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Supramolecular Chemistry of Bile Acid Derivatives: Formation of Gels

Ponnusamy Babu,[1] Neralagatta M. Sangeetha,[2] Uday Maitra*[3]

Summary: This article describes some of the exciting results obtained during the study of the gelation behavior of bile acid derivatives in the authors laboratory. The serendipitous discovery of charge-transfer interaction promoted gelation of organic solvents by (steroid)pyrene derivatives/TNF is presented. In this class of molecules, the effect of the location of the chiral center in chiral gelators on the overall chirality of the aggregates in the gel was studied. Also described are the aggregation behavior of bile acid based aqueous gelators which led us to postulate design principals to obtain bile acid based aqueous/organogelators.

Keywords: bile acids; charge-transfer; organo and aqueous gels; rotational dynamics; thermochromism

Introduction

Bile salts are a class of molecules that belong to the family of steroids and the studies on these molecules has been and is being pursued actively because of their importance in various physiological functions [1]. Bile salts formed by the conjugation of bile acids with taurine and glycine are biologically important surfactants and are involved in numerous biological functions. They solubilize lecithin and cholesterol as mixed micelles in bile [2], help in the absorption of dietary lipids and fat soluble vitamins like retinol and ∃-carotene [3], aid in digestion by removing pancreatic hydrolysis products such as fatty acids [4], and also regulate a number of cholesterol metabolizing enzymes in liver and intestine [5].

An important characteristic of the bile salts is the formation of micelles in aqueous solutions. Bile salts are facially amphiphilic possessing a steroid nucleus with a convex hydrophobic surface (∃-face) and a concave hydrophilic surface (∀-face) lined with hydroxyl groups. Structures of some of the bile acids is shown in chart 1. The aggregates formed by the association of these molecules in solution are very different from those of the classical surfactants which have well defined hydrophilic and liphophilic parts. The critical micellar concentration of these salts depends on a number of factors including their structure, pH, ionic strength, temperature *etc* [1a]. The structure of the micelles formed by bile salts have been investigated by techniques such as nuclear magnetic resonance spectroscopy [6], light scattering [7], small angle X-ray scattering [8] and fluorescence probing to name a few [6b, 9].

The unique structural features of bile acids have rendered them useful in supramolecular chemistry and many of the derivatives have been used in host-guest and biomimetic chemistry [10]. Bile acids/salts and their derivatives self-organize to give supramolecular gels which have immense application potential [11]. A majority of these bile acid derivatives have a general structure with an intact steroidal backbone and a variable side chain group (Charts 1 and 2) [12,17]. Depending on the solvation property of this side chain group, sol-

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[1] Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India
[2] No. 109, Thayappa Garden, HAL IInd Stage Kodihalli, Bangalore 560 008, India
[3] Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012, India
Fax: (+91) 80 360 0529
E-mail: maitra@orgchem.iisc.ernet.in

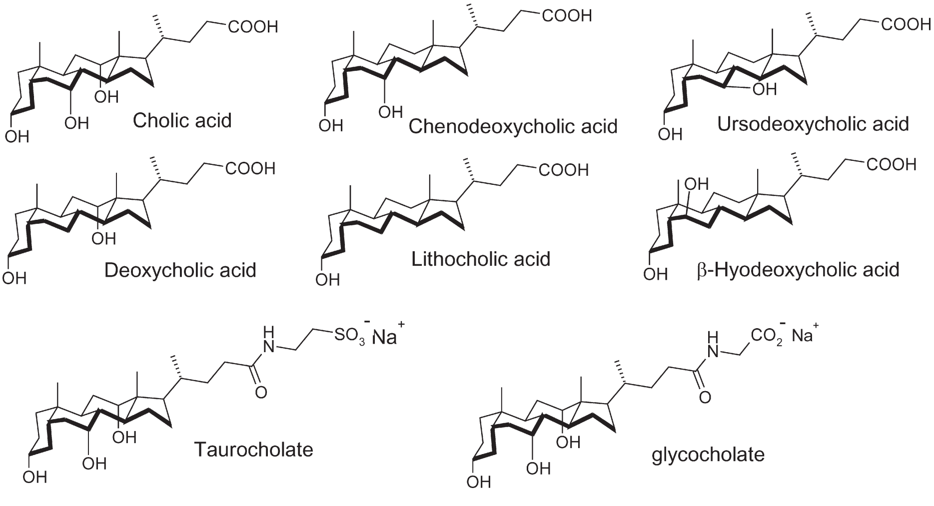


Chart 1.
Structures of some common bile acids/salts.

vents ranging from water to organics can be gelled: polar functionalities at the side chain that lead to ionic species gelate aqueous solutions, while neutral derivatives with alkyl groups gelate organic solvents. For instance, naturally occurring bile acids/salts form aqueous gels. These include calcium cholate [12], sodium deoxycholate [13], sodium lithocholate [14] and chenodeoxycholic acid [15]. On the other hand, derivatives such as *N*-isopropyl cholamide [16] and closely related *N*-cholyl amino acid alkyl esters [17] gel organic liquids.

The 'clot' (gel) formation by sodium cholate in the presence of an excess of various calcium salts ($CaCl_2$, $CaBr_2$, $Ca(NO_2)_2$ and CaI_2, *etc.*) at 50°C was reported in 1914 by Schryver [12]. This is perhaps the first and the only report that discusses cholate gels. These gels are formed only at high temperatures and are irreversible. The formation of aqueous gels by sodium deoxycholate (NaDC) and lithocholate (NaLC) at pH values close to neutrality, was first reported by Sobotka and Czeczowiczka *et al.* in 1958 [13]. They made detailed studies on the concentration and pH dependence of gel formation. In the same year, Rich and Blow published their studies on the air dried, brittle fibres drawn from a viscous solution of NaDC (which was referred as "*acomplex of sodium deoxycholate*") by X-ray diffraction and demonstrated their helical structures [18]. Subsequently in 1961, Ramanathan, Currie and Colvin showed the presence of helical microfibrils in NaDC suspensions by electron microscopic studies [19]. Of the several bile acids investigated by them (cholic, apocholic, lithocholic, hyodeoxycholic, taurocholic and glycocholic acids) only deoxycholic acid salt was found to generate super helices with a right-handed twist which eventually got transformed to

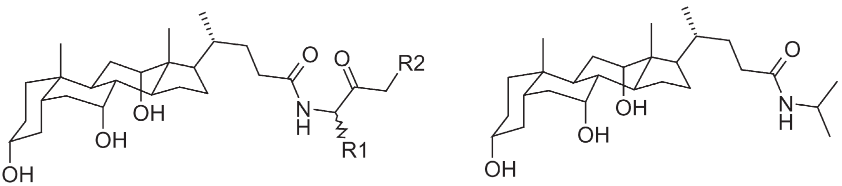


Chart 2.
Bile acid derived organogelators.

rod-like aggregates. Much later, the gelation phenomena of NaDC and the properties of the gels formed have been studied extensively [20]. Sodium lithocholate forms liquid-like suspensions of nanotubes or gels in water, depending upon concentration and pH [14]. Sodium lithocholate which is relatively non-polar as compared to other bile acids gelates organic solvents such as methanol, ethanol and ethylene glycol also [14a]. Similarly, nonpolar derivatives such as *N*-isopropyl cholamide [16] and closely related *N*-cholyl amino acid alkyl esters [17] gel aromatic solvents. In this account we describe the serendipitous discovery of donor-accepter interaction promoted organogel formation from bile acid and non-bile acid based systems. In the second part the aggregation behavior of some of the bile acid-derived gelators are discussed.

Bile Acid Appended with an Aromatic Moiety: Charge Transfer Promoted Gelation of Organic Fluids

Some of the bile acid based gelators studied in our lab were discovered serendipitously. The first of them were organogelators discovered during the course of investigation of a cholic acid based molecular tweezer [21]. It was found that aromatic donor-substituted bile acid derivatives such as **A**, **B** and **C** (Chart 3) gelatinized alcohols such as butanol, cyclohexanol, (and in the case of **A**, $CHCl_3$) *in the presence of* one equivalent of trinitrofluorenone (TNF) as the acceptor. Gelation did not occur in the absence of the acceptor and the optimum stoichiometric ratio between the donor and the acceptor was found to be 1:1 by gel to sol transition temperatures (T_g). Interestingly, moving the aromatic moiety to the other hydroxyl groups on the steroid or to the side chain resulted in the loss of gelation property. The gels were highly colored (Fig. 1) and the gelation was shown to be driven by charge transfer interaction between the aromatic unit on the steroid and the acceptor molecule. The charge transfer band (~600 nm) observed in the absorption spectrum of a sol of **B**/TNF in $CHCl_3$ increased substantially upon gelation as can be seen from the plot showing the temperature dependence of the intensity of the charge transfer band.

Figure 1.
Photograph of a gel derived from an alkyl-pyrene/TNF.

Encouraged by these findings a series of aromatic derivatives in which *n*-alkyl groups

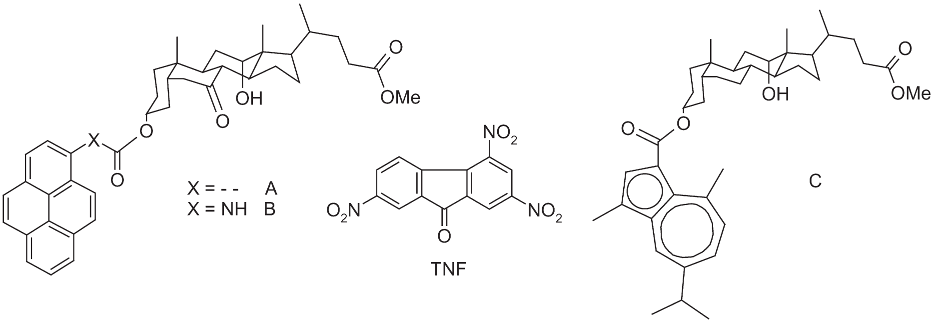
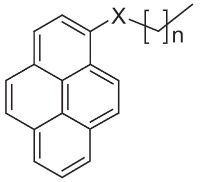
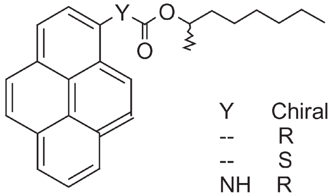


Chart 3.
Steroidal derivatives that form colored organogels in the presence of TNF.



x	n
COO	15
CH_2	14
O	13
NHCO	16
NHCOO	15
NHCONH	15

Chart 4.
n-Alkylated pyrene derivatives.



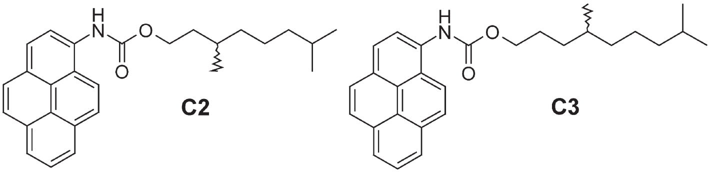
Y	Chirality
--	R
--	S
NH	R
NH	S

Chart 5.
2-Octanol derived pyrene derivatives.

replaced the steroid nucleus were designed for a similar gelling mechanism, which led to interesting results (Chart 4). This class of molecules gelled organic solvents as efficiently as their bile-acid counterparts. However, the nature of the linking group had a profound effect on the gelation characteristics [22]. Molecules with linking groups such as ester, alkyl, ether formed charge transfer gels in alcoholic and hydrocarbon solvents in the presence of TNF, while the ones with amide, urethane and urea groups gelled a variety of organic solvents *on their own via* π-π stacking and H-bonding interaction mediated self-assembly. Incidentally, chiral 2-octanol derived compounds (Chart 5) belonging to this class of gelators gave 'chiral' gels. The ester linked derivatives gave colored gels in cyclohexanol and the urethane linked derivatives gelled hydrocarbons such as cyclohexane, *n*-hexane, *n*-decane *etc.* on their own. In both cases, the gels obtained from enantiomers gave mirror image CD spectra.[22a] The SEM of these gels however did not show any helical fibers. Subsequently, urethane linked chiral gelators (Chart 6) with the chiral center placed away from the aromatic nucleus were synthesized and the effect of this on the chirality of the resulting gels was investigated. The citronellol derivatives gelled isooctane and *n*-decane. The gelation was driven by H-bonding and π-π interactions as determined by IR and fluorescence. The IR spectra of the gel showed C=O stretching frequencies corresponding to both H-bonded and non H-bonded carbonyl groups. Interestingly, the chirality of the gels decreased with increasing distance of the chiral center from the aromatic core as deduced by the decreasing molar ellipticity of the isooctane gels. The SEM of a gel of **C2** in isooctane (Fig. 2) revealed that the gel consisted of fibers entwined with each other and manifested no apparent macroscopic chirality. Figure 3 shows a model for the helical self-assembled structure of **C2**.

Modified Bile Acids: Gelation of Aqueous Liquids

A tripodal bile-acid based host (nonahydroxy tripodal cholamide, "NH", Chart 7) designed to bind polar guests was found to be an efficient gelator of weakly acidic aqueous media containing a small amount of an organic co-solvent [23]. The protonation of the tertiary nitrogen was a prerequisite to obtain gels in water and formation of stable gels required an organic cosolvent. These gels were transparent with



C2 **C3**

Chart 6.
Citronellol based chiral gelators.

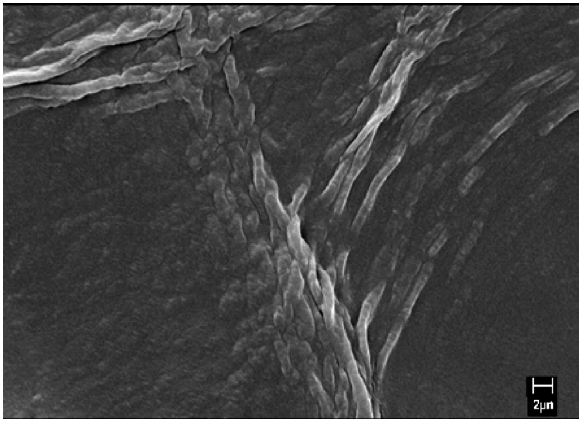


Figure 2.
SEM image of a xerogel of **C2**.

had high thermal stabilities. Ultrafast time-resolved fluorescence technique was used to investigate the rotational dynamics of polarity sensitive fluorescent probes such as ANS (8-aninlinonaphthalene-1-sulfonic acid) and DPH (1,6-diphenylhexatriene) solubilized in the gel derived from the tripodal cholamide [24]. This gave information on the structure and dynamics of the gel network.

In the gel phase at 25°C, the fluorescence anisotropy decay of ANS showed two rotational correlation times (ϕ), 13.2 ns (60 %) and 1.0 ns (40%). The longer component of ϕ represented the ANS bound or dissolved in the hydrophobic pockets of the gel fibres. The shorter component of ϕ represents the molecular motion of ANS in the solution phase of the gel (free ANS) and its value was higher than the ϕ observed in the neat solvent (<0.2 ns) used for gelation. This is possibly due to the partial immobilization of the water molecules in the aqueous phase around and between the gel fibers. Thus, the tumbling motion of the probe ANS present in both regions, *viz.*, the hydrophobic and the solvent accessible regions of the gel phase were significantly reduced from that in the pure solvent mixture. A similar study done

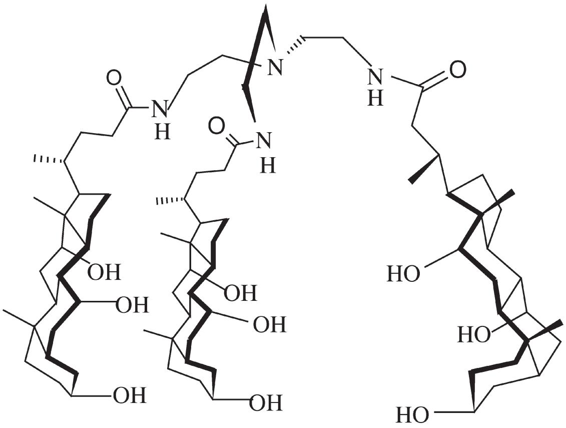


Chart 7.
Tripodal nonanydroxy ("NH") super hydrogelator.

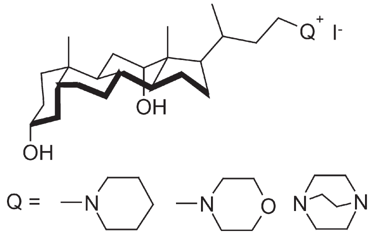
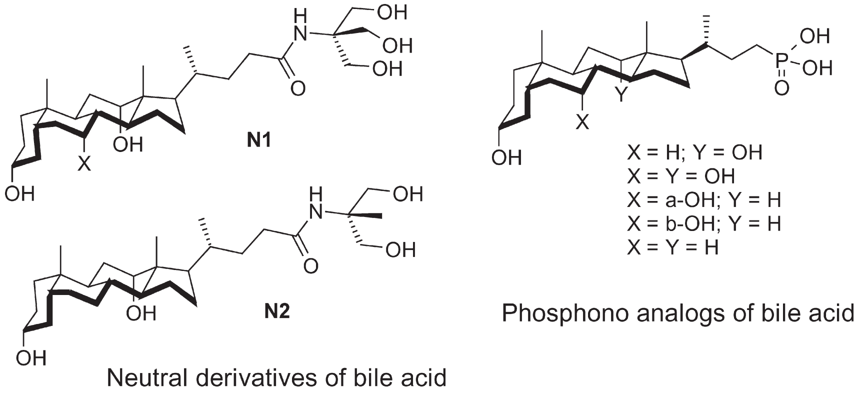


Chart 8.
Cationic analogs of bile salts.

with DPH as a probe exhibited both similarities and differences. Like ANS a faster dynamics of DPH in sol phase (1.3 ns) as compared to that in the gel (4.8 ns) was observed. However, unlike ANS only one component was observed in either of the phases suggesting that DPH is always bound to the hydrophobic pockets of the aggregates owing to its non-polar nature. There was also a substantial increase (30 fold) in the ANS fluorescence intensity in the gel phase as compared to that in the sol [23]. This observation coupled with the fact that the bulk pH of the system could be different in the sol and the gel state led to the hypothesis that incorporation of an organic dye whose pK_a lies in the proximity of the bulk pH of the gel could lead to a color change of the system during the sol-gel transition. Bromophenol blue (BPB) which exists in its yellow form at pH 3.0 and blue form at pH 4.5 was chosed as the dye of choice. When small amounts of bromophenol blue was incorporated in the system (7.5 mM gelator in 25% v/v of acetic acid/water), there was a color change from yellow in the sol phase to green in the gel as a result of differential binding of the dye molecules in the two phases. This lead to a shift in the equilibrium of the protonated and the unprotonated dye which resulted in the color difference. Additionally, an induced circular dichroism of BPB observed only in the gel phase pointed to the existence of chiral superstructures in the gel network.

Another class of gelators related to this, quaternary cationic salts of bile acids were found to gelate aqueous electrolyte solutions without the aid of any cosolvent (Chart 8) [25]. These compounds gave gels of high mechanical strength and thermal stability. This discovery opened up avenues for the design and evaluation of neutral and other anionic bile acid derivatives (Chart 9). Thus a series of TRIS derived bile acids [25a] and phosphono analogs of bile acids [26] were prepared and investigated for their gelation potential. While the neutral derivatives **N1** and **N2** gelated aqueous solutions such as $MeOH/H_2O$, $EtOH/H_2O$ and $DMSO/H_2O$, some of the phosphono analogs gelled aqueous solutions in the pH range 1.7–7.5 depending on the number and orientation of the hydroxyl



Neutral derivatives of bile acid

Phosphono analogs of bile acid

Chart 9.
Neutral and anionic analogs of bile salts.

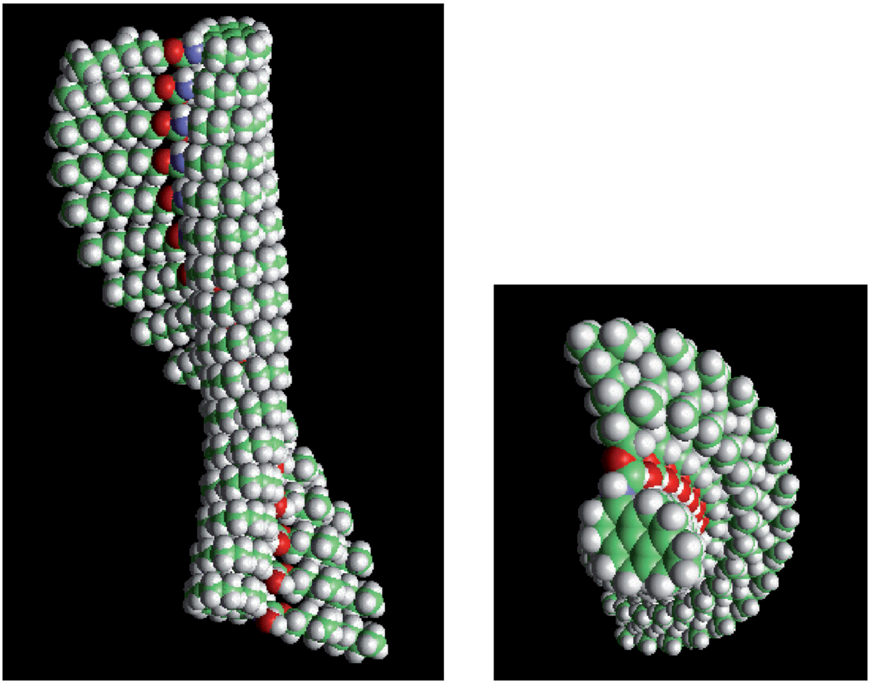


Figure 3.
Axial and equatorial views of an assembly of 32 molecules of **C2**.

Figure 4.
A thermochromic hydrogel: bluish gel at the bottom, reddish sol at the top in an upside down 1 mm path length quartz cuvette.

groups on the bile acid backbone: phosphonocholates formed gel at pH 1.7–2.5 and phosphonolithocholates formed gel at pH 7–7.5 and others between pH 3.0–6.5. In a manner similar to the observance of thermochromism in tris cholamide gel doped with BPB, the dye congo red incorporated in a gel of 23-phosphonodeoxycholate (forms gel between pH 3.1–3.4) changed its color from violet to magenta-red upon heating to the sol (Fig. 4) [26b].

Conclusions

In conclusion, this short account highlights some of the interesting results achieved in the author's laboratory on the serendipitously discovered organo and hydrogelators. A few representative examples of 'smart' gels are also mentioned. Detailed rheological, structural and other studies on some of these gels are being actively pursued in our laboratory.

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