From micelle to microemulsion: an investigation of structural changes using molecular dynamics

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Objective Although a huge number of experimental works may be observed in the literature for microemulsions, modelling reports on these nano-systems are rare. Additionally, no comprehensive work so far has detailed the changes occurring to a micellar droplet when oil molecules are introduced (i.e. obtaining microemulsions from micelles). This work aimed to simulate a micelle and a microemulsion system containing polysorbate 80 - as surfactant - and study the structural changes in the two systems.

Methods Molecular dynamics simulation employing a coarse-grained model was used.

Results The results showed that the oil molecules located in the hydrophobic core of the micelle make its size larger, while the co-surfactant molecules distribute throughout the system and enter the hydrophilic shell of the micelle.

Conclusion It was shown that insertion of oil molecules makes the droplets more spherical, while co-surfactants tend to enlarge them.

Keywords microemulsion, polysorbate 80, molecular dynamics, structural changes

Introduction

"Microemulsion" was first defined in 1981 as "a system of water, oil, and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution."[1] When preparing a mixture of oil and water, in the absence of surfactant(s), a two-phase system is formed. By addition of a surface active agent (surfactant), the surfactant molecules move to the interface of oil and water and lower the interfacial tension. The surfactant molecules then try to trap some oil (water) molecules within their aggregates and make a nanodispersion of oil (water) in the water (oil) phase. Addition of more surfactant molecules will eventually "solubilize" the whole oil (water) phase, in continuous phase.[2,3] This forms the microemulsion particles.

Structurally, microemulsions are similar to micelle droplets (i.e. aggregates of surfactant molecules,[4] while an internal phase is located within the droplet. The internal phase should be immiscible with the continuous phase. Usually, in addition to the surfactant molecules, a short chain alcohol is added to the preparation to provide flexibility of interface and promote the stability of the microemulsions. Such alcohols are called co-surfactants.[5]

It is a common practice to employ molecular simulation approaches in order to fill the gaps left by experimentalist and provide insight into dynamics and thermodynamic quantities at molecular levels. Considering the literature, an ocean of experiments can be found on microemulsions. Also, numerous simulation works have been reported on micelles. However, limited number of molecular simulation reports is available for microemulsions. In works by Baaden et al., the role and behavior of tri-n-butyl phosphate (TBP) as a surfactant, a modifier of interface, or a solute in a mixture of water and oil was studied. The results of this molecular dynamics (MD) simulation showed that hydrogen bonding at high TBP concentrations may lead to the formation of microemulsions.[6,7] A microemulsion system was simulated employing mesoscale MD simulations, by use of single site representation of water and oil.[8] Chen et al. used a similar method to investigate the structural properties of the microemulsion droplets.[9] The density profile of the microemulsion beads have also been given in an oil/water/CTAB system.[10] More recent works have focused on the structure and flexibility of interfacial surface in Aerosol OT (docusate sodium) micelles.[11,12]

Our previous work detailed coarse grained MD simulation of a micelle of polysorbate 80 along with a self-assembly study of the micelle.[13] In brief, the report detailed dynamics and structural properties of the micelle as well as a single polysorbate 80 molecule. In this work, we tend to extend our work to a microemulsion system containing glyceryl trioctanoate as oil phase, ethanol as co-surfactant, and polysorbate 80 as surfactant in water, a system which has been experimentally investigated too.[14] Since the ingredients that have been used in this work are pharmaceutically approved, this microemulsion has the potential to be used in various delivery routes. We aim to investigate the possible structural changes occurred by introducing the oil molecules into the micelle. To do so, a micellar system with and without co-surfactant was compared with the microemulsion droplet. To avoid enlarging, detailing some parameters such as diffusion constant, which are not directly related to structural changes, have been excluded from this article. The obtained data provides insight into the structural differences between a micelle and a microemulsion droplet and may be considered as a complementary work to our previous experiments.[15,16]

Methodology

Molecular Model

The molecular model of the polysorbate 80 molecule was considered, as detailed previously,[13] with modifications adopted from mapping polyethylene glycol.[17] Briefly, five bead types were employed including $-CH_n-CH_2-CH_2-CH_2-$, $-CH_n-CH_2-CH=CH-$, $-CH_2-CO-O-$, $-CH_n-CH_n-O-$, and $-CH_n-CH_n-OH$ for the surfactant molecule. The oil molecule consisted of

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$-CH_n-CH_2-CH_2-CH_2-$ and $-CH_2-CO-O-$ bead types and ethanol was taken as a single bead. The parameters of bonded and non-bonded interactions were taken from previous reports of MARTINI force field.[18,19] MD simulations were performed by DLPOLY_2.19[20] in the NPT ensemble, with pressure and temperature set at 1 bar and 310°K, respectively. The Berendsen's algorithm was employed with barostat and thermostat relaxation times fixed at 1.0 ps. The van der Waals interactions had a cutoff value of 12.0 Å and the timestep was 40fs.

Our previous experimental results have shown that in the most stable form, the intended microemulsion system contains 170 molecules of ethanol and 17 oil molecules per 60 molecules of polysorbate 80.[14] The phase diagram of the microemulsion has also been given previously.[16] To study the structural changes when moving from a micelle to a microemulsion (i.e. from having surfactant only molecules in water to having a mixture of surfactant, co-surfactant and oil molecules, dispersed in water) the following four systems were simulated:

1. a single micelle system which was previously self assembled[13] with 60 surfactant molecules (i.e. aggregation number of polysorbate 80[21] in 10323 water beads (MI),

2. a micelle plus co-surfactant system (i.e. random replacement of 170 water beads in MI with ethanol to yield MI-A),

3. a microemulsion system containing 60 surfactant molecules, 170 co-surfactants, and 8 oil molecules (i.e. having half the oil molecules of the ultimate microemulsion, ME-0.5), and

4. a microemulsion with 60 surfactant molecules, 170 co-surfactants, and 17 oil molecules to represent a whole droplet of microemulsion (ME-whole).

In other words, MI, MI-A, ME-0.5, and ME-whole represent systems containing surfactant/water, surfactant/co-surfactant/water, surfactant/co-surfactant/oil/water, and surfactant/co-surfactant/oil/water, respectively. In ME-0.5 and ME-whole, oil molecules were initially positioned randomly.

To obtain the true simulation time, the overall time was scaled by 4, as suggested by the MARTINI force field. The simulations were performed for 100.0 ns,

of which the first 5.0 ns was taken as equilibration and the remaining was used to extract the averages. During the equilibration period, parameters such as potential energy and eccentricity of formed droplets were monitored to confirm reaching the equilibrium, beyond which no substantial change is observed in these parameters.

Results and Discussion

Figures 1 and 2 illustrate variation of potential energy of the system as well as eccentricity of the assembled droplets during simulation period. As the details show, a fall in the potential energy is observed during the first ~1.0 ns, of which the first half is associated with a sharp fall, followed by a gradual decrease in the second half. It is arguable that during the first ~0.5 ns, some self-assembly processes occur, while in the time range 0.5–1.0 ns, only rearrangement of surfactant/co-surfactant/oil molecules happen to obtain the most stable droplet. A similar pattern is observed for the eccentricity of the formed droplets. Therefore, an approximate equilibration period of 1.0 ns appears to be sufficient for stabilizing the droplets.

Fig. 3 shows snapshots of the equilibrated systems under study. As shown in Fig. 3a, the micelle forms a nearly spherical shape, as reported previously.[13] In addition, polar groups of the micelle are laid in a hydrophilic shell, covering the hydrophobic moieties of the surfactants to form a typical micelle droplet. In Fig. 3b, where co-surfactant is added to the micellar system, the ethanol molecules appear to distribute throughout the system with few diffusing to the droplet. These molecules remain in the hydrophilic parts of the surfactant molecule and may contribute to form a shell around the hydrophobic core. Oil molecules then move to the centre of the droplets to form the oil-in-water microemulsion droplets and lie between the surfactant hydrophobic and hydrophilic chains (see Fig. 3c, d). These findings are verified by the details given in Fig. 4 and Tables 1 and 2, as will be discussed later.

The radii of gyration (R_g) of the whole droplets as well as the hydrophobic core, consisting of hydrophobic tails of the surfactant and the oil molecules, for the four systems under study have been given in Table 1. From the results, by introducing oil molecules, no
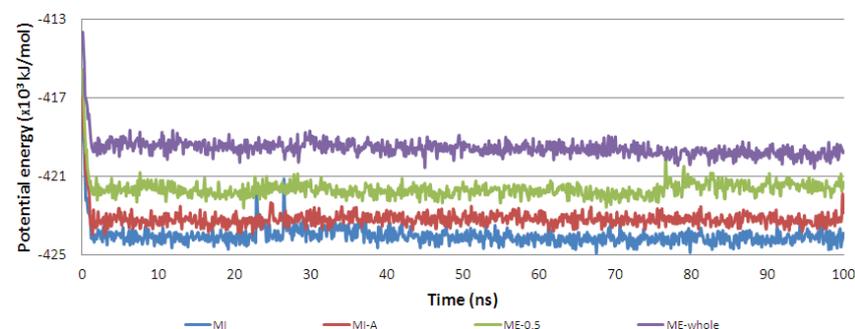

Fig. 1 **Change in potential energy of the systems during simulation time.**
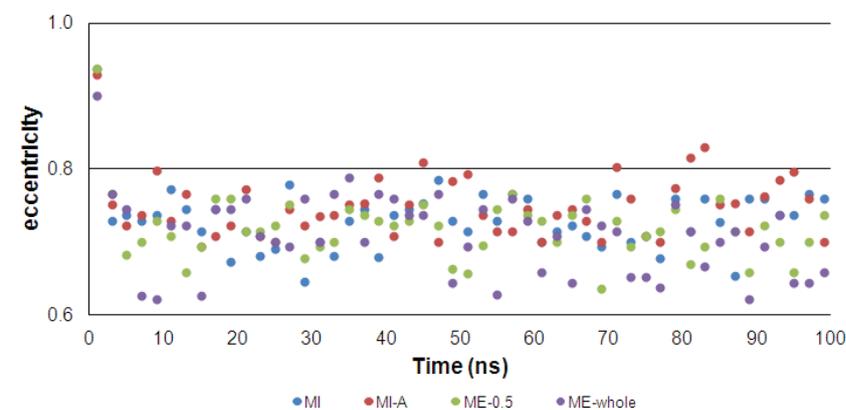

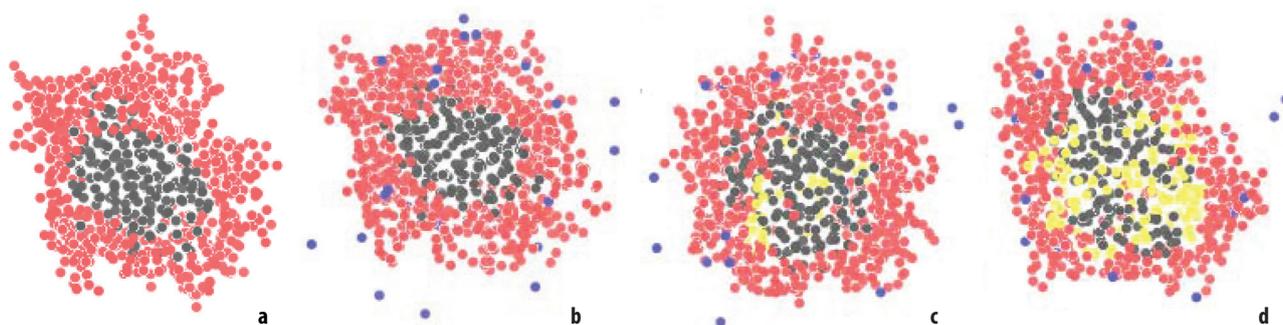Fig. 2 **Eccentricity of four systems as a function of simulation time.**

Fig. 3 Snapshots of the equilibrated droplets for the MI (a), MI-A (b), ME-0.5 (c), and ME-whole (d). The hydrophilic, hydrophobic, ethanol, and oil moieties are represented by red, black, blue, and yellow colours, respectively. A core-shell system has been formed in all the system, having oil and hydrophobic parts of surfactant in the core and hydrophilic parts in the shell, wherever applicable.
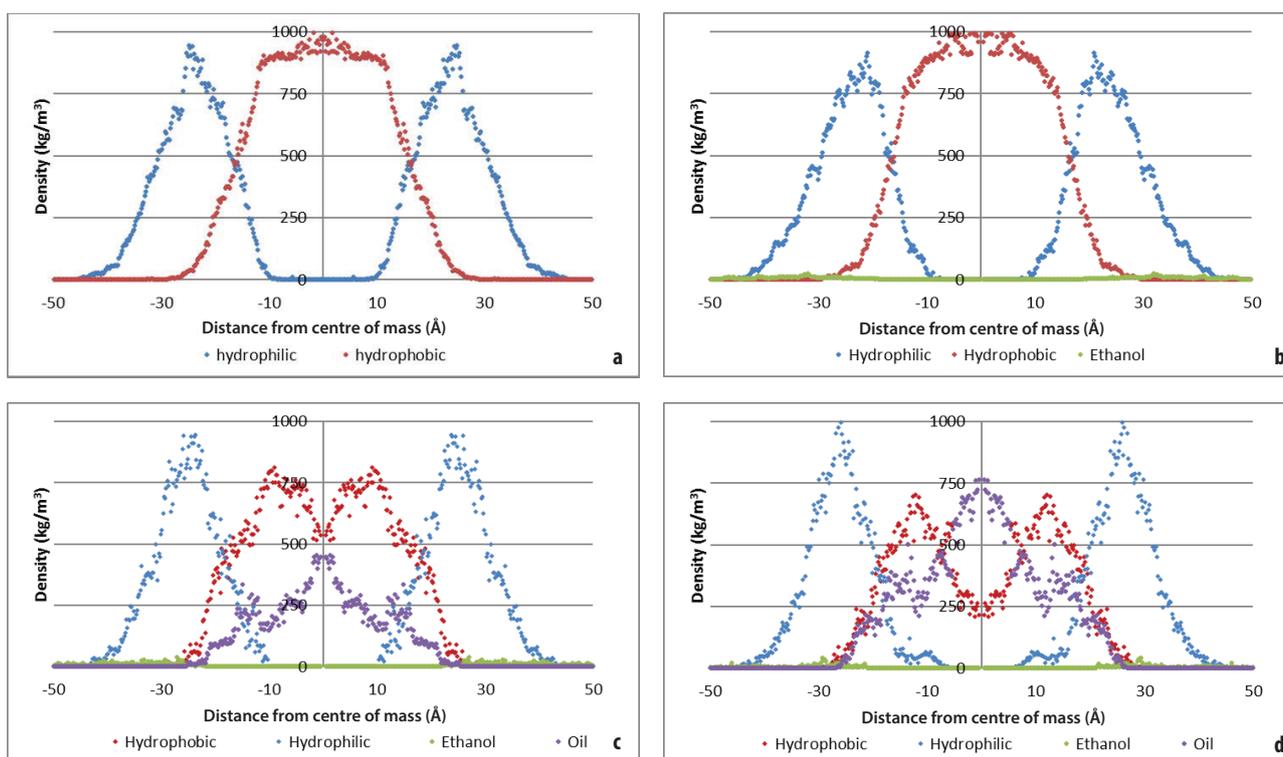

Fig. 4 Density profile of different systems as regards with the droplets centre of mass (a) MI, (b) MI-A, (c) ME-0.5, and (d) ME-whole. The centre of droplets mainly consisted of oil, covered by hydrophobic parts of the surfactant. The shell mostly contains the hydrophilic parts of the surfactant and the co-surfactant, wherever applicable.

Table 1. **Radius of gyration of the whole droplet as well as the hydrophobic core in the systems under study, averaged over the trajectory**

	Whole droplet (Å)	Hydrophobic core (Å)
MI	26.32	16.00
MI-A	26.55	15.87
ME-0.5	26.45	16.58
ME-whole	26.59	17.74

ME: microemulsion; MI: micelle.

Table 2. **The obtained ellipsoidal semiaxes (a, b, and c) and the eccentricity (e) of the systems under study**

	a (Å)	b (Å)	c (Å)	<a/c>	e
MI	38.65	35.93	26.08	1.48	0.73
MI-A	39.22	36.25	25.92	1.51	0.75
ME-0.5	39.04	34.88	27.52	1.42	0.71
ME-whole	38.74	35.65	27.62	1.40	0.70

ME: microemulsion; MI: micelle.

important change in the size of the whole droplet is observed (26.59 Å vs. 26.32 Å). This is not consistent with the general understanding about microemulsions as they are commonly known as swollen micelles.[22] This is probably due to fact that in a typical microemulsion, concentrations of oil and surfactant are more or less similar. Thus, a considerable volume of nanoemulsion droplet is expected to be occupied by oil molecules. However, in the system understudy, the total number of oil beads is 153 which is substantially smaller than the number of surfactant beads – 1680.

On the other hand, oil molecules have penetrated into the hydrophobic core and enlarged it (i.e. from 16.00 Å to 17.74 Å). The effect of this increase in the core size on the size of whole droplet is neutralized by stretching (i.e. thinning) the hydrophilic shell, thus, only a small change (i.e. <0.3 Å) is observed in the whole droplet size. The lack of a considerable change in the radius of gyration of micelle (MI) compared with microemulsion (ME-whole) has been experimentally reported in a similar system.[14]

Additionally, comparing the R_g values of MI with MI-A in Table 1 shows that when ethanol molecules are introduced to the system, a small decrease in radius of gyration of the core is observed while the whole droplets becomes somewhat larger. This is due to fact that some of the co-surfactant molecules go into the hydrophilic part of the micelle (i.e. micelle shell) which leads to expanding the shell. Simultaneously, the ethanol molecules "push" the hydrophobic parts of the surfactants together, making a degree of packing the core part of the micelle.

Using the equation $R = \sqrt{5/3}\,R_g$, the radius of the droplets in each system may be estimated as 33.98, 34.28, 34.15, and 34.33 Å for MI, MI-A, NE-.5, and ME-whole, respectively. The deviation of the radius and R_g of the micelle in current work from that of our previous report (i.e. 27.55 Å) is most probably due to the modifications performed on the mapping polyethylene glycol and alkyl parts of the surfactant.

To characterize the droplets shape, an ellipsoid was assumed for each droplet, having three semiaxes, a, b, and c. The semiaxes were computed using principal axes of inertia in the droplet $I_1 > I_2 > I_3$ by:

$$I_1 = \frac{1}{5}M(a^2 + b^2)$$
$$I_2 = \frac{1}{5}M(a^2 + c^2)$$
$$I_3 = \frac{1}{5}M(b^2 + c^2)$$

where total mass of droplet is shown by M and the eccentricity of the droplets was computed by the following equation:

$$e = \sqrt{1 - \frac{c^2}{a^2}}$$

Table 2 lists the shape parameters of the systems. Considering the details in Table 2, which lists the shape parameters of the droplets, a prolate ellipsoid is obtained for all the systems. Similar shapes have been documented for micelles with different surfactant types.[13,23,24] Furthermore, comparing MI with MI-A systems, a slight increase in the eccentricity is observed. This shows that the ethanol molecules which have entered the hydrophilic shell have made the droplets more elongated. While, addition of oil molecules has made the droplet slightly more spherical (i.e. less eccentricity and a/c values). To investigate this effect, one may consider the packing parameter, P, as a useful approach in predicting the geometry of micelles:

$$P = \frac{V}{a_0 l_c},$$

where V, a_0, and l_c represent the volume of hydrophobic tail, the headgroup area, and the maximum length of the tail.[25] Spherical micelles have P values <1/3, while non-spherical ones are characterized by higher values for P. In our previous work, a packing parameter of ~0.12 was determined for the surfactants in the micelle.[13] It is arguable that when oil molecules are introduced to the system, they enter the hydrophobic core and squeeze the hydrophobic tails of the surfactants. Therefore, the volume occupied by the hydrophobic tails decreases (i.e. V) and the packing parameter decreases too. Consequently, a more spherical micelle may be expected from the system. Similarly, ethanol molecules contribute to making smaller values for the headgroup area and larger values for P. As a result, deviation from sphere could be expected for the droplet. Undoubtedly, such changes in the micelle shape may be associated with changes in the thermodynamic stability of the system[26] and further studies are required to examine it.

Fig. 2 shows the average density profiles of the systems. As described previously,[13] the effect of the deviation of the droplets shape from sphere was ignored. In our previous work, the density profiles of the following moieties were reported for the polysorbate 80: the hydrophobic tails, the core parts of the molecule, the terminal polar droplets having hydroxyl groups, and the hydrophilic parts. In this work, to make

the comparison between the four systems easier, the molecular structure of polysorbate 80 was divided into two distinct groups: a hydrophilic part (containing –CH$_2$–CO–O–, –CH$_n$–CH$_n$–O–, and –CH$_n$–CH$_n$–OH) and a hydrophobic part (having –CH$_n$–CH$_2$–CH$_2$–CH$_2$– and –CH$_n$–CH$_2$–CH=CH–). Therefore, the density distribution of hydrophobic part, hydrophilic part, oil, and ethanol were computed.

From Fig. 4a, the core of the micelle consists merely of hydrophobic beads and the hydrophilic particles come into sight at ~8 Å from the centre of mass. Comparing the data from Fig. 4a and b, no considerable change in the distribution of surfactant droplets may be observed by the addition of ethanol molecules. The closest ethanol molecules to the centre of mass lie ~18 Å from centre. Their density then starts to increase to some extent and a peak in concentration appears to be ~32 Å off centre. This peak could be due to interactions between the ethanol molecules and the hydrophilic parts of the polysorbate 80, especially the beads having hydroxyl groups. The interaction level for these beads with ethanol is defined as level II in the forcefield,[19] showing strong attractions between them, probably due to the existence of hydrogen bonds. Remembering the important effect of co-surfactants in forming microemulsion droplets, this peak could be associated with forming stable microemulsion droplets.

Moving on to the Fig. 4c and d, the oil molecules appear to push the hydrophobic parts and locate into the centre of the droplet. Their density then starts to decrease and the oil molecules disappear at ~25 Å and ~27 Å from centre of mass for the ME-0.5 and ME-whole, respectively. Interestingly, at 12-15 Å from centre, a transient increase in the density of oil is observed, at which the density of oil and hydrophilic parts of the polysorbate 80 are more or less equal. Considering the two bead types present in the oil, the alkyl group (i.e. –CH$_n$–CH$_2$–CH$_2$–CH$_2$–) may not be the cause as the group has a strong hydrophobic nature, thus, cannot make suitable interactions with the hydrophilic parts of the surfactant. We believe that the increase in concentration of oil is due to its –CH$_2$–CO–O– bead types which could make suitable interactions with the hydrophilic parts of the surfactant (i.e. interaction level IV).
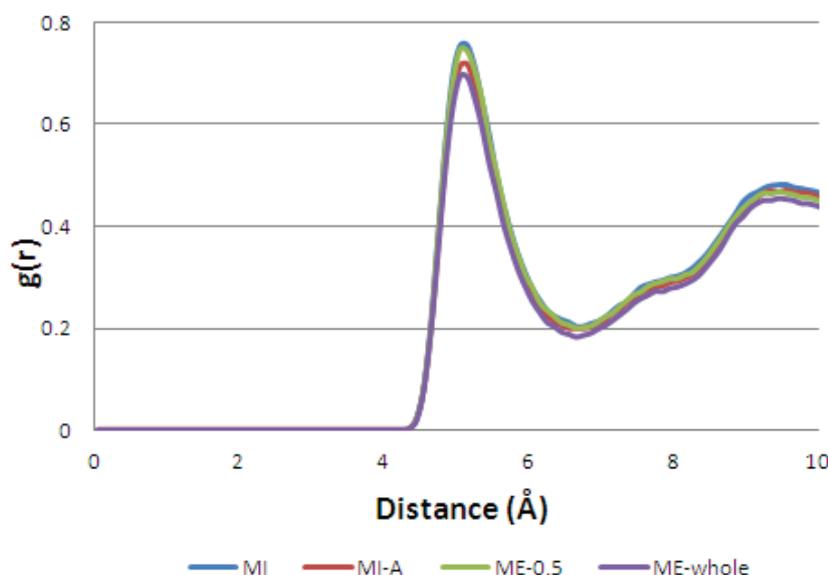
Fig. 5   **Averaged radial distribution functions for the four systems with water beads.**

shells, which are composed of surfactant molecules, in interaction with water molecules.

Conclusion

The MD simulations of a nano-system containing polysorbate 80 as surfactant with or without ethanol (i.e. co-surfactant) and oil were performed in this study to identify the possible structural changes. In general, oil molecules tend to move to the central region of the droplets, thus, making the hydrophobic core of the droplets larger. Interestingly, insertion of oil/ethanol does not influence the overall size substantially. It was also found that addition of oil molecules make the droplets more spherical, while ethanol molecules deviate the aggregate from a spherical shape.

Acknowledgment

Furthermore, by adding oil to the system, the ethanol molecules are driven from the droplet: they start to show up at ~21Å from centre of mass in ME-0.5 and ME-whole, compared with ~18Å in MI-A.

Moreover, the radial distribution function, $g(r)$, of each of the moieties was computed and reported in Fig. 5. As the details show, the hydration shells do not appear to be different in the four systems, showing the importance of the

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