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Low molecular mass cationic gelators derived from deoxycholic acid: remarkable gelation of aqueous solvents

Shreedhar Bhat[a] and Uday Maitra[a,b,*]

[a]*Indian Institute of Science, Department of Organic Chemistry, Bangalore 560012, India*
[b]*Chemical Biology Unit, JNCASR, Bangalore 560012, India*

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Abstract—During the past decade, the study of molecular self-assembly and network formation from small molecule gelators has become one of the most active areas of supramolecular chemistry. A serendipitous discovery of the gelation of a cationic bile salt (**4**) led us to investigate the aggregation properties of this new class of cationic hydrogelators. This article summarizes the recent efforts on the study of side chain structure–aggregation property relationship of cationic bile salts. Bile acid analogs with a quaternary ammonium group on the side chain were found to efficiently gel aqueous salt solutions. Some of the cationic bile salts gelled water alone and many of them gelled aqueous salt solutions even in the presence of organic co-solvents (≤20%) such as ethanol, methanol, DMSO, and DMF. These gels showed interconnected fibrous networks. Unlike natural anionic bile salt gels (reported for NaDC and NaLC), the cationic gels reported here are pH independent. Cationic gels derived from DCA showed more solid-like rheological response compared to natural NaDC gels studied earlier by Tato et al.
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1. Introduction

The self-assembly process has enabled synthetic chemists to create spectacular architectures ranging from dots and particles; rods and fibers; helices and grids; rotaxanes and catenanes to molecular motors. Supramolecular gels result from a complex self-assembly process leading to the formation of fibrous networks. Multiple supramolecular interactions[1] between the individual building blocks, for example, hydrogen bonding, metal coordination, hydrophobic interaction, etc., are believed to be critical in the gel forming process.

Gels are classified in many ways. Depending on the nature of the solvent that is being gelled, systems have been classified as organogels and hydrogels. Hydrogels are important soft materials that have been shown to have many applications in material science and biomedical engineering.[2] They are useful as drug delivery vehicles, scaffolds for tissue engineering,[3] and for controlled release of other biological agents.[4] Gels are also useful in pollutant capture and removal.[5] Recent efforts are being made to construct hydrogel based chemical sensors and enzyme inhibition assay kits.[6] In addition, gels have utility in the development of new materials that reversibly respond to various external stimuli.[7]

Gel formation in aqueous or non-aqueous media by polymers and biopolymers is a well-documented phenomenon in the literature. Besides the well-known class of synthetic or natural macromolecules,[8] which form reversible gels[9] under appropriate concentration and solvent conditions, another important class of (physical) gels is constituted by small molecules.

Low molecular weight hydrogelators offer several advantages over polymer gels, despite the fact that such hydrogels have complex intermolecular association modes that are often difficult to define. Such small gelator molecules have special chemical functionalities responsible for the auto-associative behavior operative in certain concentration, temperature, and solvent conditions. The ease of formation and the reversibility of gel formation by small molecular weight hydrogelators are attractive strategies for synthesizing new materials. The properties of a material depend both on the nature of its molecular constituents and the precise spatial positioning of functional groups, which can be manipulated in small molecules, but usually not in polymers.

Since molecular gels can have diverse structures on the microscopic and mesoscopic scales, there has been considerable interest in developing functional gels by fine tuning gelator molecules and/or doping them with molecules with additional properties.[10] Examples of low molecular weight compounds, which can gel water[11] or organic liquids[12] are abundant in the current literature. But, there are few reports

* Corresponding author. Tel.: +91 80 2293 2690; fax: +91 80 2360 0529; e-mail: maitra@orgchem.iisc.ernet.in

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on the structure–property study of a given class of self-assembled systems.

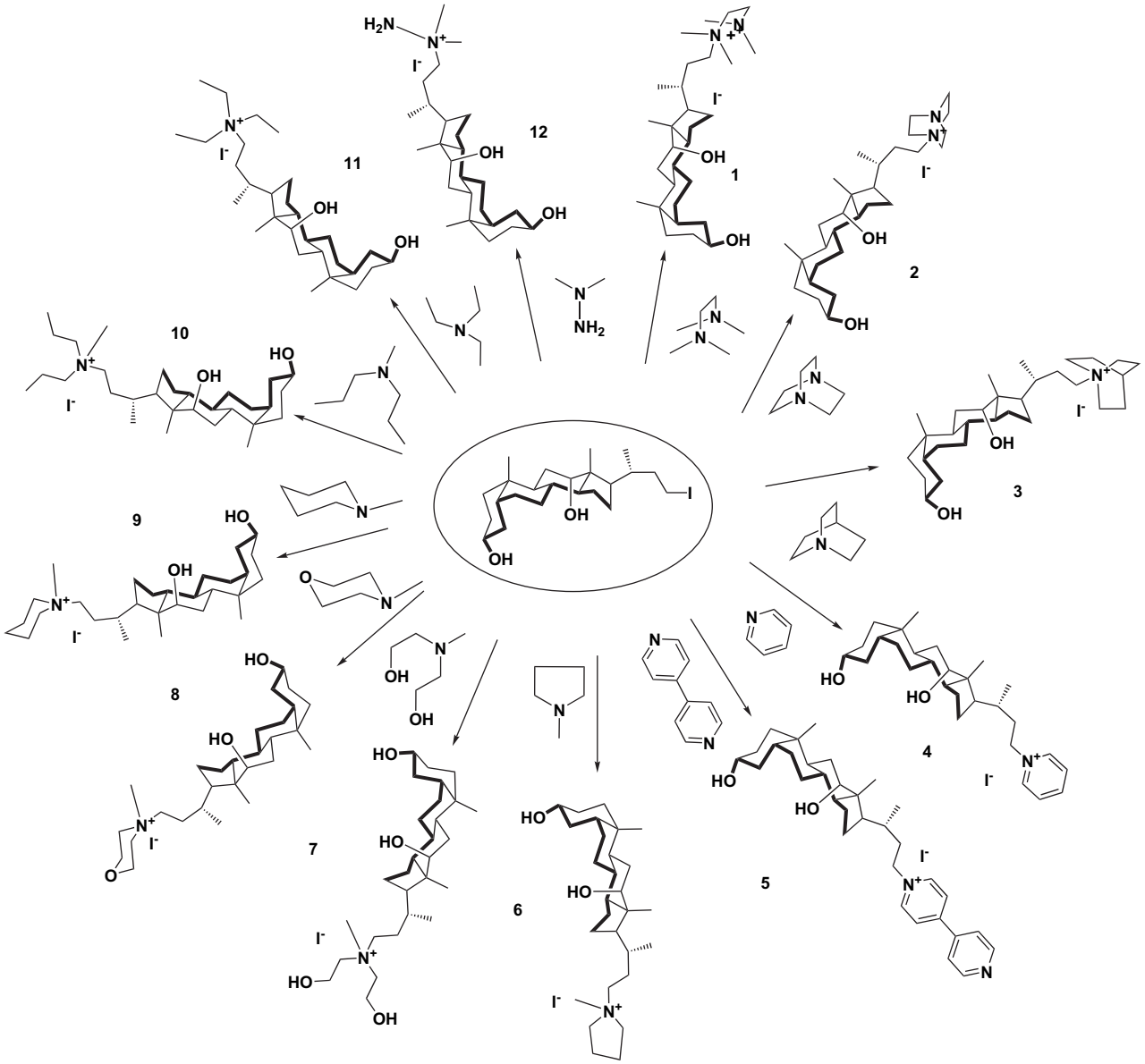
We have recently reported a few cationic analogs of bile salts as potent hydrogelators.[13] In this article we summarize the detailed structure–property study made with this novel class of cationic hydrogelators.

2. Results

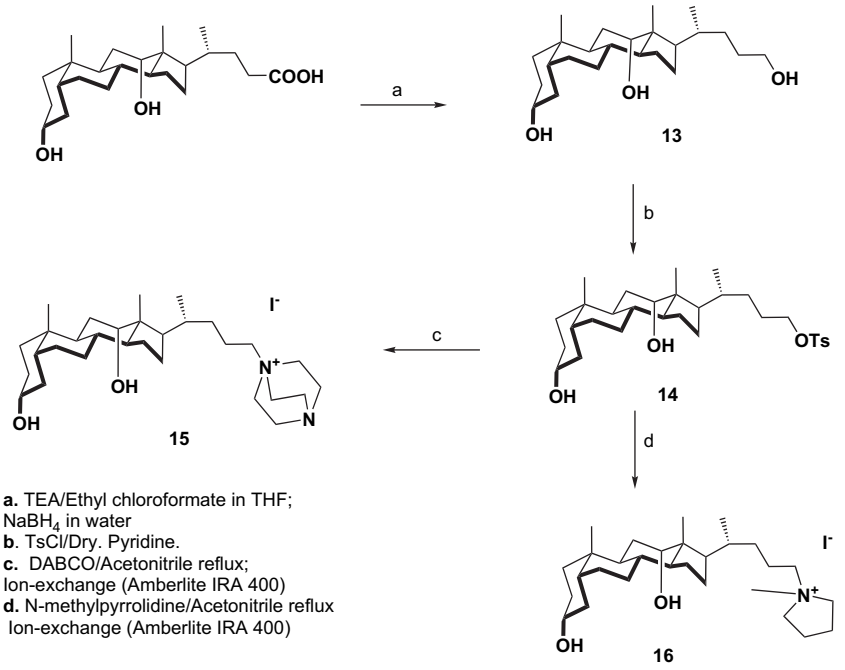
2.1. Gelation studies

All the final compounds listed in Schemes 1–3 were tested for their gelling abilities in various solvents. Each of these cationic bile salts exhibited unique aggregation behavior. Compounds **2**–**4**, **6**, **8**, **9**, and **12** formed thermoreversible gels in water in the presence of NaCl,[14] whereas other bile salts gave crystals, precipitates or solutions depending upon the gelator concentration chosen. Compounds **4**, **8**, **9**, and **11** have been previously reported in detail.[13] Compound **2** formed a stable gel in pure water at and above 0.8%. Compound **12** at 1% w/v formed a stable gel in 0–4 M aqueous NaCl solutions and the transparent gels progressively became translucent with increasing NaCl concentration. In contrast, bile salts **3**, **6**, and **9** showed an interesting aggregation behavior in 1 M NaCl: forming (a) gel in the concentration range of 0.2–1.0% w/v, (b) gel containing dispersed gelator particles above 1.5%, and (c) microcrystals at >2%.

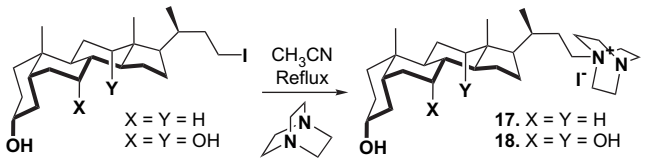
Gels formed at lower concentration of the gelator and NaCl are transparent and turned progressively translucent when the concentration of either of these is increased. There were significant differences in the optical appearance of the gels derived from cationic bile gelators. The gels made of **3**, **6**, **9**, and **12** were more translucent compared to those



Scheme 1. Synthesis of cationic bile salts from iododeoxycholane (the iodo derivative was prepared from deoxycholic acid by following the reported procedure).[13a]



Scheme 2. Synthesis of side chain elongated cationic bile salts.



Scheme 3. Mono and trihydroxy cationic bile salts.

of **2** and **8**. Also, the gels of **3**, **6**, and **9** are more rigid and resistant to mechanical shaking than are the other. A striking difference in the macroscopic cohesion of the gels was also observed. When subjected to mechanical agitation, gels of **2**, **8**, and **12** (typically from 0.5% to 1% w/v) broke into a slurry of small globular aggregates, while the gels derived from **3**, **6**, and **9** broke into larger lumps. Thus, gels of **8** and **12** exhibited lower cohesion at a macroscopic level. At this stage, therefore, the unique macroscopic aspects and behaviors that characterize each of the systems were noticeable.

For better gelation of aqueous solvents the use of NaCl with bile salt is essential. NaCl provides an ionic environment in addition to salting out effect. The concentration of NaCl seemed to have a significant influence on the gel strength of these bile salts. However, salt concentrations above 4 M were found to destroy the gelation process.[15] Gelation required at least 0.1 M NaCl, and for obtaining optically transparent hydrogels 0.5–1 M NaCl was optimum. In addition to aqueous NaCl solution, gels were obtained in 1 M aqueous solutions of NaBr, Na_2SO_4, Na_2CO_3, NaN_3, $NaNO_3$, KCl, LiCl, and $BaCl_2$, but precipitated in aqueous 1 M NaI. Compounds **3**, **6**, and **9** formed a gel in the presence of organic co-solvents such as MeOH, EtOH, DMF, DMSO, etc. (up to 20% organic co-solvent). Higher percentage of organic co-solvents resulted in fluids indicating the importance of the hydrophobic effect in the gelation process. Compounds **1**, **5**, **7**, and **10** were found to be non-gelators.

1-Anilino-8-naphthalene sulfonate (ANS) has been used for probing the formation of hydrophobic surfaces during protein folding since its fluorescence quantum yield increases in non-polar environments.[16] We have also used ANS fluorescence for probing gelation.[17] For estimating critical gel concentrations (CGC) ANS anisotropy was preferred since the fluorescence intensity enhancement was less sensitive to CGC.[18] Compounds **2**, **3**, **6**, and **12** showed critical gel concentration values of 7.0, 2.0, 2.5, and 4.5 mM, respectively, in 0.5 M NaCl. CGC decreased upon increasing the NaCl concentration, and the minimum concentration required to gel a given solvent mixture increased with an increase in the amount of the organic solvent. Additionally, lower CGC was associated with compounds with lower aqueous solubility.

2.2. T_{gel} measurements

In general, the thermal stability of gels as often characterized by T_{gel} (melting temperature of the gel) is dependent on several parameters like the surface of the gelation tube used, concentration of NaCl chosen, diameter of the tube, and the concentration of the gelator. However, under defined experimental conditions unique gel melting values were obtained.

The thermal stability of the gels increased with the concentration of the gelator, in a nearly linear fashion (data not shown). Higher thermal stability was also observed when the salt concentration was increased.[19] A comparison of T_{gel} for cationic bile salt gels is shown in Figure 1. No trivial relation between the melting temperature of the gels (T_{gel}) and the melting point of the pure cationic solids (on the order of 265–290 °C; see Section 5) was observed. The enthalpy of disaggregation was estimated from plots of ln (C) versus $1/T_{gel}$ using the Schröeder van Laar equation $\{\ln(C) = (\Delta H/RT_{gel} + \text{constant}$, where R is the universal gas constant and

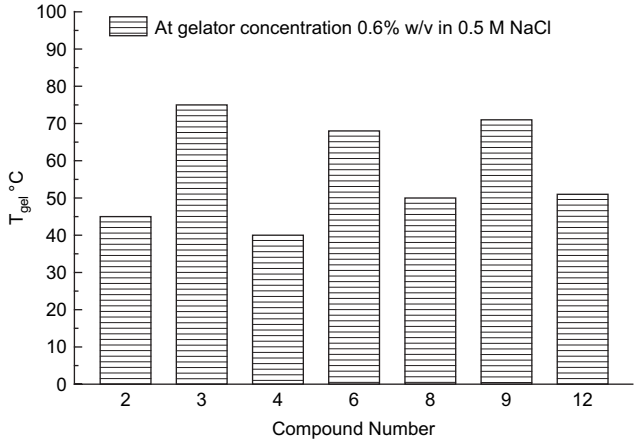


Figure 1. Comparison of T_{gel} values versus side chain structure of DC salts (experiments were performed at 0.6% w/v gelator concentration and 0.5 M NaCl).

C is the concentration of the gelator}. The estimated ΔH (gel melting) values were 50–80 kJ mol^{-1}.

T_{gel} values decreased with an increase in the percentage of the organic co-solvent, owing to the increased solubility of the gelator in the presence of higher amounts of the organic solvent. The gels of DMSO/H_2O were found to have higher T_{gel} values than those of the gels formed in EtOH/H_2O.

2.3. Microviscosity measurements

Polarization experiments carried out with 1,6-diphenylhexatriene (DPH) were made to determine the microviscosity of the cationic bile salt aggregates using the following expression[20]

$$\eta = 2P/(0.46 - P) \quad (1)$$

where, P is the polarization of DPH inside the gel.

Measured polarization and microviscosity values are listed in Table 1.

Table 1. Fluorescence polarization and microviscosity values for cationic bile salt aggregates obtained by probing with DPH (values are within 10% experimental error)

Bile salt	Head group	Concn (% w/v)	Polarization (P)	Microviscosity (η, Poise)
NaC	COOH	10	0.1	0.55
NaDC	COOH	10	0.17	1.25
2	DABCO	1	0.25	2.4
3	*N*-Methyl pyrrolidine	0.5	0.35	7.2
6	Quinuclidine	0.5	0.28	3.1
12	Dimazine	1.0	0.26	2.8

2.4. Side chain length and aggregation

The side chain of bile salts has been given primary importance in the self-assembly process of bile salts. It has been reported by Roda et al. that the CMC values increased significantly as the side chain was shortened from **C26** to **C24** to **C23** for free bile salts. Conjugation of the side chain carboxylic group with glycine or taurine, although increasing the length of the side chain, causes little change in the CMC values.[21]

To study the effect of side chain length on the aggregation of the cationic bile salts, the side chain was elongated by one carbon unit for two of the representative members of the class (compounds **15** and **16**) as shown in Scheme 2, and the aggregation properties were studied.[22] Preliminary investigation showed lowering of the CMC values upon increasing the side chain length by one carbon. For compounds **15** and **16** lowering in CMC values from 2.5 to 1.0 mM and 2.0 to 1.5 mM were found, respectively, after one carbon elongation at the side chain. However, noticeable differences were observed on the gelation properties of the cationic bile salts upon side chain elongation. The gelation abilities completely changed upon adding a carbon to the side chain. Compound **16** formed a gel in 1 M NaCl solution at a bile salt concentration greater than 0.3%. But compound **15** did not gel salt solutions under identical conditions. This suggested that C23-cationic bile salts are stronger gelators than C24 analogs under these conditions.

2.5. Electron microscopy study

Electron microscopic investigations of the xerogels of the cationic bile salts were attempted. The xerogel showed different types of fibrillar networks (shown in Fig. 2A–D). The diameter of the gel strands was of the order 3–10 μm.

The present experimental data do not allow us to postulate an exact theoretical model for the supramolecular aggregation of these molecules to form a self-aggregated gel network as could be seen from the SEM. No pronounced helicity of the fibers was observed even though the gelator molecules are all homochiral.[23]

2.6. Rheological properties

Flow properties of the gels prepared in a glass vial of defined size can be roughly characterized by visualizing their motion as a cause of weight when inverted. However, for exact quantification, rheological experiments are necessary. Linear and non-linear rheological measurements were carried out to characterize the flow behavior of the cationic bile salt gels.

2.6.1. Amplitude sweep. Amplitude sweep measurements were taken at an oscillating frequency of 1 Hz. G' (storage modulus) and G'' (loss modulus) values were determined at different gelator concentrations. Gels made from compounds **2**, **3**, **6**, **8**, **9**, and **12** in NaCl solution were characterized by their high elastic modulus G' and yield stress σ^* values (the critical stress at which the gel flows or gel loses its structure). G' was found to be larger than G'' in the LVE regime suggesting that the materials have solid-like response. G'/G'' for various cationic gelator is shown in Figure 3. A plot of elastic modulus versus gelator concentration (to verify $G' \propto C^n$) is shown in Figure 4.

The yield stress (σ^*) also increased with the gelator concentration and the magnitude was proportional to the elastic modulus (Fig. 3) and scaled as the power of the gelator concentration ($\sigma^* \propto C^n$) (Fig. 5). The values of the exponents

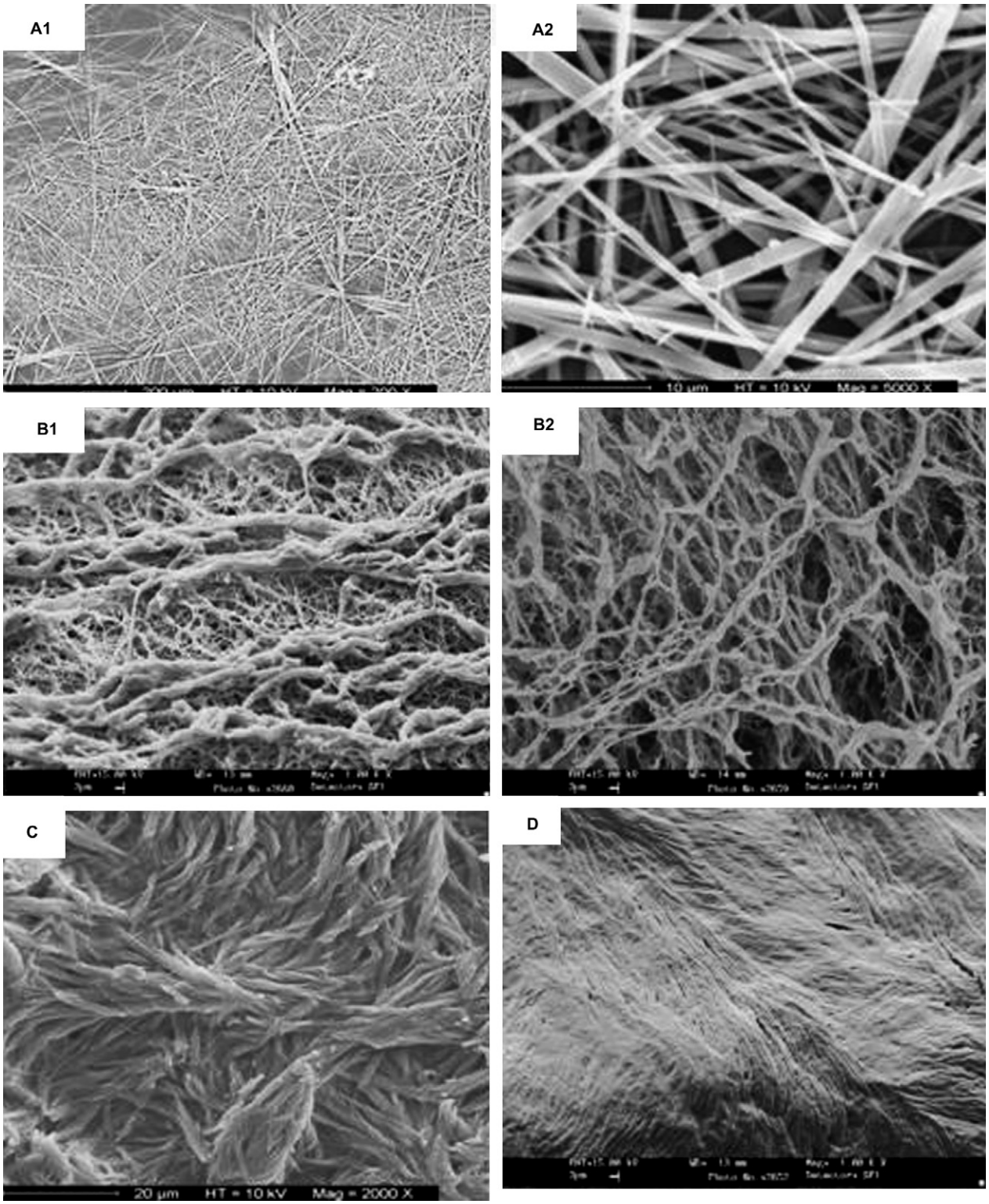


Figure 2. SEM images of cationic gels: (A) 0.5% w/v of **3** in 0.5 M NaCl (scale bars A1=200 μm and A2=10 μm), (B) 0.5% w/v of **6** in 0.5 M NaCl (scale bars B1 and B2=3 μm: B1 and B2 are different zones of the same xerogel), (C) 1% w/v **2** in 0.15 M NaCl (scale bar 20 μm), and (D) 1% w/v **12** in 0.5 M NaCl (scale bar 3 μm).

(*m* and *n*) listed in Table 2 are in good agreement with the reported values for low molecular mass gelling agents.[24,25]

The results obtained from the amplitude sweep experiments clearly indicate that individual cationic gels have unique rheological response.

2.6.2. Frequency sweep. Frequency sweep measurements were carried out on the cationic bile salt gels at applied stress amplitudes, which fall in the linear viscoelastic domains (LVE regime). The frequency sweep experiment showed a gel-like behavior over the complete range of frequencies studied. The typical rheograms obtained for the gels of

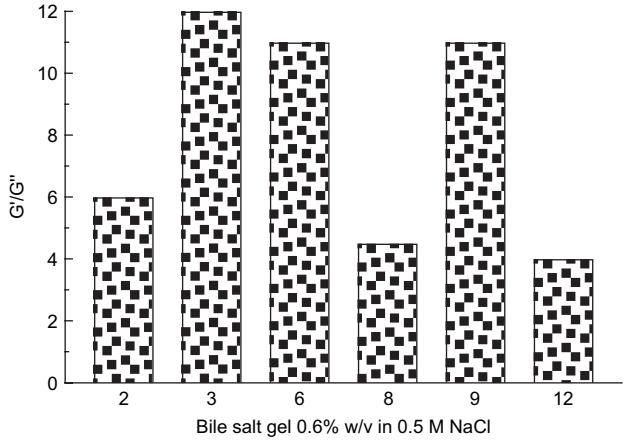


Figure 3. Comparison of G'/G'' versus side chain structure of DC salts (at 0.6% w/v gelator in 0.5 M aqueous NaCl).

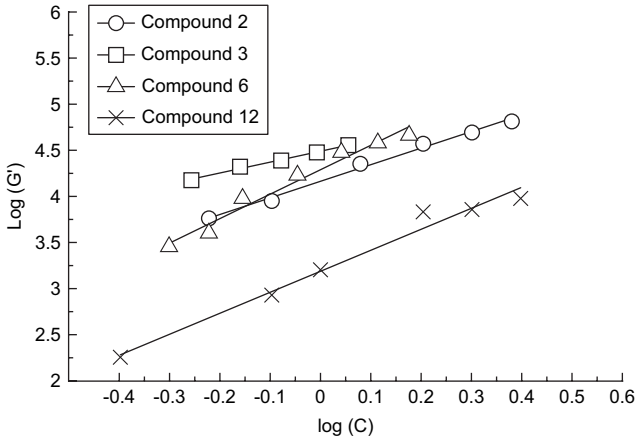


Figure 4. log–log representation of a plot of G' (elastic modulus) versus gelator concentration (0.4% w/v gelator in 1 M aqueous NaCl).

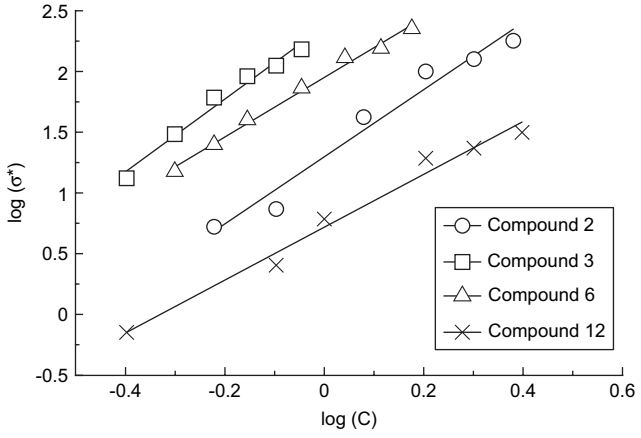


Figure 5. log–log representation of a plot of σ^* (yield stress) versus gelator concentration (0.4% w/v gelator in 1 M aqueous NaCl).

compound **2** in 1.0 M aqueous NaCl at 0.4% w/v gelator concentration are presented in Figure 6. The elastic modulus G' was an order of magnitude higher than the loss modulus G'' over the entire range of frequency, which characterizes the materials as *viscoelastic soft solids*. Nevertheless, over

Table 2. Rheological data obtained for some of the cationic bile salts (for comparison, 0.4% w/v gelator concentration was found to be ideal to gel all the four cationic bile salts)

Gelator	C (% w/v, in 1 M NaCl)	G' (Pa)	G'/G''	σ^* (Pa)	m	n
2	0.4	3500	6	7	2.1	2.5
3	0.4[a]	5200	10	35	2.2	2.7
6	0.4[a]	4100	9	20	2.6	2.4
12	0.4	460	4	2	2.3	2.2

[a] 20% MeOH was used to prepare the gel.

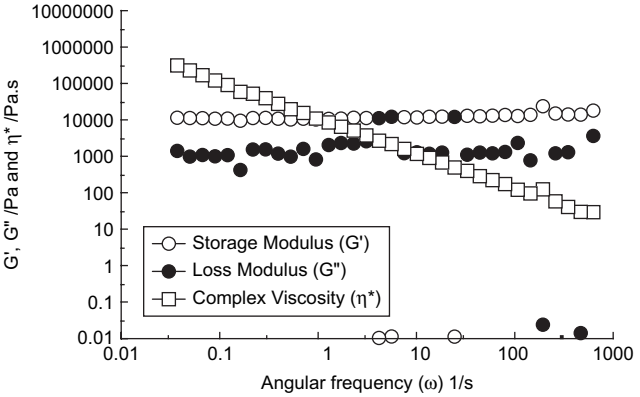


Figure 6. Frequency sweep experiment using cationic bile salt gels (0.4% w/v gelator **2** in 1 M aqueous NaCl).

long time scales (small frequencies of the applied stress), liquid-like flowing properties may prevail.

The complex viscosity decreased slowly and continuously with frequency and the slope approached −1 in a log–log representation (Fig. 7). This type of mechanical behavior is generally a characteristic observed in soft viscoelastic solids and hence many of the cationic bile salt derived materials can be rheologically classified as gels.[26]

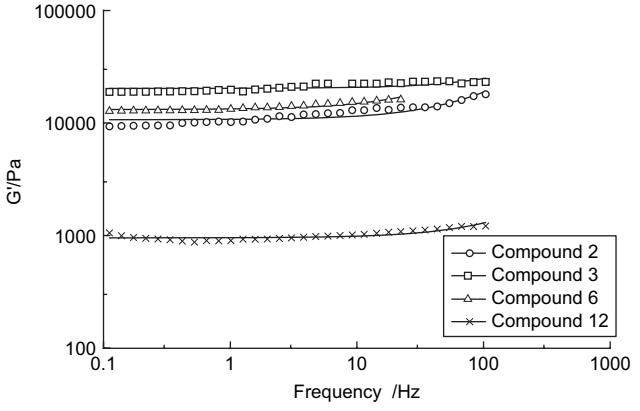


Figure 7. Combined plot of G' versus oscillating frequency for cationic bile salt gels (0.4% w/v gelator in 1 M aqueous NaCl).

2.6.3. Flow curves. Flow curves of cationic gels (at gelator concentration 0.4% w/v) were measured in 1.0 M NaCl solution, while 20% MeOH was used as a co-solvent to prepare gels of **3** and **6**. All the cationic gels studied showed a shear thinning behavior with the exponent being nearly zero from the power law fit (Fig. 8).

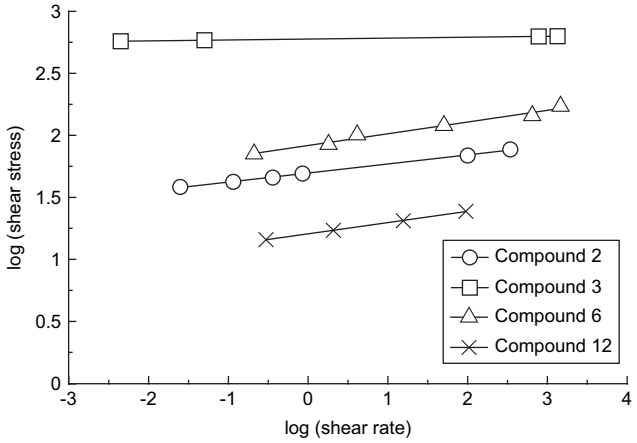


Figure 8. Power law fit for flow curves (log–log plot of shear stress versus shear rate) of cationic bile salt gels (at 0.4% w/v gelator in 1 M aqueous NaCl).

Cationic bile salt gels show a finite minimum stress point required to flow (yield value). This flattening of the shear viscosity at zero shear followed by a sharp decay with a slope approaching −1 is typical of semi-dilute solutions of rods exhibiting a solid-like behavior of complex fluids.[27]

3. Discussion

3.1. Bile salt structure and gelation property

Although gels derived from bile acids have been known for a long time,[28] they have not been systematically studied.[29] The current work presents a first attempt to analyze and correlate structure–aggregation properties derived from cationic bile salts. A large collection of cationic side chain modified bile salts was screened for gelation. Our studies highlight the importance of the deoxycholate (DC) backbone for their gelling property—presumably for having the 'right' hydrophilic–hydrophobic balance the details of which are still not understood. Cationic bile salts derived from cholic acid (compound **18**) were found to be more water soluble, hence they remained in solution under normal circumstances.[30] On the contrary, cationic bile salts derived from lithocholic acids (compound **17**) were found to be highly water insoluble with no gelation properties.[30] The gelling properties of the DC salts were found to be somewhat dependent on the side chain structure, although, most of them have high mechanical strength, thermal stability, and optical clarity.

The pyridinium salt (**4**) was the first compound studied in this series. Subsequently, the deoxycholic acid side chain was modified with various cationic head groups derived from heterocyclic, aromatic, linear, and branched compounds.

Replacement of the pyridinium cation (**4**) with a bipyridyl unit (**5**) resulted in a poorly soluble, non-gelling compound, as was observed with a quinolinium group also. Several non-aromatic heterocycles such as *N*-methyl piperidine and *N*-methylpyrrolidines were subsequently explored. To our delight, these cationic bile salts (**6** and **9**) were found to be excellent hydrogelators and gelled 1 M NaCl solution at concentrations as low as 0.25% w/v. The gels obtained were optically transparent up to 1% w/v. It was found that compound **9** had higher T_{gel} and higher storage modulus than compound **6**. It is remarkable that a subtle variation in the ring size causes such a noticeable change in their gelation properties.

'Opening' the piperidine head group of **9** resulted in a non-gelator **10** with higher aqueous solubility. On the other hand, incorporation of an oxygen atom at the 4-position of the piperidine head group of **9** resulted in compound **8**, which formed gels across wider concentration ranges (1 M NaCl, 0.5–3% w/v). The G' value measured for **8** was less than compounds **6** and **9** but similar to compound **2**. This is indicative of better solvation of the head group of compound **8**, presumably because of the additional heteroatom. The gels obtained were less strong than with a single heteroatom containing head group (**9**). Finally, 'opening up' the morpholine ring of **8** at the oxygen end resulted in highly water soluble, non-gelling compound **7**.

Further modification of the head group was made by introducing bicyclic head groups such as quinuclidine. This compound (**3**) was found to be an excellent gelator compared to all other cationic bile salts in this group. This gel had the highest G' value (see Fig. 3 or Fig. 7). Therefore, it seems clear that a rigid cationic head group leads to stronger gels. The introduction of a second nitrogen atom on the bicyclic quinuclidine head group of **3** resulted in gelator **2** with higher water solubility yielding softer gels than **3**. The gels made from **2** were similar in strength, but more transparent than the gels from compound **8**. Thus the results indicated that the bile salts containing two heteroatoms at the head group form softer gels than those with a single heteroatom.

Gels prepared from compounds **2** and **8** showed identical gel melting points and the G'/G'' values obtained were also very similar. Furthermore, an open cyclic analog of **2** resulted in a non-gelator molecule **1**, even though it has a CMC comparable to compound **2**. Finally, we made compound **12** representing two N-atoms directly linked to each other and this turned out to be a good gelator. The properties of the gels prepared from **12** were also similar to compounds **2** and **8**, except they were somewhat translucent.

3.2. Comparison of aggregation properties of quaternary bile salts derived from open chain and cyclic bases

Unlike their cyclic counterparts, most of the bile salts prepared from acyclic bases did not gel aqueous solvents, although the CMC values for bile salts derived from both open and cyclic bases were similar (Table 3). Bile salts made from cyclic bases showed large fluorescence anisotropies (ANS), up to 0.28, even at low bile salt concentrations around 0.15% w/v, which is related to medium viscosity. Under identical conditions, ANS fluorescence anisotropy observed for bile salts derived from acyclic bases remained below 0.15. These observations suggest the requirement of a rigid bile salt side chain for the growth of the smaller aggregates to obtain the gel.

Table 3. Comparison of the CMC values obtained for bile salts bearing acyclic and cyclic quaternary head groups

Bile salt	Cyclic head group	CMC (mM)	Bile salt	Corresponding open cyclic head group	CMC (mM)
2	DABCO	2.5±0.2	**1**	TMEDA	3.0±0.2
9	*N*-Methyl piperidine	2.3±0.2	**10**	*N*-Methyl dipropylamine	2.5±0.2
8	*N*-Methyl morpholine	2.3±0.2	**7**	*N*-Methyl diethanolamine	2.5±0.2

4. Conclusions

Although a wide variety of derivatives display gelation behavior, a small change in molecular structure can have a dramatic effect on the gelling capacity. Current research has provided a detailed understanding of the aggregation behavior of various cationic bile salt analogs. Importance of the side chain structure and length on the gelation of cationic bile salts has been qualitatively evaluated in the present work. The results supported the earlier postulate of strong correlation of aggregation versus side chain structure.

Most of the DC-hydrogels have been shown to behave as cellular solids. But the cationic bile salts are strong gels characterized by high elasticity and yield stress values. Flow behaviors of the cationic bile salt gels were characterized and the gels were shown to have a strong side chain structure dependent flow. Interestingly, each of the cationic systems exhibits a unique behavior in terms of its thermal, optical, and mechanical properties. Elastic moduli of gels obtained from these cationic derivatives are 2–3 orders of magnitude larger than those observed for sodium deoxycholate at comparable concentrations. The elastic moduli are however comparable to those reported for organogels derived from compounds of low mass.

SEM analysis of the dried gels shows fibers of finite radii with indeterminate lengths either bundled or intertwined, revealing no specialized branching. The gels obtained from cationic bile salts melt at moderate temperatures (50–70 °C), which opens up various commercial applications.

We believe that some of these cationic bile salts may be biologically active (antimicrobial, DNA binding, etc.). Additionally, the gels derived from them may find use in novel material and hybrid material synthesis. The gels can also serve as a useful chiral medium for manipulating chemical reactions and product selectivity. Some of these possibilities are being explored in our laboratory and the results from these experiments will be reported in due course.

5. Experimental section

5.1. Materials

Bile acids were purchased from Fluka for synthesizing different cationic bile salts. All the organic bases used were purchased from Aldrich (99%+). Pyrene (Aldrich) was used after recrystallization in methanol. Solvents used were of analytical grade or double distilled prior to use. Double distilled water was used for all spectral measurements. Glassware was rinsed with acids and cleaned with soap water prior to use.

All new cationic bile salts were synthesized by using procedures reported by Sangeetha et al. (from author's laboratory) and characterized by spectroscopic methods.[13] General method for the syntheses of 24-nor-3α-12α-dihydroxy-5β-cholane salts: to a suspension of 24-nor-23-iodo-3α-12α-dihydroxy-5β-cholane in CH_3CN heated to reflux, corresponding organic base was added in excess (2 equiv) and refluxing was continued further for a period of 6 h. Solvent was removed in vacuo and the crude product was chromatographed over silica gel column using 25–85% EtOH/$CHCl_3$ as an eluent or purified by precipitating the cationic bile salts by adding diethyl ether to an ethanolic solution.

5.1.1. *N*,*N*-Dimethyl,*N*-(dimethylaminomethyl),*N*-(3α-12α-dihydroxy-24-nor-5β-cholan-23-yl) iodide (1). Yield: 70%. Mp 251–252 °C. $[\alpha]_D^{25}$ 49.0 (*c* 1.0, EtOH). IR (KBr, cm^{-1}): 3444, 2922, 2865, 2772, 1632, 1466, 1447, 1383, 1042, 918. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.61 (s, 3H), 0.85 (s, 3H), 0.96 (d, J=5.4 Hz, 3H), 1.01–1.80 (m, steroidal CH, CH_2, 31H), 2.99 (br s, 6H), 3.11 (br s, 6H), 3.768 (s, 1H), 4.29 (d, J=3.6 Hz, 1H), 4.51 (d, J=3.6 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.29, 17.30, 23.06, 23.38, 26.07, 26.93, 26.03, 27.68, 28.55, 30.17, 32.89, 33.53, 33.78, 35.09, 35.60, 36.24, 41.53, 45.09, 45.84, 45.99, 47.49, 50.24, 50.398, 52.29, 59.54, 62.02, 69.87, 70.85, 79.20. LRMS: calcd for $C_{29}H_{55}N_2O_2$: 463.43, obsd 463.50. Anal. Calcd for $C_{29}H_{55}N_2O_2I$: C 58.97, H 9.39, N 4.74. Found: C 58.98, H 9.51, N 4.71.

5.1.2. *N*-(3α-12α-Dihydroxy-24-nor-5β-cholan-23-yl) diazabicyclo[2.2.2]octyl iodide (2). Yield: 75%. Mp 296–297 °C. $[\alpha]_D^{25}$ 39.0 (*c* 1.0, EtOH). IR (KBr, cm^{-1}): 3454, 2920, 2860, 1462, 1383, 1091, 1055, 1043, 843. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.60 (s, 3H), 0.84 (s, 3H), 0.96 (d, J=5 Hz, 3H), 1.01–1.81 (steroidal, CH, CH_2, 27H), 3.00 (br s, 6H), 3.30 (br s, 6H), 3.77 (s, 1H), 4.34 (d, J=3.6 Hz, 1H), 4.55 (d, J=3.6 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.35, 17.33, 23.08, 23.40, 26.09, 26.87, 26.95, 27.03, 28.54, 30.19, 32.91, 33.55, 33.80, 35.12, 35.62, 36.25, 41.56, 44.73, 45.74, 46.03, 47.50, 51.32, 61.44, 69.87, 70.85, 79.30. LRMS: calcd for $C_{29}H_{51}N_2O_2$: 459.39, obsd 459.46. Anal. Calcd for $C_{29}H_{51}N_2O_2I$: C 59.38, H 8.76, N 4.78. Found: C 59.58, H 8.80, N 4.91.

5.1.3. *N*-(3α-12α-Dihydroxy-24-nor-5β-cholan-23-yl) quinuclidinium iodide (3). Yield: 70%. Mp 277–278 °C. $[\alpha]_D^{25}$ 42.0 (*c* 1.0, EtOH). IR (KBr, cm^{-1}): 3430, 2936, 2860, 1620, 1462, 1382, 1043, 943. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.60 (s, 3H), 0.84 (s, 3H), 0.95 (d, J=3.3 Hz, 3H), 1.02–2.05 (m, steroidal CH, CH_2, 26H), 3.06–3.19 (br m, 15H), 3.77 (br s, 1H), 4.31 (d, J=3.6 Hz, 1H), 4.52 (d, J=3.6 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.33, 17.29, 19.09, 23.06, 23.37, 26.09, 26.95,

27.20, 28.55, 30.19, 32.90, 33.55, 33.78, 35.12, 35.61, 36.24, 41.56, 45.74, 46.00, 47.48, 53.46, 61.33, 69.88, 70.85. LRMS: calcd for $C_{30}H_{52}NO_2$: 458.39, obsd 458.40. Anal. Calcd for $C_{30}H_{52}NO_2I+H_2O$: C 59.69, H 9.02, N 2.30. Found: C 59.78, H 8.93, N 2.21.

5.1.4. *N*-(3α-12α-Dihydroxy-5β-24-nor-cholan-23-yl) 4,4′-bipyridinium iodide (5). Yield: 65%. Mp 216–217 °C. $[\alpha]_D^{25}$ 31.0 (*c* 1.0, EtOH). IR (KBr, cm^{-1}): 3414, 2959, 2924, 2861, 1634, 1471, 1458, 1380, 1300, 1251. ^1H NMR (300 MHz, DMSO-d_6) δ: 0.61 (s, 3H), 0.92 (s, 3H), 1.08 (d, *J*=6.3 Hz, 3H), 1.14–2.00 (m, steroidal CH, CH_2, 27H), 3.81 (br s, 1H), 4.31 (d, *J*=3.8 Hz, 1H), 4.50 (d, *J*=3.8 Hz, 1H), 8.04 (d, *J*=5.7 Hz, 2H), 8.62 (d, *J*=6.6 Hz, 2H), 8.87 (d, *J*=5.7 Hz, 2H), 9.26 (d, *J*=6.6 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.37, 17.22, 23.07, 23.41, 26.05, 26.93, 27.09, 28.59, 30.20, 32.90, 33.59, 33.78, 35.09, 35.60, 36.22, 37.56, 41.54, 46.06, 47.50, 58.72, 69.90, 70.91, 79.234, 121.92, 125.41, 140.81, 145.35, 150.96, 152.12. LRMS: calcd for $C_{33}H_{47}N_2O_2$: 503.36, obsd 503.4. Anal. Calcd for $C_{33}H_{47}N_2O_2I+1.5H_2O$: C 60.36, H 7.52, N 4.26. Found: C 60.40, H 7.56, N 4.12.

5.1.5. *N*-(3α-12α-Dihydroxy-24-nor-5β-cholan-23-yl) *N*-methylpyrrolidinium iodide (6). Yield: 70%. Mp 253–254 °C. $[\alpha]_D^{25}$ 39 (*c* 1.0, EtOH). IR (KBr, cm^{-1}): 3419, 2932, 2858, 1635, 1469, 1375, 1042, 930. ^1H NMR (300 MHz, DMSO-d_6) δ: 0.60 (s, 3H), 0.84 (s, 3H), 0.98 (d, *J*=5 Hz, 3H), 1.02–1.81 (m, steroidal CH, CH_2, 34H), 2.07 (br s, 4H), 2.97 (s, 3H), 3.78 (s, 1H), 4.34 (d, *J*= 3.6 Hz, 1H), 4.56 (d, *J*=3.6 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.50, 17.20, 20.04, 21.13, 23.12, 23.52, 26.12, 26.99, 27.31, 28.64, 30.25, 32.25, 32.95, 33.84, 35.09, 35.68, 36.28, 41.61, 46.04, 46.41, 47.52, 63.34, 63.52, 69.97, 71.08, 79.20. LRMS: calcd for $C_{28}H_{50}NO_2$: 432.38, obsd 432.34. Anal. Calcd for $C_{28}H_{50}NO_2I+0.5H_2O$: C 59.14, H 9.04, N 2.46. Found: C 59.14, H 9.04, N 2.50.

5.1.6. *N*-(3α-12α-Dihydroxy-5β-24-nor-cholan-23-yl) *N*-methyldiethanolammonium iodide (7). Yield: 65%. Mp 226–227 °C. $[\alpha]_D^{25}$ 35.0 (*c* 1.0, EtOH). IR (KBr, cm^{-1}): 3406, 2965, 2934, 2913, 2858, 1630, 1448, 1370, 1090, 1043, 925. ^1H NMR (300 MHz, DMSO-d_6) δ: 0.60 (s, 3H), 0.84 (s, 3H), 0.97 (d, *J*=5.1 Hz, 3H), 1.00–2.07 (m, steroidal CH, CH_2, 31H), 3.09 (s, 3H), 3.79 (br s, 5H), 5.49 (br s, 2H), 4.31 (d, *J*=3.6 Hz, 1H), 4.54 (d, *J*=3.6 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.34, 17.31, 18.57, 23.10, 23.45, 26.11, 26.99, 27.15, 27.48, 28.59, 30.21, 32.93, 33.83, 33.87, 35.16, 35.65, 36.27, 41.61, 46.07, 47.54, 49.02, 54.77, 61.18, 63.12, 69.92, 70.92, 79.30. LRMS: calcd for $C_{28}H_{52}NO_4$: 466.38, obsd 466.4. Anal. Calcd for $C_{28}H_{52}NO_4I$: C 56.65, H 8.83, N 2.36. Found: C 56.62, H 8.92, N 2.24.

5.1.7. *N*-(3α-12α-Dihydroxy-24-nor-5β-cholan-23-yl) *N*-methyl-*N*,*N*-dipropylammonium iodide (10). Yield: 75%. Mp 247–248 °C. $[\alpha]_D^{25}$ 39.0 (*c* 1.0, EtOH). IR (KBr, cm^{-1}): 3361, 2935, 2860, 1644, 1471, 1448, 1371, 1091, 1045. ^1H NMR (300 MHz, DMSO-d_6) δ: 0.61 (s, 3H), 0.85 (s, 3H), 0.98 (d, *J*=5.1 Hz, 3H), 1.01–1.80 (m, steroidal CH, CH_2, 37H), 2.94 (s, 3H), 3.16 (m, 4H), 3.78 (br s, 1H), 4.24 (d, *J*=3.6 Hz, 1H), 4.46 (d, *J*=3.6 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 10.55, 12.30, 15.16, 17.34, 23.06, 23.40, 26.08, 26.94, 27.11, 27.29, 28.52, 30.19, 32.89, 33.70, 33.78, 35.09, 35.60, 36.24, 41.55, 45.90, 46.04, 47.50, 59.17, 61.89, 69.88, 70.89. LRMS: calcd for $C_{30}H5_6NO_2$: 462.43, obsd 462.50. Anal. Calcd for $C_{30}H_{56}NO_2I+3.5H_2O$: C 64.54, H 10.80, N 2.51. Found: C 64.62, H 10.11, N 2.45.

5.1.8. *N*-(3α-12α-Dihydroxy-24-nor-5β-cholan-23-yl) hydrazyl iodide (12). Yield: 65%. Mp turns brown at 160 °C and melts near 175 °C. $[\alpha]_D^{25}$ 36 (*c* 1.0, EtOH). IR (KBr, cm^{-1}): 3471, 3389, 3249, 3139, 2934, 2862, 1621, 1448, 1378, 1044. ^1H NMR (300 MHz, DMSO-d_6) δ: 0. 59 (s, 3H), 0.83 (s, 3H), 0.95 (d, *J*=6.3 Hz, 3H), 1.00–1.80 (m, steroidal CH, CH_2, 24H), 3.16 (s, 6H), 3.77 (s, 6H), 4.27 (d, *J*=3.6 Hz, 1H), 4.50 (d, *J*=3.6 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.37, 17.40, 23.13, 23.45, 26.14, 26.99, 27.08, 28.06, 30.23, 32.98, 33.32, 33.84, 35.13, 35.68, 36.28, 41.60, 45.77, 46.05, 47.59, 55.05, 55.37, 57.38, 66.16, 69.96, 70.94. LRMS: calcd for $C_{25}H_{47}N_2O_2$: 407.44, obsd 407.39. Anal. Calcd for $C_{25}H_{47}N_2O_2+3H_2O$: C 4.34, H 8.94, N 5.05. Found: C 54.30, H 8.57, N 4.89.

5.1.9. 3α-12α-24-Trihydroxy-5β-cholane (13). To a stirring solution of deoxycholic acid (1 g, 2.55 mmol) in THF (20 mL), triethylamine (0.6 mL, 8.2 mmol) was added followed by the slow addition of ethyl chloroformate (0.4 mL, 3.24 mmol). The reaction mixture was stirred for 2 h and a solution of sodium borohydride (1.2 g, 32 mmol) in water (7 mL) was added slowly. The resulting mixture was allowed to stir at room temperature (28 °C) for 12 h. HCl (20 mL of 1 M solution) was added to the reaction mixture and was stirred for an additional 30 min. Inorganic materials were removed out by filtration and the reduction product was extracted using ethyl acetate (2×50 mL). The EtOAc layer was washed with water, dried over anhyd Na_2SO_4, and the white powder obtained was finally crystallized from ethyl acetate to yield 0.8 g of the crystalline solid product with mp 102–103 °C. $[\alpha]_D^{25}$ 54.0 (*c* 2.0, EtOH). IR (neat, cm^{-1}): 3398, 2938, 2863, 1063. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.68 (s, 3H), 0.90 (s, 3H), 0.98 (d, *J*=6.0 Hz, 3H), 1.05–1.86 (m, steroidal CH, CH_2, 39H), 2.78 (m, 2H), 3.99 (br s, 1H), 3.60 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 10.91, 12.72, 17.61, 23.15, 23.68, 26.16, 27.17, 27.58, 28.61, 29.58, 29.79, 30.36, 31.85, 33.50, 34.11, 35.29, 35.41, 35.99, 36.36, 42.07, 45.81, 47.38, 48.12, 62.96, 71.31, 72.86. LRMS: calcd for $C_{24}H_{42}O_3$+Na: 401.30, obsd 401.4. Anal. Calcd for $C_{24}H_{42}O_3+0.5H_2O$: C 74.35, H 11.18. Found: C 74.11, H 10.87.

5.1.10. 3α-12α-Dihydroxy-5β-24-(4-toluenesulfonyloxy) cholane (14). To a suspension of 3α-12α-24-trihydroxycholane (1.0 g, 2.65 mmol) in THF (20 mL), triethylamine (1.5 mL, 20.54 mmol) and *p*-toluenesulfonylchloride (1.0 g, 5.24 mmol) were added. The reaction mixture was stirred in an ice-water bath for 12 h. Crude mixture was extracted into chloroform (50 mL), washed with water (2×20 mL), and dried over anhyd Na_2SO_4. Finally, after removal of volatiles the residue was chromatographed over silica column using 25% EtOAc/$CHCl_3$ as an eluent, resulting in 1 g (70%) of the product as white solid with mp 66–68 °C. $[\alpha]_D^{25}$ 39.5 (*c* 2.0, EtOH). IR (neat, cm^{-1}): 3388, 2937, 2863, 1598, 1448, 1360, 1176, 1038, 916, 813, 737. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.64 (s, 3H), 0.90 (s, 3H), 0.92 (d,

merged, 3H), 0.97–1.83 (m, steroidal CH, CH_2, 29H), 2.45 (s, 3H), 3.61 (m, 1H), 3.96 (br s, 1H), 4.00 (m, 1H), 7.35 (d, J=7.8 Hz, 2H), 7.76 (d, J=7.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.69, 17.41, 21.64, 23.13, 23.65, 25.67, 26.13, 27.14, 27.52, 28.63, 30.40, 31.36, 33.60, 34.11, 35.06, 35.24, 36.01, 36.39, 42.07, 46.43, 47.33, 48.19, 71.19, 71.69, 73.01, 127.86, 129.81, 133.25, 144.65. LRMS: calcd for $C_{31}H_{48}O_5S$+Na: 555.33, obsd 556.0. Anal. Calcd for $C_{31}H_{48}O_5S$: C 68.72, H 9.11. Found: C 69.00, H 8.81.

5.1.11. N_1-(3α-12α-Dihydroxy-5β-cholan-24-yl) diazabicyclo[2.2.2]octanyl iodide (15). To a refluxing suspension of 3α-12α-dihydroxy-5β-24-(4-toluenesulfonyloxy) cholane (1.0 g, 1.87 mmol) in CH_3CN (10 mL), diazabicyclo[2.2.2]octane (0.42 g, 3.75 mmol) was added and refluxed for 6 h. The solvent was evaporated and the crude product was purified by reprecipitating the alcoholic solution repeatedly with diethyl ether as mentioned earlier. Amberlite IRA 400 (iodide form) was used to exchange tosylate by iodide. The pure product weighed 1.04 g (83%) as white solid. Mp 299–300 °C. $[\alpha]_D^{25}$ 39.0 (c 1.0, EtOH). IR (KBr, cm^{-1}): 3454, 2920, 2860, 1462, 1383, 1091, 1055, 1043, 843. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.57 (s, 3H), 0.80 (s, 3H), 0.94 (d, J=6 Hz, 3H), 1.12–1.69 (m, steroidal, CH, CH_2, 31H), 3.12 (m, 6H), 3.41 (m, 6H), 3.83 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.51, 17.19, 18.20, 23.12, 23.68, 26.00, 27.25, 28.62, 30.00, 32.00, 32.95, 33.83, 35.02, 35.26, 35.68, 36.42, 41.60, 43.59, 46.02, 46.25, 47.50, 50.67, 63.71, 69.93, 71.01. LRMS: calcd for $C_{30}H_{53}N_2O_2$: 473.5, obsd 473.4. Anal. Calcd for $C_{30}H_{53}N_2O_2I$+1.5H_2O: C 57.38, H 8.99, N 4.46. Found: C 57.48, H 8.70, N 4.36.

5.1.12. N-[3α-12α-Dihydroxy-5β-cholan-24-yl]-N-methylpyrrolidinium iodide (16). To a refluxing suspension of 3α-12α-dihydroxy-5β-24-(4-tolunesulfonyloxy) cholane (0.5 g, 0.94 mmol) in acetonitrile (10 mL), N-methylpyrrolidine (0.2 mL, 2.3 mmol) was added and kept at 70 °C for an additional 5 h. The crude product was purified by column chromatography using 20–80% ethanol/chloroform as an eluent giving 0.4 g (68%) of the pure salt as a white solid. Mp 258.8–259.0 °C. $[\alpha]_D^{25}$ 41.0 (c 1.0, EtOH). IR (neat, cm^{-1}): 3411, 2934, 2862, 1636, 1466, 1044, 755. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.60 (s, 3H), 0.83 (s, 3H), 0.96 (d, J=6 Hz, 3H), 1.01–1.75 (m, steroidal CH, CH_2, 33H), 2.06 (br s, 4H), 2.96 (s, 3H), 3.79 (br s, 1H), 4.28 (br s, 1H), 4.50 (br s, H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.51, 17.20, 20.04, 21.13, 23.12, 23.52, 26.12, 27.00, 27.31, 28.64, 30.25, 32.25, 32.95, 33.84, 35.09, 35.68, 36.28, 41.61, 46.04, 46.41, 47.52, 63.52, 63.39, 69.95, 71.08, 79.20. LRMS: calcd for $C_{29}H_{52}NO_2$: 446.74, obsd 447.0. Anal. Calcd for $C_{29}H_{52}$ NO_2I: C 60.72, H 9.14, N 2.45. Found: C 60.80, H 9.14, N 2.45.

5.1.13. N-(3α-Hydroxy-24-nor-5β-cholan-23-yl) diazabicyclo[2.2.2]octyl iodide (17). To a refluxing suspension of 24-nor-23-iodo-3α-hydroxy-5β-cholane[22b] (1.0 g, 2.24 mmol) in CH_3CN (10 mL), diazabicyclo[2.2.2]octane (0.49 g, 4.4 mmol) was added and refluxed for 6 h. The solvent was evaporated and the crude product was purified by reprecipitating the alcoholic solution repeatedly with diethyl ether as mentioned earlier. The pure product weighed 0.87 g (70%) as white solid. Mp 283–284 °C. IR (KBr, cm^{-1}): 3395, 2949, 1465, 1080, 1050.^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.61 (s, 3H), 0.86 (s, 3H), 0.92 (d, J= 5.2 Hz, 3H), 1.03–1.91 (m, steroidal CH, CH_2, 29H), 3.00 (br s, 6H), 3.24 (br s, 6H), 4.47 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 11.83, 18.54, 20.39, 23.28, 23.79, 26.18, 26.74, 26.87, 27.59, 30.37, 33.43, 34.20, 35.12, 35.37, 36.29, 41.47, 42.33, 44.71, 51.39, 55.00, 56.08, 61.36, 69.83. LRMS: calcd for $C_{29}H_{51}N_2O_2$: 443.1, obsd 443.5. Anal. Calcd for $C_{29}H_{51}N_2O_2I$: C 61.04, H 9.01, N 4.91. Found: C 59.72, H 8.86, N 4.45.

5.1.14. N-(3α-7α-12α-Trihydroxy-24-nor-5β-cholan-23-yl) diazabicyclo[2.2.2]octyl iodide (18). To a refluxing suspension of 24-nor-23-iodo-3α-7α-12α-trihydroxy-5β-cholane[22b] (1.0 g, 2.04 mmol) in CH_3CN (10 mL), diazabicyclo[2.2.2]octane (0.45 g, 4.0 mmol) was added and refluxed for 6 h. The solvent was evaporated and the crude product was purified by reprecipitating the alcoholic solution repeatedly with diethyl ether as mentioned earlier. The pure product weighed 0.74 g (65%) as white solid. Mp 293–294 °C. $[\alpha]_D^{25}$ 19.0 (c 1.0, EtOH). IR (KBr, cm^{-1}): 3398, 2948, 1669, 1636, 1466, 1078, 1051. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.59 (s, 3H), 0.80 (s, 3H), 0.97 (d, J=4.8 Hz, 3H), 1.01–2.26 (m, steroidal CH, CH_2, 26H), 3.01 (br s, 6H), 3.42 (br s, 6H), 3.60 (br s, 1H), 3.76 (br s, 1H), 4.04 (br s, 1H), 4.17 (br s, 1H), 4.36 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.28, 17.38, 22.64, 22.74, 26.26, 26.93, 27.18, 28.54, 30.39, 33.69, 34.38, 34.93, 35.31, 41.49, 44.76, 45.75, 45.82, 51.38, 61.56, 66.18, 70.41, 70.92. LRMS: calcd for $C_{29}H_{51}N_2O_3$: 475.7, obsd 475.4. Anal. Calcd for $C_{29}H_{51}N_2O_3I$+2H_2O: C 54.54, H 8.68, N 4.39. Found: C 54.92, H 8.36, N 4.15.

5.2. General

All steady state fluorescence experiments were performed using a Perkin–Elmer LS-50B luminescence spectrometer with a built-in system for polarization of the excited and emitted lights and calculation of grating factor and were expressed as anisotropy. The grating factor G, which is the ratio of the sensitivity of the detecting system for the vertically and horizontally polarized lights, was calculated prior to every individual anisotropy measurement. All fluorescence spectra were recorded in 2 mm or 5 mm quartz cuvettes.

5.3. CMC determination

Over 50 methods have been employed in the literature to determine the CMC values of surfactant solutions. An invasive method, which involved the use of hydrophobic dye pyrene in to the bulk of the surfactant medium, was applied in the current measurement.[31] The concentration of the cationic bile salt at which pyrene I_3/I_1 showed the first inflection point was taken as CMC. In general, the cationic bile salts showed CMC values between 2 and 4 mM in 0.5 M NaCl solution depending on the head group, which was found to be lower than sodium deoxycholate.[32]

5.4. Gelation test

Gelation tests were carried out by dissolving a known amount of the gelator in water or aqueous salt solution taken

in test tubes (d=8 mm, l=10 cm) upon heating. The clear solution obtained was kept at room temperature without any disturbance. Observations were recorded after 24 h.

5.5. CGC measurements

Fluorescence anisotropy of ANS (1-anilino-8-naphthalene sulfonate) was found to be more sensitive to gel formation. Critical gel concentration (CGC) of the cationic bile salt was determined by probing the fluorescence anisotropy of ANS. Aqueous ANS solution (10 μM) was used to make the gel/micelle. Fluorescence anisotropy was measured against gelator concentration after thermostating the samples for at least 2 h. The CGC was determined by plotting the fluorescence anisotropy of ANS as a function of bile salt concentration. The concentration at which the ANS anisotropy increased rapidly was taken as critical gel concentration (CGC). Binding of ANS fluorescent probe to macromolecules depends on the surface cationic charge and solution pH.[33]

5.6. Microviscosity measurements

Microviscosities of the cationic bile salt gels were estimated using DPH (1,6-diphenylhexatriene). An aqueous solution of DPH (10 μM) was prepared by diluting the THF stock solution (5 mM) with a known amount of water. Aqueous DPH solution was used to prepare gel/micelle. Fluorescence polarization of the DPH probe inside the micelle/gel was recorded. This polarization value was converted into microviscosity using basic mathematical equations.[34]

5.7. Sol–gel transition temperature (T_{gel})

Gels prepared in sealed Pyrex tubes (diameter 8 mm, length 10 cm; gel volume 0.5 mL) were kept upside down in a thermostated water bath and the temperature of the thermostat was raised slowly (2 °C/min). The temperature at which the gel fell under gravity was noted as the T_{gel} (Fig. 1).[35]

5.8. Scanning electron micrographs

Freshly prepared gels were dried over the sample grid. To observe the morphology of the xerogels (dried gel), 200 Å thick gold films were deposited by dc sputtering after drying, and were examined using a Leica 440*i* scanning electron microscope with a LaB_6 emitter.

5.9. Rheology

Physica (Anton Paar) MCR 300, a stress controlled rheometer with adjustable Peltier temperature controlling system was used for performing the experiments. All the measurements were carried out at 20.0±0.1 °C using cone-plate geometry (CP-25/2). The gap between cone and plate was 0.05 mm (measuring position) for all the measurements. Gel sample was introduced between the cone and the plate in the form of a hot sol. After confirmation of the complete gelation, followed by measuring the dynamic moduli as a function of time (2–3 h) at a very small-applied stress, the experiments were performed. The measuring cell used with a hydrated sponge cover limited evaporation of the solvent during the experiment. The determination of the linear viscoelastic regime of deformations and yield stress of the gels was done by dynamic measurements at a frequency of 1 Hz of the oscillatory stress.

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