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Tunable Size and Spectral Properties of Fluorescent NanoGUMBOS in Modified Sodium Deoxycholate Hydrogels

Susmita Das^a, Sergio L. deRooy^a, Atiya Jordan^a, Lin Chandler^b, Ioan I. Negulescu^c, Bilal El-Zahab^a, and Isiah M. Warner^{a,*}

^aDepartment of Chemistry, Louisiana State University, Baton Rouge, LA-70803.

^bHoriba Scientific, 3880 Park Avenue, Edison, New Jersey.

^cDepartment of Human Ecology, Louisiana State University, Baton Rouge LA-70803.

Abstract

Microstructures of sodium deoxycholate hydrogels were observed to be altered considerably in the presence of variable tris(hydroxymethyl)aminomethane (TRIS) concentrations. These observations were confirmed by use of X-ray diffraction, polarized optical microscopy, rheology, and differential scanning calorimetry measurements. Our studies reveal enhanced gel crystallinity and rigidity with increasing TRIS concentration. The tunable hydrogel microstructures obtained under various conditions have been successfully utilized as templates to synthesize cyanine based fluorescent nanoGUMBOS (nanoparticles from a Group of Uniform Materials Based on Organic Salts). A systematic variation in size (70–200 nm), with relatively low polydispersity and tunable spectral properties of [HMT][AOT] nanoGUMBOS, was achieved by use of these modified hydrogels. The gel microstructures are observed to direct the size, as well as molecular self-assembly of the nanomaterials, thereby tuning their spectral properties. These modified hydrogels were also found to possess other interesting properties such as variable morphologies ranging from fibrous to spherulites, variable degrees of crystallinity, rigidity, optical activity, and release profiles which can be exploited for a multitude of applications. Hence, this study demonstrates a novel method for modification of sodium deoxycholate hydrogels, their applications as templates for nanomaterials synthesis, as well as their potential applications in biotechnology and drug delivery.

1. Introduction

Modification of the internal structure of hydrogels self-assembly and applications of such materials are recognized as important developments in the fields of nanotechnology and materials chemistry.^{1,2} In this regard, bile salts are naturally occurring molecules possessing an amphiphilic structure with a steroidal backbone, some of which are classified as low molecular weight (LMW) gelators.^{3,4} LMW gelators are different from polymeric gels with respect to the fact that former spontaneously self-assembling due to physicochemical forces such as hydrogen bonding, π - π interactions, and hydrophobic interactions, while the latter is primarily formed as a result of chemical cross-linking.⁵ Over the past two decades, low molecular weight gelators are of considerable interest due to interesting properties such as well defined self-assembled structures, thermal reversibility, simplicity of preparation, and

*Corresponding author, iwarner@lsu.edu.

Supporting Information Available

XRD, Differential Scanning Calorimetry, Deconvoluted absorption spectra, Excitation spectra are provided in supporting informations. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

potential applications in various technologies.⁶ Since these gels are primarily formed as a result of physical forces, the mechanical strength of such gels is not as great as their polymeric counterparts. Hence, enhancing the mechanical strength of existing hydrogels is of great interest due to the potential variety of applications for such systems.

In recent years, Maitra and coworkers^{7,8} have developed numerous deoxycholic acid (DC) derivatives and the effects of temperature, pH, and salt concentration on the supramolecular structures or self-assemblies leading to gelation have been studied extensively.⁹ However, to the best of our knowledge, the effect of tris(hydroxymethyl)aminomethane (TRIS) on modification of deoxycholate hydrogels to yield strikingly interesting physical properties and its applications in tuning the properties of nanomaterials has not been reported.

Several inorganic nanomaterials^{1,10} have been synthesized using a variety of hydrogel templates. More recently, the enhanced emissions of certain organic nanoparticles, e.g. Nile red and rhodamine B, prepared in silica gels have also been studied.^{11, 12} Such enhanced emission in the solid state has been attributed to J-type of aggregation of the dye molecules in the gel microdomain.^{11,12} However, an examination of the effect of altering the internal structure of the gel and its concomitant effect on the properties of synthesized nanoparticles has not been explored in these studies.

In the studies herein reported, we demonstrate the role of TRIS in modifying the properties of sodium deoxycholate (NaDC) hydrogel and its application to tuning the properties of fluorescent organic nanoparticles (FONs). The tunable gel morphology, microstructure, mechanical strength, and anisotropy verifies the role of various concentrations of TRIS in altering the properties of the gel which can then be exploited for a variety of applications. In the present study, organic nanoparticles were prepared from a new class of materials generated in our laboratory which are termed a Group of Uniform Materials Based on Organic Salts (GUMBOS). These materials have been shown to possess multiple properties and are promising candidates with great potential in the field of materials chemistry. In this study, we have used [HMT][AOT] GUMBOS^{13,14} previously reported by our group and derived from a near-IR cyanine dye. We further demonstrate hydrogel controlled aggregation of these dye molecules within the nanoGUMBOS using the four different hydrogels obtained by variations in the TRIS concentration. As observed from transmission electron microscopy, scanning electron microscopy, and spectroscopic measurements, a systematic control of size and spectral properties of the nanoparticles is achieved by control of the morphology of the deoxycholate hydrogels. In two of our hydrogel systems, the selected nanoGUMBOS were observed to exhibit enhanced emission due to head-to-tail stacking (J-aggregation). In contrast, the other two hydrogels produced a possible card pack arrangement (H-aggregation) of the same fluorophore within the nanoGUMBOS which lead to quenched fluorescence. These observations were also confirmed using fluorescence lifetime measurements of the nanoGUMBOS in these different gel environments. The size of the nanoGUMBOS was observed to also systematically vary in these different hydrogel systems. Thus, we present a simple approach to modification of existing sodium deoxycholate hydrogels and application of these gels for tuning the size and self-assembly of embedded fluorophores. These modified DC hydrogels have also been shown to impart additional properties such as enhanced photostability to our nanoGUMBOS. Rheology, x-ray diffraction (XRD), polarized optical microscopy (POM), and differential scanning calorimetry (DSC) studies have been used to confirm variations in packing of the hydrogel with various TRIS concentrations, which in turn are responsible for the observed alterations in properties. Thus, we consider this study a noteworthy development of currently used material which could greatly expand its properties for use in drug delivery, nanotechnology, and materials science.

2. Experimental Section

2.1. Materials

1,1',3,3',3',3'-hexamethylnodotricarbocyanine (HMT) iodide (97%), bis (2-ethylhexyl) sulfosuccinate (AOT) sodium salt (99%), sodium deoxycholate (NaDC), tris(hydroxymethyl)aminomethane (TRIS) and ethanol (spectroscopic grade), 6-propionyl-2-(dimethylamino)naphthalene (PRODAN) were purchased from Sigma Aldrich and used as received. Triply deionized water (18.2 MΩ cm) from an Elga model PURELAB ultra™ water filtration system was used for sample preparation in all experiments. Carbon coated copper grids (CF400-Cu, Electron Microscopy Sciences, Hatfield, PA) were used for TEM imaging.

2.2. Synthesis and characterization of NIR GUMBOS

NIR GUMBOS (most of which are also frozen ILs) were prepared using anion exchange procedures similar to those reported in the literature.^{13,14} The synthesis and characterization of [HMT][AOT] GUMBOS has been reported in previous studies.^{13,14}

2.3. Synthesis of NIR nanoGUMBOS in DC hydrogels

A very low gelator concentration of 20mM was used for all studies. Tris buffer of four different concentrations viz. 25mM, 100mM, 250 mM, and 500mM were prepared and the pH was adjusted to 6 by use of dilute or concentrated HCl. An amount of solid NaDC equivalent to 20 mM was added to each buffer solution in separate vials, vortexed for 30 sec, and placed in a sonicator bath. To each of these microliter amounts of 1 mM ethanolic solution of [HMT][AOT] was added an amount of dye to give a final dye concentration of 20 μM. Then, sonication was continued for 5 min, after which the system was left to equilibrate for half-an-hour. Since [HMT][AOT] GUMBOS is water insoluble, nanoGUMBOS of [HMT][AOT] were formed in the hydrophilic pockets of the hydrogel. All solvents used in this study were filtered prior to nanoparticle preparation using 0.2 μm nylon membrane filters. The particles thus formed are then characterized using techniques as described.

2.4. Characterization of size and morphology of the nanoGUMBOS

The average particle size and size distribution of the prepared nanoGUMBOS were obtained by use of transmission electron microscopy (TEM). TEM micrographs were obtained using an LVEM5 transmission electron microscope (Delong America, Montreal, Canada). These NIR nanoGUMBOS were first synthesized in the gels and then spotted on TEM grids. When dry, the TEM grids were washed with water to eliminate the gelator which is water soluble. Thus, the hydrophobic nanoparticles remain on the surface of the grid after washing. The grid was then allowed to dry and TEM images of the nanoGUMBOS were obtained.

2.5. Absorption and fluorescence studies of nanoGUMBOS

Absorbance measurements were performed using a Shimadzu UV- 3101PC, and UV-Vis-near-IR scanning spectrometer (Shimadzu, Columbia, MD). Fluorescence studies were performed using a Spex Fluorolog-3 spectrofluorimeter (model FL3-22TAU3); Jobin Yvon, Edison, NJ). A 0.4 cm path length quartz cuvet (Starna Cells) was used to collect the fluorescence and absorbance against an identical cell filled with buffer as the blank. Fluorescence studies were all performed adopting a synchronous scan protocol with right angle geometry. Fluorescence spectra were corrected for inner filter effects using a standard formula.¹⁵ Photostability studies were performed using the same spectrofluorimeter with a 450 watt xenon lamp as the excitation source. The widths of the excitation and emission slits

were maintained at maximum i.e. 14/14, for maximum light exposure of the samples during the entire period of study.

2.6. Fluorescence Lifetime measurements

Fluorescence lifetime measurements were performed at Horiba Jobin Yvon, NJ using time domain mode. A picosecond pulsed excitation source of 735 nm was used and emission was collected at 760 nm with a TBX detector. The time correlated single photon counting (TSCPC) mode was used for data acquisition with a resolution of 7 ps/channel.

2.7. Characterization of Gels

Gels were characterized using Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), Polarized Optical Microscopy (POM) and fluorescence anisotropy studies using ANS as a probe. XRD was performed using Bruker/Siemens D5000 automated powder X-ray diffractometer with Rietveld analysis software. The SEM images were collected using SM-6610, JSM-6610LV high and low vacuum scanning electron microscope (JEOL USA, Inc., Peabody, MA). Polarized optical microscopic studies were performed using Olympus BH polarizing optical microscope with MD 1900 camera. For these measurements, a few microliters of the sample were dropped on a precleaned glass slide and air dried. Images were then captured at right angle between the polarizer and the analyzer (cross-polarized). Differential Scanning Calorimetry (DSC) studies were performed using Thermal Analysis (TA) instrumentation in order to obtain the sol-gel transition temperatures. Thermograms were obtained using a heating rate of 5 °C/min and the samples were heated from 20 °C to 70 °C. Rheological properties of the gels were measured by use of a Thermal Analysis (TA) advanced rheometer. The temperature of the plate was maintained at 25 °C and the plate geometry was used with a gap of 300 µm.

3. Results and Discussions

3.1. Characterization of the Modified Hydrogels

Gels of high mechanical strength are extremely desirable for various applications and several studies have focused particularly on improving this property.^{16,17} In order to obtain a detail understanding of the factors which contribute to enhanced rigidity of our modified hydrogels, several characterization techniques were followed. In this study, it was observed that rigidity of the NaDC hydrogels increased dramatically with increasing TRIS concentration at a relatively low gelator (NaDC) concentration (20 mM) (Figure 1). Gel A (20 mM NaDC+ 25mM TRIS) was found to be a soft gel with some fluidity. However, the gel rigidity consistently increased with increasing TRIS concentration [Gel B (20 mM NaDC+ 100 mM TRIS), C (20 mM NaDC+ 250 mM TRIS) and D (20 mM NaDC+ 500 mM TRIS)].

3.2. XRD studies of the gels

X-ray diffraction studies of the air dried hydrogels revealed the crystallinity of the NaDC hydrogels. An increase in TRIS concentration from 25 mM to 500 mM lead to an increase in diffraction intensity by two orders of magnitude. In addition, the diffraction pattern of the hydrogel system changed considerably (Figure S1). In Gel A, distinct peaks were obtained at 2θ 31.6° (most intense) and 45.4° corresponding to the short range spacings of 2.82 nm and 1.99 nm, respectively which were the only predominating peaks. However, in Gel B, two new peaks appear at 21.5°($d=4.1$ nm)(most intense) and 10.7°(8.3 nm) as the predominating peaks. The 31.6° peak was the less intense in this case and the peak intensity at 2θ 45.4° is extremely weak. Likewise, appearance of new peaks and alteration of their relative intensities was observed for the other two gel systems of 250 (Gel C) and 500 mM (Gel D)

tris concentrations. The significant increase in diffraction intensity is attributed to the enhanced crystallinity of the NaDC hydrogels with increasing TRIS concentration and hence explains the observed increased rigidity.¹⁷ An appreciable change in the diffraction pattern suggests that the internal structure of the hydrogel is also altered in the process.

3.3. Microscopic characterization of the gels

The observation of enhanced crystallinity due to formation of micron sized crystalline domains in the hydrogels with various TRIS concentration was further confirmed by use of Polarized Optical Microscopic (POM). It is evident from the cross-polarized optical micrographs in Figure 2 that in addition to exhibiting increased birefringence, the morphology of the hydrogels also changed significantly from fibrous to spherulites with increasing TRIS concentrations.

We believe that this significant modification of the morphology of these low molecular weight (LMW) hydrogels using an appreciably low gelator concentration and without any chemical cross-linking is of paramount importance. These physical gels with crystalline nanodomains may have potential applications in several areas, including formation of tough thin films, in contact lens, in dialysis, and as templates for synthesizing nanomaterials of desirable sizes and properties.^{17,18} Transmission electron micrographs (Figure 3) of the hydrogels also confirmed modified gel morphology with increasing TRIS concentrations.

3.4. Differential Scanning Calorimetry (DSC) studies of hydrogels

The profound effect of TRIS in modifying the NaDC hydrogel microstructures is also reflected in the DSC results (Figure S2). In those studies, it was observed that the sol-gel transition temperature (T_g) was tuned from 34 to 52 °C, in going from Gel A to Gel D. This increase in T_g is complementary to our XRD and POM results, which demonstrated enhanced crystallinity with increasing TRIS. An increase in T_g suggests that intermolecular forces are stronger with increasing TRIS concentration, and this results in more rigid and crystalline gels.

3.5. Rheology of the gels

Examination of data from rheology studies helped to understand the mechanical and viscoelastic properties of these hydrogels, which in turn allowed an estimation of the potential of these materials for different applications. For example, gels of high mechanical strength are sought for applications in bioengineering and biotechnology. In addition, hydrogels, which shear thin on application of certain shear stress and rapidly turn solid on removal of stress, are highly desirable for injectable therapeutic delivery vehicles.¹⁹ In this study, the storage modulus (G') and loss modulus (G'') were obtained as a function of frequency (Figure 4). It is observed that the storage modulus values increased with increasing TRIS concentration, suggesting that the interactions leading to gel formation are stronger at higher TRIS concentrations. Since no covalent interactions are expected in the case of these LMW hydrogels,²⁰ we believe that it is primarily hydrogen bonding interactions which are significantly enhanced²⁰ by the increased number of TRIS molecules, with the number of gelator molecules remaining the same. The TRIS molecules are believed to bridge between the deoxycholate molecules through hydrogen bonding interactions, thereby enhancing the gel rigidity. As visually observed from the relative fluidity of the gels in the inverted vials (Figure 1), their expected order of viscosities should be Gel A < Gel B < Gel C < Gel D. The G'' value, which are directly proportional to viscosity, follow nearly the same trend over the entire frequency range with the exception of Gel D. In the case of Gel D, G'' as well as η (viscosity) first increases with frequency and suddenly drops at frequencies greater than 40 rad/s. This suggests that the fluidity of this gel increases with increasing frequency. Thus, although the gel is very rigid, it tends to lose its rigidity after

oscillation for long periods of time.. Explanation of such an observation is also supported through enhanced intermolecular interaction with increasing TRIS concentration, with the exception of Gel D at higher oscillation frequencies. The ratio G'/G'' followed a slightly different trend. Other than Gel A, G'/G'' values are essentially greater than 1 (~9–14) at high frequencies and very close to one at low frequencies, suggesting their solid like properties at high frequencies. However, for Gel A, G'/G'' is less than one in the low frequency region suggesting its viscous liquid behavior. Among Gels B, C, and D, Gel B exhibits the highest G'/G'' values, followed by Gel C and then Gel D. Further studies were performed as discussed in the later sections in order to obtain a complete understanding of the gel microenvironment and better explanation to these observations.

3.6. Fluorescence probe of hydrogel microenvironment

Fluorescence anisotropy measurements were performed on these hydrogel systems using the hydrophobic fluorescent probe ANS in order to gain better insight into the gel microenvironment. ANS has been used²¹ previously to study the onset of gelation in bile hydrogels. ANS usually exhibits a sharp increase in fluorescence intensity upon onset of gelation, accompanied by higher fluorescence anisotropy values due to restricted motion of the fluorophore in the gel. The observed increased crystallinity and rigidity with increasing TRIS concentration suggested higher fluorescence anisotropy of the probe with higher concentrations of TRIS. However, the fluorescence anisotropy of ANS was found to decrease with increasing gel crystallinity which suggests the presence of larger hydrophilic microdomains that allow rapid rotational diffusion of the fluorophore (Figure 5A). This observation is consistent with the rheology results. Although larger intermolecular interactions increase the overall viscosity and rigidity, the availability of large numbers of TRIS molecules to bridge between deoxycholate molecules through hydrogen bonding interactions leads to the formation of larger hydrophilic microdomains at higher tris concentrations. The presence of larger water pockets at higher tris concentrations also explains larger fluidity upon application of higher oscillation frequency and shear for a longer time period. This picture is quite similar to a membrane which is rigid enough to form a container and at the same time fluid enough to allow lateral transport.²²

In order to further confirm this observation, another neutral fluorescent probe (PRODAN) was used so that interference from electrostatic interactions can be minimized. PRODAN is known to efficiently provide information about the hydrophobicity and hydrogen bonding capacity of a given micro-environment.^{23,24} The fluorescence emission wavelength of PRODAN exhibit a large red-shift in going from a highly non-polar to a polar solvent (Figure 5B). It is interesting to note that this study reveals that the emission maxima of PRODAN at first blue shifts from 520 nm in water to 458 nm with increased intensity in Gel A, suggesting an extremely hydrophobic and less hydrogen bonding microenvironment in this particular gel. However, in going from Gel B to Gel D, the emission peak gradually shifts back to 520 nm accompanied with a shoulder of reduced intensity at 458 nm, suggesting an increase in micropolarity and hydrogen bonding capacity of the hydrophilic pockets with increasing TRIS concentration in the gels. Such an observation reveals that the PRODAN molecule moves to a bulkier water like environment with increasing TRIS concentration in the gels, with little influence from the hydrophobic gelator molecules. This further confirms our consideration of increased hydrogen bonding and enhanced sizes of the water pockets with increasing TRIS concentrations in the gels.

3.7. Release profile of the hydrogels

The formation of larger hydrophilic domains or larger pore sizes of the hydrogels with increasing TRIS concentration was further investigated by studying the release profile of all four hydrogel systems. For these studies, the representative drug molecule was encapsulated

into the gels during their formation and allowed to stabilize for 24 hours. Then, the gels were incubated with pH 7.4 phosphate buffer system. Following this, absorbance of the supernatant buffer was measured at various time intervals. The concentration of the representative encapsulated drug molecule was chosen as such that the absorber follows Beers-Lambert's law within the total experimental volume. It was observed that the release profile of a relatively small molecule such as fluorescein was nearly identical for all hydrogels (Figure 6). However, the release of a large protein such as bovine serum albumin from the four hydrogels studied showed considerable differences from each other. It was observed that the release of BSA from gel A was the slowest, suggesting the smallest pore size. Since this hydrogel system is the least rigid, this hydrogel exhibits a burst release of the protein after 200 minutes of incubation with a phosphate buffer of pH 7.4. However, the other three hydrogels systems were highly stable for much longer periods of time and exhibited increased release rates with increasing TRIS concentration from 25–500 mM and with increasing crystallinity. This suggests the presence of larger microdomains or larger pores in these hydrogels of higher rigidity. Among various other applications, drug delivery is one of the important properties of this hydrogels.^{25, 26} Hence, this interesting modification of the NaDC hydrogels enhances its applications in biomedicine for tunable drug delivery and nanotechnology.

3.8. Application of modified hydrogels in naomaterials

Considering the remarkable potential for modification of hydrogel structures and properties, applications of these materials for synthesis of nanomaterials with controlled properties have been investigated and exploited. For example, Simmons' et al. have reported incorporation of nanoparticles such as superparamagnetic ferrites and semiconducting CdS for potential applications in new devices.²⁷ Synthesis of FON's has also been reported.^{11,12} In the present study, in addition to the other applications discussed earlier, we further demonstrate the application of these hydrogels for systematically tuning the size and spectral properties of near infrared (NIR) [HMT][AOT] nanoGUMBOS.

3.9. TEM studies of nanoGUMBOS

The nanoGUMBOS synthesized in these variable hydrogel systems were characterized using TEM. Analyses of the transmission electron micrographs revealed highly spherical nanoGUMBOS with relatively low polydispersity. The least crystalline hydrogels possessing smallest hydrophilic microdomains (gel A) yielded the smallest nanoGUMBOS with size 75 ± 9 nm. In gels B, C, and D, the size of the nanoGUMBOS respectively produced were 100 ± 6 nm, 130 ± 13 nm and 200 ± 10 nm (Figure 7). Since [HMT][AOT] GUMBOS are hydrophobic, they will tend to precipitate out in hydrophilic microdomains. Thus, an increase in size of the nanoGUMBOS in different gel systems is complementary to our previous observation of increased size of hydrophilic domains in going from gels A–D. Hence, a systematic variation in size of hydrophobic nanoparticles can be achieved using this approach.

3.10. Absorption and fluorescence studies of the [HMT][AOT] nanoGUMBOS in gels

Variations in the size of the nanoparticles in the modified hydrogels were also observed to be associated with interesting variations in spectral properties. The absorption spectra of the [HMT][AOT] nanoGUMBOS in all four hydrogel systems revealed a change in shape from gels A to D (Figure 8a). The full width at half maxima (FWHM) also increased with increased rigidity and crystallinity of the gel. This suggested that the gel microenvironment has a profound effect on the molecular self-assembly of the dye forming the nanoparticles.²⁸ It is observed that [HMT][AOT] nanoGUMBOS synthesized in gel A has a ~17 nm red shifted narrow absorption band, as compared to [HMT][AOT] nanoGUMBOS in the buffer. Broadening of the absorption spectrum was observed with a gradual blue shift for the

nanoGUMBOS synthesized in gels of increased rigidity. In a previous study,¹⁴ we have demonstrated that these nanoGUMBOS, when formed by reprecipitation in water, have a contribution from three types of molecular aggregates, i.e. J-, H-, and randomly oriented aggregates. J aggregates are formed as a result of head-to-tail stacking of the transition dipoles, leading to a red-shifted narrow absorption and enhanced fluorescence. In contrast, H-aggregates are formed as a result of card-pack or parallel stacking of the transition dipoles, exhibiting blue shifted broad absorption and quenched fluorescence. The randomly oriented aggregates do not exhibit any ordered stacking and exhibit absorption and fluorescence characteristics similar to monomeric species. The observed variations in spectral properties of these aggregates can be thoroughly explained by use of exciton theory.²⁹

An intense fluorescence emission band around 773 nm from the [HMT][AOT] nanoGUMBOS synthesized in gels A and B together with the narrow red shifted absorption band are likely attributable to significantly high J-aggregation within the nanoGUMBOS in this gel system (Figure 8b). A broadening of the absorption spectra with enhanced shoulder in the blue edge and quenching of fluorescence intensity in gels C and D are likely the result of increased H-aggregation and aggregates with higher aggregation number within the nanoGUMBOS. The observed blue shift is considered to be due to the presence of primarily H- and Random components in the nanoparticles in Gels C and D, of which the H- is non-fluorescent and the random component emits in the monomer region, which is blue-shifted to J-aggregate emission. The deconvoluted absorption spectra of the nanoGUMBOS in the various gel systems reveals that different types of aggregates are formed in the gels of different compositions and thereby resulting in tunable spectral properties (Figure S3). The twist angle ($\theta = 2 \tan^{-1}(f_1/f_2)$)³⁰ was calculated from the deconvoluted absorption spectra of [HMT][AOT] nanoGUMBOS in Gel A (97.8°) and Gel D. Evaluation of these data suggests that in gel A the aggregates are predominantly J-type where as in Gel D (69.54°) the aggregates are predominantly H-type. This observation agrees well with fluorescence emission and excitation studies and are also well supported by the lifetime studies of the [HMT][AOT] nanoGUMBOS, discussed in the following section.

An examination of the fluorescence excitation spectra (Figure S4) reveals that for [HMT][AOT] nanoparticles in gel A, the maxima is around 771 nm which is ~18 nm red-shifted from the absorption maxima of 753 nm suggesting that the maximum emission comes from excitation at 771 nm. The overlap of the excitation maxima with the emission maxima further suggests the formation of J-aggregates in gels A and B. However, with increasing gel rigidity, the maxima are blue shifted and quenched, which correlates with our interpretation of increased contribution from H- and random aggregates within the nanoGUMBOS. Thus, nanoparticles of tunable size and spectral properties can be obtained in this modified gel microenvironment.

3.11. Lifetimes of [HMT][AOT] nanoGUMBOS in hydrogels

Fluorescence lifetime measurements were also performed for [HMT][AOT] nanoparticles in aqueous and gel media. For 1uM [HMT][AOT] in water, the absorption spectrum was similar to the monomer absorption and the lifetime decay was best fitted to a double exponential decay. However, a 349 ps component contributed 99% of the observed fluorescence. This was attributed to the monomeric species.³¹ The decay of 20uM [HMT][AOT] nanoGUMBOS in water was also best fitted to a double exponential decay with two lifetime components of 75 ps (21%) and 363 ps (79%). J-aggregates are known to exhibit shorter lifetime than the corresponding monomeric species.³² In our previous study,¹⁴ we reported that the primary fluorescent components in the [HMT][AOT] nanoGUMBOS are J-aggregates and that the randomly oriented (R) components have spectral characteristics similar to the monomer. Thus, the shorter lifetime component in [HMT][AOT]

nanoGUMBOS is assigned to the J-aggregates and the longer lifetime component to randomly oriented aggregation. However, in the hydrogels, the lifetime values of the same dye nanoparticles were different. It is well established that fluorescence lifetimes strongly depend on the polarity and hydrophobicity of the solvent in which the fluorophore is present. For heptamethine cyanines, it has been observed that the fluorescence lifetimes are shortest in water and as the solvent polarity decreases, i.e. increasingly aprotic solvents, the fluorescence lifetime increases.³¹ This is attributed to the fact that in polar solvents, polymethine cyanines involve enhanced twisted intramolecular charge transfer (TICT) and hence the fluorescence is quenched.³³ However, with decreasing solvent polarity, TICT is diminished which leads to enhanced fluorescence quantum yields and lifetimes.³³ Our studies using the hydrophobicity probe ANS reveal that the gel environment was similar to that of organic solvents such as ethanol or acetone. This suggests that the lifetimes of the dye will be longer in gels as compared to those in water. In gels A and B, where the hydrophilic microdomains are smaller (revealed from smaller particle size and very slow release of BSA), the dye will experience more non-polar microenvironment as compared to gels C and D. This observation is consistent with the observed longer lifetime of the J- and R component (Table 1) as compared to the aqueous media. As observed from absorption studies in going from gels A to D, new shoulders appear in both the red and blue edges indicating formation of new H- and J-aggregates at the cost of R. Since H aggregates are non-fluorescent, J-aggregates contribute to most of the observed fluorescence. This explains the observed increase in relative amplitude of the shorter lifetime component as compared to the longer lifetime component in Gels C and D. The drop in average lifetime in going from gels A to D is again consistent with our previous assumption of increased size of the hydrophilic microdomains.

3.12. Photostability studies of [HMT][AOT] nanoGUMBOS

The [HMT][AOT] nanoGUMBOS synthesized in gels A and B were found to be highly photostable with 100% retention in fluorescence intensity for an irradiation of 2500 secs as compared to 26% loss of fluorescence intensity for [HMT][AOT] nanoGUMBOS in buffer (Figure 9). This enhanced photostability is considered to be the result of the combined effect of highly photostable J-aggregates³⁴ formed in these gels and an effect of gel environment as well. In contrast, a decrease in J-aggregate contribution or increased H-aggregation leads to decreased photostability in gels C and D. The photostability study further confirms our prediction of J-aggregation in gels A and B and predominantly H-aggregation in the other two gels. In one of our previous studies, it has been shown that [HMT][AOT] nanoGUMBOS has greater contribution from J aggregates, as compared to [HMT][BETI] nanoGUMBOS which has major contributions from H-type aggregates. An examination of the photostabilities of such nanoGUMBOS suggests that [HMT][AOT] nanoGUMBOS are more photostable (retains 74% fluorescence intensity) compared to the BETI nanoGUMBOS (retains 57% fluorescence intensity) indicating greater contribution from J-aggregates, thus enhancing the photostability of the nanoparticles.³⁴

4. Conclusions

In summary, we have demonstrated the possibility of significant modification of a known hydrogel system. These NaDC hydrogels, which are known to precipitate out at pH values lower than 6.7 have been shown to yield transparent gels with TRIS at pH 6 and significantly lower gelator concentration. Increasing TRIS concentration effectively modifies the optical, morphological, and mechanical properties of the gel enhancing the possible applications of such a system. This modification is quite different from the effect of salt or pH since the latter are primarily based on increased aggregation number of the gelator through hydrophobic interactions, while TRIS operates primarily by increasing hydrogen

bonding interactions. Two potential applications of these modified hydrogels have been demonstrated in this study, one of which involves delivery of biomolecules and the other in development of nanomaterials. With regard to the latter, these modified gels have been successfully used in obtaining nanoGUMBOS of variable size and spectral properties. These findings have broadened the application of these nanoparticle-gel hybrid materials for applications in materials science, optoelectronic, and photovoltaics. We propose that this modification of NaDC hydrogels can be exploited for other applications as discussed in the introduction⁶ which are not outlined in this manuscript.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. 20 mM NaDC hydrogels with various TRIS concentrations (A) 25 mM, (B) 100 mM, (C) 250 mM and (D) 500 mM. The blue color is imparted by the dye [HMT][AOT].

Figure 2.
Polarized Optical Microscopy of the modified hydrogels (A) GelA, (B) Gel B, (C) Gel C,
(D) Gel D

Figure 3. Transmission Electron Micrographs of modified NaDC (20 mM) Hydrogels: (A) 25 mM, (B) 100 mM, (C) 250 mM and (D) 500 mM TRIS.

Figure 4.
(A) Storage modulus (G') and (B) Loss modulus (G'') of modified NaDC gels plotted as function of oscillation frequency.

Figure 5.

(A) Fluorescence anisotropies of ANS at different wavelengths in Gels A–D compared to free ANS, $\lambda_{\text{ex}}=360$ nm. (B) Fluorescence emission spectra of PRODAN in Gels A–D, compared to that in water, $\lambda_{\text{ex}}=360$ nm

Figure 6.
Release profile of (A) Fluorescein from the modified hydrogels, (B) Bovine Serum Albumin. The Y-axis represents absorbance of the released representative drug.

Figure 7.
Transmission Electron Micrographs of [HMT][AOT] nanoGUMBOS synthesized in Gels A–D.

Figure 8. (A) Absorption spectra of [HMT][AOT] nanoGUMBOS in the modified hydrogels; (B) Normalized Fluorescence spectrum of [HMT][AOT] nanoGUMBOS in the modified hydrogels; (C) Fluorescence intensities at the emission maxima of [HMT][AOT] nanoGUMBOS in the hydrogels.

Figure 9.

(A) Photostabilities of [HMT][AOT] nanoGUMBOS in varying concentration of tris-HCl at pH 6 without NaDC, $\lambda_{\text{ex}} = 737$ nm and $\lambda_{\text{em}} = 755$ nm. (B) Photostabilities of [HMT][AOT] nanoGUMBOS in modified hydrogels, $\lambda_{\text{ex}} = 753$ nm and λ_{em} was maintained at corresponding emission maxima in each gel system.

Table 1

Sample	Lifetime	α	Relative amplitude	χ^2
1 μM in water	$\tau_1=349$ ps $\tau_2=1.56$ ns	99% 1%	99% 1%	1.17
20 μM in Water	$\tau_1=75$ ps $\tau_2=363$ ps $\tau_3=1.86$ ns	56% 44% 1%	21% 78% 1%	1.01
20 μM in Gel A	$\tau_1=658$ ps $\tau_2=1.28$	78% 22%	64% 36%	1.06
20 μM in Gel B	$\tau_1=627$ ps $\tau_2=1.23$ ns	84% 16%	73% 27%	0.98
20 μM in Gel C	$\tau_1=545$ ps $\tau_2=1.07$ ns	88% 12%	79% 21%	1.09
20 μM in Gel D	$\tau_1=522$ ps $\tau_2=1.05$ ns	90% 10%	82% 18%	1.09