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ARTICLE TYPE

Remarkable Isomer-Selective Gelation of Aromatic Solvents by a Polymorph of a Urea-linked Bile acid-Amino acid Conjugate

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We report an unusual, isomer-selective gelation of aromatic solvents by a polymorph of a urea-linked bile acid-amino acid conjugate. The gelator showed selectivity towards gelation of 1,2-disubstituted aromatic solvents.

Positional isomers of organic molecules hold the peculiarity of having slightly different carbon skeletons yet having very similar physical and chemical properties which make their separation challenging. With the development of modern analytical techniques methodologies are now available for the discrimination of such isomers.[1] But a simple and visual technique which is general for various classes of organic compounds can be highly useful.

Supramolecular gels formed from small organic molecules have gained much attention in recent years due to their self healing ability and stimuli responsive nature.[2] These gels have been used as smart materials for sensing and analytical purposes. The first report of using a gel for visual sensing of positional isomers came from the Shinkai group where they have shown colour changes of a napthalenediimide gel in the presence of positional isomers of dihydroxynaphthalene.[3] Harada and co-workers have used polymeric gels modified with β-cyclodextrin to discriminate the position of substituents on the naphthyl ring.[4] Selective disassembly of a supramolecular gel in the presence of catechol was reported by Escuder and Miravet.[5] Visual discrimination of 2,2'-bipyridine, among other positional isomers, was reported recently by Tu and co-workers.[6] All these reports deal with either the collapse or colour change of a pre-formed gel in the presence of a particular positional isomer of an analyte. Moreover, the reported methods were confined to specific examples.

Herein we report a practical, and possibly a general methodology, discovered serendipitously, for the discrimination of positional isomers of common aromatic solvents. Our studies began with the synthesis of a urea-linked bile acid-amino acid conjugate as a possible organogelator. Uredo peptides[7] and bile acid-based urea derivatives[8] have been reported in the literature. Initially, three different urea derivatives were prepare using (L)-phenylalanine and three bile acids (**1-3**). (D)-Phenylalanine based urea derivative (**4**) was also synthesized for a comparative study (Chart 1).

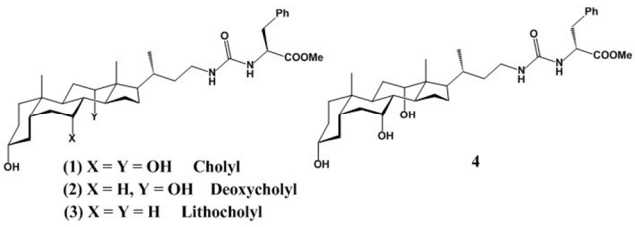

Chart 1: Urea linked bile acid-amino acid conjugates

The litho and deoxycholyl derivatives (**3** and **2**) were found to be more crystalline than the cholyl derivative (**1**) as these two precipitated out from the reaction mixture during the synthesis. Compound **3** was soluble only in hot DMSO while **2** was soluble in methanol at room temperature. The as-isolated (chromatography) cholyl derivative (**1**) seemed to be an amorphous solid and it was readily soluble in chloroform. An attempt to precipitate this compound from its chloroform solution by adding petroleum ether resulted in the formation of a crystalline white precipitate (**1-C**) which was soluble only in methanol. This suggested that the cholyl derivative **1** was exhibiting polymorphism. Detailed studies on the polymorphism of bile acid-amino acid conjugates have been reported in the literature.[9]

In order to characterize the crystalline (**1-C**) and amorphous (**1-A**) forms of **1**, both the forms were analyzed by powder XRD. The data (Fig. 1) showed sharp peaks indicating the crystallinity of the precipitated sample (**1-C**) while **1-A** showed broad peaks indicating its amorphous nature.

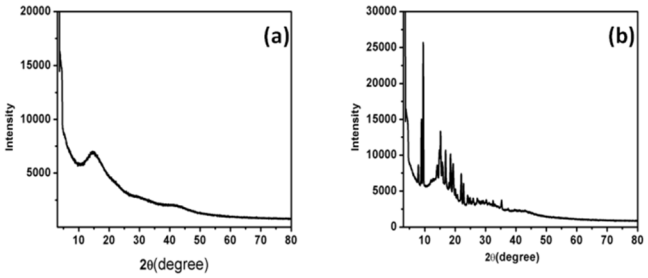

Figure 1: Powder XRD profile of (a) amorphous (**1-A**) and (b) crystalline (**1-C**) forms

Neither of the two polymorphs showed any sharp melting points. As DSC is an important tool to understand such systems, we have analyzed the DSC profiles of both forms.

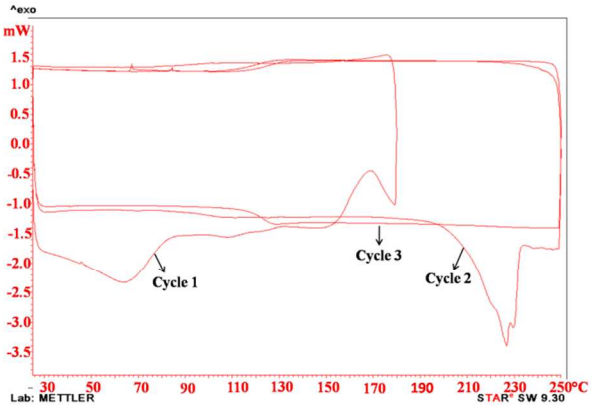

Figure 2: DSC profile of amorphous polymorph (**1-A**)

The first cycle in the DSC experiment (Fig. 2) was 25-180-25°C. In this cycle the amorphous material (**1-A**) showed a glass transition at 110°C which was followed by a crystallization at 170°C. This indicated the transformation of one polymorph into the other. In the second cycle the glass transition was still present along with a sharp endotherm around 230°C. This probably suggests the decomposition of the material at its melting point as the crystallization peak was not present in the cooling cycle. The third cycle (25-250-25°C) showed only one glass transition at 120°C.

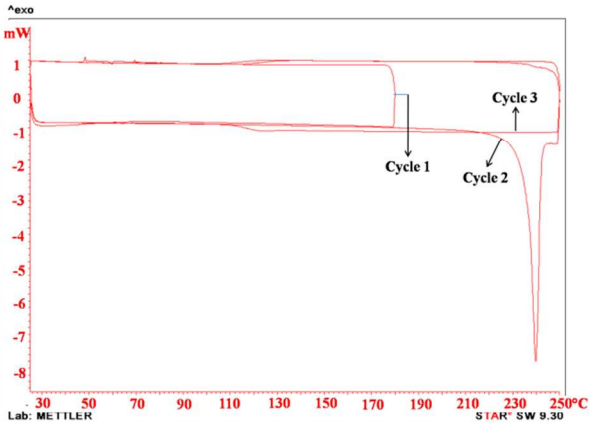

Figure 3: DSC profile of crystalline polymorph (**1-C**)

As evident from the DSC profile (Fig. 3), the crystalline form showed no glass transitions before its melting point. The sharp endotherm at 238-240°C indicated the melting, but there was no corresponding crystallization peak in the cooling cycle suggesting decomposition. Instead, a glass transition was observed at 120°C.

The DSC experiments, therefore, showed that the amorphous material (**1-A**) gets transformed into the crystalline polymorph on heating to 180°C. The second and third heating–cooling cycles of the amorphous material (**1-A**) thus, was the same as that of the crystalline material (**1-C**).

Solid state ^{13}C NMR spectroscopy (CPMAS) is another way to distinguish among different polymorphs.[9] The amorphous material (**1-A**) showed broad peaks and the crystalline form (**1-C**) showed much sharper peaks in the solid state ^{13}C NMR spectra (Fig. 4).

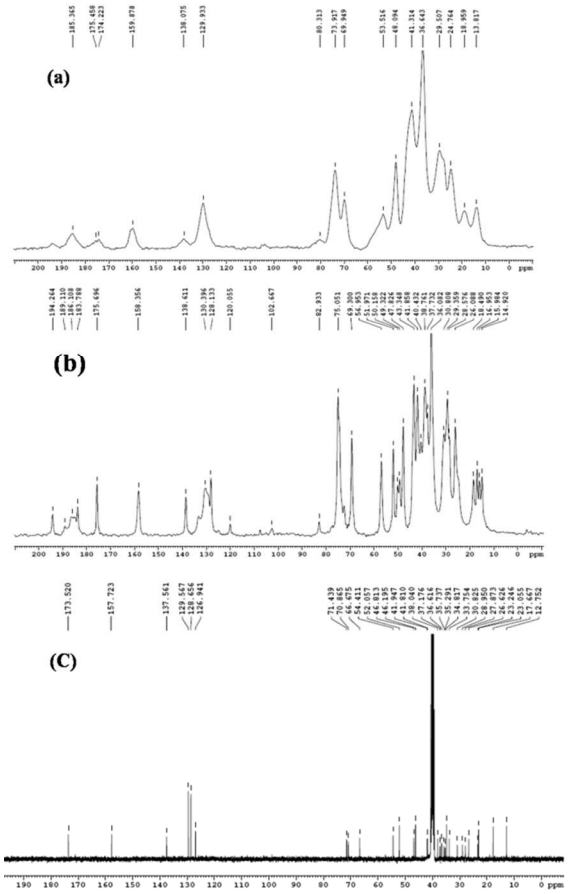

Figure 4: Solid state ^{13}C NMR spectra of (a) amorphous (**1-A**), (b) Crystalline polymorph (**1-C**) and (c) ^{13}C NMR spectrum in $CDCl_3$

Having two different polymorphs of the urea derivative **1-A** and **1-C** in hand, their aggregation and gelation properties in various organic solvents were examined. All the gelation experiments were done by heating the compounds in the respective solvents at 90°C for 5-10 min to get clear solutions, which were allowed to cool slowly. The crystalline form remained insoluble in almost all solvents tested for gelation. But the amorphous material formed transparent gels in some of the organic solvents. The most interesting observation was the isomer selectivity in gelation. The amorphous polymorph was able to gel 1,2-dimethylbenzene (1,2-DMB or *o*-xylene) but it precipitated out from 1,3-DMB (*m*-xylene) and 1,4-DMB (*p*-xylene) solutions![10]

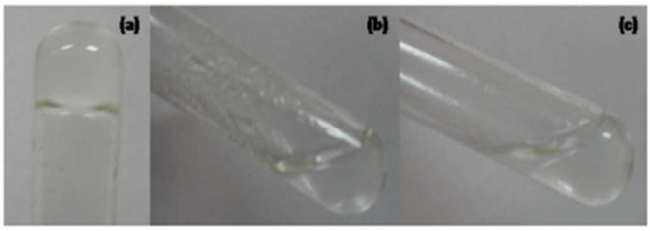

Figure 5: (a) Gel formed in 1,2-DMB and precipitate formed in (b) 1,3-DMB and (c) 1,4- DMB

The amorphous form also formed gels in 1,2- and 1,3-dichlorobenzenes. Both the gels were equally transparent. But successive heating–cooling cycles made the gels translucent and weak. The 1,3-DCB gel broke after three cycles while the 1,2-DCB gel remained as such. It is therefore clear that the gelation of 1,2-DCB is favored by the urea derivative. The turbidity developed in the gels was attributed to the transformation of amorphous material into the crystalline one, which is a non gelator, being much less soluble.

To check whether the urea derivative shows selectivity in its gelation of other disubstituted aromatic solvents we chose 2, 3 and 4-chlorotoluenes. It was again observed that the gelation of 2-chlorotoluene was favoured over the others as it gelled quickly (within 10 minutes). With 3 and 4-cholorotoluenes it took 30-45 minutes to form the gels and the gels were not so stable.

At this point we believe that the 3 and 4- isomers favour the rapid formation of the crystalline polymorph, which is a non-gelator. In the 1,2-isomer, formation of crystalline polymorph was a relatively slow process, resulting in gelation. On standing for a week this gel also broke with the precipitation of the crystalline polymorph.

Compound **4** was also used for gelation studies and it was found that this compound did not show any selectivity towards 1,2-DMB. Instead, it formed gels in all the three xylenes. This compound was able to gel all positional isomers of DCB (1,2- and 1,3-) as well as chlorotoluenes. Even though it was clear from the DSC profile that **4** showed polymorphism, we were not able to isolate any of them.

SEM analysis of both 1,2-DMB and 1,2-DCB gels of **1-A** showed fibrous morphology as well as spherical aggregates. Aggregates of various sizes were placed on the bed of fibres (Fig. 6) and majority were ~ 1 μm in diameter.

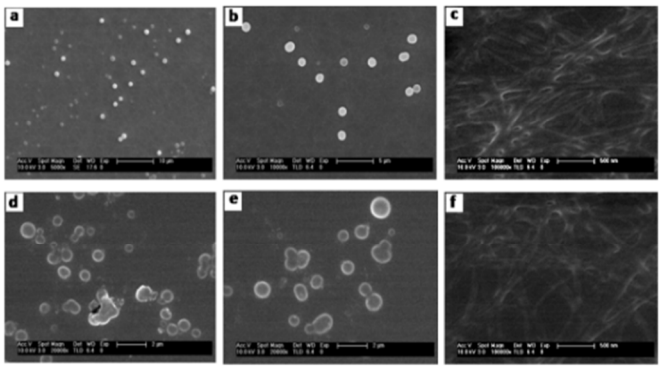

Figure 6: SEM images of (a-c) 1,2-DMB gel and (d-f) 1,2-DCB gel of **1-A**

Detailed AFM analysis of the gel samples of **1-A** were carried out to analyze their morphology. AFM in the tapping mode showed fibrous network, with the fibre/tape diameter being ~100 nm. The fibres/tapes were found to be arranged on a bed of spherical aggregates. In some places it appeared that the fibres were formed from the fusion of those spherical aggregates. The following AFM images shown in fig.7 illustrate the tape like morphology as well as the spherical aggregates present in the gel.

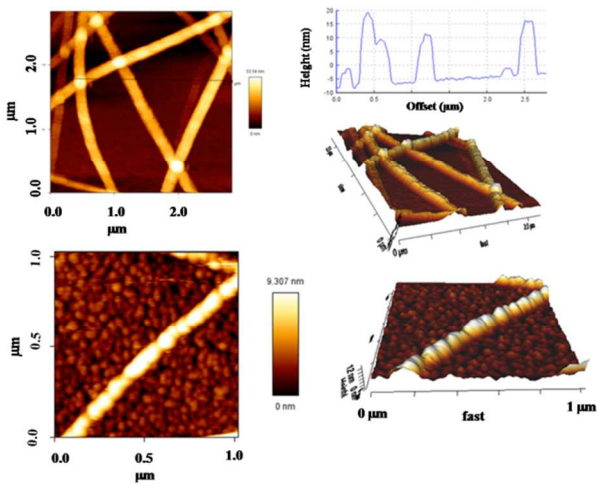

Figure 7: AFM images of 1,2-DMB gel of **1-A**

The 1,2-DCB gel of **1-A** also showed fibrous network as well as the spherical aggregates. Spherical aggregates were found to be an integral part of the gel fibres with fibre diameter ~ 50-60 nm (Fig. 8).

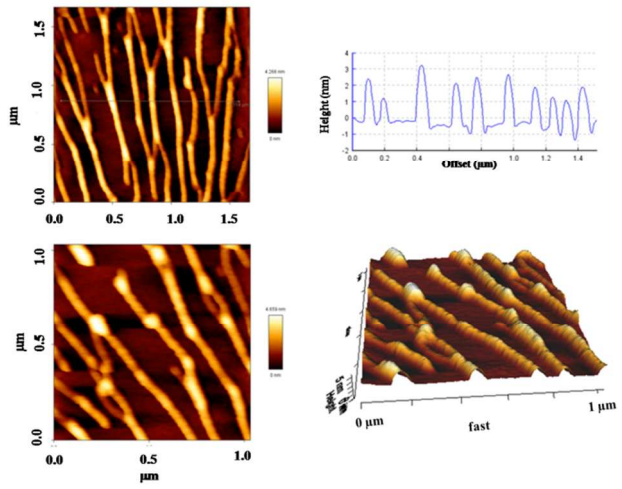

Figure 8: AFM images of 1,2-DCB gels of **1-A**

TEM analysis of the 1,2-DMB gel of **1A** also showed fibrous morphology. Bundles of fibres were seen along with spherical aggregates of various sizes (Fig. 9).

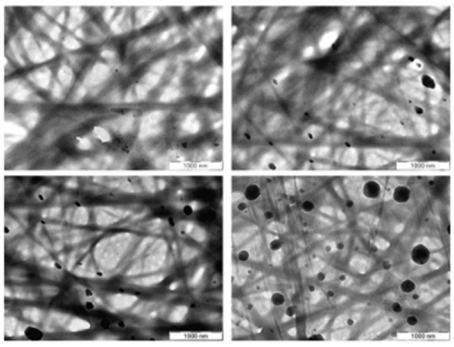

Figure 9: TEM images of 1,2-DMB gel of **1-A**

The presence of spherical aggregates in the gel suggested that at lower concentrations the urea derivative aggregated to form spherical structures which later combine and grow into tapes/fibres. This was suggested by examining the dilute solution (0.1 mM) of the gelator **1-A** in 1,2-DMB by DLS and AFM. DLS studies showed the presence of aggregates of average size around 80 nm.

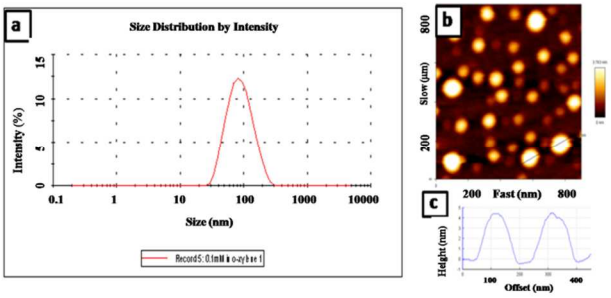

Figure 10: (a) DLS pattern (b) AFM image of 0.1 mM solution of **1-A** in 1,2-DMB; (c) Cross section of selected particles (150 nm)

It was interesting to note that compound **1-A** showed aggregation in water also. DLS pattern of 0.1 mM solution in water (sample preparation in SI) showed the presence of particles of average size 70 nm. AFM imaging also proved the existence of spherical aggregates with a broader range of sizes.

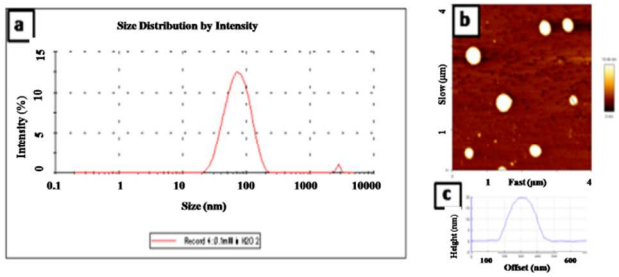

Figure 11: (a) DLS pattern (b) AFM image of 0.1 mM solution of **1-A** in water; (c) Cross section of selected particle (200 nm)

Dynamic rheology experiments are generally used to find out the mechanical strength of the gel samples. Time sweep, frequency sweep and stress sweep experiments were performed on a 1 wt % gel of **1-A** in 1,2-DMB. Experiments were carried out using 20 mm parallel plate (cross hatched) geometry at 25°C. For the time sweep experiment the frequency and oscillatory stress used were 0.16 Hz and 0.1 Pa respectively. It was found that the G' value increased from 40 Pa to 65 pa over a period of 45 minutes (Fig. 12).

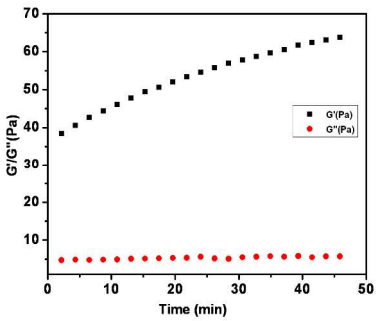

Figure 12: Time sweep experiment performed on 1,2-DMB gel of **1-A** (1 wt %)

Frequency sweep experiment (Fig. **13a**) done at an oscillatory stress of 1 Pa showed that the material was indeed a gel with G' and G" values 65 and 6 Pa respectively. A sudden drop in the G' value at an oscillatory stress of 2.5 Pa indicates the gel breakage during the stress sweep experiment (Fig. **13b**).

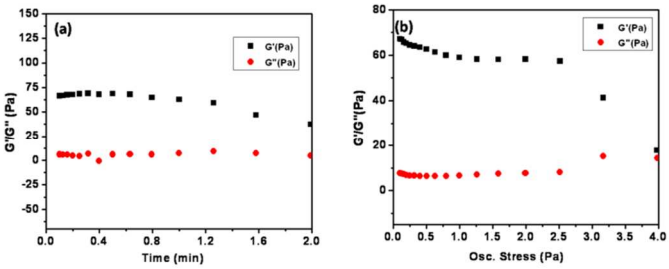

Figure 13: (a) Frequency sweep and (b) Stress sweep experiments on 1,2-DMB gel of **1-A** (1 wt %)

Conclusions

We have synthesized a novel family of urea linked bile acid-amino acid conjugates and studied the polymorphism shown by the cholyl derivative bearing (L)-phenylalanine using different techniques such as DSC, powder XRD and solid state ^{13}C NMR. Our studies have shown that the amorphous form is a gelator of organic solvents and it showed remarkable selectivity towards 1,2-DMB (*o*-xylene). This is a simple and rapid technique for the discrimination of 1,2-DMB from the other two positional isomers. We have shown that our gelator can be used to distinguish 1,2-disubstituted dichlorobenzenes and chlorotoluenes from the other positional isomers. We have done extensive microscopic studies to establish the gel morphology and have shown that the gelator molecules aggregate to form spherical structures at lower concentrations in both organic and aqueous media. Our studies showed that at higher concentrations gel fibres are formed from these aggregates. The (D)-phenylalanine based urea derivative was also found to be an organogelator, but it did not show any isomer selectivity in gelation. Thus we have developed a simple and efficient method for the discrimination of 1,2-disubstituted organic solvents. The reason for the unsual ortho selectivity is not clear at this time, but it may be related to solubility, viscosity and other bulk properties of the solvent.[11] To the best of our knowledge this is the first report of this kind. Further changes in the molecular design are being explored in our laboratory and the results from these studies will be reported elsewhere.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [synthesis and characterization of urea derivatives and AFM images of the gels of **4**]. See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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10 The minimum gelation concentration was found to be about 0.2 wt. %. The maximum we tested was 2 wt%.

11 Attempts have been made to correlate our experimental results with various physical properties such as viscosity, dielectric constant, dipole moment etc. of all three sets of solvents. At this point we believe that it is not a single property of the ortho disubtituted solvent which makes it a suitable medium for gelation. Instead it may be a combination of all the above mentioned physical properties along with higher solubility of the urea derivative that favours gelation.