

Phase evolution, structural characteristics and mechanism of vesicle formation from a synthetic amphiphile: Controlled morphology by tuning solution phase parameters

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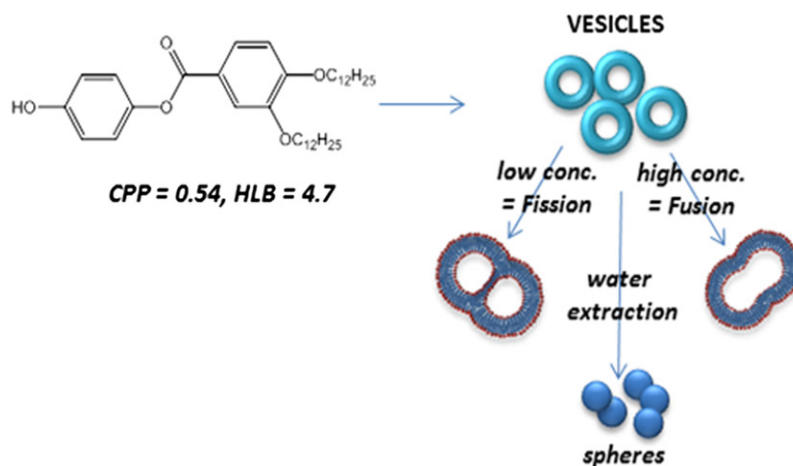
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Abstract

We report herein, the aggregation behavior of 3, 4-di(dodecyloxy)benzoic acid-4-hydroxy phenyl ester (DDBE), a synthetic amphiphile and a true non-ionic surfactant system as per the geometrical considerations. The true surfactant nature of the system stems from its hydrophilic-lipophilic-balance (HLB=4.7), comparable to that of Span-60, also a true non-ionic surfactant. This compound undergoes spontaneous vesicle formation in THF:water binary solvent mixtures which further underwent fission at lower DDBE concentrations and fusion at higher concentrations, leading to giant vesicles of the order of 3000 nm. These vesicles are sensitive to the polarity of their environment. The predominant mode of interaction as observed from the molecular dynamics simulations were found to be π - π stacking with the phenyl rings of the molecule. Further, the system, upon complete extraction into water, formed spherical aggregates of size 50 nm based on the good solvent-poor solvent combination as the necessary condition for the vesicle formation.

Graphical abstract



Keywords: Bilayer vesicle, non-ionic surfactant, binary solvent mixtures, molecular dynamics simulations, unilamellar vesicle, vesicle fission and fusion

1. Introduction

A careful interplay of non-covalent interactions in molecules such as the hydrophobic, hydrogen bonding, electrostatic, dipole-dipole, magnetic, and van der Waals interactions have led to a wide variety of supramolecular aggregates with myriad morphologies such as microemulsions, lamellae, vesicles, rods and spheres.^[1-3] This implies that the shape-complementarity with the external forces together chisel the

type of structure that molds into a nanoscale architecture. The past two decades have seen such systems containing block copolymers with potential applications in molecular electronics,^[4,5] cosmetics, drug delivery, sensors, and so on.^[2,6,7] A variety of small amphiphilic molecules have also been employed in forming aggregates of various shapes.^[8,9] In particular, since their first visualization,^[10] vesicles have been the subject of interest due to their capacity of biomimicking and hence serve as an efficient means to engineer

nano-sized functional delivery systems. By definition, “vesicles are dynamic supramolecular structures composed of amphiphilic molecules that form bilayers enclosing a small aqueous compartment”.^[8] Thus the vesicles are made up of bilayer membranes which in turn comprise the amphiphilic molecules that aggregate as a result of a balance in the forces due to their lipophilic and hydrophilic moieties. These forces are concentration and temperature dependent and hence vesicular morphologies can be tuned to the user’s needs. Theoretical studies pioneered by Israelachvili et al.^[11] and Hyde^[12] have demonstrated a fundamental correlation between the geometry of amphiphilic molecules and the shape of their self-organized structures. It was shown that controlling the shape of individual amphiphilic molecules by tuning the size of the hydrophobic part relative to the hydrophilic part allows a variation of the topology of the self-assembled structures from spherical to rod-like micelles, and finally to bilayered vesicles. The spontaneous curvature model introduced by Helfrich^[13] correlates the mechanics of mono- and bilayer thin films with the shapes of aggregate structures and has been successfully employed for example, to rationalize the spherical shape of bacteriorhodopsin vesicles.

The vesicles are not only formed by the classical phospholipids: the liposomes, but also by a wide variety of synthetic amphiphiles. In the recent past, synthetic or artificial vesicles have gained a large importance owing to the ease with which its building blocks can be designed. Further, they serve as excellent biomembrane mimics in terms of delivery vehicles for drugs and genetic matter thus finding use in pharmacology, medicine, genetic engineering, cosmetic industry and also as a support for semi-conductor materials for solar cell applications.^[14] In particular, the advent of vesicle preparation from synthetic surfactants “Ufasomes”, which later were termed “Niosomes” due to the preparation of vesicles from non-ionic surfactant molecules.^[15,16] Niosomes can be defined as the class of vesicular nanocarriers^[17,18] where, “the non-ionic surfactant vesicles obtained on the hydration of synthetic non-ionic surfactants, with or without the incorporation of cholesterol or their lipids”.^[19] The niosomes have an edge over the liposomes due to their chemical stability, easiness of surface modification, low toxicity, and compatibility with biological systems, biodegradability, and entrapment of both hydrophilic and lipophilic moieties in their compartment based on the surfactant from which they are prepared.^[20] Furthermore, non-ionic surfactants exhibit high interfacial stability and are very stable towards strong electrolytes, acids and bases.^[21] For example Span 60 surfactant vesicles have been used entrapment of Cytarabine hydrochloride, a drug used in the treatment of Lukemia with 80% entrapment efficiency.^[22] In another study, Brij-35 and Brij-58 based niosomes were prepared with the encapsulation of insulin which revealed a sustained release of the drug.^[23] Insulin encapsulation studies were also done on Span 40 and Span 60 niosomes which revealed insulin protection against proteolytic enzymes.^[24] Comprehensive reports on drug delivery studies of various niosomes formed from non-ionic surfactants can be found in literature.^[20, 25–27] On the other hand, a mixture of non-ionic surfactants can give rise to varying properties leading

to formulations of water-in-oil emulsions.^[28] Almost all the non-ionic surfactants contain a hydrophobic alkyl chain and hydrophilic groups containing alcohol, phenoxy, ester, ether, amide or sugar-based head groups.^[28] Nevertheless, in this direction, very few reports on the design, synthesis and physicochemical characterization of an amphiphile can be seen, while all the above systems have been studied on commercially available surfactants which have been modified to the user’s needs^[25–27,29] and hence we were motivated to design and study such a system for pharmacological applications.

In this article we have designed a new molecule, 3, 4-di(dodecyloxy)benzoic acid-4-hydroxy phenyl ester (DDBE) speculating on the properties expected for a non-ionic surfactant which we expect to find application in drug-carrier niosomes and/or emulsion formulations. We report the aggregation behaviour of DDBE, which in terms of geometric considerations, can be classified as a non-ionic amphiphilic molecule. The prime objective of this study is to optimize the experimental conditions and to investigate the formation and mechanism of geometrically defined self-organized aggregates from the amphiphilic DDBE in binary solvent mixture. The aggregates thus formed were investigated through absorption spectroscopy, light scattering and transmission electron microscopic techniques.

2. Materials and methods

The DDBE was synthesized according to the previously reported procedure.^[30] All the other chemicals used were of analytical grade and were used without further purification.

2.1. Preparation of the solutions

DDBE was dissolved in THF to make a 5 mM stock solution. To this a required quantity of water was added in various volume fractions to obtain three concentrations of 1, 0.1 and 0.01 mM. Furthermore, a series of solutions of DDBE in THF:water mixture were prepared with increasing water content keeping the concentration of DDBE constant.

2.2. Instrumentation

UV-Visible absorption spectra were recorded on a Varian Cary 5E double-beam spectrophotometer using a 10 mm path length Infrasil Quartz Cuvette.

Dynamic Light Scattering (DLS) measurements were performed on 90 Plus/BI-MAS (Brookhaven Instruments Corporation) equipped with diode laser operating at a power of 15 mW and at 659 nm wavelength. All the measurements were done at a scattering angle of 90° in a special dust-free light-scattering cell at 25 °C.

Transmission electron microscopic (TEM) investigations were performed using a Philips TEM instrument (CM12) equipped with a field emission gun operated at 120 kV. Microfilms for TEM studies were prepared by placing a drop of the solution on a carbon-coated copper grid and drying the solvent by evaporation at ambient temperature.

Surface tension measurements were carried out by the Wilhelmy plate method with freshly prepared test solutions at 25 ± 0.1 °C.

The **turbidity** of the solutions was measured at room temperature with a HACH 2100P Turbidimeter.

High Resolution Scanning Electron Microscopic (HRSEM) images were acquired on a FEI Quanta 200 instrument.

Molecular Dynamics Simulations were performed using Hyperchem Professional^[31] program package with geometry optimized structures using the Gaussian' 09 program^[32] at B3LYP/6-31G* level. Molecular mechanics with MM + force field was used to perform dynamics in the NVE ensemble at constant temperature in vacuo. The intermolecular potential functions comprised of short range van der Waals interactions, dispersion interactions and long range charge-charge electrostatic interactions. Simulations were carried out over 5 ps with a time step of 1 fs in order to explore the possible existing molecular interactions associated with the DDBE bilayers.

3. Results and discussion

3.1. Molecular design

It has been well understood that non-ionic surfactants/amphiphiles are generally resistant to acids, bases and electrolytes. Thus, the vesicles formed from such systems have shown good stability and low toxicity compared to their anionic/cationic/amphoteric counterparts. They have been used in a variety of biological applications owing to their tendency to maintain neutral pH.^[20,36] Keeping the above points into concert, we went ahead in designing a non-ionic surfactant. While the single chain surfactants have the tendency to form micelles, the double chain surfactants predominantly aggregate into vesicles.^[33,34] Thus, we chose to include two alkyl chains into our molecule. On the other hand, a surfactant with lower HLB has the higher tendency to form vesicles over micelles. Since the -OH containing non-ionic surfactants are fairly resistant to their surroundings, we used -OH as the end group. When $n \geq 8$ for double chain surfactants, vesicles are the preferred mode of aggregation. Further, it has been seen in literature, when $n = 12$ vesicles are quite likely formed.^[9,33-35] Based on all the above into mind, we have designed the molecule as seen in Chart 1. According to the best of our knowledge, and as mentioned in the introduction, most of the synthesized non-ionic surfactants found in literature in the past two decades only have groups introduced into a surfactant which is made more hydrophilic.^[15,16,20,21,36] But in our case, we believe that designing and synthesis of a whole surfactant will open doors for specific fine-tuning of a molecule to cater to user's exact needs.

3.2. Molecular self-assembly: effect of solvent polarity on absorption characteristics

DDBE, as synthesized according to a previously reported procedure,^[30] was found to be insoluble in water due to the lack of a typical hydrophilic group in its structure but is

readily soluble in a huge range of organic solvents such as benzene, toluene, dichloromethane, carbon tetrachloride, chloroform, hexane, and tetrahydrofuran (THF).^[30] In the present study, the focus was laid on water soluble solvents owing to the fact that a possible biological application cannot be envisaged for solvents that are water immiscible. Furthermore, benzene although being water soluble was not considered here due to its carcinogenic nature thus leaving us with THF as a solvent of choice.

The UV-Visible absorption of DDBE in THF reveals three peaks at 235, 268 and 291 nm. These peaks over a concentration range of 0.001 mM to 5 mM showed Beer-Lambert's linearity and absence of solvatochromic shifts revealing the absence of aggregation in the single component solvent media. It has been well documented in literature on the conditions for vesicle formation:

1. a small head group size order of 6 Å will possibly lead to a vesicle formation when the condition in point 3 below is satisfied;^[34]
2. when head groups are small, the electrostatic repulsion between the bilayers is also low leading to low degree of curvature thereby forming vesicles;^[33,34]
3. when the ratio between the length of the polar group to the length of the alkyl chain is small, the vesicles are formed;^[9,34]
4. non-ionic double chain surfactants predominantly aggregate into vesicles. Thus for such systems critical aggregate concentration is evidenced over critical micelle concentration (CMC).^[9,21,33]

Thus, as predicted from the above factors, DDBE with a length of polar group 6.3 Å (Fig. S3) directly forms vesicles (*vide infra*) than assembling into micelles and hence CMC could not be determined. Moreover, as explained above, they do not show any aggregation tendency in single solvent. Thus, the complete study uses the mixture of solvents THF and water.

On the other hand, the cloud point, could not be determined for the molecule as it was completely insoluble in water. As will be seen in subsequent sections, the HLB value of DDBE was calculated to be 4.7 which is similar to the Span 60 whose cloud point is unknown due to its insolubility in water.^[37]

In contrast, DDBE exhibited a marked solvatochromism in THF:water binary solvent mixtures at room temperature as visualized in Figure 1a. DDBE spontaneously formed self-organized assemblies upon addition of water to THF at a fixed composition. With increasing addition of water, a turbid solution with bluish color developed, indicating the formation of aggregates (Figure S1). The morphology and structure of the formed DDBE aggregates were investigated through TEM and DLS measurements, as will be discussed in subsequent sections.

The absorption spectrum (Figure 1a) indicates a blue shift of the 235 nm absorption feature to 225 nm and the shoulder at 289 nm is red shifted to 304 nm with a decrease in intensity with increasing water percentages. The

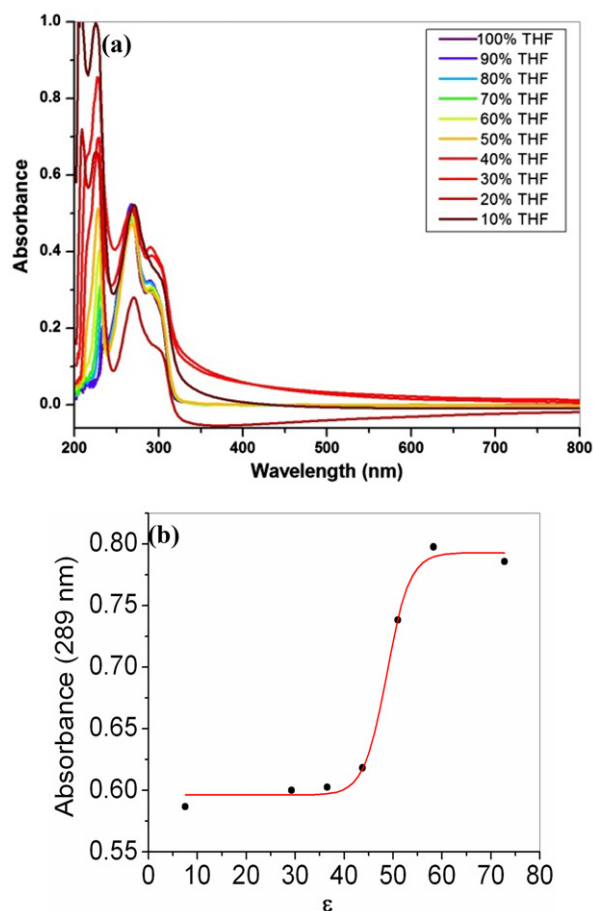


Figure 1. (a) UV-Visible absorption spectra and (b) absorbance versus ϵ_{mix} of 0.01 mM DDBE in THF: water mixture in the composition range 100 – 10% v/v.

benzoic acid precursor of the DDBE, the compound DDBA^[38] is reported to have absorption peaks at 268 and 295 nm. The inclusion of the hydroxy quinone group in the present compound DDBE, has resulted in the appearance of the peak at high energy region ~ 235 nm. The exciton theory predicts the decrease of absorption coefficient and a shift in the peak^[30,40] as aggregates are formed, which is very evident in this system that obviously undergoes aggregation with increasing polarity.

In order to explore the role of solvent polarity towards aggregation, a series of measurements of the dielectric constant, shift of the absorption peaks, turbidity and surface tension were done with increasing percentage of water in the binary solvent mixture. The dielectric constant for the binary solvent combinations, ϵ_{mix} was calculated according to the equation,^[41]

$$\epsilon_{\text{mix}} = f_A \epsilon_A + f_B \epsilon_B \quad [1]$$

where the suffixes A, B, and mix represent the solvents A, B, and mixed solvent, respectively, and f is the volume fraction of the solvent. The plots in the Figures 1b and S2 based on absorbance, surface tension and turbidity measurements unanimously indicate the critical dielectric constant to be ~ 45 which then corresponds to 40:60% v/v THF:water combination, that provides the necessary environment for the aggregation to occur.

3.2. Description of the amphiphile and molecular modeling

Molecules can undergo aggregation due to the interplay of varying parameters such as the hydrogen bonding, hydrophobic-hydrophobic van der Waals interactions, ionic interactions, π - π interactions. Not all the molecules that fall into the above category are amphiphilic. In order to classify DDBE into an amphiphile, it is important to look at its Hydrophilic-Lipophilic-Balance, HLB. The HLB is a measure of the hydrophilicity or the hydrophobicity of a surfactant amphiphile which is determined by calculating values for the different regions of a molecule as described by Griffin.^[42] The HLB value describes the partitioning behavior of a molecule between a polar and non-polar medium which is dependent upon the characteristics of the hydrophobic and hydrophilic groups present in it such as alkyl chain length, nature of head group viz., charge, polarity, pKa etc.

$$HLB = \frac{20M_H}{M_L + M_H} \quad [2]$$

where M_L and M_H are the molecular weights of the hydrophobic and hydrophilic moieties. An HLB value of zero corresponds to a completely hydrophobic molecule and a value of 20 corresponds to a completely hydrophilic molecule. In the present case, the HLB was calculated to be 4.7 which is equivalent to a non-ionic surfactant Span 60 (HLB = 4.7), indicating that DDBE can be considered a true non-ionic surfactant.

The energy and geometry optimization of DDBE was done on Gaussian 09^[32] using the density functional B3LYP/6-31G* (Figure S3). The results were analysed using Gaussview 05,^[43] and gave a dipole moment of 3.39 D.

The phenomenon of self-assembly of an amphiphile can be explained using the concept of molecular packing parameter called as the Israelachvili-Mitchell-Ninham's packing parameter or simply the critical packing parameter,^[44]

$$p = \frac{v_c}{a_o l_c} \quad [3]$$

where v_c is the volume of the hydrocarbon tail of the surfactant core or the hydrophobic volume, a_o is the hydrophilic head group area and l_c is the hydrophobic alkyl chain length or the critical chain length. The DDBE molecule with its dimensions as shown in Figure S3, has a $p \sim 0.54$ corresponding to a truncated cone shape structure and is expected to form flexible bilayers/vesicles^[44,45] and thus justifies the above explained tendency to aggregation as evidenced by the absorption spectroscopy.

The DDBE bilayers were simulated using the Hyperchem professional program package^[31] using the geometry optimized structure of DDBE using the Gaussian 09 program^[32] at B3LYP/6-31G* level. An MM+ force field was used to perform dynamics in the NVE ensemble at constant temperature in vacuo. Simulations were carried out for 1 ps on a 1:1 DDBE:DDBE bilayer arrangement, with a step time of 1 fs. As visualized in Figure 2a and Figure 2b, associative π - π interactions between the flat π -surfaces of DDBE's

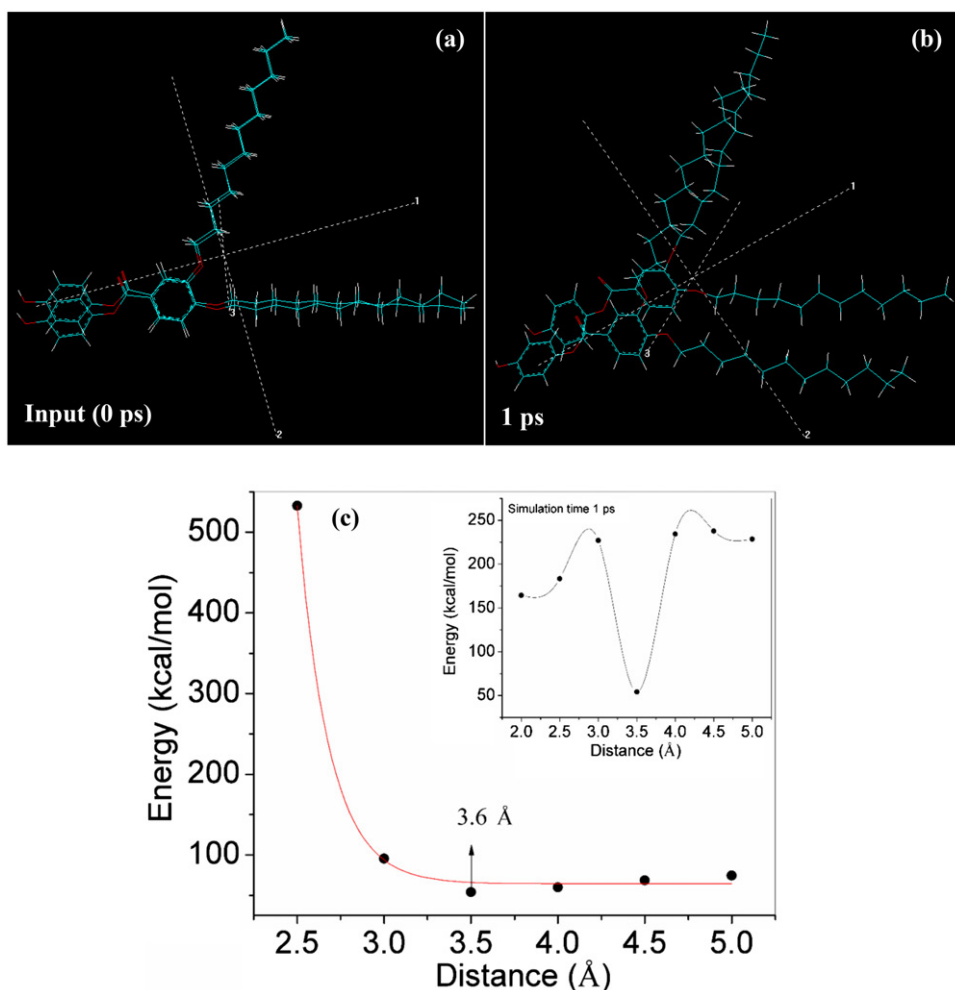


Figure 2. (a) and (b) Show the snapshots of typical interactions between phenyl rings of DDBE molecules at various simulation time intervals with a 1 fs time step. (c) Shows the total energy vs. distance between the planar 6-membered phenyl rings of DDBE.

phenyl ring were observed at different simulation times upon approach of the DDBE molecule towards one of the 6-membered rings of the other DDBE molecule. It has been reported by Hunter and Sanders^[46] that, when two aromatic molecules interact, the π -systems assume inter planar distances of 3.4 – 3.6 Å. Thus, in the present case, an equilibrium distance of 3.56 Å revealed a minimum energy configuration (Figure 2c), which complies with the above. Further, simulations done on increasing number of DDBE molecules further clarified the associative π - π interactions prevalent even on longer timescales (Figure S4). Thus π - π stacking of the DDBE molecules is the stable arrangement leading to the formation of bilayers and hence the driving force for self-assembly towards the formation of unilamellar vesicles.

3.3. Size and shape of the aggregates

Span 60, a true non-ionic surfactant forms vesicles without the addition of cholesterol and produces maltese crosses under polarized light.^[47] A similar observation was evidenced in our case, where DDBE produced maltese crosses (Figure 3) near its melting temperature revealing its inclination to form vesicles which further proves the system to be a true non-ionic surfactant. Further, the vesicles of Span 60

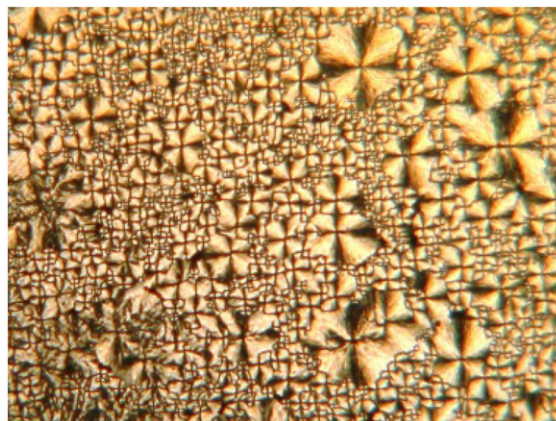


Figure 3. Formation of maltese cross for DDBE observed near its melting point under hot stage microscope fitted with polarized light.

are very stable.^[48] Further, the appearance of such structures under polarized light reveal the mesogenic character of the molecule.

As predicted from the critical packing parameter and the absorption measurements, the molecule forms aggregates and hence the dynamic light scattering (DLS) experiments were performed assuming the system to be spherical

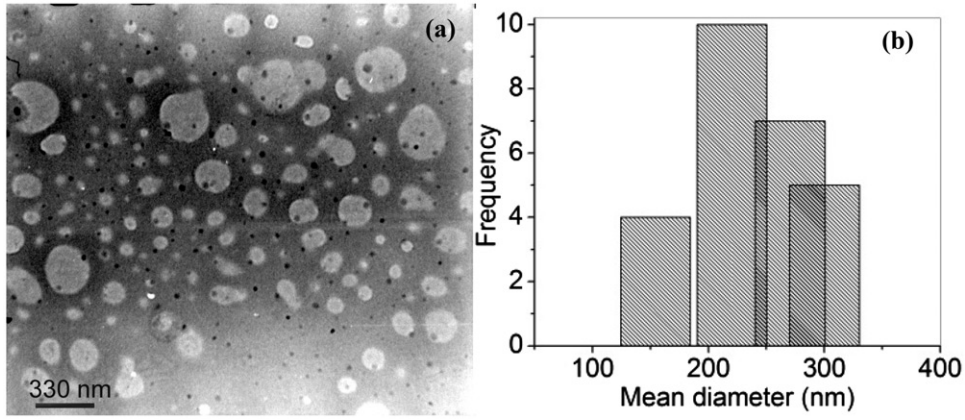


Figure 4. (a) Transmission electron micrographs of the microstructures and (b) histogram from TEM showing the size distribution of DDBE vesicles in 40:60 THF:water mixture [DDBE] = 0.01 mM.

aggregates. The auto correlation profiles of DDBE aggregates from DLS experiments revealed an exponential behavior at a scattering angle of 90° (Figure S5). The particle size distribution at 25°C revealed the mean diameter of the vesicles to be ~ 240 nm for 0.01 mM DDBE in 40:60% THF:water mixture which were corroborated by the transmission electron microscopic (TEM) experiments.

The TEM studies on the DDBE aggregates – a representative image and histogram can be seen in Figure 4a – revealed the formation of spherical vesicles with an average diameter of 240 nm for a 0.01 mM DDBE in 40:60% THF:water mixtures.

The experimental data from TEM and DLS thus evidence for the ternary system's reorganization into a bilayer vesicle with the hydrophobic moieties towards the interior, while the polar hydrophilic part of DDBE was exposed to the aqueous environment. The diffusion coefficient as calculated from the Stokes-Einstein equation^[41] was found to be $2 \times 10^{-6} \text{ cm}^2\text{s}^{-1}$ for 40:60 THF:water mixtures.

3.5. Description of vesicles

As can be seen from the above results, the vesicle formation occurs at a minimum of 40:60 THF:water solvent mixture. According to the CPP calculations, there is a clear evidence for the molecule's tendency to form vesicles owing to its truncated cone shaped structure. Considering the vesicle to have an outer dimensions where the geometric constraints arise with the outer radius R_o and thickness t_o , the minimal vesicle radius, R_{min} can be calculated from the following equation,^[45]

$$R_{min} = \frac{3 + \left[3 \left(\frac{4v_c}{a_0 l_c} - 1 \right) \right]^{1/2}}{\frac{6(1 - \frac{v_c}{a_0 l_c})}{l_c}} \quad [4]$$

Thus, the minimum vesicle size depends upon the packing parameter and the critical chain length. Using the above parameters, the R_{min} for the present system was theoretically

calculated to be 28 nm which is matching well with the value ~ 35 nm as inferred from the TEM images.

According to Antonietti and Förster,^[49] the two main contributions to the free energy of an amphiphile are the interfacial energy of the hydrophilic-hydrophobic interface and the loss of entropy when the flexible surfactants are forced to fit into the aggregate microdomains, thus forcing an amphiphile to undergo structure formation. In this regard, the geometrical shape plays a pivotal role in forcing a molecule to form a bilayer as has been evidenced from the above explanations. The vesicles are formed in a two-step process involving the formation of a bilayer which undergoes closure owing to the energy constraints. Bending of a bilayer thus requires a bending energy, $E_{bend} = 8\pi\kappa$ (when a spherical vesicle is formed from a bilayer bending), where κ is a bending constant given by the Helfrich equation,^[50, 51]

$$\kappa = \frac{k_B T}{16\pi} \left(\frac{R}{\sigma} \right) \quad [5]$$

where, R is the mean vesicle radius and σ is the standard deviation as obtained from the experiments. Using the above, the bending constant was found to be $99 k_B T$ for DDBE which is much higher than a typical value of $40 k_B T$ for block copolymer bilayers.^[51,52] Thus, when $\kappa \gg k_B T$, the vesicles are stabilized by a spontaneous curvature. Further, in accordance with the bending constant, a large bending energy of $\sim 2850 k_B T$ was estimated owing to the formation of equilibrium vesicles, which is independent of the vesicle radius thereby stabilizing it.

We have, in the present study, investigated the effect of concentration on the vesicle formation with increasing water volumes. It is known that at low concentrations, the repulsive forces between the bilayer membranes force their closure leading to the formation of vesicles.^[53]

For a concentration of 0.01 mM of DDBE, the vesicles are spontaneously formed at 60% water volume (Figure 5a). Thus the addition of water to the solvent THF is to induce the precipitation of the hydrophobic units of the amphiphile. This method has been well exploited in the case of block copolymers to induce aggregate/vesicular geometries.^[54] By

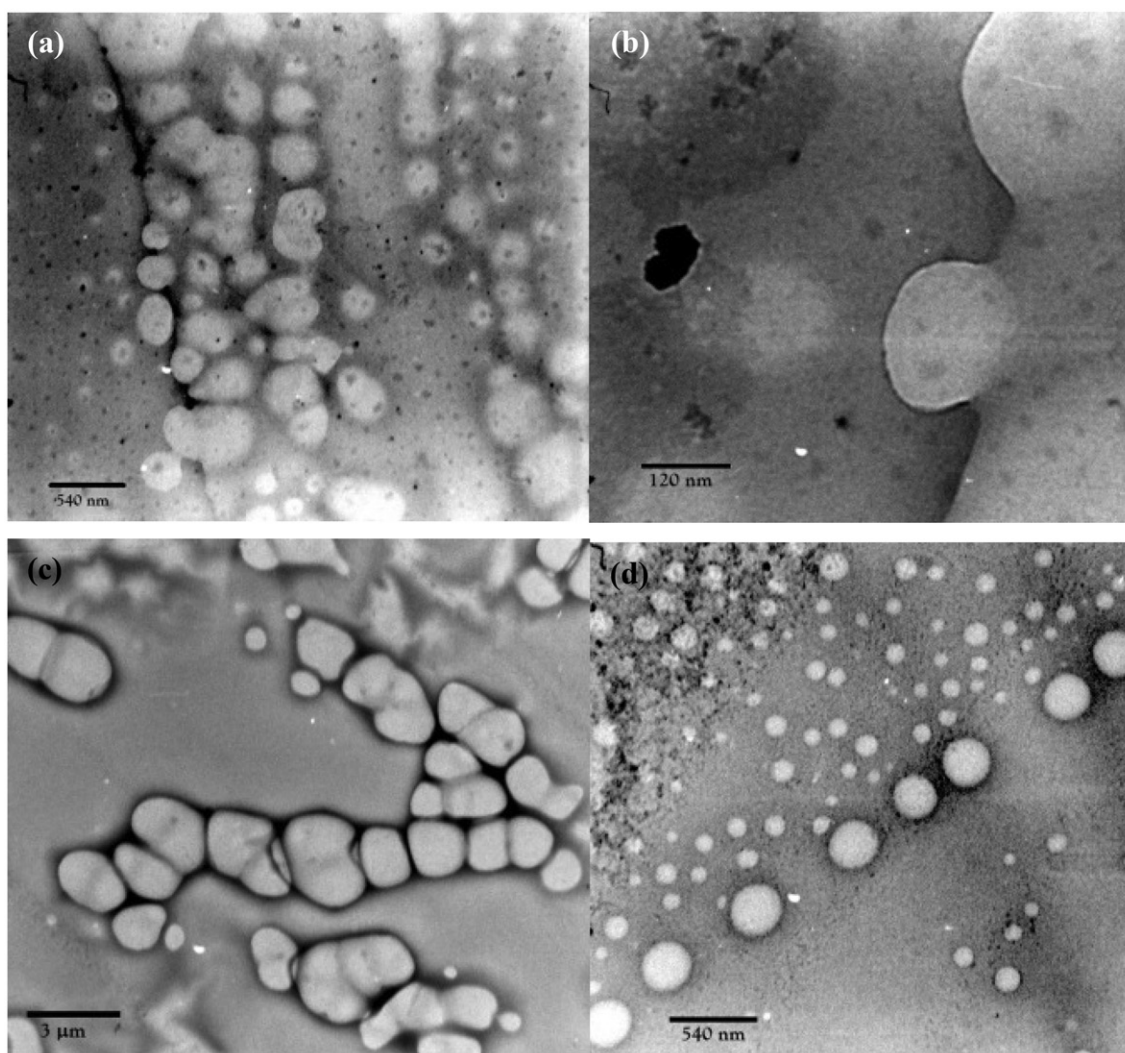


Figure 5. Transmission electron micrographs of the vesicle fission of DDBE (a & b) 40:60% THF:water showing the budding of vesicles (c & d) 50:50% THF:water showing the vesicles formed. Scale bars: (a & d) 540 nm, (b) 120 nm, (c) 3 μm .

increasing the water concentration to a 50:50 THF:water mixture the fission of vesicles is observed (Figure 5b to 5c). Completion of fission is indicated by a clear break from what is called a “pearls in a string” appearance as seen from Figure 5d leading to vesicles of decreased size (~ 200 nm). This can be understood from the fact that by increasing polarity, there is a driving force from the hydrophobic parts of the molecule to bud out into new vesicles from the defect rich regions of the aggregates. With increasing water content the size of the vesicles remained constant and the fission decreased. At this point we presume that the increasing polarity will stabilize the vesicle formed instead of a further vesicle budding.

On moving to a ten times higher concentration of 0.1 mM DDBE, the vesicles are still existent but did under-went fusion as can be evidenced in Figures 6a and 6b where a clear contact point leading to vesicle adhesion occurs which further coalesce to form the center wall. The system further undergoes the dissolution of the temporary central wall to form the new vesicle of ~ 475 nm diameter as seen in

Figure 6c. The vesicles did not undergo an increase in size with increasing water content. This can be attributed to the fact that increasing water content decreases the frequency of water collisions and decrease the alkyl chain mobility.^[54] A similar observation where the vesicles had a thermodynamic control was observed by Luo and Eisenberg,^[55] on the poly(styrene)-*b*-poly(acrylic acid) (PS-*b*-PAA) diblock copolymer vesicles which underwent fission and fusion, depending on the solvent polarity and polymer concentration.

On further increasing the concentration to 1 mM, as evidenced by Figure 7, there is an increase in fusion with vesicle sizes reaching an average of 650 nm. Such a phenomenon was also reported by Choucair et al.^[56] on poly(styrene)-*b*-poly(acrylic acid) where an increasing concentration of the solute species induces fusion in such binary solvent mixtures. The system is still under the regime of large unilamellar vesicles (LUV) which may further undergo fusion with increasing concentration leading to the formation of giant vesicles which has indeed been observed by us, where the sizes averaged to 3000 nm (Figure 8).

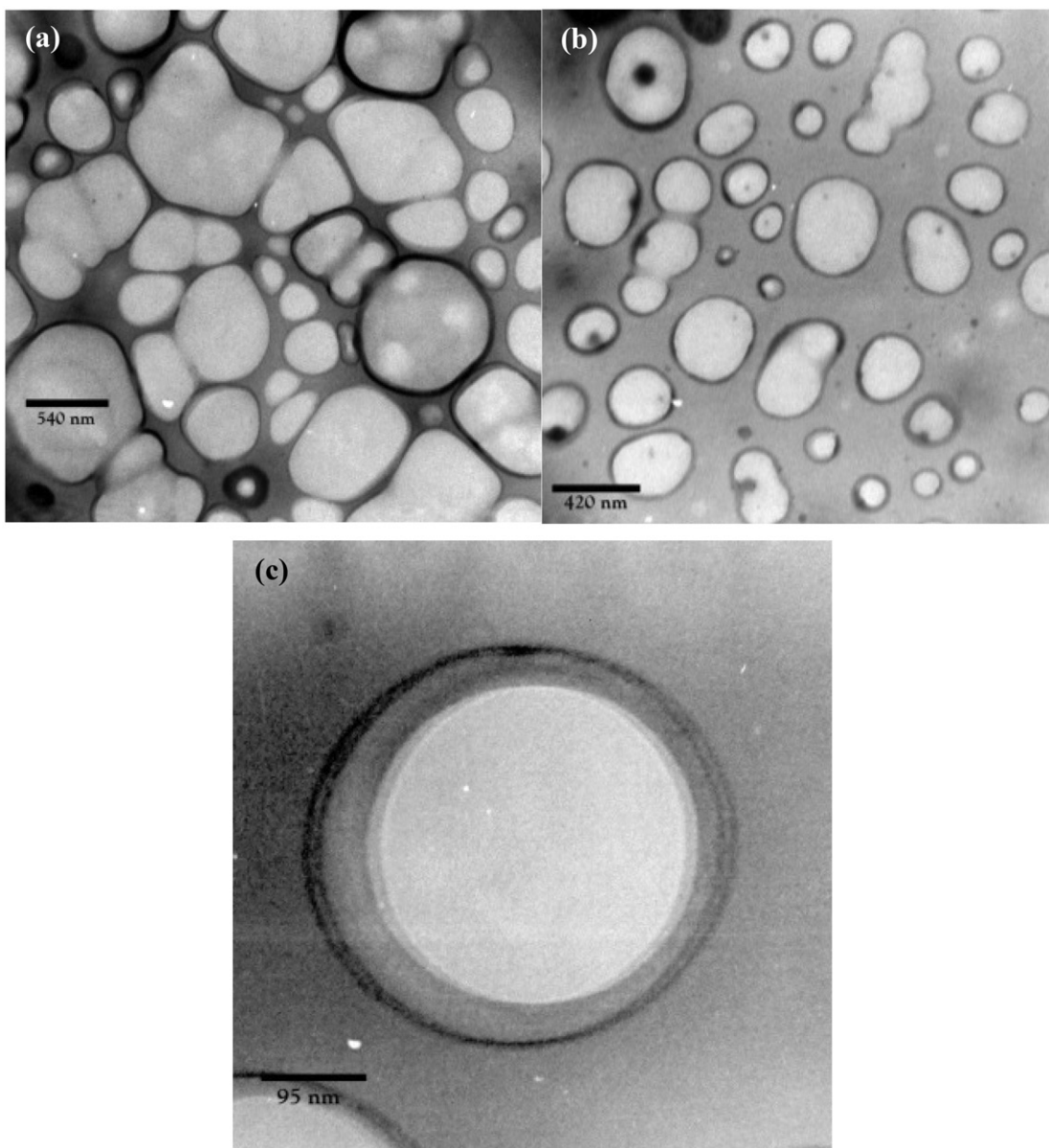


Figure 6. Transmission electron micrographs of vesicle fusion of DDBE at 40:60 THF:water mixture (a & b) the coalescence of the vesicles (c) the new vesicle formed after fusion. [DDBE] = 0.1 mM Scale bars: (a) 540 nm, (b) 420 nm, (c) 95 nm.

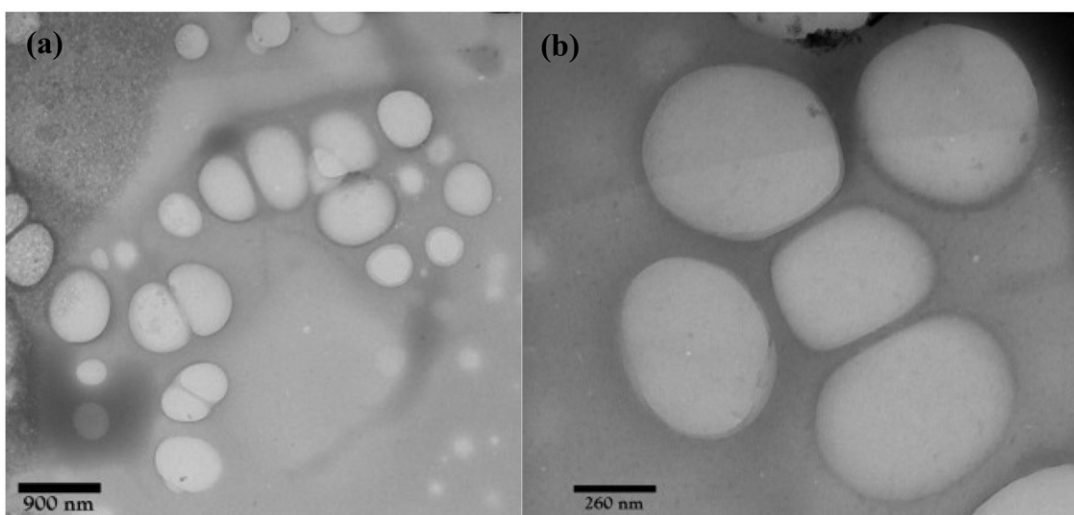


Figure 7. Transmission electron micrographs of vesicles formed at [DDBE] = 1 mM, 50:50 THF:water mixture. Scale bars: (a) 900 nm, (b) 260 nm.

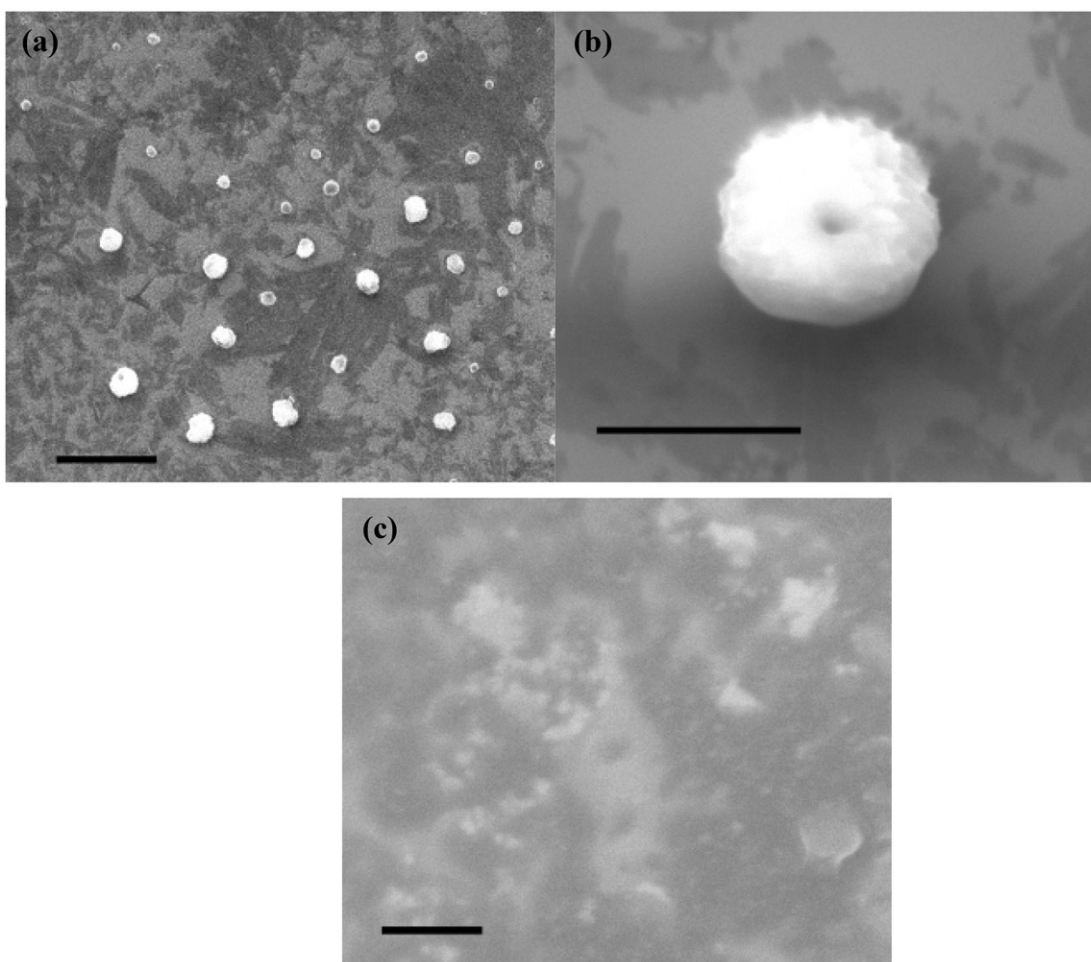


Figure 8. Scanning electron micrographs of the vesicles formed from [DDBE] = 5 mM, 40:60 THF:water mixture on (a & b) hydrophilic, (c) hydrophobized silicon substrates. Scale bars: (a) 20 μm , (b) 5 μm , (c) 2 μm .

Further, the system was sensitive to the environment where we tested it on hydrophilic and hydrophobized silicon substrates using scanning electron microscopy (SEM). On a hydrophilic substrate, the giant vesicles were clearly observed while on the hydrophobic substrates we observed crumbling of the vesicle (Figure 8c). This can be attributed to the fact that the hydrophobic substrate provides a poor medium for vesicle formation.

When the DDBE was completely extracted into water, it exhibited uniform spherical aggregates of 50 nm which clearly indicates that even though water is required for the vesicle formation, a 100% polar environment disfavours the vesicle formation and so does the 100% non-polar environment as is visualised from Figures 8c and 9.

Based on the above discussions, a route to the structural transformation of DDBE with varying solvent environments can be drawn as shown in Figure 10.

4. Conclusions

The DDBE system is proven to be amphiphilic and a true surfactant by the virtue of its mesogenic character and by the molecular packing/HLB considerations. The system aggregates in a 40:60 THF:water mixture at a minimum concentration of 0.01 mM based on the electronic absorption

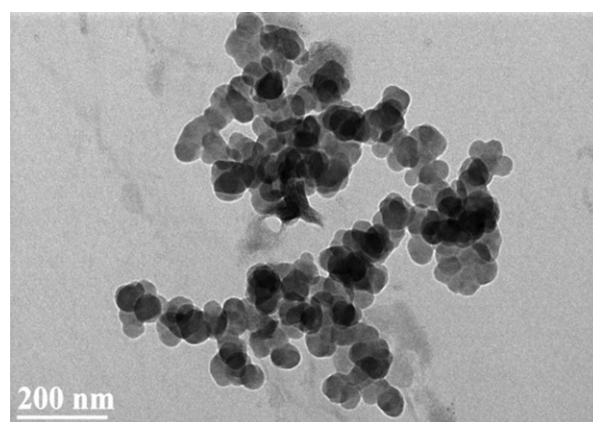


Figure 9. High resolution transmission electron micrograph of 0.01 mM DDBE extracted completely into water.

spectra. The molecular dynamics simulations revealed the interaction between the phenyl rings of the DDBE molecule leading to π -stacking. Due to its amphiphilic nature, and as predicted by the critical packing parameter and calculations, the molecule forms bilayer vesicles which undergo fission or fusion depending on the concentration and the water content. The system is further sensitive to the environment after its formation. Thus, we expect our system to find application as emollients or sustained drug delivery systems when

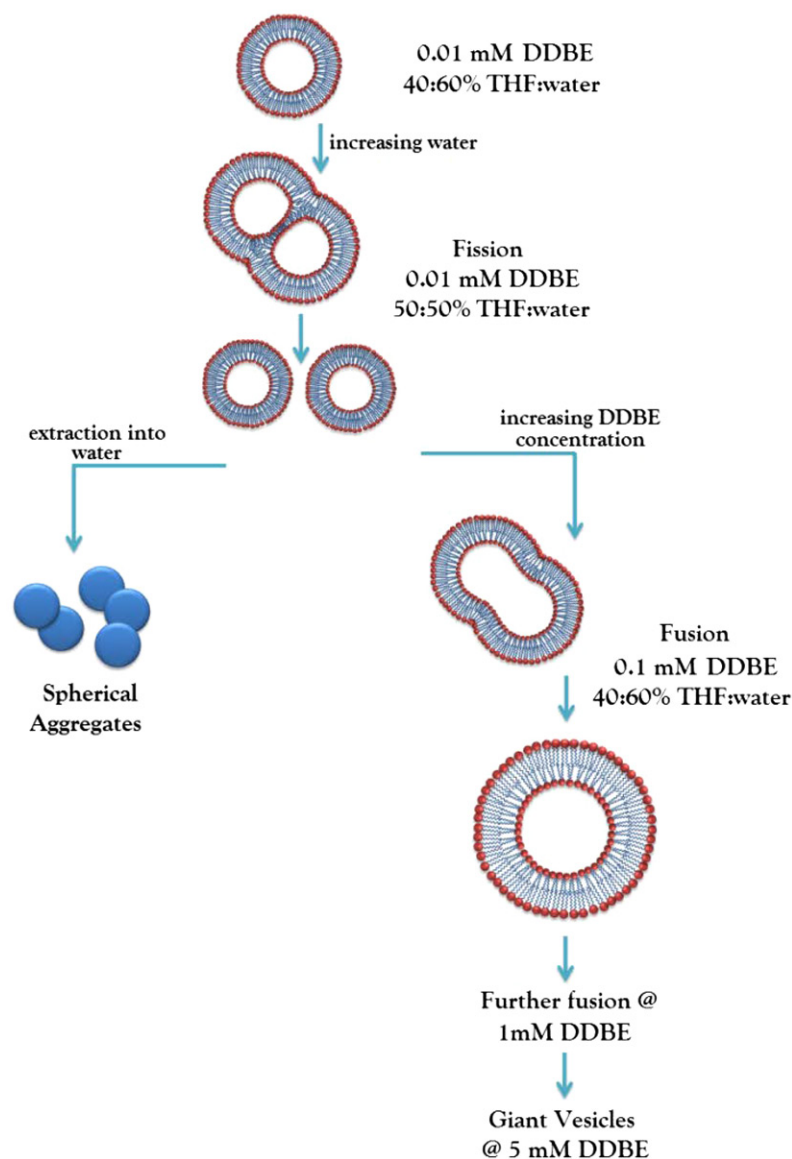


Figure 10. A cartoon representation of the sequence of transformations underwent by DDBE under the influence of environment and concentrations.

these vesicles are encapsulated with drugs, and experiments are underway for the same.

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Supporting Information

Visual snaps, Solvent polarity towards aggregation plots, geometry optimized structure of DDBE, MD Calculations, Dynamic light scattering plot. This material is available online free of charge via Figshare.

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