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ARTICLE

Can Self-Assembled Hydrogels Composed of Aromatic Amino Acid Derivative function as drug delivery carrier?

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Low molecular weight hydrogelators (LMOHGs) have attracted recent attention due to their diversified applications. In an attempt to artificially imitate their importance in the design of drug delivery carrier, we have synthesized two simple *N*-terminaly protected aromatic amino-acid derivatives that form efficient stable hydrogels at room temperature. The gelation property of the hydrogels have been thoroughly investigated by various techniques and its strength has been determined by rheological studies. Inorder to explore the efficacy of the hydrogels as tools for drug delivery, we have developed hydrogel nanoparticles (HNPs)using a surfactantand high speed homogenization approach. Interestingly, ourhydrogel nanoparticles display good entrapment efficiency and release kinetics of the model drug 5-Fluoro Uracil from the hydrogel matrix . Our experimental results reveal that hydrogel **II** displays slightly higher efficiency as a drug delivery carrier may be due to the presence of aromatic ring in the backbone incomparison to hydrogel I. Thisincreased strength may be attributed to the increase of π–π interaction when the aromatic residue is present in the backbone. Therefore the nanoparticles generated from hydrogel **II,** may have better hydrogen bonding ability with drug in comparison to the hydrogel**I**. Henceforth resulting in slightly slower release of drug from the hydrogel matrix.This fact may shed some light about the candidature of our hydrogels as future carriers for drug delivery.However, further studies to evaluate the candidature of these novel type of aromatic amino acid hydrogel nanoparticles for nano-medical applications is under investigation.

Introduction

The principle of self-assembly involves the construction of complex architectures from biological building blocks which exhibits diversified applications in drug delivery, tissue engineering, electronic devices etc.[1-4]The building blocks that have been extensively known for generation of novel structures are nucleic acids, phospholipids and large peptidyl building blocks.[5-8]Like large polypeptides, short amino acid derivatives and peptides too, can self-assemble into various nanostructures as well as nanoscale ordered hydrogels.[9-18]

Low molecular weight hydrogelators(LMOHGs) composed of amino acids and short peptides represent a promising approach to drug delivery pathways.[19]This is because of the fact that these molecules are of biological origin and possesses non-toxic behavior. They can form specific secondary, tertiary and quaternary structures which provides unique opportunities for the design of nanomaterials,that doesnot exist with traditional organic molecules.[20]

Peptide based hydrogel nanoparticles (HNPs) have gained momentum in recent years as promising nanoparticulate drug delivery system.[21-24] This occurs due to a combination of two different characterestics a) Hydrophilicity and extremely high water content of hydrogels and b) nanoparticle which is exclusively small in size. The amino acid/peptide hydrogel nanoparticles (HNPs) can be modulated rationally by controlling the hierarchical self-assembly process which includes the non-involvement of any potentially hazardous chemicals such as cross-linkers, that may affect its biocompatibility.[24-28]Moreover, their synthesis procedure is very simple and in vivo degradability is non-toxic due to the fact that they are composed of simple ecofriendly amino acids. Although, till date significant examples are documented in the literature, majority of them either involves synthetic and natural polymers or longer peptides/peptide amphiphiles as building blocks for drug delivery systems.[29-41]. The exploration of hydrogel nanoparticles from short peptides/simple aromatic amino acid derivatives, where the concept of self-assembly has been explicitly exploited remains in a rudimentary stage.[42]

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Electronic Supplementary Information (ESI) available: [details of any spectraFigures of gel, ^{1}H NMR, IR, Mass,]. See DOI: 10.1039/x0xx00000x

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As a part of the investigation, in this report our effort lies in modulating the excellent hydrogelating ability of two *N*-terminally protected aromatic amino acid derivatives, Benzoyloxy carbonyl phenylalanine (Z-Phe-OH) (Hydrogelator**I**) and Fluorenylmethoxy carbonyl meta amino benzoic acid (Fmoc-*m*-ABA-OH) (Hydrogelator**II**) (Figure 1) that forms efficient, stable hydrogel in water, at room temperature with a minimum gelator concentration of 0.02 % and 0.05 % w/v, respectively. The gelation properties of the hydrogel have been investigated by scanning electron microscopic studies, FT-IR, and rheological studies. Inorder to explore the efficacy of the hydrogels as tools for drug delivery, we have developed hydrogel nanoparticles (HNPs) using a surfactant and high speed homogenization approach. Our hydrogel nanoparticles display good entrapment efficiency and release kinetics of the model drug 5-Fluoro Uracil from the hydrogel matrix.This fact may shed some light about the candidature of our hydrogels as future carriers for drug delivery.

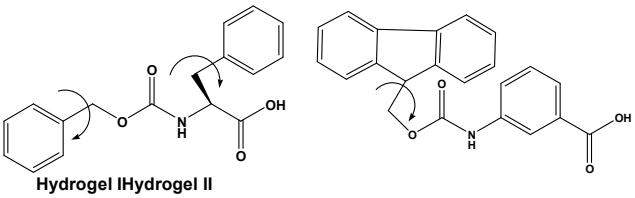


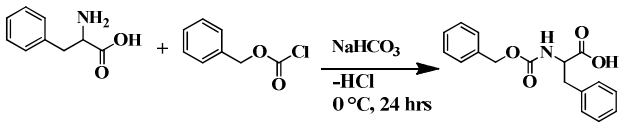
Figure 1. Chemical structures of Hydrogel**I** and **II**

Experimental

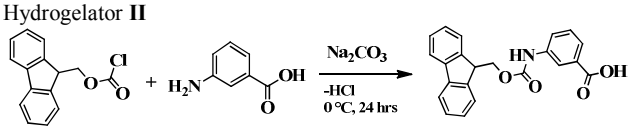
Materials and methods:

Benzoyloxy carbonyl (Z) and Fluorenylmethoxy carbonyl (Fmoc) chlorides, 5-Fluoro-uracil (5-FU) and all other chemicals were purchased from Spectrochem chemicals. The surfactant Vitamin-E-TPGS was purchased from Sigma Aldrich Chemicals Company Pvt. Ltd. All solvents used in the synthesis were purified, dried, or distilled, as required. ^{1}H NMR spectra were recorded by using Bruker Ultra shield (400MHz) spectrometer. Mass spectra were recorded in ESI-MS mode on MicroTOF-Q-II instrument manufactured by Bruker Daltonics; IR spectra were recorded by using Shimadzu, Prestige 21 FT-IR spectrometer.

Hydrogelator**I**



Hydrogelator **II**



Synthesis of Amino Acid Derivatives I and II

The amino acid derivatives**I** and **II** were synthesized using conventional solution phase methodology, with racemization free techniques. All the intermediates obtained were checked for purity by thin layer chromatography (TLC) on silica gel.The final derivatives were purified by column chromatography using silica gel (100-200 mesh) as the stationary phase and ethyl acetate and petroleum ether mixture as the eluent. The reported derivatives **I** and **II** were fully characterised by NMR, IR spectroscopy and mass spectrometry.

Z-Phe-OH (Hydrogelator I): Phenylalanine (2.0gm, 12.12 mmol) was dissolved in 20 ml of 20% sodium bicarbonate solution and 4.0 gm of benzoyl chloride was added to it and shaken vigorously in a stoppered flask. The stopper was removed from time to time since carbon dioxide is evolved. When the odour of benzoyl chloride completely disappeared, it was acidified with dilute hydrochloric acid and extracted with ethylacetate as the organic phase. The organic layer was evaporated to get a solid brownish derivative.

Yield: 3g (83%); m.p. 343 - 344°C. FTIR (KBr pellet, cm-^{1}): 1538, 1643, 1696, 3329.^{1}H-NMR ($CDCl_3$, ppm): 2.8(1H,d,C$^\beta$H of Phe), 3.08(1H,d, C$^\beta$H of Phe), 4.3(2H,m,C$^\beta$Hs of benzyloxycarbonyl), 5.0 (1H,m,C$^\alpha$H of Phe), 7-7.4(10H,m,Aromatic H's of Phe and Benzoyloxycarbonyl),7.6(1H,d,NH of Phe), 12.8(1H,br, COOH of Phe).

Fmoc-mABA-OH (Hydrogelator II):*m*ABA-OH (2 g, 14.58 mmol) was dissolved separately in basic sodium carbonate solution (36.4 ml) and cooled in an ice-water bath. Then to it, cooled solution of Fmoc-Cl (5.6 g) in dioxane (36.4 ml) was added. The reaction mixture was allowed to come to room temperature and stirred for 24 hrs. The pH was adjusted to acidic condition with 1:3 HCl and extracted with ethylacetate as the organic phase. The organic layer was evaporated to get a solid brownish product of Fmoc-mABA-OH.

Yield: 4.19 g (80%); m.p. 220-225°C. FTIR (KBr pellet, cm-^{1}):1536, 1694, 3306. ^{1}H NMR ($CDCl_3$, ppm): 4.28 – 4.30(^{1}H,m,CH of Fluoren), 4.48(1H, d, J = 6.4 Hz, CH_2 of Fluoren), 7.30-7.41 (8H,m,Aromatic protons of Fluoren), 7.41 (2H,br,*m*ABAHb and Hd),7.56-7.66(3H,m, *m*ABAHb, Hd&Hc) , 7.72-7.88 (8H,m,Aromatic protons of Fluoren), 8.13(1H,s, *m*ABA Ha), 9.89(NH of *m*ABA), 12.91(COOH of *m*ABA).

Gel-melting temperature (Tm)

The melting temperature of resultant gel in water was determined by the 'inverse flow method'. Samples were gelled in 10 mm diameter glass vials and attached inverted to a thermometer near the bulb end. This assembly was immersed in a stirred water bath, the temperature of which was raised at 5°C min $^{-1}$ using a hot plate. The temperature at which the solid mass falls from top was recorded as Tm. This procedure was repeated on three independent samples, and the average values are reported.

Field Emission scanning electron microscopic study (FESEM)

Morphology of xerogels obtained from amino acid derivatives**I** and **II**were investigated using FESEM microscope (JEOL JSM - 6700F) and were gold coated.

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Synthesis of HNPs

The amino acid derivative based hydrogel nanoparticles (HNPs) were prepared using self-assembly and modified inverse emulsion technique in which vitamin E-TPGS was used as an emulsion stabilizer. In short, HNPs were formulated by the dilution of stock solution (100 mg/mL) in pure water to a final peptide concentration of 10 mg/mL. The resulting solution was added drop-wise into 50 mL slightly warmed mineral oil containing vitamin E-TPGS at concentration of 0.4% wt/v and homogenized using high speed homogenizer. Next, nanoparticles were allowed to self-assemble for 2 h with continuous stirring at 4° C to allow the surfactant monolayer accumulation on the surface of the nanoparticle. Upon the completion of the self-assembly process, the resulting suspension was mixed with a non-polar solvent, and centrifuged to obtain phase separation. Supernatant was removed and HNPs were washed again to remove the remaining residues of mineral oil and finally vacuum dried. The obtained HNPs were used immediately, or stored at 4ºC for later use.

Nanoparticle characterization

Nanoparticle size diameter, polydispersity, and surface charge were measured using Malvern Zetasizer, with 4mW 633 He-Ne Laser, (DTS version 4.10, Malvern, U. K.) with appropriate viscosity and refractive index settings. The temperature was maintained at 25°C during the measurement.

Transmission electron microscopy (TEM)

A total of 500 µL of HNPs was prepared and suspended in PBS and placed on a 400-mesh copper grid. After 2 min, the excess of fluid were removed. Negative staining was obtained by covering the grid with 10 µL of 2% uranyl acetate in water. After 2 min, excess uranyl acetate solution was removed. Samples were viewed using a FEI-TECNAI G2 (Netherlands) TEM operating at 200 kV accelerating voltage. Images were acquired digitally using Gatan CCD camera.

Entrapment efficiency of the hydrogel HNPs

The entrapment efficiency of hydrogel nanoparticles were determined by separation of nanoparticles from the aqueous medium containing non-associated drug by centrifugation at 15000 rpm at 4°C for 40 min. The amount of free drug was measured by UV spectrophotometer at 267 nm. The entrapment efficiency of the nanoparticles were calculated as per the equation given below:

$$\text{Entrapment efficiency} = \frac{(\text{Total drug} - \text{free drug})}{\text{Total drug}} \times 100$$

In vitro drug release of 5-fluorouracil from HNPs

The in vitro release profile of the model drug 5FU from the drug loaded HNPs were performed using dialysis membrane previously soaked for 24 hrs in the dissolution membrane and stretched around at one end of the tube. The drug loaded formulations were carried out using pretreated membrane which were immersed into 30 mL of phosphate buffer solution of pH 7.4 at room temperature and magnetically stirred at 50 rpm. At selected time intervals aliquots were withdrawn from release medium and replaced with same amount of phosphate buffer (1 ml). The samples were analyzed thrice using UV-spectrophotometer at 267 nm. The percentage of cumulative drug release was plotted against time to get the release curves..

Rheology

Rheological measurements were carried out on a Rheoplus MCR302 (Anton paar) rotational rheometer with parallel plate geometry and obtained data were processed with start rheometer software. For the oscillatory shear measurements, parallel top plate with a 25 mm diameter and 1.0 mm gap distance were used. Gels (6 mg/ml) for rheological experiments were prepared on the bottom plate of the rheometer. The shear modulus (storage modulus G` and loss modulus G``) were measured against % strain from 0.001% to 1 %. Frequency sweep experiment was performed from 0.11 to 100 rad/s at constant strain of 1 %.

Results and discussion

Preparation and Characterization of the Hydrogels

From the literature documentation, it is evident that π–π interactions play a vital role in gelation. Therefore presence of aromatic groups in the molecule is an obvious choice. So our first design consists of Z-F-OH (Hydrogel **I**) (Figure 1). This molecule consists of a single aromatic unit at the *N*-terminus and another aromatic unit in the sidechain. In addition the insertion of two methylene units in between the two aromatic groups provides two rotational degrees of freedom to the aromatic units which was expected to accelerate the gelation process.[43]Inorder to decipher whether the position and number of aromatic groups present in the conjugate plays any role in gelation process, we have designed Fmoc-*m*ABA-OH (Hydrogel **II),** (Figure 1). In this conjugate the aromatic group in the sidechain has been shifted to the backbone (Phe to *m*ABA-OH) and the Z-group (1 aromatic unit) in the *N*-terminus has been replaced by Fmoc group (2 aromatic units).

In this study, the aromatic amino acid derivatives **I**and **II** undergoes hydrogelation, with a minimum gelation concentration of 0.02% w/v and 0.05% w/v respectively without any sonication at room temperature. At first the hydrogelators were dissolved in a drop of DMSO under mild heating condition and allowed to cool. Then to it required amount of water was added to maintain the volume upto 1 mL. It was allowed to stand at room temperature for 8-10 min to obtain an opaque gel.The formation of the gel was confirmed by inverted test tube method (Figure. S1).

The gels are stable over a range of pH, from pH 5.5 to 7 (water) and also over a period of six months. Sol–gel transition temperatures (Tgel) for both the hydrogelators**I**and **II** (% w/v) were plotted against different concentrations of hydrogelators(Figure 2). These plots show that the Tgel values of the aromatic amino acid derivatives increases with an increase in concentration (% w/v) of the hydrogelator until the plateau region is reached. This plateau region indicates that the

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formation of the hydrogel network has reached the saturation limit. No further addition of any molecule is capable of increasing the gel melting temperature (Tgel value).[44]

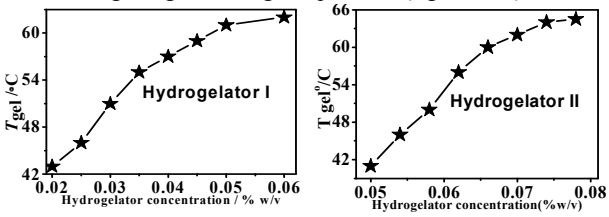

Figure 2. The change in Tgel profile of theHydrogelator **I** (left) and **II** (right) with respect to the concentration

To understand the mechanism governing the self-assembly propensities of the aromatic amino acid derivatives**I** and **II**, FTIR measurement was carried out (Figure S2). The FTIR spectra of the xerogels obtained from the amino acid derivatives show a major band at 3300–3340 cm^{-1}, a characteristic feature of hydrogen bonded NH stretching. No band was observed around 3400 cm^{-1} for both the derivatives, which indicates the absence of any free NH group. Both the xerogels of the derivatives**I** and **II** further exhibited peaks in the region 1696 , 1694 cm^{-1} (amide I) and 1538, 1536 cm^{-1} (amide II) respectively, characteristic feature of C=O stretching and NH bending frequencies respectively. These data therefore suggests the presence of β-sheet conformation for both the conjugates in their corresponding dried gel state.[45-46]

In order to gain insight about the morphology of the hydrogels, scanning electron microscopic (FESEM) investigation was carried out. The FE-SEM images (Figure 3) of the xerogels of hydrogelator **I** and **II** exhibits flat ribbon like morphology with width ranging from 600–800 nm and 100–120 nm respectively. The fibers were found to be several micrometers long. Interestingly,hydrogelator**II**exhibits dense mesh-like aggregates (Figure 3d at higher magnification)formed due to overlapping of flat ribbon like structures incomparison to hydrogelator **I**.This difference of morphology may be attributed to the less number of π-surface as well as fewer hydrogen bonding elements in hydrogelator**I**with respect to hydrogelator **II**.

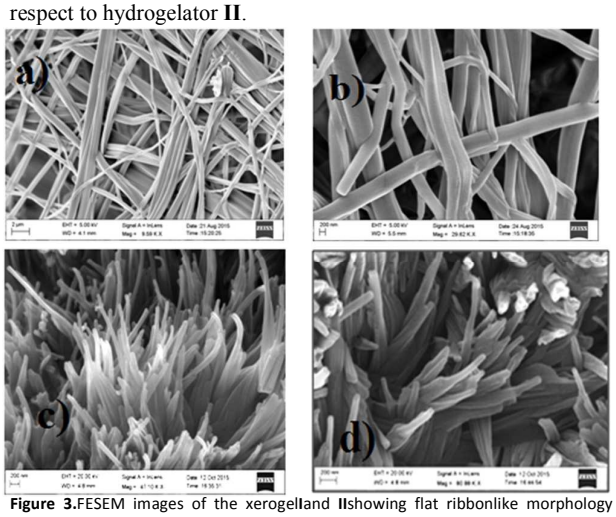

Figure 3.FESEM images of the xerogel**I**and **II**showing flat ribbonlike morphology of thefibris(a,c: lower magnification; b,d: higher magnification).

Rheology Characterisation

Inorder to address the mechanical strength and stability of the hydrogelators**I** and **II**, rheological studies were performed where the storage modulus G'(elastic response) and loss modulus G''(viscous response) were measured in frequency sweep experiments. [47]

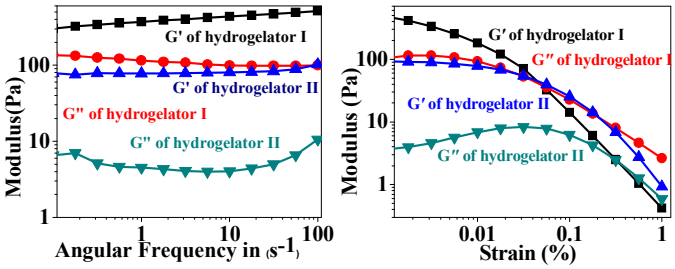

Figure 4. Rheological study of the hydrogels **I** and **II** showing the frequency sweep study (in the left)and Amplitude sweep study (in the right)

As is evident from Figure 4 (left), in the lower viscoelastic region (LVR), the storage modulus G' is higher than the loss modulus (G'') for both the amino acid derivatives. The observation suggests a soft 'solid-like' gel phase formation.[48]Moreover a cross over point is noticed in the experimental frequency regions, where the value of loss modulus G'' slightly exceeds the value of storage modulus G' and transformed into solution state (Figure 4 right). This particular point where the crossover occurs is called yield stress (σy). These gelators showed a G'(Storage modulus) value that was sufficiently larger than G''(Loss modulus); this difference remained almost constant up to about 0.5% and 0.05 % strain respectively for both the amino acid derivative **I** and **II** respectively (Figure 4 right). As the strain was increased beyond this point, both the storage modulus (G') and the loss modulus (G'') started to fall because intermolecular forces that held the gel together started being overcome by the applied strain and the fibrils were unable to withstand large deformations. The frequency-sweep experiments further showed that both the G' and G'' values were weakly dependent on the frequency (Figure 4 left), which was indicative of an entangled network-like system.

The principle guidelines of gelation involves a balance between enthalpy and entropy, the nature of weak interactions, such as hydrogen bonds and π–π forces involved, resulting in thermodynamic minimum. In Hydrogelator I, two methylene units are being inserted in between the aromatic moieties, incomparison to hydrogelatorII (that contains one methylene unit). Insertion of methylene units between aromatic groups are already known to accelerate the gelation process.[43] Although more π–π interactions are observed in hydrogelator I, due to the presence of aromatic residue in the backbone, we observe lower melting point of hydrogelator **II** than hydrogelator **I**, at same concentration. This may be attributed to the more entropic contribution from the two methylene unitsof hydrogelator **I**, which might overrule the extra stability gained due to π–π interaction,due to presence of aromatic moiety in the backbone, resulting in thermodynamic minimum

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conditions. Interestingly, the ratio between storage modulus and loss modulus of both the aromatic amino acid derivative **I** and **II** is approximately one order of magnitude higher which indicates significant mechanical stability of the hydrogels. This data prompted us to explore the candidature of our hydrogels in drug delivery application. Similar observation where gels of similar strength have been utilized for various applications has been reported by other groups.[49-53]

Preparation and characterization of HNPs

Literature documentation reveals that hydrogel nanoparticle (HNP) provides a promising alternative approach towards drug delivery applications.[16-18,34] But most of the report demonstrates the use of extremely complex techniques.

Our aromatic amino acid derivative **I** and **II**based hydrogels are prepared using the concept of self-assembly involving weak interactions. Henceforth are ecofriendly in nature. Therefore we became interested to explore whether our hydrogelscould be utilized for the synthesis of hydrogel nanoparticles. As illustrated in Figure 5 modified inverse emulsion technique (water-in-oil) was used for HNP formulation. In order to synthesize the hydrogel nanoparticles, the peptide was first dissolved in its monomeric state in organic solvent (DMSO), followed by dilution in water. This solution is considered to be the aqueous phase. This aqueous phase was drop-wise added into the organic phase containing the surfactant vitamin E-TPGS dissolved in light paraffin oil. Vitamin ETPGS was chosen as a surfactant due to its enormous advantage.[54-55]Moreover,the hydroxylic groups in vitamin E-TPGS may provide sites for the attachment of targeting units.

The heterogeneous mixture was then subjected to homogenization using a high speed homogeneizer operating at25000 rpm for ten minutes. During this process the hydrogelator**I** and **II** mixture were dispersed well and allowed to self-assemble into particle aggregates.[56]

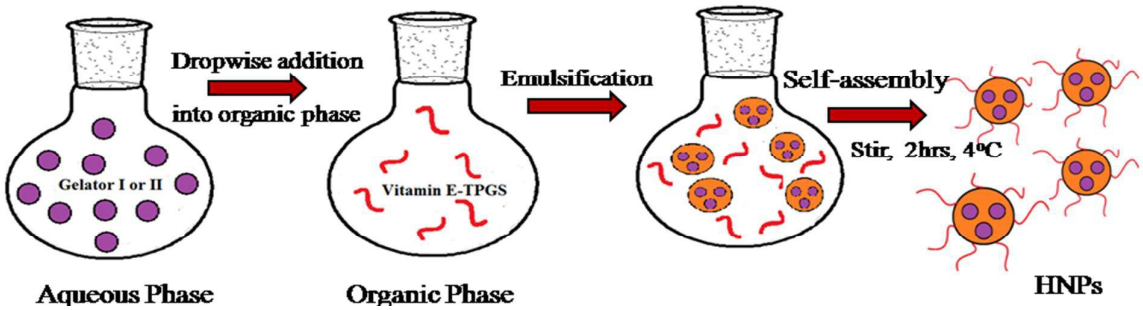

Figure 5: Schematic representation of Hydrogel Nanoparticle Formulation (HNP) preparation by modified Inverse Emulsion Method.

The mixture was then allowed to stir at 4°C for 2 hrs to allow the attachment of the surfactant along the surface of the hydrophilic core.

The self-assembled aromatic conjugate occupies the hydrophilic core of HNPs, and vitamin E-TPGS monolayer in outer hydrophobic periphery as in micelle (Figure 6).Finally, the HNPs were purified using centrifugationand a series of washings with a non-polar solvent.The particle dimensions were then recorded using a zetasizer. For pharmaceutical application, the physical dimension of the nanoparticles (like particle size, polydispersity index, zetapotential) influencesbiodistribution and in vivo efficacy.We were able to control the size of the HNPs by standardizing the parametersof the emulsion process, such as the stirring speed, stirring time,concentration of gelators and concentration of the surfactant.Our optimized formulation results reflect that the hydrogelator**I**and **II** exhibit an average particle size of 254 nm and 482 nm respectivelys. Furthermore, we analyzed the HNPs using transmission electron microscopic (TEM) analysis. The TEM images obtained demonstrates the presence of well-discrete spherical structure nanoparticles with various sizes (Figure. 7 and 8). The Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules and particles that are small enough, a highZeta potential will confer stability, i.e. the solution or dispersion will resist aggregation. When the potential is low, attraction exceeds repulsion and the dispersion will break and flocculate.The Zeta potential value of hydrogel nanoparticles for both the hydrogelators were found to be -25.5 mV for optimized formulation, which indicates significant stability of the nanoparticles.The pKa of 5-FU is 8.1. Under the conditions of the experiment, the pH of the medium remains neutral (since water has been used for gelation) or slightly acidic (which may occur due to ionization of the organic acids that has been used as hydrogelators). Henceforth the drug is expected to remain un-ionized under this condition as the pH of the medium is much below the pKa value.The negative charge of the zeta potential could be attributed to the presence of carboxyl groups in the amino acid derivatives as well as the emulsion stabilizer, vitamin E TPGS coating around the hydrophobic core.[57]

Generally, stresses caused by lyophilization may cause fusion of the HNPs.[58] Henceforth before subjecting to lyophilization,cryoprotectants are added to protect the fragile structure of the nanoparticles.In our case we have used vitamin E-TPGS, which not only serves as a surfactant but also as a cryoprotectant.[59]After lyophilisation we determined the particle size distribution after three months (Figure 9a and b). The

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results show that the particles have similar diameters and morphology even after three months, thus emphasizing the stability of the formulation. These results further indicate thatafter formulation, the particles could be readily turned into dry form, and re-suspended prior to use.

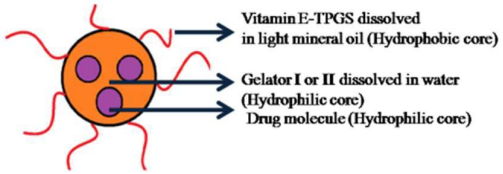

Figure 6. Cartoon diagram of drug encapsulated hydrogel nanoparticle formed after self-assembly.

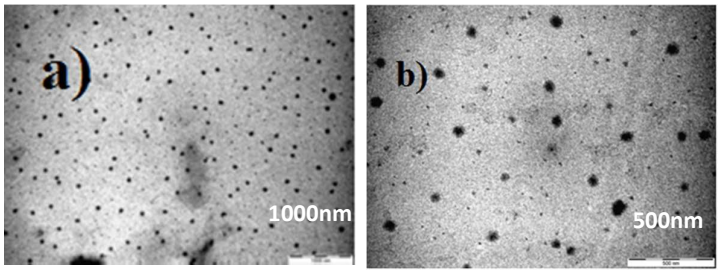

Figure 7: TEM images of the hydrogel nanoparticles (HNPs) from optimized formulation a) Hydrogelator**I** and b) Hydrogelator **II**

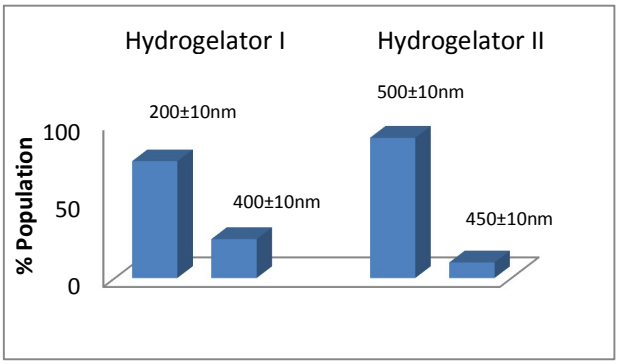

Figure 8: Particle size distributionof the hydrogel nanoparticles (HNPs) obtained from optimized formulation

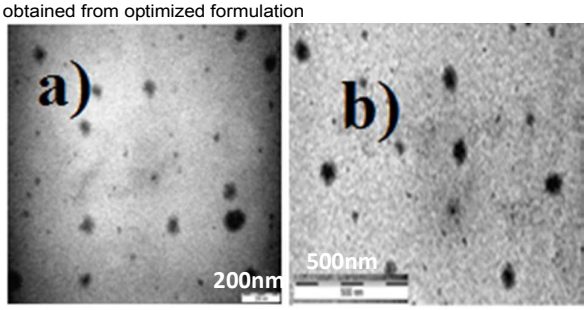

Figure **9.**TEM images of the stored hydrogel nanoparticles (HNPs) after three months a) Hydrogelator**I**and b) Hydrogelator **II**

Drug Encapsulation and *in vitro*release studies

As a part of the investigation, thereafter our idea was to explorethe efficacy of our nanoparticles for encapsulation and release studies of a model drug 5-fluorouracil (5-Fu)from the hydrogel matrix. Therefore, the drug loading efficiency and release kinetics were determined by dialysis membrane in HBS at 37°C under gentle stirring, to mimic physiological conditions. The encapsulation of the model drug 5-flourouracil (5-Fu) from the HNPs matrix showed an entrapment efficiency of 77.57% and 84.52% for hydrogelators**I** and **II** respectively. The concentration of the released drug was measured in solution over time, and quantified using UV spectroscopy. The resulting release profiles of 5-Fu from both the derivatives are presented in Figure 10. It has been noticed that the release profile of 5-Fu loaded HNPs slightly differs for both the derivatives. The release of 50% of 5-Fu from the HNPs was within 3 h/4 hr for hydrogelator**I**and **II** respectively, and after 7 h/9h the kinetics of release reached a plateau. This difference in release kinetics between 5-Fu for both the derivatives might be due to their dissimilar chemical structures. We propose that aromatic interactions and hydrogen bond formation between the amino acid derivativeand drug may play a crucial role in its efficient encapsulation. The slightly higher efficiency as a carrier of hydrogelator**II** may be attributed to the presence of aromatic ring in the backbone which may increase the extent of hydrogen bonding and π–π interaction to a greator extent with the drug molecule (incomparison to hydrogel **I**), thereby resulting in slightly slower release of drug from the hydrogel matrix.

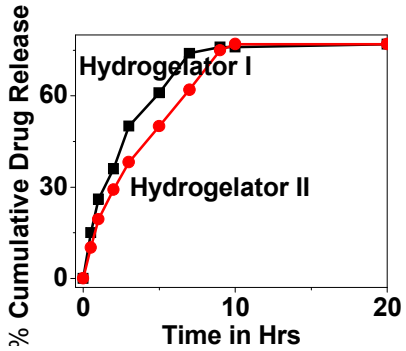

Figure 10: Release profiles of the model drug from the hydrogel matrix

Conclusions

In summary, we have demonstrated the formation of two simple amino acid derivatives that display excellent hydrogelating ability under mild conditions. The gelation property of the hydrogel was investigated by various microscopic techniques and its strength was determined by rheological measurements. Interestingly,these amino acid conjugate based hydrogels have been nicely tuned to prepare and stabilize hydrogel nanoparticles (HNPs) utilizing inverse emulsion procedure.The method proposed here is environmentally benign, as simple amino acid conjugates have been employed. The approach adopted is the concept of self-assembly utilizing weak interactions, unlike other polymers that involves harsh conditions and complex techniques for its fabrication.Till date significant examples are documented in the literature where

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synthetic and natural polymers, longer peptides/peptide amphiphiles have been used as building blocks for the drug delivery systems. However, to the best of our knowledge, thesederivatives represent one of the very few reports of HNP formation resulting from the self-assembly of simple amino acid derivatives, solely driven by an environmentally benign approach i.e. weak interactions, unlike other polymers that requires drastic conditions and complex techniques for its fabrication. The obtained HNPs were characterized and evaluated for their size, zeta-potential and post-formulation stability.Our gelators display good entrapment efficiency and *in vitro* release kinetics of a model drug 5-FU.The obtained results clearly indicate that these amino acid derivative based HNPs may be suitable as a potential drug delivery system. However, further studies to evaluate the candidature of these novel type of amino acid conjugate based HNPs for nano-medical applications is under investigation .

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Textual abstract

This result reflects the efficient candidature of our hydrogelators and nanoparticles generated therefrom as excellent carriers for drug delivery.

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