_{gel} plot it can be stated that at a particular concentration the T_{gel} is fixed i.e. this is the saturated limit.

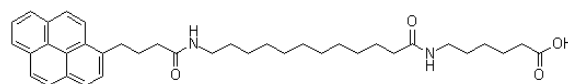


Figure 2. Chemical structure of the hydrogelator.

The hydrogelator (Figure 2) contains a fluorescence active pyrene moiety. It is interesting to note that the hydrogelation behavior has been monitored using fluorescence spectroscopy. The gelator molecules have offered distinguishable fluorescence spectrum in sol as well as in gel states (Figure 3). In sol state the hydrogelator has shown fluorescence emission peak at 434 nm region. However, the hydrogel has offered fluorescence emission peak at 463 nm (Figure 3). Interestingly, after the formation of hydrogel the fluorescence emission peak has been shifted from 434 nm to 463 nm. The red shifting clearly shows the aggregation of the hydrogelator molecules to form the hydrogel. The emission peak at 463 nm may be due to the formation of excimer complex involving the pyrene moieties. The PL and PLE spectra of the hydrogelator has been shown in Figure 4 at MGC.

The photographic view of the hydrogel in visible light as well as in presence of UV light was having wavelength 365 nm have been shown in the inset of the Figure 4. The quantum yield of the hydrogel has been calculated as 5.43 % with respect to standard dye quinine sulphate ($\Phi_s = 0.546$ in

water). The detail procedure has been given in supporting information.

The hydrogel has been characterized thoroughly using FE-SEM, TEM, X-ray diffraction (XRD), fluorescence spectroscopy and rheology. The freeze dried hydrogel sample has shown nanofibrous (flake type) network structure (Figure 5a) which is evident from the FE-SEM image. Transmission electronic microscopic (TEM) analysis has also shown nanofibrous network structure (Figure 5b).

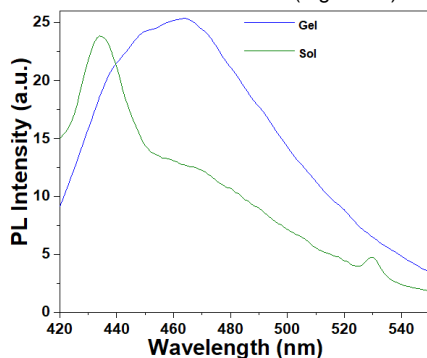


Figure 3. Fluorescence spectra of the hydrogelator in gel (0.29 % w/v) state and in sol state (10 times diluted from MGC) at an excitation wavelength of 387 nm (PLE of the hydrogel, see Figure 4).

The self-assembly nature of the hydrogel has also been characterized by means of X-ray diffraction analysis of the xerogel. Wide angle X-ray scattering has been performed to understand the molecular packing of the hydrogels. The xerogel has been obtained from the pyrene-based dipeptide

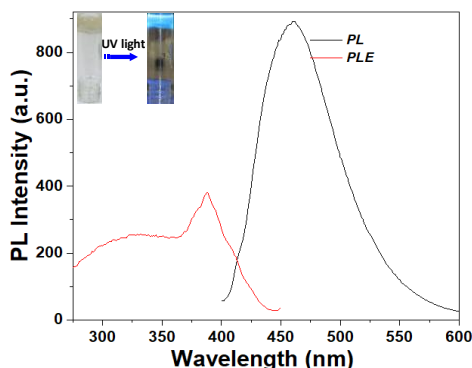


Figure 4. Fluorescence emission (PL) and fluorescence excitation (PLE) spectra of the hydrogel at MGC.

hydrogel, offer π - π stacking phenomenon within the π moieties of the pyrene. However, a broad peak pattern has been obtained from the wet gel material. This may be due to the heavy scattering of the water molecules within the hydrogel. Both in xerogel and in wet gel states the peak at region $2\theta = 25$ to 26 degree is seen and this is due to the π - π interaction between the π moieties of the gel (Figure S3).

Pristine SWCNTs dispersion and characterization: Stable pristine SWCNTs effective dispersion in water has been carried out by using mainly two ways: (1) covalent

modification of the graphene walls and (2) noncovalent wrapping of the pristine SWCNTs using oligopeptide/ biomolecules/ polymers or amphiphilic small molecules.³⁵ However, a noncovalent modification by using a suitable dispersing material is more convenient as the intrinsic properties of the graphene sheet is compromised whereas in the former process, the π cloud get disrupt indeed. Moreover, the dispersing agent should be able to disperse high amount of pristine SWCNTs and the adhesive interactions should be greater than the cohesive interaction so that proper rebundling of pristine SWCNTs can be done in water. In a typical dispersion of pristine SWCNTs in water, at first 2 mg of the hydrogelator has been dissolved in 1 mL of 1 (M) NaOH solution, then 0.5 mg of pristine SWCNTs has been added to the gelator containing clear 1 mL solution (Figure 6). The entire mass has been mixed well and it has been subjected to sonication of time 60 minutes in a bath sonicator. After the completion of sonication, it appeared like a homogeneous black solution as shown in the Figure 6. The % of SWCNTs dispersion has been calculated by using the measurement of absorption just after the sonication and after 1 h of centrifugation time at 21952 g. In both cases the absorptions have been recorded at 550 nm. Then the % of dispersion has been calculated directly by using Coleman and co-workers procedure.³⁶ The optical density value and concentration used to estimate the % of dispersion and it has been calculated to be 84 %. This value is much higher than the reported dispersion by different research groups using SDBS and other surfactants.³⁷ It is interesting to note that the superior dispersion of hydrophobic pristine SWCNTs has been achieved due to the π - π interactions of the pyrene- π cloud with the SWCNT's π system and also due to the strong van der Waals interactions with the long chain peptide molecules. The dispersed SWCNTs has been characterized by using UV-Vis-NIR study (Figure S4).

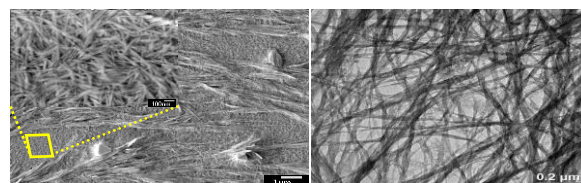


Figure 5. (a) Field emission scanning electron microscopic (FE-SEM) analysis (inset shows enlarged view of the network structure) and (b) Transmission Electronic Microscopic (TEM) analysis of the hydrogelator in the xerogel state.

Incorporation of SWCNTs into the self-assembled hydrogel network:

The pyrene-based amphiphilic dipeptide moiety has been weighed for 3 mg and then 1 mL of 1 (M) NaOH has been added. Then the total solution has been sonicated for 30 s and subjected to heat very gently keeping the temperature of the sonicator kept at about 50 °C. A measured amount of pristine SWCNTs has been added to this gelator solution in hot condition and subjected to sonicate keeping the temperature of the bath water at about 50 °C. After 30 minutes of sonication, the total mixture has been heated in a hot plate to get clear black colored solution and subjected to cool at room temperature to get a black colored

hydrogel. The SWCNTs incorporated hydrogel has been characterized by using FE-SEM, rheology and Raman spectroscopic analysis. The optimum concentration of SWCNTs is 0.075 % w/v that can be dispersed as well as incorporated into the hydrogel.

Raman Study of the hybrid hydrogel: Single walled carbon nanotube (SWCNTs) has demonstrated to be a distinctive system for the analysis and study of Raman spectra. Raman spectroscopic analysis of the hybrid hydrogel has been done to ensure the dispersion as well as the encapsulation of pristine SWCNTs into the hydrogel matrix. The Raman spectral analysis of the hybrid hydrogel ample has been shown in Figure 7. It is evident from the Figure 7 that the presence of RBM (The Radial Breathing Mode), G band (due to stretching of C-C bond of graphitic materials) and G' band (due to sp² carbon materials). In the Figure S5 RBM mode, G and G' bands are shown separately. The RBM bands are visible indicating the presence of the two peaks at 161 nm and 216 nm, where as the peak at 1665 nm and 2596 nm are corresponding to G and G' respectively. The presence of these above mentioned characteristic peaks in the hybrid hydrogel system suggest the successful incorporation of SWCNTs into the pyrene containing hydrogel material.

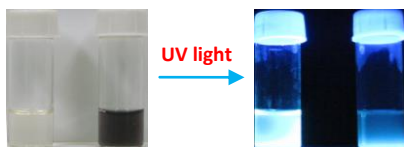


Figure 6. Photograph of the hydrogelator and SWCNTs dispersed hydrogelator solutions in presence of visible light and in presence of UV lamp having wavelength 365 nm.

Effect of SWCNTs to fluorescence into the hydrogel: It is interesting to note that after the incorporation of pristine SWCNTs within the hydrogel matrix the fluorescence emission due to the fluorophore moiety pyrene has been decreased. Here, I have studied the fluorescence-quenching phenomenon, which has been directly proportional to the amount of incorporated SWCNTs into the hydrogel. Different weight/volume ratios were achieved by changing the amount of SWCNTs and keeping the concentration of the gelator unchanged at 0.07 % w/v the fluorescence intensity has almost 83 % decreased (Figure S6). The quenching may be due to the fact the energy may be transfer from the fluorophore pyrene moiety to the SWCNTs.³⁸ This energy transfer leads to the lowering of quantum yield of the hydrogelator as well as short lived fluorescence materials.

Time resolved fluorescence spectroscopic studies: Fluorescence decays of the hydrogel and the SWCNTs incorporated hydrogel have been measured at an excitation of 340 nm. The fluorescence decay profiles of the hydrogel and the SWCNTs containing hydrogel have shown in Figure S7. The average fluorescence decay time has been calculated to be 22.83 ns for the peptide hydrogel. However, the SWCNTs incorporated hydrogel fluorescence decay time

has been found to be 3.35 ns. So, upon the inclusion of pristine SWCNTs into the hydrogel the fluorescence decay time has been significantly lowered. This may be due to the transfer of electron from the pyrene-excited state to the single walled carbon nanotube system.³⁹ Thus it can be stated that the energy transfer is indeed the dominating deactivation pathway of the pyrene-excited state in the SWCNTs incorporated hydrogel.

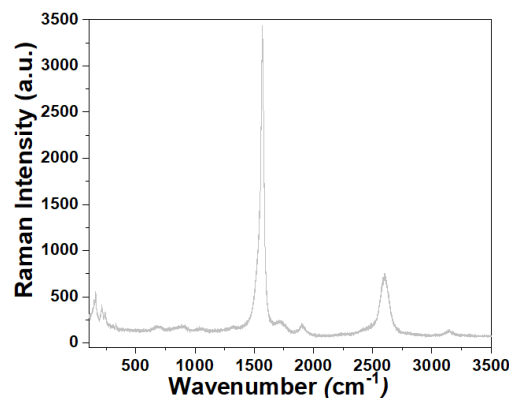


Figure 7. Raman spectroscopic analysis of the dried SWCNTs-hydrogel sample.

Morphology of the hybrid hydrogel: FE-SEM analysis of the native hydrogel has clearly demonstrated the presence of extended nanofibers network structure (Figure 5a). However, upon incorporation of pristine SWCNTs into the hydrogel matrix the morphology of the hybrid system has been changed. Actually, the native hydrogel fibrils are flakes type of network structure however, the hybrid hydrogel has been appeared as fibrillar bundle structure network in a regular fashion (Figure 8). FE-SEM image indicated a change in morphology of the pyrene containing peptide-based hydrogel upon the hybrid hydrogel formation with SWCNTs from cross-linked network structure to fibrillar directed assembled structure with pocket type structure.

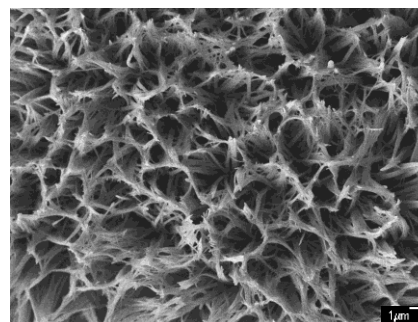


Figure 8. Field emission scanning electron microscopic (FE-SEM) analysis of the SWCNTs containing hybrid xerogel sample.

Rheological studies: It is interesting to measure the rheological property of the hybrid hydrogel to investigate the influence of the incorporated SWCNTs into the native pyrene appended dipeptide-based hydrogel and also the fate of visco-elastic property of the native hydrogel upon the incorporation of SWCNTs. Storage modulus (G') and loss

modulus (G'') have been recorded as a function of angular frequency for both the native hydrogel as well as for the SWCNTs containing hybrid hydrogel over a range of angular frequency in a frequency sweep experiments. The G' and G'' for the native hydrogel as well as for the hybrid hydrogel (Figure 9) are almost parallel to the applied frequency over the entire range of the experiments. Rheological experiment shows that there is little enhancement of gel stiffness upon the hybrid hydrogel formation.

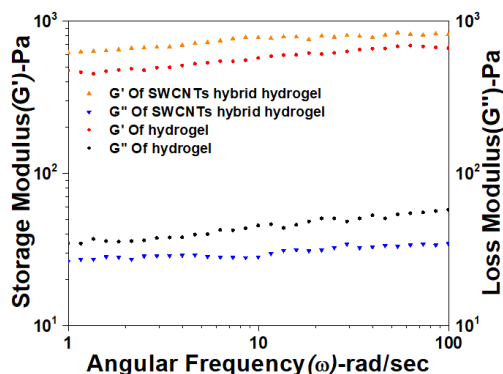


Figure 9. Storage modulus and loss modulus profile of the hydrogel and the SWCNTs containing hybrid hydrogel as a function of angular frequency.

4. Conclusions

This study has demonstrated the formation of pyrene containing dipeptide-based fluorescent supramolecular hydrogel in basic medium. This supramolecular hydrogel exhibit bluish green emissive fluorescent property. Interestingly, the pristine SWCNTs can be dispersed in aqueous media in presence of the pyrene containing peptide hydrogelator up to a maximum of 84 %. Pristine SWCNTs can be incorporated into the fluorescent hydrogel. The fluorescent property, morphology and rheological property of the native hydrogel changed after the hybrid hydrogel formation. The fluorescent hydrogel becomes rigid after the incorporation of SWCNTs into the native hydrogel.

5. Acknowledgements

SR wishes to thanks DST Inspire Faculty Award and Research Grant (IFA17-CH269).

6. Notes and References

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