Prayogik Rasayan

Determination of critical vesicular concentration (cvc) of nano-sized oleanolic acid by using hydrophobic pyerene as a fluorescence probe

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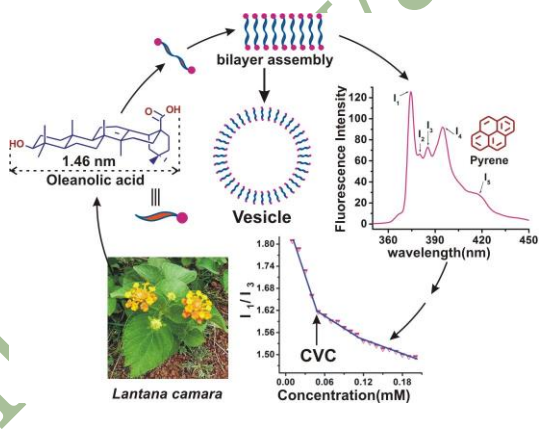
Previously we have reported the spontaneous self-assembly of renewable nano-sized oleanolic acid in aqueous-organic binary solvent mixtures to form vesicles (B. G. Bag and K. Paul, *Asian J. Org. Chem.*, 2012, 1, 150 – 154). Herein, we report the critical vesicular concentration (cvc) of oleanolic acid in DMSO-Water at varied ratio. The cvc's determined by fluorescence spectrophotometry using hydrophobic pyrene as a fluorescence probe in 2:1, 1:1 and 1:4 DMSO-water mixture were 83, 73, 47 μm respectively. The cvc values were comparable with the corresponding data of arjunolic acid.



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1. Introduction

Study of the self-assembly of natural and synthetic compounds in aqueous and non-aqueous media leading to supramolecular architectures of nano to micrometer dimensions has become an area of immense research interest during recent years.[1,2,3] Such investigations have not only helped in improving the knowledge of the structure-property relationships but also have found the use of such self-assemblies in drug delivery, cosmetics, nano-biodiagnostics, etc. [4,5,6,7] There are several reports in the literature on the vesicular self-assembly based on synthetic compounds,[8,9,10,11] But examples of the formation of vesicles from nontoxic, non-hazaradous, biocompatiable natural products are not very common. Considering the significance of the renewable nature phytochemicals, scientists have taken it as a challenge to study the self-assembly properties of different plant metabolites.[12,13,14,15,16] Terpenoids, the major plant secondary metabolites, having nanometric lengths, lypophylic backbone and several hydroxyl and or carboxyl groups in common have made them an attractive choice for the study of their self-assembly properties.[12,13]

Oleanolic acid (OA **1**, $C_{30}H_{48}O_3$) a monohydroxy triterpenoid, is present in free form in different parts of plants and anticancer, antitumor, and antiwrinkle activitiy of OA has also been demonstrated.[17,18,19] Recently we have reported the spontaneous self-assembly of OA in binary liquid mixtures yielding vesicles. The vesicular self-assemblies

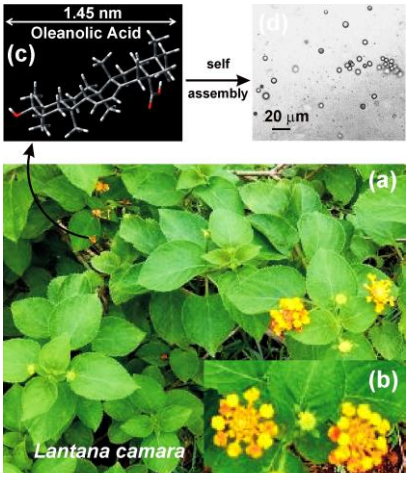

Figure 1: (a,b) *Lanata camara* leaves with flower, (c) Energy minimized DFT structure of Oleanolic acid (d) OPM micrograph of Self-aasembled Vesicles of oleanolic acid (0.2 mM) in 1:4 DMSO-water

could entrap various fluorophores including the anticancer drug doxorubicin. Realizing the significance of the self-assembly of OA in binary liquid mixtures in biology and medicine, it occurred to us that the self-assembly of OA can be investigated in DMSO-water with increasing ratio of water and study its critical vesicular concentration (cvc). previously

by us.[20] cvc has been studied by using fluorescence spectrophotometric method.[21,22,23,24,25,26] The solvent dependent vibronic band intensities (I-V) of pyrene more specifically the ratio of intensity of peak I to peak III (I_1/I_3) played crucial role to determine the cvc of oleanolic acid in DMSO-water binary liquid mixture at varied ratio.

2. Experimental

2.1 Materials and method

Dichloromethane (DCM), DMSO were purchased from SRL and pyrene was purchased from MERCK. DMSO was dry distilled by following the standard method before use.[27] Optical microscopy was carried out in native state taking colloidal self-assembled solution of oleanolic acid (0.2 mM) in DMSO-water (1:4) using Nikon Eclipse LV100POL instrument. Fluorescence spectrophotometry study was carried out in HITACHI F-4200 instrument.

2.2 Preparation of pyrene solution

Weighed amount of pyrene (1 mg) was taken in a vial and dissolved in 1 mL DCM to obtain a clear solution (4.9 mM). From this 4.9 mM pyrene solution 20 mL was taken in another vial and volume of the solution was made upto 1 mL with DCM to obtain 0.1 mM pyrene solution. Aliquots of 20 mL from 0.1 mM pyrene solution were taken in each of the twenty different clean and dry vials. Then solvent was evaporated so that each vial contain fixed amount of pyrene (0.4 mg)

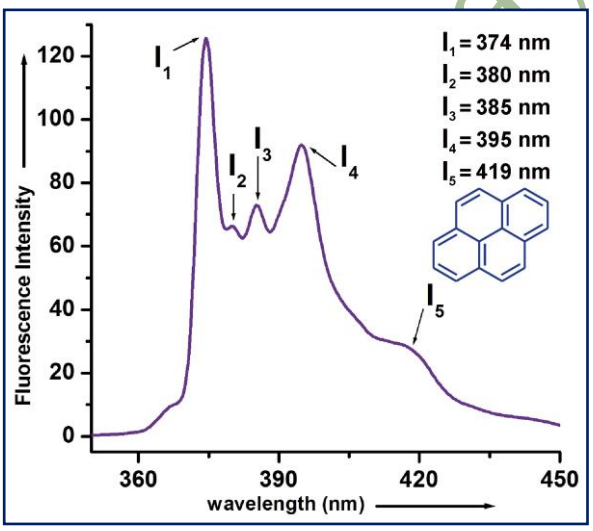

Figure 2: Fluorescence emission spectrum of 1μm Pyrene in DMSO-water (1:4)

2.3 Preparation of Oleanolic acid solution

Weighed amount of Oleanolic acid (2 mg) was taken in a vial and dissolved in 2 mL DMSO to obtain a clear solution (2.189 mM).

2.4 Fluorescence Probe Studies

To determine the cvc of **OA**, a series of twenty solutions were prepared at different concentration of Oleanolic acid from 0.01 mM to 0.20 mM .The concentration of fluorescence probe was kept fixed at 1 mM for each case. For this purpose varied amount of previously prepared Oleanolic acid solution in DMSO (2.189 mM) was added to each vial containing fixed amount of pyrene(0.4 μg). Then distilled water was added maintaining the ratio of DMSO: H2O at 2:1, 1:1, 1:4 respectively in total volume of 2 mL. Thus Oleanolic acid solution in DMSO: H2O of 0.01- 0.2 mM were prepared containing fixed concentration of pyrene (1mM). All of these samples were heated with magnetic stirring for 3-4 minutes and incubated for 24 h at room temperature before measuring the fluorescence. The fluorescence spectra were recorded using HITACHI FL- 4200 fluorescence spectrometer keeping (λ_{ex} = 337 nm, λ_{em} = 340 nm excitation band width = 2.5 nm, emission band width = 2.5 nm).

3. Results and Discussion

Pyrene, a hydrophobic aromatic probe and its water solubility is very less. Pyrene as a fluorescence probe have gained special consideration due to its fascinating photophysical properties, notably the long lifetime. Hydrophobic pyrene shows five (I-V) different vibrinic emission band intensities which are strongly sensitive to solvent micro-environment. The perturbation in the vibronic band intensities of pyrene monomer is dependent on the solvent dipole moment than the dielectric constant of bulk solvent; this is due to some specific solvent-solute or dipole-dipole interaction mechanism.[21] In the presence of vesicles and other macro molecular self-assembled systems, pyrene prefers to soluble in the inner lypophobic regions of these assemblies and hence it could be successfully employed as a fluorescence probe for studying vesicular and other membrane like self-assembled microstructures.[28] The change in intensities of the vibronic bands has been utilized to determine the cvc of **OA**. The ratio of the emission intensities of the first (I_1 = 374 nm) to third (I_3 = 385 nm) peaks were plotted against the varying concentration of OA and cvc was obtained from the inflection point (Fig. 2). Relative intensity of peak I to peak III, referred here as the I_1/I_3 ratio, explain the micro-environmental effects on fluorescence intensity of pyrene monomer. At lower concentrations, (I_1/I_3) represents the value characteristic of pyrene in aquous medium, and at higher concentrations it signifies the value of pyrene entirely in the lypophobic micro environment afforded by the vesicular membrane like structure.[28] Inflection points in I_1/I_3 vs. concentration plot were observed at 0.083, 0.073 and 0.047

mM corresponding to cvc of **1** in (1:0.5), (1:1) and (1:4) DMSO-water respectively. The obtained cvc vaues of monohydroxy oleanolic acid were compared with cvc values of arjunolic acid under identical experimental condition (Table 1). This reveals relatively lower cvc values for trihydroxy arjunolic acid[29] compared to monohydroxy oleanolic acid.

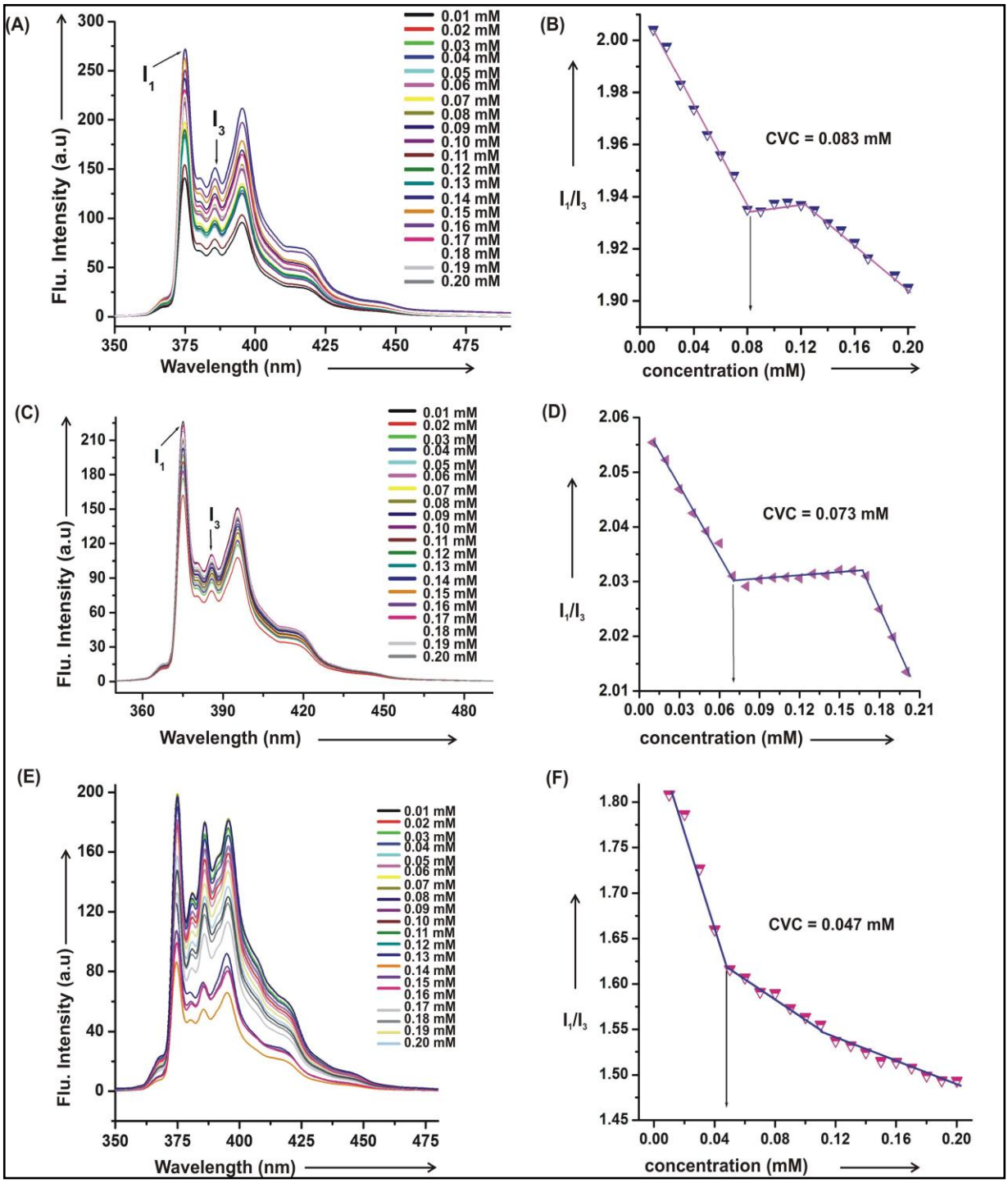

Figure 3. (A,C,E) Fluorescence emission spectra Of pyrene (B,D,F) I_1/I_3 vs. concentration plot oleanolic acid in DMSO -water (1:0.5, 1:1 and 1:4) system at 25°C respectively

Table 1: Critical vesicular concentration in varying ratio of DMSO-water

Solvent	Ratio	CVC[a] (μM)	CVC[b] (μM)
DMSO - water	2:1	83	68
DMSO - water	1:1	73	57
DMSO - water	1:4	47	49

[a] cvc of oleanolicacid; [b] cvc of arjunolic acid.

4. Conclusion:

In this paper we have reported the cvc of oleanolic acid in DMSO-Water solvent mixture in different ratio using pyrene as a fluorescence probe. The obtained value of cvc values were 83, 73 and 47 μM in 2:1, 1:1 and 1:4 DMSO-water respectively. These values were comparable to 68, 57 and 49 μM respectively obtained for arjunolic acid under identical conditions. In both the triterpeoids, decrease in cvc values were observed with increase in the percentage of water in the binary liquid mixture.

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