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Extraction of Natural Colorants using Supramolecular Solvents Composed of Triton X-114 and Ionic Liquids

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Abstract

The interest for natural colorants from microbial sources has increased in the last few years. However, the extraction of these compounds from complex biomasses/media is still a challenge for industrial applications, mainly due to the requirements of biocompatibility, sustainability, and efficiency. With this aim, supramolecular solvents (SUPRAS) composed of nonionic polyethylene glycol tert-octylphenyl ether (TX-114) and various cationic surfactants ($n$-alkyl-3-methyl imidazolium bromide ([C$_n$mim]Br, $n = 10, 12, 14, 16$), and tributyl-tetradecylphosphonium chloride ([P$_{4,4,4,14}$]Cl) ionic liquids (ILs) and cetyltrimethylammonium bromide (CTAB)) were here studied for the extraction of red polyketides colorants from the fermented broth of *Talaromyces amestolkiae*. Firstly, the influence of ILs on the SUPRAS phase behavior was determined by measuring the cloud point temperature ($T_{CP}$) and coarse-grained molecular dynamic (CG-MD) simulations. The results of extraction showed that for all SUPRAS the red colorant preferentially partitioned into the surfactant-rich (bottom) phase (partition coefficients, $K > 10$) with the highest partition using [C$_{14}$mim]Br as a cosurfactant ($K = 14.69 \pm 0.15$). The systems studied also allowed high recovery efficiency of all mixed surfactant-based SUPRAS (> 70% of red colorant recovered in a single extraction step) with selective for the separation of the red colorant from the yellow (1.52 ± 0.04) and orange (1.62 ± 0.08) counterparts present in the fermented broth. The novel SUPRAS have demonstrated remarkable potential in extracting red colorants from fermented broth, without requiring harsh operating conditions. As such, these platforms offer an effective means of concentrating and pre-purifying the red colorants, and hold promise for application to other molecules with similar chemical properties.

*Keywords*: supramolecular solvents; surfactant self-assembly; phase diagrams; coarse-grain molecular dynamic simulations; polyketide colorant extraction
Introduction

Color is the dominant sensorial attribute for consumers, being responsible for 60 to 90% of their positive/negative reactions to products [1]. Moreover, in foods, color is also used as an indicator of safety and quality [2]. For these reasons, most food industries modify or accentuate them by including colorants in their aliments [2]. Synthetic colorants represent 70% of those used in the food industry due to their higher stability and lower production costs [3,4]. However, consumers are increasingly rejecting artificial food additives, due to health and environmental concerns [5]. With this in mind, food industries are gradually replacing synthetic with natural colorants, with 80% of new drinks and foods in 2022 containing natural color additives [6]. Although they meet the desires of consumers, the food industry is finding it difficult maintaining supply and reducing the price of natural colorants, due to their complex production and purification [6]. In this sense, the development of strategies to enhance the production and extraction/purification of natural colorants from complex matrices may help address these issues.

Among natural colorants, those produced by a biotechnological route stand out, mainly because they exhibit several advantages, with emphasis on the increased production rates (high speed and yield) compared to vegetal or animal sources [7,8]. Regarding microbial producers, the colored substances synthesized by filamentous fungi hold significant value in various industries [9]. In this context, the filamentous fungus Talaromyces amestolkiae biosynthesize polyketides water-soluble yellow-orange-red colorants classified as amino-hexanedioic acid azaphilone colorants [4,10,11] with antimicrobial activity against Staphylococcus aureus and low cytotoxicity against fibroblast cells [12]. This microbial source has been raised as a favorable and very promising alternative for the industrial production of natural and functional food colorants. However, after their cultivation step, an extraction process is required to concentrate and pre-purify the fungal colorant before colorant application [13].

The level of purity of natural colorants depends on the application. For uses in the food and pharmaceutical industries, the purity requirements are high, requiring complex downstream processing to ensure proper recovery and purification of the colorants from the fermented broth. This is even more critical as industrial processes need to be economically and environmentally sustainable, making the downstream design a challenge for the industrial production of microbial colorants [3]. The bioseparation...
design must ensure the stability of the biomolecule, *i.e.* use milder and biocompatible operation units, as well as ease scaling up to achieve high processing yields and cost-effectiveness [14,15].

Among the techniques commonly employed in the food and pharmaceutical industries, liquid-liquid extraction (LLE) operations appear as a simple, fast, and accessible method to extract and separate biomolecules from complex media such as fermented broth [14]. In general LLE processes use hydrophobic organic solvents to create a biphasic system, which is then applied to extract and separate target molecules from contaminants according to their physicochemical affinities [14]. However, organic solvents are often not compatible with biomolecules leading to their degradation or a decrease in their biological activities, and/or do not have the necessary tuning degree (limited polarity array) for the selective separation of the target solutes of the complex fermentation medium [14,16].

Alternatively, aqueous biphasic systems that avoid the use of hydrophobic organic solvents are more amenable to the extraction and purification of biomolecules as they preserve the structure and function of the biomolecules. Among these are the Supramolecular Solvents (SUPRAS) from which a large number can be based only on surfactants and water avoiding the use of organic solvents [17–20].

Surfactants are amphiphilic molecules composed of a hydrophilic head and a hydrophobic moiety that can exhibit a complex variety of phase behaviors in an aqueous solution depending on their concentration and temperature [21]. Above the critical micelle concentration (CMC), specific for each surfactant, the system forms aggregates (*e.g.*, micelles, vesicles) while at higher concentrations it may present other phases in equilibrium. Depending on the nature of the surfactant it may through coacervation yield a biphasic solution above or below the critical (lower or upper) solution temperature. This phase separation is known as the cloud point temperature ($T_{CP}$) and it depends on the nature of the surfactant, its concentration, and the presence of co-surfactants, ions, or additives [22–26]. The shape and size of the aggregates located in the concentrated phase of the SUPRAS can be controlled by varying some key conditions, such as the concentration and composition (mixed systems) of the surfactants, ionic strength, pH, and temperature [27]. For instance, spherical micelles can change to prolate shape micelles, cylinders, or bilayers when the surfactant temperature or surfactant concentration is modified or after the addition of other compounds. Generally, ionic surfactants in the
absence of salts show minimal growth, forming spherical micelles due to strong electrostatic repulsion between equally charged heads. However, nonionic surfactants can present significant growth, since the repulsion between the heads is weaker due to steric nor electrostatic effect [28]. Insights into the impact of these parameters in the size and shape of micellar aggregates can be obtained experimentally or through computational tools such as molecular dynamics (MD) simulations revealing the impact of ionic surfactant and additives on the self-assembly process of nonionic micelles [29]. This understanding of the surfactant behavior in solution in presence of co-surfactants and additives is essential for the design of successful systems for the purification of biomolecules.

Considering the issues related to the production of natural colorants from complexes matrices, this work aimed to evaluate whether SUPRAS composed of the nonionic surfactant 1,1,3,3-(tetramethylbutyl)phenyl-polyethylene glycol (Triton X-114, TX-114) and the ILs, 1-alkyl-3-methylimidazolium bromide ([C\textsubscript{n}mim]Br, \(n = 10, 12, 14\) and 16) and tributyl-tetradecylphosphonium chloride ([P\textsubscript{4,4,4,14}Cl]), used as co-surfactants, are efficient for the recovery of natural polyketide colorants from the fermented broth of \textit{T. amestolkiae}. To achieve this objective, firstly, the respective phase diagrams as a function of temperature (T\textsubscript{CP}) were determined for the new SUPRAS. Then, coarse-grain molecular dynamics (CG-MD) simulations were performed to evaluate the interaction and aggregation of nonionic and some of the cationic surfactants ([C\textsubscript{n}mim]Br, \(n = 10\) and 14, and [P\textsubscript{4,4,4,14}Cl]). Finally, the ability of each SUPRAS for the recovery and separation of the natural red colorants present in the fermented broth of \textit{T. amestolkiae} was inferred. The results obtained were compared with SUPRAS formed by TX-114 and CTAB surfactant.

2. Experimental

2.1. Materials

The 1-alkyl-3-methylimidazolium bromide ([C\textsubscript{n}mim]Br, \(n = 10, 12, \) and 14) ILs (purity < 99%, determined by NMR) were synthesized by the Center for Natural and Exact Sciences, the Federal University of Santa Maria – UFSM, RS, Brazil, following previously described methods [30,31]. Tributyl-tetradecylphosphonium chloride ([P\textsubscript{4,4,4,14}Cl]) was kindly donated by Cytec (WoodlandPark, NJ). The nonionic surfactant
polyethylene glycol tert-octylphenyl ether (TX-114) and the ionic surfactant hexadecyltrimethylammonium bromide (CTAB) were purchased from Sigma-Aldrich® (St. Louis, MO, USA). All other reagents were analytical grade and used as received. The chemical structures of all surfactants and ILs are presented in Figure 1.

2.2. Microorganism cultivation and natural colorants production

Talaromyces amestolkiae DPUA 1275 was kindly provided by the DPUA Culture Collection, from the Mycology laboratory of the Federal University of Amazonas (Manaus, AM, Brazil). Cultures were preserved in sterile distilled water and reactivated in PDA-YE (g.L\(^{-1}\)): Potato Dextrose Agar (30 g.L\(^{-1}\)) supplemented with yeast extract (5 g.L\(^{-1}\)) and maintained at 30 °C for 168 h.

As previously described by our research group for the production of natural colorants [32], the inoculum was prepared in Petri plates (90 mm x 10 mm) containing 10 mL of PDA-YE medium, transferring the reactivated microorganism to the center of the plate and keeping it at 30 °C for 168 h. Erlenmeyer® type flasks (250 mL) containing 50 mL of the culture medium were prepared and then inoculated with 10 mycelium agar discs (8 mm in diameter). The composition of the culture medium was (g.L\(^{-1}\)): glucose (10), monosodium glutamate – MSG (25), MgSO\(_4\) (0.012), Fe\(_2\)SO\(_4\) (0.010) and CaCl\(_2\) (0.015). The pH of the culture medium was adjusted to a pH value of 5.0 and sterilized at 121 °C for 15 min. After the inoculation, the flasks were incubated in an orbital shaker (New Brunswick Innova 40R, Eppendorf, Inc., USA) at 30 °C for 168 h and 150 rpm. At the end of the cultivation, the fermented broth was first filtered with an 80 gm\(^{-2}\) paper filter (Whatman, UK) and then with a 0.45-μm filter (Millipore, Bedford, MA, USA). The clarified fermented broth was stored at -80 °C for the extraction studies. The chemical structure of the colorants produced is presented as Figure S1 in the Supporting Information (SI).

2.4. Determination of the SUPRAS binodal curves

Binodal curves for SUPRAS composed of TX-114 (5, 9 and 13 wt%), [C\(_n\)mim]Br \((n = 12, 14 e 16)\), [P\(_{4,4,4,14}\)]Cl or CTAB at concentrations of 1.25, 2.50, 10.00 and 50.00 mM in McIlvaine buffer (pH 6.5) were determined by the cloud point titration [23,33].
To this purpose, a stock solution of 75 mM of the respective IL was prepared and used as required. The components were added into graduated glass tubes (15 mL) to a final total mass of 3 g and homogenized for 30 min at 30 rpm in a circular homogenizer. Then, the systems were transferred to a thermostatic bath (Ethik Technology) at 20 °C, with the temperature slowly increased in 0.1 °C increments until reaching the $T_{cp}$. The assays for the determination of the binodal curve were performed in duplicate.

The kinematic viscosity (uncertainty of ± 0.35%) and density (uncertainty of 5 x $10^{-4}$ g.cm$^{-3}$) measurements of the systems were performed at atmospheric pressure and 25.00 (±0.02)°C with an automated SVM 3000 Anton Paar rotational Stabinger viscometer-densimeter, calibrated using standard solutions [34]. They are presented in Table S1 from the Supporting Information (SI).

2.5. Molecular dynamics simulations

MD simulations were performed with the GROMACS package 2019 [35] integrating the motion equations using the leapfrog algorithm [36] with a time step of 20 fs. The total potential energy was obtained as a sum of the bond stretching, angle bending, and dihedrals for the bonded contributions where bonds were constrained using the linear constraint solver (LINCS) [37]. The non-bonded contributions were the Lennard-Jones (LJ) potential and Coulomb function where long-range electrostatic interactions were assessed using the Particle-Mesh-Ewald (PME) [38]. Non-bonded interactions were computed using a Verlet cutoff scheme (potential-shift-Verlet modifier) with a cut-off of 1.2 nm. The temperature was fixed to 20 °C using the velocity-rescaling thermostat [39] with a coupling time constant of 1.0 ps. The pressure was fixed to 1 bar with the Parrinello-Rahman barostat [40] using an isotropic pressure coupling and a coupling time constant of 24.0 ps. Periodic boundary conditions were used for the triclinic simulation boxes built with Packmol [41] where all compounds were arranged randomly.

The equilibrium protocol for the initial configurations comprised a first step of energy minimization using the steepest descent algorithm to elude close contacts between molecules followed by two simulations in the NVT and NpT ensembles to set the proper temperature and density respectively before the NpT production runs. The total potential energy, pressure, temperature, and density were monitored along the equilibration to ensure that the thermodynamic equilibrium was attained. CG-MD simulations were
managed with the visual molecular dynamics (VMD) software package [42]. The aggregate distributions were analyzed using an in-house code [43] inspired by the Hoshen–Kopelman cluster counting algorithm [44].

2.5.1 Molecular modeling and coarse-grained model validation

Since a MARTINI CG model for TX-114 is not available in the literature, the TX-110 model developed by Pizzirusso et al. [45] was taken as a reference, from which five EO groups were eliminated to resemble the TX-114 moiety. In Pizzirusso et al. work [45], a comprehensive comparison between TX-100 atomic MD simulations and experimental data was carried out to develop the CG model. Despite they performed an ad-hoc fine-tuning CG parameterization to better reproduce the atomistic simulation findings, in our work we kept their original CG MARTINI parameterization as shown in Figure 1, to maintain the transferability of MARTINI. Additionally, for our TX-114 CG model, we selected three small SC1 CG beads to map the benzene ring (Figure 1), as recommended in the original MARTINI model [46] instead of the two CG beads used by Pizzirusso et al. [45] to emphasize the geometric structure of the ring. A CG-MD simulation for a 0.15% wt of TX-114 concentration (0.005 M) in an aqueous solution was run at 20 ºC to evaluate our TX-114 CG mapping. This concentration was above the critical micelle concentration (2.8·10⁻⁴ M). [47] After the minimization and equilibration steps, the system was run in an NpT ensemble for 9 microseconds of simulation time to ensure that the equilibrium was reached. 200 TX-114 molecules comprised the simulation box and yielded three small micelles and vesicles. This configuration yielding an averaged aggregation number (N_{agg}) of 67 was also observed experimentally [48] with reported aggregation numbers in the range (62-88) [47,49]. The N_{agg} was obtained using our cluster counting code throughout the trajectory of the CG-MD simulation. The final simulation snapshot and the averaged density profile of the aggregates are shown in Figure S2 in the supporting information section. The CG models for the [C_{10}mim]Cl [50], [C_{14}mim]Cl [50], [P_{4,4,4,1a}]Cl [51] ILs, chloride [46] anions, and water [46] were taken from the literature. The structures and parameters are illustrated in Figure 1.
Figure 1. The chemical structures of the compounds used in the experiments are shown on the left and the CG parameterization is displayed on the right. The TX-114 consists of three SNa CG beads (red) for the hydrophilic polyethylene oxide tail connected with the organic benzene ring (cyan) entailing three SC1 CG beads and linked to the dimethyl groups mapped with two C1 beads (green). CG beads for chlorides (Cl\textsuperscript{−}) implicitly include six water molecules emulating the first solvation shell. The CG water model uses the P4 bead type which implicitly includes four water molecules [46]. Note that the CG beads displayed are not shown in scale and MARTINI bead types are labeled inside the beads. The TX-114 vesicle shown at the top was obtained in our simulations.

2.6. Colorants extraction

SUPRAS were prepared with different concentrations of TX-114 (5, 9, and 13 wt%) and [C\textsubscript{n}mim]Br (n = 12, 14, and 16), [P\textsubscript{4,4,4,14}]Cl, or CTAB at concentrations of 1.25 and 2.5 mM which corresponded to 1.67 and 3.33 wt% of the system since a stock solution of 75 mM was used. The components were added in graduated glass tubes (15 mL) together with the fermented broth until the final absorbance of 1.0 units of absorbance (UA) at wavelength 500 nm in McIlvaine buffer (pH 6.5), considering the total final mass equivalent to 3 g. The tubes were homogenized for 30 min at 30 rpm in a circular homogenizer and incubated in an ultra thermostatic bath at a temperature established according to the binodal curves of each SUPRAS. Subsequently, the systems were
centrifuged at 3864 xg for 10 min at the same temperature used in the extraction using a refrigerated centrifuge (Universal 320R - Hettich). After centrifugation, the phases were already in equilibrium and the top (surfactant-poor phase) and bottom (surfactant-rich phase) phases were collected. The extraction time was determined by performing a previous test with the biphasic system composed of TX-114 13 wt%, [C$_{16}$mim]Br 2.5 mM, and McIlvaine buffer (pH 6.5). The incubation times for colorants partitioning were set at 1, 3, and 24 h.

The colorants extraction performance of each SUPRAS was evaluated by determining the following parameters: partition coefficient – $K$ (cf. Equation 1); volumetric ratio – $V_R$ (cf. Equation 2); red colorant recovery yields in the bottom phase – $R_B$ (cf. Equation 3) and in the top phase – $R_T$ (cf. Equation 4); Selectivity for red colorant relative to the yellow and orange colorants – $S_e$ (cf. Equation 5); mass balance – MB (cf. Equation 6):

$$K = \frac{Abs_B}{Abs_T}$$  \hspace{1cm} (1)

$$V_R = \frac{V_B}{V_T}$$  \hspace{1cm} (2)

$$R_B = \frac{100}{1 + \frac{1}{R_V K}}$$  \hspace{1cm} (3)

$$R_T = \frac{100}{1 + V_R K}$$  \hspace{1cm} (4)

$$S_e = \frac{K}{K_{O/Y}}$$  \hspace{1cm} (5)

$$MB(\%) = \left(\frac{Abs_B V_B + Abs_T V_T}{Abs_i V_i}\right) \times 100$$  \hspace{1cm} (6)

where, $Abs_i$, $Abs_B$, and $Abs_T$ correspond to the initial absorbance, and absorbance in the bottom and top phases, respectively. $V_i$, $V_B$, and $V_T$ correspond to the initial volume, bottom volume, and top volume, respectively. $K_{O/Y}$ is equivalent to the $K$ of the orange or yellow colorants.
2.6. Quantification of natural colorants

Colorants are usually expressed by color value based on the absorbance spectrum and colorimetric analysis [52]. Thus, the quantification of natural colorants was estimated by spectrophotometric analysis, following the procedure previously established by us [11,32,53]. Briefly, the natural colorants concentration was estimated by measuring the supernatant absorbance at wavelength 420, 470, and 500 nm, which correspond to the maximum absorbance for the yellow, orange, and red colorants, respectively [11]. Visual absorbance was acquired on an EnSpire Alpha Plate Reader spectrophotometer (PerkinElmer®), and colorants quantification was determined considering the dilution factor of each sample.

2.7 Statistical analysis

The extraction experiments were performed in triplicate and the results compared using analysis of variance (ANOVA) with Tukey’s HSD to verify significant differences of astaxanthin and others carotenoids at 95% confidence level (p ≤ 0.05). The analysis was performed using Origin Software version 10.0 (Northampton, Massachusetts, USA).

3. Results and discussion

3.1. Binodal curves of the TX-114 + ILs-based SUPRAS

The experimental binodal curves were determined by the cloud-point titration method with duplicates starting from 20 °C with 0.1 °C increments until reaching the T_{CP}. The results are presented as the average of the duplicate with standard deviations. The lines are guides to the eye to facilitate the comparison between the experimental data. Initially, a binodal curve of TX-114 in McIlvaine buffer pH 6.5 was determined as a reference and compared with a binodal curve described in the literature [54]. This experimental binodal curve is presented in Figure 2.a, along with the literature data [55,56]. Then, to develop platforms for the extraction of natural colorants and aim to define the SUPRAS cloud points, binodal curves were determined for the SUPRAS composed of nonionic surfactant TX-114 + ILs (detailed information in Tables S2, S3, and S4 from SI). The binodal curves were determined for systems composed of TX-114 (5, 9, and 13 wt%) in McIlvaine buffer pH 6.5 + ionic surfactants ([C_{n}mim]Br (n = 10, 12, 14, 16), [P_{4,4,4,14}]Cl and CTAB) at 1.25 mM (Figure 2.b) and 2.50 mM (Figure 2.c). The concentrations of each SUPRAS
were selected based on the initial screening for the T<sub>CP</sub> of different systems with TX-114 (5, 9, and 13 wt%) + [C<sub>10</sub>mim]Br (1.25, 2.50, 5.00, 10.00, and 50.00 mM) in McIlvaine buffer pH 6.5, as presented in Tables S3 and S4.

**Figure 2.** Binodal curves represented as the concentration of surfactant (wt%) versus temperature (ºC) for the SUPRAS composed of TX-114 in McIlvaine buffer pH 7 (from the literature [55,56], in empty square black dashed line) and pH 6.5 (from this work, in red) (a); and for TX-114 (5, 9 and 13 wt%) in McIlvaine buffer pH 6.5 + ionic surfactants at 1.25 mM (b) and 2.50 mM (c).

*Figure 2.a* shows that increasing TX-114 concentration in McIlvaine buffer pH 6.5 resulted in higher T<sub>CP</sub>, as previously observed for the system with McIlvaine buffer pH 7 [55,56]. The slight deviations between the two curves are in line with the small differences induced by the pH values of the SUPRAS [54]. After determining the TX-114 binodal curve in buffer pH 6.5, the effect of adding ionic surfactants was then evaluated, and compared with the effect induced by CTAB. As shown in Table S3 from SI, [C<sub>10</sub>mim]Br concentrations of 5.00, 10.00, and 50.00 mM caused a significant increase in T<sub>CP</sub> (from below 35 ºC at 1.25 and 2.50 mM, to above 60 ºC at 50.00 mM). A similar
temperature increase in $T_{CP}$ of the TX-114 aqueous solution was observed by Gu and Galera-Gómez [57].

Figures 2.b and 2.c reveal that the addition of different cationic surfactants led to increases in $T_{CP}$, especially at lower concentrations of TX-114. The differences observed indicate that the ratio between ionic and nonionic surfactants also plays a role in the extent of the $T_{CP}$ displacement. On the other hand, the effect of increasing the cationic alkyl chain length of the ionic surfactants added to TX-114 in McIlvaine buffer had less impact on the $T_{CP}$ of the SUPRAS under the conditions of this study. The greatest $T_{CP}$ difference found was only 0.8 °C between the SUPRAS with $[C_{10} \text{mim}] \text{Br}$ and $[C_{16} \text{mim}] \text{Br}$ at the highest concentrations (i.e., 5 wt% TX-114 + 2.50 mM of the ILs). In this case, the small increase of $T_{CP}$ with ILs with longer cationic alkyl chains appears to be a result of the more negative standard Gibbs Energy of aggregation, favoring aggregation [58].

Comparing these results with the literature, Torres et al. [33] reported an increase in the $T_{CP}$ of the SUPRAS of TX-114 with the addition of $[C_8 \text{mim}] \text{Cl}$, $[C_{10} \text{mim}] \text{Cl}$, and $[C_{12} \text{mim}] \text{Cl}$. A similar effect on SUPRAS equilibria was observed by Bender et al. [59], who reported an increase in the $T_{CP}$ in systems composed of the nonionic surfactant Tergitol 15-S-7 and dicationic ILs derived from 1, n-bis(3-methylimidazolium-1-yl)alkane bromide ([$\text{BisAlk(mim)_2}[2\text{Br}]$]) — in which $\text{Alk} = \text{butyl, hexyl, octyl, and decyl}$ at 2 wt% of IL in McIlvaine buffer (pH 7.0). Vicente et al. [24] investigated the influence of imidazolium-, phosphonium-, and quaternary ammonium-based ILs in the binodal curves of SUPRAS composed of the nonionic surfactant TX-114. They showed that the nature of ILs has an important effect on $T_{CP}$, mainly because of the relative hydrophobic/hydrophilic nature of the ILs. These authors reported a decrease in TX-114 $T_{CP}$ by using ILs with a hydrophobic nature as additives (e.g., phosphonium and quaternary ammonium families). TX-114 self-assembly yields neutral vesicles [57] and the addition of ionic surfactants creates charged vesicle surfaces, modifying their size and shape [57] whilst promoting electrostatic repulsion forces between neighbor vesicles hindering aggregation. These factors, which will be further scrutinized in the molecular dynamic simulation section below, seem to contribute to the increase in $T_{CP}$, with the intensity of the effect depending on the nature and concentration of the ionic surfactant [57].

Although the presence of ionic surfactant in SUPRAS may be favorable for the recovery of biomolecules, care must be taken with increasing $T_{CP}$ in the presence of these
additives, as high temperatures can degrade or destabilize some thermolabile molecules. In addition, it will also increase the operating costs (energy utilities) of the extraction unit [14,60]. With these aspects in mind and to extract thermolabile natural colorants, a concentration of IL was selected to obtain SUPRAS with $T_{\text{CP}}$ below 35 °C (i.e., 1.25, 2.50 mM). A temperature of 36 °C was selected for all extraction studies of natural red colorants from the fermented broth of *T. amestolkiae*. This temperature is above the binodal curve for all systems presented in Figures 2.b and 2.c and does not affect the properties of the natural colorant according to previous studies [32].

3.2. *CG-MD simulation of the TX-114 + ILs-based SUPRAS*

To shed light on the self-assembly of TX-114 in the presence of different ILs, four systems were chosen to run the CG-MD simulations. A solution of TX-114 in water at 3 wt% and its mixture with 2.5 mM of $[\text{C}_{10}\text{mim}]\text{Cl}$, $[\text{C}_{14}\text{mim}]\text{Cl}$, $[\text{P}_{4,4,4,4,14}]\text{Cl}$ were selected. The TX-114 and IL concentrations were selected bearing in mind the computational time costs since it is quite difficult to simulate diluted systems, even at the CG level. Furthermore, based on the experimental binodal curves shown in Figure 2, the high concentration of TX-114 yielded a weak impact of the IL when compared with diluted TX-114 solutions. Thus, the small differences observed in the binodal curves above ~ 8 wt% of TX-114 led us to select 3 wt% of TX-114 concentrations, where the impact of the IL was much higher and easier to capture in CG-MD simulations. In all CG-MD simulations, the temperature and pressure were fixed to 10 °C and 1 bar, respectively. The simulation boxes contain 1000 TX-114 molecules, and 130 ILs, when present. The CG-MD simulations were run and the TX-114 and IL self-assembly was visualized at different stages and their sizes were monitored with the cluster counting code. Figure 3 shows the CG-MD simulation snapshots summarizing the obtained aggregates after 3 µs of simulation time.
**Figure 3.** CG-MD simulation snapshots after 3 µs of simulation time for the TX-114 aqueous solution (a) and their IL mixtures with 2.5 mM of [C10 mim]Cl (b), [C14 mim]Cl (c) and [P4,4,4,14]Cl (d). A detailed perspective of the IL arrangement in the TX-114 vesicles is shown in the center for each system. The color code is the same as in Figure 1 for the TX-114 moieties. ILs are colored brown and purple for the alkyl-chain tail and head groups, respectively. Water molecules and chlorides were removed for clarity.

The TX-114 system without IL exhibited two equally sized vesicles as shown in Figure 3.a with an average diameter of ~ 7 nm and N_{agg} = 500. When the ILs are incorporated, the system with [C10 mim]Cl displayed one rod-like vesicle (Figure 3.b) with a size of 17 x 7 nm approx. The [C10 mim]^+ cations were arranged with the alkyl-tail immersed in the hydrophobic TX-114 region shown in green in Figure 3.b. The imidazolium rings (purple) were located at the vesicle surfaces (inner and outer) shown in red color (Figure 3.b). However, a different picture was observed when the [C14 mim]Cl was incorporated, yielding three spherical-shaped vesicles as illustrated in Figure 3.c.

Despite the arrangement of the [C14 mim]Cl moieties in the TX-114 vesicles being similar to the solution with [C10 mim]Cl, the [C14 mim]Cl seemed to play a different role in the initial stages of the aggregation yielding a different scenario as discussed in more detail below. The TX-114 + [P4,4,4,14]Cl mixture formed a prolate-like vesicle (Figure 3.d) with the IL moieties arranged in the TX-114 structure similar to the [C10 mim]Cl
mixture as can be seen in **Figure 3.b** where a rod-like TX-114 vesicle of 14 x 12 x 7 nm size was obtained. The longer alkyl-chain lengths and bigger head group volume exhibited by the [P$_{4,4,4,14}$]$^+$ cation, could be the reason behind the vesicle structure disparity when compared with the mixtures with [C$_{10}$mim]$^+$. The larger cationic head group and hydrophobic interactions with [P$_{4,4,4,14}$]$^+$ yielded bend structures which seem to be energetically favored.

To elucidate the relation between the structures formed and the role of each IL, **Figures S3, S4, S5, and S6** illustrate the initial stages of the self-assembly for the TX-114 aqueous solution and their mixtures with [C$_{10}$mim]Cl, the [C$_{14}$mim]Cl and [P$_{4,4,4,14}$]Cl, respectively. The first 1700 ns of simulation time were used to evaluate the initial stages of the self-assembly. **Figure S3** displays the formation of early small aggregates, growing by inter-micelle fusion processes up to 145 ns and finally yielding small vesicles after 360 ns. **Figures S4 and S5** show the TX-114 mixtures with [C$_{10}$mim]Cl and [C$_{14}$mim]Cl, respectively, where the TX-114 dominated the initial self-assembly with the formation of small aggregates before any IL aggregation (here completely colored in blue and rescaled to enhance the view). **Figure S4** shows that in the TX-114 + [C$_{10}$mim]Cl mixture, early TX-114 aggregates quickly absorbed [C$_{10}$mim]$^+$ cations, yielding TX-114/[C$_{10}$mim]$^+$ charged micelles increasing their size up to 145 ns. At 362 ns the system formed TX-114 vesicles that increased their size rapidly until 1700 ns, displaying a similar vesicle size distribution as in the TX-114 solution without IL (**Figure S3**). **Figure S5** shows a similar scenario for the TX-114 + [C$_{14}$mim]Cl mixture in the first 145 ns with the formation of small TX-114/[C$_{14}$mim]$^+$ charged micelles but micelle fusion processes proceed slower as can be noticed in the micelle size distribution above 362 ns, yielding smaller micelles when compared with the [C$_{10}$mim]Cl solution (**Figure S4**). In fact, at 1360 ns, the TX-114 + [C$_{10}$mim]Cl formed vesicles which can be barely found in the TX-114 + [C$_{14}$mim]Cl solution (**Figure S4**).

**Figure S6** shows the TX-114 + [P$_{4,4,4,14}$]Cl mixture clearly showing that the TX-114 dominated the self-assembly in the first 17 ns, and yielded larger aggregates when compared with the imidazolium solutions shown in **Figures S4 and S5**. This could be related to the stearic character of the butyl head groups in [P$_{4,4,4,14}$]$^+$ which enhances inter-micelle interactions, compared with the less screened imidazolium charged ring and promoting inter-micelle fusion events, as previously discussed in the literature [61,62]. As a matter of fact, after 145 ns, micelle fusion events yielded even bigger vesicles than
those found in the solution without IL (Figure S3). Thus, the early stages of aggregation show that the TX-114 aqueous solution (Figure S3) and the [C\textsubscript{10}mim]Cl mixture (Figure S4) displayed similar sizes and shapes of spherical vesicles. The [C\textsubscript{14}mim]Cl mixture yielded smaller vesicles, being their formation was delayed compared with the TX-114 aqueous solution or the mixture with [C\textsubscript{10}mim]Cl. Conversely, faster aggregation was observed in presence of [P\textsubscript{4,4,4,14}]Cl, producing a flattened vesicle structure in contrast with the spherical vesicles obtained for the other systems. Thus, at 3 wt% of TX-114, the nonionic surfactant dominates the self-assembly in the IL mixtures, with the IL cations being absorbed by TX-114 aggregates before being able to aggregate into IL micelles. The impact of [C\textsubscript{10}mim]Cl in the TX-114 vesicle formation was similar to the [P\textsubscript{4,4,4,14}]Cl above ~300 ns, despite the stronger charged character of the imidazolium ring compared with the [P\textsubscript{4,4,4,14}]\textsuperscript{+} cation. Thus, it seems that the shorter alkyl-chain length of [C\textsubscript{10}mim]\textsuperscript{+} played also a significant role in the arrangement of the IL around the TX-114 structure. [C\textsubscript{14}mim]Cl and [P\textsubscript{4,4,4,14}]Cl mixtures exhibited a completely different scenario despite having the same alkyl-chain length. The different nature of the head group, charge vs steric in [C\textsubscript{14}mim]Cl and [P\textsubscript{4,4,4,14}]Cl, respectively, played a major role in the TX-114 mixed vesicle formation.

3.3. Extraction of red natural colorant using TX-114 + ILs-based SUPRAS

After defining the work conditions for all SUPRAS, i.e., 36 ºC and 1.25 and 2.50 of ionic surfactant, the minimum equilibrium time for the partition of natural colorants was established. Considering that viscosity is an important factor for slowing down mass transfer in biphasic liquid-liquid extractions [63], the system composed of TX-114 (13 wt%) + [C\textsubscript{16}mim]Br (2.50 mM)) was selected for initial screening of the natural colorant partition and to determine the extraction time due to its high viscosity compared to the other systems evaluated. Table S5 from SI shows a steep increase in the $K$ of the red colorants for the surfactant rich-phase of the SUPRAS between 1 ($0.78 < K < 0.91$) and 3 h ($3.43 < K < 5.31$), while minor variations in partition coefficients were observed from longer extraction times (at 24 h: $3.88 < K < 5.42$). For this reason, 3 h was defined as a suitable time for the natural colorant extraction experiments.

The partition of natural colorants was evaluated by determining the corresponding $K$ values at different absorbance wavelengths, considering the different color fractions
found during the characterization of the *T. amestolkiae* fermented broth (yellow – 420 nm; orange – 470 nm; and red – 500 nm). However, as our main objective is the recovery of red colorants (corresponding to absorbance at 500 nm), the extraction results were presented as a function of red colorant absorbance, as well as the selectivity relative to the yellow and orange colorants. The results of the partition of the red colorant at 500 nm for each SUPRAS are shown in Figures 4.a and 4.b and detailed experimental data with respective std of red, orange, and yellow colorants are presented in Table S6 from SI.
Figure 4. The partition coefficient with absorbance at wavelength 500 nm ($K_{500\text{ nm}}$) of red colorants from the fermented broth of *T. amestolkiae* ($K_{500\text{ nm}}$) using SUPRAS composed of TX-114 (5, 9, and 13 wt\%) + CTAB (a) or ILs *i.e.*, (b) [C$_{10}$mim]Br, (c) [C$_{12}$mim]Br, (d) [C$_{14}$mim]Br, (e) [C$_{16}$mim]Br, (f) [P$_{4,4,4,14}$Cl] at 36 ºC, after 3 h of equilibrium time. The ILs or CTAB were added to the systems at concentrations of 1.25 mM and 2.50 mM. The partition coefficients of red colorants for the TX-114-based SUPRAS without cationic surfactants are between 0.7 and 1.5, as previously reported by Torres et al [28]. The error bars represent 95% confidence limit for the trials, and the means followed by the same letter do not differ significantly by the Tukey test (p < 0.05).
Figure 4 shows that the red colorant partitioned preferentially into the surfactant-rich phase (bottom), for all the systems studied, with $K$ ranging from 6.1 to 14.7. All cationic ILs and CTAB promoted a positive effect on the partition of the red colorant, in comparison with the system of only TX-114, which exhibited a maximum $K$ of 2.16 at 3 wt% [33]. Interestingly, the increase of TX-114 concentration reduced the $K$ values for both concentrations of cationic additives (1.25 mM and 2.50 mM) being the best performance usually obtained for SUPRAS composed of 5.0 wt% of TX-114. The higher the TX-114 concentration, the more hydrophobic the surfactant-rich-phase is, thus, the partition of colorant to the surfactant-rich phase is decreased, leading to lower $K$ values.

Regarding the effect of the surfactant nature, it can be observed that the addition of surfactant with the shortest and longest cationic alkyl chain lengths (i.e., $\text{[C}_{10}\text{mim}]\text{Br}$, $\text{[C}_{16}\text{mim}]\text{Br}$) led to worse performance for red colorants partition suggesting that dodecyl and tetradecyl chains are optimal for this application. The fermented broth of *T. amestolkiae* presents a mixture of amino-hexanedioic acid azaphilone colorants [11]. These colorants present the same chromophores, which represent the red color, and regardless of the variable side group, they are considered hydrophilic with negative octanol/water partitioning values at pH 6.5 [64]. The partition must thus be influenced by electrostatic interactions. The presence of cationic surfactants induced the formation of positively charged aggregates, which seems to favor the interactions with the red colorants (which are negatively charged up to pH values of 7.0). However, although the presence of cationic surfactant improves the partition of the red colorant into the surfactant-rich phase, high amounts of these surfactants promote a decrease in the $K$ values, this tendency is more pronounced with larger surfactants. As exposed in the CG-MD simulations, the addition of different cationic surfactants generates aggregates (e.g., micelles, vesicles) with different shapes and sizes. Therefore, the affinity of red colorants for the surfactant-rich phase will be dependent on the structural features of these aggregates.

These results confirm that TX-114 + ILs SUPRAS have great potential for the extraction of biomolecules from fermented broth, requiring low concentrations of the cationic and nonionic surfactants, which is very interesting from an industrial point of view (fewer costs with extractants and additives for the extraction). As can be confirmed in Table S7 from SI, the recovery of the red colorant in the surfactant-rich (bottom) phase
varied from 80 to 97%. These high concentrations of red colorants in the bottom phase can be also confirmed through visual inspection as shown in **Figure S9**.

Comparing our findings with those reported by Santos *et al.* [64], using colloidal gas aphrons (CGA) generated by CTAB to recover red colorants from fermented broth, the mixed SUPRAS here studied revealed to be more efficient since the highest partitioning coefficient obtained by CGA technique was only 5.39.

Regardless of the good performance of SUPRAS composed of TX-114 + ionic surfactants for the partition and recovery of the red colorant, we also aimed to evaluate their potential application for the selective separation and purification of natural colorants. For that, it was evaluated the selectivity of the partitioning of the red fraction of the fermented broth relative to the yellow (Se<sub>500/420 nm</sub>) and orange (Se<sub>500/470 nm</sub>) fractions (**Table 1**).
Table 1. Selectivity of extraction (Se) of the red colorant relative to the yellow (\(\text{Se}_{500/420\ \text{nm}}\)) or orange (\(\text{Se}_{500/470\ \text{nm}}\)) colorants for the TX-114 (5, 9, and 13 wt%) + ILs or CTAB SUPRAS (1.25 and 2.50 mM) during the extraction of red colorants from fermented broth at 36 ºC and after 3 h of equilibrium time. Results are the average of a duplicate and are presented with their respective standard deviations (std).

<table>
<thead>
<tr>
<th>TX-114 (wt%)</th>
<th>Ionic surfactant</th>
<th>Ionic surfactant concentration (mM)</th>
<th>1.25</th>
<th>2.50</th>
<th>1.25</th>
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<tr>
<td></td>
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<td>(\text{Se}_{500/420\ \text{nm}}) (yellow) ± std</td>
<td>(\text{Se}_{500/470\ \text{nm}}) (orange) ± std</td>
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<td>5</td>
<td>CTAB</td>
<td>1.57 ± 0.03</td>
<td>1.74 ± 0.02</td>
<td>1.77 ± 0.04</td>
<td>1.95 ± 0.00</td>
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<td>([\text{C}_{10}\text{mim}]\text{Br})</td>
<td>1.31 ± 0.04</td>
<td>1.68 ± 0.01</td>
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<td>1.87 ± 0.02</td>
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<td>([\text{C}_{12}\text{mim}]\text{Br})</td>
<td>2.58 ± 0.11</td>
<td>1.63 ± 0.18</td>
<td>1.88 ± 0.01</td>
<td>1.89 ± 0.18</td>
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<tr>
<td></td>
<td>([\text{C}_{14}\text{mim}]\text{Br})</td>
<td>1.56 ± 0.01</td>
<td>1.78 ± 0.03</td>
<td>1.91 ± 0.01</td>
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<td>([\text{C}_{16}\text{mim}]\text{Br})</td>
<td>1.43 ± 0.01</td>
<td>1.65 ± 0.02</td>
<td>1.73 ± 0.00</td>
<td>1.97 ± 0.01</td>
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<td>([\text{P}_{4414}]\text{Cl})</td>
<td>1.43 ± 0.07</td>
<td>1.62 ± 0.02</td>
<td>1.65 ± 0.16</td>
<td>1.67 ± 0.05</td>
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<td>9</td>
<td>CTAB</td>
<td>1.45 ± 0.01</td>
<td>1.52 ± 0.09</td>
<td>1.89 ± 0.36</td>
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<td>([\text{C}_{10}\text{mim}]\text{Br})</td>
<td>1.45 ± 0.00</td>
<td>1.59 ± 0.01</td>
<td>1.67 ± 0.01</td>
<td>1.77 ± 0.03</td>
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<td>([\text{C}_{12}\text{mim}]\text{Br})</td>
<td>1.57 ± 0.08</td>
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<td>1.69 ± 0.04</td>
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<td>([\text{C}_{14}\text{mim}]\text{Br})</td>
<td>1.42 ± 0.11</td>
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<td>([\text{C}_{16}\text{mim}]\text{Br})</td>
<td>1.66 ± 0.05</td>
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<td>1.83 ± 0.02</td>
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<td>([\text{P}_{4414}]\text{Cl})</td>
<td>1.47 ± 0.01</td>
<td>1.47 ± 0.01</td>
<td>1.71 ± 0.00</td>
<td>1.55 ± 0.02</td>
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<tr>
<td>13</td>
<td>CTAB</td>
<td>1.43 ± 0.05</td>
<td>1.47 ± 0.03</td>
<td>1.61 ± 0.04</td>
<td>1.50 ± 0.18</td>
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<td>([\text{C}_{10}\text{mim}]\text{Br})</td>
<td>1.43 ± 0.02</td>
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<td>([\text{C}_{12}\text{mim}]\text{Br})</td>
<td>1.43 ± 0.01</td>
<td>1.49 ± 0.01</td>
<td>1.58 ± 0.01</td>
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<td>([\text{C}_{14}\text{mim}]\text{Br})</td>
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<td>1.55 ± 0.02</td>
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<td>([\text{C}_{16}\text{mim}]\text{Br})</td>
<td>1.44 ± 0.01</td>
<td>1.41 ± 0.02</td>
<td>1.61 ± 0.03</td>
<td>1.63 ± 0.01</td>
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<td>([\text{P}_{4,4,4,14}]\text{Cl})</td>
<td>1.41 ± 0.07</td>
<td>1.49 ± 0.09</td>
<td>1.48 ± 0.11</td>
<td>1.64 ± 0.11</td>
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The results shown in Table 1 suggest that the systems presented some ability for selective separation of red colorant from both the yellow and orange colorant (Se ranging from 1.3 to 2.6). There was almost no difference in selectivity between most of the experimental assays (Se from 1.3 to 2.0), with exception of the SUPRAS composed of 5 wt % TX-114 + 1.25 mM \([\text{C}_{12}\text{mim}]\text{Br}\), which showed a \(\text{Se}_{500/420\ \text{nm}}\) of 2.6. In this way,
the mixed SUPRAS can concentrate and pre-purify the fungal colorant, removing extracellular metabolites presented in the fermented broth such as sugars and proteins, although, in one single stage, it is still not able to separate molecules with a similar chemical structure like red, orange, and yellow colorants produced by *T. amestolkiae*. It is important to note that the red colorants are produced by the amination of the orange colorants, which are the first to be produced during the fungi metabolism, while the yellow colorants are produced by the reduction of the orange colorants. These colorants are quite similar chemically; therefore, their fractionation and purification require multi-stage liquid-liquid extraction operations or high-resolution chromatographic downstream techniques.

The volumetric ratio ($V_R$), an important parameter to be determined during the development of SUPRAS for the extraction of biomolecules - as it indicates the potential of these systems to concentrate the target products in reduced volumes, was also evaluated (detailed results are reported in Table S8 from Supporting Information). The $V_R$ is lower for the systems with a reduced concentration of TX-114. Consequently, these SUPRAS have concentrated the natural colorants in the surfactant-rich phase. The molarity of the ionic surfactants also appeared to influence this parameter, since the increase of this parameter led to higher $V_R$ values. The different ionic surfactants had little impact over the $V_R$ for TX-114 at 5 and 9 wt%; however, there were some variations at 13 wt%. For example, the $[P_{4,4,4,14}]\text{Cl}$ at 1.25 mM reduced the $V_R$ with TX-114 wt% when compared to the other ionic surfactant, while the $[C_n\text{mim}]\text{Br}$ with $n = 10$ and 12 at 2.50 mM increased the $V_R$. Thereby, solutions entailing lower concentrations of anionic and ionic surfactants enhance the partition of natural colorants into the micellar-rich phase.

These new SUPRAS showed a great ability to recover red colorants from fermented broth, while also presenting mild work conditions (*e.g.*, the temperature of 36 °C, kinematic viscosities ranging from 24 to 146 mm²/s, low concentrations of phase-forming agents). Therefore, these systems can be used as platforms to concentrate and pre-purify the red colorants and may be further applied to other molecules with similar chemical characteristics. Besides, as the colorant partitioned to the surfactant rich-phase, it could be recovered using microfiltration technology using an appropriate membrane. However, as the colorant can be applied in different fields such as pharmaceutical, cosmetic or dye, the intention is to use the surfactant-IL in the formulation that the
colorant will be applied. In this sense, the surfactant-IL can help in the solubilization of colorant promoting a high interaction with different matrices.

**Conclusions**

In this work, we studied the effect of cationic surfactants of the imidazolium, phosphonium, and ammonium families on the self-assembly of SUPRAS based on TX-114, and the impact of this on the ability of these SUPRAS to extract natural colorants from a fermentation broth. It was shown that the cationic surfactants increased the $T_{CP}$ on the binodal curves of TX-114 in McIlvaine buffer pH 6.5, which seems to be due to the contribution of the ILs to the electrostatic repulsions in the aggregates, which increased the energy barrier required for the phase separation. This was confirmed by the CG-MD simulations showing that 3 wt% TX-114 solutions form vesicles in water whose structure incorporates and is significantly affected by the presence of cations surfactants.

The novel TX-114-based SUPRAS studied in this work seems to be good candidates for the separation of natural colorants from fermented broth, with very high recoveries ($R_B > 80\%$) and partitioning ($K > 6$). In particular, the SUPRAS with relatively low TX-114 concentrations (i.e., ~ 5 wt%) favored the partitioning of the red colorant for the micelle-rich phase. While the introduction of a charge into the vesicles seems to be the main factor driving the colorants extraction the alkyl chain length seemed to have also an effect, albeit smaller, with dodecyl and tetradecyl chains showing the best results. Thereby, it seems important to consider not only the charge but also the diverse shapes and sizes of the aggregates formed in TX-114 + IL aqueous solutions since they affect the affinity of colorants for the surfactant-rich phase modifying their partitioning. The use of judiciously chosen cationic surfactants can enhance the ability of SUPRAS to recover colorants from fermented broths and help to create novel platforms for increased production of natural food colorants.

**Credit authorship contribution statement**

Cecília Naomi Nakamura: Data curation, Formal analysis, Investigation, Methodology. Nathalia V. Veríssimo: Investigation, Formal Analysis, Writing - original draft, Writing - review & editing. Fernanda Oliveira: Investigation, Formal Analysis, Writing - original
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.

Abbreviations

(tetramethylbutyl)phenyl-polyethylene glycol (TX-114); 1-decyl-3-methyl imidazolium bromide ([C\textsubscript{10}mim]Br); 1-dodecyl-3-methyl imidazolium bromide ([C\textsubscript{12}mim]Br); 1-hexadecyl-3-methyl imidazolium bromide ([C\textsubscript{16}mim]Br); 1-tetradecyl-3-methyl imidazolium bromide ([C\textsubscript{14}mim]Br); absorbance in the bottom phase (Abs\textsubscript{B}); absorbance in the bottom phase (Abs\textsubscript{T}); aqueous micellar two-phase systems (SUPRAS); averaged aggregation number (N\textsubscript{agg}); bottom volume (V\textsubscript{B}); cetyltrimethylammonium bromide (CTAB); Cloud point temperature (T\textsubscript{CP}); coarse-grained molecular modeling (CG-MD); colloidal gas aphrons (CGA); critical micellar concentration (CMC); initial absorbance (Abs\textsubscript{i}); initial volume (V\textsubscript{i}); ionic liquids (ILs); linear constraint solver (LINCS); liquid-liquid extraction (LLE); mass balance (MB); molecular dynamics (MD); n-bis(3-methylimidazolium-1-yl)alkane bromide ([BisAlk(mim)\textsubscript{2}][2Br]); Particle-Mesh-Ewald (PME); partition coefficient (K); partition coefficient with absorbance at wavelength 500 nm (K\textsubscript{500 nm}); partition coefficient of the orange or yellow colorants (K\textsubscript{O/Y}); point temperature (T\textsubscript{CP}); radial distribution functions (RDFs); selectivity of extraction (Se); selectivity of extraction of the red colorant relative to the orange colorant (Se\textsubscript{500/470 nm}); selectivity of extraction of the red colorant relative to the yellow colorante (Se\textsubscript{500/420 nm}); sodium dodecyl sulfate (SDS); standard deviations (std); supporting information (SI); top
volume ($V_T$); tributyl-tetradecylphosphonium chloride ([P$_4$$_4$$_4$$_4$,14]$\text{Cl}$); visual molecular dynamics (VMD); volumetric ratio ($V_R$).

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References


Highlights

- Molecular dynamics (MD) confirmed that shape, size and charge of TX-114 vesicles changes with IL addition.
- High recovery (> 80 %) and partitioning ($K > 6$) of colorants with TX-114 + IL SUPRAS.
- SUPRAS composed of cationic surfactants can promote an increase in colorants recovery.
- TX-114 + IL SUPRAS provides selectivity in the partitioning of red colorants from yellow/orange counterparts.
**Credit authorship contribution statement**

Cecilia Naomi Nakamura: Data curation, Formal analysis, Investigation, Methodology.
Nathalia V. Veríssimo: Investigation, Formal Analysis, Writing - original draft, Writing - review & editing.
Fernanda Oliveira: Investigation, Formal Analysis, Writing - original draft, Writing - review & editing.
Bruna L. Kuhn: Investigation.
Clarissa P. Frizzo: Conceptualization, Writing - review & editing.
German Perez Sanchez: Investigation, Formal analysis, Writing - original draft, Writing - review & editing.
João A. P. Coutinho: Funding acquisition, Investigation, Writing - review & editing.
Jorge F. B. Pereira: Investigation, Writing - original draft, Writing - review & editing.
Valéria C. Santos-Ebinuma: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing - review & editing.
CG-MD
TX-114 (3 wt%) vesicles in water

No IL

IL 2.5 mM
([C₅H₅n)im]Cl)

Supramolecular solvents (SUPRAS)

TX-114 (5 - 13 wt%) +
IL (1.25 - 2.50 mM)

$K > 6$
Recovery > 80 %
Selectivity (red colorant) > 1.3

Surfactant-rich phase
Natural colorant extraction
(yellow, orange, red)
Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: