Journal of Molecular Liquids 440 (2025) 128844

Contents lists available at ScienceDirect

Journal of Molecular Liquids

journal homepage: www.elsevier.com/locate/molliq

Role of food additives on properties of Polysorbate 60 solutions

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ARTICLE INFO

Keywords:
Preservatives
Polysorbate-60
Micelle shape
Viscosity

ABSTRACT

Polysorbates are hydrophilic, nonionic surfactants widely used in food, cosmetic, and pharmaceutical products. Often, these emulsifiers are used in combination with different preservatives, and it is essential to conduct an in-depth study of the influence of such additives on the properties of Polysorbate 60 solutions. In the current study, we investigated the effect of various food additives: citric acid, sodium benzoate, potassium sorbate, a mixture of citric acid and sodium citrate, and propylene glycol on the stability and micellar properties of Polysorbate 60 by using different experimental methods such as GC, DSC, SAXS, DLS, optical observations in polarised light, and NMR. When stored at room temperature without additives, solutions of Polysorbate 60 slowly undergo phase separation over time. Our results show that citric acid, a mixture of citric acid and sodium citrate, and propylene glycol increase the rate of this phase separation. In contrast, sodium benzoate and potassium sorbate are incorporated into the mixed micelles. They localize within the palisade layer, a region of the micelle where the surfactant tails begin, effectively preventing the phase separation. As a result, Polysorbate 60 solutions with these specific additives remain transparent and stable for over a year.

1. Introduction

Ethoxylated sorbitan esters, e.g. Polysorbates, are hydrophilic nonionic surfactants often applied in food, cosmetic and pharmaceutical products because of their low toxicity, good emulsifying properties, good solubilization capacity, etc. [1–4]. The ability of Polysorbate micelles to solubilise and transport nonpolar molecules through the aqueous phase is essential for the practical application of these surfactants. As a consequence of their nature, nonionic surfactants are less sensitive to pH and ionic strength but sensitive to changes in temperature, i.e. increasing the temperature leads to a decrease in the solubility of the surfactant in the aqueous solution [5]. However, additives can affect the phase and micellar behaviour of nonionic surfactants based on their localisation in the surfactant micelles or based on the dehydration of micellar associates and competing for water [6–9]. For example, Deng et al. [7] demonstrate that the hydrophobic molecule thymol can be solubilised in the micelles of Polysorbate 80 without a significant change in micelle size. The NMR analysis indicates that the thymol molecules are located between the hydrophilic head group and the hydrophobic tail group of the surfactant. On the other hand, according to the work of Bide et al. [6], the hydrophilic molecule of choline chloride can be incorporated in Polysorbate 80 micelles, leading to an increase in the hydrodynamic diameter of the micelles from 9.95 nm to 11.08 nm. The authors propose that the choline chloride molecules interact with Polysorbate 80 head group by hydrogen bond and Van der Waals' force [6]. The increased solubilization of hydrophobic moieties in Polysorbate 80 micelles in the presence of propylene glycol (PG) is reported in the works of Aboutaleb and Abdelzaher [10] and Rao and McClements [11]. Yagmur et al. [12] analyse the behaviour of Polysorbate-containing microemulsions in the presence of PG and limonene. They demonstrate that water-soluble PG is incorporated into the interface, leading to transformation from prolate to quite globular droplets and also shrinkage in the micellar size. Garti et al. [13] show that an isotropic microemulsion region is formed by five component system of fatty alcohols or ethoxylated sorbitan fatty esters, ethanol, R(+)-limonene, and PG, which is characterised by a single continuous microemulsion.

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https://doi.org/10.1016/j.molliq.2025.128844
Received 18 September 2025; Received in revised form 18 October 2025; Accepted 29 October 2025
Available online 2 November 2025

In our previous study on processes for preparing transparent flavour oil emulsions for beverage applications [14], we found that citric acid and sodium citrate; sodium benzoate or potassium sorbate can be used to prepare stable transparent nanoemulsions of lemon oil and lemon and orange oil mixture in the presence of Polysorbate 60 or Polysorbate 80 without using synthetic co-solvents such as propylene glycol.

There is limited information in the literature on the impact of these preservatives on surfactant properties. Castro et al. [15] reported that the antimicrobial efficiency of potassium sorbate decreases when the surfactant Polysorbate 20 is added to the emulsion. This result is attributed to a decrease in the concentration of free preservative molecules in the aqueous phase due to the partition between surfactant micelles and water. Several authors report the use of citric acid and sodium benzoate as hydrotropic agents, which improve the solubility of inadequately water-soluble drugs [16,17]. One of the dominating hypotheses for the mechanism behind this phenomenon is that the hydrotropic agents form oriented clusters, in which the solutes (drugs) are solubilised [16]. Khimani et al. [18] show that micelles of moderately hydrophilic PEO-PPO-PEO triblock copolymers transform from spherical to prolate-ellipsoidal shape with an increased aggregation number in the presence of *p*-hydroxybenzoic acid. The cloud point of the polymers also decreases with increasing acid concentration. However, the sodium salt of the acid increases cloud point and shows only a marginal decrease in aggregation number even at higher concentrations. The latter is explained by sodium hydroxybenzoate acting as a "water structure breaker". As a result, more water molecules are available to interact with the micelles.

Our previous work on processes for preparing transparent flavour oil emulsions for beverage applications [14] established that Polysorbate 20 failed to stabilise citrus oil nanoemulsions due to its shorter chain and more hydrophilic nature, whereas Polysorbate 60 and 80 successfully formed stable flavour oil emulsions with certain additives. However, Polysorbate 80 has an unsaturated bond in its tail, which makes the molecules more susceptible to oxidation. Given these findings, the current study focuses on Polysorbate 60, as information about the effect of various additives on its properties is limited. The current study aims to investigate the effect of preservatives potassium sorbate, sodium benzoate, citric acid, and a mixture of citric acid and sodium citrate on the micellar behaviour and properties of Polysorbate 60 solutions. The effect of the co-solvent propylene glycol was also studied. We specifically focus on these preservatives because, in our previous work [14], we obtained transparent *flavour* oil nanoemulsions by using some of these additives. However, the mechanism behind these observations was not clear. The main goal of the current study is to determine how these additives affect the micellar properties of Polysorbate 60 and, consequently, the stability of the prepared solutions. This work tests two main hypotheses: (1) The additives alter the chemical stability of the surfactant against hydrolysis; (2) The additives modify the solubilization capacity of the surfactant micelles for diester molecules present in the Polysorbate solution and prevent their precipitation over time. To achieve our aim, we applied several different methods and strategies: (1) To evaluate the changes in the shape and size of Polysorbate 60 micelles, we performed viscosity, DLS, and SAXS measurements; (2) To understand the interaction between the additives and surfactant molecules, as well as their localization relative to each other, we carried out NMR analysis; (3) To find the causes of the appearance of precipitates in some of the solutions, we performed optical observations under polarised light, chemical analysis by GC, and DSC measurements of the sediments in the unstable solutions.

2. Materials and methods

2.1. Materials

The nonionic surfactant polyoxyethylene sorbitan monostearate (Polysorbate-60, product of TCI, CAS: 9005-67-8, CMC 0.02 mM [19]) is used and its concentration is fixed to 15 wt% in the aqueous phase. Gas chromatography analysis shows that Polysorbate 60 contains C18/C16 in the hydrocarbon chain at 1:1 ratio (Fig. S1), which agrees very well with the results reported by Mustan et al. [20]. According to the work of Abrar and Trathnigg [21], among the monoesters, Polysorbate 60 also contains diesters, triesters, and tetraesters.

The studied additives are citric acid (CA, Sigma, CAS: 77–92-9), sodium benzoate (Na-benz, Sigma, CAS: 532–32-1), potassium sorbate (K-sorb, Teokom, Bulgaria, CAS: 24634–61-5), sodium citrate (Na-citr, $Na_3C_6H_5O$ 5.5H_2O, Teokom, Bulgaria, CAS: 6858-44-2) and 1,2-propylene glycol (PG, Teokom, Bulgaria, CAS: 57–55-6). The solubility, partition coefficients, and concentrations of the studied additives are given in Table S1. Deionised water purified by the Milli-Q Organex system (Millipore, USA) is used for solution preparation. The additives are dissolved in water, then Polysorbate 60 is added, and the mixture is stirred at 50 °C until complete dissolution. The concentrations of the additives in the solutions correspond to those in the systems with the best results obtained in the work by Ahtchi-Ali et al. [14]. Additionally, Polysorbate 60 solutions with the same concentration of additives (0.7 M) are prepared for comparison.

2.2. Optical observations of heated solutions and solutions after storage

15 wt% solution of Polysorbate 60 and a certain additive (0.7 M K-sorb, 0.7 M Na-benz, 3.9 M PG, 1.1 M CA or 2.56 M CA + 0.16 M Na-Citr in the aqueous phase) is prepared via magnetic stirrer mixing. A thin capillary with a height of 100 μm is filled with each solution and then transferred to a thermostatic chamber, which is connected to a thermostat set to 95 °C. The solutions are kept at 92 ± 1 °C and observed in polarised transmitted light via an optical microscope Axioplan (Zeiss, Germany), equipped with an objective Zeiss Epiplan 50×, a digital camera, and a video recorder. The scale bar in the pictures included in the article is 20 μm.

Additionally, heat treatment is applied by stirring the solutions for 30 min in a water bath set to 90 ± 2 °C. Afterwards, the hot solutions are rapidly cooled (16 °C/min) to room temperature by homogenising in a cold-water bath with $T = 17 \pm 1$ °C. Then these solutions are observed in polarised transmitted light (objective Zeiss Epiplan 50×). The solutions prepared after mild heating, as described in Section 2.1, are also observed under the microscope after 1 week of storage at ambient conditions.

2.3. Measurements of the micelle sizes, viscosity and density

The sizes of the micelles of Polysorbate 60 are determined via Zetasizer Nano ZS (Malvern Instruments), set at 173° scattering angle with a wavelength of 633 nm and temperature during measurements set to 25 °C. Intensity weighted mean hydrodynamic diameter, Z_{AVE} and mean volume diameter (d_V) are used as characteristics of the micelle size. The effect of the additives on the viscosity of the solutions is considered for the measurements. The viscosities of the aqueous solutions are measured via a capillary viscometer at 25 °C. Before the measurement, the solutions are kept at 25 °C in a water bath for at least 5 *min*. Three measurements are performed for each system and the average value of the viscosity is used. The mass density of the aqueous solutions is measured via DMA35 Portable density meter (Anton Paar, Austria) at 25 ± 0.1 °C. The accuracy of the measurement is ±0.001 g/cm^3.

2.4. Small angle x-ray scattering (SAXS) analysis

SAXS measurements of the micellar solutions are carried out on an in-house X-ray scattering system (XEUSS 3.0 SAXS/WAXS System, Xenocs, Sassenage, France) with a CuKα X-ray source operating at λ = 0.154 nm (Xeuss 3.0 UHR Dual source Mo/Cu, Xenocs, Sassenage, France) and Eiger2 4 M detector (Dectris Ltd., Baden Deattwil,

Switzerland) with slit collimation. The sample-to-detector distance (SDD) of 1500 mm allowed to access the q-range of 0.02–0.5 $Å^{-1}$. The data acquisition time is set to 30 *min*. Silver behenate is used as a standard to determine the SDD and the coordinates of the beam centre on the detector. Samples are enclosed in a vacuum-tight thin glass capillary with an outer diameter of 1 mm and a thickness of 10 μm. The scattered intensity is normalised to the incident intensity, corrected for the background scattering from the capillary and calibrated to the absolute scale. The measurements are performed at an ambient temperature of 25 °C.

Temperature gradient measurements are conducted by the following protocol: first, the solution is placed in a glass capillary and the measurement is performed at an ambient temperature 25 °C and 1000 mm SDD for 20 min. Then the temperature is raised consecutively up to 50 °C and 70 °C with 10 °C/min rate and the measurement is repeated at these two temperatures. Further, the heating continues to 75 and 80 °C with 5 °C/min followed by measurements at the respective temperatures. Then, thermal equilibration at 80 °C for 15 min is applied to the system and the measurement is then repeated. After that, the temperature is gradually decreased using the same protocol applied for heating, and the measurements are repeated.

The analysis of the reduced SAXS data is performed by the SASView software. The SAXS intensity has two contributions coming from the particle form factor P(q) and the interparticle interference factor S(q). The data is *analyzed* by different model functions (core-shell cylinder, ellipsoid and sphere, microemulsion) implemented in SasView. The applied structure factor corresponds to hard sphere interactions according to the Percus-Yevick equation for non-charged systems. The SLD values are determined via NIST NCNR SLD calculator [22] by accounting for the scattering length of each component and the volume it occupies. In some cases, a combined model is shown to describe the experimental data.

2.5. NMR analysis

The NMR study is carried out on a Bruker Avance III HD 500 MHz spectrometer (Rheinstetten, Germany) fitted with a high-resolution broadband probe-head with Z gradient. Experiments are conducted at $T = 25$ °C. The studied samples are prepared as described in section 2.1. 0.5 ml of the sample and 0.1 mL of deuterium oxide (99.8 atom % D) with TMSP-Na-2,2,3,3-d_4 as internal standard are mixed before measurement. Topspin 3.6.5 software package (Bruker) is used for spectrum collection and data analysis.

3. Experimental results and discussion

The experimental results are shown in the following order: Section 3.1 describes the experimental results about the Polysorbate 60 solutions prepared under a mild heating procedure (the solutions are stirred at 50 °C and stored afterwards). Section 3.2 presents the experimental results about the effect of heat treatment (up to 90 °C) of solutions. Section 3.3 provides the overall discussion of obtained results from various methods employed.

3.1. Polysorbate 60 solutions prepared at 50 °C

3.1.1. pH and stability of prepared solutions

Aqueous solutions of 15 wt% Polysorbate 60 were prepared with and without various additives: propylene glycol (PG), citric acid (CA), a mixture of citric acid and sodium citrate (CA + NaCitr), potassium sorbate (K-Sorb), and sodium benzoate (Na-Benz). The additives were used at a concentration of 0.7 M, as well as at specific concentrations from our patent (28.*3* % PG or 3.9 M, *20* % CA or 1.1 M, and *40* % CA + 4.*7* % Na-Citr, for a total concentration of 2.7 M). These concentrations are based on our previous work [14], which developed the method for preparing transparent flavour oil emulsions. The higher concentrations of Polysorbate 60 and additives studied here represent their levels in the concentrated flavour oil emulsions, and are calculated to achieve the desired concentrations in the final, highly diluted product. Note that these concentrated emulsions are diluted (100- to 1000-fold) during subsequent production steps [23] to yield transparent soft drinks with the required flavour and preservative concentrations [24].

As shown in Table 1, the measured pH values of the prepared solutions indicate that both K-Sorb and Na-Benz increase the pH of Polysorbate 60 solutions. The pH of a solution without additives rises from 6.8 to 8.2 with K-Sorb and to 7.8 with Na-Benz. This effect is due to the fact that K-Sorb and Na-Benz are salts of a strong base (NaOH or KOH) and weak acids (sorbic and benzoic acid, respectively). In contrast, solutions containing 1.1 M CA or 2.7 M CA + NaCitr have a much lower pH of 1.0 and 2.2, respectively. The presence of 3.9 M PG has no significant impact on pH, causing only a slight decrease to 6.4.

The solutions were prepared using the procedure described in section 2.1 and stored at 25 °C for up to one year. We observed that solutions containing K-Sorb and Na-Benz remained stable and transparent for at least a year. In contrast, all other solutions formed precipitates during storage. The approximate time until precipitate formation is shown in Table 1. The microscope images of stored Polysorbate 60 solutions reveal the presence of small crystals in solutions without additives, whereas lamellar structures are seen in the solutions containing PG, CA or CA + Na-Citr (see Fig. 1).

To investigate whether the increased pH was responsible for the enhanced stability of the K-Sorb and Na-Benz solutions, we adjusted the pH of a solution containing 3.9 M PG to 8 by adding sodium hydroxide. This pH adjustment slowed the precipitation process, and the solution remained transparent for approximately one week before precipitation. The pH of the solution's serum was measured again after storage and remained close to 8. This result indicates that while a higher pH can delay precipitation, it alone is not enough to account for the long-term stability of solutions prepared with Na-Benz and K-Sorb. Therefore, the higher stability must be attributed to other factors, such as the specific interaction of these additives with the Polysorbate 60 molecules.

The decreased pHs of Polysorbate 60 solutions containing CA and CA-NaCitr could facilitate the acidic hydrolysis of the surfactant molecule and formation of reaction products, which are less water-soluble [25–27]. However, Dwivedi et al. [25] investigated the hydrolysis of Polysorbate 20 and Polysorbate 80 at pH of 1, 7 and 11 at three temperatures (5, 25 and 40 °C) and showed that at pH 1 and pH 7 at 25 °C after 50 h of storage there is almost no changes in the fraction of esterified molecules for Polysorbate 80, see Fig. 9 in Ref. [25]. Bates et al. [28] show that the rates of acidic hydrolysis of Polysorbate 40, 60 and 80 are similar, concluding that the chemical structure of the polysorbate has a minor effect on the process. The authors also show that the rate of the acidic hydrolysis decreases significantly when the surfactant

Table 1
pH and time for formation of precipitates in 15 wt% Polysorbate 60 solutions prepared by mild heating at 50 °C upon storage at room temperature.

Aqueous phase	pH	Time after which precipitates are formed
No additive	6.8	2–3 days
0.7 M Na-benzoate (10 wt%)	7.8	Stable*
0.7 M K-sorbate (10 wt%)	8.2	Stable*
0.7 M propylene glycol (5.3 wt%)	6.5	2 days
3.9 M propylene glycol (28.3 wt%)	6.4	24 h
0.7 M CA (13.45 wt%)	1.3	24 h
1.1 M CA (20 wt%)	1.0	24 h
0.66 M CA + 0.04 M Na-citrate (12.66 wt% CA + 1.46 wt% Na-citrate)	2.2	24 h
2.56 M CA + 0.16 M Na-citrate (40 wt% CA + 4.7 wt% Na-citrate)	2.2	24 h

* The solutions are stored for at least a year and no precipitates are formed within them.

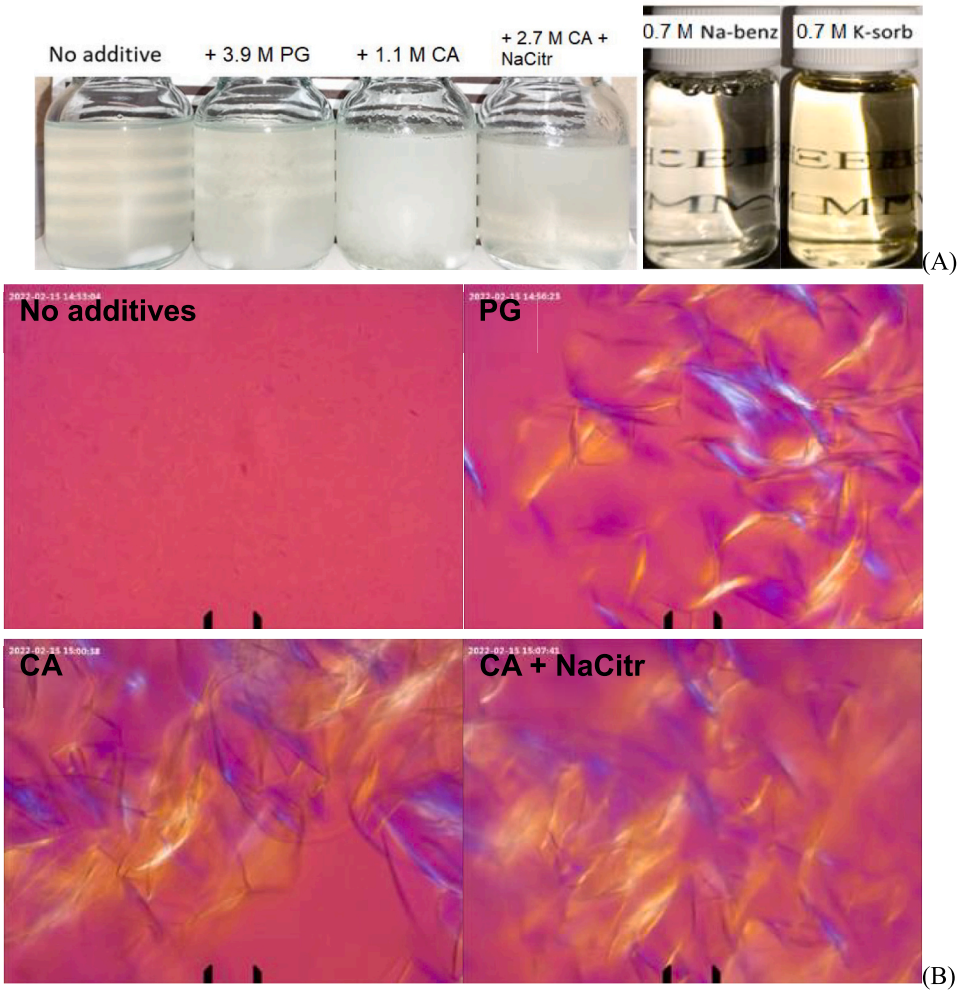

Fig. 1. (A) Pictures of *15 %* Polysorbate 60 without additives, + 3.9 M PG; + 1.1 M CA; +2.7 M CA + NaCitr after 1-week storage and *15 %* Polysorbate 60 + 0.7 M NaBenz; + 0.7 M KSorb after 6 months. (B) *Polarised* light images of the solutions of *15 %* Polysorbate 60 (from left to right): no additives; + 3.9 M PG, + 1.1 M CA; +2.7 M CA + NaCitr after 1-week storage.

concentration is increased. Therefore, we can conclude that the precipitates formed in the presence of additives are not significantly affected by the formation of water insoluble reaction products due to surfactant hydrolysis.

Note that the hydrolysis phenomenon is discussed only for Polysorbate 60, because precipitate formation is observed even in solutions without any additives, which means that the precipitates are primarily formed from the surfactant used. The faster precipitation in solutions containing propylene glycol, citric acid, and a mixture of citric acid and sodium citrate cannot be due to the hydrolysis of these additives, as none of them hydrolyze in aqueous solutions under the studied conditions.

3.1.2. Viscosity and size of formed aggregates in freshly prepared solutions

All freshly prepared solutions were transparent, and their mass densities and viscosities were measured using a densitometer and a capillary viscometer, respectively. The experimental data from these measurements are presented in Table 2. The measured viscosities depend on the concentration of the additive and the presence of Polysorbate 60. To isolate the contribution of Polysorbate 60, we calculated the relative viscosity, defined as the viscosity of the solution containing Polysorbate 60 divided by the viscosity of the solution in which Polysorbate 60 is dissolved. The presence of Polysorbate 60 micelles in an aqueous solution without additives leads to a threefold increase in viscosity. This increase is significantly higher than the *37 %* rise expected if spherical, non-interacting micelles were formed, as predicted by the

Table 2
Bulk properties measured at 25 °C of freshly prepared solutions: mass density, ρ, solution viscosity, η, and relative viscosity defined as a ratio of the viscosity of Polysorbate 60 solutions and the viscosity of the solution without added surfactant.

Aqueous phase	No surfactant		*15 %* Polysorbate 60 + additives		Relative viscosity, η_r
	ρ, g/cm^3	η*, mPa.s	ρ, g/cm^3	η, mPa.s	
No additive	0.997	0.89	1.012	2.8	3.1
0.7 M Na-benzoate	1.045	1.33	1.054	4.6	3.5
0.7 M K-sorbate	1.036	1.24	1.046	3.9	3.1
0.7 M propylene glycol	1.001	1.02	1.017	3.0	3.0
3.9 M propylene glycol	1.020	3.20	1.035	7.7	2.4
0.7 M CA	1.06	1.23	1.067	3.9	3.2
1.1 M CA	1.085	1.72	1.099	5.6	3.3
0.7 M CA + Na-citrate	1.067	1.26	1.072	4.1	3.3
2.7 M CA + Na-citrate	1.239	6.4	1.228	39.1	6.1

* Data shown for viscosity of the solutions without surfactants are obtained with solutions containing the same ratio between water and additives as in the solution containing 15 wt% Polysorbate 60.

Einstein equation. This discrepancy suggests the formation of elongated micelles that interact through excluded volume interactions, a conclusion supported by the SAXS data analysis. The relative viscosity is the highest for solutions containing 2.7 M CA + NaCitr, showing that the impact of Polysorbate 60 micelles is most pronounced for this system. This indicates a substantial change in the shape and size of the Polysorbate 60 micelles in the presence of these preservatives. It is also notable that while the viscosities of PG and CA + NaCitr solutions are similar without the surfactant, the addition of Polysorbate 60 creates a significant difference between them, highlighting that the shape of the formed aggregates varies depending on the specific additives used.

To gain more detailed information about the micelles formed in the different solutions, their sizes were measured using both Dynamic Light Scattering (DLS) and Small-Angle X-ray Scattering (SAXS). The DLS results, presented in Table 3, show that the mean micellar diameter for the Polysorbate 60 solution without any additives is 5.7 ± 0.1 nm. Upon addition of Na-Benz, the micellar diameter remains almost the same of 5.8 ± 0.2 nm. The micellar size decreases down to 4.8 ± 0.1 nm with the addition of 3.9 M PG, which agrees with the relative viscosity data, where the micelle contribution drops from 3.1 to 2.4. The DLS method was not suitable for measuring the micelle size in a solution containing 2.7 M CA + NaCitr due to the high viscosity of the solution.

The micellar shape and size were analyzed by evaluating SAXS spectra, see Fig. 2. Most of the spectra show a broad maximum, Fig. 2A, characteristic for micelles formation [29–31] with the only exception of 2.7 M CA + NaCitr. The experimental x-ray scattering curves were fitted by models incorporated in SASView software. First, model-independent analysis was applied via Indirect Fourier Transform (GIFT) in order to obtain Pair Distance Distribution Functions (PDDFs). This gives information about the objects overall size without making assumptions about scattering length densities (SLD) of the hydrophobic core and hydrophilic shell of the micelles. As shown in Fig. S2, the PDDF functions exhibit consecutive maximum and minimum, which is typical for core-shell structures, like micelles. This pattern is related to the scattering contrast between the hydrophobic core and the hydrophilic shell [29]. The core radius (R_{core}) is determined from the inflection point between the first maximum and minimum of the PDDF [29]. For the present systems the R_{core} is approximately 2.5 ± 0.1 nm for Polysorbate 60 solutions with 0.7 M of the studied additives. This parameter is affected only by the presence of 1 M CA and 2.7 M CA + NaCitr which lead to increase up to 2.8 nm and 4.2 nm, respectively. The changes indicate a significant alteration in the shape of the micelles formed within these systems.

Then different models were tested accounting for the core-shell structure of the micelles [29]. The SLD of the tails was fixed to 7.52 × 10^{-6} $Å^{-2}$ (the value corresponds to that of hexadecane molecule with a mass density of 0.77 g/ml). The SLD of the shell was also fixed at 10.4 × 10^{-6} $Å^{-2}$, which was calculated by using NIST software [22], as described in Section 2.4. A hard sphere structure factor was incorporated to account for excluded volume interactions of non-ionic objects. Initially, the micellar volume fraction (Φ) was fixed to 0.15 which corresponds to calculated volume fraction for Polysorbate 60 with 1.0 g/ml mass density within the micelles. This is a reasonable assumption because manufacturer-provided mass density of Polysorbate 60 is 1.044 g/ml. Once a reasonable fit was achieved, the volume fraction was allowed to vary as an adjustable parameter. The variation of the solvent SLD was also accounted by calculating the values for the used additives with the respective mass densities, taken from Table 2. The resulting solvent SLD values are presented in Table 3.

The simplest core-shell model (for sphere) was not able to fit the experimental data which led to the application of an ellipsoidal model. Note that elongated micelles are reported for Tween solutions [30,31]. Ellipsoidal micellar model describes the experimental data for most of the studied systems with well-defined broad peak, located in the range between 0.05 and 0.2 $Å^{-1}$. The model for ellipsoidal particles includes two rotational axis and their ellipsoidal core radius (R_{xcore}) and polar core radius (R_{ycore}) with the respective shell thicknesses (t_x and t_y). In the present model, the shell thickness was assumed to be uniform across the entire ellipsoid, in order to minimize the number of fitting parameters. The applied core-shell ellipsoid model shows good fitting with a small χ^2 index. Further optimization by using cylindrical model did not improve the fit. The final results from the best fits are shown in Table 3, and the corresponding fits are presented as continuous curves in Fig. 2. The SAXS fit of the solution of Polysorbate 60 yields a prolate ellipsoid with an axial ratio (R_{ycore}/R_{xcore}) of ~1.7, which agrees very well with the 3.1-times increase in the solution's viscosity, also strongly suggesting formation of elongated micelles.

For the solutions containing 2.7 M CA + Na-Citr, the ellipsoidal model was not able to describe the curves at low q values. This particular system shows very different scattering spectra compared to the others: no broad peak and the scattering intensity decay as q^{-4} in a wider range of scattering vectors. Taubner-Strey model developed for microemulsions predicts such behaviour [32]. It is designed for nonionic surfactants and provides information on the periodicity (d) and correlation length (ξ) of hydrophilic and hydrophobic domains [3,33–35]. Despite that this model was able to predict the shape of the curve, the fit was not sufficiently good again. The best fit was obtained by applying combined model that sums core-shell ellipsoid and Taubner-Strey. The accuracy of fitting was measured by the χ^2 index which is obtained by SASView software. For 2.7 M CA + Na-Citr system, the periodicity and correlation length were determined to be 8.6 nm and 5.8 nm, respectively, while the parameters of ellipsoid are summarized in Table 3. The

Table 3
Micelles dimensions in Polysorbate 60 solutions as determined by SAXS spectra analyzed with core shell ellipsoid ± Taubner-Strey model in SASView: equatorial and polar core radiuses, shell thickness, micellar volume fraction and by DLS: mean hydrodynamic diameter and mean volume diameter.

15 % Polysorbate 60 +	Core-shell ellipsoid with hard sphere interactions from SAXS measurements					DLS	
	SLD solvent, 10^{-6} $Å^{-2}$	R_{xcore}, nm	R_{ycore}, nm	*t*, nm	φ	Z_{AVE}, nm	d_V, nm
No additive	9.44	2.06 ± 0.05	3.44 ± 0.05	1.87 ± 0.05	0.21	7.7 ± 0.1	5.7 ± 0.1
0.7 M PG	9.47	2.05 ± 0.05	3.30 ± 0.08	1.77 ± 0.07	0.21		
0.7 M K-sorb	9.68	1.92 ± 0.05	2.90 ± 0.05	1.69 ± 0.07	0.20	7.1 ± 0.1	5.3 ± 0.3
0.7 N Na-benz	9.73	1.88 ± 0.07	3.06 ± 0.08	1.70 ± 0.05	0.16	10.3 ± 0.6	5.8 ± 0.2
0.7 M CA	9.90	1.81 ± 0.05	3.12 ± 0.08	2.20 ± 0.05	0.19		
0.7 M CA + NaCitr	9.93	1.83 ± 0.05	3.68 ± 0.08	2.20 ± 0.05	0.22		
3.9 M PG	9.64	1.95 ± 0.05	3.46 ± 0.08	1.70 ± 0.08	0.18	6.3 ± 0.1	4.8 ± 0.1
1.1 M CA	10.1	1.80 ± 0.05	3.00 ± 0.08	2.50 ± 0.05	0.26	8.1 ± 0.1	6.2 ± 0.4
2.7 M CA + NaCitr	11.0	1.87 ± 0.03	7.85 ± 0.08	0.77 ± 0.08	0.30		
Serum after centrifugation							
3.9 M PG	9.64	1.68 ± 0.05	4.70 ± 0.10	2.15 ± 0.05	0.17		
1.1 M CA	10.1	1.70 ± 0.05	2.88 ± 0.08	2.33 ± 0.05	0.20		
2.7 M CA + NaCitr	11.0	1.77 ± 0.05	6.80 ± 0.08	0.95 ± 0.08	0.27		

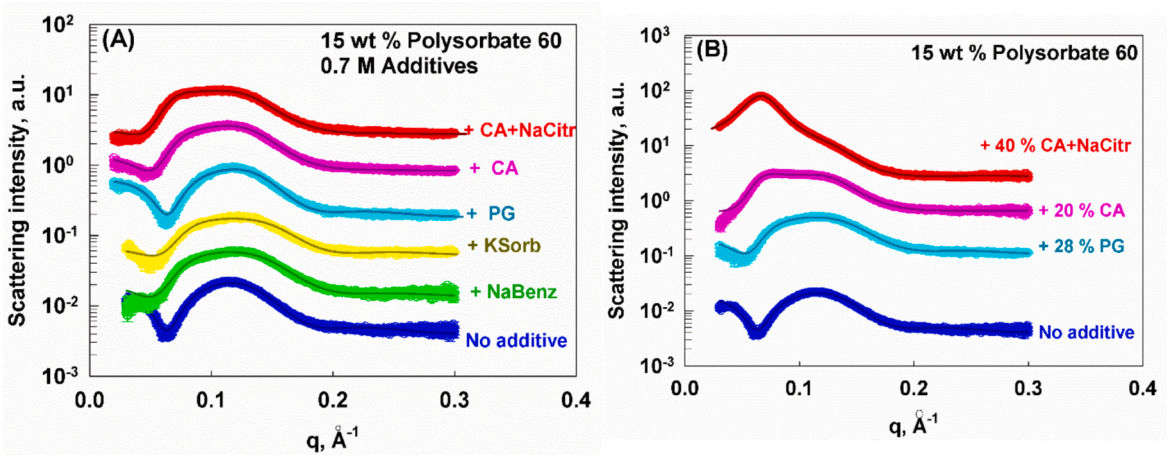

Fig. 2. Scattering intensity as a function of scattering vector for 15 wt% Polysorbate 60 solutions in presence of (A) 0.7 M additives and (B) higher concentrations of additives. Samples are denoted as follows: no additive (blue); + NaBenz (green); + KSorb (yellow); + PG (cyan); + CA (pink), + CA + NaCitr (red). Solid lines represent the fit by core-shell ellipsoid with hard sphere interactions. *(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)*

aspect ratios of the ellipsoid radii of the micelles in the solutions with additives show again formation of elongated micelles, the most significant effect observed in the presence of 2.7 M CA + NaCitr with R_{ycore}/R_{xcore} of 4.2.

The equatorial core radius of Polysorbate 60 micelles without additives was determined to be approximately 2.06 nm, which aligns well with the 2.05 nm predicted by the Tanford formula [36] for a surfactant with a 17-carbon atom tail. This radius remains unchanged with the addition of 0.7 M PG. However, it decreases to 1.9 nm in the presence of 0.7 M Na-Benz or 0.7 M K-Sorb, and further to 1.8 nm with CA or CA + Na-Citr. These small changes likely relate to the distribution of the C16 and C18 surfactants within the micelles, with longer C18 chains possibly moving to the polar regions in the presence of CA and CA + Na-Citr, thereby reducing the equatorial core radius. The polar core radius for Polysorbate 60 solutions without additives is 3.4 nm. It decreases to 3.0 nm with the addition of 0.7 M K-Sorb or Na-Benz, but remains almost unchanged when PG is added, regardless of its concentration. A significant change is observed with the addition of 2.7 M CA + Na-Citr, where the polar core radius increases to approximately 7.7 nm, indicating a major change in micelle shape.

The shell thickness without additives is approximately 1.9 nm, which is consistent with literature data for Polysorbate 80 micelles. This thickness slightly decreases to 1.7 nm with 0.7 M K-Sorb or Na-Benz. Conversely, it increases to 2.2 nm in the presence of 0.7 M CA or CA + Na-Citr, possibly due to the formation of hydrogen bonds between citric acid and the ethylene oxide (EO) groups of Polysorbate 60. Increasing the CA concentration to 1.1 M further thickens the shell to 2.5 nm. However, a more significant increase in the CA + Na-Citr concentration to 2.7 M drastically reduces the shell thickness to 0.8 nm. This dramatic change suggests a significant reduction in the number of free water molecules available to hydrate the EO groups, leading to a dehydrated shell.

From these experiments, we can conclude that the presence of 0.7 M K-Sorb and 0.7 M Na-Benz in the aqueous phase leads to the formation of Polysorbate 60 micelles with smaller equatorial and polar core radii and a slightly reduced shell thickness. The presence of 0.7 M PG does not significantly alter the properties of the Polysorbate 60 micelles, while a higher concentration of 3.9 M PG causes a slight reduction in shell thickness. The addition of 0.7 M CA, 0.7 M CA + NaCitr, and 1.1 M CA decreases both the equatorial and polar core radii but increases the shell thickness. However, a significant reduction in free water in the 2.7 M CA + NaCitr system leads to a dramatic elongation of the surfactant micelles and a substantial decrease in the shell thickness.

3.1.3. Stored solutions

After being stored for three days, the turbid solutions were centrifuged, and the resulting clear phases (serums) and precipitates (sediments) were collected for analysis. DLS analysis of the serum separated from Polysorbate 60 solutions without additives revealed the presence of micelles with a $d_V = 5.8 \pm 0.2$ nm, which is very close to the size measured in freshly prepared solutions (5.7 ± 0.1 nm). Following five months of storage of this serum, further sedimentation occurred. However, the size of the remaining clear solution only increased slightly to 6.1 ± 0.1 nm. The serum separated from the Polysorbate 60 solution containing 3.9 M PG exhibited a mean micellar size of 4.8 ± 0.2 nm. This size remained virtually unaffected at 4.9 ± 0.4 nm even after five months of serum storage and the subsequent precipitation of molecules from the solution.

The SAXS spectra of the transparent solutions show a typical micellar structure, similar to freshly prepared solutions (Fig. 3A). In contrast, the SAXS spectra of the separated sediments reveal the presence of sharp correlation peaks at a 1:2:3 ratio, which correspond to a lamellar phase with a characteristic distance of approximately 9.3 nm (Fig. 3B). The fitting parameters for the serums (Table 3) show that their equatorial core radius is around 1.7 nm. This indicates that the longer-chain surfactants have been expelled from the micelles and likely formed the lamellar phases observed in the sediment spectra. The remaining micelles in serums from solutions with 1.1 M CA and 2.7 M CA + Na-Citr have a slightly smaller polar core radius than those in the freshly prepared solutions. However, the micelles in the serum from the 3.9 M PG solution have a larger polar core radius, suggesting that more molecules precipitate over time, an observation confirmed by further precipitation upon longer storage of the separated serums.

DSC analysis of the serums showed no peaks in their thermograms, indicating no phase transitions. In contrast, the sediments underwent both crystallization and melting transitions upon temperature changes, Fig. S3. The melting peak for all sediments appeared between 38 °C and 42 °C. The enthalpy of this transition was highest (≈ 7.5 J/g) for the sediment from the PG-containing solution, intermediate (≈ 5.0 J/g) for the solution without additives and with CA, and lowest (≈ 2.3 J/g) for the sediment from the solution with 2.7 M CA + Na-Citr.

From this series of experiments, we can conclude that the turbidity of the solutions is caused by the formation of lamellar phases that coexist with micellar aggregates. The presence of K-Sorb and Na-Benz in the solution prevents the formation of these precipitates over time, keeping the solution clear and stable. In contrast, CA, PG, and CA-NaCitr mixture accelerate the segregation of the surfactants, which in turn leads to the

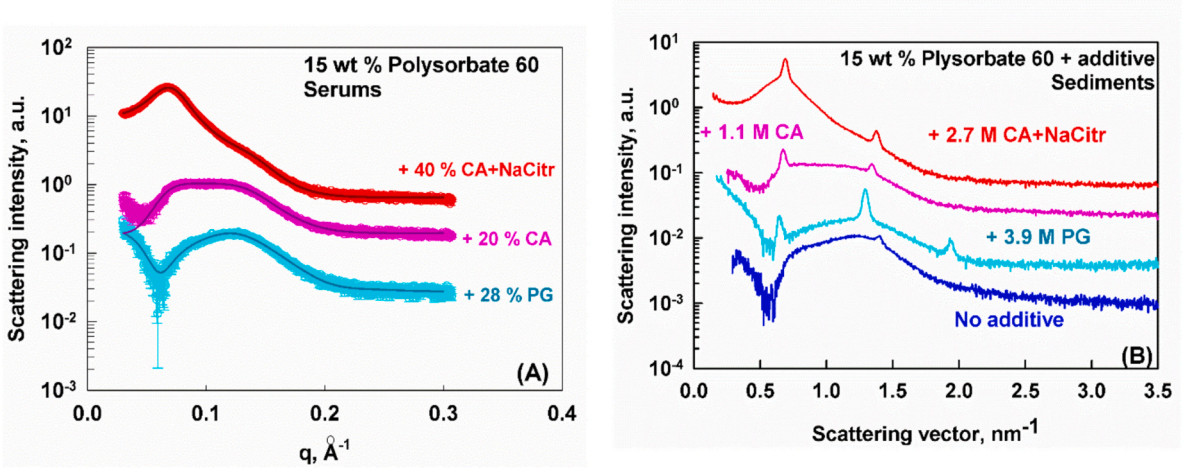

Fig. 3. Scattering intensity as a function of scattering vector from SAXS measurements of the solutions of *15 %* Polysorbate 60 solutions (A) Serums; (B) Sediments separated after centrifugation of solutions without additives (blue); + 3.9 M PG (cyan); + 1.1 M CA (pink); + 2.7 M CA + NaCitr (red). *(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)*

faster formation of the lamellar phases and visible precipitation.

3.1.4. NMR analysis of the freshly prepared and stored solutions

The samples were analyzed using 1D NMR experiments, including ^{1}H with water suppression and ^{13}C, as well as 2D NMR experiments such as DOSY and, in some cases, NOSY. These techniques were used to further investigate the effect of the additives on Polysorbate 60 micelles. The ^{1}H water-suppressed NMR spectrum of a freshly prepared Polysorbate 60 solution without any additives shows six characteristic peaks (see Fig. 4 and Table 4): four peaks for H-atoms from the surfactant's tail: at 0.88 ppm (CH3), 1.29 ppm (CH2 groups), 1.59 ppm (β − CH2), and 2.32 ppm (α − CH2). Two signals for H-atoms from the surfactant's head: at 3.70 ppm and 4.21 ppm. In the presence of the studied additives, chemical shifts were observed for some of these peaks (Fig. S4 and Table 4).

The position of the CH3 peak, which appears at 0.88 ppm in Polysorbate 60 solution without additives, is significantly affected by the presence of 0.7 M Na-Benz, shifting to 0.97 ppm. This notable change indicates that Na-Benz is incorporated into the surfactant micelles, with its benzene ring influencing the shielding of the H-atoms in the CH3 group. The effect of K-Sorb is much smaller, with the peak appearing at 0.90 ppm. This suggests that K-Sorb is also incorporates into the micelles but its influence on the core is less pronounced than that of Na-Benz. In contrast, 3.9 M PG has no significant impact on the position of the CH3

Table 4
Position of characteristic peaks in ^{1}H spectra of Polysorbate 60, in the presence and absence of additives in the solution.

Aqueous phase	Peak position, ppm				
	Tail				Head group
No additive	0.88	1.29	1.59	2.32	3.70
3.9 M PG	0.87	1.27	1.58	2.31	3.69
0.7 M Na-benzoate	0.97	1.37	1.64	2.36	3.65
0.7 M K-sorbate	0.90	1.31	1.60	2.33	3.69
1.1 M CA	0.86	1.26	1.56	2.29	3.66
2.7 M CA + Na-citrate	0.86	1.26	1.56	2.29	3.66

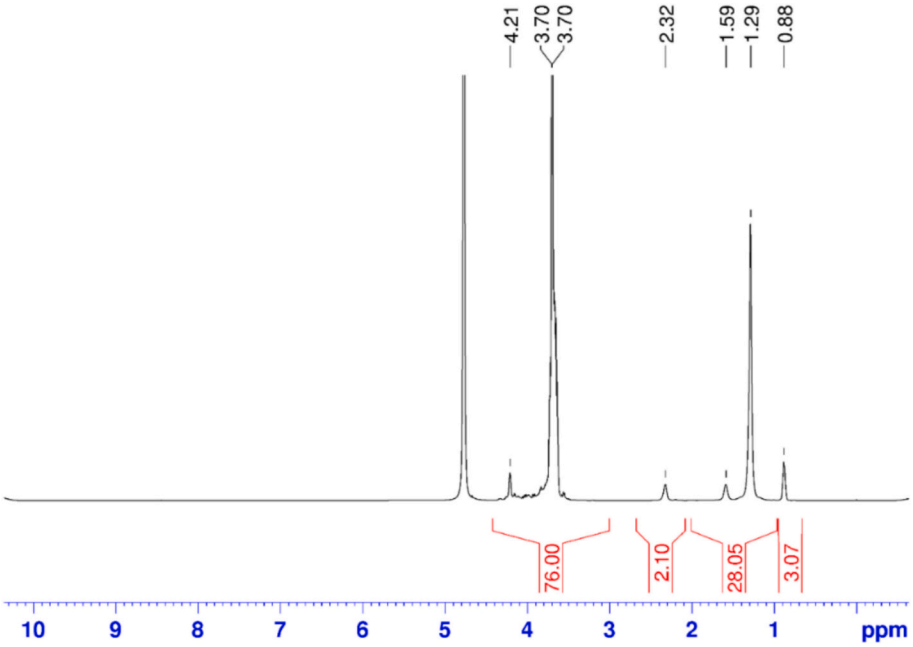

Fig. 4. ^{1}H NMR with water suppression spectrum, acquired at 25 °C, of a freshly prepared *15 %* Polysorbate 60 solution.

peak, indicating that it remains in the aqueous phase and does not interact with the micelle core. The presence of 1.1 M CA and 2.7 M CA + Na-Citr has the opposite effect, shifting the peak to 0.86 ppm.

A similar trend is observed for the H-atoms of the CH2 groups in the hydrophobic tail. The shift is strongest for Na-Benz from 1.29 to 1.37 ppm and less pronounced for K-Sorb (1.31 ppm). In contrast, additives that induce precipitation cause a shift in the opposite direction: 1.27 ppm with PG and 1.26 ppm with CA and CA + Na-Citr.

In conclusion, Na-Benz and K-Sorb are incorporated into the Polysorbate 60 micelles, leading to the deshielding of nearby protons in the surfactant tail. Conversely, PG, CA, and CA + Na-Citr remain in the aqueous phase, where they facilitate better packing of the molecules within the micelles, leading to a more shielded environment for the protons in the tail.

To check further this conclusion, we acquired 1D *NOE* spectra with different zones saturated by selective irradiation (Fig. 5). The *NOE* experiments provide direct evidence for the spatial proximity between sodium benzoate and the surfactant. Selective irradiation of the aromatic resonances of benzoate (δ 7.5–8.0 ppm) induces *NOE* enhancements in the ethoxy protons (δ 3.5–4.0 ppm) and the CH_2 protons (δ 1.3–1.5 ppm) of Polysorbate 60. Irradiation of the ethoxy and tail regions results in detectable intensity changes in the aromatic resonances of benzoate. These reciprocal *NOE* effects unambiguously confirm that benzoate anions are located in close contact with the oxyethylene headgroups at the micelle–water interface and near the tails of the core. This, combined with the change in chemical shifts of the signals for the CH_3 and CH_2 protons in the mixture of polysorbate 60 with sodium benzoate, points to a localization consistent with a model in which sodium benzoate is located in the palisade layer rather than buried in the hydrophobic micellar core.

Similar measurements are also performed in the presence of potassium sorbate (Fig. 6). In this case, selective irradiation of the vinyl protons of sorbate (δ 5.8–6.5 ppm) induces *NOE* responses both in the ethoxy region of Polysorbate 60 (δ 3.5–4.0 ppm) and in the methylene protons of the hydrophobic chains (δ 1.2–1.5 ppm). Reciprocal effects are also observed: Irradiation of the surfactant headgroup protons leads to changes in the sorbate resonances, while irradiation of the aliphatic

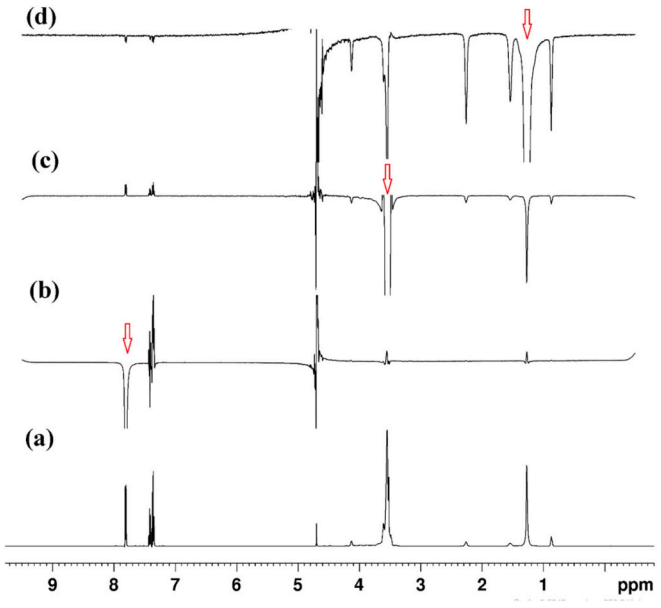

Fig. 5. 1D *NOE* experiments performed with a solution of freshly prepared *15 %* Polysorbate 60 + 0.7 M NaBenz, acquired at 25 °C: (a) ^{1}H NMR with water suppression, (b-d) 1D *NOE* spectra with different selective irradiation frequencies (b – 3906 Hz; c – 1767 Hz; d – 634 Hz). The position of the irradiation is denoted with red arrow. *(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)*

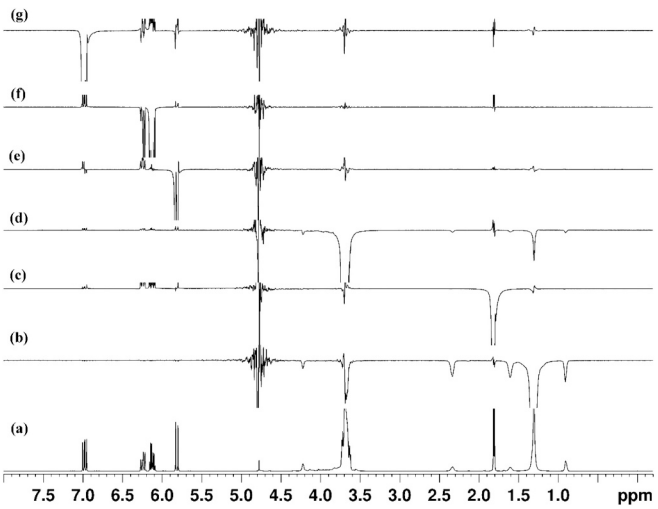

Fig. 6. 1D *NOE* experiments performed with a solution of *15 %* Polysorbate 60 + 0.7 M KSorb, acquired at 25 °C: (a) ^{1}H NMR with water suppression, (b-g) 1D *NOE* spectra with different selective irradiation frequencies (b – 614 Hz; c – 867 Hz; d – 1808 Hz; e – 2869 Hz; f – 3027 Hz; g – 3452 Hz).

chain region produces weaker, but still detectable, enhancements in the sorbate vinyl signals. These findings indicate that potassium sorbate can approach both the headgroups and the chains of Polysorbate 60, suggesting partial penetration into the palisade layer. The obtained correlation data for both additives therefore support the conclusion that sodium benzoate and potassium sorbate are situated in the palisade layer of the micelles.

The NOSY spectrum of the mixture Polysorbate 60 with CA + Na-Citr show correlation between protons from the α CH_2 of the citric acid and protons from the head region of Polysorbate 60 but no correlation with the protons from the tail of the surfactant. This is in good agreement with the proposed position of CA and Na-Citr which remain in the aqueous phase close to the shell of the micelles.

We acquired ^{1}H NMR spectra of the freshly prepared mixtures and the supernatant (serum) to investigate the effects of precipitate formation. The results, presented in Table S2, show the ratio of protons from the head and tail regions of the surfactant. No change in this ratio was observed when sodium benzoate was used as a preservative, because there are no precipitates. Comparing the spectra of Polysorbate 60 without additives before and after centrifugation reveals a slight change in the signal intensity from the surfactant's tails. This suggests a loss of protons in the tail region, which is associated with the precipitation of longer-chain molecules and diesters. This effect is even more pronounced in the presence of citric acid (with and without sodium citrate), where the quantity of protons in the tail region is noticeably diminished.

We also investigated the ^{13}C NMR spectra of the solutions to determine the location of the preservatives within the surfactant micelles. The stacked ^{13}C NMR spectra are shown in Fig. S5, and the most notable signals are summarized in Table 5. The peak position of the C-atoms

Table 5
Chemical shift of some of the ^{13}C signals.

System: *15 %* Polysorbate 60	chem shift, ppm	Δ, Hz	chem shift, ppm	Δ, Hz	chem shift, ppm	Δ, Hz
No additives	13.95	_	30.13	_	69.67	_
+ 3.9 M PG	13.95	0.0	30.05	−10.5	69.73	6.4
+ 0.7 M NaBenz	13.96	2.0	30.04	−11.8	69.50	−22.0
+ 0.7 M KSorb	13.99	5.4	30.08	−6.4	69.65	−3.8
+ 1.1 M CA	13.93	−2.5	30.07	−7.4	69.65	−3.6
+ 2.7 M CA + NaCitr	13.91	−3.8	30.03	−12.9	69.62	−7.2

from the terminal methyl groups is shifted by +2 Hz and + 5.4 Hz in the presence of Na-Benz and K-Sorb, respectively. In contrast, a negative shift is observed for micelles formed with CA and CA + Na-Citr, indicating a more hydrophobic micelle core in these cases. Propylene glycol has a minimal effect on the terminal methyl group peak, suggesting its influence is less significant than that of CA and CA + Na-Citr. PG slightly affects the chemical shift of the C-atoms in the hydrophilic part of the molecule. Na-Benz has the most pronounced effect on the hydrophilic region's chemical shift, indicating its location in the micelle's palisade layer. The effect of K-sorb is smaller than Na-Benz's but still notable. The presence of CA and CA + NaCitr causes significant changes in the chemical shifts of C-atoms in both the hydrophobic and hydrophilic regions, confirming a substantial change in the shape and size of the surfactant micelles, as also demonstrated by SAXS measurements.

From this series of experiments, we can conclude that Na-Benz and K-Sorb are distributed between surfactant micelles and aqueous solutions. Most probably, they are situated in the palisade layer of the micelles and that is why they affect the peak positions from the atoms coming from the tails and from the head group of the surfactant. CA, Na-Citr and PG, which induce the formation of lamellar phases, are situated mainly in the aqueous phase, and they induce the compaction of the core of the micelles, which facilitates the formation of lamellar phases from diesters and long chain monoesters.

3.2. *Effect of heat treatment on the properties of Polysorbate 60 solutions with additives*

Next, we studied the effect of heat treatment by stirring the solutions at 90 °C for 30 min and then rapidly cooling them to room temperature. The latter mimics the conditions for nanoemulsion preparation described in our previous work [14] and the work of Rao and McClements [11]. The cloud point of a 15 wt% Polysorbate 60 solution is determined to be around 90–92 °C, as indicated by visual opalescence and the formation of droplets from separated surfactant, as observed under the microscope (Fig. 7). The studied additives increase the cloud point of Polysorbate 60, as no changes are detected in the samples upon heating to 95 °C. An increase in cloud point of silicone surfactants in the presence of propylene glycol is reported by Soni et al. [8] and of PEO-PPO-PEO triblock copolymers in the presence of sodium hydroxybenzoate by Khimani et al. [18].

The liquid crystalline phase appears in the solutions of Polysorbate 60 in the presence of PG and citric acid within minutes when the solutions are subjected to heat treatment and rapid cooling (Fig. 8). The solutions of Polysorbate 60 in the presence of potassium sorbate or sodium benzoate remain stable and transparent after the heat treatment. Note that the solution containing 1.1 M CA is opalescent after heat treatment due to the smaller size of the aggregates and slower process of precipitation.

To further investigate the solutions, we measured the SAXS spectra of Polysorbate 60 solutions (both without additives and with 2.7 M CA + NaCitr) after they were heated to 90 °C and rapidly cooled to 25 °C. The SAXS spectra were then analyzed by using a core-shell ellipsoid model and core-shell ellipsoid+Taubner-Steray combined model, respectively. The results (shown in Fig. S6 and Table S3) indicate no significant change in the characteristics of the micelles in either solution. However, the spectra for the CA + NaCitr system showed the formation of a liquid crystalline phase, indicated by weak peaks, suggesting that the solution's turbidity is caused by lamellar clusters that form upon heating.

We also conducted SAXS measurements on solutions containing 0.7 M K-Sorb and 2.7 M CA + NaCitr as they were heated in the SAXS instrument. The spectra (Fig. S7) were analyzed using aforementioned models. To account for changes in the scattering length density (SLD) of the core, shell, and solvent with temperature, we used experimental density data for water [37], hexadecane [38], and PEG 400 [39]. The parameters used for the fitting are included in Table S4, and the results are shown in Fig. 9. A significant decrease in the equatorial core radius was observed as the temperature increased for the CA + NaCitr solutions. This suggests that after passing the transition temperature of the long-chain molecules around 45 °C as shown by DSC, these molecules are expelled from the micelle cores and likely begin forming lamellar phases. While a decrease in equatorial core radius was also detected for the micelles in the 0.7 M K-Sorb solution, the effect was much less pronounced. As shown by the polar radii in Fig. 9, these micelles are significantly smaller with a higher curvature, making it more difficult for the long-chain molecules to be expelled and form well-organized lamellar phases.

3.3. *Discussion*

Table 6 summarises the effect of different additives on the micelle size and shape, relative viscosity, NMR chemical shifts, and solution stability. The relative viscosity of the Polysorbate 60 solution decreases from 3.1 (no additive) to 2.4 (in presence of 3.9 M PG), which accompanies the decrease of d_V from 5.7 nm to 4.8 nm measured by DLS. However, the aspect ratio determined from the analysis of SAXS data increases from 1.67 to 1.77, showing the formation of more elongated micelles in the presence of 3.9 M PG. There is no shift in the ^{1}H-atom from the methyl group of the surfactant tail, showing that this additive remains mainly in the aqueous phase, which is in good agreement also with its negative value of LogP. All these results show that 3.9 M PG is not included in the surfactant micelles, but changes the interaction between their head groups by changing the H-bonds between the surfactant head and water molecules and, as a consequence, leads to the formation of elongated micelles. In these elongated micelles, the probability for interaction between the diester molecules increases, and as a consequence, upon storage, these molecules form lamellar phases, which precipitate and separate from the solution. The preparation of these solutions with heating to high temperature and rapid cooling

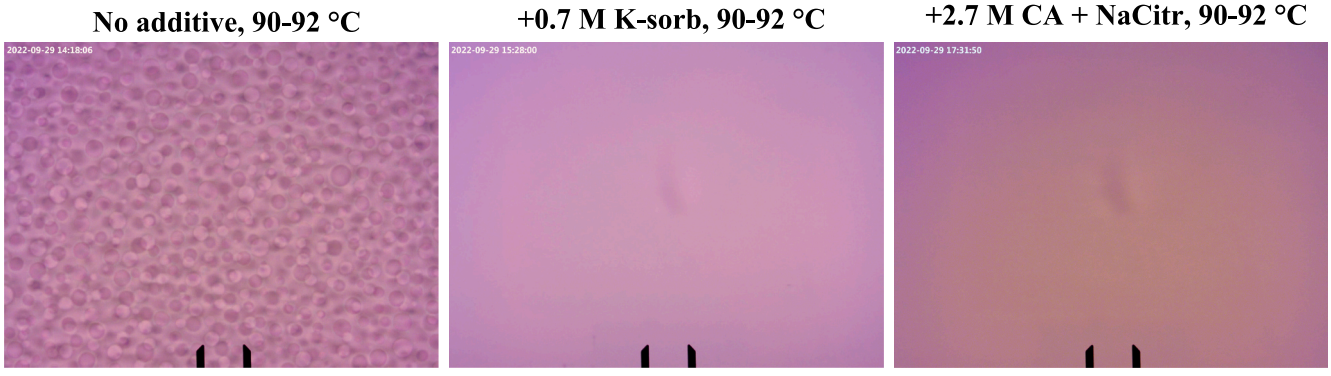

Fig. 7. Polarised light images of *15 %* Polysorbate 60 (from left to right): without additives, +0.7 M potassium sorbate and + 2.7 M citric acid + sodium citrate at 90–92 °C.

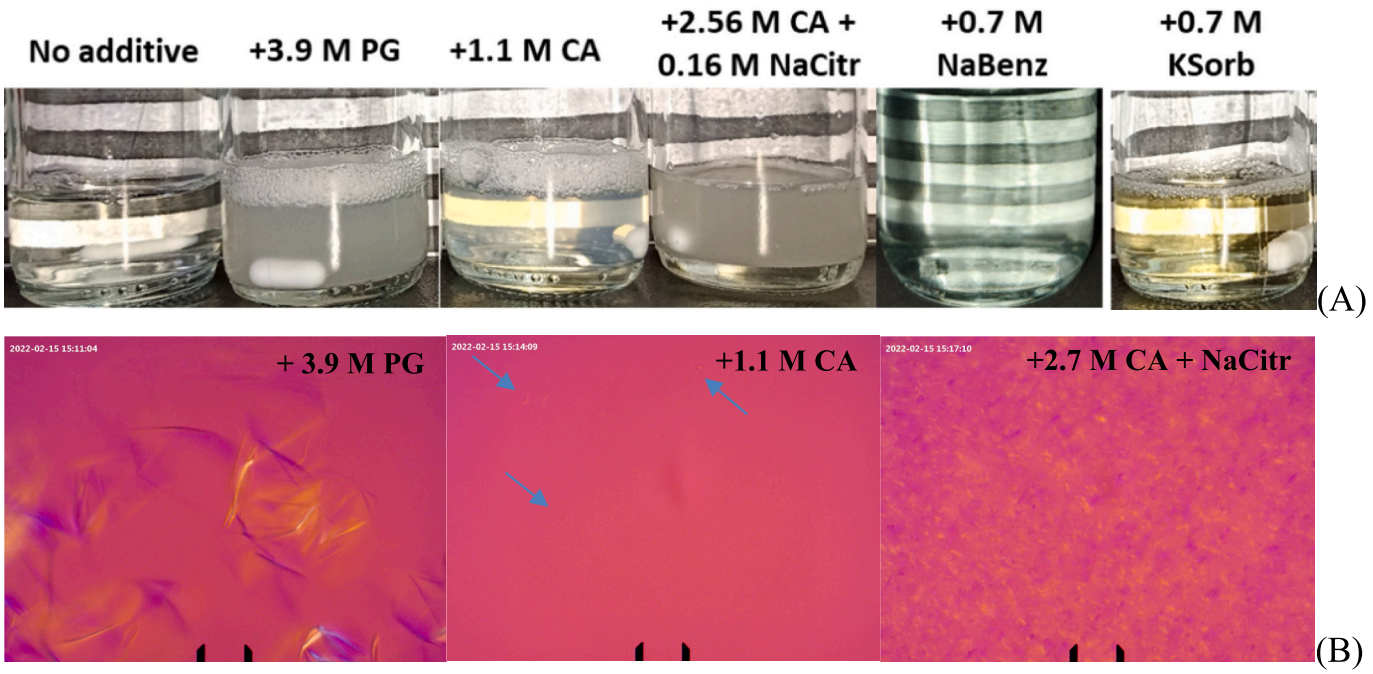

Fig. 8. (A) Picture of the solutions of *15 %* Polysorbate 60 without additives, + 3.9 M PG; 1.1 M CA; 2.7 M CA + NaCitr; 0.7 M NaBenz; 0.7 M KSorb 10 *min* after heat treatment. (B) Polarised light images of the solutions of *15 %* Polysorbate 60 (from left to right): + 3.9 M PG, 1.1 M CA; 2.7 M CA + NaCitr after heat treatment.

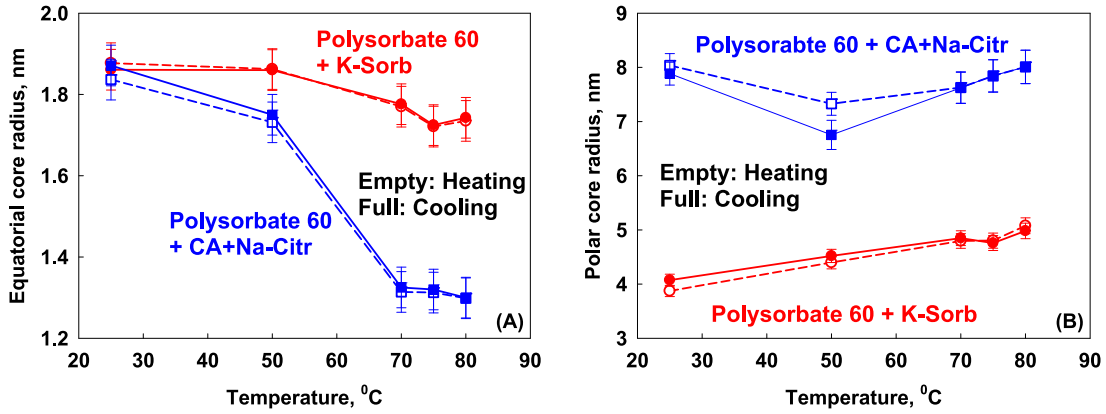

Fig. 9. (A) Equatorial core radius and (B) Polar core radius as a function of temperature for Polysorbate 60 micelles formed in presence of 0.7 M K-Sorb (red circles) and in presence of 2.7 M CA + NaCitr (blue squares) upon heating from 25 to 80 °C (empty points) and upon cooling from 80 °C down to 25 °C (full points). The solutions are kept for 15 min at 80 °C. *(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)*

accelerated the process of lamellar phase formation, and phase separation occurred minutes after their cooling to room temperature.

The addition of 0.7 M Na-Benz to the Polysorbate 60 solution increases the relative viscosity from 3.1 to 3.5, decreases the mean volume micellar diameter from5.7 to 5.3 nm, decreases the aspect ratio of the elongated micelles from 1.67 to 1.61 and induces a significant shift in the ^{1}H-atom from the methyl group of the surfactant tail, and *NOE* correlation is observed between the additive and both the head and tail of the surfactant. These results indicate that this molecule is situated in the palisade layer of the surfactant micelles. The LogP of Na-Benz is −2.3, while the LogP of benzoic acid is 1.87 and its pKa is 4.2. The studied solutions containing sodium benzoate have a pH of 7.8. This means that the concentration of benzoic acid in the surfactant micelles can reach a concentration of 0.21 mM, whereas the concentration of sodium benzoate, calculated under the assumption that its distribution between the aqueous solution and the palisade layer of the micelles is the same as between octanol and water, is estimated to be ≈ 34 mM. This estimated concentration is approximately three times lower compared to the concentration of the Polysorbate 60 molecules included in the micelles. The intercalation of sodium benzoate molecules within the palisade layer of the micelles induces electrostatic repulsions between the molecules. This repulsion prevents the segregation of diesters from monoesters and, consequently, inhibits the formation of lamellar phases. In this manner, the diester molecules remain evenly distributed within the micelles, and the prepared solutions remain stable for more than a year, see Fig. 10. Furthermore, solutions containing Na-Benz that are formed by heating up to 90 °C and rapid cooling also remain stable for a year.

The addition of 0.7 M K-Sorb to Polysorbate 60 changes neither the relative viscosity nor the mean micellar size of Polysorbate micelles alone, but it leads to a significant decrease in the aspect ratio of the elongated micelles from 1.67 to 1.51. This additive is also incorporated into the palisade layer of Polysorbate 60 micelles, as can be seen from the shift in the ^{1}H-atom from the methyl group of the surfactant tail and

Table 6
Summary of the relative viscosity, mean volume micellar size, aspect ratio of formed micelles in Polysorbate 60 solution in presence of various studied additives along with main shifts in peaks in NMR, determined position of the additives in the micelles and stability of prepared solution during storage at room temperature after mild heating at 50 degrees.

Aqueous phase	Relative viscosity, η_r	d_V, nm	R_{ycore}/R_{xcore}	NMR	Position of the additives in the micelles	Stability
No additive	3.1	5.7 ± 0.1	1.67 ± 0.05	–	–	Precipitation after 2–3 days
0.7 M Na-benz	3.5	5.3 ± 0.3	1.61 ± 0.06	+ 0.09 ppm Shift in the CH_3 ^{1}H; - 22 Hz Shift in the OCH_2 ^{13}C; *NOE* correlation between the additive and the head and tails of the surfactant	In the palisade layer	Stable
0.7 M K-sorb	3.1	5.8 ± 0.2	1.51 ± 0.05	+ 0.02 ppm Shift in the CH_3 ^{1}H; - 3.8 Hz Shift in the OCH_2 ^{13}C; *NOE* correlation between the additive and the head and tails of the surfactant	In the palisade layer	Stable
3.9 M PG	2.4	4.8 ± 0.1	1.77 ± 0.06	No Shift in the CH_3 ^{1}H; + 6.4 Hz Shift in the OCH_2 ^{13}C;	Mainly in the aqueous phase	Precipitation after 24 h
1.1 M CA	3.3	6.2 ± 0.4	1.67 ± 0.06	- 0.02 ppm Shift in the CH_3 ^{1}H; - 3.6 Hz Shift in the OCH_2 ^{13}C;	Mainly in the aqueous phase	Precipitation after 24 h
2.7 M CA + NaCitr	6.1	–	4.20 ± 0.08	- 0.02 ppm Shift in the CH_3 ^{1}H; - 7.2 Hz Shift in the OCH_2 ^{13}C; *NOE* correlation between the additive and the head of the surfactant only	Mainly in the aqueous phase - close to the micelle shell	Precipitation after 24 h

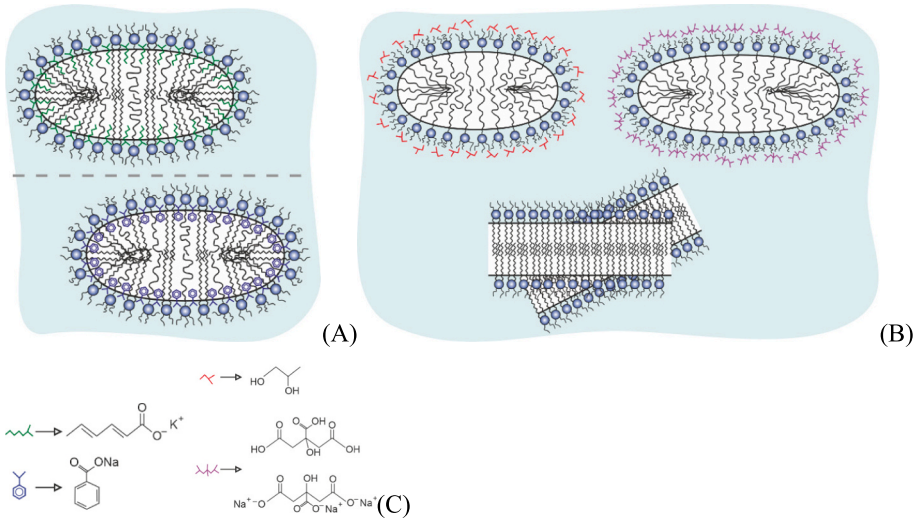

Fig. 10. Schematic illustration of the main conclusion of the current study: (A) potassium sorbate and sodium benzoate increase the *solubilization* of long chain molecules in Polysorbate 60 and significantly improve the stability of the solutions to precipitation, whereas (B) long-chain molecules from Polysorbate 60 precipitates in the solutions without additives, with added propylene glycol and citric acid due to poor incorporation of these molecules in the micelles. (C) Legend of the additives.

the observed *NOE* correlation between the K-sorb and both the head and tail of Polysorbate 60. From these measurements, we can conclude that K-sorb is also able to intercalate between surfactant molecules in Polysorbate 60 micelles and to induce electrostatic repulsions. These repulsions prevent the formation of lamellar phases by diester molecules, and the solutions remain stable for a year, independent of the preparation procedure (mild heating or heating to 90 °C and rapid cooling).

The presence of 1.1 M CA in the aqueous solution leads to almost negligible change in the relative viscosity, mean micellar size, and aspect ratio of elongated micelles. A negative shift in the ^{1}H-atom from the methyl group of the surfactant tail is explained by the better packing of surfactant tails in presence of CA in the aqueous phase. This additive remains in the aqueous solution and competes with the ethoxy groups for water molecules. As a consequence, the surfactant molecules are more tightly packed in the micelles, which makes easier for diesters to form a lamellar phase. The precipitation is faster and occurs within 24 h after solution formation at mild heating.

The Polysorbate 60 solutions containing 2.7 M CA + Na-Citr have a much higher relative viscosity compared to the Polysorbate 60 solution alone (3.1 vs. 6.1) due to the formation of significantly more elongated micelles with an aspect ratio of 4.2 instead of 1.67. The NMR spectra show that these additives interact only with the surfactant head group, indicating that they are close to the micelle shell but do not incorporate into the palisade layer of the micelles. The significant change in the viscosity and the much higher aspect ratio are at least partially related to the absence of free water molecules to hydrate the ethoxy groups of Polysorbate 60 molecules, which decreases the shell thickness of the micelles, as seen from data shown in Table 2. The formation of elongated micelles facilitates the formation of lamellar phases by diester molecules, and precipitation occurs within 24 h after preparation of these solutions under mild heating and within minutes after heating to 90 °C and rapid cooling. The faster precipitation in the latter case is related to the fact that upon heating, the tails of these molecules are melted and they can be separated more easily from other molecules during heating.

As is well known from the literature, the shape of the micelles is governed mainly by the surfactant packing parameter, which is the ratio

of the volume of the hydrophobic tail to the area per molecule's head group multiplied by the tail length [40]. The presence of diester molecules in Polysorbate 60 increases the volume of the hydrophobic tail, thereby increasing the packing parameter and leading to the formation of elongated micelles, see Table 6. On the other hand, the intercalation of K-Sorb and Na-Benz into the palisade layer of the Polysorbate 60 micelles increases the area per molecule and decreases the packing parameter, resulting in the formation of less elongated micelles. In these micelles, the diester molecules are evenly distributed and cannot be separated from the monoesters as lamellar phases. As a consequence, the stability of these solutions is much higher compared to the stability of solutions in which the formation of elongated micelles is more favorable, such as in the case with the other three studied additives (PG, CA, and CA + Na-Citr), which remain primarily in the aqueous solution and do not incorporate into the palisade layer of the micelles. Therefore, the additives that are able to incorporate into the palisade layer of the micelles increase the stability of solutions containing longer chain molecules (like diesters) that are prone to form lamellar phases.

4. Conclusions

The effects of preservatives, including sodium benzoate, potassium sorbate, citric acid, and a mixture of citric acid and sodium citrate, on the phase and micellar behaviour of the nonionic surfactant Polysorbate 60 in aqueous solution were studied. It was shown that Polysorbate 60 solutions undergo slow precipitation of long-chain molecules over 2–3 days of storage at room temperature. This process is facilitated by the addition of citric acid, a mixture of citric acid and sodium citrate, or propylene glycol, with precipitates forming after 24 *h* of storage. The process is further accelerated after heating above the melting temperature of long-chain surfactants and rapid cooling. The presence of sodium benzoate and potassium sorbate leads to the formation of stable solutions for more than a year.

It was also shown that Polysorbate 60 forms elongated micelles with an aspect ratio of 1.7, which increases up to 4.2 upon the addition of a mixture of citric acid and sodium citrate. In contrast, a slight decrease in the aspect ratio to 1.6 and 1.5 is observed for solutions containing sodium benzoate and potassium sorbate. The latter effect is explained by the incorporation of these molecules in the palisade layer of Polysorbate 60 micelles. They are able to prevent the formation of lamellar phases from long-chain molecules present in Polysorbate. Therefore, potassium sorbate and sodium benzoate increase the solubilization of the diesters from Polysorbate 60 and significantly improve the stability of the solutions to precipitation, both before and after heating.

The practical significance of the current study is related to the fact that the obtained results clearly show that food additives can be useful tools for changing the properties of formulations containing Polysorbate molecules.

The scientific significance of the current study is related to the fact that small molecules with a well-pronounced hydrophobic part can be used as efficient additives to prevent the precipitation of long-chain surfactants, owing to their ability to increase the solubilization capacity of Polysorbate 60 micelles.

CRediT authorship contribution statement

Dilek Gazolu-Rusanova: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Zlatina Mitrinova:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Nevena Pagureva:** Investigation, Formal analysis. **Nikola Burdzhiev:** Investigation, Formal analysis. **Slavka Tcholakova:** Writing – review & editing, Visualization, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Funding

This study was funded by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No BG-RRP-2.004-0008.

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.

Acknowledgments

The support of the Centre of Competence "Sustainable Utilization of Bio-resources and Waste of Medicinal and Aromatic Plants for Innovative Bioactive Products" (BIORESOURCES BG), project BG16RFPR002-1.014-0001, funded by the Program "Research, Innovation and Digitization for Smart Transformation" 2021-2027, co-funded by the EU, is greatly acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2025.128844.

Data availability

Data will be made available on request.

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