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Using coarse-grained molecular dynamics to understand the effect of ionic liquids on the aggregation of Pluronic copolymer solutions

Germán Pérez-Sánchez\textsuperscript{a,}\textsuperscript{*}, Nicolas Schaeffer\textsuperscript{a}, André M. Lopes\textsuperscript{b}, Jorge F. B. Pereira\textsuperscript{c} and João A. P. Coutinho\textsuperscript{a}

\textsuperscript{a}CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-1933 - Aveiro, Portugal
\textsuperscript{b}Faculty of Pharmaceutical Sciences, University of Campinas, Campinas, SP, Brazil.
\textsuperscript{c}Univ Coimbra, CIEPQPF, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II – Pinhal de Marrocos, 3030-790 Coimbra, Portugal

\textsuperscript{*}Corresponding author:
Prof. Germán Pérez-Sánchez
gperez@ua.pt (Phone: +351-234-370200)
Aveiro Institute of Materials, Department of Chemistry,
University of Aveiro
Abstract

This study is aimed to enhance the understanding of the interaction between ionic liquids (ILs) and non-ionic Pluronic triblock copolymers in aqueous two-phase micellar systems (ATPMS) used for selective separation/purification of hydrophobic biomolecules. The ILs allow a precise control of the cloud point phase separation temperatures (CPT), particularly important when the stability of the molecule is highly dependent on temperature. The effect of choline-based ILs, with two different counter anions, chloride and hexanoate, was evaluated by molecular dynamics simulations (MD) for F-68 and L-35 Pluronic aqueous solutions. The simulations revealed the role played by the anions during the Pluronic self-assembly, with choline chloride hindering the Pluronic aggregation, while the choline hexanoate favoured micelle formation and coalescence, in agreement with the experimental data. A detailed study of the accessible surface area of Pluronic showed a progressive dehydration of the Pluronic hydrophilic micelle corona in choline hexanoate mixtures promoting inter micelle interactions and, consequently, micelle coalescence. With the addition of choline hexanoate, it was observed that the hydrophilic segments, which form the micelle corona, twisted towards the Pluronic micelle core. The electrostatic interaction is also shown to play a key role in this IL-Pluronic aqueous solution, since the hexanoate anions are accommodated in the Pluronic micelle core while the choline cations are hosted by the Pluronic micelle corona, with the ions interacting with each other during the self-assembly process. In addition, a comparison study of F-68 and L-35 aqueous solutions, shows that the IL impact depends on the length of the Pluronic hydrophilic segment. This work provides a realistic microscopic scenario of the complex interactions between Pluronic copolymers and ILs.

Keywords: MARTINI model, choline and hexanoate coarse-grain model, cloud point temperature, molecular dynamics, Polymeric micelles.
1. Introduction

The interest in amphiphilic block copolymer aqueous solutions is growing due to the formation of diverse self-assembly morphologies and, consequently, their use in novel applications namely drug delivery, bio-imaging/sensing tools and highly efficient biocatalytic and separations platforms. Among the wide array of commercial amphiphilic polymers, a class of ABA triblock copolymers commercially known as Pluronic® (or Poloxamer®) is of particular interest since it offers a wide variety of amphiphilic characters. Specifically, Pluronic copolymers are composed by three blocks in A-B-A conformation: a hydrophobic poly(propylene oxide) block (PPO, part B) sandwiched between two hydrophilic poly(ethylene oxide) blocks (PEO, part A).

Pluronic copolymers in an aqueous environment self-assemble into micellar-type nanostructures, i.e., polymeric micelles, at concentrations above the critical aggregation concentration (CAC, equivalent to the critical micellar concentration - CMC). Since these micelles are composed of a hydrophobic core and a hydrophilic surface, they are significantly more stable than surfactant-based micelles, e.g., do not dissociate immediately after extreme dilution because of their remarkably low CAC (~10^{-5} – 10^{-7} mol dm^{-3}). In addition, they can create a well-defined biphasic solution, namely, by increasing the temperature above the polymer lower critical solution temperature (LCST) commonly known as cloud-point temperature (CPT), two immiscible water-rich environments (a micelle-rich (polymer-rich) phase and a micelle-poor (diluted) phase) are formed. Due to the thermo-responsive character of the micelles, these can be employed as aqueous two-phase micellar systems (ATPMS) for selective extraction of biomolecules, in alternative to the aqueous two-phase systems (ATPS) composed of polymers and salts.

The morphology of the Pluronic self-assembled system can be classified according to the volume fraction of PEO (hydrophilic portion) block of the copolymer ($f_{\text{PEO}}$ parameter). In general, the larger is the PEO moiety of the copolymer, the higher is the CPT in aqueous solution (i.e., higher LCST values). For example, Pluronic copolymers with $f_{\text{PEO}}$ values of 50 and 70-80% have CPT values of > 70 and > 100 °C, respectively. In fact, the high CPT of Pluronic-based ATPMS have initially prevented their use as viable options for the formation of self-
assembled polymeric micelles, particularly, because of operational difficulties in working at high temperatures (e.g. high energy consumption, difficulty in encapsulating drugs, precipitation of drugs and/or copolymers, and partial/total degradation of the target biomolecules). To overcome the temperature issues, the use of ionic adjuvants (e.g., ionic liquids - ILs), have been largely studied, since depending on the salting-in or salting-out ability of the adjuvant, an increase or decrease of the LCST values of the ATPMS can be achieved. In addition to the changes on CPT, the use of ILs as adjuvants can also provide other interesting features, namely a fine adjustment of the hydration properties of the system to act as facilitators for the solubilization of molecules; and the adjustment of the shape and size of the micelles that influence the interaction and encapsulation rates of molecules. Considering the eco-friendly and biocompatible characteristics of choline-based ILs, these appear as excellent choices for developing volatile organic solvents (VOC)-free protocols for the encapsulation of hydrophobic drugs in polymeric micelles at room temperature.

As recently demonstrated by our research group for Pluronic copolymers [such as L-35 (PEO_{11}-PPO_{16}-PEO_{11}) and F-68 (PEO_{76}-PPO_{29}-PEO_{76})], the use of choline ([Ch]+)-based ILs as ionic additives may be quite effective to enhance the micelle-micelle interactions, enlarging the biphasic region (i.e., lowering the CPT values) as well as improve the drug encapsulation within Pluronic + IL-based ATPMS, in comparison with the nano formulations without ionic additives. The influence of [Ch]+-based ILs on decreasing the CPT of Pluronic copolymers (L-35 and F-68) is in accordance with the increase of the anionic alkyl chain length, with following trend: choline hexanoate ([Ch][Hex]) ≈ choline butanoate ([Ch][But]) > choline propanoate ([Ch][Pro]) > (choline acetate) [Ch][Ac] ≈ (choline chloride) [Ch]Cl. Despite the clear trend found in the experimental studies, the mechanisms and interactions behind the impacts of choline-based ILs on Pluronic aqueous solutions occurring at molecular level and, consequently, the respective binodal curves are still unclear and many questions remain, such as: “How are the ILs ions interacting with the Pluronic structure (i.e. with PEO or PPO blocks)?”; “Are these ions rearranging the water surrounding the Pluronic copolymers?”; “How are the ILs affecting the inter micelle interactions between the Pluronic molecules?”; “Are the anions of the more hydrophobic ILs (e.g., [Ch][Hex]) creating cross-linked micelles with Pluronic polymers?”
To obtain further answers, studies using computational calculations (e.g., molecular dynamics – MD) and/or small- and wide-angle X-ray scattering (SAXS/WAXS) experiments are required. For example, as demonstrated by Perez-Sanchez et al., MD can be used to clarify the amphiphilic characteristics and micelle morphologies of diluted non-ionic Pluronic aqueous solutions. In this work, it has been revealed that the micelle morphology and inter micelle interactions play an important role in controlling the CPT, describing the formation of physically cross-linked micelles in reverse Pluronic (B-A-B), as well as the mobility of micelles and aggregates. Thus, according previous results from MD simulations, the computational tools will help not only to reveal the impact of choline-based ILs on the self-assembly process of Pluronic micelles, but also to provide further insights about the size, type and shape of these micellar aggregates. For this purpose, the MARTINI-based coarse-grained (CG) model for diluted Pluronic copolymers aqueous solutions was used for the L-35 and F-68 Pluronic copolymers. For the choline-based ILs, there are no CG MARTINI models available in the literature despite being extensively experimentally and computationally investigated. In this work, a new [Ch]Cl and [Ch][Hex] CG models based in MARTINI were developed and validated. Fortunately, the atomistic models have been extensively used to describe these compounds where various atomistic force fields, based in OPLS, AMBER or GROMOS, are available for choline ([Ch]+), chloride (Cl\(^-\)) and hexanoate ([Hex]-). The radial distribution functions and densities of atomistic models were here taken as a reference and used to find a suitable topology for the CG versions as previously reported for other ILs. It must be highlighted that modelling relatively small molecules is difficult with MARTINI mainly due to the 4:1 CG mapping (4 heavy atoms per interaction bead). Once the CG models for the ILs were obtained, F-68 and L-35 aqueous solutions (taken as a references) and their mixtures with [Ch]Cl and [Ch][Hex] ILs were analysed by MD simulations and the obtained results compared with previous experimental data. The very initial stages of micelles formation at room temperature were addressed with the aim to shed light into the complex interactions between Pluronic moieties and the ILs in the critical initial stages of the micellar formation.

2. Methodology
2.1 Simulation details

The MD simulations were carried out with the GROMACS package v. 2019.1\textsuperscript{30} adopting the leapfrog algorithm\textsuperscript{31} to integrate the equations of motion with a 20 fs time step. The non-bonded interactions included the Lennard-Jones (UJ) potential and the Coulombic term with the potential shift-verlet modifier in both with a cut-off radius of 1.2 nm. The long-range electrostatic interactions were assessed using the Particle-Mesh-Ewald (PME).\textsuperscript{32} The temperature was fixed to 298K using the velocity-rescaling thermostat.\textsuperscript{33} The Parrinello-Rahman barostat\textsuperscript{34} and isotropic pressure coupling were selected in both, the equilibration and production runs at 1 bar. Bonded interactions involved bond stretching, angle bending and dihedral torsion terms. The bonds were constrained using the LiNear Constraint Solver (LINCS).\textsuperscript{35} Triclinic simulation boxes were selected with periodic boundary conditions in all directions using random initial configurations arranged with Packmol.\textsuperscript{36}

The simulation protocol consisted of an energy minimization step using the steepest descent algorithm to avoid close contacts between atoms in the initial configurations followed by a short equilibrium steps in the NVT and NpT ensembles, consecutively, prior the NpT production run. The systems were evaluated by visual inspection of the simulation snapshots and monitoring the total energy along the equilibration to ensure that a proper thermodynamic equilibrium is attained.

2.2 Molecular modelling and coarse-grained model validation

The CG model for diluted Pluronic aqueous solutions previously published\textsuperscript{19} was used for the Pluronics F68 (PEO\textsubscript{76}−PPO\textsubscript{29}−PEO\textsubscript{76}, M\textsubscript{w} ≈ 8400 g mol\textsuperscript{-1}) and the L-35 (PEO\textsubscript{11}−PPO\textsubscript{16}−PEO\textsubscript{11}, M\textsubscript{w} ≈ 1900 g mol\textsuperscript{-1}) involved in this study. The choline chloride ([Ch]Cl) and the sodium hexanoate (Na[Hex]) CG versions were mapped under the MARTINI\textsuperscript{20} v.2 model. The CG parameters were selected taking the [Ch]Cl and Na[Hex] All-Atom (AA) systems as a reference. The MD-AA model (CHARMM force field) and the [Ch]Cl concentration of 20 %wt in aqueous solution developed by Maginn et al.\textsuperscript{24} was chosen since was able to reproduce the most
relevant thermodynamic properties. On the other hand, the OPLS\textsuperscript{37} force field was selected for the Na[Hex] system since exhibited a good agreement with the experimental and computational results shown by Fotouhabadi et al.\textsuperscript{26} In this case, the Na[Hex] concentration was 0.5 mol dm\textsuperscript{-3} in aqueous solution to compare with their results. The ILs CG mapping was as follows; the [Ch]\textsuperscript{+} was represented with two CG beads with a low polar P1 and charged no hydrogen bond capability of Q\textsubscript{0} bead for the polar and the nitrogen charged center as shown in Figure 1. Similarly, the [Hex]\textsuperscript{-} was mapped with two CG beads, the apolar C\textsubscript{1} and the charged hydrogen bond acceptor capability of Q\textsubscript{a} for the alkyl-tail and the carboxyl group, respectively (Fig. 1). The above CG beads were chosen to replicate the main physico-chemical features of [Ch]Cl and Na[Hex] and reproduce their AA counterparts. More details about the CG mapping and the topologies used in this study can be consulted in the ESI.

Figure 1. Coarse-grain mapping scheme for the systems involved in this study. The CG beads of choline and hexanoate, [Ch]\textsuperscript{+} and [Hex]\textsuperscript{-}, respectively, are overlaid over the atomistic moieties. The poly(propylene oxide) (PPO) and the poly(ethylene oxide) (PEO) segments in the Pluronic L-35 and F-68 are shown in black and orange colour, respectively. The L-35 and F68 micelles were taken from our MD simulations. More details about the Pluronic CG model can be found in a previous publication.\textsuperscript{19} Chloride Cl\textsuperscript{-} and sodium ions Na\textsuperscript{+} CG beads are surrounded by six implicit water molecules emulating the first solvation and the CG bead for water includes four water molecules.\textsuperscript{20} The CG beads displayed are not shown to scale and the MARTINI bead types are labelled inside the beads. The labelling inside the beads correspond to the selected parameters of the MARTINI energy matrix of interactions.
Thus, a simulation box containing 100 [Ch]Cl with 3,103 water molecules and 20 Na[Hex] with 2,200 water molecules were prepared in both, AA and CG. The trajectories of the NpT production runs were analysed after 5 ns of simulation time. The system density and the radial distribution functions (RDFs) of selected ILs groups in [Ch]^+ and [Hex]^-, respect their counterions and water, were compared between both AA and CG systems. It must be pointed out that the AA and CG comparison is not straightforward since the CG bead includes around four heavy atoms (mapping 4:1 hydrogen atoms are not taken into account) conversely to the single point of the AA reference. Also, the counterions (Cl^- and Na^+) in MARTINI contain, implicitly, the first solvation layer of six water molecules. Thus, the CG water RDFs intensities were normalised by a factor of 4 to fairly compare the AA and the CG curves. With the above CG MARTINI beads, the ILs RDFs showed a reasonable qualitative good agreement with the AA counterparts (Figures S1 and S2 of the ESI). It must be noticed that the CG bead volume around their interaction center induces some uncertainty in the distances when compared with the AA curves and this fact must be taken into account. The CG bead size is 0.47 nm and this fact limits the screening of the RDF as can be seen in the maximum displayed by the CG RDFs (Figures S1 and S2 of the ESI). To provide more insight in the comparison, the system density obtained in the AA and CG simulations were compared with literature data (Table S1 of the ESI). The density obtained in the CG systems agreed fairly well with literature data and our AA simulations as displayed in Table S1 of the ESI).

The RDF and the solvent accessible surface area (SASA) were obtained with the gmx rdf and the gmx sasa GROMACS tools. The Visual Molecular Dynamics (VMD) package was used to visualise the simulation trajectories and produce the simulation snapshots. The micelle density profiles and aggregation number ($N_{agg}$) were obtained by using an in-house cluster-counting code based on the Hoshen-Kopelman algorithm. This code, which somehow resembles the radial distribution function tool, reads the trajectory of all the molecules (mapped by different CG beads as shown in Figure 1) along the simulation and calculates the number of CG beads around a group of beads selected as a reference. For instance, to obtain the Pluronic micelle density profile, the Pluronic CG beads coloured in black (hydrophobic core) are taken as reference representing the micelle center of mass (CoM) and the radial distance to the rest of
CG beads in the system is estimated and plotted. This procedure easily illustrates the position of all the molecules respect the CoM.

3. Results and discussion

3.1. Effect of the ionic liquids on the aggregation of F-68 aqueous solution

The initial stages of F-68 micelles formation are investigated to shed light on how F-68 unimers arrange to form micelles in aqueous solution in the absence and presence of choline-based ILs. Three systems composed of F-68 + H$_2$O, F-68 + [Ch]Cl + H$_2$O and F-68 + [Ch][Hex] + H$_2$O (details in Table S2 in the ESI) were prepared for the MD simulations. The concentrations of 1 wt% of F-68, 2.75 mol dm$^{-3}$ for [Ch]Cl and 0.6 mol dm$^{-3}$ for [Ch][Hex] were chosen to allow a direct comparison with previous experimental data. The final system simulation snapshots are shown in Figure 2 for the three runs. The system without IL exhibited three bulky F-68 micelles with $N_{agg}$ of 3, 4 and 8 obtained with the cluster counting code. The micelles density profile of this system is depicted in Figure S3a of the ESI exhibiting a micelle diameter of around $\varnothing \approx 3.0$ nm – taking the micelle radius as the maximum of the PEO (orange) curve. The main effect of the IL presence was a clear slowdown in the F-68 aggregation, more significant in the system with the chloride$^-$ as anion. In this system, the F-68 remained as unimers as can be seen in Figure 2 and also by the lack of a clear micelle density profile shown in Figure S3b of the ESI. A different scenario was observed in the system with [Hex]$^-$ as counter anion where the system formed a few small F-68 micelles with $N_{agg} \approx 3$ and $\varnothing \approx 2.1$ nm, and spherical aggregates as illustrated in Figure S3c of the ESI. In this system, it must be highlighted that the F-68 aggregates were arranged close to each other contrary to what was observed in the F-68 aqueous solution and the [Ch]Cl mixture. This suggests that the system could be in the biphasic region as observed in the CPT experiments reported by Kurnik et al. Inversely to the CPT increase induced by the addition of ILs (and references therein), the addition of [Ch][Hex] to F-68 CPT induces a CPT decrease when compared with the F-68 aqueous solution. The simulation screenshots in Figure 2 seem to follow this tendency, with the F-68 + H$_2$O displaying bulky micelles whereas the addition of [Ch][Hex] promoted micelle coalescence. It must be
noticed that the addition of [Ch][Hex] did not promote a micelle growth \( (N_{\text{agg}} \approx 3) \) but instead enhanced the inter micelle aggregation.

As pointed out by Kurnik et al.,\textsuperscript{17} hydrophobic interactions between the F-68 and the [Ch][Hex] seem to play an crucial role. The [Hex]\(^-\) displays a relatively large alkyl chain, exhibiting some surfactant-like behaviour, being in this manner prone to interact with the hydrophobic PPO moieties at the Pluronic micelle core. The potential hydration of the different compounds can shed light into this issue. The SASA was calculated for all compounds in the three MD simulation runs.

![screenshots](image)

**Figure 2.** Screenshots for the MD simulations of a) system without IL, b) system with [Ch]Cl and c) system with [Ch][Hex] revealing the effect of the IL and the nature of the anion in the F-68 aggregation in aqueous solution. H\(_2\)O and ILs molecules were removed to allow a clear vision of the F-68 aggregates. F-68 PEO and PPO segments are shown in orange and black, respectively.

The SASA profiles of the PEO, PPO, [Ch]\(^+\), Cl\(^-\) and [Hex]\(^-\) moieties were obtained and are presented in **Figure 3.** **Figure 3a** indicates that the SASA of the PEO groups in the system without IL (black) is higher compared with their ILs mixtures alongside the F-68 aggregation process. However, while in the [Ch]Cl and the F-68 aqueous solutions the SASA profile of PEO groups remained constant along the simulation, in the [Ch][Hex] mixture they exhibited a progressive loss of PEO hydration when the system is transitioning towards the clustered
micellar phase. More details about the PEO hydration are discussed below with the aid of the RDFs. Conversely, Figure 3b showing the SASA of the PPO core shows that ILs hamper the F-68 aggregation (as shown in Figure 2), producing smaller aggregates with the F-68 PPO hydrophobic core more exposed to the solvent. The PPO in the F-68 mixture with [Ch][Hex] displayed a higher contact with the solvent than observed for the systems with the [Ch]Cl, in particular prior to the F-68 micelle formation with a remarkable PPO SASA decrease after 600 ns of simulation. It must be highlighted that despite the system with [Hex]− having less anions per Pluronic compared with the mixture with chloride as counter anion, the [Hex]− exhibited a stronger impact in the micelles formation. Conversely, the SASA curve is approximately flat in the system with [Ch]Cl since the F-68 remained as unimers up to at least 1000 ns of simulation time. If we focus in the SASA of the [Ch]+ and the Cl− in the F-68 system, both ions display similar and constant SASA values, slightly higher for the [Ch]+ as illustrated in Figure 3c – a difference of ≈ 0.14 nm² in the accessible area. Conversely, this difference in the [Ch][Hex] mixture was higher, ≈ 0.4 nm², where the [Hex]− anion exhibited a higher SASA along the simulation as illustrated in Figure 3d. Like the systems containing [Ch]Cl, no changes were observed in the SASA during the simulation for both ions. The SASA difference between [Ch]+ and [Hex]− ions results from the larger alkyl-chain of [Hex]−. The micelle density profile in Figure S3c of the ESI shows that the [Hex]− anions (purple) were mainly linked to the PPO moieties (black) of the F-68 PPO micelle core.
Figure 3. Solvent accessible surface area (SASA) profiles for the F-68 aqueous solution and the mixtures with the [Ch]Cl and [Ch][Hex], runs 5, 6 and 7, respectively after 1000 ns of simulation time. Y-axis is the solvent accessible surface area in nm² units. The accessible surface areas were obtained in all systems for the following groups: a) PEO, b) PPO, c) the [Ch]^+ and Cl⁻ in run6 and d) [Ch][Hex] in run7.

The RDF between the PPO moieties and the [Ch]^+ (black) and [Hex]⁻ (red) ions are shown in Figure 4a. The RFDs show that more [Hex]⁻ can be found around the hydrophobic PPO micellar core of the F-68. This indicates that the hydrophobic-hydrophobic interactions between the PPO and the alkyl chain of the [Hex]⁻ are the main driving force as suggested by Kurnik et al. The RFDs between [Hex]⁻ and [Ch]^+ with water (Figure 4b) displayed a similar behaviour, with the [Hex]⁻ showing an expectable somewhat lower interaction. The [Hex]⁻ interaction with water might be explained by the behaviour of the F-68 PEO moieties. In Figure 2c, the PEO branches (solid line) are arranged towards the PPO core, conversely to the bulkier PEO branches exhibited in the system without ILs (dashed line) (Figure 2a). Thus, the key factor on this system could be a different organization of the PEO chains promoted by the presence of [Ch][Hex]. The degree of PEO bending, towards the PPO core in both systems (with and without IL), can be estimated from the RFDs between the PPO and the outermost CG bead of the PEO segment. Figure 4c demonstrates that more PEO terminal groups can be found around the PPO core taken as a reference, despite the micelles in the [Ch][Hex] system being smaller, as shown in Figure 2 (N_agg of 5 vs 3 without and with IL, respectively). Figure 4d displays the RDF between
the PEO and water in both systems highlighting the dehydration of PEO in the system with [Ch][Hex] as a consequence of this PEO arrangement closer to the PPO F-68 core. Figure S4a of the ESI compares the RDFs between the PEO-[Ch]+ and [Hex]-[Ch]+ showing that [Ch]+ are mostly attached to the PEO branches (also shown in the micelle density profile in Figure S3 of the ESI), but [Hex]- is still somehow linked with [Ch]+. The electrostatic attraction between the [Hex]- accommodated in the micelle core and the [Ch]+ hosted in the PEO branches seems to play a key role in this particular PEO arrangement towards the PPO micelle core.

![Figure 4](image)

**Figure 4.** RDFs for the F-68 + [Ch][Hex] + H$_2$O (run7) system: a) RDFs between the PPO and both ions [Ch]+ (black) and [Hex]- (red), b) RDFs between the water molecules and the [Ch]+ (black) and [Hex]- (red). c) RDFs between the F-68 PPO and the last CG bead of the F-68 PEO segment in the F-68 + H$_2$O (dashed line) and the F-68 + [Ch][Hex] + H$_2$O (solid line) systems. d) RDF between the water molecules and the F-68 PEO for the F-68 + H$_2$O (dashed line) and the F-68 + [Ch][Hex] + H$_2$O (solid line) systems.

### 3.2. Effect of the ionic liquids on the aggregation of L-35 aqueous solution

The effect of the [Ch]Cl and [Ch][Hex] in the aggregation of L-35 in aqueous solution was also investigated. The L-35 displays half of the F-68 PEO/PPO ratio – 1.2 vs 2.6 for L-35 and F-68, respectively – and its molecular weight is four times smaller. Three systems were prepared with
a L-35 concentration of 1 wt% in aqueous solution and two solutions with 1.0 mol dm\(^{-3}\) of [Ch]Cl and 0.15 mol dm\(^{-3}\) of [Ch][Hex] (cf. details reported in Table S2 of the ESI). The L-35 and the ILs concentrations were selected to allow a direct comparison with the experiments carried out by Kurnik et al.\(^{18}\)

The same simulation protocol used before was followed for the MD production runs and the final simulation snapshots are shown in Figure 5. The L-35 aqueous solution (Figure 5a) produced two micelles with \(N_{\text{agg}}\) of 10 and 5 in agreement with the Pluronic model development results.\(^{19}\) The micelle density profile is shown in Figure S5a of the ESI depicting a diameter of \(\bar{\sigma} \approx 3.0\) nm. The effect of the ILs in the L-35 micellization followed a similar pattern to what was observed for the F-68. The system containing [Ch]Cl shown in Figure 5b produced mostly unimers after 1 \(\mu\)s of simulation. However, some small L-35 aggregates (\(N_{\text{agg}} \approx 3\)) were formed as can be also noticed in the micelle density profile displayed in Figure S5b of the ESI. This can be caused by the lower PEO/PPO ratio in L-35 (1.2 vs 2.6), where the PPO content is more prominent, and the aggregation proceeds faster. The density profile also shows that the [Ch]\(^+\) (green) and Cl\(^-\) (red) were arranged at outside of the L-35 aggregates, with Cl\(^-\) ions closer the L-35 surface (orange). The L-35 aqueous solution with [Ch][Hex] formed one micelle (Figure 5c) after 1 \(\mu\)s of simulation time. However, two Micelles coexisted alongside the simulation exhibiting a similar shape and size to the system without IL as can be seen in the micelle density profile shown in Figure S5c of the ESI – further details on the micelle evolution for this system are explained below with the SASA analysis. The density profile shows how the [Hex]\(^-\) (purple) was placed well inside the L-35 micelle and linked to the PPO core (black) driven by the relatively long alkyl-chain in the [Hex]\(^-\) anion. The [Ch]\(^+\) (red) was arranged in the PEO L-35 micelle corona (orange). It must be noted that two micelles in the L-35 aqueous solution were well solvated whereas in presence of [Ch][Hex] it formed one micelle with \(N_{\text{agg}} = 15\) and \(\bar{\sigma} \approx 3.5\) nm (Figure 5c). This could indicate, as observed for the F-68 system, that the mixture is in the biphasic region as shown by the experimental CPT results.\(^{18}\)
Figure 5. Screenshots for the MD simulations of a) system without IL, b) system with [Ch]Cl and c) system with [Ch][Hex] highlighting the effect of the IL and the nature of the anion in the L-35 aqueous solution. Water and ILs molecules were removed for clarity. The L-35 PEO and PPO moieties are shown in orange and black colour, respectively.

The spectrum of the SASA data displayed in Figure 6a shows that the solvent accessible area of PEO groups with the addition of ILs. The differences in the SASA between the L-35 selected groups and the ions were less marked compared with the F-68 system. Conversely, the differences in the SASA of PPO moieties are more evident as shown in Figure 6b. In both mixtures with ILs the PPO exhibited higher water accessible areas than for the system without IL, as observed for the F-68 system (Figure 3). Contrary to the F-68 system in which the self-assembly proceeds slower due to the higher PEO/PPO ratio and the high molecular weight, a brief comparison of the L-35 PPO SASA profile shown in Figure 6b and the trajectories obtained along the simulations, allowed us to discern how was the L-35 self-assembly process in detail. In the first 200 ns, the L-35 remained mostly as unimers and then proceed to form two micelles, initially dispersed in the fluid bulk but rapidly becoming closer to coalescence until 800 ns. Above that, the two coalesced micelles finally merged to form a single aggregate, clearly shown by the abrupt decrease in the SASA profile at 800 ns, caused by the dehydration triggered by the fusion of the two PPO micelle cores. The SASA of the ions in each IL mixture is displayed in Figures 6c and 6d for the [Ch]Cl and [Ch][Hex] systems, respectively. The [Ch]+ possesses a
higher accessible area than Cl- in the [Ch]Cl system whereas in the [Ch][Hex] system, the [Hex]- exhibited a higher SASA, as it was also observed in the F-68 systems.

Figure 6. SASA profiles for the L-35 aqueous solution and the mixtures with the [Ch]Cl and [Ch][Hex], runs 8, 9 and 10, respectively after 1000 ns of simulation time. Y-axis is the solvent accessible surface area in nm² units. The accessible surface areas were obtained in all systems for the following groups: a) PEO, b) PPO, c) the [Ch]^+ and Cl- in run9 and d) [Ch][Hex] in run10. Red arrows in b) highlight when the main micelle fusion processes occurred.

The RDFs for the L-35 PPO and the [Ch]^+ and [Hex]- ions were obtained and are shown in Figure 7a. The RDF profiles confirm that the [Hex]- anions were arranged closer to the PPO when compared with the [Ch]^+ cations, analogously to the F-68 system. The RDF of [Ch]^+ and [Hex]- with water are also displayed in Figure 7b, denoting a similar water layer around both ions.

Following the PEO rearrangement analysis carried out in the F-68 system, the degree of PEO bending towards the PPO was also analysed in the system with [Ch][Hex]. The RDFs between the PPO and the terminal bead of the PEO segment are shown in Figure 7c. The terminal PEO in the system with the [Ch][Hex] (solid line) exhibited a higher interaction with the PPO than in the L-35 system without IL (dashed line). The RDFs between the PEO segments and water for these systems are also compared in Figure 7d. The RDFs intensities at 0.5 nm indicate a slight dehydration around the PEO groups in presence of [Ch][Hex]. This could happen as a
consequence of the arrangement of the PEO segments towards the micelle core as denoted by the RDFs in Figure 7c and similar to the F-68 system (Figure 4c). Figure S4b of the ESI compares the RDFs between the PEO-[Ch]+ and [Hex]-[Ch]+ showing the plausible electrostatic screening between [Ch]+ linked to the PEO (as can be also noticed in the micelle density profile of Figure S5 of the ESI) and interacting with [Hex]-. Thus, the electrostatic attraction between these ions in the Pluronic micelle formation explains this singular PEO arrangement, promoting the dehydration of PEO and enhanced inter micelle interactions as expected in the CPT.

Figure 7. RDFs for the L-35 + [Ch][Hex] + H₂O (run7) system: a) RDFs between the PPO and both ions [Ch]+ (black) and [Hex]- (red), b) RDFs between the water molecules and the [Ch]+ (black) and [Hex]- (red). c) RDFs between the L-35 PPO and the last CG bead of the L-35 PEO segment in the L-35 + H₂O (dashed line) and the L-35 + [Ch][Hex] + H₂O (solid line) systems. d) RDF between the water molecules and the PEO group in L-35 + H₂O (dashed line) and L-35 + [Ch][Hex] + H₂O (solid line) systems.

3.3. Discussion

The micelle density profiles provided some clues on how two different choline-based ILs participate in the Pluronic self-assembly and phase transition towards the biphasic regime, as well as on understanding the arrangement of the IL ions around the Pluronic copolymers. In
both F-68 and L-35, the [Ch]+ and Cl− species were arranged around the Pluronic micelles but not contributing to the self-assembly, producing some small micelles in the L-35 system. The [Ch]Cl is more akin to a conventional salt yet delays micellization. At 2.75 mol dm−3, close to 40 wt%, the micellization of non-ionic surfactants is pushed to higher surfactant concentrations as shown in protic ILs due to the increase in solvophobic interactions delaying aggregation.41,42 In our systems, the [Ch]+ and Cl− species were arranged out of the micelle surfaces, generating an electrostatic repulsion between them as it was revealed in a previous work,19 hindering the micelle fusion processes and further micelle coalescence.

A completely different scenario was observed by changing the Cl− by [Hex]− anions, especially due to the different role played by the longer anionic alkyl-chain of the [Hex]− in the Pluronic self-assembly. As shown by the density profiles, the [Hex]− was arranged well inside the micelle core, cooperating in the formation of micelles. Despite the [Ch]+ cations being placed at the micelle surface, the hydrophobic-hydrophobic interactions between the [Hex]− and the PPO of the micelle core promoted the micelle growth and allowed the micelles to coalesce, as experimentally observed with the formation of a biphasic region.17,18 Furthermore, the strong adsorption of the [Hex]− inside the micelle yielded a more screened charge, also promoting a CPT decrease — conversely to larger alkyl-chain IL-Pluronic mixtures where electrostatic repulsion between micelles occurs, namely, 1-methyl-3-tetradecylimidazolium chloride [C14mim]Cl or the phosphonium-based tributyltetradecylphosphonium chloride [P4,4,4,14]Cl ILs.28

Thus, the studied F-68 and L-35 Pluronic copolymers exhibited a similar behaviour in presence of [Ch]Cl, barely yielding a few small aggregates, but in the [Ch][Hex] system instead they formed clustered Pluronic micelles. The ILs surrounded the F-68 and L-35 Pluronic aggregates in both mixtures with the [Ch]Cl placed outer the Pluronic aggregate and both ions in the same position relatively to the PPO core. Conversely, in the [Ch][Hex] mixture, the [Hex]− anions were arranged well inside the Pluronic micelles, linked to the PPO core and cooperating in the Pluronic micelle formation and coalescence, sign of a biphasic regime characterised by two coexisting micelles-rich and -poor phases (i.e., water-poor and rich phases, respectively) at room temperature in good agreement with previous experiments.18
It has been found a singular behaviour of the Pluronic hydrophilic corona (PEO) in presence of the [Ch][Hex]. The PEO segments were twisted or bent towards the micelle core contrary to the straighter arrangement in the system without IL – more evident in the F-68 system since the PEO branches are considerable much longer than in L-35 (76 vs 11 PEO groups). The bending of PEO is a consequence of the cooperative role of the $[\text{Hex}]^-$ anion in the Pluronic micelle self-assembly with the $[\text{Hex}]^-$ moieties linked to the Pluronic PPO micelle core as shown in the micelle density profiles. In addition, as soon as the $[\text{Hex}]^-$ is absorbed during the Pluronic micelle formation, the hydrophilic-hydrophilic interactions between the $[\text{Hex}]^-$ charged region and PEO could aid the PEO bending towards the micelle core. No micelle size increase was observed with addition of these ILs in both systems, with exception of the L-35 system with [Ch][Hex] which produced a micelle larger than the system without IL.

These results demonstrate that the MD simulations are powerful to reveal the interactions occurring at molecular level in micelles, and consequently to allow a better prediction and design the experimental approaches. In fact, the full understanding of IL-based micelles self-assembled structures will allow to adjust separation processual conditions (i.e., temperature and concentration of copolymers and/or ionic adjuvants) and the micelles concentration in coexisting phases. Therefore, all the other extraction parameters, such as purification factor, selectivity, and recovery, can also be adjusted. Particularly, as here demonstrated, depending of the nature of the ILs used as adjuvants in Pluronic aqueous solutions, a different impact on micelles structure and on phase separation behaviour will occur, with a direct consequence in further partition studies using ATPMS, e.g., due to larger and hydrophobic micelles [Ch][Hex]-Pluronic-based ATPS may be more adequate for hydrophobic solutes, while [Ch]Cl-based ATPMS may be more viable for the selective partition of solutes with a higher hydrophilicity. On the other hand, with the characterization of the micelles self-assembled structures a more precise design of encapsulation and drug release studies can also be achieved.

4. Conclusions
The results reported in this work demonstrate the ability of MD simulations to provide a microscopic picture of relevant interactions in the initial stages of aggregation between non-ionic surfactants and ILs, providing a new insight into the experimental data previously reported. A new coarse-grained model, based in the MARTINI force field, was developed for choline-based ILs. Atomistic simulations and experimental data found in the literature were used to successfully validate the coarse-grained model. The densities of the ILs coarse-grained systems were compared with the atomistic simulations and the experimental values found in the literature, providing, for the first time, a new coarse-grained model for choline-based ILs with Cl⁻ and [Hex]⁻ as counter anions. This model opens the door to explore the effect of relatively small ILs in non-ionic aqueous solutions.

The simulations showed that the addition of choline-based ILs affected differently the Pluronic micelle formation. While the [Ch]Cl kept the Pluronic moieties as unimers, the [Ch][Hex] favoured micelle formation. In this system, the simulation snapshots denoted how these polymer micelles coalesce in the F-68 system and fused into a big micelle in the L-35 system, forming a micelle-rich phase as expected in the biphasic region, in agreement with the experimental results of the CPT decrease obtained in Pluronic aqueous solutions using [Ch][Hex] as adjuvant. The MD simulations captured the impact of the different anionic nature of choline-based ILs in the CPT, providing further and fundamental insights on the role of these ionic species in the self-assembly of polymeric aqueous structures.

The analysis of the density profiles of the initial micelles obtained in the simulations revealed the role of the IL in the formation of the Pluronic micelle. In the F-68 system, the [Ch]Cl arranged around the small aggregates formed (mostly unimers). In the L-35 system, the [Ch]Cl was placed out of the micelle core, with the ions mostly solvated, with some around the PEO groups which form the Pluronic micelle corona as also reported in the literature for similar systems. The [Ch][Hex] played a different role in the Pluronic micelle formation. The [Hex]⁻ were arranged well inside the micelle core whereas the [Ch]⁺ in the micelle corona in both Pluronic systems. Furthermore, in the [Ch][Hex] mixtures, it was observed how the PEO segments which form the micelle corona twisted towards the micelle core, promoting a micelle dehydration leading the system to a phase separation. The [Hex]⁻ and the [Ch]⁺
accommodation, in the PPO and PEO, respectively, and their mutual electrostatic interactions during the Pluronic self-assembly process played a key role in this singular PEO arrangement when compared with the system without IL.

This work covers the lack of coarse-grained models for relatively small ILs opening the door to explore their impact in the Pluronic micelle formation. With the advent of more computational resources, this model can be used to study later stages of Pluronic micelle formation in ILs mixtures for ATPMS application as well as in nano formulations for drug delivery systems.

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References


11. E. Ruel-Gariépy and J.-C. Leroux, In situ-forming hydrogels—review of temperature-


41 T. L. Greaves, S. T. Mudie and C. J. Drummond, Effect of protic ionic liquids (PILs) on the
formation of non-ionic dodecyl poly(ethylene oxide) surfactant self-assembly structures
and the effect of these surfactants on the nanostructure of PILs, Phys. Chem. Chem. Phys., 2011, 13, 20441.

E. C. Wijaya, F. Separovic, C. J. Drummond and T. L. Greaves, Micelle formation of a non-
ionic surfactant in non-aqueous molecular solvents and protic ionic liquids (PILs), Phys.