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ARTICLE

Self-assembly of sodium and potassium betulinates into hydro- and organo-gels: Entrapment and removal studies of fluorophores and synthesis of gel-gold nanoparticle hybrid materials

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Sodium and potassium salts of a renewable nano-sized triterpenoid betulinic acid have been prepared and their self-assembly properties in water and aqueous solvent mixtures have been studied. Both the salts were excellent hydrogelators as well as gelators of aqueous solvent mixtures. The morphologies of the self-assemblies, characterized by optical and electron microscopy, revealed fibrillar networks having fibers of nano- to micrometer diameters. The thermodynamic parameters calculated for the gel to sol transition indicated the stability of the gels. Hydrogels of both sodium and potassium betulinates have been utilized for the entrapment and removal of rhodamine-B in aqueous medium. Gel-gold nanoparticle hybrid materials have also been prepared by in-situ generated gold nanoparticles at room temperature.

1. Introduction

Study of the self-assembly of small molecules in different liquids via non-covalent forces yielding gels has become a rapidly expanding area of research because the supramolecular gels thus obtained have various potential and realized applications.[1,2,3] Self-assembly of molecules yielding hydrogels are of special significance due to their range of applications in drug delivery, tissue engineering, cosmetics, removal of toxic chemicals, etc.[4,5,6,7,8] Moreover, research investigations utilizing compounds from renewable resources have gained increasing importance in recent years because such studies aim at meeting the current needs of the society without compromising the needs of the future.[9,10,11] Self-assembly of various types of renewable compounds such as fatty acids,[12,13,14] sophorolipids,[15,16,17] short peptides,[18,19,20] etc. have been reported. We have recently reported that *triterpenoids*, the C30 subset of the major plant secondary metabolite *terpenoids*, are renewable functional nano-entities having varied rigid and flexible lengths.[21] This prompted us to use them as such, even without functional modifications, for their self-assembly studies. Interestingly, self-assembly studies with several naturally occurring triterpenoids such as arjunolic acid, betulinic acid, oleanolic acid, glycyrrhetinic acid, all having rigid triterpenoid backbone, one carboxyl group and varied number of hydroxyl groups spontaneously self-assembled in different liquids yielding gels via the formation of vesicles, fibers, spheres, flowers, etc. of nano- to micro-meter dimensions.[22,23,24,25,26] However, because of the presence of large lypophilic backbone and inadequate number of hydrophilic groups, the compounds studied were insoluble in water. We anticipated that the alkali metal salts of the triterpenic acids will have better solubility in water as well as aqueous solvent mixtures enabling us to study their self-assembly properties in such liquids. Hence, it occurred to us that self-assembly properties of sodium and potassium betulinates can be studied in water and aqueous solvent mixtures (Figure 1).

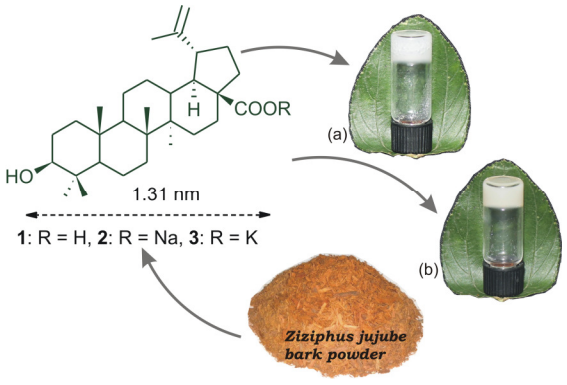

Fig. 1 Schematic representation showing gels from the salts of renewable betulinic acid **1** extracted from the bark of *Z. jujube*. Inverted vials showing (a) hydrogel of potassium betulinate **3** and (b) a gel of sodium betulinate **2** in DMSO-water (1:1) with *Z. jujube* leaf in the background.

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Betulinic acid **1** is a nano-sized pentacyclic monohydroxy triterpenic acid extractable from the barks of *Ziziphus jujube (Z. jujube)*, *Betula papyrifera* (White birch), etc. having tremendous medicinal significance as anticancer, antitumor, anti-diabetic and anti-HIV activities.[27,28,29,30] Excellent gelation ability of betulinic acid in different liquids via the formation of fibrillar networks has been reported by us.[22] Herein we report the self-assembly properties of sodium and potassium salts of betulinic acid **2** and **3** respectively in water as well as aqueous solvent mixtures along with the morphological characteristics of the self-assemblies. Both the salts **2** and **3** spontaneously self-assembled into fibers of nano to micrometer diameters yielding supramolecular gels. The hydrogels of sodium and potassium betulinates have been utilized for entrapment and removal of toxic dye such as rhodamine-B (rho-B). Synthesis of gel-gold nanoparticle hybrid materials by utilizing the hydrogels of both sodium and potassium betulinates and in-situ generated gold nanoparticles, obtained by the reduction of Au (III) with the bark extract of *Z. Jujube* (see experimental section), have also been demonstrated at room temperature.

2. Results and Discussion

Betulinic acid was isolated from the bark of *Z. jujube* and its sodium and potassium salts were prepared by reacting with sodium and potassium hydroxides (see experimental section).

Table 1: Gelation test results of **2** and **3**

Liquid[a]	**2**[b]	T_{gel}[c] (°C)	**3**[b]	T_{gel}[c] (°C)
Water	G/1.7	50	G/1.7	54
DMSO/water	G/1.9	58	G/2.3	48
DMF/water	G/1.9	51	G/2.3	51
EG/water	G/2.1	35	G/2.8	60
Ethanol/water	VS/5.0[d]	..	G/2.3	55

[a] organic liquid : water = 1:1, [b] G = gel, Minimum gelator concentrations (MGCs) are in % w/v, [c] Gel to sol transition temperatures are provided at MGC, [d] VS = viscous suspension; concentration in % w/v.

2.1 Gelation study

As anticipated, both the salts were more soluble than the parent acid in water as well as aqueous solvent mixtures. Gelation tests were carried out by dissolving the compounds (usually 5 mg) in a liquid under hot condition and then allowing the resulting solution to cool. No gravitational flow of the material, when observed by turning the vial upside down, indicated the formation of a gel. Interestingly, both the salts **2** and **3** were excellent hydrogelators as well as gelators of aqueous DMSO, DMF, EG, ethanol at a ratio of 1:1 (v/v) (Table 1). In most of the cases, opaque gels were obtained almost instantly at 5% (w/v) and the gels once formed were stable for several months on storing at room temperature under sealed conditions. Minimum gelator concentrations (MGCs), determined by the minimum concentration of the solute at which the liquid concerned could be gelled at room temperature, were determined in all the cases (Table 1). With sodium betulinate **2** as the gelator, the MGCs were same (1.9% w/v) both in DMSO-water (1:1) as well as DMF-water (1:1). With potassium betulinate **3** as the gelator too, the MGCs were same (2.3% w/v) in both DMSO-water (1:1) and DMF-water (1:1).

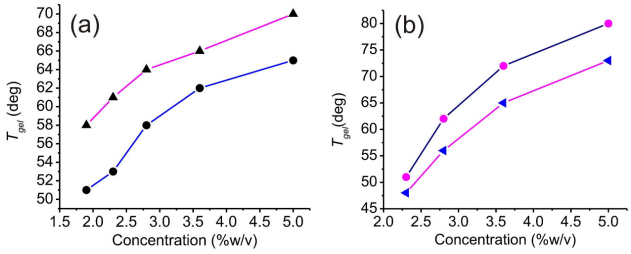

Fig. 2 T_{gel} (°C) vs concentration (% w/v) plot of (a) sodium betulinate **2** in DMSO-water (◄) and DMF-water (●) (1:1 v/v); (b) potassium betulinate **3** in DMSO-water (◄) and DMF-water (●) (1:1 v/v).

Table 2: Thermodynamic parameters (ΔH^o, ΔS^o, ΔG^o) for gel to sol transition of a gel of potassium betulinate **3** in different liquids at 298° K

Liquid	ΔH° kJ/mol	ΔS°J/mol/°K	ΔG° kJ/mol
DMSO-water	28.2	61.9	9.7
DMF- water	24.1	48.6	9.7

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 (Fig. 2). The T_{gel} values increased with increasing concentration of the solutes indicating stronger intermolecular interactions at higher concentrations. The T_{gel} values of the gels of **2** in DMSO-water (1:1) were higher compared to that of **2** in DMF-water (1:1) at identical concentrations indicating a much stronger gel in DMSO-water (Figure 2a). On the contrary, the T_{gel} values of the gels of **3** in DMF-water (1:1) were higher compared to that **3** in DMSO-water (1:1) at identical concentrations indicating a much stronger gel in DMF-water (Figure 2b). Based on the relationship of T_{gel} with concentration, the thermodynamic parameters (ΔH^o, ΔS^o, ΔG^o) at 298 °K were calculated (Table 2 and Table S1 and Fig. S4, ESI†). The positive free energy changes (ΔG^o) during gel to sol transformations in all the cases indicated the stability of the gels.

2.2 Morphological Characteristics of the self-assemblies

The morphologies of the self-assemblies were studied by optical microscopy (OM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

2.2.1 Optical microscopy images

Optical microscopy of self-assembled potassium betulinate **3** in water (1.67 % w/v) revealed the formation of fibrillar network having fibers of uniform diameter of ca. one micrometer and several micrometer lengths (Fig. 3 and Fig. S1a and S1b, ESI†). Such a fibrillar network was also observed in the case of a viscous suspension of **3** in EG-water and DMF-water (Fig. S1c, d ESI†). Fibrillar network was also observed in the viscous suspension of sodium betulinate **2** in water as well as aqueous solvent mixtures (Fig. S2, ESI†).

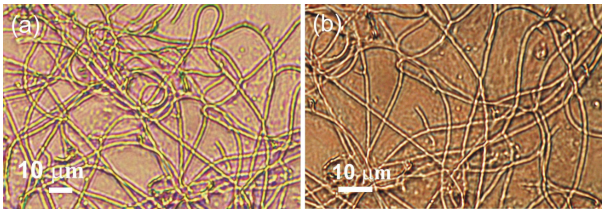

Fig. 3 Optical microscopy images of **3** in water (1.67 % w/v).

2.2.2 SEM images of the dried self-assemblies

Scanning electron microscopy images of the dried self-assemblies of potassium betulinate **3** obtained from its viscous suspension in water (0.83% w/v) revealed a dense fibrillar network having fibers of 15 - 20 nm diameters and micrometer lengths (Fig. 4). The length of the molecule being 1.31 nm, the dimensions of the fibers indicate that there are several molecules present in the length and the breadth of the fibers. Densely packed fibrillar network was also observed in the dried self-assemblies of sodium betulinate **2** prepared from its viscous suspension in water (0.72% w/v) (Fig. S3, ESI†).

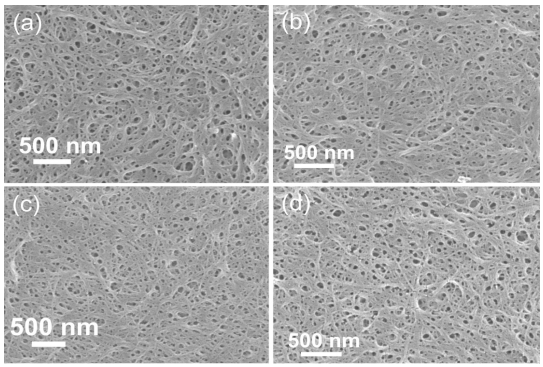

Fig. 4 SEM images of dried self-assemblies of potassium betulinate **3** obtained from its viscous suspension in water (0.83% w/v).

2.2.3 FTIR study

In FTIR of betulinic acid, the -COOH stretching frequency appears at 1686 cm^{-1} (Fig. S5a and S6a, ESI†). This peak disappeared in the FTIR of both **2** and **3** with the appearance of new peaks in the 1540 – 1559 cm^{-1} region confirming the formation of the salts.[31] To investigate the driving force for gelation, we have compared the FTIR spectra of powder samples of **2** and **3** and their dried self-assemblies prepared from water. The stretching vibrational band of carboxylates ($-COO^-$) appeared at 1541 and 1559 cm^{-1} for powder samples of **2** and **3** respectively (Fig. S5c and S6c, ESI†). However, in the FTIR spectra of the dried self-assemblies prepared from the corresponding hydrogels, the peaks for the carboxylates appeared at 1539 and 1548 cm^{-1} respectively (Fig. S5b and S6b, ESI†). The decrease in the carboxylate stretching frequencies clearly indicated strong electrostatic and dipole-dipole interactions that may have played a vital role for the formation of an ordered structure yielding gels.

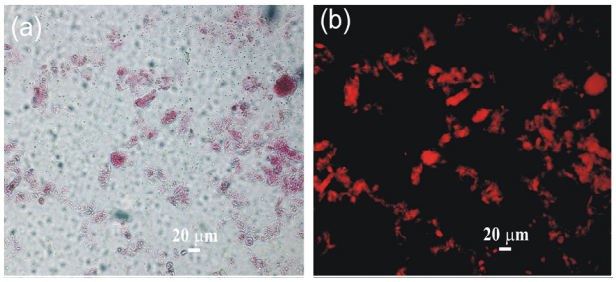

Fig. 5 Self-assemblies of sodium betulinate **2** loaded with rhodamine B in water: (a) under normal light and (b) under fluorescence light.

2.3 Study of entrapment of fluorophores

To investigate whether fluorophores can be entrapped by the self-assemblies of sodium and potassium betulinates **2** and **3** respectively, entrapment studies were carried out with a cationic fluorophore rhodamine B. Interestingly, the self-assemblies of both the salts were capable of entrapping the fluorophore. For example, when a hot solution of **2** in water (0.2 mL, 30 mM) was mixed with an aqueous solution of rhodamine B (0.01 mL, 2.1 mM) and allowed to cool at room temperature and morphologies formed were examined by epifluorescence microscopy after 14 h, reddish fluorescence were observed from the self-assemblies indicating the fluorophore entrapment (Fig. 5). Similarly, the self-assemblies of the salt **3** also entrapped the cationic fluorophore under identical conditions (see experimental section and Fig. S7, ESI†). The fibrillar network with very large surface area allows adsorption of the dyes with additional electrostatic interaction between the betulinate anion and the cationic dyes.[32]

2.4 Utilization of hydrogel in the removal of dyes

The majority of the dyes is toxic and even carcinogenic and cause damage not only to aquatic life but also to humans. Rhodamine B, a xanthine dye, is carcinogenic and it is used in the textile, printing, and paint industries.[33,34,35] For the removal of toxic dyes from contaminated water activated charcoal, carbohydrate based polymers, porous silica, etc. are used conventionally.[36,37] To investigate whether the hydrogels can be utilized for the removal of toxic dyes, we examined the removal of the rhodamine-B (rho-B), 5(6)-carboxyfluoroscein (CF) and neutral red (NR) in aqueous phosphate buffer by a hydrogel of sodium batulinate **2** and potassium betulinate **3** as a model system. Initially a hydrogel (2.5 % w/v of each) of sodium betulinate **2** and potassium betulinate **3** was prepared and an aliquot of rhodamine B (0.8 mL, 0.03 mM) in phosphate buffer (0.01 M, pH = 7.2) was placed carefully on the upper surface of the gel. The mixture was kept at room temperature and the absorbance of the phosphate buffer was measured by UV-visible spectroscopy after certain time intervals. Interestingly, 80.2% of rhodamine B was removed by a hydrogel of sodium betulinate **2** within 4 h. Similarly, 90% of rhodamine B was removed by a hydrogel of potassium betulinate **3** within 10 h 20 min (Fig. 6). The rho-B dye removal ability of the hydrogel was also examined by fluoroscence spectrophotometer. A decrease in fluoroscence intensity of the buffer solution with time confirmed the removal of the cationic dye (Fig. S8, ESI†). To verify whether other wellknown dyes can also be removed by the hydrogels of **2** and **3,** studies were carreid out with the solutions of CF or NR in aqueous phosphate buffer (0.01 M, pH = 7.2). But only 12 and 4 % of CF was removed by the hydrogels of **2** and **3** (2.5 % w/v) respectively after 24 h of equilibriation indicating that the hydrogels are capable of removing the cationic dye (rho B) more efficiently than the anionic dye CF. This selectivity towards the removal of the cationic dye is probably due to the anionic nature of the gelators **2** and **3**. Identical studies carried out with NR showed a change in color of the gel probably due to the change in pH inside the gel (Fig. S9 c,d, ESI†) with concomitant change in the peak pattern in UV-visible spectrum and hence the removal of NR dye could not be quantified even after 24 h of equilibration.

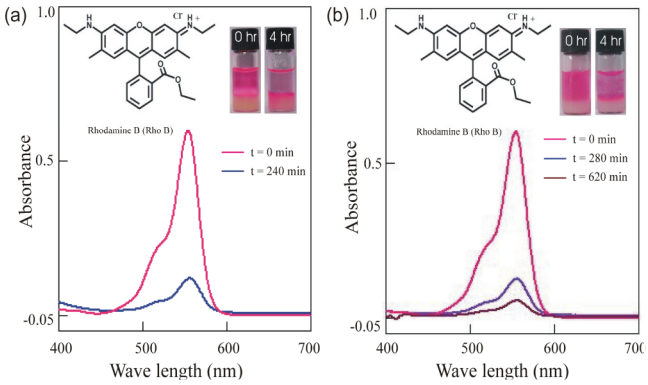

Fig. 6 UV-visible spectroscopy of phosphate buffer solutions at various time intervals during removal of rhodamine B studies by the hydrogels of (a) sodium betulinate **2** and (b) potassium betulinate **3**. Inset. *left* structure of rhodamine B and *right* photographs of vials cotaining hydrogel covered with PBS solution.

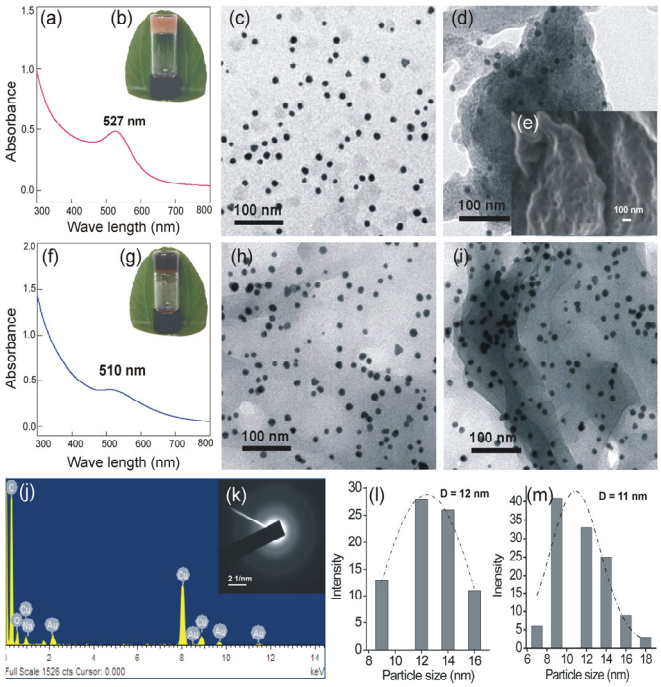

Fig. 7 (a, f) SPR band of AuNPs in gel–gold nanoparticle hybrid material prepared from sodium betulinate **2** and potassium betulinate **3** respectively, (b, g) Inverted vials containing gel–gold nanoparticle hybrid material prepared from **2** and **3** respectively with the leaves of *Z. jujube* in the background, (c-d) TEM images of gel–gold nanoparticle hybrid material prepared from **2**, (e) SEM image of dried self-assemblies of gel-gold nanoparticle hybrid material prepared from **2**, (h-i) TEM images of gel–gold nanoparticle hybrid material prepared from **3**, (j) elemental composition of hybrid material by energy dispersive X-ray analysis (EDX), (k) selected area electron diffraction pattern (SAED) obtained from gold nanoparticle and (l-m) particle size histograms of *in situ* prepared gold nanoparticle in the hybrid material prepared from **2** and **3** respectively.

2.5 *In situ* generation of gel–gold nanoparticle hybrid material

The development of gel–gold nanoparticle hybrid material is an emerging area of research due to its applications in the areas of nanobiotechnology, biomedicine, biosensors, etc.[38,39] Many of such applications require the synthesis and stabilization of gold nanoparticles (AuNPs) from non-toxic, biomolecules (via the reduction of Au (III) to Au (0)) under very mild reaction conditions. The bark extract of *Z. jujube*, is rich in polyphenolic compounds including various types of antioxidants, flavanoids and the polyphenolic compounds[40] (Fig. S10, ESI†). Thus, these phenolic compounds can be used as an effective reducing agent for Au (III) which has a high oxidation–reduction potential. Hence, it occurred to us that it can be utilized for the *in-situ* synthesis of a gel–gold nanoparticle hybrid material. Initial investigations on whether a gel can be prepared from a mixture of **2** or **3** with the bark

extract of *Z. jujube*, an aqueous solution of **2** or **3** (2.5% w/v) was mixed with the bark extract (0.2% w/v), Indeed, a opaque gels were obtained within 1 h with both **2** and **3**. Inspired by this observation, an aqueous solution of **2** or **3** (0.9 mL, 0.3% w/v) contained in a vial was treated with an aqueous solution of *Z. jujube* bark extract (1 mL, 0.1% w/v) and Au (III) colloid (0.1 mL, 10.42 mM) at room temperature. Appearance of reddish violet color almost instantly indicated the formation of gold nanoparticles. Interestingly, a reddish violet colored gel-gold nanoparticle hybrid material was formed within 1 hour at room temperature (Fig. 7b and 7g). A characteristic Surface Plasmon Resonance (SPR) band at 527 nm and 510 nm in gel–gold nanoparticle hybrid materials prepared from **2** and **3** respectively, confirmed the formation of the AuNPs (Fig. 7a and 7f). The gel–gold nanoparticle hybrid material was stable at room temperature for several months under sealed condition. HRTEM images of the hybrid material (1.1 mM) prepared from both **2** and **3** by drop-casting, showed the presence of gold nanoparticles along with self-assemblies (Fig. 7 c,d,h,i). Selected area electron diffraction (SAED) image (Fig. 8k) and energy disperse X-ray (EDX) spectra (Fig. 7j) also supported the formation of AuNPs. Average particle size of *in situ* prepared AuNPs from both **2** and **3** are 12 and 11 nm respectively (Fig. 7l and 7m). The scanning electron microscopy (SEM) of the gel-gold-nanoparticle hybrid material showed the presence of self-assembled fibrillar networks of nano to micrometer dimensions indicating that the morphology of the hybrid materials is identical to the morphology of the dried self-assemblies of **2** and **3** (Fig. 7e).

3. Conclusions

We have reported the formation of fibrillar self-assemblies of sodium and potassium betulinates in water and aqueous solvent mixtures yielding supramolecular gels in most of the liquids. According to our knowledge, this is the first report of hydrogels based on sodium and potassium salts of betulinic acid. Entrapment of fluorophore such as rhodamine B on the self-assemblies has been demonstrated by epifluorescence microscopy. Removal of the toxic dye in a phosphate buffer by the hydrogels of sodium and potassium betulinates have also been demonstrated. Preparation of gel-gold nanoparticle hybrid materials by utilizing the hydrogels of both sodium and potassium betulinates and the in-situ generated gold nanoparticles, obtained by the reduction of Au (III) with the bark extract of *Z. jujube,* have also been demonstrated by us. The renewable nature of betulinic acid with its nanometric length and the tremendous applications of hydrogels open up its use in advanced materials and nanobiotechnology.

4. Experimental Section

4.1 Extraction, purification and isolation of betulinic acid
Betulinic acid **1** was extracted from the bark of *Z. jujube* and purified as reported by us previously.[22]

*4.2 Synthesis and Characterization of sodium betulinate **2** and potassium betulinate **3**:*
Betulinic acid **1** (0.30 g, 0.66 mmol) was treated with ethanolic sodium hydroxide (10% w/v, 0.5 mL) and diluted to 6.5 mL with ethanol. The reaction mixture was allowed to stir at room temperature for 24 hours. Then the volatiles were removed under reduced pressure to afford sodium betulinate **2** as a powdered solid. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 4.73 (1H, s), 4.56 (1H, s), 3.17-3.02 (1H, m), 1.69 (3H, s), 1.0-0.9 (6H, s), 0.94 (3H, s), 0.83 (3H, s), 0.72 (3H, s) ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 179.90 (28 - COONa), 152.47, 109.04, 77.27, 56.51, 55.46, 50.66, 49.63, 47.40, 42.53, 40.76, 38.97, 38.79, 38.23, 37.72, 37.22, 34.64, 33.98, 31.27, 29.98, 28.59, 27.65, 25.86, 21.17, 19.56, 18.51, 16.70, 16.47, 16.28, 14.85. FTIR: υ_{max} (KBr, cm^{-1}) 3366(b), 2941(s), 2864(s), 1639(s), 1578 (-COO$^-$), 1450, 1374.

Betulinic acid **1** (0.30 g, 0.66 mmol) was treated with ethanolic potassium hydroxide (10% w/v, 0.5 mL) and diluted to 6.5 mL with ethanol. The reaction mixture was allowed to stir at room temperature for 24 hours. Then the volatiles were removed under reduced pressure to afford potassium betulinate **3** as powdered solid. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 4.70 (1H, s), 4.56 (1H, s), 3.17-3.02 (1H, m), 1.70 (3H, s), 0.97 (6H, s), 0.95 (3H, s), 0.85 (3H, s), 0.73 (3H, s) ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 178.48 (28 - COOK), 152.53, 108.97, 77.25, 56.60, 55.49, 50.67, 47.31, 42.50, 40.77, 40.76, 38.97, 38.80, 38.23, 37.62, 37.22, 34.62, 34.02, 31.28, 30.00, 28.60, 27.66, 25.88, 21.15, 19.62, 18.50, 16.66, 16.47, 16.28, 14.84. FTIR: υ_{max} (KBr, cm^{-1}) 3391(s), 2942(s), 2864(s), 1642(s), 1550 (-COO$^-$), 1451, 1374.

4.3 T_{gel} value determination. A gel sample contained in a vial was heated over a hot plate and the temperature was monitored by a digital thermometer placed in the gel. The temperature at which the gel started flowing when observed by tilting the vial was measured as the gel to sol transition temperature T_{gel}.

4.4 Entrapment of rhodamine B. A solution of potassium betulinate (0.2 mL, 0.01% w/v) in water was mixed with rhodamine B solution (0.01 mL, 2.1 mM) and stirred magnetically under hot condition to obtain a clear solution. The mixture was allowed to cool at room temperature for 14 h and then the mixture was examined by epifluorescence microscopy. Reddish fluorescence was observed from the self-assemblies.

4.5 Removal of dyes. Sodium betulinate **2** *and* Potassium betulinate **3** (10 mg each) contained in a separate vial was dissolved in water (0.4 mL) by heating with magnetic stirring and the resulting solution was allowed to cool at room temperature to obtain an opaque hydrogel (2.5% w/v) with in 2 h. For the preparation of dye solutions, a weighed amount of dye (1 mg) was dissolved in appropriate amount of phosphate buffer solution (PBS, 0.01 M, pH = 7.2) to get various dye solutions of 0.03 mM. As a representative procedure with rho-B, an aliquot of rhodamine B in phosphate

buffer (0.8 mL, 0.03 mM) was carefully placed on the hydrogels of both sodium and potassium betulinates. For monitoring the removal of fluorophore from the PBS buffer, the buffer solution (0.6 mL) was carefully removed and the absorbance was measured by UV-visible spectroscopy using a 2 mm quartz cuvette. After every absorbance measurement, the buffer solution was placed back on gel. The absorbance at 554.5 nm for rhodamine B in phosphate buffer (pH = 7.2) was monitored. The hydrogel of sodium betulinate **2** could remove 80.2% of rhodamine B in 4 h and the hydrogel of potassium betulinate **3** could remove 90% of rhodamine B in10 h and 20 min from the buffer solutions. An identical procedure was followed for the removal of CF and NR dyes.

4.6 Preparation of Z. jujube bark extract. *Z. jujube* bark were collected from the campus of Vidyasagar University and dried in air. Finely powdered bark of *Z. jujube* (13.5 g) was taken in a soxhlet apparatus (60 mL) and extracted with distilled ethanol (120 mL) for 5 h and filtered (fluted filter paper). The volatiles were removed under reduced pressure to afford a pale brown colored bark extract (1.66 g).

4.7 In-situ synthesis of gel-gold nanoparticle hybrid material. A colloidal suspension of **2** in water (0.3 mL, 2.5% w/v) and the bark extract of *Z. jujube* (0.1 mL) contained in a vial was heated over a hot plate with magnetic stirring to obtain a semi-transparent suspension. Then an aliquot of Au (III) solution (0.02 mL, 10.42 mM) was added and stirred at room temperature. Appearance of reddish color almost instantly indicated the formation of stabilized AuNPs. A strong hybrid-gel was formed on storing the mixture at room temperature for 1 h. Similarly, a hybrid material was also prepared with the potassium betulinate **3**. High resolution transmission electron microscopy (HRTEM) and scanning electron microscopy (SEM) were used to characterize the gold nanoparticles embedded hydrogels of sodium and potassium betulinates. For HRTEM study an aliquot of diluted samples were carefully placed over a carbon-coated cupper grid (300 mesh). Initially the grid was allowed to dry in open air for overnight and then under reduced pressure. Then the grid was used for the study of morphology of *in-situ* prepared gel-gold nanoparticle hybrid material. For SEM study an aliquot of hybrid material was pasted on a small glass plate and dried in air. Then the glass plate was dried under high vacuum and examined.

4.8 Method of sample preparation and characterization: For gelation tests, 5–10 mg of compound of **2** and **3** contained in a vial (1 cm id) was heated with a liquid with magnetic stirring over a hot plate until a clear solution was obtained. The solution was then allowed to cool down at room temperature (24–25°C) and the gel formation was checked after 2–4 h by turning the vial upside down. No gravitational flow of the liquid indicated the formation of a gel. Scanning electron microscopy samples were prepared by placing a dilute solution of the sample on a glass plate and then allowing it to dry initially in air for 24 h and then under reduced pressure for 12 h and then sputter coated with Au before use for 120 s and studied using a Zeiss Scanning Electron Microscope (EVO 18). For optical microscopy, an aliquot of sample was taken on a glass plate and covered with a cover slip and examined using a Nikon LV100 POL microscope with D-FL Epi-fluorescence attachment. For the measurement of fluorescence intensity of rhodamine B in PBS buffer, an aliquot of PBS buffer was taken in a cuvette and intensity was measured by fluorescence spectrophotometer (Hitachi F 7000). Upon excitation at λ = 554 nm, an emission spectra appeared at λ = 581.4 nm. Interestingly, the decrease in intensity of the emission spectra with time indicated the removal of the dye from the PBS buffer solution. FTIR spectra of the neat powder and dried self-assemblies were analyzed by using a Perkin Elmer Spectrum Two model with KBr pellet.

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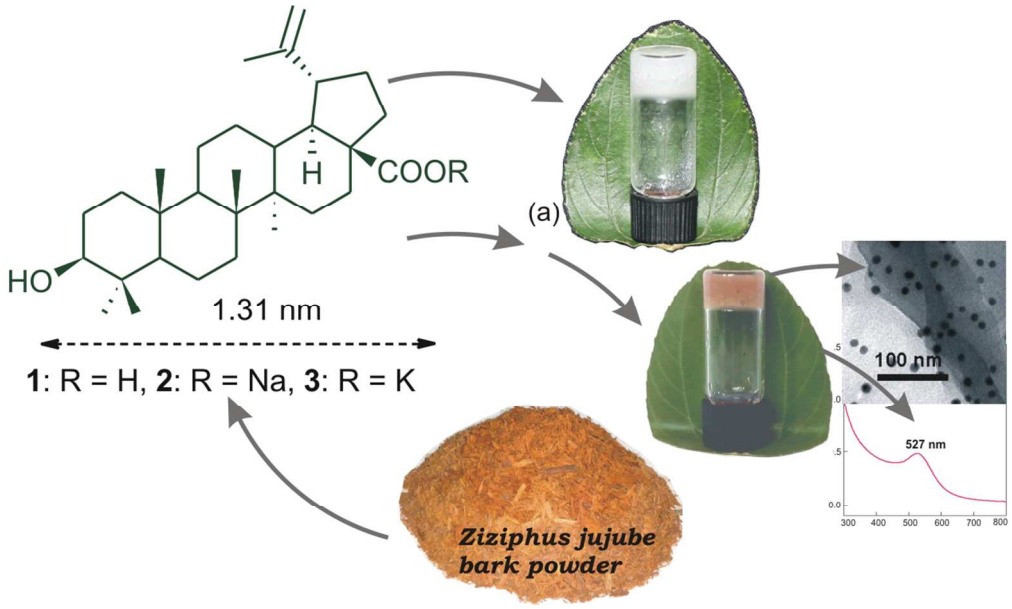
HO
H
COOR
1.31 nm
1: R = H, 2: R = Na, 3: R = K
(a)
100 nm
527 nm
Ziziphus jujube
bark powder