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NSAID solubilisation promotes morphological transitions in Triton X-114 surfactant micelles

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1. Introduction

Drug delivery systems are used to control the precise delivery of therapeutics to a biological target. With advances in nanotechnology, drug carriers are continually improving, providing better solubilisation properties and more precise control over where drug molecules are delivered. A wide range of materials are used to manufacture drug delivery vehicles (DDV), including polymers, lipoproteins, nanoparticles, and surfactants. One major family of DDVs are self-assembled nanostructures. Self-assembly enables a simple and cost-efficient method for creating nanoparticles. During the self-assembly process, the materials organize into ordered nanostructures through non-covalent interactions. In the work reported here, we focus on surfactant-based DDVs. Surfactants (a portmanteau of ‘surface active agents’) are amphiphilic molecules, which have two covalently linked moieties - a hydrophilic headgroup and a hydrophobic tail. As a result, they self-assemble in aqueous solutions into structures such as micelles or liposomes. Because of these unique properties, surfactants are commonly used as detergents, emulsifiers, antimicrobial agents, protein denaturation agents and DDVs. Non-ionic surfactants have become attractive materials for the latter, because of their biodegradability and their lower toxicity relative to the charged surfactants. With their high drug-loading capacity, cost-efficiency and simplicity of production, surfactant-based micelles are attractive candidates as DDVs.

In this manuscript, we are investigating the non-ionic surfactant Triton X-114 (TX-114). TX-114 surfactants consist of a hydrophilic polyethylene oxide (PEO) headgroup, comprising eight EO units, and a 4-(1,1,3,3-tetramethylbutyl)-phenyl hydrophobic tail. TX-114 is well-known to self-assemble into micelles in aqueous solution and is commonly used in cell lysis and protein extraction. However, only a small number of previous studies have investigated this surfactant and its self-assembly using computational methods. Yordanova et al. reported optimized CHARMM parameters for TX-114, which we have used in this work.
Generally, Ritter et al. described the prediction of the micelle water partition coefficients for different micelles including those made from TX-114, [24] using the COSMOmic software package [25].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are therapeutic agents used primarily for the treatment of pain and inflammation [26–29]. The poor solubility of NSAIDs complicates the development of oral or injectable pharmaceutical formulations used to deliver these drugs and restricts their application in oral and parenteral applications [30–34]. Therefore, the design of formulations for NSAIDs is challenging, requiring investigations into the solubilisation of this class of drugs in the presence of surfactants [35–42]. Understanding the atomistic interactions that underlie the solubilisation of NSAIDs within surfactant micelles, as revealed in this work, provides important information that is necessary to further improve these formulations.

In this study, we use all-atom molecular dynamics (MD) simulations to investigate the solubilisation of ibuprofen (C13H18O2) and indomethacin (C19H16ClNO4) sodium ions (Fig. 1), and their effect on the structural properties of TX-114 micelles. Both of these drugs are deprotonated at neutral pH. Nevertheless, despite this ionisation, these drugs are poorly soluble in aqueous solution, and in order to increase their bioavailability, DDVs are used. Our simulations show that significantly more indomethacin than ibuprofen is solubilised in the Triton X-114 micelles. This increased solubilisation of indomethacin is found to be driven by strong interactions between indomethacin molecules in the core of the micelle, which leads to large aggregates of drug molecules within the micelle. As a result, we find that the solubilisation process of the indomethacin ions results in the Triton X-114 micelle finally splitting into two daughter micelles, which are unequal in size.

2. Methods

2.1. Molecular dynamics simulations

We have studied three systems: a TX-114 micelle in aqueous solution, and a TX-114 micelle interacting with each of the two NSAIDs, ibuprofen and indomethacin (Fig. 1a & b, respectively). The CHARMM36 General Force Field (CGenFF) was used to model ibuprofen and indomethacin [43]. The TX-114 molecules were modelled by the CHARMM parameters reported by Yordanova et al. [23]. Water was modelled by the CHARMM-modified TIP3P potential [44]. All simulations were performed using the GROMACS 2020 simulation engine [45].

The TX-114 micelle was pre-assembled into a spherical structure using Packmol [46]. The micelle-only simulation was conducted with a box of initial dimensions 120 × 120 × 120 Å³.

![Fig. 1. Chemical structures of molecules studied by MD simulations. (a) Ibuprofen, (b) Indomethacin, and (c) Triton X-114 (for which n = 8).](image)
The shape of the micelle was characterized by its ellipticity ($\epsilon$), which is defined as:

$$\epsilon = \frac{I_{\text{max}}}{I_{\text{min}}}$$

(2)

where $I_{\text{max}}$ and $I_{\text{min}}$ are the largest and smallest moments of inertia of the micelle. We note that in the case of a perfect sphere, $\epsilon = 1$ and $\epsilon$ increases as the micelle becomes more non-spherical. The shape of the micelle can be further characterized by comparing the moments of inertia with respect to principal axes of the micelle. The micelle is oblate, if two values are close to each other and larger than the third, prolate, if smaller than the third, spherical if all values are approximately the same and triaxial, if all values differ. The solvent-accessible surface area (SASA) of the micelles was calculated using the Shrae-Rupley algorithm with a probe radius of 1.4 Å [54,55].

The intrinsic core–shell interface (ICSI) algorithm was used to investigate the internal and interfacial structure of the TX-114 micelle in solution, as previously applied to polymer micelles made from Tetronic 904 and Pluronic L64 [56,57]. The intrinsic density of each micelle component (in this case, the hydrophobic TX-114 tails, its hydrophilic PEO chains, and water) is expressed as follows, using a spherical polar coordinate $(r, \theta, \phi)$ basis:

$$\hat{\rho}(r) = \frac{\sum_i \delta[r - (r_i - \zeta(\theta, \phi))]}{S_i(r)}$$

(3)

Here $r_i$ is the $r$-position of atom $i$ and $\zeta(\theta, \phi)$ is the $r$-position of the intrinsic core–shell interface. Since the normalisation factor, the

<table>
<thead>
<tr>
<th>System</th>
<th>NSAID</th>
<th>Surfactant</th>
<th>Water</th>
<th>TX-114 Conc. [moldm$^{-3}$]</th>
</tr>
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<tr>
<td>TX-114</td>
<td>0</td>
<td>150</td>
<td>51958</td>
<td>0.14</td>
</tr>
<tr>
<td>TX-114 - IBUP</td>
<td>100</td>
<td>150</td>
<td>74779</td>
<td>0.11</td>
</tr>
<tr>
<td>TX-114 - INDO</td>
<td>100</td>
<td>150</td>
<td>74437</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Fig. 2. Structure of the TX-114 micelle. (a) Intrinsic density of the TX-114 micelle obtained using the ICSI method: hydrophobic tails (red), hydrophilic PEO chains (orange) and water (blue). (b) Snapshot of TX-114 micelle. Probability distributions of the different values of (c) ellipticity and (d) the solvent accessible surface area (SASA).
The average volume of each shell, $S_i(r)$, cannot be found analytically, we use Monte Carlo integration to find this quantity as

$$S_i(r) = \frac{n_i \nu_{\text{box}}}{N},$$

(4)

Note that $n_i$ is the number of random points identified in the shell in which atom $i$ is found and $\nu_{\text{box}}$ is the average volume of the simulation box (both averaged over all of the different frames analysed). $N$ is the total number of random points used in the normalisation process. Given the highly non-spherical and disordered nature of the TX-114 micelles in the presence of the drug molecules, it was not appropriate to use this method, with its spherical polar coordinate basis, to investigate these systems in the same way.

**Drug solubilisation within the micelles.** To evaluate drug solubilisation, the distances between the centers of masses (COM) of drug molecules and the TX-114 surfactants were calculated. If a drug is found within 5 Å of the nearest surfactant, it is considered to be solubilised (Fig. S15). The cut-off distance of 5 Å was selected by measuring the minimum distance between reference atoms within ibuprofen and indomethacin molecules and in Triton X-114 surfactant molecules.

**Fig. 3.** Solubilisation of drugs within TX-114 micelles. The number of drug molecules solubilised within the micelle as a function of time for the (a) ibuprofen and (c) indomethacin systems. (e) The number of indomethacin molecules solubilised within the smaller daughter micelle (SM), the larger (LM) daughter micelle, and the total number of indomethacin in both micelles after the original micelle splits. Snapshots of the systems: (b) TX-114-IBUP, (d) TX-114-INDO before splitting and (f) TX-114-INDO after splitting.
The hydration of the drug molecules was characterized by calculating the number of water molecules as a function of their radial distance from the nearest surfactant $D_r = r_d - r_i$, where $r_d$ and $r_i$ are the radial distances of the drug molecule and the hydrophobic tails of its nearest surfactant, respectively, from the micelle’s center of mass. A value of $D_r > 0$ indicates that the drug molecules are outside of the hydrophobic core of the micelle, while $D_r < 0$ implies the drug is buried within the micellar core. This analysis is useful since the disordered structures of the micelles in the presence of NSAIDs are otherwise challenging to analyse. The radial distribution function (RDF) is defined as

$$g(r)_{ij} = \frac{\rho(r)_{ij}}{\rho_j}$$

where $g(r)_{ij}$ is the probability of finding a particle of type $j$ at a distance $r$ from a reference particle of type $i$, $\rho(r)_{ij}$ is the density of type $j$ particles at a distance $r$ from a type $i$ particle and $\rho_j$ is the average density of type $j$ particles. The coordination number of water around selected drug atoms of interest was calculated using the position of the first minimum in their respective RDFs (Fig. S1). Values of 3.5 Å and 7.2 Å were used for the chlorine and nitrogen atoms in indomethacin, respectively. A value of 2.8 Å was used for the carboxylate oxygen atoms found in both drugs.

Contact maps were generated to characterise the drug-drug and drug-surfactant interactions in detail. The distances between all of the non-hydrogen atoms of the corresponding molecules were calculated, with any distance less than 5 Å deemed to indicate atoms in contact. These calculations were averaged over the final portion of the trajectories, where the number of solubilised drug molecules had reached stationarity. The contacts maps are normalised independently, with a value of 1 identifying the most frequent interaction in each case. The distribution of drug cluster sizes at different times during the simulation was calculated using a graph-theoretical approach described previously [56]. Here, we used a cutoff distance of 7.5 Å between N atoms (for indomethacin) and C8 atoms (for ibuprofen).

3. Results

3.1. Pure Triton X-114 micelle

The Triton X-114 micelle is stable in an aqueous environment, as demonstrated by the distribution of the number of surfactant molecules found in the micelle shown in Fig. S2, which shows at most a few molecules are found isolated in solution at any one time. The distribution of PEO headgroups and the hydrophobic tails of the TX-114 molecules within the micelle are shown in the plot of the intrinsic density (Fig. 2a). We note that the core of the micelle...
consists predominantly of the hydrophobic tails of TX-114, as expected for the micelles formed by a typical amphiphile. The peaks in the hydrophobe intrinsic density for \(-10 \text{ Å} < r < 0\) Å are indicative of the reordering of the tails at the interface with water. We also observe a significant population of PEO chains within the core of the micelle, which has been proposed to occur previously by Elworthy et al.\[58\]. This is different to the analogous observation for micelles made of amphiphilic block copolymers with larger hydrophobic blocks: a more effective partitioning is observed in the case of Pluronic L64 and Tetronic 904\[57\], where very little PEO density is observed in the core of the micelle. We do, however, observe the exclusion of water from the hydrophobic core of the micelle. We also see a small peak in the density of water at approximately 3.5 Å, which is a characteristic signature of water ordering at a hydrophobic interface. Additionally, from the application of the ICSI method, we are able to determine the average core radius of the micelle as 19.8 ± 0.4 Å.

The ellipticity of the TX-114 micelle, which remains approximately constant during the course of the production simulation (Fig. S4a), is 1.6 ± 0.1 (Fig. 2c). Therefore, the micelle has adopted a triaxial shape, where the length of its primary axis is found to be 101.2 ± 0.2 Å. The surface area of the micelle is determined by measuring its solvent accessible surface area (SASA), which also remains approximately constant throughout the production simulation (Fig. S6a) and has an average value of 4.745 ± 0.009 × 10⁴ Å² (Fig. 2d).

### 3.2. Solubilisation of NSAIDs within the TX-114 Micelle

After ibuprofen and indomethacin are placed into the aqueous environment around a TX-114 micelle, we see that approximately 40 drug molecules solubilise in the micelle within 40 ns (Fig. 3a & c). In the case of ibuprofen we find that there is a slight increase in the amount of drugs solubilised within the micelle and then after 200 ns the amount of drug in the micelle remains approximately constant (51 ± 4). Approximately 64% of the ibuprofen molecules are solubilised within the hydrophobic core of the micelle, while the remainder are solubilised within the EO hydrophilic corona of the micelle. The solubilisation of ibuprofen within the micelle results in the micelle transitioning to a prolate, rod-like shape (ellipticity ~ 2.53 ± 0.03, Fig. S4b). With this transition in shape of the micelle, the major axis of the micelle is 36% longer than the pure micelle (137.7 ± 7.3 Å) and the solvent accessible surface area increases to 5.621 ± 0.007 × 10⁴ Å² (Fig. S6b).

During the solubilisation of indomethacin, we observe that the amount of drug solubilised in the micelle continues to increase until t ≈ 900 ns. During this time, 80 ± 3 indomethacin molecules are solubilised within the micelle, which is approximately 1.4 times larger than found with ibuprofen. We find that approximately 66% of the indomethacin molecules are solubilised in the hydrophobic core of the micelle, with the remaining 34% located in the corona of the micelle. As with the ibuprofen-loaded micelle, we observe that the micelle transitions to a rod-like shape (elliptic-

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**Fig. 5. Interactions between pairs of neighbouring drug molecules.** Contact maps which show the amount of contact between two atoms on two neighbouring drug molecules for (a) ibuprofen and (c) indomethacin. Diagrams of (b) ibuprofen and (d) indomethacin molecules which have been coloured by their amount of contact with neighbouring drug molecules, where the colours are consistent with those used in the contact maps. Atom labels used within the contact maps are those shown in Fig. 1.
ity $\sim 3.71 \pm 0.02$, Fig. S4c). As a result of this change in shape, the solvent accessible surface area of the micelle also increases to $6.575 \pm 0.018 \times 10^4 \text{Å}^2$ (Fig. S6c).

Unlike with ibuprofen, we find that the solubilisation of indomethacin destabilises the drug-loaded micelle within the simulation timescale. We note that dynamic morphology changes occur for surfactant micelle with and without the solubilisation of small molecule,[59,60] however only as a result of indomethacin solubilisation are we able to observe this phenomenon here directly on a millisecond timescale. The original TX-114 micelle splits into two daughter micelles. The two resulting micelles have different aggregation numbers, where the larger micelle consists of 100 surfactant molecules and the smaller micelle consists of 50. Indomethacin molecules are solubilised in both of the daughter micelles with 43 ± 2 and 32 ± 2 drug molecules in the larger and smaller micelle, respectively. In both of the daughter micelles, 70% of the drug molecules are solubilised in the hydrophobic core of the micelle and the rest are found in the hydrophilic corona of the micelle.

Both of the daughter micelles are more spherical than the parent micelle was when it destabilised, with the smaller daughter micelle having a spherical shape (ellipticity $\sim 1.32 \pm 0.01$) and the larger one remaining prolate (ellipticity $\sim 1.88 \pm 0.01$) as shown in Fig. S4d. As shown in Fig. S6d, the larger daughter micelle has a major axis length of 109.7 ± 0.9 Å and a solvent accessible surface area of 3.960 ± 0.007 × 10^4 Å², while the smaller micelle has a major axis length of 73.4 ± 0.2 Å and a SASA of 2.207 ± 0.004 × 10^4 Å².

3.3. Specific Interactions between NSAIDs and TX-114 Micelles

Fig. 4 shows the contacts between the drug molecules and the TX-114 surfactant molecules, where each axis is labeled with the atom names shown in Fig. 1. For both drugs we find that they primarily interact with the same regions of the TX-114 surfactant: the 4-(1,1,3,3-tetramethylbutyl) group (atoms C5 & C6) and the benzene ring (atoms C1A - C1F) found in the hydrophobic tail of the surfactant molecule. Also, we find that the regions of the two drug molecules that interact with the surfactant molecules are largely driven by the fact that the drug molecules orient themselves within the core of the micelle so that their carboxylate groups remain hydrated by the water at the interface of the core of the micelle (Fig. S5). As a result, the carboxylate groups of the ibuprofen and indomethacin ions have only minimal contact with the EO monomers in the hydrophilic headgroups of the surfactant molecules (Fig. 4b & d). The methyl groups of the ibuprofen molecules are the primary portion of the drug molecule that is in contact with

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**Fig. 6. Evolution of drug clustering during the simulations.** Distribution of cluster sizes for ibuprofen during the initial stages (a) and the final stages of the simulation (b). Distribution of cluster sizes for indomethacin during the initial stages of the simulation (c) and the final stages of the simulation (d). We see that solubilisation does not particularly affect the aggregation of ibuprofen, while the assembly of large indomethacin aggregates are promoted by solubilisation.
the surfactant molecules. As for indomethacin, we see that the Cl atoms and the C12 - C14 carbons in the neighbouring benzene ring make significant contact with the hydrophobic tails of the surfactant molecules (Fig. 4d).

In Fig. 4, we show that during the solubilisation process of each drug there is some interaction of the drug molecules with the EO headgroups of the TX-114 surfactant molecules (atoms CA1 - CB2 & O1 - O3). For both ibuprofen and indomethacin the contact with the EO groups is distributed across the whole drug molecule, which indicates that there is no preferential interaction between the drugs and the EO groups, only that the drugs pass by them while moving towards the core of the micelle.

3.4. Aggregation of NSAIDs within Triton X-114 micelles

In order to determine the amount of aggregation of the two different NSAIDs within the core of the micelles, we have measured the amount of contact between the solubilised drug molecules. In Fig. 5a, we see that the ibuprofen molecules interact with one another via the methyl groups (atoms C9 - C12) on one end of the drug molecule (Fig. 5b). It is worth noting that the same region of ibuprofen is involved in the interactions with other ibuprofen molecules as well as the surrounding surfactant molecules.

Meanwhile the interactions between solubilised indomethacin molecules show that significant interactions are found between the chlorine (Cl) atom, the atoms in the neighbouring benzene ring (atoms C9 - C14) and then the ketone group (atoms O2 & C1) (Fig. S13a). As such, the primary mechanism of interaction between the indomethacin molecules is π–π stacking of their aromatic rings. Then the indomethacin molecules interact via their Cl atoms with the surfactant molecules.

Fig. 6 shows the distribution of drug cluster sizes at the start and end of each simulation. As we have demonstrated in previous sections, at the start of each simulation, most drug molecules are found free in solution, while by the end of each simulation, these molecules are mostly interacting with the TX-114 micelles. We see that at early stages of the simulations, ≈ 75% of ibuprofen clusters contain only a single molecule, while only ≈ 45% of indomethacin clusters correspond to free molecules at the same time. Interestingly, we note that interactions with the micelle do not particularly promote aggregation of ibuprofen, while a range of larger indomethacin aggregates are formed later in the simulations, driven by interactions with the micelle.

To prove these observations in more detail, we calculated the average drug cluster size as a function of its degree of solubilisation within the micelle (denoted by the number of contacts between each drug molecule and non-hydrogen surfactant atoms). We used a rather large interaction cutoff definition (10 Å) when measuring drug-surfactant contacts so as to be sensitive to the overall local environment of the entire drug molecule. These results are presented in Fig. S16. We note that significant clustering of indomethacin is promoted by contact with a relatively small number of TX-114 interactions and that its clustering is reduced as indomethacin molecules come into contact with a greater number of TX-114 atoms. The dependence of clustering upon the degree of TX-114 contacts is not seen as dramatically in the case of ibuprofen but follows a similar overall trend.

4. Conclusions

In this manuscript, we report on the size, shape and structural properties of a Triton X-114 micelle with an aggregation number of ~ 150 molecules. Previously, Yordanova et al. investigated the self-assembly of TX-114 micelles with MD simulations and determined the eccentricity values for micelles of various aggregation numbers [23]. Their results show that micelles with an aggregation number of more than 33 are non-spherical, which is consistent with the observation made here for our significantly larger micelles.

We have also investigated how the solubilisation of NSAIDs affect the structural properties of Triton X-114 micelles. In doing so, we have observed that TX-114 micelles solubilise more ibuprofen molecules than their TX-100 counterparts [61], during the same timescales. This trend is consistent with the experimental observation that for a variety of NSAIDs more drug molecules are solubilised in Triton X-114 micelles than those consisting of Triton X-100 [41,42]. In those experimental studies, the authors have suggested that this is due to the larger aggregation number found for Triton X-114 micelles in comparison to Triton X-100 micelles, and therefore a larger micelle size that accommodates more drug molecules. However, our two investigations, in which we have chosen to use the same aggregation number for each surfactant, suggest that it is not merely due to the difference in aggregation number and instead is a result of the smaller number of ethylene oxide monomers in the hydrophilic headgroups of the Triton X-114 surfactants, which allow for faster diffusion of the drug molecules to the core of the micelle where they are solubilised. In the case of indomethacin, we observed that as the micelle solubilises indomethacin molecules it becomes increasingly large in size and increasingly rod-like in shape until eventually it divides into two unequally sized daughter micelles. These daughter micelles are then, of course, smaller than the pure Triton X-114 micelles. This was also observed in the case of our previous work in which we investigated indomethacin-loaded Triton X-100 micelles [61]. Experimentally, Ullah et al. have found that Triton X-100 and X-114 micelles reduce in size when loaded with Meloxicam and Celecoxib, two other NSAIDs, unlike micelles made of other nonionic surfactants, including Tween 20, Tween 80, Brij 30 and Brij 35, whose size remains unchanged [42]. Our observation of the micelle division into smaller micelles upon loading of indomethacin provides a potential mechanistic description of the process by which this decrease in size occurs.

We find that both of the NSAIDs that we have studied primarily solubilise in the hydrophobic core of the micelle (64% of ibuprofen & 70% of indomethacin) but both drugs have significant numbers solubilised within the hydrophilic corona of the micelle as well. Within the hydrophobic core of the Triton X-114 micelles, we find that both drugs take preferential orientations. We find that they align such that their carboxylate groups are at the interface of the hydrophobic core of the micelle where they can remain hydrated by surrounding water molecules, and the rest of the drug molecules are located deeper within the core of the micelles where they form hydrophobic interactions with the hydrophobic tails of the Triton X-114 molecules and the hydrophobic groups of neighbouring drug molecules. The interactions we observe between the carboxylate group and the 4-(1,1,3,3-tetramethylbutyl) group of the ibuprofen molecules and the benzene ring of the Triton X-114 molecules are consistent with the experimental findings of Rub which showed that the same two molecules interact via their respective hydrophobic regions [40].

Importantly, we show here that the interactions between the drug molecules themselves and between the drugs and surfactants within a drug delivery vehicle are both important to consider when designing a stable formulation. As in the case of the indomethacin-loaded TX-114 micelles, we observe that there is significant aggregation of the drug molecules within the core of the micelle, which leads to the destabilisation of the micelle. The ibuprofen molecules do not form large clusters within the core of the micelle, and the micelle remains stable with the larger aggregation number. This insight provides important information for the further optimisation of Triton-based drug delivery formulations.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.molliq.2022.119050.

References