# Molecular Dynamics Simulations of Nanoemulsions: Effect of the Initial Geometry and Concentration

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**Abstract.** MD simulations were used to characterize the behavior of a natural antioxidant, δ-tocopherol, in a linoleic acid micelle in the presence of β-cyclodextrin as a surfactant and surrounded by water. Four different simulations were performed with different starting points and number of molecules in order to assess the importance of the initial structure and to evaluate the influence of concentration on the geometric disposition of the antioxidant with respect to the micelle. The results indicate that the surface area of the constructed oil droplet is enough to harbor the number of molecules that match the experimental molecular concentration, but that is not the case when the number increases to twice the experimental value. Also, even though the δ-tocopherol moves to the interior of the droplet across the simulations, its hydrophilic head moves out. The resulting data show the dispersive nature of the forces bonding the δ-tocopherol and β-cyclodextrin.

**Keywords:** Molecular Dynamics · Nanoemulsions · Micelles · Antioxidants

## 1 Introduction

Since the last decades of the twenty century, classical molecular dynamics (MD) has become a key methodology to address computationally the study of biomolecular systems and the characterization of the effects in surfaces. This technique provides atomic-level insights into different features of these systems, like conformational dynamics, folding pathways, and interfacial interactions. Although there is a considerable amount of studies that apply MD simulations to analyze the behavior and properties of micelles [1–6], it is scarcely used compared to the more established fields of biomolecular systems and surfaces. This disparity may be due partly to the challenges that arise when accurately modeling the dynamic amphiphilic nature of micelles. They include modelling, among others features, their size polydispersity, the shape fluctuations, and the solvent-exposed hydrophobic cores. This involves using sophisticated force fields and long simulation timescales, together with specialized protocols to achieve reproducible self-assembly and thermodynamic equilibrium. All these question have limited the use of MD simulations in micelles until recent times, when the advances in computational power and sampling techniques have boosted this use.

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O. Gervasi et al. (Eds.): ICCSA 2026 Workshops, LNCS 16766, pp. 291–299, 2027.
https://doi.org/10.1007/978-3-032-30539-8_22

The importance of micelles in different scientific fields is undeniable. They play a pivotal role in essential areas like drug delivery, cosmetics, food technology, environmental remediation and biochemistry. They are usually employed as nanocarriers that solubilize hydrophobic molecules in water via their hydrophobic cores and hydrophilic coronas. Antioxidants, such as polyphenols or phenolic compounds, are usually combined with micelles because they placed themselves mostly at the surfactant-water interface [7–9]. Positioned in this area they can scavenge reactive oxygen species and prevent lipid peroxidation—a process implicated in oxidative stress-related diseases or in food degradation. Although experimental techniques can been used to elucidate the general morphology of micelles and the incorporation of antioxidants, they are not able to provide dynamical information about the atomic interactions that rule the behavior of antioxidants in micelles. The outcome of this interactions comes as a result of a subtle balance of electrostatic and van der Waals forces, which is well described using MD with explicit solvent models and all-atom force fields like CHARMM [10] or AMBER [11]. Thus, for example, MD studies have shown that spontaneous micelle formation around membrane proteins or drugs occurs due to Lennard-Jones interactions and that the micelle stability can be tuned using additives or electric fields.

Despite all collected knowledge, there are still gaps about how specific antioxidants influence micelle dynamics. For example, how critical micelle concentration and aggregation numbers are modified. By performing MD simulations, structural properties, such radial distribution functions, and dynamical properties, as diffusion coefficients, can be calculated, which can shed light on the antioxidants behavior within the micelle. In this study, MD simulations are used to characterize the behavior of a natural antioxidant, δ-tocopherol, in a linoleic acid micelle in presence of β-cyclodextrin as surfactant and surrounded by water. This work is a new example of MD applied to soft colloidal systems, focusing in analyze how important is the assembly of the starting systems and the concentration of species in the final outcome of the simulations.

## 2 Computational Details

### 2.1 System Preparation

In order to represent the experimental preparation of the nanoemulsions and to evaluate the importance of the molar concentration of the involved species, two different systems and two routes to build them were adopted. Thus, the nanoemulsions were created using two different sets of molecules that differ in the their number:

1. 900 molecules of linoleic acid plus 4 molecules of δ-tocopherol and 13 molecules of β-cyclodextrin, surrounded by water molecules. This number of employed molecules is proportional to the expected number of molecules present when following the experimental molecular concentration and water/oil ratio.
2. 900 molecules of linoleic acid plus 9 molecules of δ-tocopherol plus 30 molecules of β-cyclodextrin, surrounded by water molecules. This is about twice as concentrated as the experimental ratio.

Regarding the way in which we built up the micelle that forms part of the nanoemulsion, we have adopted two strategies:

I. To use the Packmol package [12] to create a spherical oily droplet with the δ-tocopherol and the linoleic acid molecules. The δ-tocopherol molecules were placed randomly, and the linoleic acid molecules under the condition that the external surface of the oily droplet, which is in contact with water, was made by the hydrophilic group of these molecules. Then, the structure was minimized with the molecular mechanics modules of the ORCA package [13]. This package was used because of its features to perform minimizations and to include AMBER parameters. The resulting structure was introduced again in Packmol to randomly add the β-cyclodextrin molecules to the surface of the oily droplet. Finally, a new optimization of the whole system was performed.
II. The second strategy is analogous to strategy I, but the oily droplet is created only with the linoleic acid molecules. The δ-tocopherol molecules are placed on the surface of the oily droplet together with the β-cyclodextrin molecules.

Strategy I tries to simulate the conditions of an experimental procedure in which linoleic acid and δ-tocopherol are solvated together in water and the emulsion is formed, and then the β-cyclodextrin is mixed in and solvated. Strategy II represents an experimental procedure in which micelles of linoleic acid are produced first and then, δ-tocopherol and β-cyclodextrin are solvated. By combining the two sets of molecules and the two strategies to build the systems, four different models were obtained, each of them was simulated using molecular dynamics.

### 2.2 Molecular Dynamics Simulations

The same procedure was used to perform the simulation of all the proposed systems. Thus, the aqueous phase (solvating water) was simulated by employing the LEAP module of the AMBER suit of programs, with a TIP3P water truncated octahedron box surrounding the oily droplet so that the distance between any point of the oil droplet and any size the octahedron box was not less than 23 Å. This distance was chosen to limit the aqueous volume and get reasonable computational calculation times. A representation of the resulting model is depicted in Fig. 1. The generalized AMBER force field (gaff) parameters were used to describe the interactions between linoleic acid, δ-tocopherol and β-cyclodextrin molecules, and the TIP3P model [14] was used to describe the water molecules. These are commonly used force fields . Before starting MD simulations, the nanoemulsion geometry was minimized without restraints with the conjugate gradient method at 0 K using periodic boundary conditions in a NVT ensemble. Long-range electrostatic interactions were calculated based on the particle mesh Ewald's method with a cut-off value of 12 Å. Next, a heating procedure was performed. This procedure consist in a MD simulation that was carried out for 20 ns and where temperature was increased in a NPT ensemble from 0 K to 300 K. The time-step of this simulation was 1 fs and a Langevin thermostat collision frequency of 1.0 was used to control the temperature. The pressure was maintained at 1 atm by using the Monte-Carlo barostat with a relaxation time of 2 ps. The SHAKE algorithm [15] was applied to constrain all bond interactions involving hydrogen. The same technical specifications were used in the subsequent equilibration of the system. The simulation was performed for 80 ns in a NPT ensemble with a time-step of 2 fs. After the equilibration, a MD production simulation with no changes

in technical parameters was performed. This production run was carried out for 1 μs and the trajectory was stored every 4 ps to a total of 250000 stored snapshots. The MD simulations and the analysis of the collected snapshots were performed with the AMBER package [11]. The visualization of the structures and trajectories resulting from the MD simulations were performed with the VMD package [16].

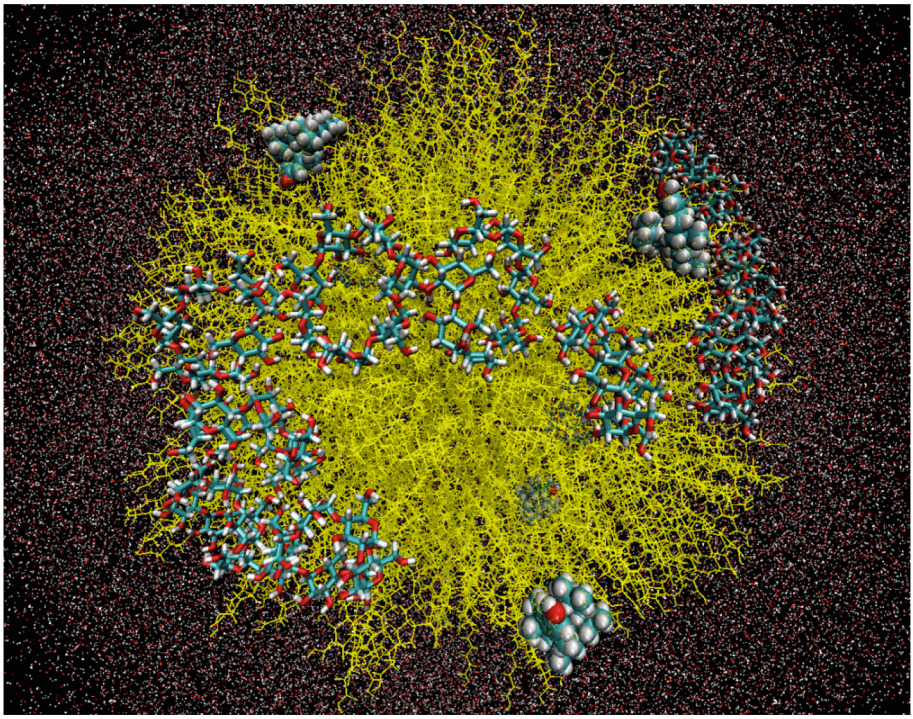

**Fig. 1.** Picture of one of the initial geometries employed in the molecular dynamics simulations. Water molecules are represented by points. Linoleic acid molecules are represented by lines. Van der Waals representation is used to depict δ-tocopherol molecules. β-cyclodextrin molecules are shown using a licorice representation.

## 3 Results and Discussion

As been already said, four simulations were performed to evaluate the influence of the starting points and the molecular concentration on the characterization of the studied system by molecular dynamics. From now on, we will label the simulation as 4Toc-I, 4Toc-II, 9Toc-I, and 9Toc-II. 4Toc corresponds to the system with four δ-tocopherol and thirteen β-cyclodextrin molecules, and 9Toc corresponds to nine δ-tocopherol and thirty β-cyclodextrin molecules. I and II correspond to strategies I and II, respectively. Thus, by comparing 4Toc-I and 9Toc-I, or 4Toc-II and 9Toc-II the effect of the molecular concentration can be analyzed. Also, if the comparison is between 4Toc-I and 4Toc-II

or between 9Toc-I and 9Toc-II, then it is the effect of the starting point of the simulation the subject that is evaluated.

Figure 2 depicts the density of probability for each of the components of the nanoemulsion. It can be seen in this figure that the thickness of the interface increases when the number of δ-tocopherol and β-cyclodextrin molecules is increased, regardless of the strategy used to build the initial structure. However, if the same number of δ-tocopherol and β-cyclodextrin molecules are considered, it can be seen that strategy II, to start the simulations with these molecules at the surface of the oil droplet, decreases the size of the interface. This may be as a consequence of δ-tocopherol and β-cyclodextrin interacting and intertwining, creating a more stable net on the surface of the droplet that produces a clearer boundary between the interface and the aqueous phase.

For this kind of nanoemulsions, it is of great interest to analyze how the δ-tocopherol is placed with respect to the surface of the droplet, since when it is localized at the interface, it can play efficiently its role as an antioxidant. Even when the starting configuration involves the antioxidant at the surface of the oil droplet, Fig. 2 shows that the δ-tocopherol moves to more profound positions across the simulation. The largest part of the tocopherol population is located within the droplet for all simulations. Yet, there are still some perceptible differences in the interface population between simulations performed with strategy I and those performed with strategy II. Although not too big, these differences may be a consequence of the intermolecular interactions between δ-tocopherol and β-cyclodextrin molecules. More precisely, due to the attractive forces between the hydrophilic head of δ-tocopherol and the hydroxyl groups of β-cyclodextrin.

When comparing the density of probability of δ-tocopherol for the 4Toc-II and 9Toc-II simulations, it can be observed that, of the two, the model with the higher molecular concentration is more populated inside the droplet. It seems to suggest that the surface area of the droplet is not enough to hold a concentration as high as that represented by model 9Toc, although from the proportions depicted in Fig. 1, it may look otherwise. This effect combines with the others already discussed, resulting in the translation of the δ-tocopherol molecules to the interior of the oil droplet.

In the previous paragraphs, the distribution of δ-tocopherol as a whole molecule has been discussed. In order to perform that, the center of mass of the molecule was used to obtain the density distribution. However, different observations can be obtained by analyzing the distribution of the hydrophilic head of δ-tocopherol instead of the whole molecule. This can be done by calculating the density of probability with respect to the oxygen atom of this hydrophilic head. Thus, when simulations with strategy I are considered, it can be seen that, even though most of the population is inside the droplet, the density presents one peak in the interface in both simulations. This may reflects the fact that, although the hydrophilic head started inside the droplet at the initial point, the simulation process moved this moiety out for some of the δ-tocopherol molecules. This behavior is opposite of what is observed for the molecule as a whole. Because the hydrophobic chain of the tocopherol gradually penetrates deeper into the micelle. Figure 2 shows that, when simulations 4Toc-II and 9Toc-II are analyzed, the largest population of the hydrophilic head is in the interface for 4Toc-II, and that 9Toc-II has a high peak at the interface, but it is counterbalanced by two peaks inside the droplet that, when added up, present a larger population than the one in the interface. This feature of

simulation 9Toc-II seems to support the observation that the surface area of the drop is not big enough for the high molecular concentration that this system represents.

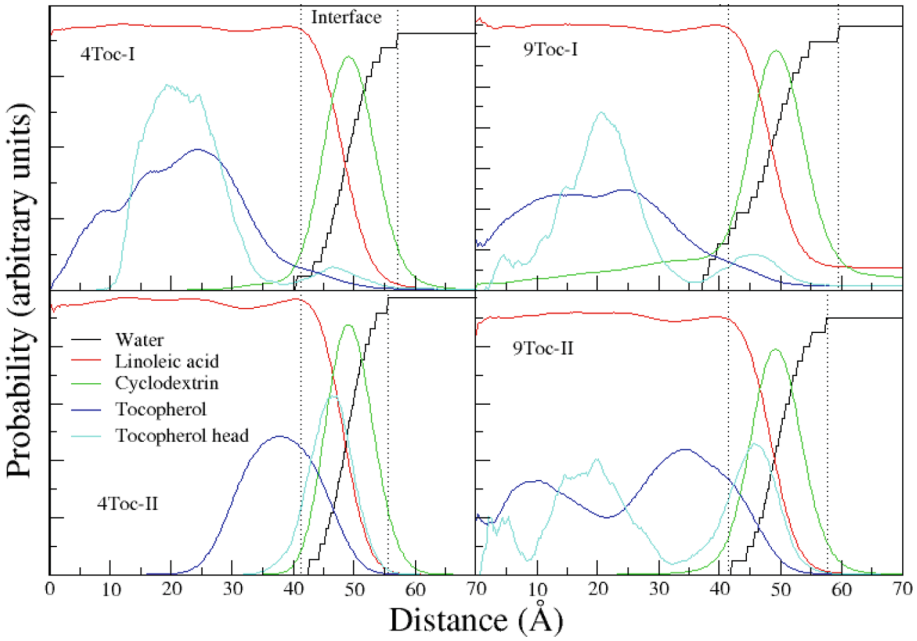


**Fig. 2.** Density of probability of the individual components of the nanoemulsion, plus the hydrophilic head of δ-tocopherol, with respect to the center of mass of the oil core of the nanoemulsion droplet for each of the four molecular dynamics simulations performed. The interface is marked by the dotted lines. See the text for an explanation of the labeling.

According to our results, the β-cyclodextrin molecules are placed mainly in the interface. By taking this into account, the number of hydrogen bonds that the δ-tocopherol establishes with the linoleic acid and the cyclodextrin may provide complementary information about the location of the antioxidant. Table 1 gives the percentage of hydrogen bonds formed by the hydroxyl hydrogen of one δ-tocopherol molecule with the carboxylic oxygen of the linoleic acid and any oxygen of the cyclodextrin. When considering the two simulations performed with strategy I, the results show that the one with the higher concentration has a lower percentage of hydrogen bonds with linoleic acid and a higher percentage with cyclodextrin. This observation agrees with the density curves in Fig. 2, where the peak in the interface is higher for 9Toc-I than for 4Toc-I. If simulations 4Toc-II and 9Toc-II are considered, the percentage of hydrogen bonds increases with concentration for both: linoleic acid and cyclodextrin, reflecting the shape of the curves in Fig. 2, where the tocopherol head is mainly in the interface for the 4Toc-II model but for the 9Toc-II model it has two peaks inside the droplet.

**Table 1.** Percentage of hydrogen bonds established by the hydrogen atom of the hydrophilic head of one δ-tocopherol molecule with the carboxyl oxygen of linoleic acid molecules and the oxygen atoms of β-cyclodextrin molecules.

| | 4Toc-I | 9Toc-I | 4Toc-II | 9Toc-II |
|---|---|---|---|---|
| $O_{Linoleic}$ | 49.0% | 37.4% | 14.1% | 23.2% |
| $O_{Cyclodextrin}$ | 0.0% | 2.3% | 1.9% | 3.1% |

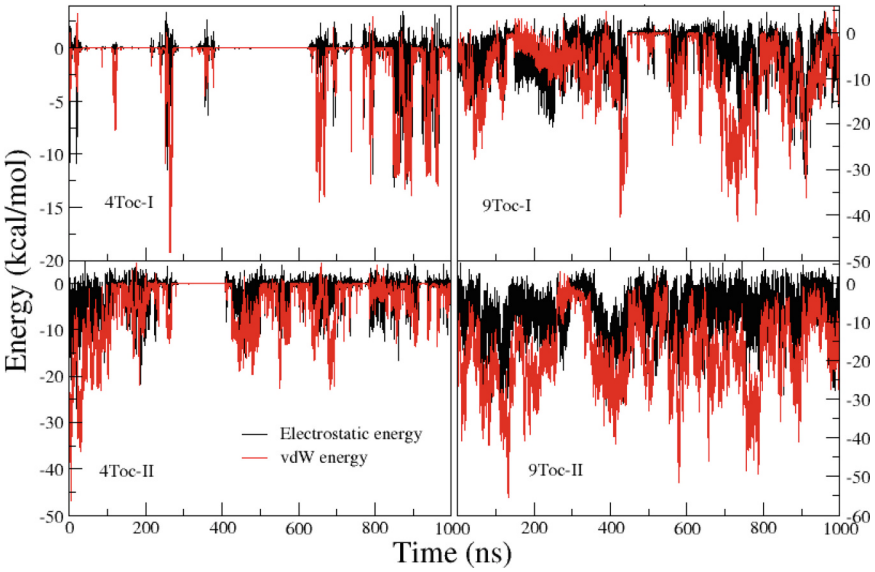


**Fig. 3.** Interaction energy between the δ-tocopherol and the β-cyclodextrin molecules split in the electrostatic and van der Waals terms along the simulation. See the text for an explanation of the labeling.

According to the literature [17, 18] δ-tocopherol and β-cyclodextrin can form an intermolecular complex in which the tocopherol is inserted into the cyclodextrin ring. In our simulations, we did not evaluate directly the existence of this kind of complex from a geometric point of view. Yet, we have obtained the interaction energy between tocopherol and cyclodextrin. Figure 3 presents the electrostatic and van der Waals contributions to the interaction energy between δ-tocopherol and β-cyclodextrin along the simulations. This figure shows that the higher the concentration, the stronger the interaction energy. Moreover, when using strategy II, with both groups of molecules initially on the surface, the interaction is stronger. The stronger the interaction, the more likely is that the molecules form intermolecular complexes. Although strategy II favors stronger interactions between these molecules, Fig. 3 illustrates that there are some parts of the simulation of the 4Toc-II model in which the interaction energy is zero or close to it. Suggesting that, along the simulation, the molecules move closer together, then move apart to a distance at which they cannot see each other, and then move closer again.

Finally, Fig. 3 shows that, along the simulations, the van der Waals term is usually more important to the interaction energy than the electrostatic term. This indicates that the interaction represented by these curves is more closely related to dispersive forces than to electrostatic forces.

## 4 Conclusions

Molecular dynamics simulations were used to characterize the behavior of a natural antioxidant, δ-tocopherol, in a linoleic acid micelle in the presence of β-cyclodextrin as a surfactant and surrounded by water. In order to check the effect of the molecular concentration and the importance of the assembly of the starting systems four different sets of simulations were performed. The strategy for building up the initial geometries was designed to represent the difference between an experimental procedure in which linoleic acid and δ-tocopherol are solvated together in water, resulting in the formation of an emulsion, and another procedure in which the emulsion is created only with linoleic acid and subsequently the δ-tocopherol is solvated.

It was found that the thickness of the interface decreases in simulations in which δ-tocopherol and β-cyclodextrin were placed on the surface of the droplet at the start of the simulation. This can be due to the two groups of molecules intertwine to create a stable network on the surface of the droplet that contributes to a more defined boundary of the interface.

Although it is not entirely clear, it seems that the behavior of the δ-tocopherol as a whole and that of its hydrophilic head are opposite to each other. Along the simulation, the δ-tocopherol appears to move toward the interior of the droplet, while the hydrophilic head moves toward the interface. This may suggest that the δ-tocopherol positions itself to interact with the cyclodextrin and the aqueous medium.

The analysis of the interaction between the cyclodextrin and the tocopherol shows that of dispersive forces predominate over electrostatic interactions. Moreover, this analysis reveals that the molecules can arrange themselves in such a way that they cannot see each other. This results in sections of the simulation in which the interaction energy is zero.

**Acknowledgments.** This study was funded by Ministerio de Ciencia e Innovación (grant number PID2022-136443OB-I00) and Xunta de Galicia (grant number GRC2024/27).

**Disclosure of Interests** The authors have no competing interests to declare that are relevant to the content of this article.

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