

Short communication

Physicochemical characterization of aqueous micellar systems formed by environmentally friendly salts



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ABSTRACT

In this work, environmentally friendly aqueous micellar two-phase systems containing nonionic surfactants (Triton X-114, Triton X-100 and Genapol X080) and organic salts (sodium citrate and sodium tartrate) were characterized. In order to accomplish this objective, the binodal diagrams (cloud point vs. surfactant concentration) were obtained for each condition. Additionally, critical micelle concentration (CMC) and micellar hydrodynamic diameter (D_H) were determined for each system. According to the obtained results, it was found that the presence of salts lowered the CMC (Δ CMC up to 0.15 mM) and cloud point values (Δ CP up to 18 °C) following the sequence: sodium citrate > sodium tartrate. In addition, the hydrodynamic diameters of the micelles were notoriously increased in presence of the studied salts, showing the high sensitivity of the described aqueous micellar two-phase systems to the medium condition. These results open perspectives for the use of greener aqueous micellar two-phase systems for bioseparation purposes.

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1. Introduction

Surfactants comprise a group of amphiphilic molecules composed of a hydrophilic moiety and a hydrophobic moiety, known as head and tail, respectively. The surfactants can be classified onto anionic, cationic, zwitterionic or nonionic [1] according to the structural characteristic of their head. When these molecules are dissolved in a polar solvent above a critical micelle concentration (CMC), they are able to form nanometer-sized aggregates in which the hydrophilic heads remain on the outer surface and the hydrophobic tails flock to the interior in order to minimize their contact with the solvent [2,3]. The size and shape of these aggregates depend on surfactants properties and on the medium conditions such as temperature, total surfactant concentration, ionic strength, and pH.

For some micellar systems, a temperature increase [4,5] promotes a spontaneous phase separation, resulting in an aqueous micellar two-phase system (AMTPS). The mentioned phase-

separation process, between a micelle-rich phase and a micelle-poor phase, is attributed to the temperature effect on the thermal motion of water molecules, thus affecting the interaction/solvation of micelles. At increasing temperature, micelles start to interact with each other, thus resulting in a micellar network [4]. The phase-separation temperature, so-called cloud point (CP), depends basically on the surfactant structure and concentration. The presence of additives such as inorganic salts, biopolymers, fatty acids, aliphatic alcohols, and phenols also affects the CP strongly [6–8]. Particularly, the addition of certain salts, drastically lower the phase-separation temperature due to strong electrostatic interactions between salts and water molecules, which prevail against the hydrogen bonds between the surfactant polar heads and water molecules [9]. This makes polar head–polar head (micelle–micelle) interactions more favorable than the polar head–solvent (micelle–water) ones.

The use of aqueous micellar two-phase systems (AMTPS) has been considered to be an attractive alternative in liquid–liquid extraction for years [10–13]. The first application of AMTPS as a separation methodology was reported by Watanabe and Tanaka for the concentration of zinc ions [14]. Afterwards, Bordier [15] demonstrated the differential partitioning of proteins within AMTPS phases.

Up to date, the use of AMTPS has been extended to the purification of different molecules such as aromatic hydrocarbons, viruses and antibodies [10,12]. Triton X-114 is one of the most

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Nomenclature

| | |
|------------------|---|
| AMTPS | Aqueous micellar two-phase system |
| ANS | 1-Anilino-8-naphthalene sulfonate |
| Cit | Citric acid |
| CMC | Critical micelle concentration |
| CP | Cloud point |
| Δ CMC | Critical micelle concentration variation |
| Δ CP | Cloud point variation |
| ΔG_{agg} | Free energy change associated with surfactant aggregation |
| D_H | Micellar hydrodynamic diameter |
| ΔS_{agg} | Entropic change associated with surfactant aggregation |
| GX080 | Polyethylene glycol monoalkyl ether (Genapol) X-080 |
| Tart | Tartaric acid |
| T_c | Critical temperature |
| TX100 | Polyethylene glycol <i>tert</i> -octylphenyl ether (Triton) X-100 |
| TX114 | Polyethylene glycol <i>tert</i> -octylphenyl ether (Triton) X-114 |

widely used surfactant because of its slight protein denaturation index and biodegradability [16–18]. For example, Triton X-114 in combination with McIlvaine buffer (citrate/phosphate buffer) and an affinity ligand [19] has recently been used to purify an anti-electronegative LDL single-chain antibody fragment (recovery of 88% and purification factor of 2). On the other hand, surfactants belonging to the Genapol series are also widely used due to their low toxicity. In fact, the use of Genapol X-080 has been approved by the Food and Drugs Administration (FDA), extending their application to the processing of edible and pharmaceutical products. For example, liquid–liquid extraction with AMTPS, also known as cloud point extraction, using Genapol X-080 and NaCl has been used to purify polyphenols from wine sludge with recovery values close to 76% [20].

Despite of the successful results above mentioned, the use of non biodegradable salts should be avoided because of their negative environmental effect. The replacement of inorganic salts by biodegradable and non-toxic ones, such as citrate and tartrate, has been reported to be a good alternative [21–23]. For example, sodium citrate and sodium tartrate has been widely used in aqueous two phase systems with polyethylene glycol (PEG), ionic liquids (IL) and Ucon [22–24]. Nevertheless, up to our knowledge, the use of these organic salts to form AMTPS with nonionic surfactants has been poorly explored. There are just a few reports about liquid–liquid equilibrium of Tween 20/sodium citrate [25] and Triton X-100/sodium citrate [26] aqueous two-phase systems

in which the reported phase separation data only involve the concentration of the system components at a fixed temperature [2,27]. However, the knowledge of temperature effect on phase-separation behavior, necessary for a better understanding and exploitation of the AMTPSs in purification processes, has not been further explored.

Taking into account the mentioned above, the main aim of this work was to determine the main characteristics of AMTPSs containing nonionic surfactants (Triton X-114, Triton X-100 and Genapol X080) and organic salts (sodium citrate and sodium tartrate). To accomplish that, the binodal diagrams (cloud point vs. surfactant concentration) were obtained for each case. Additionally, critical micelle concentration (CMC) and micellar diameter (D_H) were determined for each system. This characterization represents a start point to a further application of these systems in bioseparation purposes.

2. Materials and methods

2.1. Materials

The nonionic surfactants, polyethylene glycol *tert*-octylphenyl ether (Triton) X-100 and X-114 (TX100 and TX114, respectively) and polyethylene glycol monoalkyl ether (Genapol) X-080 (GX080), were purchased from Sigma–Aldrich and used without further purification (see Table 1). Tartaric acid (Tart), citric acid (Cit), sodium hydroxide and 1-anilino-8-naphthalene sulfonate (ANS) were supplied by Sigma–Aldrich and used as received. All the other reagents were of analytical grade and used without further purification.

Sodium citrate and sodium tartrate stock solutions (500 mM) were prepared by dissolving the acid in water and adjusting the pH to 5.00 with sodium hydroxide.

2.2. Experimental procedure

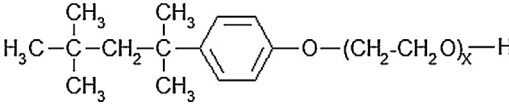
2.2.1. Critical micelle concentration (CMC)

The critical micelle concentration of the surfactant was determined by using 1-anilino-8-naphthalene sulfonate (ANS) as hydrophobic probe ($\lambda_{excitation}$ 382 nm, $\lambda_{emission}$ 470 nm, [ANS] 0.1 mM, temperature 22 °C) [28]. Fluorescence measurements were performed on an Aminco Bowman S2 spectrofluorometer with a thermostated circulating water bath and each measurement was performed by triplicate.

2.2.2. Cloud point (CP) determination

Cloud point determination of surfactant solutions was performed by the method described by Watanabe and Tanaka [14], which consist in visually identifying the temperature at which solutions with known concentrations of a given surfactant become cloudy. To accomplish that, 5 mL-systems containing surfactant (0–8% w/w) and sodium citrate/sodium tartrate (50, 100 and

Table 1
Chemical structure and purity of surfactants.

| Compound | Chemical structure | Mass fraction purity |
|---|--|----------------------------|
| Triton X-100, $x = 9-10$ Triton X-114, $x = 7-8$ |  | ≥ 0.99 ≥ 0.99 |
| Genapol X-080, $x = 8$; $Y = 12$ | $H_3C-(CH_2)_Y-O-(CH_2-CH_2O)_x-H$ | ≥ 0.99 |

200 mM) were prepared by mixing the pure surfactant, the stock solutions of sodium citrate/sodium tartrate pH 5.00 and water at 8 rpm for 1 h at room temperature. Then, the systems were transferred to a refrigerated bath at 8 °C (temperature at which the systems exhibited a single and clear phase). Subsequently, the temperature was slowly raised (by 0.1 °C). The temperature at which the solution first became cloudy, indicating the onset of phase separation, was taken as the cloud point. The observed values of CP were then plotted as a function of the corresponding surfactant concentration. The procedure was repeated three times to warrant reproducibility.

2.2.3. Apparent hydrodynamic diameter (D_H)

Micelle hydrodynamic diameter was estimated by means of dynamic light scattering (DLS) measurements. These experiments were performed using Zetasizer Nano ZS-100 (Horiba) with He-Ne laser operating at a wavelength of 633 nm and 90° scattering angle. Micellar solutions of fixed surfactant concentration (5% w/w) and different salts composition were previously filtered using a membrane filter of 0.2 μm pore size. Each measurement was performed at least three times.

3. Results and discussion

3.1. Critical micelle concentration (CMC)

Fig. 1 shows ANS fluorescence variation at increasing TX100 concentration in either water or in NaTart solutions (50, 100 and 200 mM). No significant changes in the fluorescence intensity (FI) of the probe were appreciated at low surfactant concentration (below 0.1 mM) in all the cases. However, an abrupt increase in FI is observed at TX100 concentration above 0.1 mM. This is a well known behavior, related to the incorporation of the hydrophobic probe into the interior of micelles. This allows to detect the minimal concentration at which the surfactant begins to aggregate, also known as critical micelle concentration (CMC) [28]. Similar results were observed for the other surfactant solutions (see Supplementary Figs. 1 and 2).

Supplementary material related to this article found, in the online version, at <http://dx.doi.org/10.1016/j.fluid.2015.03.011>.

CMC estimation was carried out by using the method presented by Zana and et al. [29] which consists in calculating the intercept of the extrapolation of the nearly horizontal part of the plot FI vs.

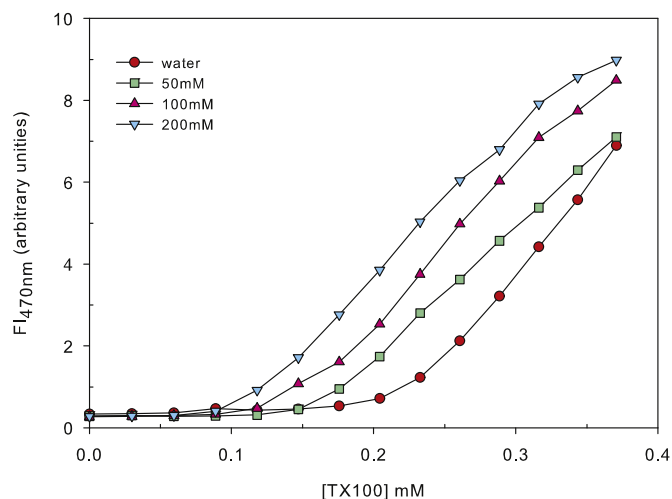


Fig. 1. Dependence of ANS (0.1 mM) fluorescence intensity on TX100 concentration in water and in NaTart solutions of different salt composition (50, 100 and 200 mM). $\lambda_{\text{excitation}}$ 360 nm, $\lambda_{\text{emission}}$ 480 nm, temperature: 22 °C, pH 5.00.

Table 2

CMC values (mM) of nonionic surfactants determined in water and in biodegradable salt solutions. Temperature: 20 °C; pH 5.00. The presented data are the average of triplicates with their standard deviation.

| | CMC (mM) | | |
|-------------|-------------|-------------|---------------|
| | TX100 | TX114 | GX080 |
| Water | 0.20 ± 0.01 | 0.18 ± 0.03 | 0.081 ± 0.003 |
| NaCit (mM) | | | |
| 50 | 0.14 ± 0.02 | 0.16 ± 0.02 | 0.065 ± 0.005 |
| 100 | 0.11 ± 0.01 | 0.11 ± 0.02 | 0.063 ± 0.002 |
| 200 | 0.05 ± 0.01 | 0.12 ± 0.01 | 0.051 ± 0.004 |
| NaTart (mM) | | | |
| 50 | 0.15 ± 0.03 | 0.14 ± 0.02 | 0.083 ± 0.005 |
| 100 | 0.13 ± 0.02 | 0.15 ± 0.01 | 0.061 ± 0.002 |
| 200 | 0.10 ± 0.01 | 0.10 ± 0.01 | 0.054 ± 0.003 |

Standard uncertainties s are $s(\text{cmc}) = \pm 2 \times 10^{-6}$ (M).

surfactant concentration in Fig. 1 and the rapidly varying part of that plot. The micellization process is believed to be caused by the so-called hydrophobic effect. When dissolving a surfactant – amphiphilic molecule – in water, the formation of ordered water cages around the hydrophobic tails of the surfactant occurs in order to minimize repulsion forces. The micelle formation is a mechanism that allows eliminating the contact between non-polar chains and water since the hydrophobic tails flock to the interior of micelle. A positive entropic change (ΔS_{agg}), associated to the release of structured water molecules from the hydrophobic moiety, contributes favorably to the free energy change (ΔG_{agg}) of the self-aggregation, and drives the process [30]. As can be seen from Table 2, the estimated CMC values of TX100 and TX114 in water were found to be 0.20 and 0.18, respectively, agreeing with literature results [31,32]. On the other hand, it can also be appreciated that the presence of NaCit and NaTart induces a CMC decrease, whose magnitude depends on salt concentration. This trend has already been observed by other authors and it is generally attributable to a change in the structured water degree [33]. According to the Hofmeister series, citrate and tartrate are kosmotropic (order-making) anions that interact more strongly with water than water with itself. Due to their strong tendency to hydrate, these anions compete for water with the surfactants chains, thus promoting the release of water molecules from their non-polar tails. This additional entropic contribution, associated to the salt presence, favors the micellization process and decreases the CMC value.

On the other hand, Table 2 also shows the CMC values of GX080. The presented data (0.05–0.08 mM) are in good agreement with literature results [20]. Clearly, the CMC of GX080 is much lower than those of TX100 and TX114. This is a consequence of the “aliphatic” hydrophobic tail of this surfactant that is reported to lower the CMC more strongly than the “branched alkylphenyl” hydrophobic tails, present in TX100 and TX114 [30]. In this case, the addition of salts did not affect notoriously the CMC of the surfactant when comparing with the effect observed for the Triton X surfactants. This can be explained on the basis of the following approximate relationship which relates ΔG_{agg} with the CMC [30]:

$$\Delta G_{\text{agg}} = RT \ln \text{CMC} \quad (1)$$

By differentiation:

$$d\Delta G_{\text{agg}} = RT \frac{d\text{CMC}}{\text{CMC}} \quad (2)$$

According to previous works [34], the change in the ΔG_{agg} ($d\Delta G_{\text{agg}}$), caused by a given salt at a certain concentration, is the same irrespective the non-polar tail of the surfactant, being aliphatic or aromatic. Consequently, the change in CMC ($d\text{CMC}$), caused by salt, depends on the magnitude of CMC. This explains

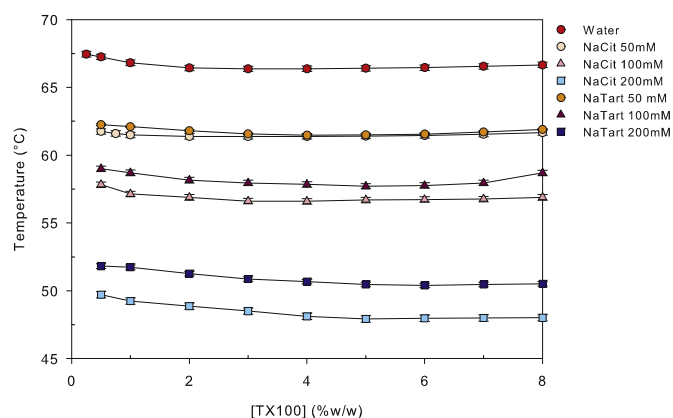


Fig. 2. Cloud point temperatures of Triton X-100 aqueous micellar systems in pure water and in presence of NaCit and NaTart, pH 5.00, at different concentration (50 mM, 100 mM and 200 mM). The presented data are the average of triplicates and the error bars are presented in each experimental point.

that the lower changes, observed for GX080, are determined by its lower CMC value respect to those of TX100 and TX114.

3.2. Cloud point (CP) determination

Clouding phenomenon is an important feature of nonionic surfactants and is related to the way in which surfactant–water interactions occur [35]. Even though phase separation temperature mainly depends on surfactant structure and concentration [36], the presence of additives, such as salts and ligands [4,20,37,38], is known to strongly affect the surfactant cloud point and, as a consequence, the physicochemical properties of the aqueous micellar two phase systems. As such, it is of considerable importance to acquire knowledge of the clouding behavior of the surfactant solution [36].

Figs. 2 and 3 show the dependence of TX100 and Tx114 cloud points on their concentration in water and salt solutions. In all cases, the coexistence curves presented similar characteristics to those previously reported by other authors [32,38], i.e., with increasing surfactant concentration the cloud point decreases in dilute regime and, subsequently, increases after reaching a minimum cloud point value, also known as critical temperature (T_c) [39]. On the other hand, it can also be seen that TX100 presented CP values higher than those of TX114, behavior that has already been attributable to the fact that the latter surfactant has less ethylene oxide units per molecule [32] (see

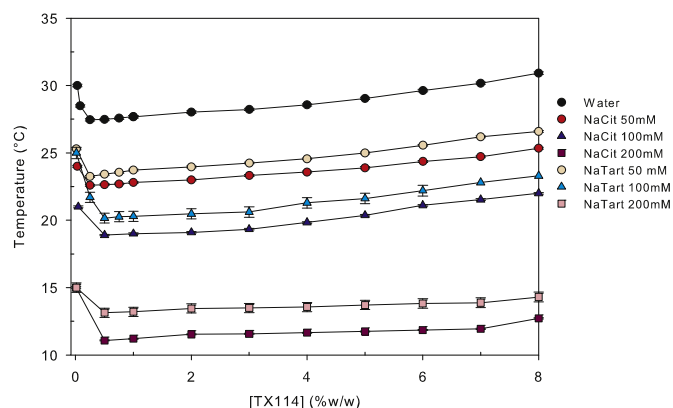


Fig. 3. Cloud point temperatures of Triton X-114 aqueous micellar systems in pure water and in presence of NaCit and NaTart, pH 5.00, at different concentration (50 mM, 100 mM and 200 mM). The presented data are the average of triplicates and the error bars are presented in each experimental point.

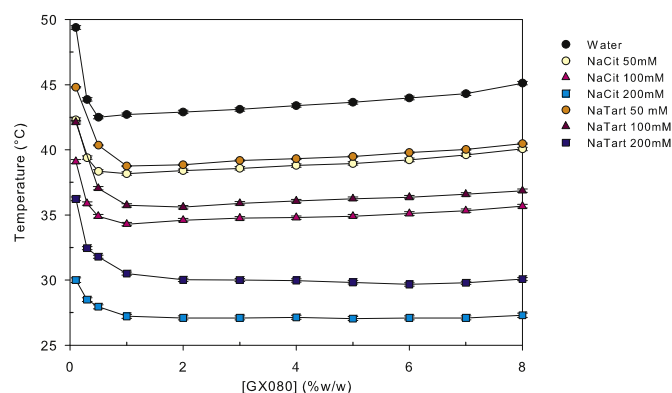


Fig. 4. Cloud point temperatures of Genapol X-080 aqueous micellar systems in pure water and in presence of NaCit and NaTart, pH 5.00, at different concentration (50 mM, 100 mM and 200 mM). The presented data are the average of triplicates and the error bars are presented in each experimental point.

Table 1). Note that CP values depend strongly on the polyoxyethylene chain length but it is less influenced by the hydrophobe size [30].

Cloud points vs. surfactant concentration curves for GX080 are depicted in Fig. 4. This surfactant belongs to the well-known family of polyethylene glycol alkyl ethers, which exhibits significant differences in its CMC and absorptivity respect to the surfactant of the Triton X-series (polyethylene glycol alkylphenyl ethers) [17]. Particularly, the phase separation behavior of this detergent has not been deeply investigated. In fact, the published cloud points greatly differ between each other, belonging to an extremely wide range of temperatures (from 34.5 to 75 °C) [17]. Nevertheless, according to the results presented in Fig. 4, the aqueous micellar system composed of GX080 and water presents a T_c value of 42.5 °C, which is in good agreement with the results presented by Schrader et al. [17] and Chatzilazarou and et al. [20].

When analyzing the effect of organic salts on CP, it can be seen from Figs. 2–4 that the added electrolytes exert an important reduction on the cloud point of the surfactant solutions. CP is strongly dependent on cosolutes. Different ions may either increase or decrease the CP and may be named as “salting in” or “salting out”, respectively. This different effect may be understood from the interaction between the surfactant and the cosolute. The effect is more pronounced for the anions. Chaotropic anions (i.e., SCN^- , I^-) break the water structure in bulk water and increase the concentration of free water molecules that can form hydrogen bond with the surfactant chains. Others, such as Cl^- and SO_4^{2-} , tend to strongly hydrate and accumulate in the bulk water relative to the vicinity of oxyethylene groups. These anions, known as kosmotropic, decrease the micellar hydration shell, induce a closer interaction among micelles and consequently, favor the phase separation [10,35,36]. Clearly, the citrate and tartrate, used in this work, belong to this last type of cosolutes since both anions decrease the CP of AMTPSs [4,9]. It can also be appreciated that in presence of NaCit CP values resulted to be lower (higher decreases in CP relative to AMTPS in water) than those observed in presence of NaTart [9]. This agrees with the Hofmeister series which indicates that citrate possesses a higher kosmotropic character than tartrate. Notice that this series allows the prediction of the extent of the anion and cation effects on cloud point based on their influence in water structure [4].

3.3. Apparent hydrodynamic diameter (D_H)

Micellar size is a critical parameter that is directly related to the viscosity and other rheological features of micellar solutions [35].

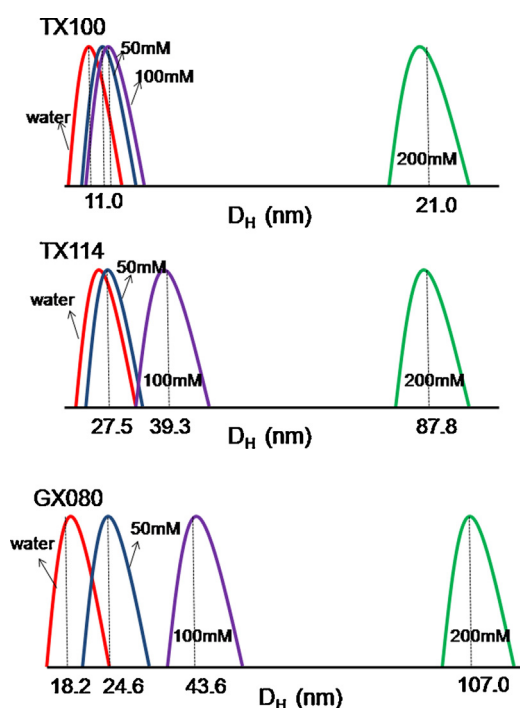


Fig. 5. Distribution of micellar hydrodynamic diameters of surfactants (TX100, TX114 and GX080; 5% w/w) in absence and presence of NaCit at different concentrations (50, 100 and 200 mM). pH 5.00. Temperature: 22 °C for TX100 and GX080, 10 °C for TX114.

These system characteristics determine both, phase separation and partitioning behavior of AMTPS. In this context, it is of considerable importance to acquire knowledge of the effect of NaCit and NaTart on micellar size.

As shown in Fig. 5, the distribution profiles for the hydrodynamic diameters of nonionic surfactants (5% w/w) were estimated from DLS in absence and presence of different concentrations of NaCit. The apparent hydrodynamic diameter (D_H) of TX100 and TX114 in water were about 11.0 nm and 27.5 nm, respectively, agreeing with bibliographic values [31,35]. Micelles of GX080 in water presented an average D_H value of 18.5 nm. In this case there is not enough bibliographic information to compare it.

In all cases, it can also be seen that in presence of salt, the micelles experienced a significant size growth, being this behavior dependent on salt concentration and on the surfactant characteristic. One of the most accepted explanation for this process is that the added salts disrupt the water structure around the micelle, thus leading to a favoring surfactant interaction and consequently to a micelle growth [35]. On the other hand, the differences observed between the different surfactants, i.e., the most hydrophilic ones (TX114 and GX080) were more affected for salt addition than TX100, could be attributable to both, the way in which the surfactants are hydrated or the proximity between the working temperature and the T_c value (see Fig. 5) [35]. Similar results were observed with NaTart.

4. Conclusion

The physicochemical characterization of AMTPS composed of water + nonionic surfactants (Triton X-100, Triton X-114 and Genapol X-080) + biodegradable salts (sodium citrate or sodium tartrate) was carried out in this work. The CMC values as well as the cloud points showed to be affected by salts addition. More importantly, the T_c resulted to be drastically lowered by high concentration of salts which is advantageous in terms of

purification of temperature-sensitive molecules. Additionally, the micellar size also resulted to be altered in presence of electrolytes, indicating that the systems characteristics can be highly regulated by adding citrate or tartrate salts. These properties and several additional advantages such as low cost and fast phase separation make the assayed systems promising, versatile, and attractive tools in the field of bioseparation.

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