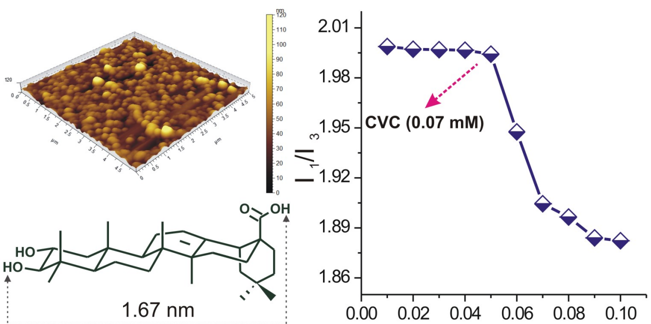
Prayogik Rasayan

Critical vesicular concentration of maslinic acid in aqueous binary liquid mixture by using hydrophobic pyrene as a fluorescent probe

Sk Nurul Hasan and Braja Gopal Bag*

Department of Chemistry and Chemical Technology, Vidyasagar University, Midnapore 721102, West Bengal, INDIA,
Email: brajagb@gmail.com

Received- March 10, 2019 | Accepted: March 21, 2019 | Published online – March 23, 2019



Maslinic acid, a naturally occurring 6-6-6-6-6 dihydroxy triterpenic acid is extractable from the fruits of olive *(Olea europaea)* as a white crystalline solid. This molecule can undergo spontaneous self-assembly in different aqueous-organic binary liquid mixures to yielding vesicles of nano to micrometer dimensions (B.G. Bag, S.N. Hasan, S. Ghorai, S.K. Panja, *ACS Omega*, DOI: 10.1021/acsomega.8b03667*)*. Herein, we report our detailed investigation of the critical vesicular concentration (CVC) of self-assembled vesicles obtained from maslinic acid in different aqueous-organic binary liquid mixtures by using pyrene as a fluorescent probe. We choose two binary liquids mixture such as DMF-water, DMSO-water and also varying the solvent ratio of DMSO-water. The CVC value of DMF-water is 90 μm and for DMSO-water at 2:1 v/v, 1:1 v/v, 1:1.5 v/v and 1:4 v/v are 70 μm, 58 μm, 60 μm and 50 μm respectively.

Keywords: CVC, maslinic acid, self-assembly, vesicle, fluorescence, pyrene.

1. Introduction

Self-assembly, a process by which the individual components spontaneously assemble to form an ordered structure in different liquids without external direction has been in the forefront of research during the last decade.[1,2,3,4,5,6] Self-assembly studies of amphiphilic molecules such as fatty acids[7], amino acids[8], steroids[9], sophorolipids[10] etc. have been reported in aqueous as well as aqueous-organic binary liquids in recent years. However, self-assembly studies of naturally occurring nano sized triterpenoids based amphiphilic molecules gained renewed interest because of its renewable nature in contrast to the petroleum based chemicals that are depleting fast.[11,12,13] Triterpenoids are the special class of amphiphilic molecules with the C30 subset are the major plant secondary metabolite having varied rigid and flexible lengths with different functional groups at different dispositions. These molecules can undergo spontaneous self-assembly in different aqueous and aqueous-organic binary liquids yielding supramolecular architectures such as fiber, vesicle, tube, ribbons, helix and flower etc. Among these architectures, vesicles gain enormous interest due to its membrane structure. There are many methods of describing stability of membranes, from permeability to encapsulation ability. One of the most common method of assessing the likelihood of membrane formation is critical vesicle concentration (CVC) i.e the minimum concentration of amphiphilic molecules is necessary to form vesicles[14]. Thus CVC is an important parameter to gain the better knowledge about the formation of vesicles and have enormous importance in various applicable fields of vesicle such as drug delivery carrier, gene therapy, magnetic resonance imaging, *etc.*

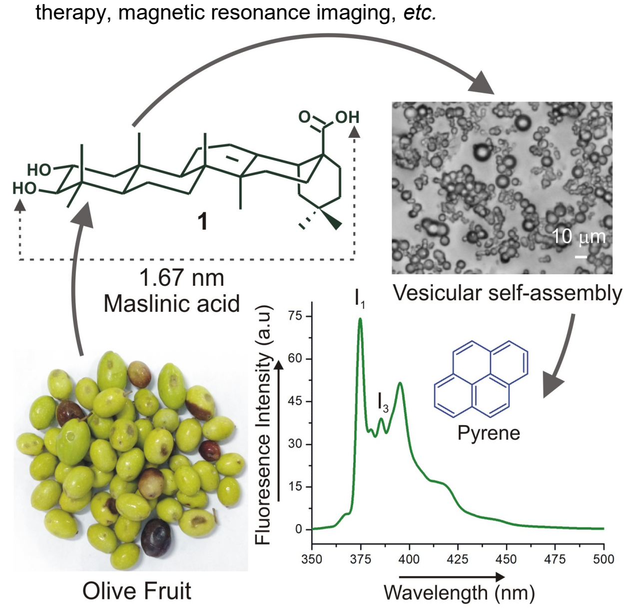


Figure 1: Schematic representation of vesicular morphology of Maslinic acid obtained from olive fruit and its critical vesicular concentration.

Maslinic acid **1**, a naturally occurring amphiphilic molecule having two hydroxyl and one carboxyl group at opposite ends of the rigid 6-6-6-6-6 pentacyclic triterpenoid backbone obtained from the fruit of olives (*Olea europaea*). This molecule can undergo spontaneous self-assemblies in different aqueous-organic binary liquids yielding vesicles (Figure 1 and 2).[15] In this manuscript, we report our detailed investigation on determination of the minimum concentration of maslinic acid necessary to form vesicles i.e critical vesicular concentration (CVC) of self-assembled vesicles obtained from maslinic acid in different aqueous-organic binary liquids by using pyrene as a fluorescent probe. According to our knowledge, this is the first report on determination of critical vesicular concentration (CVC) of maslinic acid in aqueous-organic binary liquids. Here we choose two binany liquids mixture such as DMF-water, DMSO-water and also varying the solvent ratio of DMSO-water. The CVC value of DMF-water is 90 μm and for DMSO-water at 2:1 v/v, 1:1 v/v, 1:1.5 v/v and 1:4 v/v are 70 μm, 58 μm, 60 μm and 50 μm respectively.

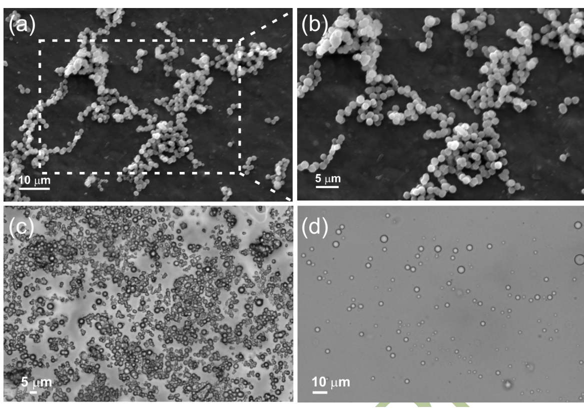


Figure 2: (a,b) Sem images of **1** prepared from the colloidal suspension of **1** in DMF-water (13.9 mM, 2:1 v/v); Optical micrograph of **1** (c) in THF-water (26.5 mM, 1:1 v/v), (d) in DMSO-water (28.2 mM, 2:3 v/v).

2. Experimental

2.1 Materials and Method

DMSO, dichloromethane were purchased from SRL and pyrene was purchased from MERCK. DMSO, was dry distilled by following the standard procedure[16] prior to use. Optical microscopy was performed using Nikon Eclipse LV100POL instrument. Fluorescence study was carried out in HITACHI F-4200 instrument.

2.2 Preparation of Pyrene solution

Pyrene (1.2 mg) was weighed in a vial and dissolved in dichloromethane (1 mL) to obtain a clear solution (6.42 mM). An aliquot of 15.5 μL (6.42 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).

2.3 Preparation of Maslinic acid solution

Maslinic acid (1 mg) was taken in a vial and dissolved in DMSO (1 mL) to obtain a clear solution (2.1 mM).

2.4 Fluorescence Probe Studies

To determine the cvc, a series of solutions were prepared at different concentration of maslinic 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 maslinic acid solution in DMSO (2.1 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 v/v, 1:1 v/v, 1:4 v/v respectively in total volume of 2 mL. Thus maslinic 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.

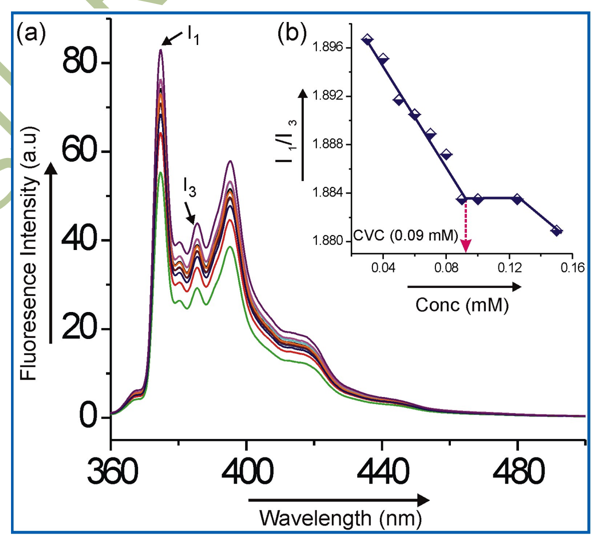


Figure 3: Determination of critical vesicular concentration of Maslinic acid in DMF-water system (2:1 v/v).

3. Results and Discussion

To investigate the critical vesicular concentration of maslinic acid, fluorescence probe analysis is becoming an important area in biophysical studies of multimolecular assemblies such as micelles and vesicles[17]. Pyrene is one of the few condensed aromatic hydrocarbons which show significant fine structure (vibronic bands) in its monomer fluorescence spectra in solution phase. Studies with pyrene as a fluorescence probe have received special consideration. Due to presence of such several interesting photophysical properties which make it suitable for use as an effective fluorescence probe. The five predominant peaks are numbered as I_1 to I_5 and variations in the intensities of the

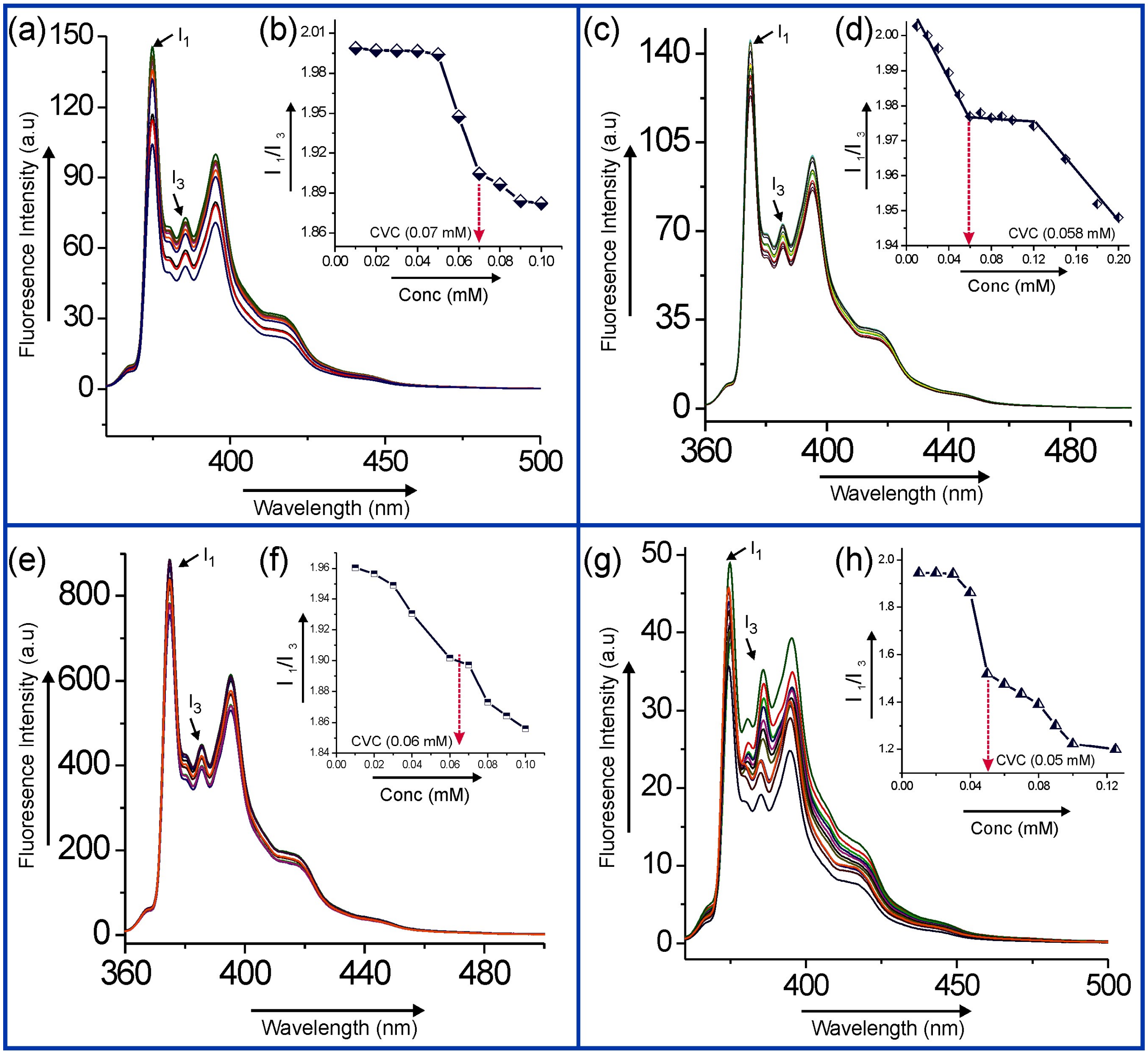


Figure 4: Determination of critical vesicular concentration of Maslinic acid in DMSO-water system at different solvent ratio (a, b) 2:1 v/v, (c, d) 1:1 v/v, (e, f) 1:1.15 v/v, (g, h) 1:4 v/v.

various bands are observed during change of concentration of maslinic acid from 0.01 mM to 0.20mM.

All fluorescence spectra were measured using a Hitachi fluorometer (F-4200) at 25° C. Here we prepared two binany liquids mixture such as DMF-water, DMSO-water and also varying the solvent ratio of DMSO-water. To determine the cvc, a series of solutions were prepared at different concentration of maslinic acid from 0.01mM to 0.20 mM and small amount of the fluorescent probe was added so that the concentration of probe is fixed at 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 I_3 shows maximum variations relative to the 0-0 band, thus the relative intensity of peak I_1 and I_3, referred as the I_1/I_3 ratio, will be used to explain the micro environmental effects on fluorescence of pyrene monomer. The micropolarity of self-assembled structures of Maslinic acid could be conveniently studied using pyrene as the suitable fluorescent probe. A plot of I_1/I_3 with varying concentration of maslinic acid is shown in Figure 1. The break point obtained from this plot called critical vesicular concentration. The CVC value of DMF-water is 90 μm (figure 3) and for DMSO-water at 2:1 v/v, 1:1 v/v, 1:1.5 v/v and 1:4 v/v are 70 μm, 58 μm, 60 μm and 50 μm respectively (Figure 4). This decreasing order is quite comparable with other two amphiphilic molecules such as arjunolic acid[18](having two secondary, one primary hydroxyl group and one carboxyl group at opposite ends of the rigid 6-6-6-6-6 pentacyclic triterpenoid backbone) and oleanolic acid[19] (having one primary hydroxyl group and one carboxyl group at opposite ends of the rigid 6-6-6-6-6 pentacyclic triterpenoid backbone) in DMSO-water binary liquids mixture.

4. Conclusion

This is the first report of determination of critical vesicular concentration of naturally ocuring triterpenoids, maslinic acid .The critical vesicular concentration of maslinic acid in DMF-water, DMSO-water medium and also vary the ratio of DMSO-water was successfully determined using pyrene as an effective fluorescence probe. From these consecutive experiments we observed that the critical vesicular concentration of maslinic acid is decreases with increasing the concentration of water in DMSO-water binary liquids mixture.

5. Acknowledgement

BGB thanks Science and Engineering Research Board (SERB), India (ref. EMR/2016/001123), India Srilanka project (DST/INT/SL/P25/2016) and Vidyasagar University for financial support and infrastructural facilities. SNH thanks UGC to provide research fellowships.

6. References

1 C. A. E. Hauser, S. Zhang, *Nature* **2010**, 486, 516-517.
2 S. Bhattacharya, S.K. Samanta, *Chem. Rev.* **2016**, 116, 11967-12028.
3 N. Amdursky, M. Molotskii, E. Gazit, G. Rosenman, *J. Am. Chem. Soc.* **2010**, 132, 15632-15636.
4 E. Busseron, Y. Ruff, E. Moulin, N. Giuseppone, *Nanoscale* **2013**, 5, 7098-7140.
5 C. Rest, R. Kandanelli, G. Fernández, *Chem. Soc. Rev.* **2015**, 44, 2543-2572.
6 H. Y. Hsueh, C. T. Yao, R. M. Ho, *Chem. Soc. Rev.* **2015**, 44, 1974-2018.
7 M. Delample, F. Jerome, J. Barrault, J.P. Douliez, *Green Chem.*, **2011**, 13, 64-68
8 D. Das, T. Kar, P. K. Das, *Soft Matter*, **2012**, 8, 2348-2365.
9 E. Carretti, M. Bonini, L. Dei, B.H. Berrie, L.V. Angelova, P. Baglioni, R.G.Weiss, *Acc. Chem. Res.*, **2010**, 43, 751-760.
10 N. Baccile,N. Nassif, L.Malfatti, I.N.A. VanBogaert, W.Soetaert, G. P. Arnaudet, F. Babonneau, *Green Chem.*, **2010**, 12, 1564-1567.
11 B. G. Bag, R. Majumdar, *Chem. Rec.*, **2017**, 17, 841-873.
12 P. K. Vemula , G. John, *Acc. Chem. Res.*, **2008**, 41, 769.
13 G. John, B.V. Shankar, S.R. Jadhav, P.K.Vemula, *Langmuir*, **2010**, 26,17843.
14 A. M. E.Sayed, A. Sherbini, H. Mahmoud, A. Kader, R.Y. Hamzah, *Colloids and Surfaces A*: *Physicochem. Eng. Aspects*, **2001**, 194, 133-142.
15 B.G. Bag, Sk N. Hasan, S. Ghorai, S.K. Panja, *ACS Omega*, **2019**, DOI: DOI: 10.1021/acsomega.8b03667
16 Purification of Laboratory Chemicals, W. L. F. Armarego and D. D. Perrin, Read *Educational and Professional Publishing Ltd*, **1996**, 4 th Ed.
17 K. Kalyanasundaram , J. K. Thomas, *J. Am. Chem. Soc.*, **1977**, 7, 2039-2044.
18 S. Ghorai, K. D. Wijesekera, M. T. Napagoda, B. G. Bag, *Prayog. Ras.* **2018**, *2(1),* 24-27.
19 S. K. Panja, K. D. Wijesekera, M. T. Napagoda, B. G. Bag, *Prayog. Ras.* **2018**, *2(2),* 5-8.