

Self-assembly study of ammonium oleanolate and generation of gel-gold nanoparticle hybrid material

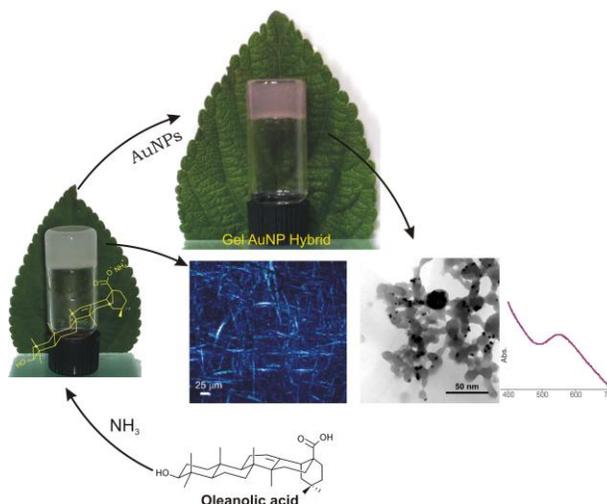
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Abstract:

A solution of oleanolic acid, a renewable nano-sized monohydroxy triterpenic acid in tetrahydrofuran, transformed into a gel instantly at room temperature on treatment with aqueous ammonia. Study of the self-assembly properties of ammonium oleanolate in water and aqueous solvent mixtures indicated that it self-assembles in the aqueous solvent mixtures forming gels in DMSO-water and DMF-water. Fibrillar networks with optical birefringence properties were observed by polarized optical microscopy. Scanning electron microscopy of the dried gel samples indicated fibrillar networks with fibers of nano to micrometer diameters. The thermodynamic parameters calculated from the gel to sol transition temperatures indicated the stability of the gels. A gel-gold nanoparticles hybrid material has also been prepared and characterized by HRTEM, EDX, SAED and surface Plasmon resonance studies.



Keywords: Triterpenoid; oleanolic acid; renewable; ammonium oleanolate, self-assembly

1. Introduction:

Self-assembly of small molecules in liquids via non-covalent interactions has been an area of tremendous research interest during the last decade because, the nano- to micro-sized architectures such as vesicles, fibers, tubes, flowers that are formed, have both potential and realized applications in the areas drug-delivery, cosmetics, painting, nano-biotechnology, pharmacology, etc.^{1,2,3,4,5} Of significance is the self-assembly of molecules in aqueous solvents which helps in the applications of the self-assemblies in biological studies in addition to other applications. Triterpenoids with nano-

metric lengths having varied rigid and flexible parts are common in nature.⁶ The renewable nature of most of the triterpenoids having several hydroxyl and carboxyl groups makes them interesting candidates for the study of their self-assembly as renewable functional nano-entities. Recently, we have demonstrated that the pentacyclic triterpenoids betulinic acid,⁷ oleanolic acid,⁸ glycyrrhetic acid⁹, arjunolic acid¹⁰ and betulin¹¹ spontaneously self-assemble in different liquids without any functional transformations and observed that, minute structural variation in the triterpenoid back-bone and functional group dispositions resulted in drastic changes in their self-assembly properties.

However, due to the presence of a large lipophilic backbone and lack of sufficient number of polar groups, solubility in water and aqueous solvent mixtures were difficult. We reasoned that the the carboxyl functional group present in the triterpenic acids can be converted to salts rendering them soluble in aqueous solvents. Hence it occurred to us that the self-assembly properties of ammonium salt of oleanolic acid can be studied.

Oleanolic acid is a 6-6-6-6-6 pentacyclic monohydroxy triterpenic acid, extractable from the root bark of *Lantana camara*^[12] having tremendous medicinal importance including anticancer and antitumor activities.^[13] We have recently reported the self-assembly properties of oleanolic acid **1** in different liquids. It shows spontaneous self-assembly in a range of organic liquids forming mostly vesicular structures along with a small percentage of fibrillar networks.⁸ Herein we report the self-assembly properties of ammonium oleanolate in water and aqueous solvent mixtures. The salt preferentially formed fibrillar network structures, as evident by optical and electron microscopy studies, yielding gels in aqueous DMSO and aqueous DMF. Strong gels were also obtained in aqueous solvents in the presence of antioxidant rich bark extract of *Lantana camara*. By utilizing this phenomenon, we have demonstrated the generation of gel-gold nanoparticle hybrid material and characterized by HRTEM, EDX, SAED and surface Plasmon resonance studies.

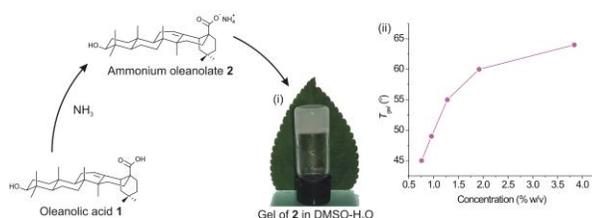


Figure 1: Structures of renewable oleanolic acid **1** and its ammonium salt **2**. (i) Inverted vial containing a gel of ammonium oleanolate in DMSO-water (10:3) with the leaf of *Lantana camara* in the background, (ii) Plot of gel to sol transition temperature T_{gel} vs % concentration of **2**.

2. Result and discussion:

2.1 Study of self assembly properties

Oleanolic acid was extracted from the root bark of *Lantana camara*. The ammonium salt **2** was prepared by treatment of a solution of oleanolic acid in tetrahydrofuran with concentrated aqueous ammonia solution. Self-assembly studies were carried out dissolving **2** (0.005 g) usually in a liquid under hot condition and then allowing the resulting solution to cool at room temperature. No gravitational flow of the material, as observed by turning the vial upside down, indicated the formation of a gel. Interestingly, the salt formed opaque gels in aqueous-DMSO and aqueous-ethanol (Table 1) though it was almost insoluble in water and formed a precipitate from DMF-water (10:2). Minimum gelation concentrations (MGCs), determined by the minimum concentration of the salt at which the liquid concerned could be gelled at room temperature, were 0.76% in DMSO-

Serial no.	Medium (V/V)	State	MGC (%)	T_{gel} (°C)
1	water	I	-	-
2	DMSO-water (10:3)	G	0.76	45
3	Ethanol-Water (10:6)	G	2.3	55
4	DMF-water	P	-	-

water (10:3) and 2.3% in ethanol-water (10:6).

P = precipitation; I = insoluble; G = gel; MGC = minimum gel concentration; T_{gel} are provided at MGC.

Thermoreversibility of the gels, confirmed by repeated heating and cooling allowed us to plot the gel to sol transition temperature T_{gel} vs % gelator concentration. The T_{gel} values increased with increasing concentration of the solutes indicating stronger intermolecular interactions at higher concentrations. Based on the relationship of T_{gel} with concentration, the thermodynamic parameters at 298 °K were calculated to be ΔH° 11 kJ/mol, $\Delta S^{\circ} = 21.4$ J/mol/°K and $\Delta G^{\circ} = 4.6$ kJ/mol. The positive free energy change during gel to sol transition indicated the stability of the gels.

2.2 Morphological characteristics of the self-assemblies

The morphology of the gel was studied by optical microscopy (OM) and scanning electron microscopy (SEM). Optical microscopy of the gel of **2** in DMSO-water and Ethanol-water indicated the formation of fibrillar network with fibers of submicron diameters and several micrometers in length (Figure 2). Optical birefringence was observed in a gel of **2** in DMSO-water (10:3, 0.7% w/v) (Figure 2f). Scanning electron microscopy carried out with the dried gels samples of **2** in ethanol-water (10:6; 2.3% w/v) and DMSO-water (10:3, 0.76% w/v) also indicated a fibrillar network morphology with fibers of nano to micrometer diameters and several micrometer lengths (Figure 3).

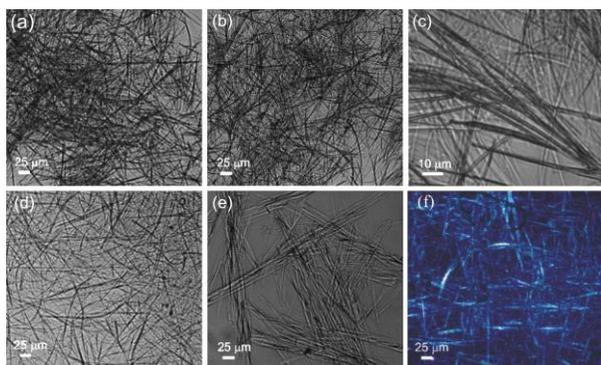


Figure 2: (a-c) optical microscopy image of ammonium salt in EtOH-water, (d-f) optical microscopy image of ammonium salt in DMSO-water

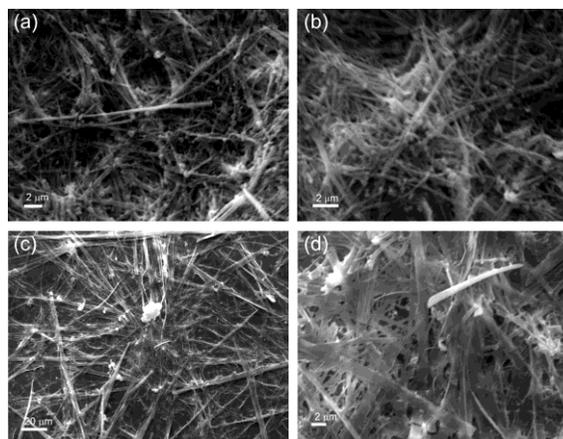


Figure 3: SEM images of xerogel of **2** in (a,b) EtOH-water and (c,d) DMSO-water.

2.3 Gel-gold nanoparticle hybrid material

Gel-gold nanoparticle hybrid material is an emerging area of research because of its tremendous applications in the areas of pharmacology, biodiagonestics, medicine, drug-delivery, catalysis, nanobiotechnology, biomedicine, biosensors etc. , The stem bark of *Lantana camara* is rich in polyphenolic compounds including antioxidants and preliminary investigations showed that the bark extract can be utilized for the green synthesis of gold nanoparticle. Moreover, we were successful in preparing a gel-plant extract-hybrid material from a mixture of **2** in DMSO and an aqueous solution of the stem bark of *Lantana Camara*, maintaining the ratio of DMSO-water (10:3). Hence, it occurred to us that it can be utilized for the synthesis of a gel-gold nanoparticle hybrid material under identical solvent composition. Interestingly, when a freshly prepared gold nanoparticle colloid, synthesized using the stem bark extract of *Lantana camara* (2 g/lit), was mixed with a solution of **2** in DMSO under hot condition maintaining the same ratio of DMSO:water (10:3), and then the mixture was allowed to cool at room temperature, a reddish violet colored hybrid material was obtained indicating the formation of gel-gold nanoparticles hybrid material. The gel-gold nanoparticle hybrid material was stable at room temperature (Figure 4h). Surface Plasmon

Resonance studies showed a strong band at 553 nm indicating the presence of AuNPs (Figure 4g). HRTEM images of samples prepared by drop-casting of a diluted sample of the hybrid material indicated flex-type morphologies containing gold nanoparticles embedded in the gel-matrix (Figure 4). Selected area electron diffraction (SAED) studies and energy dispersive X-ray (EDX) spectra also showed evidence of AuNPs. Previously we have demonstrated with arjunolic acid based gel-gold nanoparticle hybrid material¹⁰ and sodium and potassium betulinate based gel-gold nanoparticle hybrid material that the morphology of the self-assemblies is not affected by the hybrid formation. In the current study also, the morphology is unaffected by the gel-gold nanoparticle hybrid formation.

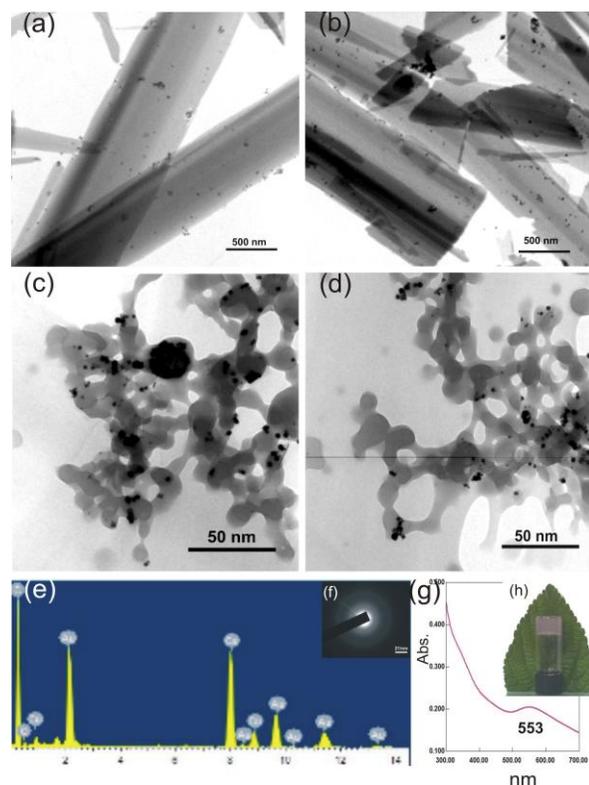


Figure 4: (a-d) TEM images of gel-gold nanoparticle hybrid material (in DMSO-water system (10 : 3) ratio), (f) elemental composition of hybrid gel by energy dispersive X-ray analysis (EDX), (e) selected area diffraction pattern (SAED) obtained from gold nanoparticle (g) SPR band of AuNPs in gel-AuNPs hybrid material (h) photograph of gel-gold nanoparticle hybrid material.

3. Conclusions

In conclusion, we have reported the spontaneous self-assembly of ammonium oleanolate in aqueous solvents yielding supramolecular gels. The morphologies of the self-assemblies have been characterized by optical and electron microscopy. A composite gel from ammonium oleanolate and antioxidant rich bark extract of *Lantana camara* was also obtained in the aqueous solvent mixtures. By utilizing this phenomenon, a gel-gold nanoparticle hybrid material was generated from ammonium oleanolate and stabilized gold nanoparticles synthesized using the bark extract of *Lantana camara*. The hybrid material was characterized by surface Plasmon resonance, HRTEM, EDX and SAED studies. The studies revealed the formation of almost spherical shaped gold nanoparticles on the surface of flex and fiber like self assemblies. Further applications of the hybrid material are in progress in our laboratory and will be reported in due course.

4. Experimental Section:

Synthesis of ammonium salt of oleanolic acid:

Oleanolic acid (0.102 g, 0.219 mmol) taken in a round bottom flask was dissolved in freshly distilled THF (1.10 mL) and the clear solution was treated with concentrated ammonia solution (25% w/v, 0.152 mL). The mixture turned into a gel instantly during addition of ammonia solution and the pH of the mixture was measured to be 10.3. The mixture was diluted with THF (0.4 mL) and stirring was continued overnight. Then the reaction mixture was dried under reduced pressure to afford a white solid.

¹H NMR (400MHz DMSO-d₆) δ: 5.15 (1H, t), 3.00 (1H, q), 1.90 -1.01 (20H, m), 1.04(3H,s), 0.91 (3H, s), 0.87 (3H, s), 0.71 (3H, s), 0.69 (3H, s), 0.67 (3H, s); ¹³C NMR (100 MHz, DMSO-d₆) δ: 179.1, 144.4, 121.9, 77.3, 55.3, 47.6, 45.9, 42.1, 41.3, 40.6, 38.8, 38.5, 37.1, 33.8, 33.3, 33.0, 32.6, 30.9, 28.7, 28.5, 27.9, 27.4, 26.1, 23.8, 23.4, 23.1, 18.5, 17.3, 16.5, 15.6;

5. Acknowledgement

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6. References

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