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Research paper

Lyso-phosphatidylcholine as an interfacial stabilizer for parenteral monoclonal antibody formulations

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ABSTRACT

Therapeutic proteins suffer from physical and chemical instability in aqueous solution. Polysorbates and poloxamers are often added for protection against interfacial stress to prevent protein aggregation and particle formation. Previous studies have revealed that the hydrolysis and oxidation of polysorbates in parenteral formulations can lead to the formation of free fatty acid particles, insufficient long-term stabilization, and protein oxidation. Poloxamers, on the other hand, are considered to be less effective against protein aggregation. Here we investigated two lyso-phosphatidylcholines (LPCs) as potential alternative surfactants for protein formulations, focusing on their physicochemical behavior and their ability to protect against the formation of monoclonal antibody particles during mechanical stress.

The hemolytic activity of LPC was tested in varying ratios of plasma and buffer mixtures. LPC effectively stabilized mAb formulations when shaken at concentrations several orders of magnitude below the onset of hemolysis, indicating that the potential for erythrocyte damage by LPC is non-critical. LPC formulations subjected to mechanical stress through peristaltic pumping exhibited comparable protein particle formation to those containing polysorbate 80 or poloxamer 188. Profile analysis tensiometry and dilatational rheology indicated that the stabilizing effect likely arises from the formation of a viscoelastic film at approximately the CMC. Data gathered from concentration-gradient multi-angle light scattering and isothermal titration calorimetry support this finding. Surfactant desorption was evaluated through sub-phase exchange experiments. While LPCs readily desorbed from the interface, resorption occurred rapidly enough in the bulk solution to prevent protein adsorption. Overall, LPCs behave similarly to polysorbate with respect to interfacial stabilization and show promise as a potential substitute for polysorbate in parenteral protein formulations.

1. Introduction

Protein drugs play an indispensable role in modern therapy. More than half of the biopharmaceuticals approved between 2019 and 2022 were monoclonal antibodies (mAbs) [1]. Therapeutic proteins are susceptible to chemical and physical degradation, and are exposed to various stress factors throughout their lifecycle. One significant contributing factor is exposure to various interfaces. Due to their amphiphilic character, protein molecules can adsorb onto these interfaces, leading to interfacial film formation and partial protein unfolding. Rupture of the interfacial film can result in the formation of large aggregates in solution [2–5]. These particles not only affect the quality of the drug product, but also impact its safety and efficacy [6–8].

Surfactants are often added as excipients to counteract protein adsorption. The mechanism of stabilization has been put forth in several theories. The most prevalent theory suggests that surfactants, being smaller amphiphilic molecules, diffuse and adsorb more rapidly. This effectively hinders protein adsorption and prevents the formation of the

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problematic interfacial film [9,10]. Other studies state that surfactants can directly interact with proteins. Surfactants could, in this case, sterically hinder protein self-interaction or solubilize the protein in solution, preventing interfacial adsorption [11–13].

Common in parenteral formulations is the use of polysorbates (PS) 20 and 80. Approximately 90 % of surfactant-containing formulations include one of these two molecules. Other formulations utilize poloxamer (PX) 188 or 171, although these are considered less effective in protein stabilization [14]. Poloxamer 188 has been associated specifically with the formation of visible protein-PDMS particles during long-term storage in glass vials with siliconized stoppers [15–17]. However, PSs can undergo hydrolysis and oxidation during long-term storage, leading to the formation of undesired byproducts that can trigger additional protein or free fatty acid particle formation [9,15–18]. Furthermore, PSs are associated with hypersensitivity or anaphylactic reactions [18,19]. Therefore, there is a need to provide suitable alternatives to these standard surfactants.

PEG-based surfactants can exhibit comparable efficacy to PS in stabilizing protein formulations, but they are also subject to oxidation and can induce an immunogenic response [20]. Cremophor EL was less effective than PS80 in preventing IgG aggregation during mechanical agitiation [21]. While polyoxyethylene fatty ethers could prevent protein aggregation in some formulations and are resistant to photo-oxidation, they provided less stabilization in other formulations and exhibited hemolytic activity at low concentrations [20,22,23]. The use of non-ester sugar-based surfactants, such as dodecyl maltoside, remains under-investigated in terms of protein stabilization, but show initial promising results [20,24]. Certain trehalose fatty acid esters demonstrated the ability to reduce protein particle formation, but displayed cell membrane disruption at concentrations 5–10 times below their critical micelle concentration (CMC) [25]. Sugar esters require further toxicology data and are likely to be equally susceptible to hydrolysis as PS. Cyclodextrins (CD) have been highly effective in stabilizing protein therapeutics against interface induced aggregation, but they do not exhibit the physiochemical characteristics of typical surfactants and their mode of action requires further elucidation [20,26–29]. Concerns with respect to manufacturing, excipient interactions, and high concentrations formulations must also be addressed [29].

Lecithins, specifically lyso-lecithins, offer a potential alternative. Lyso-phosphatidylcholine (LPC) is a monoacyl component found in natural lecithins. LPC can be enzymatically derived from natural sources, such as soybean or egg, or synthesized at the *sn*-1 position [30]. Depending on the fatty acid composition, LPCs present enhanced solubility and reduced susceptibility to hydrolysis compared to diacyl lecithins [31,32]. This study focuses on S LPC 80, a lecithin derived from soybean with 80 % monoacyl content, primarily composed of a linoleic acid chain and LMPC RS, a synthesized reference standard with myristic acid.

While diacyl lecithin is often used in parenteral applications (oil-in-water emulsions, liposomes, and mixed micelle formulations), the hemolytic activity of LPCs has previously prevented their consideration for liquid parenteral application [33–35]. And yet, it has been established that lipids bind to serum albumin, preventing LPCs from penetrating erythrocyte membranes [36]. Consequently, evaluating hemolysis in whole blood is more biologically relevant than experiments using isolated erythrocytes in buffer [37]. Therefore, we assessed the hemolytic activity of LPCs using whole blood in plasma; specifically comparing hemolysis with respect to buffer concentration. We used this information to determine if the concentrations required for protein stabilization could be critical upon injection, considering the further dilution of LPCs *in vivo*.

Proteins can adsorb and aggregate when exposed to various interfaces relevant during biologics manufacturing and application, including air–liquid interfaces, glass, rubber stoppers, silicone oil, or tubings. As illustrative examples, we subjected a mAb formulation to mechanical stress by shaking it in vials and pumping it through tubings before studying the stabilizing effect of S LPC 80 and LMPC RS compared to PS80 and PX188. Protein particle formation was quantified using micro flow imaging and turbidity.

Possible stabilization in bulk solution through protein-surfactant interactions was investigated using concentration-gradient multi-angle light scattering (CG-MALS) and isothermal titration calorimetry (ITC). Colloidal stability with the addition of surfactants was assessed using dynamic light scattering (DLS).

Profile analysis tensiometry was used to investigate the adsorption rate and surface activity of LPCs at the air–liquid interface. As small zwitterionic molecules, LPCs were expected to adsorb faster than PS80 and generate higher surface pressure than both PS80 and PX188. Changes to these properties with the addition of mAb to the formulations were also examined to detect the presence of protein at the interface. Moreover, the interfacial rheology of antibody films was analyzed, as it often differs characteristically from surfactant films [38]. Using oscillating pendant drop methodology, surfactant and surfactant–protein films were characterized. Again, differences between these films would indicate mAb influence at the interface despite surfactant adsorption. Finally, the reversibility of surfactant adsorption and the ability of mAb to actively remove surfactants from the interface were evaluated through sub-phase exchange experiments.

2. Materials and methods

2.1. Materials

Monoclonal IgG1 antibody at 33.3 mg/ml in 20 mM histidine buffer pH 5.4 and human growth hormone (hGH) at 8.9 mg/ml in PBS were used as stock protein solutions. Bovine serum albumin (BSA) was dissolved to 15.3 mg/ml in 20 mM L-His pH 5.4. Lyso-phosphatidylcholine from soybean with 80 % monoacyl lecithin content (S LPC 80) and lyso-myristyl-phosphatidylcholine reference standard (LMPC RS), primarily linoleic acid in the *sn*-1 position, were generously donated by Lipoid (Ludwigshafen, Germany). Poloxamer 188 (Kolliphor® P 188) was obtained from BASF (Ludwigshafen, Germany) and polysorbate 80 (Tween® 80) was obtained from VWR (Darmstadt, Germany). The main component of the chemical structure of the surfactants is depicted in Fig. 1 with important characteristics listed in Table 1 [39–45]. Buffers were prepared using L-Histidine from Sigma Aldrich (Steinheim, Germany) and highly purified water (HPW) from an Arium pro DI Ultrapure Water System (Sartorius Stedim Biotech GmbH, Goettingen, Germany). The pH was adjusted using hydrochloric acid (VWR). Buffers were filtered through 0.2 μm cellulose acetate filters (47 mm ø, Sartorius Stedim Biotech GmbH) and all solutions were sterile filtered using a 0.2 μm polyethersulfone (PES) membrane syringe filters (VWR). Fresh plasma from whole blood donation was purchased from the blood donation service of the Bavarian Red Cross (Munich, Germany) and whole blood was kindly donated from volunteers in-house. Dulbecco's Phosphate Buffer Saline (PBS) was purchased from VWR, and Triton-X 100 from Sigma-Aldrich.

2.2. Hemolytic activity of surfactants in presence of serum

Hemolysis testing of surfactants was conducted using PBS alone, 25 % plasma:75 % PBS, and 95 % plasma:5 % PBS. Whole blood (3.8 μl) was admixed to 100 μl plasma/PBS and combined with 100 μl of each surfactant stock solution in a Nunclon Delta-treated MicroWell™ 96-well, flat-bottom microplate (Thermo Fischer Scientific GmbH, Steingrund, Germany), resulting in a final blood concentration of 1.9 % (1.9 x 10^7 cells/well). The final surfactant concentration ranged from 0-20 mg/ml.

After incubation at 37 °C from 60 min, plates were centrifuged at 500 g for 5 min. Absorbance at 541 nm (Spark® Multimode Microplate Reader, Tecan, Männedorf, Switzerland) of 100 μl of supernatant was measured.

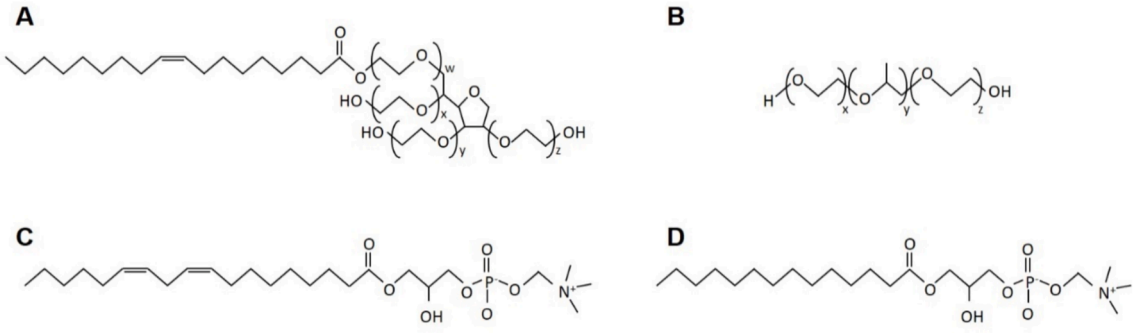

Fig. 1. Characteristic chemical structure of A) PS80, B) PX188, C) the main component of S LPC 80, D) of LMPC RS.

Table 1
Characteristics of surfactants used.

	MW [g/mol]	CMC [mg/ml]	HLB
PS80	1,310	0.01	15
PX188	8,400	0.1–4	29
S LPC 80	468	0.01	9.1–11.3
LMPC	520	0.01	–-

Wells with 20 mg/ml of surfactant, but no erythrocytes, were taken as negative controls, whereas samples with 20 mg/ml Triton-X and 1.9 % whole blood were considered positive controls. With these controls, a linear relationship between hemoglobin absorbance at 541 nm and % hemolysis was established. A sigmoidal fit of the % hemolysis against surfactant concentration was employed to calculate the surfactant concentration at which 5 % (HC5) and 50 % (HC50) hemolysis occurred. The hemolytic activity of S LPC 80 was tested at 0, 25, and 95 % plasma, whereas PS80, PX188, and LMPC RS were exclusively tested at 95 % plasma.

2.3. Mab stabilization by Lyso-PC upon shaking

3 Ml of a 1 mg/ml mAb solution in 20 mM l-his pH 7.4, with varying surfactant concentrations, was subjected to reciprocal shaking at 235 rpm with a 1 cm amplitude using a HS 260 basic shaker (IKA®-Werke GmbH & co. KG, Staufen, Germany) for 24 h in 6R vials (Schott AG, Müllheim, Germany) sealed with with borosilicate glass stoppers made in-house (n = 3). Particle formation was quantified using turbidity (TL2360 LED Turbidimeter, Hach, Düsseldorf, Germany) and sub-visible particle analysis via micro-flow imaging (FlowCam® 8100, yokogawa fluid imaging Technologies Inc, Scarborough, USA). FlowCam samples of 150 µl were analyzed at 0.15 ml/min using a 10x magnification cell. Images were collected at a rate of 28 frames/s for 60 s

2.4. Mab stabilization by Lyso-PC upon peristaltic pumping

Antibody formulations (6 ml, n = 3) in 20 mM L-His pH 5.4 were pumped at 180 rpm for 1 h through Pt-cured silicone tubings (ID 1.6 mm, Accusil Watson-Marlow, Falmouth, UK) using a peristaltic pump (Flexicon PD12 with MC 12 control unit, Watson-Marlow, Ringsted Denmark). Tubings were washed with 1 L HPW at 80 °C and steam sterilized (121 °C, 15 min, 2 bar) prior to beginning with pumping experiments. MAb-free control solutions (6 ml) were circulated first to prime the tubings with other formulation components. Formulations contained 0, 0.001 or 0.01 mg/ml of PS80, PX188, S LPC 80, or LMPC RS. Protein aggregation was determined via turbidity measurements and sub-visible particle analysis (FlowCam® 8100).

2.5. Protein stabilization via Protein-Surfactant Interactions

We tested for direct LPC-mAb interactions using isothermal titration calorimetry (MicroCal iTC200, malvern panalytical Ltd, Malvern, UK). The sample cell was loaded with dialyzed 0.25 mM mAb in 20 mM His pH 5.4, and injections of 2 µl with 2 mM LPC were performed every 300 s with a duration of 0.5 µl/s stirred at 750 rpm. Titration into pure buffer served as a negative control, while 1 mM S LPC 80 was titrated into 0.23 mM BSA as a positive control. Experiments were conducted at both 25 and 40 °C. Raw heat power peaks were integrated and normalized using NITPIC Software (University of Texas, Dallas, USA) to yield the observed heat in kJ/mol [46].

Additionally, we aimed to investigate LPC-mAb interactions using concentration-gradient multi-angle light scattering (CG-MALS). However, because the size of mAb molecules obscures surfactant molecules and micelles during light scattering, we instead tested interactions between hGH and LPC 80 or PS80 micelles. Details of these experiments are provided in the Supplementary Material.

2.6. Colloidal stability of protein solution

Changes in hGH and mAb colloidal stability were evaluated using DLS. MAb experiments used 20 mM His pH 5.4 as a buffer system, while hGH measurements were performed with PBS. Samples with 0.001 mg/ml surfactant (PS80, S LPC 80, LMPC RS) and 1–8 mg/ml protein were centrifuged for 15 min at 13,300 rpm. 25 µl of each sample solution were pipetted into a 384 microwell plate (Corning, New York, USA) and centrifuged at 2,000 rpm for 2 min. Prior to analysis, wells were sealed with silicone oil to prevent evaporation. Samples were measured using a DynaPro plate reader III (Wyatt Technology, Santa Barbara, CA) at 25 °C with 20 acquisitions of 5 s. Protein interaction parameter (k_D) was calculated from the protein concentration dependence of the diffusion coefficient (D):

$$D = D_0(1 + k_D c) \quad (1)$$

where D_0 is the diffusion coefficient at infinite dilution and c is the protein concentration. From k_D, the osmotic second virial coefficient A_2^* of mAb was determined using the TIM equation to estimate the net attractive or repulsive interactions[47]:

$$A_2^* = \frac{k_D + 6.29\,[mL/g]}{1.19M} \quad (2)$$

where M represents the molar mass of the protein.

2.7. Analysis of interfacial behavior by profile analysis tensiometry

Interfacial tension (IFT) of 0.001–1 mg/ml surfactant solutions in 20 mM L-His pH 5.4 (with and without mAb) was assessed using profile analysis tensiometry (PAT1M, Sinterface Technologies, Berlin, Germany) in pendant-drop mode with a 15 µl drop measured every second.

After reaching equilibrium adsorption, five 10 % volumetric oscillations at varying frequencies were carried out to determine storage and loss modulus of the surfactant films. Software provided by Sinterface Technologies was used to calculate viscoelastic properties using a Fourier Transform based on the amplitude of the IFT sinewave during oscillation and the phase-angle between the IFT and volume sinewaves. The resulting equation can be summarized as (Eq. (3)).

$$E^* = \frac{d\gamma}{dln\delta A} = E' + iE''(3)$$

where E^* represents the dilatational viscoelastic modulus, $d\gamma$ is the change in interfacial tension, $dln\delta A$ is the natural logarithm of change in area, E' is the elastic or storage modulus, and E'' is the viscous or loss modulus.

Desorption of surfactant films was investigated using a coaxial capillary in pendant drop mode. First surfactant samples with a concentration of 0.1 mg/ml were allowed to adsorb for 100 min. Subsequently the sub-phase of the pendant drop was replaced with 1 ml of either histidine buffer or a 1 mg/ml mAb solution. If mAb was used as the exchange solution, a second exchange step with 3 ml buffer was performed. 0.2 µl of the sub-phase was aspirated every 0.5 s. Following equilibrium adsorption of each step, viscoelatic properties were analyzed using the aforementioned oscillations.

3. Results

3.1. Hemolytic activity of surfactants in presence of serum

LPCs have been shown to lyse cell membranes at concentrations ranging from 0.00345-0.525 mg/ml in saline buffer, which, at first glance, makes them unfavorable for use in parenteral applications. [33,34] However, the question arises, whether this is also the case in whole blood. To better mimic hemolysis *in vivo*, we analyzed the hemolytic activity of S LPC 80 in mixtures of plasma and PBS. Fig. 2 displays the substantial decrease in hemolysis with rising plasma concentration. HC50 increases from 0.1 to 6.89 mg/ml in 95 % plasma compared to pure buffer (Table 2). LMPC RS, with its saturated fatty acid chain, displayed slightly higher hemolytic activity, with an HC5 of 1.3 mg/ml and HC50 of 2 mg/ml. PS80 and PX188 did not display hemolytic activity at concentrations < 20 mg/ml.

3.2. Mab stabilization by Lyso-PC upon shaking

To assess the stabilizing effect of LPCs on proteins against stress at the air–liquid interface, we subjected mAb solutions to shaking. Prior to shaking, all formulations contained less than 2,000 particles > 1 µm/ml. Shaking of surfactant-free mAb solutions generated 40,000 particles > 1 µm/ml and 3,500 particles > 10 µm/ml. At a concentration of 0.01 mg/ml, LPCs prevented protein aggregation. S LPC 80 provided a protective effect comparable to that of PS80 and PX188, with less than 1000 particles > 1 µm/ml and less than 200 particles > 10 µm/ml. Samples with 0.01 mg/ml LMPC RS yielded 13,500 particles > 1 µm/ml and 470 particles > 10 µm/ml. At higher concentrations, LMPC RS also afforded similar stabilization to PS80 and PX188. At a concentration of 0.001 mg/ml, LPCs did not prevent protein aggregation, resulting in more than 90,000 particles > 1 µm/ml (Fig. 3). Except for LMPC RS at 0.001 mg/ml, the turbidity of surfactant-containing formulations after shaking was very low, specifically lower than that of the surfactant-free mAb solutions. Samples with a higher turbidity correlated to those with a high number of large particles (>10 µm).

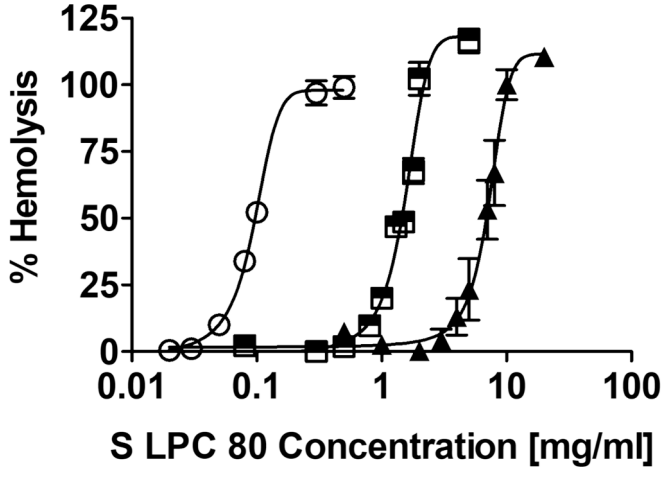

Fig. 2. Percent hemolysis at different concentrations of S LPC 80 in pure PBS (), 25% plasma:75% PBS (), and 95% plasma:5% PBS (). Solid lines represent the sigmoidal fit used to calculate HC5 and HC50.

Table 2
Hemolytic activity of S LPC 80 expressed as HC5 and HC50 in PBS, 25% plasma:75% PBS, and 95% plasma:5% PBS.

	HC5 [mg/ml]	HC50 [mg/ml]
PBS	0.04	0.10
25 % plasma:75 % PBS	0.57	1.45
95 % plasma:5% PBS	2.46	6.89

3.3. Mab stabilization by Lyso-PC upon peristaltic pumping

MAb formulations can also encounter peristaltic pumping during manufacturing processes, calling for an evaluation of protein aggregation as a consequence of this stress. Prior to pumping, all formulations had < 2,000 particles > 1 µm/ml. As seen in Fig. 4, surfactant-free formulations exhibited substantial particle formation, with over 1 million particles > 1 µm/ml and 2,000 particles > 10 µm/ml after 1 h of pumping, yielding highly turbid solutions (40 NTU). Surfactants at a concentration of 0.001 mg/ml failed to prevent particle generation, as evidenced by similar particles counts (>400,000 particles > 1 µm/ml, >1,000 particles > 10 µm/ml) and comparable turbidity (>30 NTU). However, at 0.01 mg/ml, both S LPC 80 and PS80 effectively inhibited aggregation, yielding considerably reduced particle counts (<16,000 particles > 1 µm/ml, <1,000 particles > 10 µm/ml). PX188 and LMPC RS provided more stabilization than at lower concentrations, but did not entirely hinder protein aggregation, with approximately 100,000 particles > 1 µm/ml. Notably, the formation of particles > 10 µm did not change, although LMPC RS lead to fewer large particles overall (~1,000 particles > 10 µm/ml). The turbidity of formulations containing 0.01 mg/ml PX188 or LMPC RS approached that of PS80 samples.

3.4. Protein stabilization via Protein-Surfactant interactions

The direct interaction between BSA and S LPC 80 at 40 °C, as reported in literature and evident in Fig. 5A, was confirmed [36]. The heat of injection (HOI) increased gradually from −20 kJ/mol to −13 kJ/mol with the addition of S LPC 80 to the BSA sample. Contrastingly, titrating S LPC 80 into mAb at 40 °C, resulted in a slightly endothermic change to HOI with low concentrations, which leveled to a plateau. This decrease corresponds to the CMC at this temperature [48]. We observed the same behavior upon injecting S LPC 80 into pure buffer, substantiating the argument, that any change in HOI is triggered by micelle formation at this concentration. LMPC RS illustrated similar HOI patterns, albeit with a more pronounced change at the CMC. After reaching the CMC, HOI stabilized around zero, overlapping with the negative control (S LPC 80 titration into buffer). A similar trend was observed at 25 °C, although the CMC shifted to a lower concentration. This temperature dependence of the CMC corresponds well with literature [48]. Additionally, if heat had

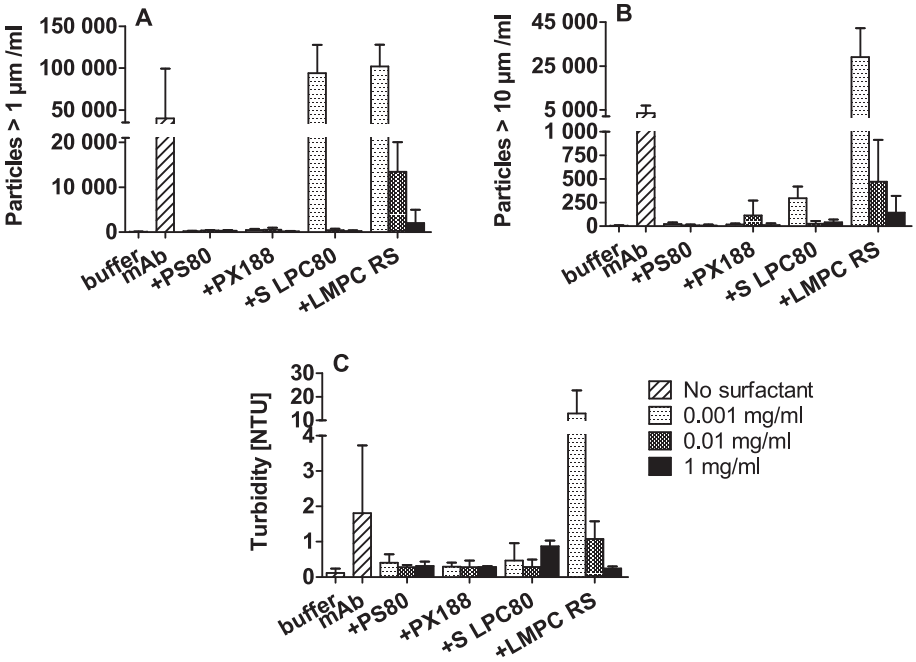

Fig. 3. Particle formation measured via micro-flow imaging and turbidity of mAb and surfactant-mAb solutions following 24 h shaking.

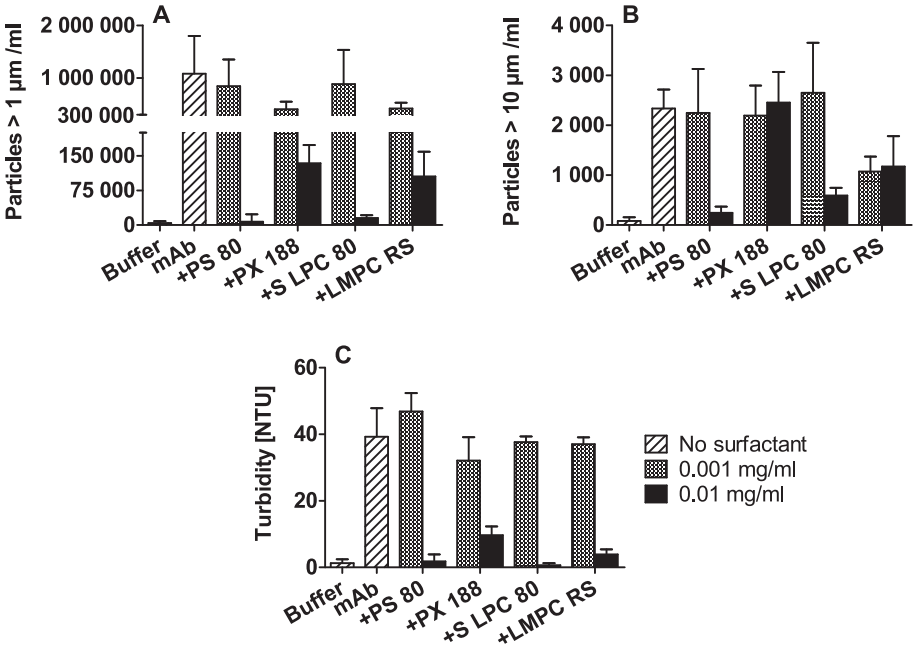

Fig. 4. Particle formation measured via micro-flow imaging and turbidity of mAb and surfactant-mAb solutions following 1 h peristaltic pumping.

been released due to mAb-LPC binding, a shift in the observed CMC compared to LPC-buffer measurements would have occurred [49].

3.5. Colloidal protein stabilization

The colloidal stability of mAb and hGH was characterized using k_D, which for antibodies can be transferred into A_2* (Table 3). Calculations for k_D were done using a molecular mass of 156 kDa for mAb and 20 kDa for hGH. Without surfactants, mAb exhibited net repulsive behavior, whereas hGH showed attraction. The addition of LPCs or PS80 did not largely affect the interactions of either protein.

3.6. Analysis of interfacial behavior by profile analysis tensiometry

3.6.1. Adsorption speed and interfacial tension of surfactants with and without antibody at the Air-Liquid interface

To understand the mechanism of protein protection, differences in surfactant behavior, and the potential coexistence of protein and surfactants at the interface, we monitored the IFT over time at various surfactant concentrations. With increasing surfactant concentration, adsorption quickened and the equilibrium surface tension fell (Fig. 6). PX188 and both LPCs adsorbed faster than PS80, and they maintained a steady IFT throughout the experiment. In contrast, PS80 showed a steady decline in IFT over time.

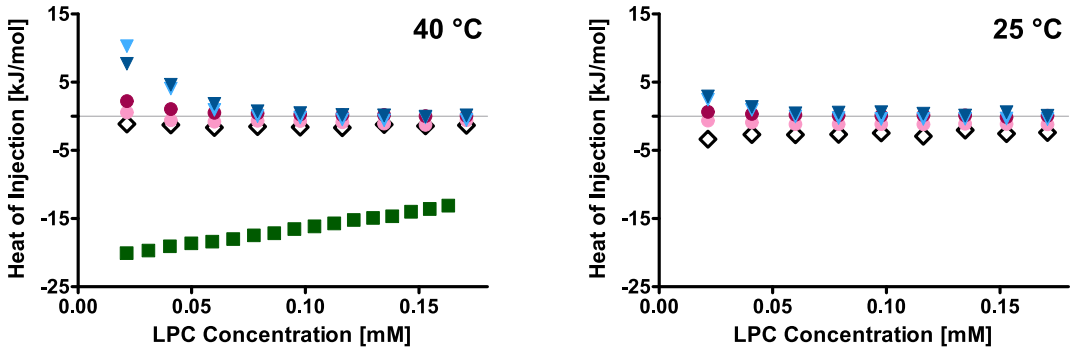

Fig. 5. ITC results presented as heat of injection resulting from titration of S LPC 80 into mAb (), buffer (), or BSA (); LMPC RS into mAb () or buffer (); and buffer into mAb () at 40 °C and 25 °C.

Table 3
Protein interaction parameter k_D and second virial coefficient A_2* of mAb and hGH in surfactant solutions acquired via DLS.

	k_D [mL/g]	A_2* [mL•mol/g^2] • 10^{-4}
mAb	21.7 ± 2.4	1.5 ± 0.1
mAb + PS80	16.3 ± 1.4	1.2 ± 0.1
mAb + S LPC 80	14.3 ± 0.6	1.1 ± 0.0
mAb + LMPC	18.7 ± 2.5	1.3 ± 0.1
hGH	−13.9 ± 0.7	n/a
hGH+PS80	−16.0 ± 3.0	
hGH+S LPC 80	−19.0 ± 1.8	
hGH+LMPC	−20.1 ± 7.6	

Above the CMC (0.01 mg/ml for PS80, S LPC 80, and LMPC RS, 0.1–4 mg/ml for PX188)[39–44], the reduction in IFT was directly related to hydrophobicity. At a surfactant concentration of 0.1 mg/ml, S LPC 80 revealed the lowest IFT (37.7 mN/m), followed by LMPC RS (39.5 mN/m), PS80 (44.8 mN/m) and PX188 (52.8 mN/m). At 0.001 mg/ml, the IFT of PX188 decreased over time, whereas the IFT of PS80 and S LPC 80 at this concentration did not significantly differ from pure buffer. At a heightened concentration of 0.01 mg/ml, PS80 and S LPC 80 solutions began to demonstrate adsorption effects.

Co-adsorption experiments were conducted using 1 mg/ml mAb and 0.01 mg/ml, 0.1 and 1 mg/ml surfactant. Pure mAb showed an IFT of only 69 mN/m after 100 min, which further decreased to 64 mN/m after 10 h. Overall, the co-adsorption of mAb lead to slightly faster adsorption (Fig. 7). At all concentrations, LMPC RS, PS80, and PX188 exhibited similar IFT with or without the co-adsorption of mAb (Table 4), indicating minimal to no mAb adsorption. Conversely, the IFT of S LPC 80 dropped by 14.7 mN/m at 0.01 mg/ml with co-adsorption. As the concentration increased above the CMC, the effect of mAb adsorption subsided.

3.6.2. *Viscoelastic properties of surfactant and Surfactant-Antibody films*

Oscillating dilatational rheology provides additional insights into interfacial composition. MAbs form predominantly elastic films, whereas the rigidity of surfactant films is highly dependent on concentration [38]. In Fig. 8, we confirmed the elasticity of 1 mg/ml mAb,

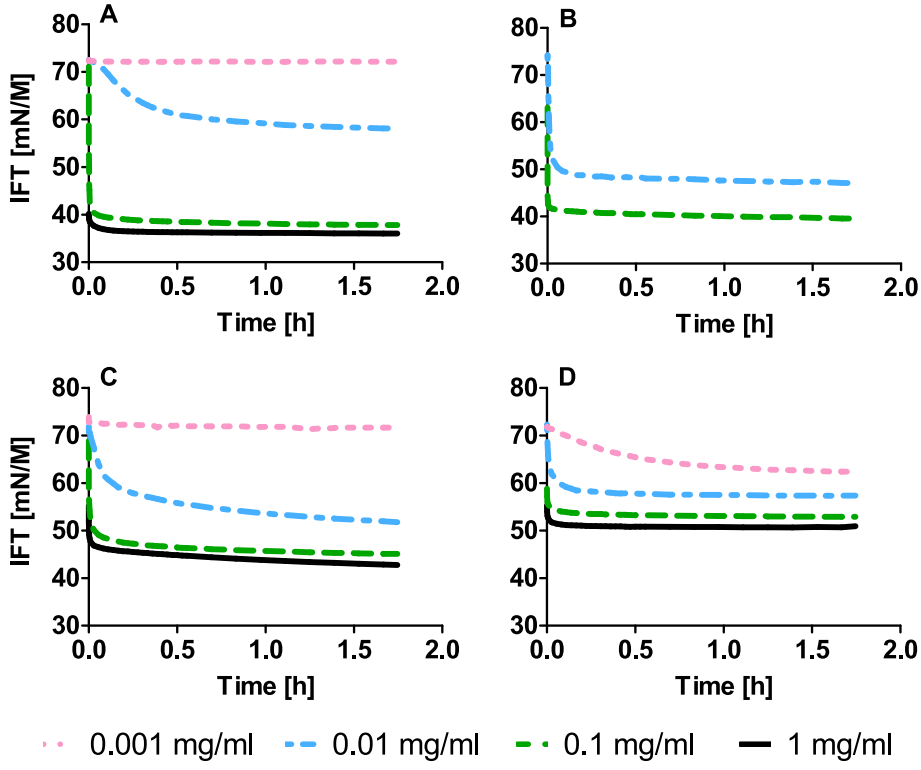

Fig. 6. Time-dependent IFT of A) S LPC 80, B) LMPC RS, C) PS80, and D) PX188 in buffer at varying concentrations using profile analysis tensiometry.

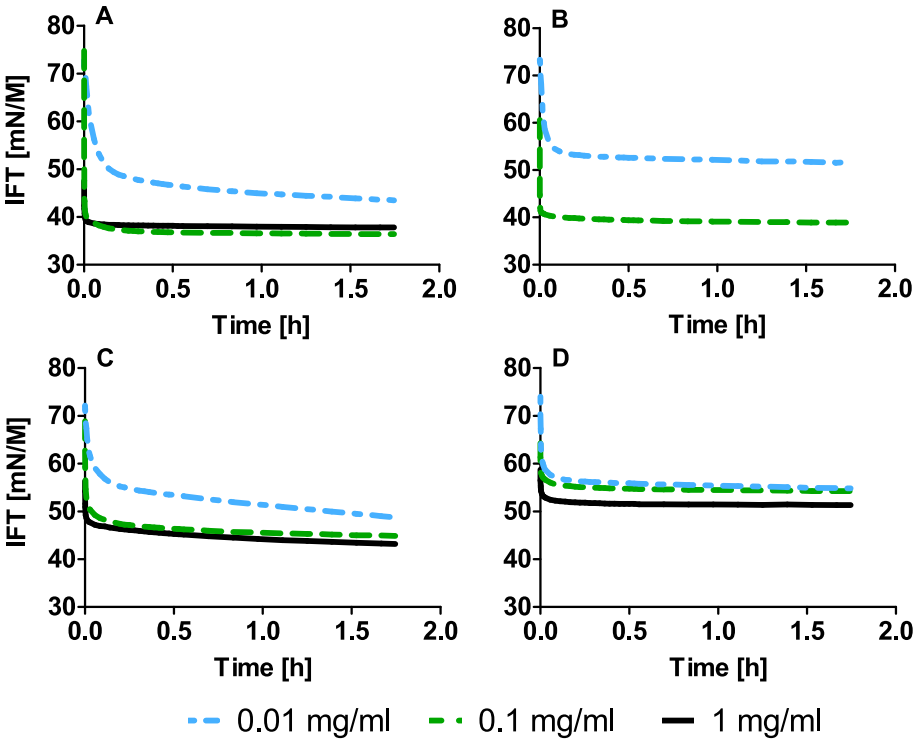

Fig. 7. Time-dependent IFT of A) S LPC 80, B) LMPC RS, C) PS80, and D) PX188 in 1 mg/ml mAb solutions at varying concentrations using profile analysis tensiometry.

Table 4
Difference in equilibrium IFT [mN/m] between surfactant solutions in buffer and protein solution at varying concentrations. ($\Delta IFT = IFT_{surf} - IFT_{mab-surf}$).

Surfactant	Concentration [mg/ml]		
	0.01	0.1	1
S LPC 80	14.7 ± 0.2	1.4 ± 0.0	−1.7 ± 0.2
LMPC RS	−4.5 ± 0.3	0.5 ± 0.3	n/a
PS80	3.5 ± 0.4	0.2 ± 0.4	−0.4 ± 0.0
PX188	2.7 ± 0.1	−1.3 ± 0.0	−0.5 ± 0.1

which had a negligible viscous component. After 100 min, E' at 0.1 Hz was 25 mN/m, which drastically rose to 53 mN/m after 10 h of adsorption.

S LPC 80 first formed a viscoelastic film at 0.01 mg/ml, which coincides with the concentration that initially provided interfacial protection during shaking and pumping, and with the CMC (Fig. 9A). At 0.1 mg/ml, E' dropped from 50 mN/m to 14 mN/m (at 0.1 Hz), while E" rose from 4 mN/m to 10 mN/m (Fig. 10A). Since the viscous modulus was negligible at all other concentrations, E" appeared to reach its maximum at 0.1 mg/ml.

Co-adsorption of mAb at all concentrations resulted in negligible changes in E' and E". The largest difference was observed at 0.01 mg/ml, but these changes lie within the range experimental variability (Fig. 9A). As the concentration increased, the effect diminished.

LMPC RS also revealed the presence of an elastic film at 0.01 mg/ml, with more viscous influence than that of S LPC 80 (Fig. 10B). As the concentration increased to 0.1 mg/ml, both E' and E" decreased, presenting similar characteristics to the S LPC 80 film at 1 mg/ml. Again, the co-adsorption of mAb has the most effect at 0.01 mg/ml (Fig. 9B).

Polysorbate followed a similar trend, although the viscous modulus remained less than 5 mN/m at all concentrations (Fig. 9C). The storage modulus again presented a maximum (29 mN/m at 0.1 Hz) around the CMC, along with the most significant contribution of co-adsorption at this concentration. However, the drop in surface tension with mAb co-

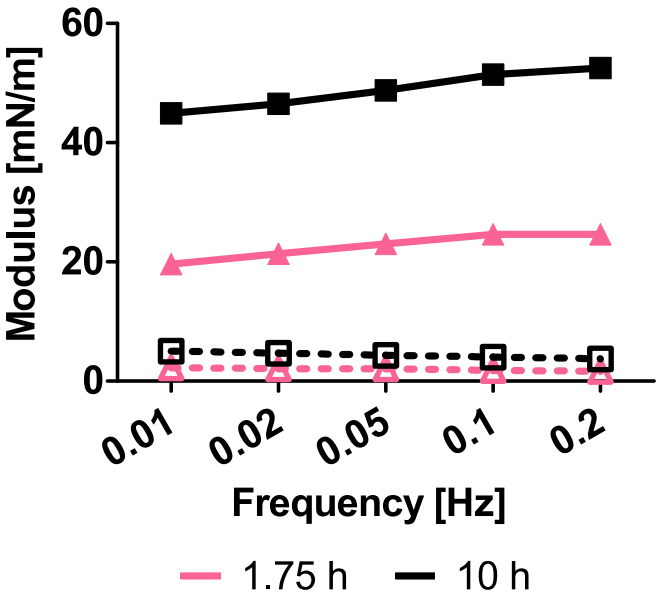

Fig. 8. Elastic E' (closed symbols with solid lines) and viscous E" (open symbols with dashed lines) modulus of mAb solutions, after 1.75 h and 10 h of adsorption, acquired via profile analysis tensiometry. Lines are a visual aid and do not represent measured data points.

adsorption was significantly smaller for PS80 than for S LPC 80 (3.5 mN/m versus 14.7 mN/m). This suggests an enhanced level of interfacial protection by PS80; a small increase in the elastic modulus was still observed.

Interestingly, PX188 had the lowest elastic modulus among the tested surfactants (maximum of 16 mN/m at 0.1 Hz) and showed the least variation between concentrations (Fig. 9D). As observed with IFT, a poloxamer film already formed at 0.001 mg/ml. Amplifying the

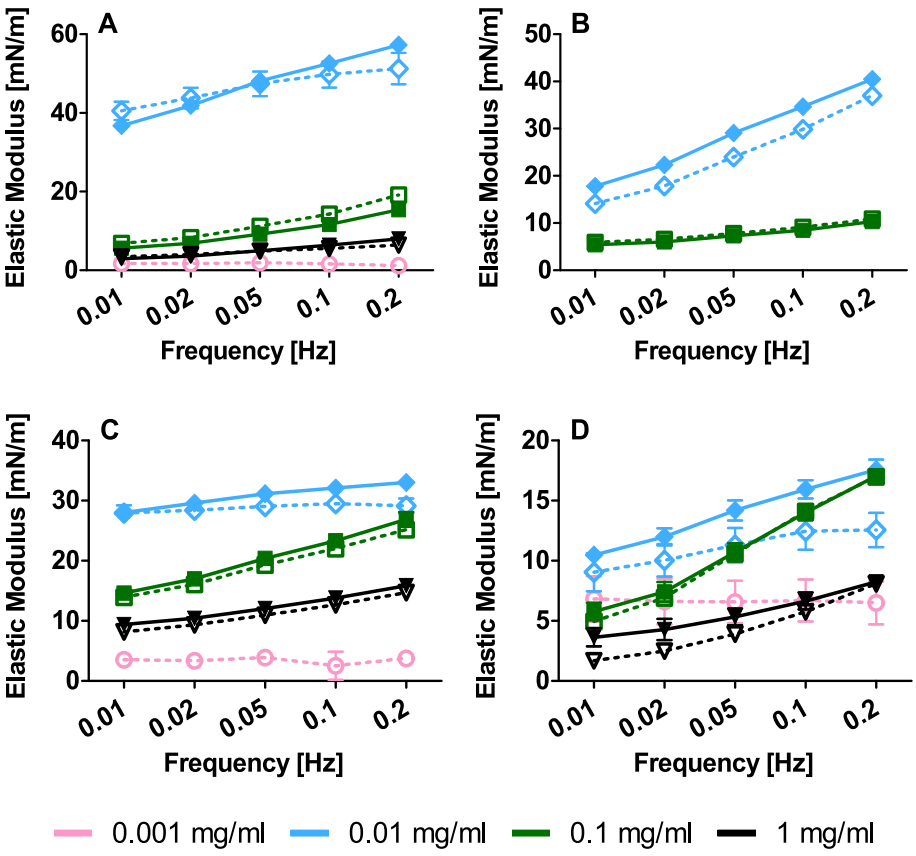

Fig. 9. Elastic modulus E' of A) S LPC 80, B) LMPC RS, C) PS80, and D) PX188 in buffer (open symbols with dashed lines) and mAb solutions (closed symbols with solid lines) acquired via profile analysis tensiometry. Lines are a visual aid and do not represent measured data points.

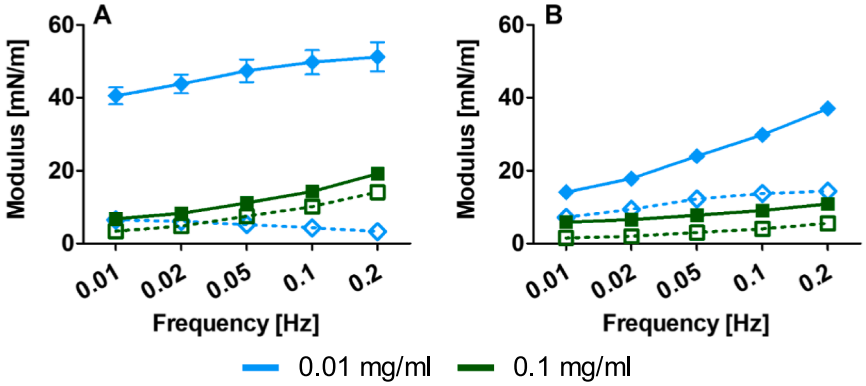

Fig. 10. Elastic E' (closed symbols with solid lines) and viscous E" (open symbols with dashed lines) modulus of A) S LPC 80 and B) LMPC RS in buffer, at 0.01 and 0.1 mg/ml, acquired via profile analysis tensiometry. Lines are a visual aid and do not represent measured data points.

concentration led to higher E' values, similar to PS80 and S LPC 80. However, a stronger frequency dependence was evident at 0.1 mg/ml compared to other concentrations. At 0.05 Hz, the values for 0.01 and 0.1 mg/ml overlapped, and at higher frequencies, 0.1 mg/ml displayed higher E' than 0.01 mg/ml. Regarding mAb adsorption, the greatest effect was seen at 0.01 mg/ml, and increasing the concentration resulted in smaller differences between surfactant and surfactant-mAb films.

3.6.3. *Desorption behavior of surfactant films after sub-phase exchange*

To determine whether the elastic behavior of the surfactant film directly affects its resistance to desorption, we performed sub-phase exchange experiments. When S LPC 80 was exchanged with buffer, IFT increased by 14 mN/m, which was accompanied by a surge of 72 mN/m in E'. Similar changes were noted when S LPC 80 was exchanged with mAb, although the boost in E' was only 46 mN/m. Subsequent exchange with buffer induced negligible changes. S LPC 80 was readily desorbed upon sub-phase exchange (Table 5). LMPC RS similarly desorbed upon sub-phase exchange, with an increase of 19 mN/m in IFT and of 37 mN/m in E'. No difference between mAb and buffer exchange was detected with regards to IFT, although E' only rose by 29 mN/m.

PS80 exhibited more resistance to exchange, displaying only a minor increase in surface tension with each exchange step. The rheological data also mirrored this behavior. However, due to experimental error, it is difficult to discern whether mAb molecules actively contributed to PS80 desorption. As no interactions could be detected between PS80 and

Table 5
IFT and Elastic modulus E' at 0.1 Hz of surfactant solutions in buffer after equilibrium adsorption (Eq), the first (1st ex), and second (2nd ex) sub-phase exchange step.

	IFT [mN/m]			E' [mN/m]		
	Eq	1st	2nd	Eq	1st	2nd
PS80	42.3 ± 0.8	43.6 ± 2.0		31.5 ± 2.3	42.3 ± 4.4	
PS80 + mAb	45.5 ± 2.7	49.6 ± 3.8	55.2 ± 5.0	29.5 ± 2.4	41.9 ± 4.0	37.3 ± 5.5
PX188	54.1 ± 1.0	55.9 ± 0.5		14.6 ± 2.9	18.8 ± 1.4	
PX188 + mAb	53.5 ± 2.0	52.5 ± 3.5	58.2 ± 3.2	15.9 ± 3.2	18.9 ± 1.7	28.4 ± 11.9
S LPC 80	34.5 ± 0.4	48.7 ± 1.0		18.1 ± 2.0	88.8 ± 11.6	
S LPC 80 + mAb	35.8 ± 0.7	48.4 ± 5.4	52.3 ± 3.0	17.2 ± 1.8	63.1 ± 2.0	61.4 ± 7.5
LMPC RS	33.6 ± 0.1	52.7 ± 2.6		13.9 ± 1.4	51.1 ± 5.1	
LMPC RS+mAb	33.8 ± 0.1	51.3 ± 1.0	53.5 ± 0.2	13.0 ± 0.6	41.8 ± 4.2	45.4 ± 1.9

mAb, likely mAb did not influence this behavior. PX188 behaved the most consistently throughout the exchange processes, with only a minor increase observed following the second exchange step (mAb to buffer). The presence of protein had no effect on the desorption properties of PX188. In all cases, the E' values after mAb exchange were higher than those of the co-adsorbed solutions at 0.1 mg/ml surfactant evaluated in the previous section (Fig. 9). The cause being surfactant was desorbed, resulting in a lower surface concentration than was originally adsorbed at 0.1 mg/ml.

4. Discussion

Previously, researchers have shied away from the use of lyso-lecithins in parenteral formulations due to their hemolytic activity in buffer *in vitro* [33,34]. Our studies illustrate that the presence of plasma prevented hemolysis at low concentrations. In plasma, lyso-lecithins bind to the lipid binding pocket of albumin, rendering the surfactant unavailable for interaction with the cell membrane [36]. Thus, although S LPC 80 and LMPC RS induced cell damage at lower concentrations than PS80 and PX188, the HC5 value in the presence of serum remained well above typical surfactant concentrations used in parenteral protein formulations [14].

It is understood that the predominant mechanism of surfactant-mediated protein protection is competitive adsorption [9,10]. This theory posits that surfactants adsorb more rapidly to the interface and, above the CMC, hinder protein adsorption, prohibiting film and aggregate formation. Notably, following 24 h of shaking, PS80 and PX188 demonstrated sufficient stabilization below their respective CMCs (0.01 mg/ml and 0.1–4 mg/ml) [39–43]. While this suggests the contribution of an additional mechanism in protein stabilization by these surfactants, it is important to note that the CMC is a parameter that varies greatly depending on the assessment method, both PX and PS are heterogenous mixtures, and PS specifically can form micelles at very low concentrations [40,50]. Conversely, S LPC 80 and LMPC RS stabilization became apparent only when the surfactant concentration approached the CMC of 0.01 mg/ml. All surfactants proved first stabilizing against pumping stress also at 0.01 mg/ml. Nonetheless, this concentration is 100–250 times lower than the HC5 value of LPCs in plasma. Moreover, it is important to consider that the concentrations mentioned here refer to those in the formulation prior to administration, and dilution takes place upon injection. Overall, S LPC 80 and LMPC RS hold promise as suitable excipients for intravenous applications. However, further studies evaluating *in vivo* compatibility are necessary to assess the feasibility of other administration routes.

Protein stabilization via direct interaction between specific proteins and surfactants have been suggested in literature [11–13]. However, our investigation employing CG-MALS and ITC did not reveal evidence of direct protein-surfactant interactions, neither on an individual molecule nor surfactant micelle level. While PS80 and S LPC 80 may provide some colloidal stability for both mAb and hGH, as indicated by the transition from an attractive to a repulsive second virial coefficient, the values exhibited considerable variability and should be interpreted with caution.

The formation of a highly elastic film combined with the mAb stabilization at 0.01 mg/ml supports the notion that LPC prevents protein aggregation by competitive adsorption. Nevertheless, we see a significant drop in IFT when mAb is present with S LPC 80 at this concentration, while the interfacial rheology of the film showed minimal changes. This observation suggests a substantial change at the interface. As the IFT of pure mAb is higher than that of S LPC 80, it is unlikely that mAb molecules also adsorbed to the interface [26]. Possibly mAb presence in the bulk changed the adsorption behavior of S LPC 80, leading to a higher surface concentration of S LPC 80 than in mAb-free solutions [38,43,51,52]. More detailed information about the composition of this interfacial film is required to fully comprehend the mechanism by which LPC stabilizes mAb. Future experiments involving ellipsometry and x-ray scattering could shed light on this matter.

At higher concentrations, the stabilization capacity of LPC persisted. However, at 0.1 mg/ml there was a significant drop in E' compared to 0.01 mg/ml, and a convergence of E' and E". This apparent maximum in the loss modulus signifies a time-delayed film repair mechanism, where the bulk concentration is sufficiently high to repair gaps in the interface following expansion. Increasing the concentration to 1 mg/ml accelerated the film repair process, resulting in decreased values for both E' and E"[38,53,54]. Accordingly, the influence of mAb co-adsorption diminished. Likely, the improved film repair also contributed to the ongoing stabilization of mAb during shaking.

As expected for concentrations just below the CMC, PS80 and LMPC RS also show low IFT with a maximum E' at 0.01 mg/ml. S LPC 80, however, demonstrated more extreme values for both IFT and E' at 0.01 mg/ml. Compared to PS80, this suggests that the ionic nature of LPCs plays a substantial role in their rheological behavior at the interface [38,55]. Overall it seems that the interfacial behavior of S LPC 80 shows a more sudden change of CMC, whether it be protein stabilization during mechanical stress or film characteristics. LMPC RS results in more gradual effects over changing concentrations. We notice also the higher effect of frequency on the rheological properties of LMPC RS films.

PX188 differs in its behavior at the interface. Poloxamer molecules can fold in a way to maximize both interfacial coverage and concentration. Initially, they adsorb in a flat conformation, rapidly reaching a stable IFT and forming a surfactant film. As more molecules adsorb, the hydrophilic PEO blocks extend away from the interface, creating a brush-like structure. A similar process takes place during compression, whereas expansion allows desorbed PEO segments to migrate back toward the interface, explaining the oscillatory frequency dependence of PX188 [43,56,57]. While these conformational adaptations affected IFT and viscoelastic moduli, the interface remained saturated, resulting in less drastic changes with concentration compared to the more classically structured surfactants.

Due to its ability to adsorb in a flat conformation, PX188 quickly saturates the interface. For this reason, PX188 exhibited the fastest adsorption despite its high molecular weight and hydrophilicity [58]. As time goes on, PX188 folds to accommodate additional molecules without inducing significant changes in IFT [57]. S LPC 80, LMPC RS, and PS80, on the other hand, adsorb in a fixed conformation by filling gaps in the interfacial film. The absence of repulsive forces between PS80 molecules at the interface allows for an improved packing density, which explains the continuous decline in IFT, regardless of the concentration [59].

S LPC 80 and LMPC RS films were easily removed during sub-phase exchange, regardless of the solution used. Combining this information with equilibrium IFT and E' data from co-adsorption experiments, we can conclude that a high elastic modulus does not directly correlate with improved interfacial protection. Rheological data provides insights into the composition of the interface, but cannot solely be used to evaluate protein stabilization.

To provide clarification, at high concentrations, all surfactants demonstrated improved protein protection accompanied by decreased E' and E". At very low concentrations of 0.001 mg/ml, E' and E" are also low, although the least interfacial stabilization was observed during shaking. Considering the equilibrium IFT, low values of E' and E" coupled with a high surface pressure could offer enhanced stabilization. However, as exemplified by both LPCs at 0.1 mg/ml, susceptibility to desorption does not necessarily indicate poor protein stabilization. There is a continuous exchange of molecules between the bulk and interface, and in a concentrated solution, a sufficient number of molecules are present to quickly fill the gaps generated by stress, thus prohibiting protein adsorption [38]. Therefore, it remains prudent to test interfacial protection through agitation studies.

5. Conclusion

This study demonstrated that S LPC 80 and LMPC RS are not hemolytically critical upon injection at concentrations below 2 mg/ml. Furthermore, both LPCs effectively stabilized mAb solutions against stress at the air–liquid and tubing-liquid interfaces, and stabilization was comparable to that achieved by PS80 and PX188 at concentrations $\geq$ 0.01 mg/ml. The formation of a viscoelastic film at 0.01 mg/ml played a crucial role in this stabilization process. No proof of interaction between surfactants and proteins was found. It is important to note that despite their surface activity, LPCs readily desorbed by flow in the subphase. Nevertheless, the interfacial film could be repaired with sufficiently high bulk concentrations, providing stability even under stress conditions. Therefore, the combination of interfacial rheology with agitation studies proved to be a valuable approach in characterizing interfaces and gaining insights into the mechanism and extent of protein stabilization. In conclusion, S LPC 80 and LMPC RS emerge as promising alternatives to polysorbate as interfacial stabilizers in parenteral protein formulations and are worthy of further investigation. Future studies will investigate the chemical stability of LPCs compared to PS under stressed conditions and the effects of long-term storage in the presence of mAb.

CRediT authorship contribution statement

Eleni Papadopoulos: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Betharie Cendera Arrahmani:** Visualization, Validation, Investigation, Formal analysis. **Katharina Beck:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis. **Wolfgang Friess:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2024.114514.

References

[1] G. Walsh, E. Walsh, Biopharmaceutical benchmarks, Nat. Biotechnol. 40 (2022) (2022) 1722–1760, https://doi.org/10.1038/s41587-022-01582-x.
[2] S. Rudiuk, L. Cohen-Tannoudji, S. Huille, C. Tribet, Importance of the dynamics of adsorption and of a transient interfacial stress on the formation of aggregates of IgG antibodies, Soft Matter 8 (2012) 2651–2661, https://doi.org/10.1039/c2sm07017k.
[3] S. Ghazvini, C. Kalonia, D.B. Volkin, P. Dhar, Evaluating the role of the air-solution interface on the mechanism of subvisible particle formation caused by mechanical agitation for an IgG1 mAb, J Pharm Sci 105 (2016) 1643–1656, https://doi.org/10.1016/j.xphs.2016.02.027.
[4] E. Koepf, S. Eisele, R. Schroeder, G. Brezesinski, W. Friess, Notorious but not understood: How liquid-air interfacial stress triggers protein aggregation, Int J Pharmaceut 537 (2018) 202–212, https://doi.org/10.1016/j.ijpharm.2017.12.043.
[5] N. Deiringer, W. Friess, Proteins on the rack: mechanistic studies on protein particle formation during peristaltic pumping, J Pharm Sci 111 (2022) 1370–1378, https://doi.org/10.1016/j.xphs.2022.01.035.
[6] A.S. Rosenberg, Effects of protein aggregates: An immunologic perspective, Aaps J 8 (2006) E501–E507, https://doi.org/10.1208/aapsj080359.
[7] E.M. Moussa, J.P. Panchal, B.S. Moorthy, J.S. Blum, M.K. Joubert, L.O. Narhi, E. M. Topp, Immunogenicity of therapeutic protein aggregates, J Pharm Sci 105 (2016) 417–430, https://doi.org/10.1016/j.xphs.2015.11.002.
[8] S. Zölls, R. Tantipolphan, M. Wiggenhorn, G. Winter, W. Jiskoot, W. Friess, A. Hawe, Particles in therapeutic protein formulations, Part 1: Overview of analytical methods, J Pharm Sci 101 (2012) 914–935, https://doi.org/10.1002/jps.23001.
[9] M.T. Jones, H.-C. Mahler, S. Yadav, D. Bindra, V. Corvari, R.M. Fesinmeyer, K. Gupta, A.M. Harmon, K.D. Hinds, A. Koulov, W. Liu, K. Maloney, J. Wang, P. Y. Yeh, S.K. Singh, Considerations for the use of polysorbates in biopharmaceuticals, Pharmaceut Res 35 (2018) 148, https://doi.org/10.1007/s11095-018-2430-5.
[10] H.J. Lee, A. McAuley, K.F. Schilke, J. McGuire, Molecular origins of surfactant-mediated stabilization of protein drugs, Adv Drug Deliver Rev 63 (2011) 1160–1171, https://doi.org/10.1016/j.addr.2011.06.015.
[11] P. Garidel, C. Hoffmann, A. Blume, A thermodynamic analysis of the binding interaction between polysorbate 20 and 80 with human serum albumins and immunoglobulins: A contribution to understand colloidal protein stabilisation, Biophys Chem 143 (2009) 70–78, https://doi.org/10.1016/j.bpc.2009.04.004.
[12] N.B. Bam, J.L. Cleland, J. Yang, M.C. Manning, J.F. Carpenter, R.F. Kelley, T. W. Randolph, Tween protects recombinant human growth hormone against agitation-induced damage via hydrophobic interactions, J Pharm Sci 87 (1998) 1554–1559, https://doi.org/10.1021/js980175v.
[13] X. Yuan, S. Krueger, E. Shalaev, Protein-surfactant and protein-protein interactions during freeze and thaw: a small-angle neutron scattering study of lysozyme solutions with polysorbate and poloxamer, J. Pharm. Sci. 112 (2023) 76–82, https://doi.org/10.1016/j.xphs.2022.08.017.
[14] V. Gervasi, R.D. Agnol, S. Cullen, T. McCoy, S. Vucen, A. Crean, Parenteral protein formulations: An overview of approved products within the European Union, Eur J Pharm Biopharm 131 (2018) 8–24, https://doi.org/10.1016/j.ejpb.2018.07.011.
[15] K. Soeda, K. Arai, T. Yamamoto, K. Ofuji, M. Fukuda, D. Hashimoto, Y. Yamanaka, Mechanism of protein–PDMS visible particles formation in liquid vial monoclonal antibody formulation, J. Pharm. Sci. 112 (2023) 653–664, https://doi.org/10.1016/j.xphs.2022.09.027.
[16] K. Soeda, M. Fukuda, M. Takahashi, H. Imai, K. Arai, S. Saitoh, R.S.K. Kishore, N. S. Oltra, J. Duboeuf, D. Hashimoto, Y. Yamanaka, Impact of poloxamer 188 material attributes on proteinaceous visible particle formation in liquid monoclonal antibody formulations, J Pharm Sci (2022), https://doi.org/10.1016/j.xphs.2022.04.012.

[17] C. Grapentin, C. Müller, R.S.K. Kishore, M. Adler, I. ElBialy, W. Friess, J. Huwyler, T.A. Khan, Protein-polydimethylsiloxane particles in liquid vial monoclonal antibody formulations containing poloxamer 188, J Pharm Sci 109 (2020) 2393–2404, https://doi.org/10.1016/j.xphs.2020.03.010.
[18] Walter B. Shelley, N. Talanin, E.D. Shelley, Polysorbate 80 hypersensitivity, Lancet 345 (1995) 1312–1313, https://doi.org/10.1016/s0140-6736(95)90963-x.
[19] E.A. Coors, H. Seybold, H.F. Merk, V. Mahler, Polysorbate 80 in medical products and nonimmunologic anaphylactoid reactions, Ann Allergy Asthma Immunol 95 (2005) 593–599, https://doi.org/10.1016/s1081-1206(10)61024-1.
[20] A.J.C. Ruiz, M.A.S. Boushehri, T. Phan, S. Carle, P. Garidel, J. Buske, A. Lamprecht, Alternative excipients for protein stabilization in protein therapeutics: overcoming the limitations of polysorbates, Pharm 14 (2022) 2575, https://doi.org/10.3390/pharmaceutics14122575.
[21] D. Vidanovic, J.M. Askrabic, M. Stankovic, V. Poprzen, Effects of nonionic surfactants on the physical stability of immunoglobulin G in aqueous solution during mechanical agitation, Pharmazie 58 (2003) 399–404.
[22] L. Yue, Z. Yan, H. Li, X. Liu, P. Sun, Brij-58, a potential injectable protein-stabilizer used in therapeutic protein formulation, Eur J Pharm Biopharm 146 (2020) 73–83, https://doi.org/10.1016/j.ejpb.2019.12.001.
[23] M. Katakam, L.N. Bell, A.K. Banga, Effect of surfactants on the physical stability of recombinant human growth hormone, J. Pharm. Sci. 84 (1995) 713–716, https://doi.org/10.1002/jps.2600840609.
[24] E.T. Maggio, Alkylsaccharides: circumventing oxidative damage to biotherapeutics caused by polyoxyethylene-based surfactants, Ther Deliv 4 (2013) 567–572, https://doi.org/10.4155/tde.13.19.
[25] L. Schiefelbein, M. Keller, F. Weissmann, M. Luber, F. Bracher, W. Frieß, Synthesis, characterization and assessment of suitability of trehalose fatty acid esters as alternatives for polysorbates in protein formulation, Eur. J. Pharm. Biopharm. 76 (2010) 342–350, https://doi.org/10.1016/j.ejpb.2010.08.012.
[26] M.P. Zoeller, S. Hafiz, A. Marx, N. Erwin, G. Fricker, J.F. Carpenter, Exploring the protein stabilizing capability of surfactants against agitation stress and the underlying mechanisms, J. Pharm. Sci. 111 (2022) 3261–3274, https://doi.org/10.1016/j.xphs.2022.09.004.
[27] T. Stolzke, F. Krieg, T. Peng, H. Zhang, O. Häusler, C. Brandenbusch, Hydroxylpropyl-β-cyclodextrin as potential excipient to prevent stress-induced aggregation in liquid protein formulations, Molecules 27 (2022) 5094, https://doi.org/10.3390/molecules27165094.
[28] T. Serno, J.F. Carpenter, T.W. Randolph, G. Winter, Inhibition of agitation-induced aggregation of an IgG-antibody by hydroxypropyl-β-cyclodextrin, J. Pharm. Sci. 99 (2010) 1193–1206, https://doi.org/10.1002/jps.21931.
[29] H.H. Wu, P. Garidel, B. Michaela, HP-β-CD for the formulation of IgG and Ig-based biotherapeutics, Int. J. Pharm. 601 (2021) 120531, https://doi.org/10.1016/j.ijpharm.2021.120531.
[30] S. Drescher, P. van Hoogevest, The phospholipid research center: current research in phospholipids and their use in drug delivery, Pharm 12 (2020) 1235, https://doi.org/10.3390/pharmaceutics12121235.
[31] P. van Hoogevest, Review – An update on the use of oral phospholipid excipients, Eur J Pharm Sci 108 (2017) 1–12, https://doi.org/10.1016/j.ejps.2017.07.008.
[32] M. Trotta, M. Gallarate, F. Pattarino, M.E. Carlotti, Investigation of the phase behaviour of systems containing lecithin and 2-acyl lysolecithin derivatives, Int J Pharmaceut 190 (1999) 83–89, https://doi.org/10.1016/s0378-5173(99)00281-1.
[33] S.-R. Weltzien, Slow-reacting hemolytic phosphatides: Benzylated lysolecithins, BBA (1973) 6–14.
[34] F.C. Reman, R.A. Demel, J.D. Gier, L.L.M.V. Deenen, H. Eibl, O. Westphal, Studies on the lysis of red cells and bimolecular lipid leaflets by synthetic lysolecithins, lecithins and structural analogs, Chem Phys Lipids 3 (1969) 221–233, https://doi.org/10.1016/0009-3084(69)90014-0.
[35] P. van Hoogevest, X. Liu, A. Fahr, M.L.S. Leigh, Role of phospholipids in the oral and parenteral delivery of poorly water soluble drugs, J. Drug Deliv. Sci. Technol. 21 (2011) 5–16, https://doi.org/10.1016/s1773-2247(11)50001-2.
[36] A.E.A. Thumser, D.C. Wilton, The binding of natural and fluorescent lysophospholipids to wild-type and mutant rat liver fatty acid-binding protein and albumin, Biochem J 307 (1995) 305–311, https://doi.org/10.1042/bj3070305.
[37] W. Hadnagy, B. Marsetz, H. Idel, Hemolytic activity of crystalline silica – Separated erythrocytes versus whole blood, Int J Hyg Envir Heal 206 (2003) 103–107, https://doi.org/10.1078/1438-4639-00200.
[38] R. Miller, E.V. Aksenenko, V.S. Alahverdjieva, V.B. Fainerman, Cs. Kotsmar, J. Krägel, M.E. Leser, V. Pradines, V.I. Kovalchuk, B.A. Noskov, Proteins in Solution and at Interfaces: Chpt 20 Thermodynamics, adsorption kinetics and rheology of mixed protein–surfactant interfacial layers, 2013. doi: 10.1002/9781118523063.ch20.
[39] B.A. Kerwin, Polysorbates 20 and 80 used in the formulation of protein biotherapeutics: Structure and degradation pathways, J Pharm Sci 97 (2008) 2924–2935, https://doi.org/10.1002/jps.21190.
[40] A. Tomlinson, I.E. Zarraga, B. Demeule, Characterization of polysorbate ester fractions and implications in protein drug product stability, Mol Pharmaceut 17 (2020) 2345–2353, https://doi.org/10.1021/acs.molpharmaceut.0c00093.
[41] M. Almeida, M. Magalhães, F. Veiga, A. Figueiras, Poloxamers, poloxamines and polymeric micelles: Definition, structure and therapeutic applications in cancer, J Polym Res 25 (2017) 31, https://doi.org/10.1007/s10965-017-1426-x.
[42] S.A. Maskarinec, J. Hannig, R.C. Lee, K.Y.C. Lee, Direct observation of poloxamer 188 insertion into lipid monolayers, Biophys J 82 (2002) 1453–1459, https://doi.org/10.1016/s0006-3495(02)75499-4.
[43] A. Torcello-Gómez, M.J. Santander-Ortega, J.M. Peula-García, J. Maldonado-Valderrama, M.J. Gálvez-Ruiz, J.L. Ortega-Vinuesa, A. Martín-Rodríguez, Adsorption of antibody onto Pluronic F68-covered nanoparticles : link with surface properties, Soft Matter 7 (2011) 8450–8461, https://doi.org/10.1039/c1sm05570d.
[44] Sigma-Aldrich, Product Information Lysophosphatidylcholine, (2001).
[45] F. Otto, P. van Hoogevest, F. Syrowatka, V. Heinl, R.H.H. Neubert, Assessment of the applicability of HLB values for natural phospholipid emulsifiers for preparation of stable emulsions, Die Pharm. 75 (2020) 365–370, https://doi.org/10.1691/ph.2020.9174.
[46] T.H. Scheuermann, C.A. Brautigam, High-precision, automated integration of multiple isothermal titration calorimetric thermograms: New features of NITPIC, Methods 76 (2015) 87–98, https://doi.org/10.1016/j.ymeth.2014.11.024.
[47] T. Menzen, W. Friess, Temperature-ramped studies on the aggregation, unfolding, and interaction of a therapeutic monoclonal antibody, J Pharm Sci 103 (2014) 445–455, https://doi.org/10.1002/jps.23827.
[48] H. Heerklotz, R.M. Epand, The enthalpy of acyl chain packing and the apparent water-accessible apolar surface area of phospholipids, Biophys. J. 80 (2001) 271–279, https://doi.org/10.1016/s0006-3495(01)76012-2.
[49] L.C. Vormittag, H. Heerklotz, Extending the pseudo-phase model of detergent-lipid dispersions by a detergent-binding protein, Langmuir 38 (2022) 15592–15603, https://doi.org/10.1021/acs.langmuir.2c02234.
[50] H. Knoch, M.H. Ulbrich, J.J. Mittag, J. Buske, P. Garidel, H. Heerklotz, Complex micellization behavior of the polysorbates tween 20 and tween 80, Mol. Pharm. 18 (2021) 3147–3157, https://doi.org/10.1021/acs.molpharmaceut.1c00406.
[51] M.A. Bos, T. van Vliet, Interfacial rheological properties of adsorbed protein layers and surfactants: a review, Adv Colloid Interfac 91 (2001) 437–471, https://doi.org/10.1016/s0001-8686(00)00077-4.
[52] H.L. Kim, A. Mcauley, J. Mcguire, Protein effects on surfactant adsorption suggest the dominant mode of surfactant-mediated stabilization of protein, J Pharm Sci 103 (2014) 1337–1345, https://doi.org/10.1002/jps.23908.
[53] F. Ravera, G. Loglio, V.I. Kovalchuk, Interfacial dilational rheology by oscillating bubble/drop methods, Curr Opin Colloid in 15 (2010) 217–228, https://doi.org/10.1016/j.cocis.2010.04.001.
[54] E. Santini, F. Ravera, M. Ferrari, C. Stubenrauch, A. Makievski, J. Krägel, A surface rheological study of non-ionic surfactants at the water–air interface and the stability of the corresponding thin foam films, Colloids Surfaces Physicochem Eng Aspects 298 (2007) 12–21, https://doi.org/10.1016/j.colsurfa.2006.12.004.
[55] A.P. Gerola, P.F.A. Costa, F. Nome, F. Quina, Micellization and adsorption of zwitterionic surfactants at the air/water interface, Curr Opin Colloid in 32 (2017) 48–56, https://doi.org/10.1016/j.cocis.2017.09.005.
[56] B.R. Blomqvist, T. Wärnheim, P.M. Claesson, Surface rheology of PEO−PPO−PEO triblock copolymers at the air−water interface: comparison of spread and adsorbed layers, Langmuir 21 (2005) 6373–6384, https://doi.org/10.1021/la0467584.
[57] A. Torcello-Gómez, M. Wulff-Pérez, M.J. Gálvez-Ruiz, A. Martín-Rodríguez, M. Cabrerizo-Vílchez, J. Maldonado-Valderrama, Block copolymers at interfaces: Interactions with physiological media, Adv Colloid Interfac 206 (2014) 414–427, https://doi.org/10.1016/j.cis.2013.10.027.
[58] E. Russo, C. Villa, Poloxamer hydrogels for biomedical applications, Pharm 11 (2019) 671, https://doi.org/10.3390/pharmaceutics11120671.
[59] K. Szymczyk, A. Zdziennicka, B. Jańczuk, Adsorption and aggregation properties of some polysorbates at different temperatures, J Solution Chem 47 (2018) 1824–1840, https://doi.org/10.1007/s10953-018-0823-z.