eProceedings Chemistry 4 (2019) 149-157

eISSN 2550-1453 http://eproceedings.chemistry.utm.my/

Solubilization of Violacein in Water/Sodium Dodecyl Sulfate System: Molecular Dynamics Study

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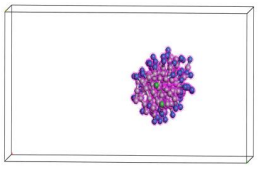
Article history:
Received 27 June 2019
Accepted 25 July 2019

GRAPHICAL ABSTRACT



Violacein assimilated inside the spherical shape micelle at 91.60 ns

ABSTRACT

Violacein, a well-known microbial pigment isolated from *Chromobacterium violaceum* that shows a wide range of application in medicine, cosmetics and textile industries. Its production has attracted many attentions for research and application due to its diverse biological activities including antibacterial and antioxidant properties. However, violacein was reported to be insoluble in water in which it is commonly dissolve in harmful solvent such as dimethyl sulfoxide and methanol. The solubility of violacein in water could be improve with the aid of surfactant by which at above the critical micelle concentration (CMC), the surfactant will form a micelle and allow the violacein to assimilate inside it, making the violacein to be soluble in water. This study focuses on the solubility of violacein in water/surfactant system through molecular dynamics approach where the surfactant used was sodium dodecyl sulfate (SDS). In details, the coarse grain method was applied to stimulate the solubilization behaviour of the violacein, where all the molecules involve are represented as beads. Each bead was assigned with charges and force field based on their natural behavior and structure. Consequently, at above CMC point, the SDS formed a spherical micelle at 91.60 ns and been able to solubilize the violacein in water. From the radial distribution function (RDF) analysis, higher peak was obtained for interaction of violacein with the hydrophobic part of SDS at radius equals to 5.125 Å compared to the hydrophillic part. In conclusion, the violacein beads were more attracted towards the hydrophobic part of surfactant compared to the hydrophillic part. In short, the coarse grain method can be applied to study the solubility of violacein in water/surfactant system. The finding may help to study the interaction of violacein with other materials which would widen its application in industries.

Keywords: Violacein, sodium dodecyl sulfate, coarse grain, bilayer micelle, radial distribution function analysis

1. INTRODUCTION

Natural pigments can be obtained from ores, insects, plants and animal to be used as colorants since prehistoric period. It has huge advantages that include rapid growth, easy processing, and independence of weather condition. Other than colorant, bacterial and fungal pigments possess numerous biological properties such as antioxidant, antimicrobial and anticancer activity [1]. Chromobacterium violaceum [2] is an example of the microorganism that is capable to produce pigment called violacein. Violacein is soluble in most common organic solvent such as ethanol but insoluble in water [2]. Addition of surfactant can be used to alter the solubilization of violacein in water where the surfactant can remain in at higher concentration by orienting themselves in aggregates called micelle that produce an aqueous solution of insoluble molecules [3]. For this study, the surfactant used was an anionic surfactant called sodium dodecyl sulfate (SDS). At above critical micelle concentration (CMC), the SDS formed micelle and helped to increase the solubilization power of violacein in water. In this study, a molecular dynamics simulation was done on violacein to study its solubilization behaviour in water/surfactant system using Material Studios 2.0 Software. In details, coarse grained method was applied to stimulate the solubilization behaviour of the violacein where, all the molecules involved were represented as beads using Mesocite module embedded in the software. Each bead was assigned with charges and force field based on their natural behaviour and structure using MARTINI force field model. Coarse grain method was more preferable than the atomistic simulation as it results in remarkable improvement for computational efficiency for simulating system that involve broad range of large time and scales. Focus of this study is to comprehend the mechanism of the solubilization process of violacein in water/surfactant system using computational approach. By this approach, the mechanism of this process can be understood easily. Simulation in this study is necessary as it bridge with the related experimental approach and the other previous simulation research done. Many other parameters that can be calculated and discover the factors that can be calculated and discover the factors thant can affect the solubility of violacein. This is the first study reported on molecular dynamics study on the solubilization of violacein in water using coarse grained of SDS surfactant.

2. EXPERIMENTAL

2.1 Coarse grain model molecules

Four main types of interaction sites are considered in the MARTINI 2.0 force field: polar (P), nonpolar (N), apolar (C), and charged (Q). Each type of interaction particle has subtypes, distinguished either by letter, denoting the hydrogen-bonding capabilities (d= donor, a= acceptor, da=both, 0=none), or by numbers, which indicate the degree of polarity (from 1, low polarity, to 5, high polarity). Table 1 shows the mapping on the SDS, violacein and water molecules where all the molecules radius and mass were set to 3.555 Å and 72 amu respectively.

Table 1. Type of force field assigned onto violacein, SDS and water molecules

Bead type	MS MARTINI force field	Charge	Mapping	Radius (Å)	Mass (amu)
Carbon tail	C1	0	Four to one		
Sulfate head	Qa	-1.0	-	3.555	72.0
Sodium	Qd	+1.0	-		
Violacein	C2	0	Two to one		
Water	P4	0	Four to one		
Simulation box ($Å^3$)		150 x 75 x 75			

2.2 SDS Micelle Development in Water

Sodium dodecyl sulfate (SDS) is one of the most widely studied anionic surfactants applied in fundamental scientific studies and industrial products. As the SDS undergoes change in the concentration, the structure of the SDS-based formulations will also be affected where at CMC point. Bilayer micelle was favoured at higher concentration than the spherical micelle that formed under low SDS concentration. Surfactants at high concentration can be simulated accurately by molecular dynamics simulations that will provide an atomistic-level insight of interactions between surfactants, cosurfactants, solvent and counterions. This method emphasizes more on how these factors govern the properties of the formulations.

The diameter of the bead is 7.11 Å which means the radius for a bead used were 3.555 Å. A simulation box was created with size 150 Å x 75 Å x 75 Å to perform all the calculations with periodic boundary conditions applied in all directions giving the total volume of the box, V_x was 843750 $Å^3$. The enclosed system was a compose of beads of water, dodecyl sulfate and sodium ions. Thus, the total number of beads, N_b in the system should be 7054 beads that consists of SDS,violacein and water beads in one large simulation box.

Mesostructure tools was used to pack the membrane molecules that consist of SDS into a slab. Slab, 12.05 Å was used to force the formation of micelles in the solvent that also contain the violacein bead. For the slab calculation, a total area of the simulation box, 5625 $Å^2$ was involved as well as the SDS area per molecule was 50 $Å^2$ [4]. The shape of micelle formed depends on the concentration of the surfactant used in the water/surfactant system.

2.3 Violacein-SDS Micelle Interaction In Simulated System

System created was geometrically optimized using the geometry optimization task provided in the Mesocite module along with algorithm smart with a fine quality as the purpose was to remove close contacts and any initial stresses in system. The default values of the convergence thresholds between optimization cycles for the fine quality in the Mesocite module are; Energy: 1×10^{-4} kcal/mol, Force: 0.005 kcal/mol$Å^{-1}$, Stress: 0.005 GPa, Displacement: 5×10^{-5} Å. NVT (number, volume, temperature) Mesocite Dynamics was carried out at a temperature of 298.15 K in order to bring system towards the equilibrium. A small-time step, 15.08 fs for 6073800 steps and carry out total simulation of 91.60 ns with The Nosé Hoover thermostat of Q ratio 1.0 [5]. Quality level will sets the non-bonded cutoff distance in the case of the bead-bead method to 15.5 Å to determine the parameters that control the simulation speed and accuracy.

In molecular dynamics, consist of different thermodynamic ensembles. In NVT (number, volume, temperature) ensemble, it uses constant number of particles, total volume and also fixed temperature. This ensemble is suitable for large system as it can help to make it stable more stable compared to other ensembles available. For instance, when the density of the system keeps on reducing itself thus, it is advisable to use this ensemble. In the Materials Studio Software, the dynamics are modified to permit the exchange of heat with the environment at controlled temperature. Among the thermostats available for this condition are Nosé, Anderson, Berendsen and velocity scale.

Ruiz and Romero (2018) used NVT ensemble for the bulk viscosity and critical micelle study of SDS in aqueous solution [5]. Shang *et al.* (2008) used this ensemble for the simulation of SDS in water and investigated the micellar structure, characteristics and the counterion distribution.

The experiment was divided into four main stages. The first stage was synthesized the alumina supported calcium based with chromium catalyst using modified and unmodified wetness impregnation methods. The second stage was optimized the preparation catalyst based on several calcination temperatures and concentration of sapodilla leaves which act as a binder in catalyst to avoid leaching between reactant and catalyst. The third stage was measured the performances of the catalytic activity and test the leaching properties over prepared catalyst. Then, the final stage was the characterization of prepared catalyst using various spectroscopic techniques.

3. RESULTS AND DISCUSSION

3.1. Coarsed Grained Model of SDS, Violacein and Water Molecules

Coarse grained model of each molecules involved in this study were constructed based on their natural behaviour and structure (noted the type of bead and its characteristics as listed in Table 1). The part of molecules were represented as beads with different color coding as shown in Table 2 For the surfactant (Table 2 (a)) the hydrophobic dodecyl sulfate pairs was mapped into two types of bead type, Qa (blue) for the sulfate group with charge -1 and function as charge acceptor while type C1 was assigned for the carbon groups with zero charge and shows low apolarity property. The mapping used for the SDS and water beads were similar to a study done by Ruiz and Romero (2018). Here, one bead represents four carbon atoms in the dodecyl chain. Meanwhile, the hydrated sodium was assigned as type Qd (purple) and function as charged donor with a charge +1. Table 2 (c) shows that the water bead where one bead represents four water molecules and described as MARTINI type P4.

Table 2 (b) shows one bead of violacein represent two molecules of violacein. The bead was assigned with C2 bead and show zero charge molecule. The assignation of the molecule as C2 bead is due to the lactam and isatin ring in the structure that contribute to its hydrophobicity and the existence of carbonyl, amine and hydroxyl groups that make the violacein dissolve in polar solvent. This is also explains the nature of the violacein which shows low polarity property due to it does not dissolve in water [2]. .

One water is equal to four water molecules and so did the SDS tail beads as they clustered based on the four to one mapping. While for ring-like violacein molecule, were mapped to a higher resolution that is two-to-one mapping. Therefore, the actual number of violacein in this system is twice the number of the violacein beads. On the other hand, the sulfate head and the sodium only consist of their actual amount as they were not considered as heavy atoms [6].

Table 2. Coarse graining SDS, violacein and water structure

Structure of molecules	Coarse grained structure bead
(a) Sodium dodecyl sulfate	
(b) Violacein	

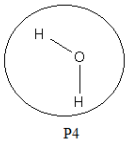
(c) Water	
	

Table 3. Number of beads in the simulation system

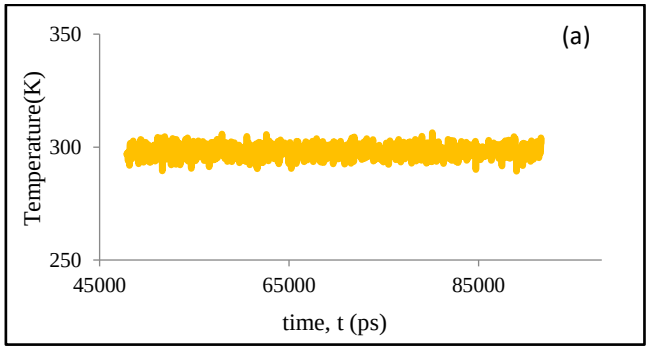
Study Phase	**Simulation**	**No. of bead before running the simulation**			
		Water	DS^- (dodecyl sulfate)	Na^+ (sodium)	V (violacein)
SDS-Water-Violcain	Multiple SDS	-	284	283	-
	Multiple violacein	-	-	-	7
	Micelle	-	284	-	-
	Violacein encapsulation	6480	284	283	2
Total beads	7054				

From Table 3, the total number of beads created for the simulation were 7054, which in consequent represents the member of molecules involved in the system.

3.2. Optimization of SDS/Violacein/Water System

The beads created were packed into the simulation box for optimization process in order to achieve the best condition before they undergo simulation. The optimization occurred under energy, 0.0001 kcal/mol, force of 0.005 kcal/mol/A, and stress of 0.005 GPa. Summation method by bead based was chosen with cubic spline truncation of non-bond energy terms. Spline width applied was 1Å, buffer width of 0.5 Å with a long-range correction in the truncation of the van der Waals term.

Figure 1 (a) shows the temperature were kept constant throughout the simulation. The constant temperature was due to the NVT ensemble that was set before the simulation begins. The dynamics were modified to allow heat exchange with the environment at a controlled temperature using Nosé-Hoover thermostats techniques that is an efficient method for constant temperature molecular dynamics simulations.



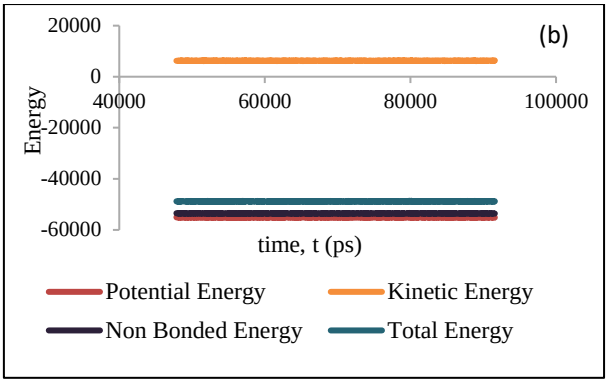


Figure 1. (a) Mesocite dynamics temperature at 298 K, (b) Mesocite dynamics energies (potential energy (brown), kinetic energy (orange), non-bonded energy (purple) and total energy (blue))

From Figure 1 (b), indicate that the energies were in minimum state where the energy were adjusted until it found to be stabilized and equilibrated. This phase is called energy minimization that includes in geometry optimization. This step is necessary as to achieve minimum conformation of a molecule by adjusted the parameters until it was found to be stabilised and equilibrated. Under constant energy, the properties of a system were obtained. The optimized system resulted in an optimized intermolecular interaction by kept the internal confirmation rigid and it reduced the beads overlapping probability by introducing unrealistic bond length or angles.

3.2. Formation of SDS Micelle in Water

Theoretically, micelles formed when the free surfactant monomer concentration reaches a critical concentration called critical micelle concentration (CMC). At a certain point, the surfactant monomer will remain constant at the system already reaches its CMC point where the number of micelles will increase upon the addition of surfactant monomer at high concentration.

When micelles formed at the CMC point, several surfactant properties will change abruptly such as the conductivity, surface tension, osmotic pressure and fluorescence as a function of concentration. CMC is crucially important physical parameters for surfactant as it reflects the interaction between the surfactants and solvent. Numerous methods available in order to establish the CMCs of surfactants such as surface tension, light scattering, fluorescence spectrophotometry, cyclic voltammetry, nuclear magnetic resonance (NMR), speed of sound and capillary electrophoresis. Depending on the type of surfactant and the temperature, the micelles normally will change its size and structure with increase in concentration where at first they form small and spherical micelles made up from around 100 surfactant then, as the concentration increased, the size will also get bigger along with the change in their shape. Surfactant CMC value is known widely in various applications related to solubilization, stabilization, isolation, and crystallization.

Figure 2 shows that the surfactants are able to form a stable spherical micelle at long simulation run which was at 91.6 ns. It was observed that for both micelle the hydrophillic part of SDS (blue beads) favors the water causes them to face outward towards the water beads. In contrast, the hydrophobic part of the surfactant accumulated inside the micelle to keep away from the water beads. At 91.6 ns all the violacein pigment were assimilated inside the spherical micelle formed. In contrast at 1.5 ns, only two violacein pigment entered the bilayer micelle. This is because at 1.5 ns, the system has not yet stabilized.

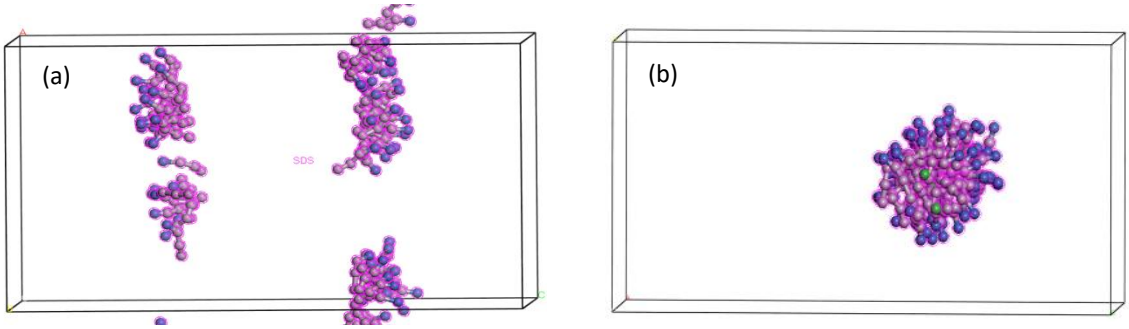


Figure 2. Formation of SDS micelle in SDS/water system at (a) 1.5 ns and (b) 91.60 ns

The micelle conformation was based on the concentration of the number of beads of the SDS. For this simulation, the concentration of the beads was unknown. Based on a study conducted by (Wang and Larson, 2015) where the simulation was conducted using GROMACS 4.5.6 showed the same micelle shape, that is a spherical micelle at a low concentration of SDS which was 72.8mM. The same observation was obtained using GROMOS45A3 by Goh *et al.* (2015) where at 100ns, the surfactants form a spherical shape of micelle at very low concentration. However, different micelle shape was obtained at high concentration of SDS in which they formed a bilayer shape micelle after a long simulation run, 100 ns as reported by Goh *et al.* (2015). From that, in this study, it can be justified that the formation of spherical micelle was due to a low concentration of SDS surfactant set for the system.

3.3. Location of Violacein Inside the SDS Micelle /Water system

From Figure 3, show the interaction of violacein beads with the SDS surfactant. All the green violacein beads favors to be stabilized at the the hydrophobic part of the spherical micelle rather than the hydrophillic head. This is due to the structure of the violacein itself where it consists of isatin and lactam ring which contributes to the hydrophobicity of the violacein towards water. Also, water is very polar with a dielectric constant, 80.1 which is higher than the polar solvents that can dissolve the violacein.

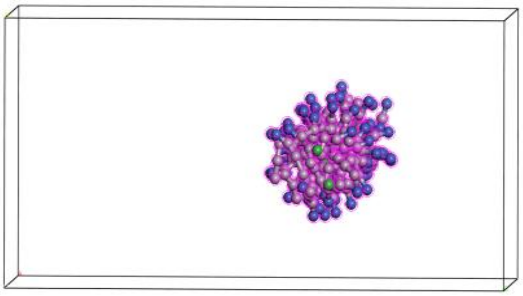


Figure 3. Interaction of violacein with SDS micelle in water system

This is however, in contrast with the experimental results obtained by Hamzah *et al.*, (2018), Singh (2012), Tehrani-Bagha and Holmberg (2013) where they study the interaction of violacein with SDS using ultraviolet-visible (UV-Vis) spectrophotometer. Singh (2012) concluded that the location of dye/pigment can be determine from Ultraviolet-Visible spectrum [9]. The violacein were considered as polar when their adsorption was slightly similar to the polar solvent and vice versa. By Hamzah *et al.* (2018) the λ_{max} value showed that the violacein in SDS that is equals to 573 nm was almost the same as the values for violacein in polar solvent which are methanol (573 nm) and ethanol (571 nm). They concluded that from their observation, violacein nanoparticle is in contact with the hydrophilic head [10].

Also, it can be observed all the violacein were solubilized inside the spherical micelle compared to when the bilayer micelle formed at initial of 1.5 ns, only two beads of violacein were found to be inside the micelle

(Figure 4.3 (a)). This is due to the long simulation time set for the large system until the SDS surfactants able to form a stable micelle that allows the all the violacein to assimilate inside it. Same observation obtained by Mobasheri *et al*. (2015), they analysed the encapsulation on AmB (Amphotericin B) molecule into P80 (Polysorbate 80) micelle at different simulation time. Their results shows that at the early of the simulation which is at 10 ns, only small amount of AmB entered the micelle [11]. Unlike at larger simulation time (600 ns), AmB were found to be inside the micelle with comparable level of interaction with both head and tail groups [11].

3.4. Radial Distribution Function Analysis of SDS, Violacein, and Water Systems

3.4.1. Solubilization of Violacein Into Coarse Grained SDS Micelle

The analysis was to study the ability of SDS surfactants as solubilizing agent for the violacein molecule. The result shows that the solubility of violacein was depends on the present of micellar conformation. The micelle which consist of 283 number of SDS beads were able to dissolve the violacein beads.

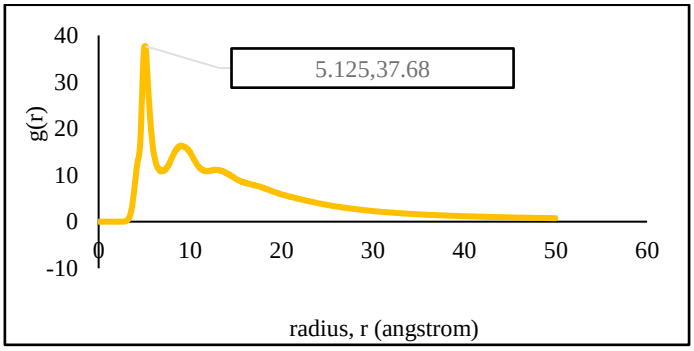


Figure 4. Average radial distribution function (RDF) of violacein with SDS micelle

Based on Figure 4, the interaction of the violacein beads with the spherical micelle was observed using RDF analysis. For this analysis, 284 number of SDS beads were clustered to form spherical micelle. As it can be seen clearly that a maximum of probability value of violacein, g(r) was peaked at 37.68, formed at radius, 5.125 Å. This indicated that at this point, the violacein beads were already present and were highly abundance at certain part of the micelle (Figure 4) surrounded with water beads after the equilibrium was reached. Also, at this point, the violacein beads were able to soluble in the water as it entered the micelle.

3.4.2. Interaction of Violacein Bead with SDS

To observe at which part of SDS that is in contact with the violacein bead, RDF analysis was also been used as the result can be interpreted easily. It can be estimated by the peak height of the RDF graph.

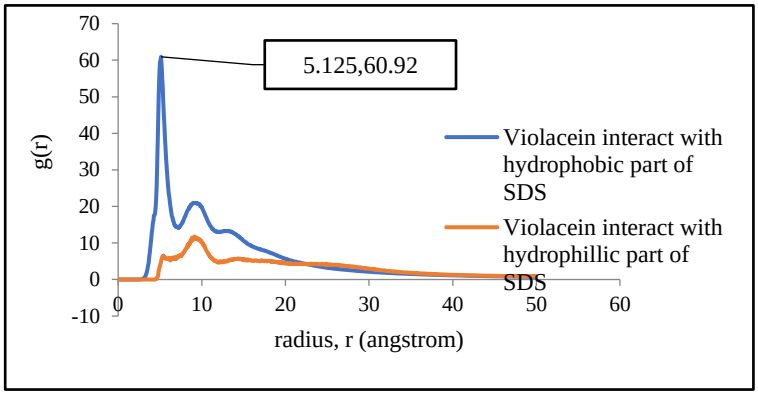


Figure 5. Radial distribution function (RDF) of interaction of violacein with different part of SDS

From Figure 5 the head and tail groups show significant difference in peak height and peak position from RDF pattern. These data provide information on the solvation behaviour of violacein in the micelle under polar environment. A sharp peak of the tail series was observed at 5.125 Å with peak height, up to 60.92 of average density at certain distance, g(r) shows that at that distance, violacein has greater affinity towards the surfactant hydrophobic part compared to the hydrophilic part of the micelle. Consequently, the violacein was considered to be dissolved in water as it entered the hydrophobic region of the micelle. The findings obtained may be due to force field assigned onto the violacein which is C2, low polarity which cause them to drive away from the water, indicates that it is immiscible in polar environment. Plus, lower water density nearby the hydrophobic tail created a less favourable interactions of violacein with water compared to its interaction with the inner part of the micelle. Therefore, the violacein was indeed assimilated inside the palisade layer of the micelle.

4. CONCLUSION

Theoretical studies on the behavioural of violacein solubilization in water was successfully developed via molecular dynamics simulation approach. For identifying this unique feature, we examined the distribution of violacein inside the micelle by radial distribution function analysis. Also, the results obtained in this study can be compared with the previous experimental and computational results by which can help in advancement in application of violacein. Generally, all the objectives for this molecular dynamic study were achieved based on the interpreted data analysis and discussion made in the chapters. The coarse grain of sodium dodecyl sulfate (SDS), violacein and water were developed using the coarse-grained simulation method embedded in Mesocite module. The process involved was assigning the charges and also the force field onto the molecules using MARTINI force field that consist of four-to-one mapping and two-to-one mapping for ring like structure. The beads were then differentiated based on the color that were automatically assigned by the module. By that, the interaction between the beads were easily observed for the data interpretation. The formation of coarse grained SDS micelle were found to be a stable spherical micelle at 91.60 ns. The shape of micelle depends on the concentration of the SDS inside the system. As in this simulation, the exact concentration was not specifically known thus, we assumed that the SDS beads were low concentration that caused the spherical micelle to form. The simulated solubilization process of violacein in water using SDS was used to determine which part of the SDS micelle will interact strongly with the violacein to allow it to be soluble in water. Overall, the results from the radial distribution function shows that at 5.125 Å the violacein beads preferred the SDS hydrophobic part rather than the hydrophillic part with the density probability, g(r) equals to 60.92 and 4.81 respectively. The violacein attached itself into the palisade layer of the micelle formed rather than in the stern layer. Many other parameters that can be calculated and discover the factors that can affect the solubility of the violacein. This is the first study reported on molecular dynamics study on the solubilization of violacein in water using coarse grained of SDS surfactant.

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