Prayogik Rasayan

Critical vesicular concentration (CVC) of arjunolic acid in DMSO-water using hydrophobic pyrene as a fluorescent probe

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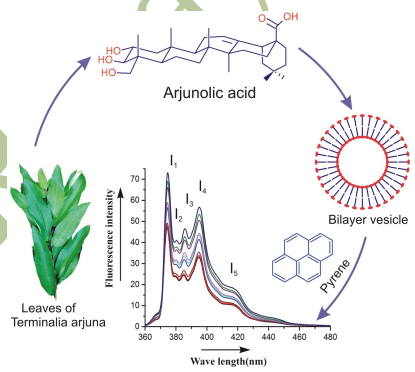
Self-assembly of arjunolic acid, a naturally occurring 6-6-6-6-6 trihydroxytriterpenic acid, in bilary liquid mixtures has been reported yielding vesicles of nano to micrometer diameters (B.G. Bag, R. Majumdar, RSC Adv., 2014, 4, 53327-53334). Critical vesicular concentration (cvc) of arjunolic acid is reported here using the fluorescence probe of pyrene monomer. The cvc determined by this method in DMSO-water binary liquid mixtures at 2:1, 1:1, and 1:4 v/v ratio were determined to be 68 μM, 57 μM and 49 μM respectively showing a lowering in the cvc's with increasing percentage of water.



Keywords: Arjunolic acid, self-assembly, vesicle, fluorescence, cvc, Pyrene

1. Introduction

Arjunolic acid is a nano-sized, 6-6-6-6-6 pentacyclic triterpenoid having three hydroxyl and one carboxyl group at opposite ends of the rigid triterpenoid backbone. The compound is extractable from the heavy wood of *Terminalia arjuna* (*T. arjuna*) as the free acid.[1] Previously we reported the spontaneous formation of vesicular self-assembly of arjunolic acid in binary liquid mixtures at low concentrations.[2] The vesicles were capable of entrapping various fluorophores such as rhodamine B, 5,6-carboxyfluorescein and also the anticancer drug doxorubicin in binary aqueous solvent mixtures.[3,4] The vesicular self-assemblies were observed in the arjunolic acid derived salts and hybrid materials.[56] Controlled release of the entrapped drug molecules carried out at physiological pH indicated their usefulness as drug delivery vehicle.

Critical vesicular concentration (cvc), defined as the minimum concentration necessary to form vesicles in a given liquid at room temperature. Literature study reveals that there is no report of determination of critical vesicular concentration (cvc) of arjunolic acid in any medium. Ease of formation of the vesicular self-assemblies and its utilization in drug deliver applications inspired us to study the cvc of arjunolic acid. Herein, we report the determination of cvc of arjunolic acid in DMSO-water binary liquid mixtures at 2:1, 1:1 and 1:4 v/v ratio using pyrene as a fluorescence probe.

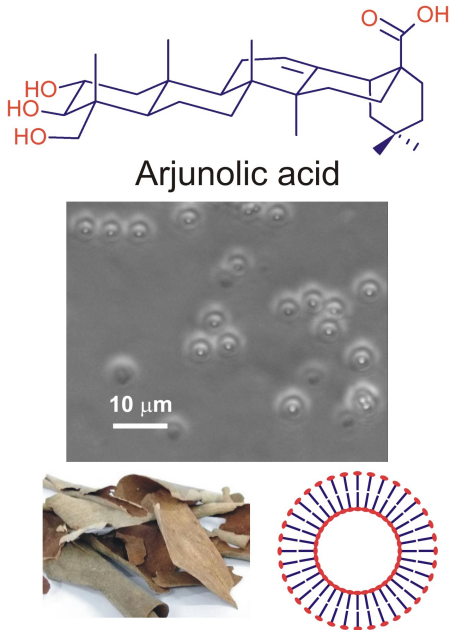


Figure 1: Generation of vesicular self–assemblies from naturally occurring arjunolic acid in DMSO-Water.

2. Experimental

Materials and Method

DMSO, dichloromethane were purchased from SRL and pyrene was purchased from MERCK. DMSO, was dry distilled by following the standard procedure[7] prior to use. Optical microscopy was performed using Nikon Eclipse LV100POL instrument. Fluorescence study was carried out in HITACHI F-4200 instrument.

2.1 Preparation of Pyrene solution

Pyrene (1 mg) was weighed in a vial and dissolved in dichloromethane (1 mL) to obtain a clear solution (4.9 mM). An aliquot of 20.5 μL (4.9 mM) was taken in another vial and the volume of the solution made upto 1 mL to obtain a solution of concentration 0.1 mM. Aliquots of 20 μL each of of 0.1 mM pyrene solution were placed in twelve different clean and dry vials and the solvent was evaporated so that each vial contain fixed amount of pyrene (0.4 μg).

2.2 Preparation of Arjunolic acid solution

Arjinolic acid (2 mg) was taken in a vial and dissolved in DMSO (2 mL) to obtain a clear solution (2.046 mM).

2.3 Fluorescence Probe Studies

To determine the cvc, a series of solutions were prepared at different concentration of arjunolic acid from 0.01 mM to 0.20 mM and the concentration of probe is fixed at 1 μM for each case. For this purpose varied amount of previously prepared arjunolic acid solution in DMSO (2.046 mM) was added to each vial containing fixed amount of pyrene. Then distilled water was added maintaining the ratio of DMSO: H_2O at 2:1, 1:1, 1:4 respectively in total volume of 2 mL. Thus arjunolic acid solution in DMSO: H_2O of 0.01 mM to 0.2mM containing pyrene (1μm) was prepared. All of these samples were heated with stirring and incubated for 24 h at room temperature before measuring the fluorescence. The excitation wavelength was 340 nm.

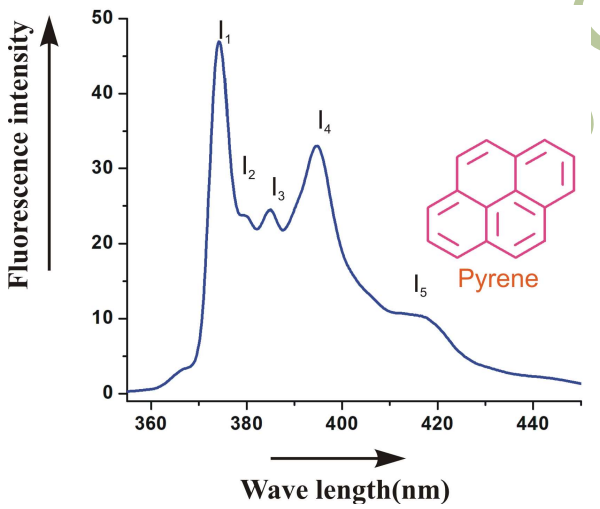


Figure 3 Fluorescence emission spectrum of pyrene in DMSO-Water (1:4, 1μM) λ_{ex} = 340 nm.

3. Results and Discussion

Arjunolic acid is a very poorly soluble amphiphile in common organic liquids as well as water due to the presence of a rigid pentacyclic backbone with the polar hydroxyl and carboxyl groups present at the two extreme ends of the molecule. But it was comparatively more soluble in polar liquids such as DMSO, DMF, THF, ethanol, etc. Hence, self-assembly was studied in aqueous DMSO, DMF, ethanol, ethylene glycol binary liquid mixtures.[2] Fluorescence probe analysis is becoming an important area in biophysical studies of multimolecular assemblies such as micelles and vesicles.[8,9,10,11,12,13] Pyrene is one of the few condensed aromatic hydrocarbons which shows significant fine structure (vibronic bands) in its monomer fluorescence spectra in solution phase. When pyrene is excited at 340 nm, five predominant peaks are observed in fluorescence emission spectrum at 375 nm, 380 nm, 385 nm, 395 nm and 418 nm which are denoted as I_1 I_2, I_3, I_4 and I_5 respectively (Figure 3).

The peak intensities are highly sensitive to the micro-environment of the fluorophore. This property has made it useful as an effective fluorescence probe.[14,15,16] As increasing ratio of water content in DMSO-water binary liquid mixtures will have drastic effect on the hydrophobicity of the medium, we resorted to study of the self-assembly of arjunolic acid in DMSO-water binary liquid mixtures at 2:1, 1:1, 1:4 DMSO-water (v/v).

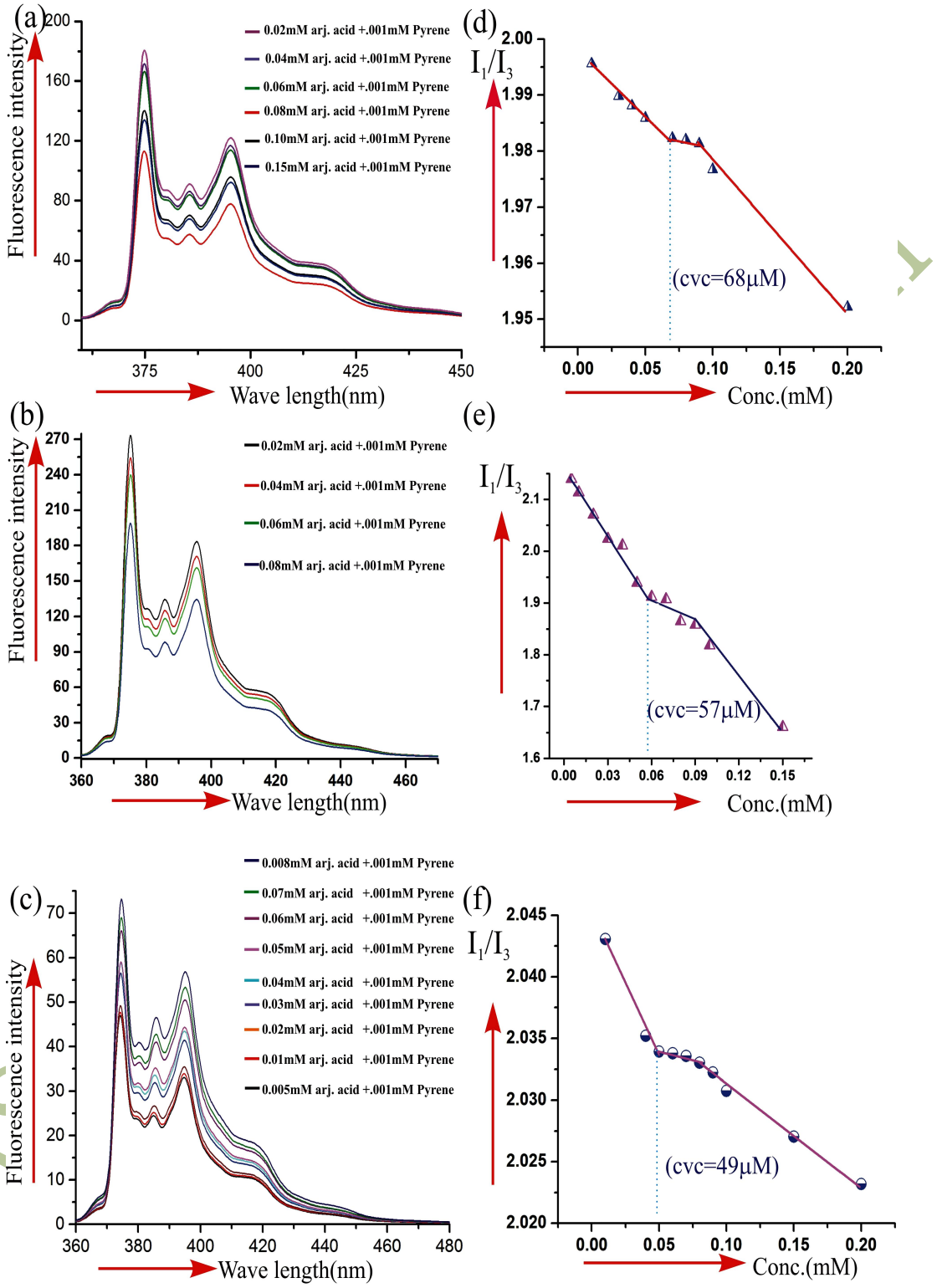


Figure 2: Fluorescence emission spectra of pyrene monomer ($\lambda_{max(ex.)}$ 340 nm) with different arjunolic acid concentration in (a) 2:1 DMSO- Water (b) 1:1 DMSO- Water,(c) 1:4 DMSO- Water and Variation of the I_1/I_3 ratio as a function of the concentration for the arjunolic acid in (d)DMSO -water(2:1) and (e) DMSO –water(1:1), (f) DMSO –water(1:4) system at 25°C

Variations in the intensities of the various bands

were observed during change in concentration of arjunolic acid from 10 μM to 200 μM in DMSO-water (2:1) Fig.2a. The CVC determined by this method in DMSO-water binary liquid mixtures at 2:1, 1:1, and 1:4 v/v ratio were determined to be 68 μM, 57 μM and 49 μM respectively showing a lowering in the cvc's with increasing percentage of water (Figure 2d, e, f). All fluorescence spectra were measured using a Hitachi fluorometer (F-4200) at 25° C. A stock solution of the fluorescent probe (pyrene) at a concentration of 0.01mM in dicholomethane was prepared.To determine the cvc, a series of solutions were prepared at different concentration of arjunolic acid from 0.01mM to 0.20mM and a small amount of the fluorescent probe was added so that the concentration of probe is fixed, 1μM for each case. The samples were incubated for 24 h in the dark at room temperature before measuring the fluorescence. The excitation wavelength was 340 nm. As the intensity of the peak III, I_3 shows maximum variations relative to the 0-0 band, thus the relative intensity of peak I to peak III, referred to hereafter as the I_1/I_3 ratio, will be used to explain the microenvironmental effects on fluorescence of pyrene monomer.The micropolarity of self-assembled structures of arjunolic acid could be conveniently studied using pyrene as the suitable fluorescent probe. A plot of I_1/I_3 with varying concentration of arjunolic acid is shown in Figure 2. Two break points are observed at 68 and 100 μM for CVC, respectively (Figure 2d). The second break in the I_1/I_3 ratio vs concentration plot suggesting the probe to be completely cordoned off from the bulk solvent by getting localized in the hydrophobic part of the vesicles. Before such a point is reached, the probe molecule is still exposed partially to the surrounding solvent.

4. Conclusion

This is the first report of determination of critical vesicular concentration of naturally occurring triterpenoids, arjunolic acid .The critical vesicular concentration of arjunolic acid in DMSO -water medium with different ratio of DMSO to water was successfully determined using pyrene as a effective fluorescence probe. From these consecutive experiments we observed that the critical vesicular concentration of arjunolic acid was decresed with increasing percentage of water in DMSO-water mixture.

5. Acknowledgement

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