Molecular Dynamics Simulation of Palm-Based Nano-emulsion System

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Abstract: - Nano-emulsions of oleyl oleate possess the potential to act as penetration enhancers in pharmaceutical applications. However, information on the phase behavior of oleyl oleate nano-emulsions which obtained through laboratory experiments was insufficient to describe the structural mechanism of the self-assembled system. In this research, the structural and dynamical properties of the palm-based nano-emulsions to act as penetration enhancer were studied by computer simulations. The structural and dynamical information of the nano-emulsions were obtained using molecular dynamics (MD) simulation method. The nano-emulsions system comprised of oleyl oleate (oil) with Span20 and Tween80 as the non-ionic surfactants was simulated in the presence of water molecules (oil-in-water system). The effect of lipid composition in the nano-emulsions system was determined by 5 series of MD simulations up to 2.5 ns. The simulation results showed that different shapes of the nano-emulsions were formed as the composition of oil was increased. The critical micelle concentration was found at 20% – 30% of lipid composition while the higher concentrations lead to the formation of lamellar system. Larger system with random initial configuration was used to simulate the self-assembly characteristics of the nano-emulsions system. The aggregation and disaggregation of the oil and surfactant molecules were observed throughout the simulation.

Key-Words: - Molecular Dynamics, Palm-based, nano-emulsion, micelle, self-assembly

1 Introduction

Nano-emulsions are known for its capability as permeation enhancer for transdermal drug delivery system [1]. However, the structural behavior of the nano-emulsions system is hard to obtain with experimental work. This can be overcome by doing the computational work to investigate the structural and dynamical properties of the nano-emulsions in nano-scale region. Computational approach is proven to be able to predict and simulate the behavior of these chemical penetration enhancers at greater detail level [2]. Nano-emulsions of palm-oil esters are self-assembled micellar system in aqueous solution which formulation consisted of palm-oil ester, non-ionic surfactant and water as the solvent.

The assembly of molecules into an aggregate is not entropic-encouraging and occurs when it is energetically favored. However, isotropic molecules such as dissolved hydrocarbons in water can associate into large droplets which, above the solubility limit, grow into a separate phase [3]. This association is due to the strong hydrophobic attraction between hydrocarbon molecules in water. The self-assembly of the nano-emulsions is primarily driven by the hydrophobic effect supplied by the polar and non-polar group of OE and S20 molecules. The resulting structure preferably to be a micelle structure. The formation of micelle however is dependant on the presence of water in large quantities relative to the lipids. Theoretically, the surfactants molecules are holding the role of minimizing the energetically...
unfavorable interaction of its hydrophobic tail plus the OE molecules with water [4]. Based on the structural physico-chemical properties of OE, its addition will increase the non-polar region in the core of the aggregate that formed while decreasing the solubility of the nano-emulsions. Thus the shape, size and polydispersity of the aggregate formed which are intimately related and sensitive to the concentration, temperature, and solution conditions will be affected. The relationship between the head-group area (α_o) and chain-length (l_c) to the shape of the resulting aggregate can be defined as:

\[ p = \frac{v}{\alpha_o l_c} \quad (1) \]

where \( p \) is the packing parameter that theoretically determine the shape of structure that formed by the self-assembly process and \( v \) is the total volume [3]. The presence of OE which has two hydrocarbon tails will increase the overall volume \( v \) and chain-length \( l_c \), but not the \( \alpha_o \) value, hence increasing the \( p \) value. This will led to the estimation on the formation of cylindrical or cubic micelle, lamellar and even inverted structure instead of spherical micelle. The formation of spherical micelle is however, most favorable due to its lowest surface area and size for a given volume.

There are internal forces acting on the nano-emulsions system that should be taken into considerations such as bond, angle and dihedral, while external forces such as attractive and repulsive forces between molecules in an aggregate and between aggregates and solvent. The movement of the molecules in the nano-emulsions structure is largely determined by the interactions between OE and S20 molecules due to non-covalent, electrostatic forces including the van der Waals interactions between aggregates of the nano-emulsions. These forces, which were used to calculate the total energy (\( E \)), potential energy (\( U \)) and kinetic energy (\( K \)), can be described as:

\[
\begin{align*}
&f_{\text{OE}}(\text{bond}) \quad \text{and} \quad f_{\text{Surf}}(\text{bond}) \\
&f_{\text{OE}}(\text{angle}) \quad \text{and} \quad f_{\text{Surf}}(\text{angle}) \\
&f_{\text{OE}}(\text{dihedral}) \quad \text{and} \quad f_{\text{Surf}}(\text{dihedral}) \\
&f_{\text{OE}}(\text{rep}) - f_{\text{OE}}(\text{att}) \quad \text{and} \quad f_{\text{Surf}}(\text{rep}) - f_{\text{Surf}}(\text{att}) \\
&f_{\text{OE}}(\text{rep}) - f_{\text{OE}}(\text{rep}) \quad \text{and} \quad f_{\text{Surf}}(\text{rep}) - f_{\text{Surf}}(\text{rep})
\end{align*}
\]

where the bond, angle and dihedral are internal forces while the external consist of electrostatic and van der Waals repulsive (rep) and attractive (att) forces.

Molecular Dynamics Simulation

For the past decades, molecular dynamics (MD) simulation has been widely used for predicting structural and computing dynamical information of molecules. This computational approach use statistical calculations of selected force fields according to Newton’s law of motion. Simulations now are performed to even larger systems with more realistic boundary conditions and better productivity [5]. MD can be a useful tool for predicting micellar behavior at higher level compared to experimental work [6]. Other available functions also can be used to calculate the physical properties of the molecules with higher precision compared to experimental approach.

By running MD simulations, many molecular properties of the OE/S20 nanoemulsion can be determined at nano level. The internal properties such as energy and geometrical deviations of the nanoemulsion can be computed statistically from the trajectories of the simulations. The resulting structures of OE/S20 nanoemulsion then can be easily stored, edited, duplicated, and deposited for the public to use. Moreover, the structural properties obtained by computational technique can be beneficially used to aid the experimental approach as it has a lot of detailed information about the structure and the dynamics properties. This research will bring an improvement on the study of palm–based chemical penetration enhancers through statistical computational approach.

2 Methods

The system was identified by a ternary phase diagram in which the suitable concentration (in percentage) of OE and S20 were obtained [7]. The nano-emulsions were prepared by packing NOE of oleyl oleate and NS20 of Span20 together using the Packmol software [8]. The nano-emulsions then solvated in a 100 nm x 4.64 x 4.64 simulation box with water molecules as the solvent. All MD simulations were performed using GROMACS v3.3 software with OPLS-AA force field applied for the lipid molecules and SPC water model for the solvent molecules [9][10]. The system was energetically minimized using both conjugated-gradient and steepest-descent algorithm until reaches 50.0 kJ mol\(^{-1}\). Equilibration simulation was performed to familiarize the system for 100 ps. All simulations were performed with step size of 2 fs. The electrostatic interactions were cut-off at 0.9 nm using Particle Mesh Ewald algorithm, and the van der Waals interactions were also cut-off at 0.9 nm. The temperature was...
coupled to an external bath using Berendsen method at 300 K with \( \tau_T \) equals to 0.1 for each type of molecules and the pressure coupled with the same method at 1.0 bar with \( \tau_p \) of 1.0 [11]. Periodic boundary condition was applied to all direction of the system with constraint applied to all bonds with H atoms [12].

Determination of Critical Micelle Concentration (CMC)

Simplistic method was used to generally predict the CMC of the nano-emulsions system. Five series of MD simulations were performed which comprised of 10, 20, 30, 40 and 50 percent of lipid composition in the nano-emulsions. The simulations were performed in constant NPT up to 2.5 ns in a 100 nm\(^3\) simulation box. The average surface tension value was calculated. The CMC of the nano-emulsions were determined by the plot of surface tension versus lipid percentage. Different kinds of shape of the nano-emulsions system were predicted as the concentration of lipid increases [13]. At the CMC region, the nano-emulsions were predicted to form a micellar structure (spherical, cylindrical, or cubic).

Simulation with different temperature

The best configuration from previous result was used to detect the effect of simulation with different temperature to the nano-emulsions system. 4 sets of simulation were performed with various temperatures at 250 K, 300 K, 350 K and 400 K respectively. The potential energy, volume, density were observed as well as the movement of the molecules that were analyzed using radius of gyration measurement of the system over simulation time.

Self-assembly of nano-emulsions

Larger scale MD simulation was performed in order to investigate the self-assembly of the nano-emulsions system. The non-ionic surfactant was changed to Tween80 (T80) due to its higher HLB value (15 ± 1.0) than S20 which theoretically easier to self-assemble with OE molecules. 5 molecules of OE and 27 molecules of T80 were packed and solvated in a 1000 nm\(^3\) simulation box. All the molecules were randomly placed in the box with no position-restriction applied to the chains of the molecules. Equilibration simulation performed until 100 ps with constant NPT to obtain proper volume and density of the system. This was followed by a 3 ns of production simulation with constant NVT. The aggregation and desegregation of the molecules were observed from the output trajectory. This was followed by analysis with radius of gyration and solvent accessible surface area measurement.

3 Results and Discussion

3.1 Critical micelle concentration

The average surface tension value was calculated for each of the 5 MD simulations consisted of 10%, 20%, 30%, 40% and 50% of lipid concentration in the system. If the CMC of pure amphiphiles is known, then CMC (\( C_M \)) of a mixed system:

\[
\frac{1}{C_M} = \frac{x_1}{C_1} + \frac{1-x_1}{f_2C_2} \tag{2}
\]

the mole fraction of \( x_1 = x_1/x_1+x_2 \) and \( x_2 = (1-x_1) \), \( C_1 \) and \( C_2 \) are pure CMC of \( N_1 \) and \( N_2 \), and \( f_1 \) and \( f_2 \) are the activity coefficient [14]. For nonionic surfactant, \( f_1 \) and \( f_2 \) are unity because of same type of interaction. Here we consider OE as a nonionic co-surfactant and so the concentration of both molecules can be summed up and termed lipid concentration or fraction. The ratio of OE and S20 were determined as OE : S20 equals to 1:3 from the experimental ternary phase diagram [7]. The results are as is Figure 1:

![Surface Tension vs Lipid Fraction](image)

**Fig. 1:** Average Surface tension versus lipid fraction.
The simulation results revealed that the average surface tension value of the system drop drastically from 10% to 20% of lipid concentration and the later value were almost constant from 20% to 50% (Figure 1). The change of the trend which was at the region of 20% to 30% was determined as the CMC region of the nano-emulsions system. The reason for this is that almost all physico-chemical properties of self-assembled system including surface tension will show an abrupt change in the slope when plotted against lipid concentration [15].

These results were supported by the graphical trajectory of each of the simulations (Figure 2). The structure at 20% and 30% of lipid concentration exhibited a micellar system. The trajectory at 30% showed a nearly-perfect cylindrical micelle structure. This showed that the micelle formation occur from 20% to 30% of lipid concentration. In agreement with the experimental results, the 20% to 30% of lipid concentration was in the isotropic region in the ternary phase diagram [7]. At 40% of lipid concentration, the system after 2.5 ns of MD simulation showed the formation of separated layers of OE and S20 which, be given enough time scale, can form a lamellar structure. However, at 50% of lipid concentration, lamellar structure already formed after 2.5 ns of simulation.

3.2 Simulation at different temperature

We carried out 4 constant NPT MD simulations with different temperature coupling value. The nano-emulsions and solvent were coupled separately but with same value. The temperature coupled system ranging from 250 K, 300 K, 350 K and 400 K were simulated up to 10 ns in a 100 nm³ simulation box to investigate the temperature effect to the structural and dynamical properties of the nano-emulsions. The initial configuration was taken from previous analysis which was the system at 30% of lipid concentration.

The temperature affected the potential energy, average density and the volume of the system after the simulation. The system was moving to more unstable state as the temperature rise. The radius of gyration plot (Figure 3) showed that when the temperature increased above 300 K, the structure was expanding as the simulation went on. At 350 K, the structure started to expand after 8 ns of simulation while at 400K, the structure expanded as twice earlier (4 ns).

![Fig. 3: Radius of gyration (Rg) of the nano-emulsion system at different temperature as the function of time.](image)

It is known that at the HLB temperature, the nano-emulsions are kinetically stable and formed in narrow size distribution and small droplet size [16]. At higher temperature, kinetic energy of the molecules increased which contributed to the expansion of the system. This expansion produced bigger gaps between molecules thus permitted water penetrations into the nano-emulsions system.
3.3 Self-assembly of the nano-emulsions

We investigated the self-assembly of the palm-based nano-emulsions system by running MD simulation on the system with larger system size. This is important in order to allow all the possible structure shapes to develop during the simulation. OE and T80 molecules were randomly scattered around the system in the initial configuration (Figure 4). The molecules can self-assemble into micelles when the concentration of the molecules (particularly T80) is larger than the CMC.

Fig. 4: Initial trajectory of the system.

Fig. 5: Trajectory of the system after 3 ns.

Micelles are known to exhibit a size distribution rather than one particular aggregation number at the equilibrium state [17]. At the end of 3 ns of the simulation, 5 aggregates formed with 3 molecules having aggregation and 3 free molecules observed from the trajectory (Figure 5). Unfortunately, the simulation time of 3 ns seemed not sufficient to obtain the reliable aggregation number and size distribution pattern of the system. Here we analyzed the largest aggregate which from now on named Agg1 (Figure 6). Trajectory observation showed that at 1 ns, there were no really aggregate formed where none of the molecules were in contact to at least 2 other molecules. This changed at 2 ns, where Agg1 had 7 molecules aggregated, while other aggregates still in formation progress. After 3 ns, 1 molecule of T80 exited agg1 which left the aggregate with 1 molecule of OE and 5 molecules of T80. The positioning of the molecules in Agg1 was explained further by the analysis using radius of gyration of Agg1 throughout the simulation (Figure 6).

Radius of gyration (Agg1)

Fig. 6: Radius of gyration for Agg1.

The radius of gyration plot indicated that the close-packed structure of Agg1 was found at around 2.25 ns to 2.75 ns. However, the close-packed structure was not stable due to huge fluctuations of $R_g$ around this time scale. Longer simulation time is required in order to obtain a stable aggregate from the self-assembled system. In drug permeation, the extremely small size of the nano-emulsions droplet play crucial role in order to penetrate the skin barrier efficiently. Smaller droplet also indicates that the structure is stable and the permeation of water molecules into the nano-emulsions is at the lowest level. It is important that the simulation produce aggregates size that are as minimum as possible in order to accurately determine the behavior of these structures as penetration enhancers structurally and dynamically.

4. Conclusion

The system with stable configuration obtained from the simulation was in agreement with the experimental
result. The system also was examined to be not tolerated to high temperature simulation which can lead to expansion and unstable structure. Clearly 3 ns simulation time and number of molecules simulated were not enough to provide more detailed explanation to the self-assembly of the palm-based nano-emulsions system. Given enough time-scale and molecules, MD simulation can provide the size distribution and aggregation pattern as well as formation and reverse-formation of the micelle with the option of obtaining different shape of aggregates in the system. More and longer time-scale structural and dynamical studies of the palm-based nano-emulsions system will be carried out in the future in order to increase the understanding of the system’s behavior at higher level.

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