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Anion Effects on the Liquid-Liquid Equilibrium Behavior of Pluronic L64 + Water + Sodium Salts at Different pH: Determination of Thermodynamic Parameters

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Abstract: Two-phase aqueous systems have replaced and simplified steps in extraction and purification processes, especially for biocompounds. Thus, this study evaluated the liquid-liquid equilibrium behavior of Pluronic L64 + water + sodium salts at pH 5.0, 7.5, and 10.0. Sodium sulfate demonstrated the greatest phase separation, followed by sodium citrate and sodium tartrate. Higher pH values resulted in larger biphasic regions. The polymer distribution coefficients increased with the addition of salt. As pH increased, there was a tendency for Pluronic L64 to migrate to the polymer-rich phase. The Gibbs energy of micellization between $-11{,}000$ and $-25{,}000$ kJ mol^{-1} indicated the spontaneity of the process micellization for all systems, showing lower values for the systems with sodium sulfate. This parameter was related to the anion speciation at each pH. Besides, the effects of water structuring around ions and ion-polymer interaction influenced the phase separation.

Keywords: Gibbs energy of micellization; aqueous biphasic systems; pluronic L64; solvation

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1. Introduction

In biotechnology, there is a great concern with the purification techniques used since the cost of this step represents 50 to 90% of the total cost of production [1]. Pharmaceutical products applied in diagnostic analyses and treatments of humans and animals require a high degree of purity. Therefore, reducing the number of steps necessary to obtain the pure final product is advantageous and must be preceded by an adequate extraction and purification design [2]. Furthermore, economically viable purification techniques, with high levels of recovery and product purity, result in great resource savings and make the final product more accessible.

Extraction systems with two liquid phases in equilibrium have been used in biotechnological processes [3–6]. In those systems, phase separation occurs due to rapid mass transfer caused by low surface tension, providing selective separation without or with little denaturation [7]. Therefore, it is a non-aggressive technique for extracting biocompounds and does not influence the product of interest, presenting a favorable environment for biological compounds [8].

The phenomenon of phase separation depends on the molecular interactions of the constituents, which are responsible for the physicochemical characteristics of both phases [9]. In this context, researchers have studied the separation of biomolecules with pharmaceutical and biotechnological interests [10]. Coelho et al. [11] compared the systems polyethylene glycol (PEG) 4000 (g mol^{-1}) + water + phosphate and PEG 4000 (g mol^{-1}) + water + ammonium sulfate in the extraction of the enzyme bromelain from pineapple residues. The first system showed the highest recovery (87%). The second had the highest purification yield. Likewise, the purification of β-galactosidase protein demonstrated high efficiency in PEG 6000 (g mol^{-1}) + water + sodium phosphate and PEG 8000 (g mol^{-1}) + water + sodium phosphate systems [12]. The recovery index and purification factor for the first system were 95% and 8.0, while for the second, 72% and 9.77.

The polymeric molecules in aqueous two-phase systems have hydrophilic and hydrophobic regions. In solution, the reorganization of polymeric surfactant tends to maximize the contact of the hydrophilic part with water and minimize this contact with the hydrophobic part, giving rise to micelles. The reorganization and formation of micelles help to form a new polymer-rich phase. Partitioning of water molecules from the polymer-rich phase, in which the monomers were solvating, results in their migration to the salt-rich phase. The micelles formed in solution have different sizes and shapes depending on the polymer and the conditions of the medium. These structures result in a significant change in their physical and chemical properties [13].

Protein separation and purification processes use various polymers, such as PEG, polypropylene glycol (PPG), and dextran. However, this study used Pluronic L64, an unconventional copolymer composed of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) arranged in triblock in the form PEO-PPO-PEO. Its advantages include low toxicity, cost, and critical micelle concentration [14]. Salts are commonly found in cell culture media, fungi, algae, and animal and plant cellular environments. These electrolytes change the cloud point and the phase separation region, affecting the phase equilibrium behavior of polymeric solutions [15,16].

Although the literature has reported studies with Pluronic L64 and sodium salts, the effect of ions and the change in pH on the behavior of liquid-liquid equilibrium was poorly explored, mainly as to the determination of thermodynamic parameters in the separation process. Studies have focused on evaluating the influence of temperature on phase equilibrium [17,18], protein extraction [19], and the partitioning of hydrophilic and hydrophobic molecules [20].

In this context, the study evaluated the effect of sulfate (SO_4^{2-}), citrate ($C_6H_5O_7^{3-}$), and tartrate ($C_4H_4O_6^{2-}$) anions on the liquid-liquid equilibrium of a system containing Pluronic L64 + water + sodium salts at pH, 5.0, 7.5, and 10.0. In addition, quantitative thermodynamic parameters presented new perspectives on the phenomenon of phase separation, as determined by the Gibbs free energy of micellization (ΔG_{mic}) as a function of pH, volume of anion hydration (V_{hyd}), the volume of the electrostriction layer (V_{el1}), volume around the electrostriction layer (V_{el2}), Gibbs free energy of anion hydration (ΔG_{hyd}), and Gibbs free energy of electrostatic interaction in hydration shell and the shell around it (ΔG_{el12}).

2. Materials and Methods

2.1. Experimental Procedure

Pluronic L64 (Sigma Aldrich, >99.0%); sodium sulfate (Synth, >99.0%); sodium citrate (Synth, >99.0%); sodium tartrate dihydrate (Synth, >99.0%); sulfuric acid (Dinâmica, 97.0–98.0%); citric acid (Dinâmica, >99.0%); tartaric acid (Dinâmica, >99.0%); sodium hydroxide (Dinâmica, 99.0%), and demineralized water were used. The systems were Pluronic L64 + water + sodium sulfate, Pluronic L64 + water + sodium citrate, and Pluronic L64 + water + sodium tartrate at 293.15 K at pH 5.0, 7.5, and 10.0. Figure 1 displays the molecular structures of the surfactant and salts.

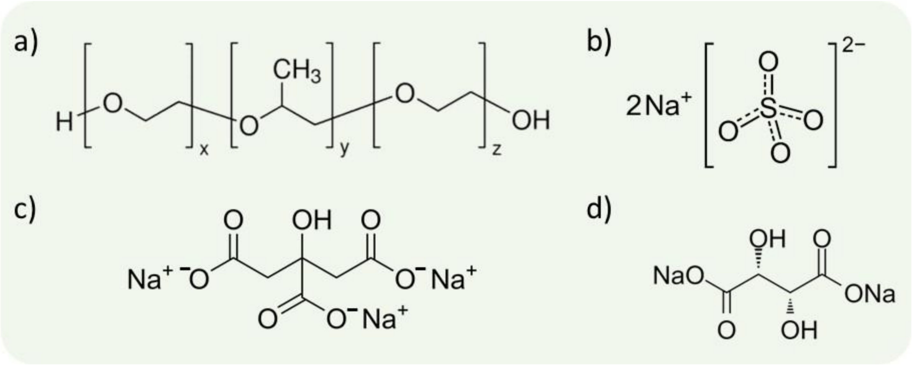

Figure 1. Molecular structures—(**a**) Pluronic L64; (**b**) Sodium sulfate; (**c**) Sodium citrate; (**d**) Sodium tartrate.

The binodal curves were obtained by turbidimetric titration using a saline solution (10% (*w*/*w*) for sodium sulfate and 30% (*w*/*w*) for sodium citrate and tartrate) in a mixture of polymer and water contained in a jacketed glass cell, connected to a thermostatic bath (Tecnal, model TE-2005) at 293.15 K. A magnetic stirrer stirred the system. The cloud point in the system determined the end of the titration. At this point, 40 drops of water were added, making the system single-phase, composed of a clear and transparent mixture. Then, the titration retook place until the cloud point formation, repeating the procedure until covering the regions between 1% and 50% (*w*/*w*) of polymer. An analytical balance (Shimadzu, model AUY-220, 0.0001 g accuracy) determined the masses in the syringes before and after adding water and saline solution to obtain the mass fractions of the components.

Buffer solutions, sulfuric acid or sodium hydroxide controlled the pH of the system. The pH was measured on a pH meter (Tecnal, model TEC-7). In systems with Pluronic L64 + water + sodium sulfate, the adjustment to pH 5.0 occurred with the addition of sulfuric acid. The adjustment occurred before and after adding 40 drops of water to all points of the binodal curve. In the Pluronic L64 + water + sodium citrate and Pluronic L64 + water + sodium tartrate systems, adjustment to pH 5.0 occurred with buffer solutions of sodium citrate/citric acid or sodium tartrate/tartaric acid, respectively. The adjustment to pH 10.0 in all systems occurred by adding sodium hydroxide since sodium citrate and sodium tartrate do not buffer the systems.

According to the method proposed by Merchuk et al. [21], the compositions of the salt-rich phase (SRP) and the Pluronic L64-rich phase (PRP) provided the tie lines. They were determined from the mass fractions of the three components (overall composition points—OC) in the biphasic region of the binodal curve. The components were weighed and added to Falcon tubes and shaken by a vortex mixer (Gehaka, model AV-2) for homogenization of the mixture. The tubes were rested in a thermostatic bath at 293.15 K for 48 h. According to the clarity of the phases and the interface completely defined, the upper and lower phases were separated with Pasteur pipettes and weighed separately to determine the compositions of Pluronic L64 and salt.

2.2. Determination of Thermodynamic Parameters

The polymer and salt mass fractions in the PRP and SRP in each tie line were determined through Equations (1)–(4) using the Solver program of Microsoft Excel. The method proposed by Merchuk et al. [21] considered the ratio between the mass of the polymer-rich phase and the total initial mass of the mixture, according to Equation (5). The Statgraphics software adjusted the parameters of Equations (3) and (4).

$$\mathrm{w}_{\mathrm{polymer}}^{\mathrm{PRP}} = \left(\frac{\mathrm{w}_{\mathrm{polymer}}^{\mathrm{OC}}}{\alpha}\right) - \left(\frac{1-\alpha}{\alpha}\right)\mathrm{w}_{\mathrm{polymer}}^{\mathrm{SRP}} \tag{1}$$

$$w_{salt}^{PRP} = \left(\frac{w_{salt}^{OC}}{\alpha}\right) - \left(\frac{1-\alpha}{\alpha}\right) w_{salt}^{SRP} \tag{2}$$

$$w_{polymer}^{PRP} = ax^3 + bx^2 + cx + d \tag{3}$$

$$w_{polymer}^{SRP} = ax^3 + bx^2 + cx + d \tag{4}$$

$$\alpha = \frac{mass^{SRP}}{mass^{OC}} \tag{5}$$

where w—mass fraction of the component (polymer or salt) in PRP or SRP, OC—initial mixing point selected in the biphasic region, and $f(w_{polymer}^{PRP})$ and $f(w_{polymer}^{SRP})$ refer to the equations of adjustment of the binodal curves, which demonstrate the relationship between the polymer mass fraction as a function of the salt mass fraction. Considering a single polynomial, the adjusted coefficients were the same for both phases (Table S1).

After determining the mass fractions of the components in each phase, their values were converted into molar fractions. As Corkil et al. [22] proposed, Equation (6) provides the Gibbs energy of micellization for micellar systems.

$$\Delta G_{mic} = RT\ln\left(X_{Polymer}\right) \tag{6}$$

where R—Universal gas constant ($kJmol^{-1}\ K^{-1}$), T—Temperature (K), and $X_{Polymer}$—Mole fraction of Pluronic L64.

Marcus [23] reported that the ion's standard partial volume results from a sum of four terms (Equation (7)).

$$\overline{V^0} = V_{hyd} + V_{el1} + V_{el2} + V_{str} \tag{7}$$

where V_{hyd}—Volume of hydrated anion by disregarding the electrical charge effect, V_{el1}—Lower volume caused by electrical charge (electrostriction) in the hydration shell, V_{el2}—Decreased volume caused in the water around the hydration shell due to the electrical effect, V_{str}—Volume increases due to the water molecules structured around hydrophobic groups.

The V_{str} is used for large hydrophobic anions with alkyl or aryl groups resulting in water molecules around them structuring themselves with an open, clathrate-like structure of positive values. V_{str} is assigned as 6.1, 5.4, and 23.2 $cm^3\ mol^{-1}$ for CH_3, CH_2, and C_6H_5 groups [24]. Therefore, the value of 23.2 $cm^3\ mol^{-1}$ for the $C_6H_5^-$ group in citrate anion was considered.

Equations (8)–(11) determined each volume contribution for salt anions at 293.15 K [25]:

$$V_{hyd} = (4/3)\pi N_{Av} + (r + \Delta r)^3 \tag{8}$$

$$V_{el1} = -59.27|z_i|\Delta r/(r + \Delta r) \tag{9}$$

$$V_{el2} = -417.5\ z^2\ [1/(r + \Delta r)] \tag{10}$$

$$\Delta r = \left(9.46 \times 10^{-4}\frac{|z|}{r} + r^3\right)^{1/3} - r \tag{11}$$

where z—Anion charge, N_{Av}—Avogadro number, Δr—Thickness of immobilized water spherical shell surrounding the ion, r—Effective radius of anions determined by the apparent molar volume at 298.2 K. Values equal to 72.0 $cm^3\ mol^{-1}$, 60.1 $cm^3\ mol^{-1}$ [25] and 36.5 $cm^3\ mol^{-1}$ [26], respectively for $C_6H_5O_7^{3-}$, $C_4H_4O_6^{2-}$, and SO_4^{2-} (Table S2).

Equations (12)–(15) provide the parameters of ΔG_{hyd} at 298.2 K [27]:

$$\Delta G_{hyd} = \Delta G_{neu} + \Delta G_{el12} + \Delta G_{uns} \tag{12}$$

$$\Delta G_{neu} = 41 - 87r + 1200r^2 \tag{13}$$

$$\Delta G_{el12} = -64.5z^2\left[0.44\frac{\Delta r}{r} + 0.987\right]/(r + \Delta r) \tag{14}$$

$$\Delta G_{uns} = 120rz^3 \tag{15}$$

where ΔG_{neu}—Gibbs free energy of the neutral interaction between anions and water, ΔG_{el12}—Gibbs free energy of the electrostatic interaction in and around the hydration shell, ΔG_{uns}—Gibbs free energy of an unsymmetrical charge effect.

Equation (16) provides the distribution coefficient:

$$k = X_{Polymer}^{PRP} / X_{Polymer}^{SRP} \tag{16}$$

where $X_{Polymer}^{PRP}$—Polymer molar fraction of PRP and $X_{Polymer}^{SRP}$—Polymer molar fraction of SRP. Both were determined through their mass balance (Equations (1)–(4)).

3. Results and Discussion

Figure 2 displays the binodal curves and tie lines, considering different pH for Pluronic L64 + water + sodium sulfate/sodium citrate/sodium tartrate systems. The change in pH influenced the composition of the binodal curves and the slope and length of the tie lines. In addition, the pH interfered with the dissociation of electrolytes, modifying the interactions between the constituents and, consequently, influencing the solvation of anions and cations, as reported by Silva and Loh [28].

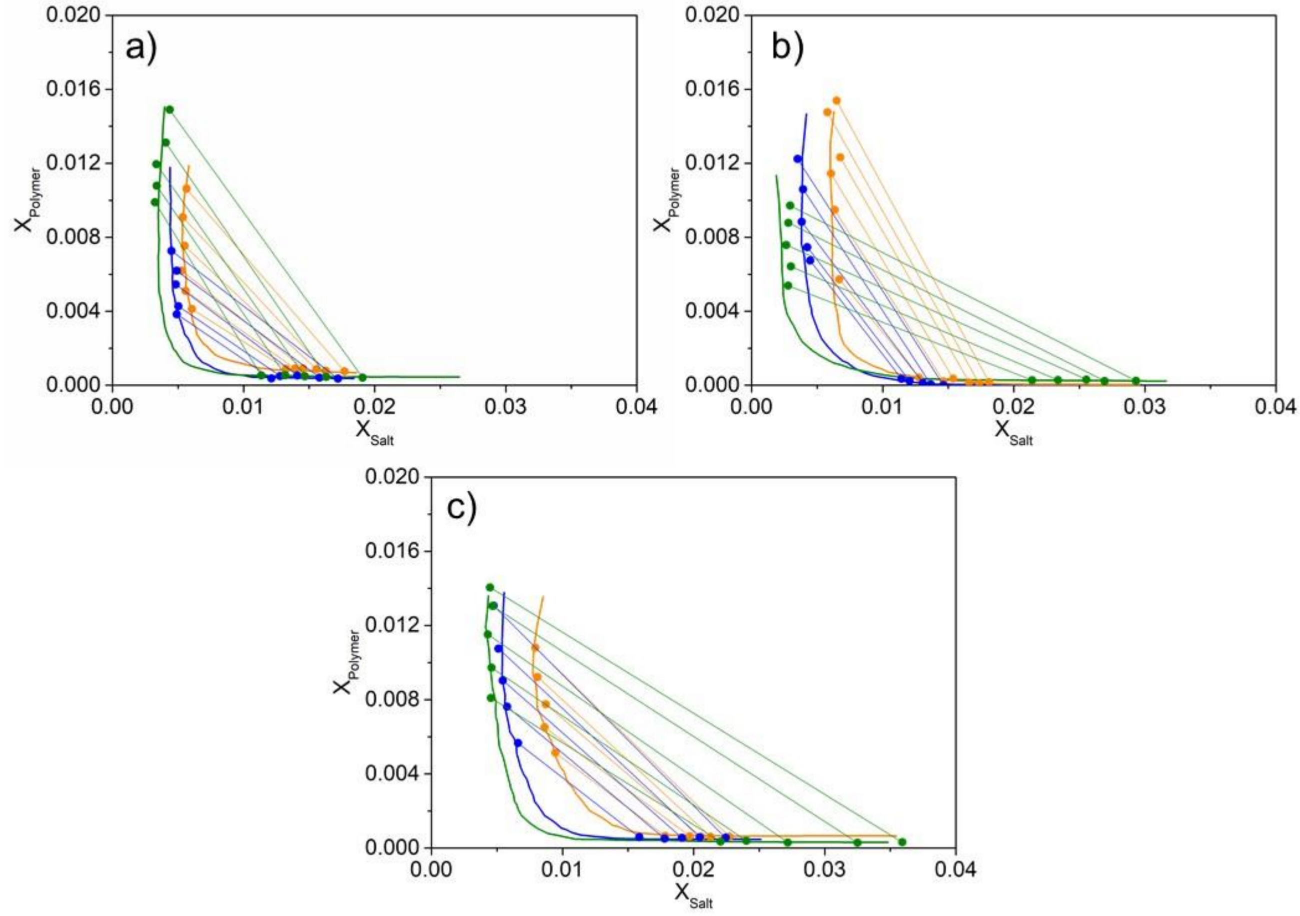

Figure 2. Binodal curves and tie lines at pH (●) 5.0, (●) 7.5, and (●) 10.0: (**a**) Pluronic L64 + water + sodium sulfate; (**b**) Pluronic L64 + water + sodium citrate; (**c**) Pluronic L64 + sodium water + tartrate.

In all systems, the increase of pH increased the biphasic region. Therefore, lower polymer and salt molar fractions allow phase separation. According to Barani et al. [29], the increase in the salting-out effect caused by the increase in pH results from the decrease in the hydrogen interactions of the water with the polymer groups due to the negative ions. Likewise, Nascimento et al. [16] concluded that the anions are less protonated at higher pH. Ions with higher valences compose the system and have a higher hydration capacity, causing an increase in the salting-out effect compared to ions with lower valences. Thus, a higher hydration capacity of the ion results in larger biphasic regions. Dan et al. [30] verified that salts could form species in solutions at different pH by changing the charges of the ionizable groups in the salt molecules, contributing to the different behavior of the binodal curves. Complete deprotonation of citrate and tartrate anions occurred at pH 7.5 and 10.0. On the other hand, at pH 5.0 for the same anions, the following species distribution occurred: $C_6H_5O_7^{3-}$ (25%), $C_6H_6O_7^{2-}$ (50%), and $C_6H_7O_7^{1-}$ (25%) and $C_4H_4O_6^{2-}$ (50%) and $C_4H_5O_6^{1-}$ (50%). Therefore, there was an increase in the phase separation region as the pH increased, mainly for systems containing citrate and tartrate salts.

Grundl et al. [31] analyzed the salting-out and salting-in effects of organic compounds in different extraction processes. Pentasodium phytate (Phy^{5-}, $5Na^+$) showed the most efficient salting-out effect. The salt was investigated for liquid-liquid extraction of 5-hydroxymethylfurfural, ethanol-water, and glycerol-water separation. The increase in salting power occurred due to the strong binding of the phosphate functions of phytic acid to water molecules. In another study, Videira and Aires-Barros [32] reported similar results with this study. The authors analyzed systems containing ${H_2PO_4}^-$ and ${HPO_4}^{-2}$ anions. The decrease in pH resulted in higher protonation of anions, with a decrease in the concentration of multivalent anions, requiring higher concentrations of salts to form the biphasic region.

As shown in Figure 3, the polymer distribution coefficient increased as the molar fraction of salt increased. In general, at pH 10, the systems demonstrated higher distribution coefficients for the polymer, while at pH 5, they were lower. For example, for systems containing sodium citrate, the polymer distribution coefficient for a molar salt fraction of 0.011 was 6.78, 10.27, and 24.88 for pH 5.0, 7.5, and 10.0, respectively. Similarly, for systems containing sodium tartrate, the values for the same molar fraction of salt were 12.90, 16.92, and 38.74 for pH 5.0, 7.5, and 10.0, respectively. This behavior indicated a tendency of Pluronic L-64 migration to the PRP with the pH increasing. The increase in the distribution coefficient corroborates with an increase in the biphasic region due to the increase in pH, which is determined by the speciation of citrate and tartrate ions at pH 5. Belchior et al. [33] reported that the protonation and decrease in the charge and charge density of the anions led to a decrease in their salinization effect and, consequently, in the partition of the polymer between the two liquid phases.

The addition of salt increases the competition of the ions of this species with the polymer for water molecules [34]. After adding saline ions, the water molecules previously solvating the polymer molecules were structured around these ions, expelling the polymer, which was partitioned from the ion-rich SRP to the PRP. This process increases the polymer concentration in the PRP and the salt concentration in the SRP.

As shown in Figure 4, the ΔG_{mic} evidenced the spontaneous process since the values were all negative, between −11,000 and −25,000 kJ mol^{-1}. The high salt molar fractions resulted in lower ΔG_{mic}, confirming that saline ions facilitated the polymer micelles formation. At the three pH levels, the micellization was more spontaneous in the sodium sulfate systems, presenting lower ΔG_{mic}. The higher values of ΔG_{mic} for the systems containing citrate and tartrate salts occurred at pH 5, while at pH 7.5 and 10, the values were similar for each salt and lower than at pH 5.

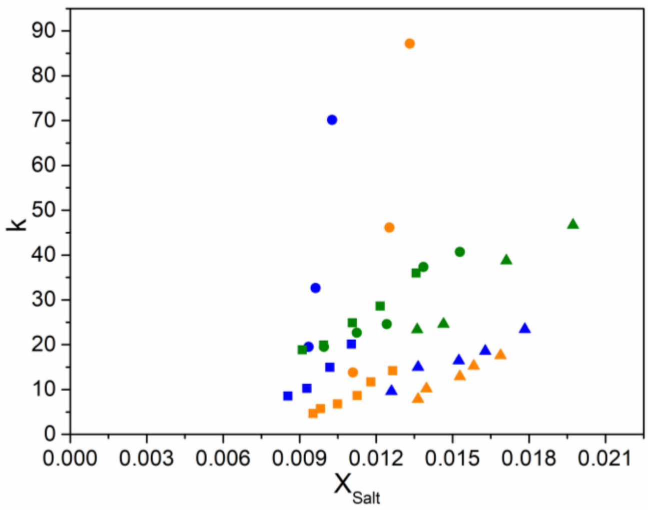

Figure 3. Polymer distribution coefficients for systems with Pluronic L64 + water + sodium sulfate at pH (●) 5.0, (●) 7.5, and (●) 10.0; Pluronic L64 + water + sodium citrate at pH (■) 5.0, (■) 7.5, and (■) 10.0; Pluronic L64 + water + sodium tartrate at pH (▲) 5.0, (▲) 7.5, and (▲) 10.0.

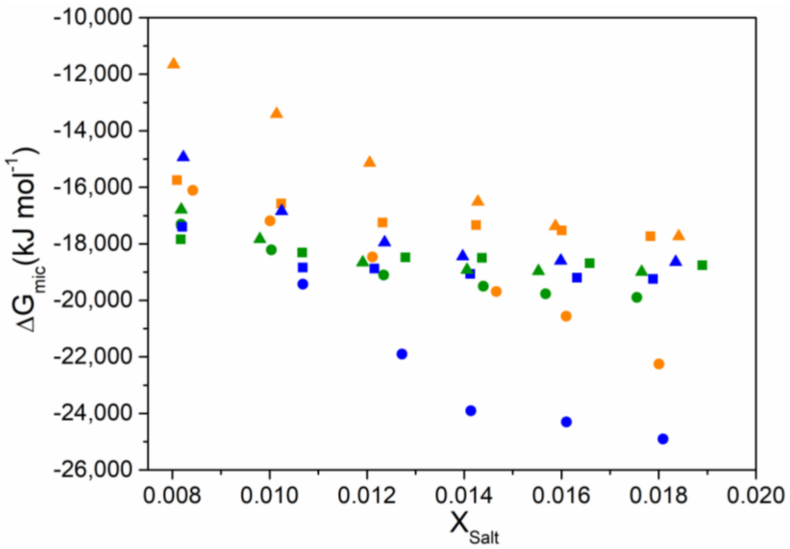

Figure 4. Gibbs free energy of micellization for systems with Pluronic L64 + water + sodium sulfate at pH (●) 5.0, (●) 7.5, and (●) 10.0; Pluronic L64 + water + sodium citrate at pH (■) 5.0, (■) 7.5, and (■) 10.0; Pluronic L64 + water + sodium tartrate at pH (▲) 5.0, (▲) 7.5, and (▲) 10.0.

The Gibbs–Helmholtz equation (Equation (17)) demonstrates that lower ΔG_{mic} results from decreasing the enthalpy of the system and/or increasing entropy with micellization. Positive ΔS_{mic} reveals that the order added to the systems by the formation of micelles is smaller than the disorder generated by polymer dehydration. On the other hand, negative ΔH_{mic} demonstrates that micellization releases energy.

$$\Delta G_{mic} = \Delta H_{mic} - T\Delta S_{mic} \tag{17}$$

Andrade et al. [18] and Rodrigues et al. [17], studying liquid-liquid equilibrium with the temperature of systems containing Pluronic L64 + water + sodium citrate or sodium tartrate and Pluronic L64 + water + sodium sulfate, reported the phase separation phenomenon as exothermic. Additionally, the systems containing sodium citrate and sodium tartrate indicated a positive entropic change due to increased translational entropy of the released water molecules. The results support the negative ΔG_{mic}.

The thermodynamics of Pluronic L64 micellization comprises the following hypotheses. Previous studies reported that hydrated oxyethylene groups of non-ionic surfactants induced the structuring of the liquid phase, and the loss of water molecules bonded to such polar groups increased the overall randomness in the systems and their entropy [35,36]. Therefore, the lowest absolute values of ΔG_{el12} among the three anions may indicate higher (and positive) ΔH_{mic} and/or lower negative ΔS_{mic} values for tartrate anions. Although $C_4H_4O_6^{2-}$ competes with polymer molecules for water, the bonds between water and this anion are weaker than those for $C_6H_5O_7^{3-}$ and SO_4^{2-}. Therefore, $C_4H_4O_6^{2-}$ anions in the system present less polymer dehydration, resulting in less energy release and less micelle formation. Weaker solvation of $C_4H_4O_6^{2-}$ also leads to less organization in the systems, providing high ΔS_{mic} values. This behavior corroborates the lower biphasic region for the systems containing tartrate anions (Figure 4).

The partial hydration or dehydration of the polymer according to the anion can occur by the interaction of hydrophilic groups of the molecules, such as oxyethylene groups, with water in the micellization of anionic surfactants [37]. The charge density of the anion provides conditions for creating a cavity to accommodate the hydrophobic tail of the polymer [38]. High charge densities result in a well-structured solvent-solvent interaction. Therefore, there is less bond breakage between water and Pluronic L64 in the presence of tartrate anion due to its lower charge density compared to citrate and sulfate anions. Besides, water cavities formed by the surfactant consume less energy, decreasing the energy absorbed in the micellization phase with higher energy dissipation. The lower solvation of the tartrate anion provides a high disorder of less structured water molecules, with a consequent increase in the entropy of the system compared to other anions.

According to Figure 5a, ΔG_{hyd} of citrate, sulfate, and tartrate anions were all negative, indicating that hydration was a spontaneous phenomenon. In absolute values, the parameter was higher for $C_6H_5O_7^{3-}$ than for SO_4^{2-} and slightly higher than that of $C_4H_4O_6^{2-}$. Similarly, the ΔG_{el12} for the three anions were negative, following the same sequence for ΔG_{hyd}, demonstrating that the water structuring around the anions due to the electrical charge was also spontaneous. Although $C_6H_5O_7^{3-}$ has a higher electrical charge than SO_4^{2-}, the charge density of the first is lower than that of the second, resulting in a similar V_{el1} (Figure 5b). On the other hand, V_{el2} followed, in absolute values, the order $C_6H_5O_7^{3-} > SO_4^{2-} > C_4H_4O_6^{2-}$, indicating the highest and the lowest water structuring surrounding the V_{el1}, respectively, for citrate and tartrate anions.

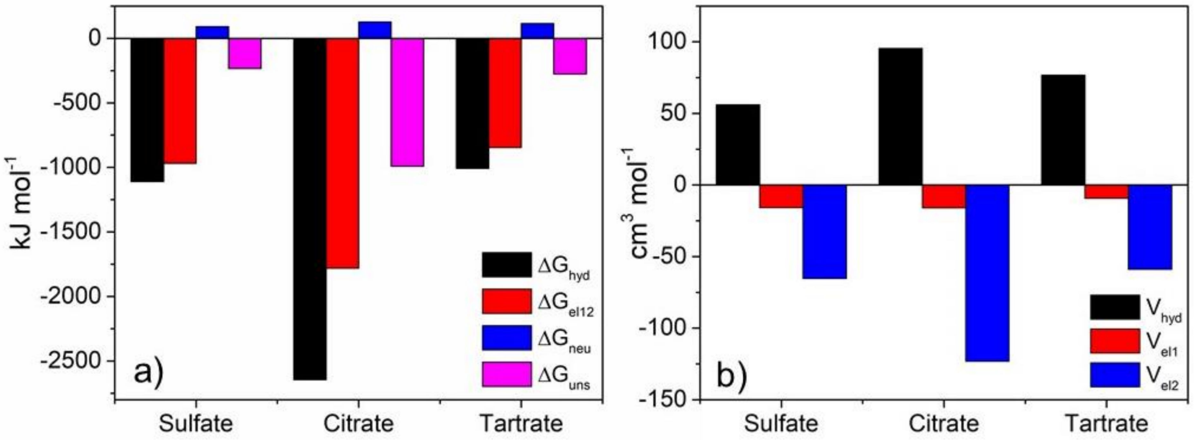

Figure 5. Thermodynamic parameters: (**a**) Gibbs free energies; (**b**) Volume contribution for salt anions.

The water structuring given by V_{el1}, V_{el2}, ΔG_{el12}, and the ion-polymer interaction must be considered in the polymer micellization and, consequently, in the phase separation. The tendency in the effectiveness of sulfate salts to induce phase separation is due to ion-polymer interactions [28,39]. The mixture of polymer with the salts leads to the interaction of the ions with the polymeric groups, releasing some water molecules that solvate them in a process driven by the increase in entropy. This ion binding continues as more electrolytes are added until a point of saturation, where no entropy gain occurs, and two-phase formation becomes more favorable.

In this context, the present research brought new approaches and important analyses of a biphasic aqueous system. Studies have reported the potential of aqueous biphasic systems for biocompound separation processes. Vicente et al. [40] studied two-phase aqueous systems of Pluronic L35 + water + dipotassium phosphate/monopotassium phosphate/tripotassium phosphate. Phase separation was directly proportional to the amount of K^+ ions in the system. In addition, the system with tripotassium phosphate showed a greater separation of phases, followed by the system with dipotassium phosphate and monopotassium phosphate. Another study reported 95% recovery of the bromelain enzyme in PEG 113-b-PNIPAM 149 + water + salts systems ($K_3C_6H_5O_7$, $K_2C_4H_4O_6$, $K_2C_2O_4$, $Na_2C_4H_4O_6$, and $(NH_4)_2C_4H_4O_6$) [41]. The enzyme activity after separation was 77% of the initial activity. In this sense, most studies used PEG in the system. Therefore, knowledge of the behavior of liquid-liquid equilibrium with other polymers is crucial for developing new techniques for separating biocompounds.

4. Conclusions

The phase separation region increased with increasing pH for systems with Pluronic L64, following the increasing order of anions $SO_4^{2-} > C_6H_5O_7^{3-} > C_4H_4O_6^{2-}$. Higher amounts of salt in the systems caused greater partitioning of the polymer into the polymer-rich phase. The negative Gibbs free energy of micellization indicated the spontaneity of the process micellization for all the systems, demonstrating the lower values for the systems with sodium sulfate. Therefore, there was a decrease in enthalpy and/or an increase in the entropy of the system. Besides, The Gibbs free energy of micellization was related to the anion speciation at each pH. The structuring of water around the ions and the ion-polymer interaction affected the phase separation, increasing the polymer concentration in the PRP and the salt concentration in the SRP. Besides, according to the values of Gibbs free energy, enthalpy, and entropy of the clouding process, the increase in entropy was the driving force for the formation of the biphasic region.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/colloids7010004/s1. Table S1. Coefficients from binodal curves for the salt and polymer rich-phase; Table S2. Thickness of immobilized water spherical shell surrounding the ion and effective radius of anions.

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Conflicts of Interest: The authors declare no conflict of interest.

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