Preparation and study of Organo-Gel derived from Ester derivatives of Arjunolic acid

Dr. Shaishab Kumar Dinda
Assistant Professor in Chemistry
Govt. General Degree College Dantan-II, PaschimMedinipur
E-mail: shaishab.orgchem@gmail.com

Abstract:Triterpenes, an important class of *plant secondary metabolites* derived from C30 precursor squalene are nano-sized molecules having varied lengths of rigid and flexible parts.We have utilized these nano-sized triterpenoids as *renewables* in the design of molecular receptors, supramolecular architectures and functional nano-materials.[1-7]Arjunolic acid, the major component of the extracts of the heavy wood of *TerminaliaArjuna* became the first choice for such investigations.

The various derivatives of the nano-sized chiral triterpenic acid could immobilize various organic solvents at low concentrations and self-assembled in organic media to form nano-sized vesicles and nano-fibers with concomitant hardening of the media. Benzyl, (naphthyl) methyl and (anthryl) methyl arjunolate were synthesized and their gelation abilities were tested. (Naphthyl) methyl and (anthryl) methyl arjunolate formed thermo reversible transparent gels efficiently mostly with aromatic solvents while benzyl arjunolate did not form gel with any one of the aromatic solvents except cyclohexane. Electron micrographs of the xero-gels revealed nano vesicles and also fibrous network consisting of fibers of micrometer lengths and nanometer diameters.

Index Terms **- renewable, triterpenes,nano science, arjunolic acid.**

1. INTRODUCTION

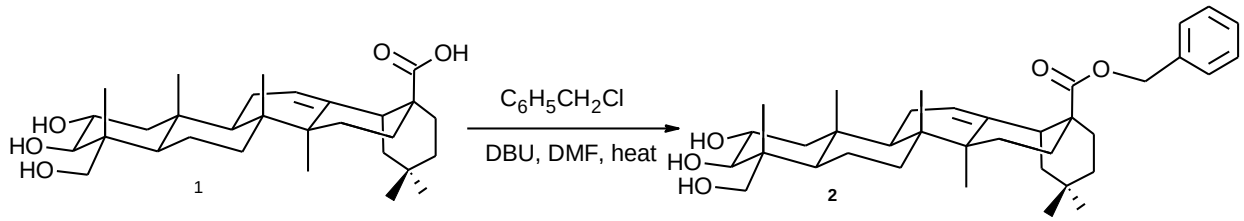
Utilization of *plants* as *renewable* resource of valuable chemicals has become a significant area of investigation in recent years towards the development of a sustainable chemical strategy.[1-7]Triterpenes, an important class of *plant secondary metabolites* derived from C30 precursor squalene are nano-sized molecules having varied lengths of rigid and flexible parts.[8]We have utilized these nano-sized triterpenoids as *renewables* in the design of molecular receptors, supramolecular architectures and functional nano-materials. Arjunolic acid (1), the major component of the extracts of the heavy wood of *TerminaliaArjuna* became the first choice for such investigations.

Terminaliaarjuna has also been used as a medicinal plant in India from the Vedic age. The leaves, fruit and bark of it have been used as a medicine for the treatment of low blood pressure, blood dysentery, Cardiac etc.[10] Recent investigations indicated that pulverized bark of TermrnaliaArjuna augmented endogeneous antioxidant compound of heart and also prevented oxidative stress.[11-13]Moreover, hypocholesterolaemic effect the pulverized bark has been reported.[13,14]Arjunolic acid (1) a pentacyclictrihydroxytriterpenic acid, is the major component of extracts of the heavy wood powder of *Terminaliaarjuna*. So one may expect that the various derivatives of the arjunolic acid should be used as a medicine. Compounds 2, 3 and 4 derivatives of arjunolic acid give the gelation properties and these gels might also be used as a medicine for the various treatment and especially the vesicles formed in the gel could be used as a drug delivery agent in near future.

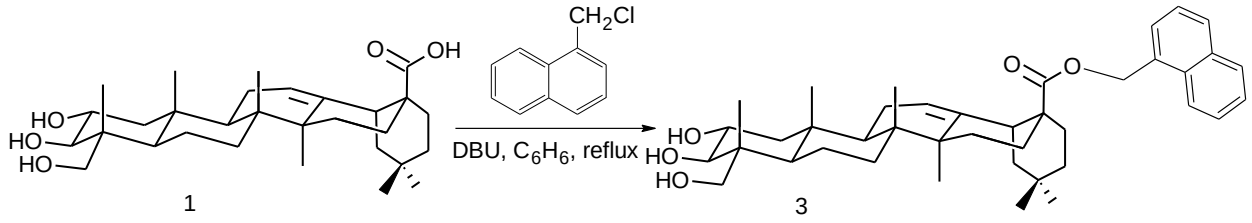
1. RESULTS AND DISCUSSION

Ester derivatives of arjunolic acid having attached aromatic units were synthesized and their gelation abilities were tested. Among three derivative synthesized, two compounds 3 and 4 are excellent gelators of various aromatic solvents and one (2) is only for cyclohexane. Morphology of the gels were studied by optical microscopy, electron microscopy and atomic forece microscopy revealed nano-sized vesicles and self-assembled fibriller networks.

2.1 Syntheses

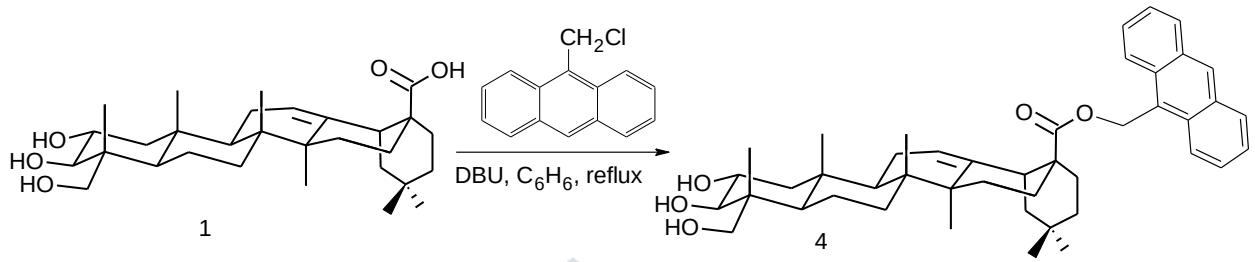


Scheme A: Reagent and condition: Benzyl chloride, DBU, DMF and heat (at 60°C), 90 %.



1 3

Scheme B: Reagent and condition: 1-Chloro methyl-naphthalene, DBU, Benzene and Reflux, 86 %.



1 4

Scheme C: Reagent and condition: 9-Chloromethyl-anthracene, DBU, Benzene and Reflux, 89 %.

Compounds **2, 3** and **4** were synthesized from Arjunolic acid **1** as shown in *scheme* ***A- C*** respectively. Benzyl arjunolate**2** was synthesized from arjunolic acid **1** by heating with benzyl chloride in DMF in the presence of DBU in 90% yield. Arjunolic acid **1**on refluxing about 8 hours with 1-Chloro methyl-naphthalene and DBU in benzene solvent gives 86 % yield of naphthyl methyl arjunolate**3**. In a similar way anthrcene-9-yl methyl arjunolate**4** was synthesized from arjunolic acid **1** by refluxing with 9-Chloromethyl-anthracene and DMF in benzene solvent about 7 hours in 89 % yield.

2.1.1 Synthesis of Benzyl arjunolate (2):

A mixture of crystalline arjunolic acid[6] (0.210 g, 0.430 mmol) and benzyl chloride (0.075 ml, 0.652 mmol) contained in a dry 5 mL round-bottom flask was suspended in dry DMF (0.20 mL) and treated with DBU (0.097 mL, 0.649 mmol). The mixture was heated over oil bath at 60 ˚C for 24 hours with continuous magnetic stirring. The reaction mixture was treated with an additional amount of benzyl chloride (0.038mL, 0.326 mmol) and the reaction mixture was heated for another 7 hours. The volatiles were removed under reduced pressure and the crude product was purified by column chromatography (Si-gel, 100-200 mesh, 1.0 X 12.5 cm) using 55% ethyl acetate/chloroform as eluant. The product was obtained as a white solid (0.230 g, 90% yield), mp 105 - 108 ºC.

^{1}HNMR (500 MHz, $CDCl_3$) δ: 7.37 - 7.28 (m, 5H, Phe-H's), 5.28 (br. app. t, J = 3.5 Hz, 1H), 5.09 (d, J = 12.5 Hz, 1H, -O-C H_a-Ph), 5.04 (d, J = 12.5 Hz, 1H, -O-C H_b-Ph), 3.78 - 3.70 (br, m, 1H, 2-H), 3.64 (d, J = 10.5 Hz, 1H, 3-H), 3.60-3.44 (br. s, 1H), 3.41 (s, 1H, 23-H_a), 3.39 (s, 1H, 23-H_b), 2.90 (dd, J_1= 13.7 Hz, J_2= 3.5 Hz, 1H, 18-H), 2.80 - 2.50 (br. s, 2H), 2.02 - 0.84 (m, terpenoid H's, 20H), 1.12 (s, 3H), 0.98 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.86 (s, 3H), 0.60 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.85, 144.15, 136.81, 128.82, 128.38, 128.32, 122.64, 80.75, 70.73, 69.13, 66.35, 49.44, 47.94, 47.12, 46.46, 46.25, 42.87, 42.16, 41.75, 39.71, 38.59, 34.27, 33.50, 32.77, 32.75, 31.10, 27.98, 26.36, 24.04,23.85, 23.43, 18.73, 17.41, 17.33, 17.31. $[\alpha]_D^{300.5}$ = + 45.88 (c 0.632 g /100 mL, $CHCl_3$). FTIR (neat): 3382, 3090, 2944, 1724, 1661, 1587, 1495, 1456, 1386, 1367, 1319, 1301, 1259, 1237 cm^{-1}. UV (2% $CHCl_3$ / MeOH, log ε) λ = 257.2 nm (3.22), 249.2 nm (3.24).HRMS (ESI): m/z calcd ($C_{37}H_{55}O_5$) 579.4049, found 579.4048 $[M + H]^+$.

2.1.2 (Naphthyl) methyl arjunolate(3):

A mixture of crystalline arjunolic acid[6] (0.31 g, 0.634 mmol) and 1-Chloro methyl-naphthalene (0.161 g, 0.911 mmol) contained in a dry 5 mL round-bottom flask was suspended in dry benzene (1.20 mL) and treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.14 mL, 0.912 mmol). The mixture was refluxed for 8 h with continuous stirring. The volatiles were removed under reduced pressure and the crude product was purified by column chromatography (Si-gel, 100-200 mesh, 1.0 X 12.5 cm) using 70% ethyl acetate/petroleum ether as eluant. The product was obtained as a foamy solid (0.342 g, 86% yield), mp124 - 127 ºC.

^{1}HNMR (500 MHz, $CDCl_3$) δ: 8.06 (d, J = 8.1Hz, 1H), 7.87 – 7.86 (t, 1H), 7.82 (d, J = 8.2 Hz, 1H), 7.55 – 7.49 (m, 3H), 7.43 – 7.40 (t, 1H), 5.57 (d, J = 8.2 Hz, 1H), 5.48 (d, J = 12.4 Hz, 1H), 5.12 (s, 1H), 3.74 – 3.69 (br. m, 1H), 3.61(d, J = 10.6 Hz, 1H), 3.39 – 3.36 (dd, J_1 = 10.6 Hz, J_2= 4.3 Hz, 2H), 2.90 – 2.86 (dd, J_1 = 13.7 Hz, J_2 = 3.9 Hz, 2H), 1.10 – 0.91 (m, terpenoid H's, 20H), 1.05 (s, 3H), 0.91 (s, 6H), 0.88 (s, 3H), 0.84 (s, 3H), 0.31 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.94, 143.83, 134.17, 132.31, 132.28, 129.43, 128.95, 128.04, 126.63, 126.22, 125.66, 124.38, 122.75, 80.78, 70.78, 69.10, 64.96, 49.42, 47.84, 47.30, 46.40, 46.24, 42.84, 42.03, 41.69, 39.57, 38.51, 34.24, 33.49, 32.87, 32.64, 31.10, 27.86, 26.29, 24.01, 23.69, 23.37, 18.68, 17.31, 17.03, 13.17. . $[\alpha]_D^{297.7}$= + 39.92 (c 0.2 g /100 mL, $CHCl_3$). FTIR (neat): 3387, 3091, 2943, 2883, 1719, 1667, 1460, 1386, 1366, 1320, 1301, 987, 962, 761cm^{-1}. UV (c = 0.002703 M in 2% $CHCl_3$ / MeOH, log ε) λ = 391.0 nm (0.007), 344.0 nm (0.015), 293.2 nm (0.881).

2.1.3 (Anthryl) methyl arjunolate (4):

A mixture of crystalline arjunolic acid[6] (0.200 g, 0.409 mmol) and 9-Chloromethyl-anthracene (0.121 g, 0.532 mmol) contained in a dry 5 mL round-bottom flask was suspended in dry benzene (0.80 mL) and treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.08 mL, 0.534 mmol). The mixture was refluxed for 7 h with continuous stirring. The volatiles were removed under reduced pressure and the crude product was purified by column chromatography (Si-gel, 100-200 mesh, 1.0 X 12.5 cm) using 80% ethyl acetate/petroleum ether as eluant. The product was obtained as a foamy solid (0.247 g, 89% yield), mp153 - 156ºC.

^{1}HNMR (600 MHz, $CDCl_3$) δ: 8.47 (s, 1H), 8.39 (d, *J* = 9.0 Hz, 2H), 8.0 9 (d, *J* = 8.4 Hz, 2H), 7.56 – 7.53 (m, 2H), 7.49 – 7.47 (t, 2H), 6.20 (d, *J* = 12.6 Hz, 1H), 6.03 (d, *J* = 12.6 Hz, 1H), 4.92 (s, 1H), 3.74 – 3.70 (br. m, 1H), 3.60 (d, *J* = 10.8 Hz, 1H), 3.37 – 3.35 (m, 2H), 2.84 – 2.81 (dd, J_1 = 14.1 Hz, J_2= 3.6 Hz, 1H), 1.90 – 0.80 (m, terpenoid H's, 20H), 0.10 (s, 3H), 0.87 (s, 3H), 0.85 9 s, 3H), 0.84 (s, 3H), 0.14 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 177.75, 143.15, 131.38, 131.04, 128.88, 128.80, 126.94, 126.24, 125.01, 124.36, 122.26, 80.24, 80.18, 70.10, 68.66, 58.67, 48.84, 47.31, 46.85, 45.89, 45.68, 42.41, 41.51, 41.28, 39.02, 38.00, 33.75, 33.06, 32.42, 32.13, 30.66, 27.36, 25.78, 23.55, 23.15, 22.80, 18.19, 16.79, 16.46, 12.77. $[\alpha]_D^{297.7}$= + 47.82 (c 0.2 g /100 mL, $CHCl_3$). FTIR (neat): 3390, 2944, 2881, 1715, 1669, 1462, 1386, 1365, 1319, 1302, 1259, 1229, 1050, 985, 960, 755 cm^{-1}. UV (2% $CHCl_3$ / MeOH, log ε) λ = 385.5 nm (0.045), 366.0 nm (0.049), 347.5 nm (0.033), 255.0 nm (0.854).HRMS (ESI): m/z calcd ($C_{45}H_{58}O_5Na$) 701.4182, found 701.4182 $[M + Na]^+$.

2.2 Gelation studies

During synthesis of various derivative of arjunolic acid, it has been observed that the compound **2** forms thermoreversible gel in very limited organic solvents. The gelation properties of the compound has been studied basically in cyclohexane solvent. By dissolving small amount of compound (about 5 mg) in the organic solvent by heating. The heated solution was allowed to cool at room temperature and after some time it was found that the complete volume of organic solvents were immobilized and it formed gel. The gel formation was confirmed by the inverted vial method. The compound **2** has been found to be a very efficient organogelators for Cyclohexane solvent at very low concentration even less than 0.5% (w/v). A single gelator molecule **2** is capable to entrap more than three moleculesof cyclohexane solvent (when the critical gel concentration is ~80ºC). It is also found that in cyclohexane solvent, the gels are very stable. The results of gelation have been summarized in table 1. The efficiency of gelators has been obtained from the minimum gelation concentration (0.50 % w/v) and from the plot of TgelVs. Concentration in Cyclohexane solvent as shown in table 2 and figure 5 respectively.

Table 1: Gelation tests with **2 – 4** in a variety of liquids.[a] [a]*G = gel, PG = partial gel and S = solution,*

Entry	Solvents	Compound 2	Compound 3	Compound 4
1.	Benzene	S	G	G
2.	Toluene	S	G	G
3.	o-Xylene	S	G	G
4.	m-Xylene	S	G	G
5.	p-Xylene	S	G	G
6.	Mesitylene	S	G	G
7.	Cyclohexane	G	S	S

All the gels were thermoreversible. Hence the gel to sol transition temperature Tgel was plotted against the % gelator concentration (*Figure **1***). With increase in the concentration of the gelator the Tgel increased indicating stronger intermolecular interactions and increased branching at higher concentrations. The Tgel value for the Cyclohexane gel was 59ºC at 0.50% (w/v) concentration and increased to 80ºC (*Figure **1***) at 2% (w/v) concentration.

Table 2: TgelVs Concentration study of Benzyl arjunolate in Cyclohexane**.**

Entry	Amount of compound(mg)	Amount of solvent(mL)	Conc.(%w/v)	State	Tgel(ºC)
1.	2.0	0.40	0.50	G	59
2.	8.0	1.60	0.75	G	64
3.	5.5	0.50	1.10	G	72
4.	5.4	0.40	1.35	G	76
5.	5.5	0.30	1.83	G	78
6.	5.4	0.27	2.00	G	80

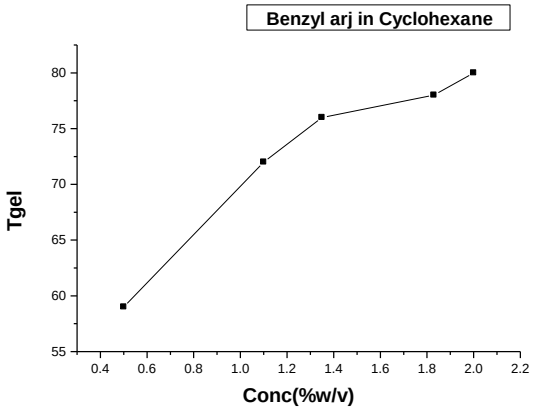


Figure 1: Plot of TgelVs. Conc (% W/V) of Benzyl arjunolate (**2**) in Cyclohexane solvent.

2.3 Microscopic studies

2.3.1 Scanning electron microscopic (SEM) studies

Morphological studies of the reported compound were investigated using scanning electron microscope (SEM). The scanning electron microscopic studies of the gel was done using a thin slice of gel on microscopic slides and drying at room temperature, finally under reduced pressure for several hours. The dried sample was platinum coated and observed under the SEM instrument. The SEM pictures were taken in a SEM instrument (Jeol Scanning Microscope-JSM-5200 and Jeol Scanning Microscope-JSM-6700F). The SEM images show the fibrillar network structures having intertwined fibers of 50 - 70 nm diameter and micrometer lengths were obtained from a gel of naphthyl methyl arjunolate**3** in o-xylene (5.0 % w/v) (*Figures **2a,b***). The dimensions of the fibers indicate that there are several molecules present both in lengths and breadths of the fibers.

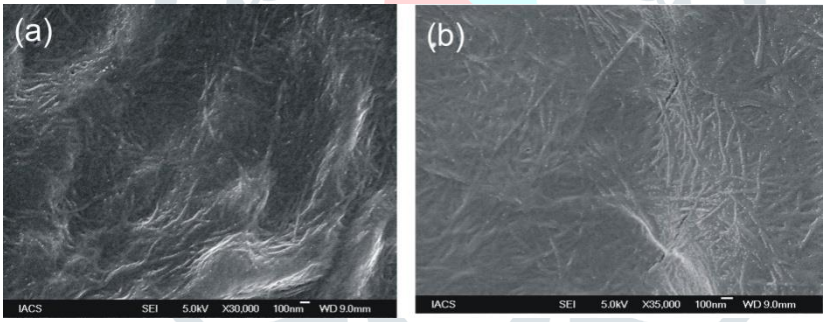


Figure 2: (a, b) Scanning Electron Microscopic images of compound **3** (naphthyl methyl arjunolate) in o-xylene (5.0 % w/v) show the nano-fiber, A thin layer of gel sample was taken on a glass plate, dried slowly in air and then under reduced pressure and sputter coated with Pt for 90 seconds and analyzed.

2.3.2 Transmission electron microscopic (TEM) studies

Morphological studies of the reported compound were investigated using transmission electron microscope (TEM). The transmission electron microscopic images of the gel was recorded in JEM-2100 with an acceleration voltage of 200 kV. A solution of compound **3** and **4** was allowed to cool at room temperature for 4 h and then an aliquot was dried on a 300 mesh Cu coated with carbon for 24 hours.

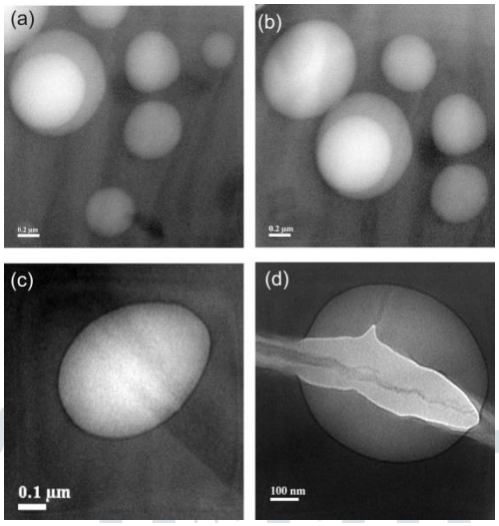


Figure 3: Transmission Electron Microscopy images (TEM) of compound **3** (naphthyl methyl arjunolate) in o-xylene (7.3% w/v). Figure (a), (b) and (c) show both the nano vesicles; figure (d) show the deformed vesicles (having hollow inner).

Figure **3(a, b & c)** show nano vesicles only. The vesicles formed are of different diameters having a range of 200 nm to 1000 nm. Figure **3d** the deformed vesicles (having hollow inner). It is really problematic to tell frankly whether these are vesicles or sphere; but the TEM images solved the problem, in the image Figure **3d**, where we have deformed vesicles having hollow inner. This is the direct evidence in favour of vesicles. Also by the further optical microscopic experiment, we have been able to prove the hollow inner of the vesicle (Figure **5**); by encapsulating a fluorescence active material (rhodamine-B) into the vesicle. We have been able to determine the thickness of the vesicle membranes, determined by TEM images (Figure **3a, b & c**), was about 3.5 nm.

2.3.3 Atomic Force Microscopic (AFM) studies

Images were collected from atomic force microscopy VEECO, diCP-II, Model no. AP0100. A hot solution was allowed to cool at room temperature for 4 hours and then coated on a glass plate and allowed to dry in air. Nano-sized vesicles were observed. Atomic Force Microscopy (height image) of compound **3** (naphthyl methyl arjunolate) in o-xylene (7.7% w/v)shows (*Figure **4***)the height of the vesicle is about 25nm – 26nm and the diameter of the vesicle is about 160nm – 162nm.

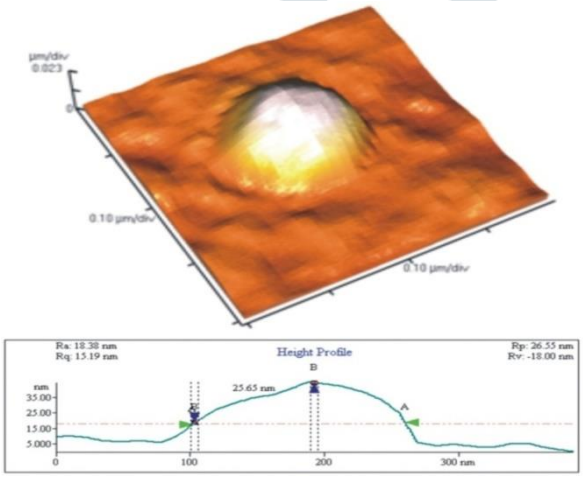


Figure 4: Atomic Force Microscopy (height image) of compound **3** (naphthyl methyl arjunolate) in o-xylene (7.7% w/v), shows the height of the vesicle is about 25nm – 26nm and the diameter of the vesicle is about 160nm – 162nm.

2.3.4 Proof of hollow inner of the vesicles

To prove the hollow inside in a vesicle; a special experiment has been carried out. We have tried to encapsulate a fluorescence active material into the vesicle inner cavity. During vesicle formation we have injected rhodamine-B in a 0.01 molar ratio, then fluorescence optical microscopic images were collected after one hour of the gel formation and the images clearly shown that the vesicles become fluorescence active i.e., the fluorescence active rhodamine-B was encapsulated into the inner hollow portion of the vesicles during its formation.

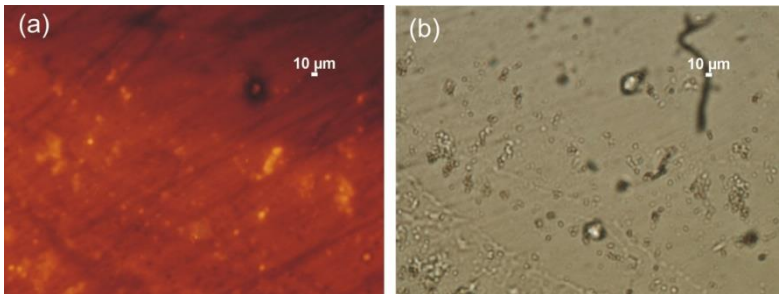


Figure 5: (a) Fluorescence optical microscopic images (20X magnification) of the rhodamine-B encapsulated gel of compound **4** in o-xylene (concentration of gel 7.5%w/v), fluorescent image (a) and phase contrast bright field image (b).

3. CONCLUSION

Arjunolic acid, a chiral functional triterpenoid, with a rigid pentacyclic backbone, offers a great opportunity for the construction of supramolecular architectures of nanometric dimensions and nanomaterials. The organogelators**2, 3** and **4** could gel various organic solvents at low concentrations. These low molecular mass organogelators are self-assembled in the solvents by the noncovalent interactions to form a fibrous network consisting of fibers of micrometer lengths and nanometer diameters and nano vesicles which have been visualized by SEM, TEM, AFM and even by optical microscopy (*Figure **2 – 5***).The height and diameter of the nano vesicles have been measured by taking height image in atomic force microscopy. The solvent molecules are basically entrapped inside the network to form a gel. The interacting groups that are present in gelator molecules are aromatic groups, hydroxyl groups in addition to the rigid lypophilicterpenoid unit. Systematic variations of the substituents in the aromatic units are necessary for getting insight about the various noncovalent forces that are involved in the self-assembly processes. Studies along this direction might pave the way for newer functional nanostruetures.[10-19] Arjunolic acid being 1.32 nm long, generation of functional nanomaterials will be no longer a dream in near future [1-9].

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