Supporting Information

Vesicular self-assembly of a natural triterpenoid arjunolic acid in aqueous medium: study of entrapment properties and in situ generation of gel-gold nanoparticle hybrid material

Braja Gopal Bag and Rakhi Majumdar

Department of Chemistry & Chemical Technology
Vidyasagar University
Midnapore 721102
West Bengal, India
Fax: 0091 3222275297
Email: braja@mail.vidyasagar.ac.in

Table of Contents **Page**

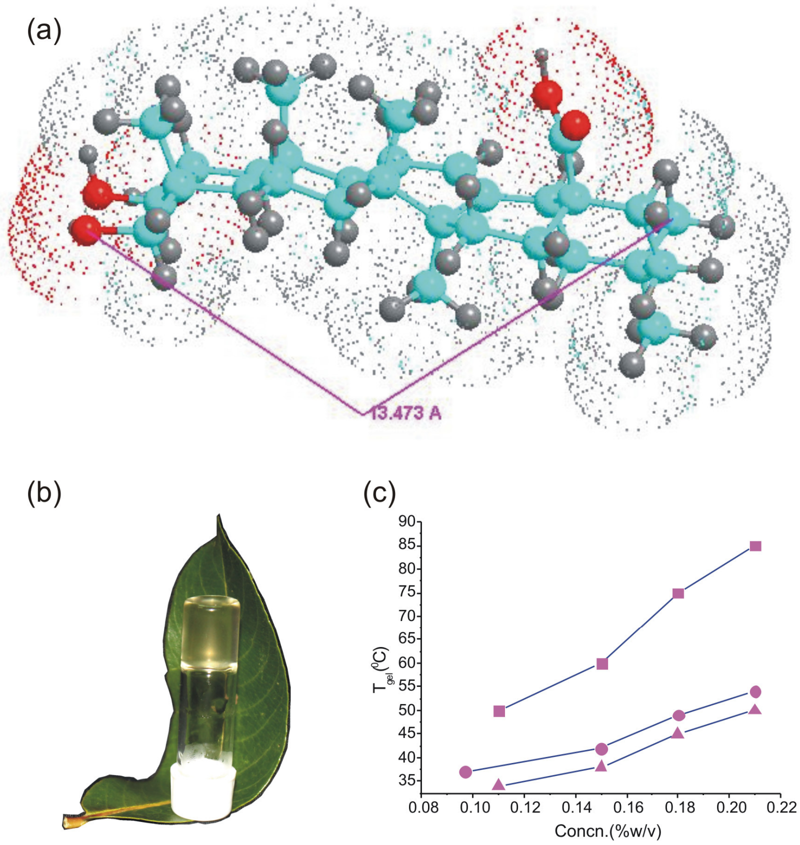

Figure S1: (a) Energy minimized structure of arjunolic acid using PCModel version 9.2; (b) Inverted vial containing a gel of a branded whisky (42% alcohol) with arjunolic acid (0.14% w/v), (c) *Tgel* vs concentration plot of arjunolic acid in (i) goldnaparticle containing hybrid gel(-■-) (ii) ethanol- bark extract of *T. arjuna* (-●-) (iii) ethanol- water(-▲-).

Table TS1: Study of self-assembly of arjunolic acid 1 in EtOH-H_2O mixtures								
Vial Nos	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
Volume of H_2O (mL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Volume of ethanol solution (mL) of 1 (0.5% w/v)	0.025	0.050	0.075	0.100	0.125	0.150	0.175	0.2
Concentration of 1 (% w/v)	0.056	0.10	0.14	0.17	0.19	0.21	0.23	0.25
State[a]	CS	CS	VL	VS	G	G	VS	VS
[a] CS = cloudy suspension, VL = viscous liquid, G = gel, VS = viscous suspension								

Table TS2: Study of self-assembly of arjunolic acid 1 in MeOH-H_2O mixtures								
Vial Nos	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
Volume of H_2O (mL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Volume of methanol solution (mL) of 1 (0.5% w/v)	0.025	0.050	0.075	0.100	0.125	0.150	0.175	0.2
Concentration of 1 (% w/v)	0.056	0.10	0.14	0.17	0.19	0.21	0.23	0.25
State[a]	CS	CS	VL	VL	VL	VL	VL	VL
[a] CS = cloudy suspension, VL = viscous liquid								

Table TS3: Gelation test results of arjunolic acid 1 in DMSO-H_2O mixtures

Serial No.	Solvent 1	Solvent 2	CGC (%)	State[a]	T_{gel} (°C)
1	DMSO	H_2O	7.14 (5:5)	P	…
2	DMSO	H_2O	7.14 (5:4)	G	67-68
3	DMSO	H_2O	7.14 (5:3)	G	42
4	DMSO	H_2O	7.14 (5:2)	G	39
5	DMSO	H_2O	7.14 (5:1)	VS	...

[a] G = gel, VS = viscous suspension, P = precipitate

Table TS4: Gelation test results of arjunolic acid 1 in DMF-H_2O mixtures

Serial No.	Solvent 1	Solvent 2	CGC (%)	State[a]	T_{gel} (°C)
1	DMF	H_2O	7.14 (5:4)	P	….
2	DMF	H_2O	7.14 (5:3)	VS	….
3	DMF	H_2O	7.14 (5:2)	G	60
4	DMF	H_2O	7.14 (5:1)	VS	....

[a] G = gel, VS = viscous suspension, P = precipitate

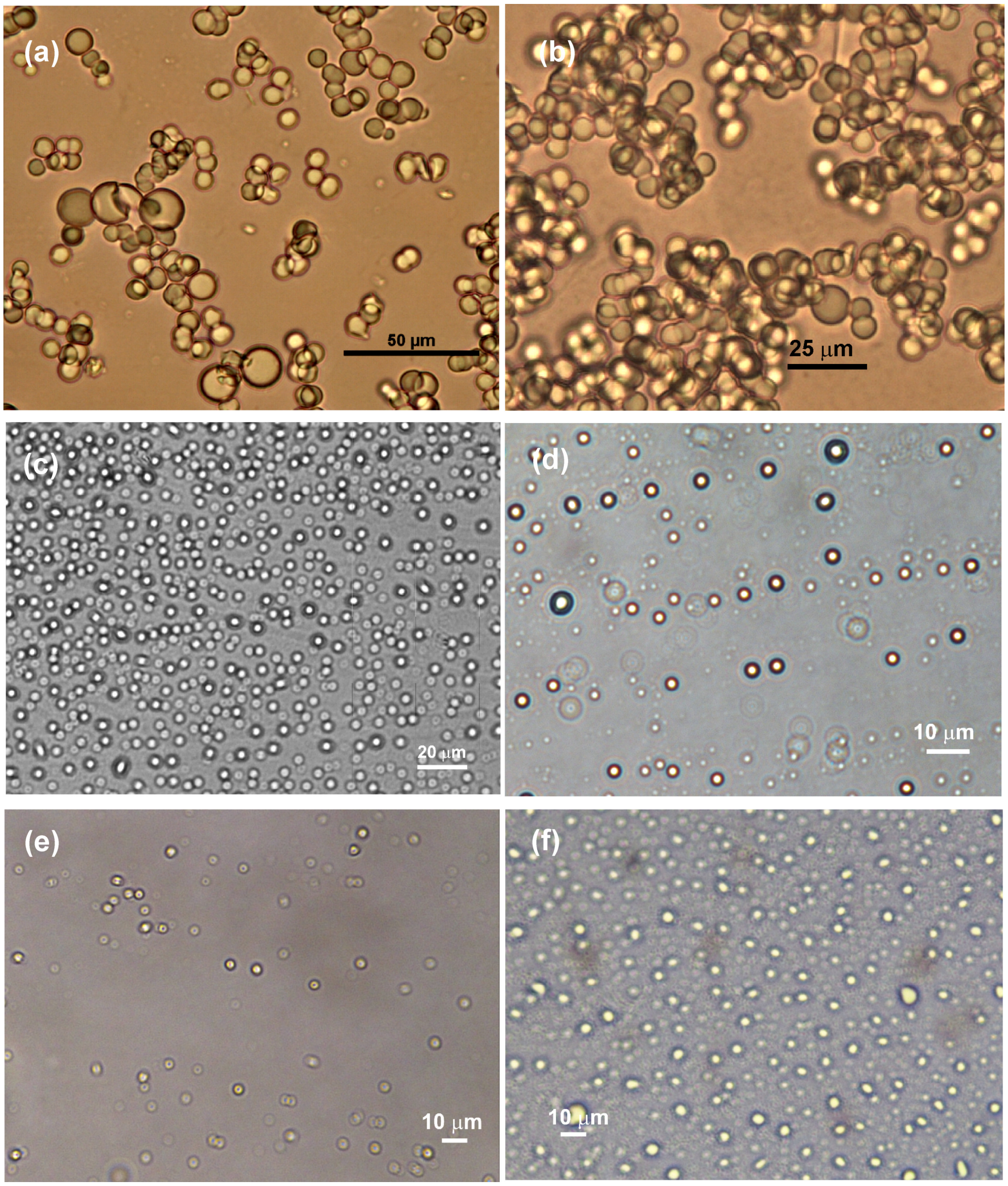

Figure S2: Optical microscopy images of spherical self–assemblies of arjunolic acid (a) in DMSO- H_2O system (5:4, 5.55 % w/ v); (b) in DMSO- H_2O system (5:2, 7.10 % w/ v); (c) in DMSO- acetonitrile (3.33 % w/v, 1:2); (d) in ethanol water (3:4, 0.21 % w/v); (e) in gel –gold nano hybrid material in DMSO- H_2O (1.7 :1 , 0.3 % w/ v) (f) in gel –gold nano hybrid material in EtOH-H_2O. (3:4 , 0.043 % w/ v)

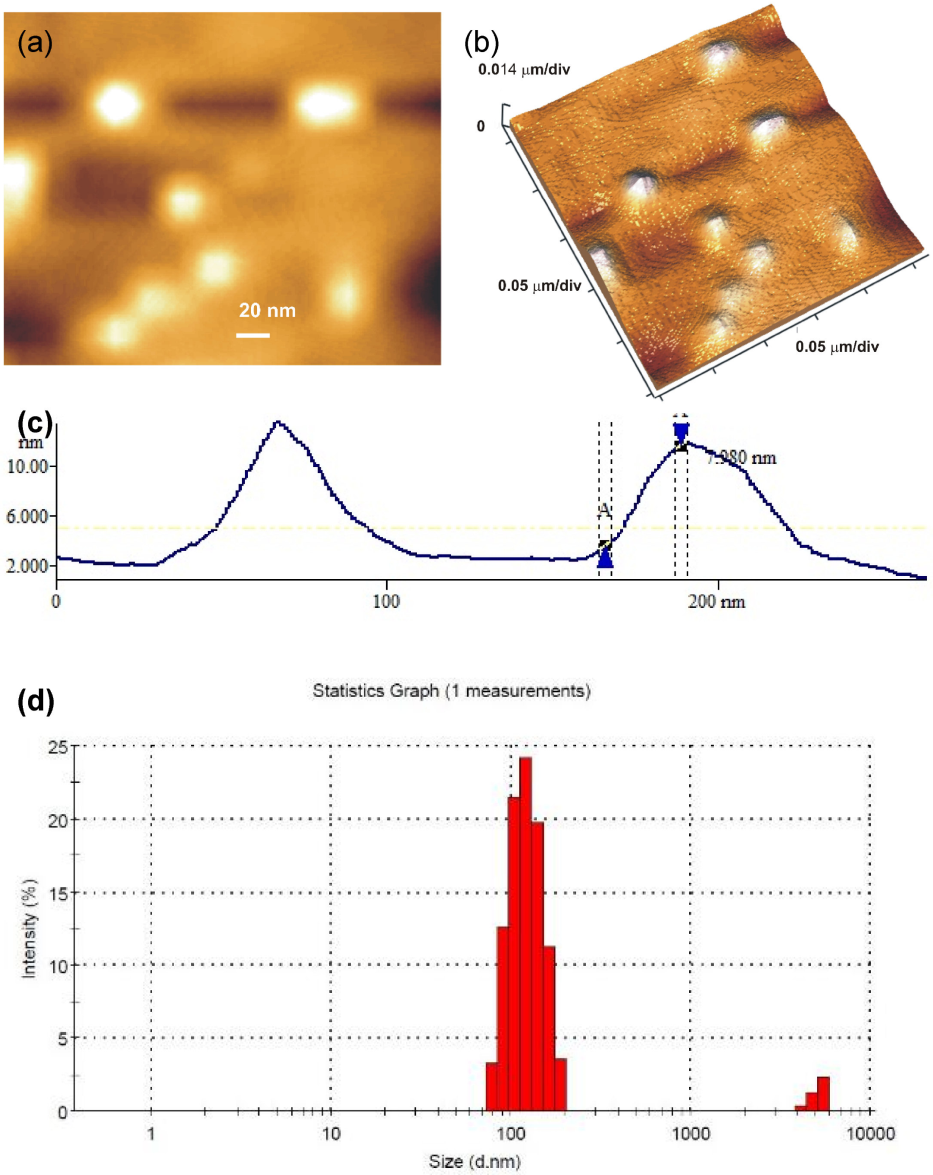

Figure S3: (a-c) AFM images of self-assembled arjunolic acid in DMF-water (0.23% w/v, 5:2 ratio); (d) **Statistical graphs obtained by DLS studies:** Arjunolic acid in Ethanol-water (3:4, 0.043 % w/v) average diameter 135.3 nm;

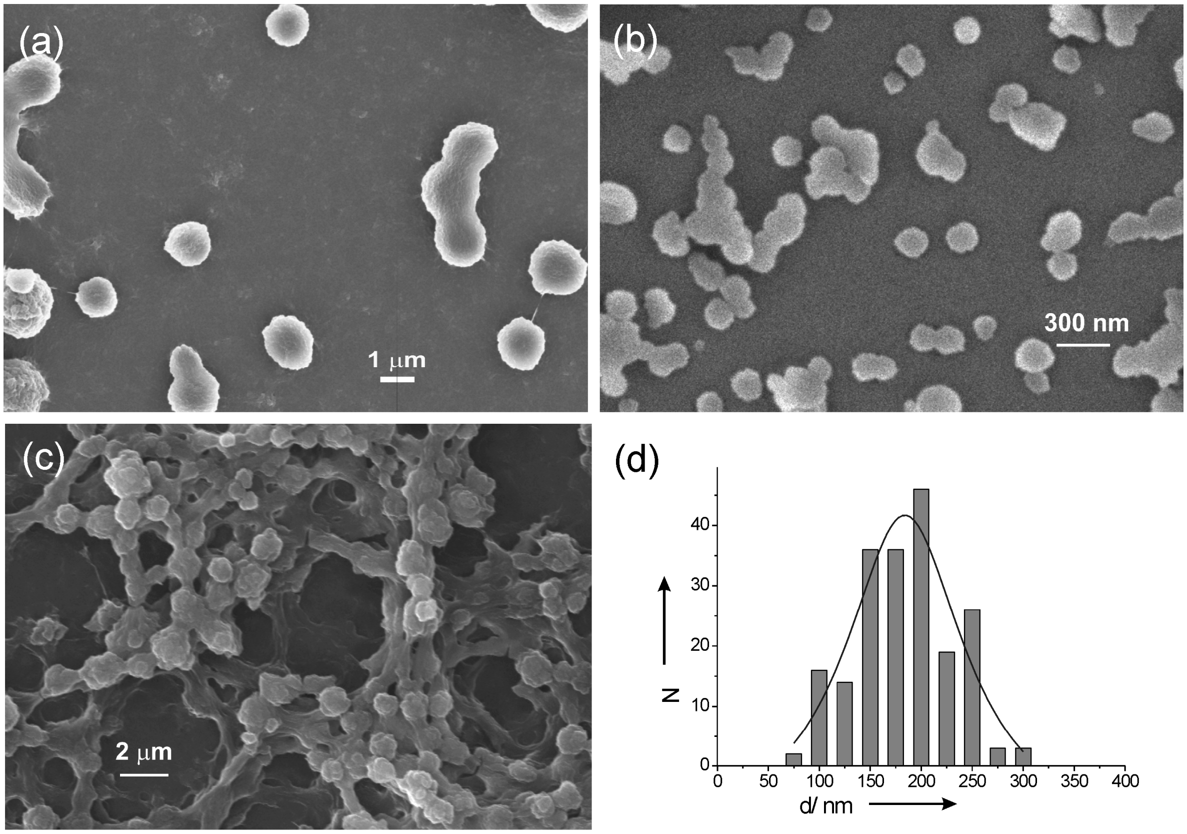

Figure S4: FESEM images of dried self-assemblies of *arjunolic acid* in (a) DMF-water (2.5 :1 , 0.71 % w/v); (b,c) gel-gold nanoparticle hybrid material (0.11% w/v); (d) histogram of the spherical objects observed by SEM of the dried self-assemblies of **1** in ethanol water (3:4, 0.11 % w/v) indicating average diameter as 185 nm.

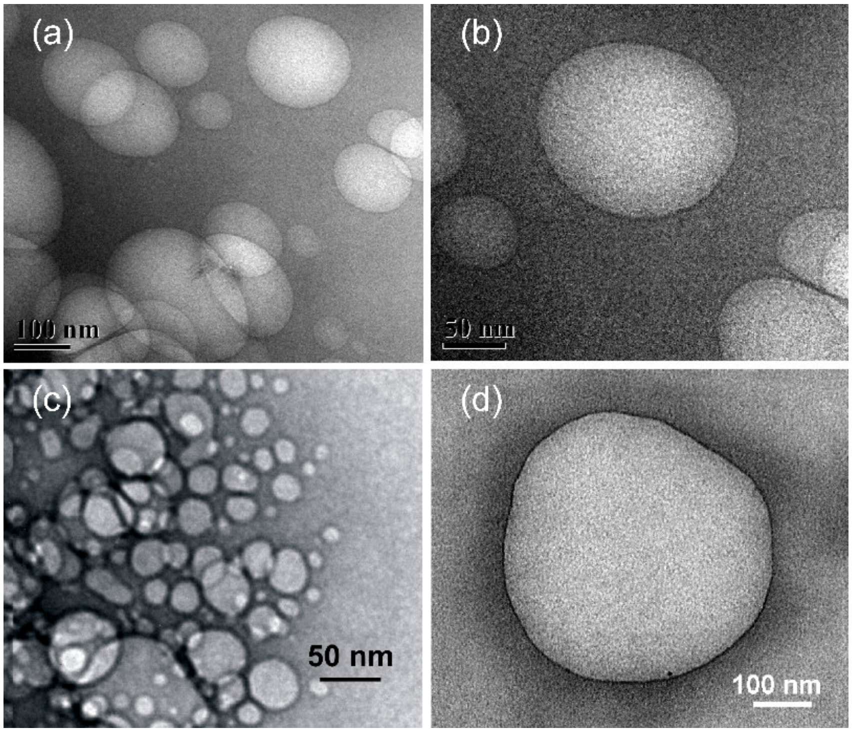

Figure S5: TEM images of dried self-assemblies of arjunolic acid in (a-b) DMSO – water system (1:1 ratio, 0.0214 % w/v), (c) DMF – water (5:2 ratio, 0.23 % w/v); (d) in a diluted hybrid gel of arjunolic acid in EtOH-water system (3:4 ratio, 0.043 % w/v).

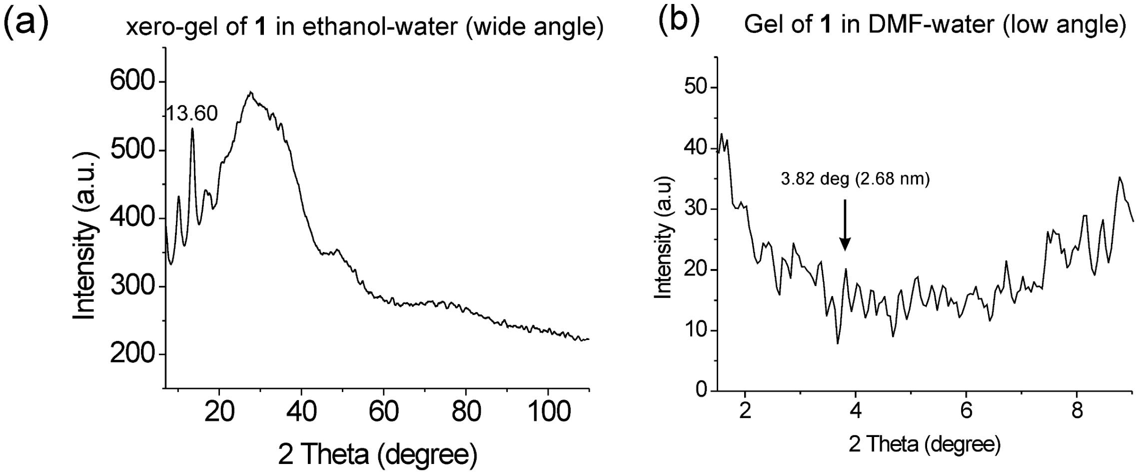

Figure S6: X-RAY diffractograms have been recorded in a **Panalytical X'pert Pro** X-ray diffractometer at room temperature (25 °C) using Co Kα filament (= 1.789 Å) . (a) The xerogel of arjunolic acid in ethanol-water. (b) Gel of **1** in DMF- water. The bilayer nature of the vesicular membranes is supported by 2θ values of 3.82 degree corresponding to a d spacing of 2.68 nm.

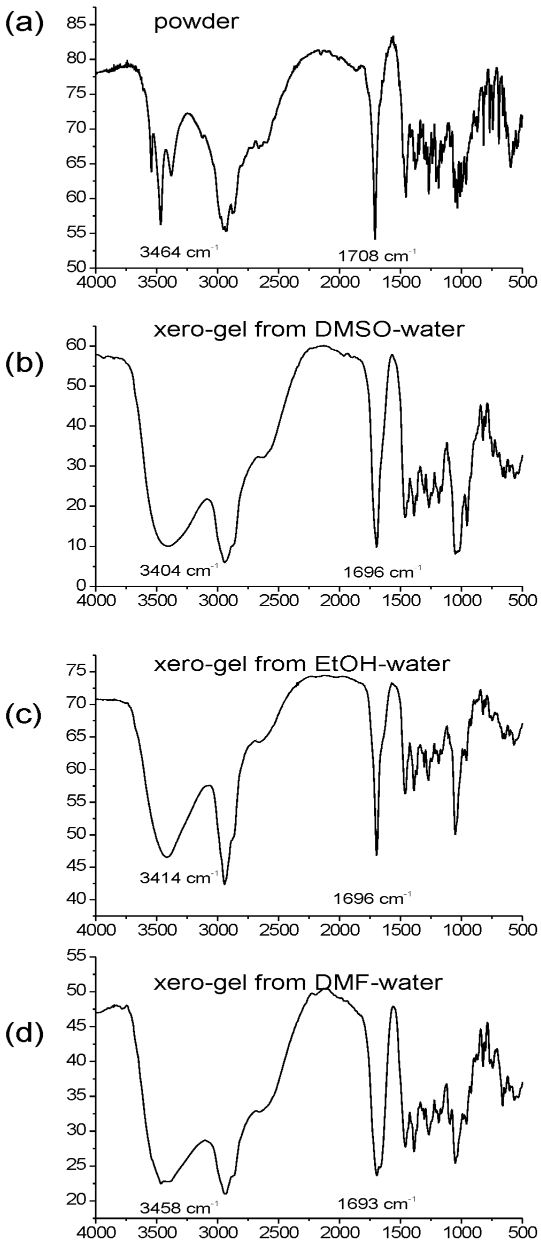

Figure S7: Overlay of FTIR spectra (KBr) of arjunolic acid: (a) powder sample, (b) xero-gel from DMSO-water (5:3), (c) xero-gel from ethanol-water (3:4), (d) xero-gel from DMF-water (5:2).

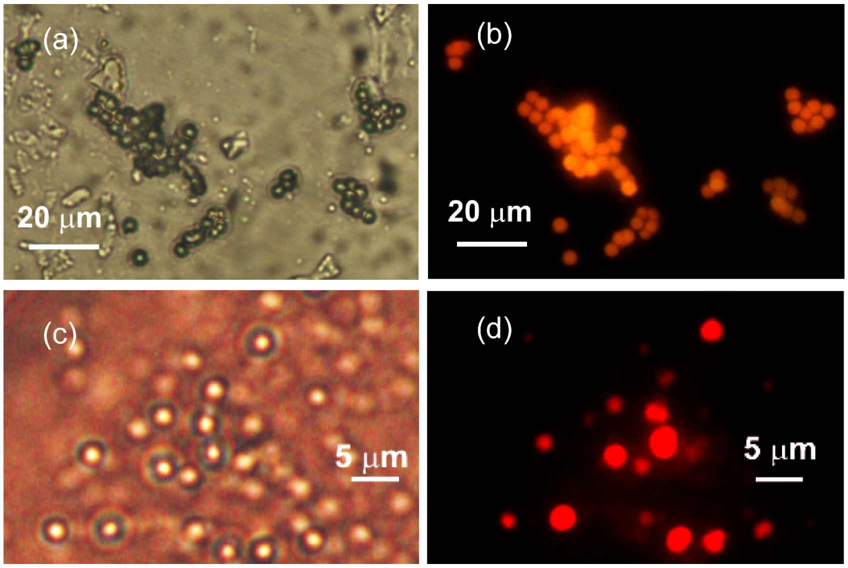

Figure S8: Epifluorescence microscopy images of (a-b) self- assembled arjunolic acid (10.23 X 10 $^{-2}$ M) in DMSO- water (7:3) containing rhodamine B (5 X 10 $^{-3}$ mM), (c-d) self- assembled arjunolic acid (4.384 mM) in ethanol-water (3:4) containing rhodamine B (2.5 X 10 $^{-3}$ mM). (a, c)under normal light, (b, d) under fluorescence light.

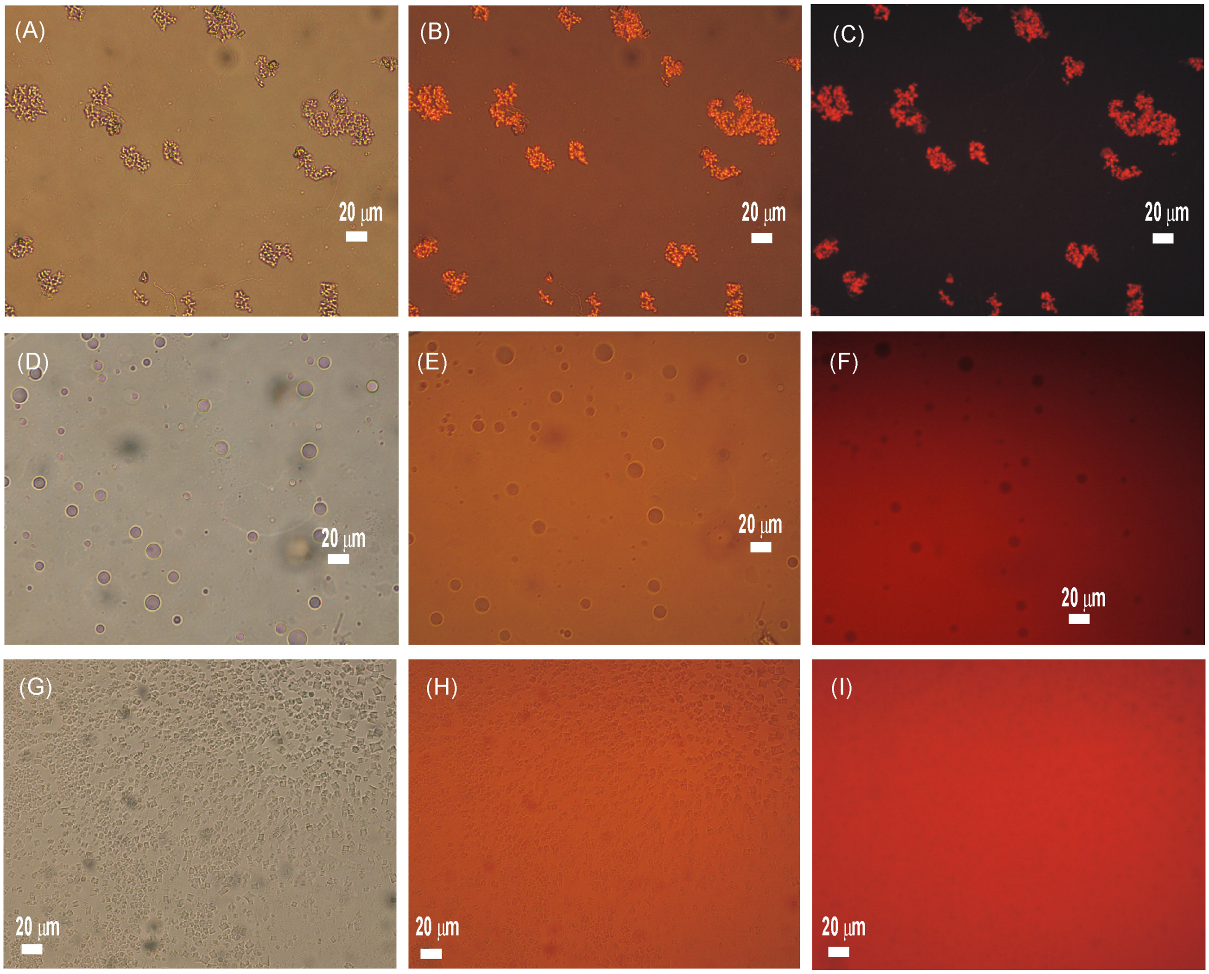

Figure S9: (A,B,C) Optical microscopy images of rhodamine B (3.57X 10^{-6}M) entrapped vesicles via self-assembly of arjunolic acid(7.31 x 10^{-2} M) ; (D,E,F) 20 minutes after the addition of Triton X-100(1.1 x 10^{-6}M) into the rhodamine B entrapped vesicular self- assembly of arjunolic acid; (G, H,I) 22 hours after the addition of Tritonx-100 into the rhodamine B entrapped vesicular self- assembly of arjunolic acid. (A, D,G) under normal light, (B,E,H) overlay images, (C, F, I) under fluorescence light.

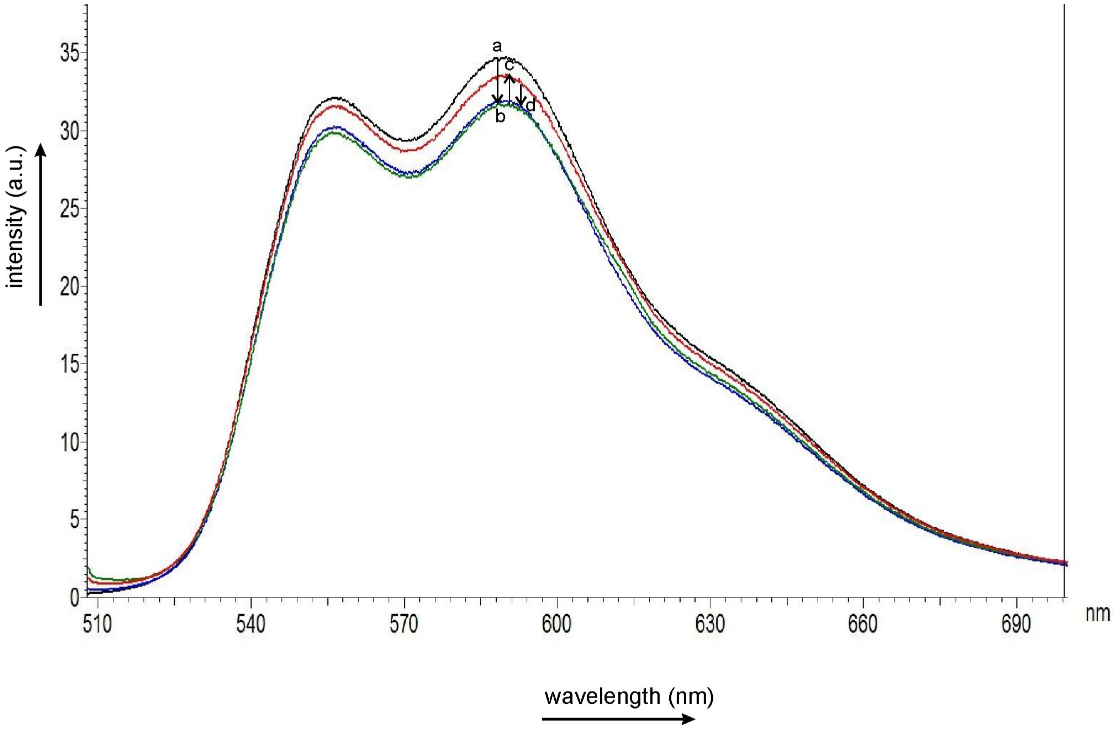

Figure S10: Fluorescence Emission Spectra of doxorubicin (c = 0.045 mM) in ethanol-water (3:4): (a) in the absence of arjunolic acid; (b, c,d) in the presence of arjunolic acid (4.38 mM) after 20 min: (b) before sonication, (c) immediately after sonication, (d) after 20 min of sonication. Fluorescence emission intensity decreases from (a) to (b) due to entrapment of the fluorophore doxorubicin inside the vesicles. The increase of fluorescence intensity from (b) to (c) is due to release of the fluorophore after sonication. The decrease in fluorescence intensity is observed again after keeping the mixture for 20 min at room temperature due to re-entrapment of the drug molecules inside the self-assemblies.

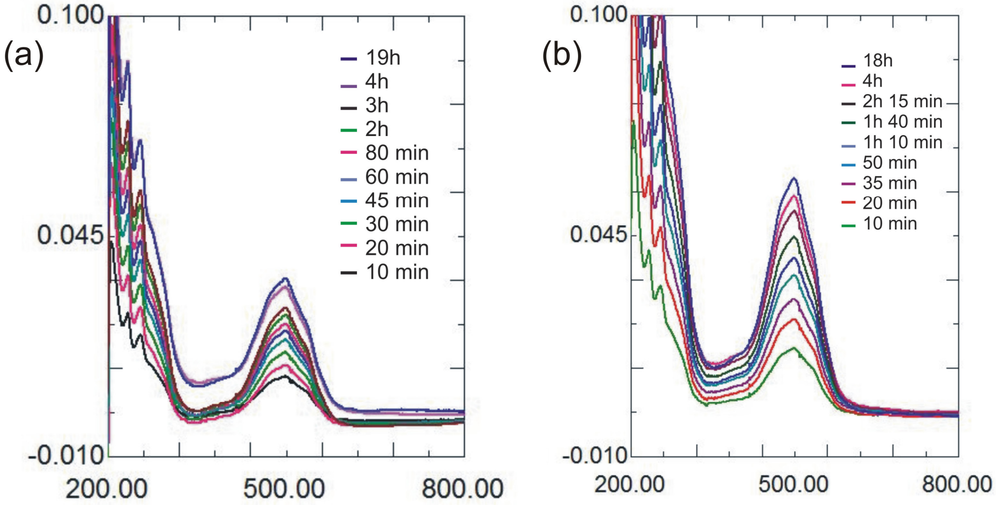

Figure S11: Release of the arjunolic acid (4.39 mM) gel-entrapped anticancer drug doxorubicin (0.31 mM): overlay of the UV-visible spectra of the released doxorubicin to buffers at (a) pH 6.6 and (b) pH 7.2 at various time intervals.

Thermodynamic parameters[1]

The various thermodynamic parameters (ΔH^o, ΔS^o and ΔG^o) during gel to sol phase transitions were calculated from the variation of T_{gel} with concentrations (Table TS1, TS2 and TS3). The positive free energy changes (ΔG^o values) obtained in all the cases studied during gel to sol transition indicated the stability of the gels. The free energy changes during gel to sol transition of compound **3** in water, n-butanol, n-heptanol and n-octanol were identical though their enthalpy and entropy changes were different (Table TS2). The free energy changes during gel to sol transition of compound **2** in water and n- octanol were also identical (Table TS3).

Calculation:

The thermo-reversibility of a gel can be expressed as:

$$\text{Gel} \rightleftarrows \text{liquid}$$

The equilibrium constant can be expressed as:

$$K = [\text{Gelator}]/ [\text{Gel}]$$

Assuming unit activity of the gel, the equilibrium constant can be expressed as :

$$K = [\text{Gelator}]$$

The Gibbs free energy change during gel melting can be expressed as:

---

[1] Rizkov, D.; Gun, J.; Lev, O.; Sicsic, R.; Melman, A. *Langmuir* 2005, **21**, 12130.

$\Delta G^0 = - RT\ln K = \Delta H^0 - T\Delta S^0$, Hence, $\ln K = -\Delta H^0/R. (1/T) + \Delta S^0/R$

The gel melting temperature (T_{gel}) increases with increasing concentration of the "solutes". A plot of lnK vs 1/T allowed us to calculate the thermodynamic parameters.

Table TS5: Thermodynamic parameters (ΔH°, ΔS°) and free energy (ΔG°) at 298 °K for arjunolic acid gels in ethanol- water (3:4), ethanol – bark extract (3:4) and Au nanoparticle containing hybrid gel in ethanol – bark extract (3:4) systems

Gel	ΔH°/ $KJ.mol^{-1}$	ΔS°/$J.mol^{-1}k^{-1}$	ΔG°/ $KJ.mol^{-1}$
ethanol- water gel of **1**	24.18	29.79	15.3
Composite gel of **1** and BETA in ethanol- water	39.07	75.05	16.71
Gel-gold nano composite	17.72	4.39	16.42

A representative plot for a gel in ethanol- bark extract is given in the figure below:

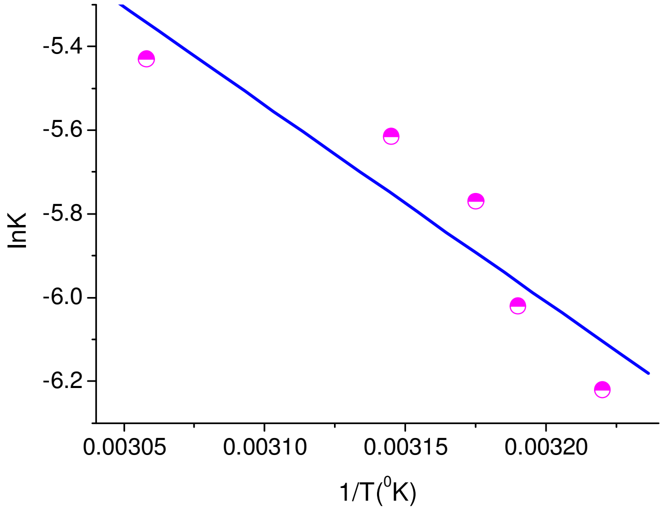

Figure S12: Plot of lnK vs 1/T for a composite gel of arjunolic acid in ethanol containing the bark extract of *T. arjuna*

From the slope we obtain $-\Delta H^0/R = - 4698.7941$ and from the intercept we obtain $\Delta S^0/R = 9.02583$

The calculated thermodynamic parameters are: $\Delta S^0 = 75.05$ J/mol/°K, $\Delta H^0 = 39.07$ kJ/mol and $\Delta G^0 = 16.71$ kJ/mol