

COMPETITIVE INTERFACIAL BEHAVIOR IN SODIUM CASEINATES- HYDROXYPROPYL CELLULOSE MIXE SYSTEMS

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Abstract.

In this work, interactions between hydroxypropyl cellulose (HPC) and sodium caseinates (Na-cas) at the air-water interface were studied. The surface tension, surface rheology, and film compatibility of the mixed protein/polysaccharide system were investigated. Tensiometry experiments showed that HPC is more surface-active than proteins, and is preferentially adsorbed at the interface. This molecule is able to migrate to the interface faster than Na-cas despite its larger size. At low HPC concentration, proteins adsorb first but HPC being more surface-active will eventually substitute them at the interface. The use of the pendant drop tensiometer allowed the real-time monitoring of protein displacement; an immediate decrease in surface tension was observed when HPC was added to a Na-cas solution. The analysis of the compression isotherms of Na-cas/HPC mixed films revealed partial miscibility between the two species within the monolayer; the films were less expanded than expected from the ideal behavior of binary mixtures. The dilatational rheology experiments showed that the presence of a very small amount of HPC had a significant effect on the viscoelastic properties of the interface. This work reported for the first time the competitive adsorption between Na-cas and HPC at the A/W interface.

Keywords: Sodium caseinates, Hydroxypropyl cellulose, Adsorption, Competitive displacement, Interfacial rheology.

1. Introduction

Proteins are commonly used as functional ingredients for various dispersions or emulsion formulations. Protein-stabilized emulsions are typically stable against aggregation through a combination of electrostatic and steric repulsion (Delahaije, Wierenga, Van Nieuwenhuijzen, Giuseppin, & Gruppen, 2013; McClements, 2004). However, in practice, these emulsions are sensitive to many stresses, including pH change, high ionic strength, heat treatment, etc. (McClements & Gumus, 2016). Hydrocolloids, such as polysaccharides, are widely applied in the food industry for their thickening and stabilizing properties, contributing to the development of food structure (Goff & Guo, 2019). Despite these great functionalities, many edible polysaccharides have only limited emulsifying ability due to their poor surface activity (McClements & Jafari, 2018). To overcome the limitation encountered with the emulsifiers traditionally used, the combination of proteins and polysaccharides has been considered. Mixed protein-polysaccharide systems have been extensively studied over the last decade because of the potential synergy that can occur between these two types of biopolymers, both in the bulk and at fluid interfaces. Proteins can anchor at the interface due to their amphiphilic properties while the hydrophilic group of polysaccharides can extend to the aqueous phase, and provide steric repulsion (Dickinson, 2008).

The combined use of proteins and polysaccharides is widespread in the manufacture of processed dispersions, with applications in food, pharmacy, and cosmetics industries (Benichou, Aserin, & Garti, 2002; Rodriguez Patino & Pilosof, 2011). Consequently, the study of the interactions between these two species finds many applications, particularly in the development of new formulations (Ghosh & Bandyopadhyay, 2012). Polysaccharides and proteins can form various physical complexes depending on a number of factors including the pH, the ionic strength, and the polymer charge. The interactions between the two molecules can be achieved through formation of covalent bonds (Maillard conjugate) or non-covalent bonds (electrostatic and hydrophobic interactions, H bonds). The application of protein-polysaccharide Maillard conjugates as emulsifiers was the subject of a recent review (Kan, Chen, Zhou, & Zeng, 2021). The adsorption of certain complexes at the interface was found to be more efficient than the initial compounds at stabilizing O/W emulsions (Harnsilawat, Pongsawatmanit, & McClements, 2006). It has been acknowledged that the formation of proteins-polysaccharides complex can have significant improvements in emulsifying and foaming properties (H. Chen et al., 2016; Hu et al., 2020; Liu, Zhao, Zhao, Ren, & Yang, 2012; Xu et al., 2020), and in emulsion stability (Dickinson, 2019). This very important topic has been studied in detail and has been the subject of a complete review (Evans, Ratcliffe, & Williams, 2013).

Beyond complex formation, protein and polysaccharides can interact through competitive mechanisms. If the polysaccharide is sufficiently surface-active, both species can compete at fluid interfaces. The knowledge and control of interfacial characteristics are essential in many applications, such as foams and emulsions. The stability and texture of the products are greatly impacted by the structural and mechanical properties of fluid interfaces (Dickinson, 2001; Wilde, 2000). The study of competition for the interface has attracted a lot of interest in recent years. These phenomena have been widely studied at the air/water (A/W) and oil/water (O/W) interfaces and are well described in the literature. However, most of the studies highlighting interfacial competition phenomena focus on

interactions between proteins and low-molecular weight surfactants such as polysorbates (Rabe, Kerth, Blume, & Garidel, 2020; Zhang, Yan, Jiang, & Ding, 2021), sucroesters (Rouimi, Schorsch, Valentini, & Vaslin, 2005; Tual et al., 2006; Zhao et al., 2014) or mono- and diglycerides of fatty acids (Munk, Larsen, Van Den Berg, Knudsen, & Andersen, 2014). Interfacial competition between proteins and polysaccharides has often received less attention, probably owing to the poor surface properties of the latter, which prevents them from adsorbing at interfaces. Due to their hydrophilic nature, most polysaccharides are not, or only slightly, surface-active.

There are however some exceptions, including hydroxypropyl cellulose (HPC). This polymer is derived from cellulose, where substituents have been grafted along the anhydroglucose backbone. The hydrophobic character of the substituent coupled with the more hydrophilic nature of the anhydroglucose backbone provide these molecules with good surface properties. HPC has received considerable interest from various application sectors due to its film forming ability (Adden, Hübner-Keese, Fortsch, " & Knarr, 2020; Arca et al., 2018; Wuestenberg, 2014), water holding capacity (Martin-Pastor & Stoyanov, 2020), viscosity enhancing properties (Arca et al., 2018), and its stabilizing properties in emulsion and foam (Lepine, Mezdoor, Erazo-Majewicz, & Michon, 2008). The low surface and interfacial tensions of HPC combined with its rheological properties, gives it excellent dual functionality in foam products both as stabilizer and emulsifier.

The use of HPC in foam-type products is often accompanied by other surface active material such as proteins or lecithin, acting as emulsifiers. Its interactions with lecithins have been reported (Lepine et al., 2008; Mezdoor, Lepine, Erazo-Majewicz, Ducept, & Michon, 2008). In spite of the common use of HPC with caseinates, the knowledge about their interactions is still limited. Na-cas is widely employed in the formation of food emulsions and foams due to its excellent functionality; it has substantial surface activity, and provides good stabilization through electrostatic and steric effects, protecting newly formed droplets against flocculation and coalescence. Although the functionality of these two molecules are already well described individually, the prediction of their behavior in a mixed system is more challenging and there is a lack of knowledge about this in the literature. In this context, a study of their interfacial interactions is relevant. The combination of several emulsifiers type may result in better or worse performance than singles ones (McClements & Jafari, 2018), which emphasizes the need for an in-depth understanding of the behavior of mixed systems. Due to its high surface properties, HPC is able to preferentially adsorb at the interface. As a result, interactions and competition phenomena between HPC and proteins could exist at the interface, and their effects must be elucidated.

Such a phenomenon has been reported with related cellulose ethers. It was demonstrated that methylcellulose and hydroxypropylmethyl cellulose can displace proteins (β -casein and β -lactoglobulin) through a competitive adsorption mechanism (Arbolea & Wilde, 2005). Other research groups have subsequently demonstrated that hydroxypropylmethyl cellulose can also have competitive interaction with whey proteins (Perez, Carrera-Sanchez, Rodríguez-Patino, & Pilosof, 2007) and soy proteins (Martinez, Carrera Sanchez, Pizones Ruiz-Henestrosa, Rodríguez Patino, & Pilosof, 2007) at the air/water interface.

In view of this, we herein investigate the dynamic interactions that exist between proteins (Na-cas) and HPC at the air/water interface. A combined approach was taken to better understand the protein-HPC interactions at the interface. Short-term and long-term adsorption dynamics have been established, a Langmuir film balance was used to analyze the physical behavior of the monolayer films, and surface dilatational experiments were performed to investigate the rheological behavior of the interface. This paper provides strong evidence of a competitive adsorption behavior between these two molecules. To the authors' knowledge, no article had demonstrated this type of effect between caseinates and HPC until now.

2. Material and methods

2.1. MATERIAL

Na-cas was obtained as a lyophilized powder ($\geq 88.5\%$ proteins). Commercial HPC used are characterized by an average molecular weight of 620,000 Da, a viscosity of 200–400 mPa s (2%, 25 °C) and a molar substitution of 3.5–4.1. Both Na-cas and HPC were kindly provided by Puratos NV.

2.2. SAMPLE PREPARATION

Stock solutions of Na-cas and HPC were prepared by dispersing the required amount of powder in ultrapure water of a resistivity of $18.2 \text{ M}\Omega \text{ cm}^{-1}$ (RephiLe Bioscience, Boston, USA) at ambient temperature (21–22 °C). Solutions were kept under agitation for at least 2 h and stored overnight to ensure complete hydration before use. Binary mixtures were prepared by mixing the stock solutions in different proportions to yield different Na-cas/HPC weight ratios (100:0, 75:25, 50:50, 25:75, and 0:100). Concentrations are expressed in mass units (wt %). All measurement were performed at pH 7.

2.3. TRANSMITTANCE MEASUREMENT

Transmittance measurements were performed at 600 nm using a UV–vis spectrophotometer (Hitachi U 2900, Tokyo Japan) equipped with a 1 cm path-length optical cuvette at ambient temperature. Na-cas/ HPC mixtures were prepared with the same method as described in paragraph 2.2, and the pH was adjusted to 6 and 7 using 0.5 and 1.0 M HCl and NaOH to minimize the effect of dilution.

2.4. DYNAMIC ADSORPTION KINETICS

In order to gain insight into both kinetic of adsorption and behavior close to equilibrium, dynamic surface tension (SFT) of solutions was investigated using two complementary techniques. The instruments were cleaned with milliQ water prior to each measurement. The cleanliness of the equipment was checked by ensuring that the surface tension of the water was $72.0 \pm 0.5 \text{ mN/m}$.

2.4.1. MAXIMAL BUBBLE PRESSURE METHOD

The behavior under very short adsorption times was measured using a bubble pressure tensiometer (Krüss BP 100, Hamburg, Germany). The bubble age ranged from 10 to 10,000 ms, and measurements were carried out at 25 °C. This device measures the maximum pressure within an air bubble formed in a liquid. Knowing the radius of the capillary, the measured pressure can be related to the surface tension by the Young- Laplace equation.

Lag times, diffusion coefficients, and adsorption rates were derived from these experiments. The lag time was calculated as the time required to decrease surface tension to a value worth 95% of the initial value. The adsorption rate was determined as the slope of the decrease in surface tension after the lag phase, as described by [Li, Blecker, and Karboune \(2021\)](#). Surface-active molecules diffuse to the interface due to Brownian motion; at short times and low concentrations, the diffusion is the rate determining step. In such conditions, the diffusion coefficients (D_s) can be calculated according to Joos and Rillaerts equation ([Joos & Rillaerts, 1981](#)). D_s were calculated by the LabDesk software (Krüss, Hamburg, Germany). The molecular weight considered for the calculations were 25,000 and 620,000 Da for Na-cas and HPC respectively.

$$\sigma_t = \sigma_0 - 2RTc\sqrt{D_s t/\pi} \quad (\text{Eq. 2.1})$$

where σ = surface tension (mN/m),

R = universal gas constant (J/mol.K),

T = temperature (K),

c = molar concentration (mol/l),

t = surface age (ms),

D_s = diffusion coefficient (m²/s).

2.4.2. DROP VOLUME METHOD

The drop volume tensiometer was used to investigate surface tension on a larger time scale (~10–100 s). Measurements were conducted using a drop volume tensiometer (Krüss DVT50, Hamburg, Germany). The apparel was equipped with a 250 µl syringe and a 2.0 mm diameter metal capillary. Eight different drop formation rates were used, and three drops were formed at each rate. The drop creation speed was varied from 0.12 to 3.0 s/µl. The equilibrium SFT was determined from the values obtained for the last 4 drops, by extrapolating the surface tension to time $t \rightarrow \infty$ in the $\sigma - t^{-1/2}$ plots ([Razafindralambo, Blecker, Delhaye, & Paquot, 1995](#)). Surface tension measurements were carried out at 25 °C.

2.5. LANGMUIR FILM BALANCE

Film compression isotherms, also known as π -A isotherms, were recorded using a Langmuir trough (Biolin Scientific KSV NIMA, Gothenburg, Sweden). This measurement allows to study the packing of molecules in the form of a monolayer at the liquid/air interface. 30 µl of Na-cas/HPC solutions were

carefully spread at the surface of an ultrapure water subphase (18.2 MΩ cm, RephiLe Bioscience, Miami, USA) using a micro syringe. The concentration of the Na-cas solutions was 0.1 wt% while mixed and HPC solutions were used at a concentration of 0.05 wt%. The concentrations and volumes used were determined through preliminary tests. Different volumes and concentrations were tested to ensure that the surface spreading was quantitative. The use of buffer was avoided to minimize the risk of contamination. The trough was cleaned before each isotherm until a surface pressure value < 0.5 mN/m was achieved after a full compression of the trough barriers. A waiting time of 15 min between sample deposition and the start of compression was observed to allow time for the film to form and stabilize. Compression of the monolayers was carried at 25.0 °C with constant barrier speed of 10 mm/min. Any change in surface tension was recorded through a Wilhelmy plate which was connected to an electro- balance. The results are expressed as a surface pressure-area isotherm. The onset of surface pressure increase, denoted A_0 , was calculated as the area when the surface pressure of the monolayer reached 1 mN/m, and the elasticity (E) was calculated from the slope of the π -A isotherm as $E = -A(d\pi/dA)$.

2.6. FILM MISCIBILITY

The study of a monolayer formed by spreading a solution containing the two substances is a valuable tool to understand the mechanical properties of an interface covered by proteins and polysaccharides. For a binary film, it is considered that if the two substances mix ideally or are completely immiscible equation (2.2) is verified. A deviation from the above mentioned equation indicates at least partial miscibility of the two components.

$$A_{ab} = x_a A_a + x_b A_b \quad (\text{Eq. 2.2})$$

where A = mean molecular area ($\text{\AA}^2/\text{molecule}$), x = molar ratio,

a and b refers to the two components while ab refers to the mixed film.

2.7. SURFACE DILATATIONAL RHEOLOGY

A pendant drop tensiometer (IT Concept, Longessaigne, France) was used to investigate the interfacial rheology of A/W interface stabilized by Na-cas, HPC, or their mixture. The instrument was equipped with a U- shaped capillary attached to a 0.5 ml syringe. In this configuration, the syringe is filled with air and capillary is immersed in the glass cuvette filled with the solution. Two bubbles were created and expelled from the capillary before each analysis to eliminate possible impurities. A rising bubble of 5 μl was created at the tip of the capillary and a sinusoidal variation of its area was applied to it. The amplitude was set to 5% with a frequency of 0.1 Hz and the deformation was started after the drop reached its equilibrium surface tension. A water bath was used to maintain a constant temperature during the whole experiment and measurements were carried out in duplicate at 25 °C. The pendant drop tensiometer calculates surface tension by applying the Young-Laplace equation to the drop profile. The instrument is equipped with a monochrome CCD camera that records images of the bubble over time. Raw digital images of the camera can be analyzed and displayed to determine

surface tension and area of the bubble. During the sinusoidal deformation, the Young-Laplace equation can be applied to the shape of the oscillating drop for measuring the viscoelastic properties of the interface. The dilatational modulus is then derived from the amplitude of the deformation following the equation:

$$|E| = A(\Delta\sigma/\Delta A) \quad (\text{Eq. 2.3})$$

The storage modulus E' and loss modulus E'' are calculated from $|E|$ and the phase angle ϕ between $\Delta\sigma$ and ΔA :

$$E' = |E| \cos \phi \quad (\text{Eq. 2.4})$$

$$E'' = |E| \sin \phi \quad (\text{Eq. 2.5})$$

2.8. STATISTICAL ANALYSIS

Measurements were performed in triplicate unless otherwise stated. Data were statistically analyzed using Graphpad Prism 8 software (GraphPad Software Inc, San Diego, USA). Multiple t-tests were performed with a significant level of 0.05 to detect significant differences between means.

3. Results and discussion

3.1. COMPATIBILITY OF NA-CAS/HPC MIXTURES

The study of the phase behavior was carried out to evaluate the presence of interactions and the compatibility between the biopolymers in the bulk. The transmittance of Na-cas solutions with increasing HPC concentration is plotted in Fig. 1. A visual assessment of the solutions after 7 days of storage at 15 °C did not detect any change in appearance, such as phase separation or precipitate formation. The results indicate that at the pH considered in this study, i.e pH = 7, Na-cas and HPC are co-soluble.

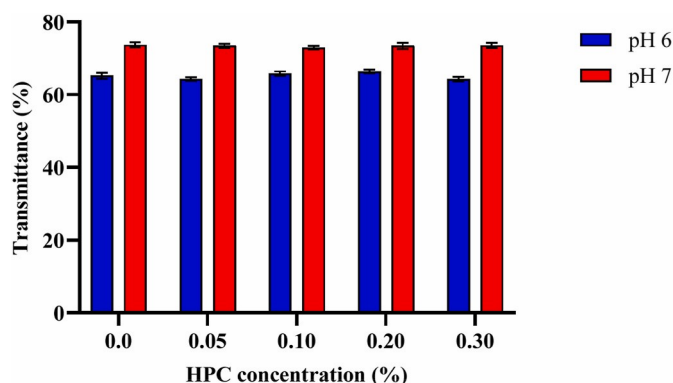


Fig. 1. Transmittance (%) at 600 nm of 0.77 wt% Na-cas solutions with increasing HPC concentrations (0.0–0.3 wt%) at different pH (6.0–7.0).

3.2. INTERFACIAL ADSORPTION KINETICS AT THE A/W INTERFACE

In order to elucidate the mechanism of interaction between Na-cas and HPC at the interface, the dynamic evolution of surface tension, surface rheology and monolayer behavior of individual components and mixed systems was studied. The adsorption rate, the ability to reduce surface tension, as well as the viscoelastic properties conferred to the interface are key parameters for a surfactant because they are determinant in the formation and stabilization of foams and emulsions (McClements, 2015).

3.2.1. SHORT ADSORPTION TIMES

The monitoring of the SFT evolution of the single components and the Na-cas/HPC mixed system was used to study the presence of possible interactions at the A/W interface. The adsorption kinetics curves in very short adsorption times (10–10,000 ms) of Na-cas (0.77 wt%), HPC (0.025–0.2 wt%) and their mixed system are represented at Fig. 2. Major differences were observed between Na-cas and HPC; both molecules demonstrated high surface activity, causing a decrease in SFT over time but with different kinetics and intensities.

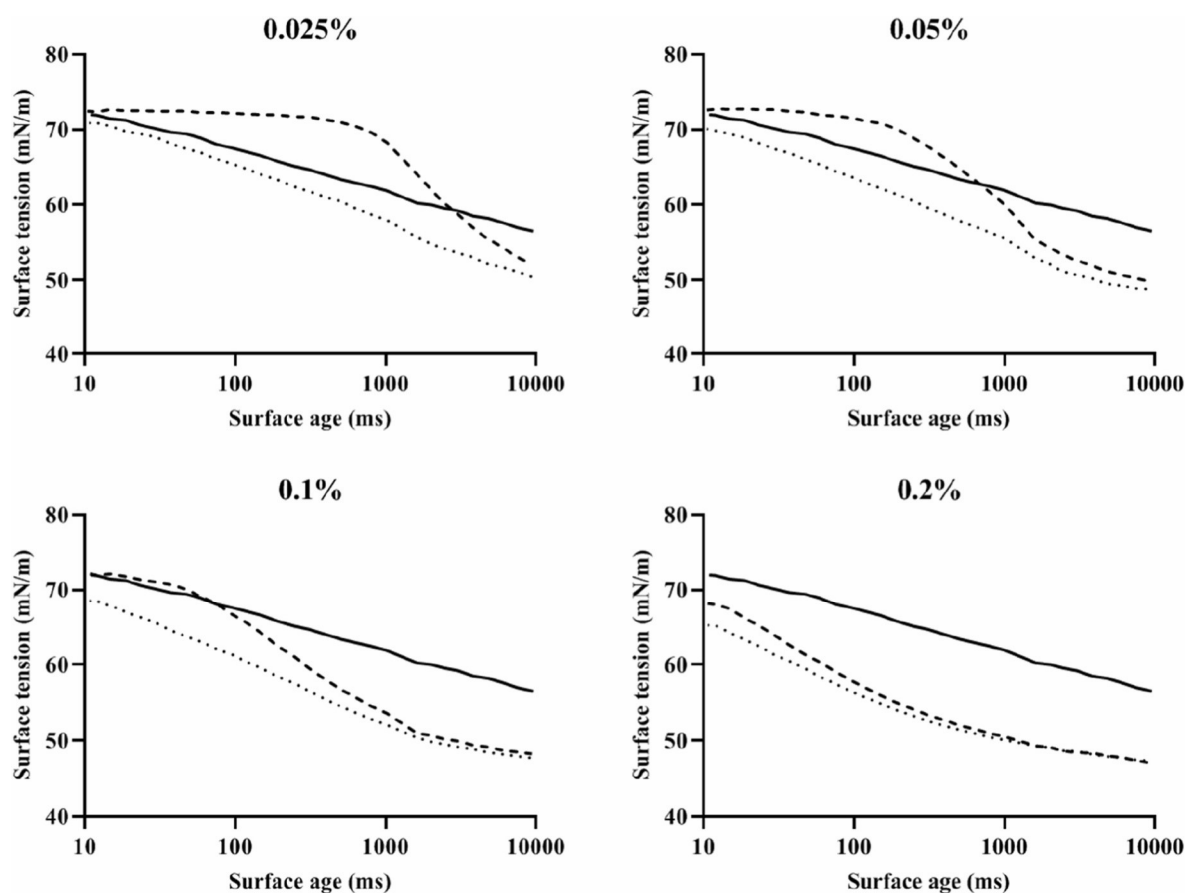


Fig. 2. Dynamic surface tension (25 °C) as a function of surface age for Na-cas (—), HPC (---), and mixed system (···). The Na-cas concentrations is kept constant at 0.77 wt% while HPC concentration is increased from 0.025 to 0.200 wt%.

The SFT of Na-cas solutions (0.77 wt%) was equal to that of water (≈ 72 mN/m) after 10 ms, and immediately started to decrease after this short time, until reaching a value of about 56 mN/m after 10,000 ms. The adsorption kinetics of HPC follows a three-step process, as previously described by [Mezdour, Cuvelier, Cash, and Michon \(2007\)](#). The first phase is a latency phase, during which no significant change in SFT is noticed. The second phase corresponds to a sharp decrease in SFT, followed by a phase where the SFT decreases less markedly. This last phase is related to the progressive rearrangement of the molecules within the surface layer (Avranas & Tasopoulos, 2000). At 0.025 wt% HPC concentration, an important lag time of about 1000 ms was observed before the SFT started to decrease. This indicates that the molecules are not yet present at the interface in this short period of time. SFT was then reduced to a value of 50 mN/m after 10,000 ms. This lag time is reduced by increasing the concentration of HPC, until there is no lag time at all at 0.2%. At this concentration, the SFT at 10 ms is already significantly reduced (≈ 65 mN/m), suggesting a fast surface coverage of the A/W interface. The SFT then continued to decrease until it reached a value of 48 mN/m after 10,000 ms.

For the four concentrations of HPC tested, the mixed system exhibited the lowest SFT across the whole time interval. The magnitude of the SFT decrease was larger than what would be expected based on the behavior of the single molecules (sum of each individual component). This suggests that these two molecules decrease SFT in short adsorption times in a synergistic way. The presence of polysaccharide in the bulk phase could create an osmotic driving force promoting the aggregation of proteins at the interface ([Baeza, Carrera Sanchez, Pilosof, & Rodríguez Patino, 2005](#); [Perez et al., 2007](#)). The difference in SFT between the mixed system and pure HPC was highest at short adsorption times and then tended to decrease with time. The final SFT was not significantly different from that of HPC alone (p-value > 0.05).

Overall, these results show that HPC has a higher surface activity and decreases SFT more strongly and rapidly compared to Na-cas, and is effective at lower concentration. The four concentrations of HPC investigated indicate that the mixed system evolves towards a state where HPC is dominant at the interface as the surface age increased. For HPC concentrations of 0.1 wt% or less, a lag time is observed before HPC starts to decrease the SFT ([Fig. 2](#)). Na-cas already settled at the interface during this time interval. However, the SFT of the mixed system evolves to a value similar to that of HPC alone, indicating that HPC eventually substitutes the previously adsorbed proteins at the interface. At 0.2 wt% HPC, it is likely that HPC adsorbs at the A/W interface before Na-cas, and subsequently impedes their adsorption. HPC will take over the proteins at the interface with a concentration-dependent effect.

The study of the kinetics of diffusion is of great importance in the investigation of interfacial competition because the species arriving first at the interface could have an advantage on the second species. The properties of the interfacial layers formed by a mixture of biopolymers are affected by the chronology of adsorption of its different constituents at the interface ([Santos, Okuro, Fonseca, & Cunha, 2022](#)). Two distinct scenarios can be distinguished: a simultaneous adsorption or a sequential adsorption. In order to compare the diffusion kinetics, the diffusion coefficients were calculated according to Joos and Rillaerts and the lag times were measured as well. The presence of a lag time is related to the time required for adsorption of a sufficient amount of surface active material to allow

molecular interactions (Ruíz-Henestrosa, Carrera Sanchez, & Rodríguez Patino, 2007). From this moment, the decrease of SFT is driven by the adsorption of the molecules rather than their diffusion. The coefficients were calculated for both compound and the values are expressed in Table 1. The first striking observation is that the diffusion kinetics were not of the same order of magnitude; Na-cas diffused at the interface with a speed of about 10^{-10} m²/s whereas HPC diffused with a speed of 10^{-5} m²/s. This confirmed that HPC molecules migrates to the interface much faster than Na-cas despite their larger molecular weight. The diffusion coefficient is highest at 0.025 wt % HPC, and significantly decreases as the HPC concentration increases. The greater number of molecules in the bulk, and the greater viscosity of the solution could explain the lower diffusion rate of the molecules towards the interface. The adsorption rate was determined after the lag time. Quite logically, the concentration had a significant impact on the adsorption process. It is positively correlated with concentration; the more concentrated the HPC, the higher the adsorption rate.

These values provides a better understanding of the dynamic of adsorption kinetics at short adsorption times. At HPC concentration ≤ 0.1 wt%, Na-cas 0.77 wt%, proteins saturates the interface more quickly and settles there first. HPC being more surface active will eventually replace the proteins and tend towards a state close to when it is used alone (sequential adsorption). At higher HPC concentration, it saturates the interface faster than Na-cas and dominates the interfacial properties.

Table 1
Diffusion/Adsorption kinetic parameters for Na-cas and HPC.

	Na-cas	HPC			
	0.77 wt%	0.025 wt %	0.05 wt%	0.10 wt%	0.20 wt%
Lag time (ms)	68 ± 1 ^b	1005 ± 22 ^d	275 ± 7 ^c	70 ± 4 ^b	<10 ^a
Diffusion coefficient (m ² /s)	2.0 ± 0.2E-10 ^a	4.1 ± 0.1E-5 ^e	3.5 ± 0.2E-05 ^d	2.4 ± 0.1E-05 ^c	1.2 ± 0.2E-05 ^b
Adsorption rate (mN/m/ms)	0.037 ± 0.002 ^c	0.006 ± 0.001 ^a	0.016 ± 0.001 ^b	0.052 ± 0.002 ^d	0.243 ± 0.046 ^e

Within the same line, means with different letters are significantly different at $P \leq 0.05$.

3.2.2. LONG ADSORPTION TIMES

In the previous experiment, all curves were still in a downward trend after a time of 10, 000 ms, indicating that the steady state had not yet been reached. A drop volume tensiometer was used to study the kinetics over a longer period of time and approach the equilibrium. The evolution of the SFT of Na-cas, HPC, and their mixed system as a function of time is presented at Fig. 3. The equilibrium SFT values were derived from these measurements. Results confirm that HPC is more surface- active than Na-cas, with equilibrium SFT values ranging from 43.12 ± 0.08 mN/m (0.025 wt%) to 42.67 ± 0.01 mN/m (0.2 wt%) compared to 46.01 ± 0.15 mN/m for caseinates. The low SFT of HPC is consistent with data already published (Cash & J.Caputo, 2010; Mezhdour et al., 2007). The combination of Na-cas and HPC led to the lowest equilibrium SFT for all HPC concentrations investigated. Differences with the

corresponding solutions of HPC alone are small but significant (p -value < 0.05). The difference between the mixed system and HPC alone decreases as the concentration of HPC increases. These results suggest a cooperative adsorption behavior, with HPC having the dominant effect on the surface properties.

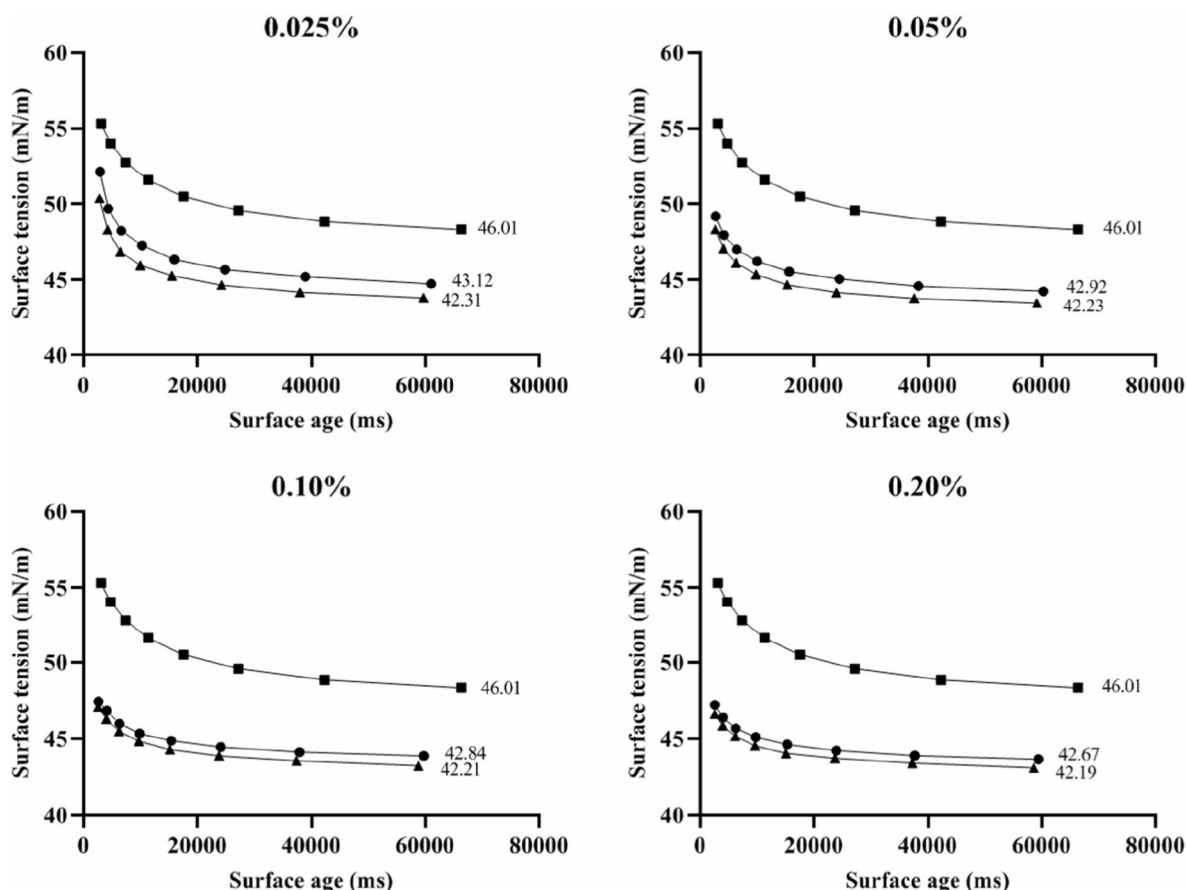


Fig. 3. Evolution of the dynamic surface tension (25 °C) as a function of time for: Na-cas (■), HPC (●), and mixed system (▲). The Na-cas concentrations is kept constant at 0.77 wt% while HPC concentration is increased from 0.025 to 0.200 wt%.

Fig. 4 shows data from the pendant drop tensiometer. This type of instrument was used to record the SFT of a Na-cas stabilized interface to which an amount of HPC was added after a certain period of time. A bubble was created in the Na-cas solution and a period of 600 s was observed in order to allow time for the proteins to adsorb at the interface and reach a state close to equilibrium. After this time, 350 μ l of 1 wt% HPC solution was added to the cuvette to reach a final HPC concentration of 0.05 wt%. The result is striking since the addition of HPC caused an almost immediate decrease in the SFT; it decreased from approximately 47 mN/m (i.e. the equilibrium value of the proteins) to about 43–44 mN/m (i.e. the equilibrium value of HPC). This sudden decrease in SFT reflects a major change in species present at the interface and illustrates the ability of HPC to substitute proteins initially present at the interface.

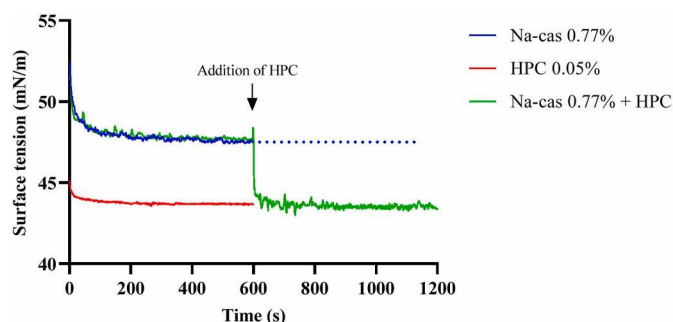


Fig. 4. Evolution of surface tension (25 °C) as a function of time for Na-cas 0.77 wt% (blue), HPC 0.05 wt% (red), and Na-cas 0.77 wt% with added HPC after 600 s. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The tensiometry results provide valuable information regarding interactions between Na-cas and HPC at the A/W interface. HPC is more surface-active than Na-cas, and has the dominant effect on SFT when used in combination with Na-cas. This is likely to be due to the faster adsorption of this molecule; HPC arrives first at the interface, which hinders the adsorption of proteins afterwards. In addition to this kinetic aspect of interfacial competition, results show that HPC is able to adsorb at the interface even if proteins were previously adsorbed. The lower the concentration of HPC, the longer it takes for it to dominate the interface, but it eventually has a dominant effect on SFT in the concentration range investigated. This effect is thought to be due to the higher surface activity of HPC compared to Na-cas. Interfaces covered by HPC will have a lower Gibbs free energy, which is more favorable from a thermodynamic point of view.

It has been previously acknowledged that the tendency of a surfactant to displace proteins depends on the rheological properties of the interface (Zhang et al., 2021). The surfactant-induced protein displacement is described by the orogenic model. Defects in the proteins film can serve as adsorption sites for the surfactant. The surfactant can adsorb into defects in the protein film, which serve as adsorption sites (Gunning et al., 2004; Mackie, Gunning, Wilde, & Morris, 2000). When the amount of surfactant adsorbed in these irregularities of the protein film irregularities increases, a growing interfacial pressure will develop, eventually leading to the collapse of the protein film. This results in a detachment of the proteins from the interface, which are thus dispersed in the bulk. Therefore, the more cohesive and elastic the film formed by the proteins on the surface, the more resistant it will be to displacement by a surfactant. Because caseinates are mainly in random coil conformation, and form less elastic interfaces compared to globular proteins such as whey proteins (Ridout, Mackie, & Wilde, 2004), they will be more easily displaced from the interface (Arboleña & Wilde, 2005; Dickinson, Rolfe, & Dalgleish, 1989; Walstra & De Roos, 1993).

3.3. EVALUATION OF INTERFACIAL FILM PROPERTIES OF NA-CAS/HPC MIXTURES

3.3.1. SURFACE PRESSURE – AREA ISOTHERMS

The film balance was used to measure the surface pressure-area isotherms. This allowed to study the arrangement of surface-active molecules within a monolayer. Fig. 5 shows the compression isotherms of Na-cas, HPC, and their mixture in different proportions. The compression isotherm was the most expanded for HPC, and the least expanded for Na-cas. Amphiphilic molecules start to develop a two-dimensional pressure in the monolayer once a certain surface coverage is reached, and distance between molecules becomes sufficiently small (Mezdour et al., 2008). The more expanded film of HPC reflects its ability to cover a larger interface. The surface pressure developed by HPC increased gradually with decreasing surface area, following a sigmoidal curve characteristic of a condensed monolayer, consistent with data published elsewhere (McNally & Zografi, 1990). The film collapsed at an area of 1.05 m²/mg; compressing the film beyond this point hardly increased the surface pressure. Na-cas had a rather straight curve, also in agreement with previous studies (Rodríguez Patino & Sanchez, 2004). The π -A isotherms of the mixed component films were found between the extremes defined by the two pure components. The increase in the proportion of HPC was associated with a shift of the curves to larger areas, indicating that the films were increasingly expanded. Characteristic points of the compression isotherms are found at Table 2.

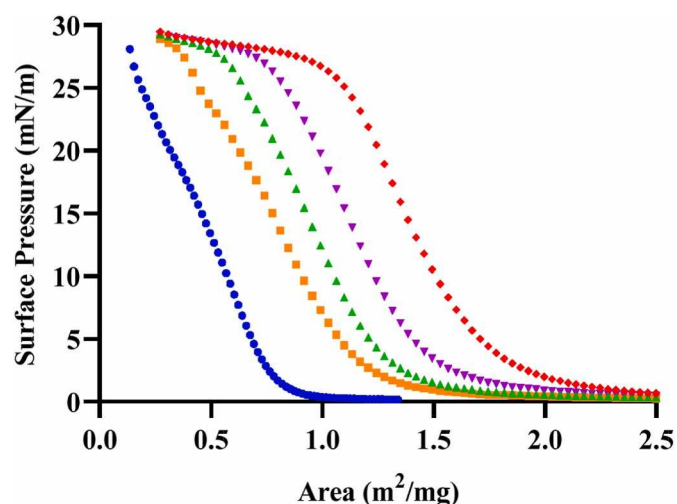


Fig. 5. Langmuir film balance compression isotherms of Na-cas/HPC mixed system: 100:0 (●), 75:25 (■), 50:50 (▲), 25:75 (▼), and 0:100 (◆) (25 °C).

Table 2

Characteristic points of the compression isotherm graph for Na-cas, HPC, and their mixtures at different molar ratios at the air/water interface.

Na-cas:HPC	100:0	75:25	50:50	25:75	0:100
A_0 (m ² /mg)	0.85 ±0.06 ^a	1.48 ±0.05 ^b	1.66 ±0.02 ^c	1.94 ±0.12 ^{cd}	2.30 ±0.15 ^d
π_f (mN/m)	28.10 ±0.60 ^a	28.92 ±0.06 ^b	29.28 ±0.04 ^c	29.23 ±0.08 ^{cd}	29.45 ±0.01 ^d

A_0 : area at the start of the compression isotherm; π_f : surface pressure at maximal compression.

Within the same line, means with different letters are significantly different at $P \leq 0.05$.

Fig. 6 below depicts the evolution of elasticity as a function of surface pressure obtained from the compression isotherm. For all solutions, the elasticity displayed a maximum peak, corresponding to a change of structure during compression. The curves were divided into two phases: a phase in which the elasticity increased with compression, and a phase in which the elasticity decreased with compression. During the first stage (start of the compression), the previously adsorbed molecules got closer and the interfacial layer was progressively covered with molecules. This increased structuration of the interface led to an increased elasticity. The decrease in elasticity observed in the second stage may be interpreted as a change in conformation at the interface and a loss of structure of the monolayer upon compression, eventually leading to formation of a multilayer (Rodríguez Patino, Sanchez, Rodríguez Niño, & Fernandez, 2001).

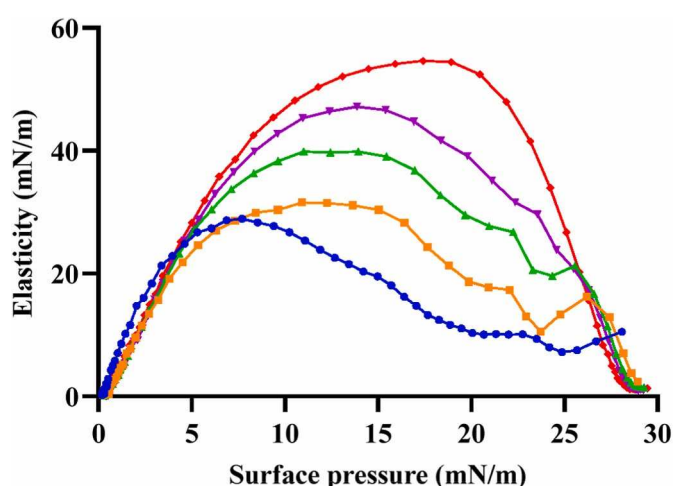


Fig. 6. Elasticity of the monolayer of Na-cas:HPC mixed system: 100:0 (●), 75:25 (■), 50:50 (▲), 25:75 (▼), and 0:100 (◆) as a function of surface pressure (Water subphase, 25 °C).

The maximum elasticity of Na-cas monolayer was 29.20 ± 0.54 mN/m, and was reached at lower surface pressure compared to system containing HPC. The film composed solely of HPC exhibited the highest maximum elasticity (54.91 ± 1.40 mN/m), and this was achieved at higher surface pressures. The shape of the E- π curve of HPC is in good agreement with what has already been reported for this compound by [Mezdour et al. \(2007\)](#). Mixed systems had intermediate behaviors between the two individual components. The presence of polysaccharide in the monolayer promoted a remarkable increase in elasticity. Increasing HPC proportion was associated with increasing maximum elasticity, and the peak was reached at increasingly higher surface pressures.

The interactions among the molecules in the monolayer led to different phase transitions. The elasticity versus surface pressure graph provides a more clear evidence of transitions occurring in the monolayer, and the characteristic of the peak maximum are shown in [Table 3](#). Na-cas had a profile with a main peak accompanied by two shoulders, while HPC had a profile with a single peak. The shoulders visible in the protein film were also visible in the mixed film, and their intensity decreased as the proportion of Na-cas decreased supporting the hypothesis that they were related to the presence of proteins in the monolayer. A slight increase in HPC proportion shifted the curve to higher areas but did not lead to a large increase in maximum elasticity. Further increase in HPC proportion led to larger areas and increasing elasticity. The maximum elasticity and HPC content were well correlated in all systems containing HPC ($R^2 > 0.999$). The surface pressure ([Fig. 5](#)) and elasticity ([Fig. 6](#)) of the mixed systems approach the values observed for pure HPC at the end of compression, again indicating the dominance of the polysaccharide at the interface.

Table 3

Maximum elasticity, and corresponding area and surface pressure during the compression isotherm.

Na-cas: HPC	100:0	75:25	50:50	25:75	0:100
E	29.20 ± 0.54^a	31.74 ± 0.25^b	39.89 ± 0.79^c	46.84 ± 0.66^d	54.91 ± 1.40^e
A	0.63 ± 0.01^a	0.85 ± 0.01^b	0.97 ± 0.03^c	1.17 ± 0.05^d	1.33 ± 0.08^e
π	8.11 ± 0.10^a	11.60 ± 0.13^b	12.82 ± 0.52^{bc}	13.21 ± 0.12^c	18.18 ± 0.29^d

A: area of the film (m^2/mg); E: elasticity of the film (mN/m); π : surface pressure (mN/m).

Within the same line, means with different letters are significantly different at $P \leq 0.05$.

[Fig. 7](#) shows plot of experimentally determined mixed films areas as a function of Na-cas molar ratio at a surface pressure of 15 mN/m. This type of representation is useful to study interactions in monolayers. The ideal behavior predicted by Eq. (2.2) is represented as a straight line for comparison purpose. In case of perfect miscibility or immiscibility between the compounds, the relationship between the area occupied by the film and the molar ratio should be linear. The area occupied by the

protein monolayer was considerably smaller than that occupied by the HPC monolayer. A negative deviation from the ideal behavior was observed; the area occupied by the mixed films was lower than the predicted area, indicating partial miscibility between the two biopolymers within the monolayer. Similar trends were observed at surface pressure of 10 and 20 mN/m (data not shown). These results suggest that the cohesive interactions between Na-cas and HPC are greater than those in the films formed by the pure individual compounds (Eftaiha & Paige, 2011).

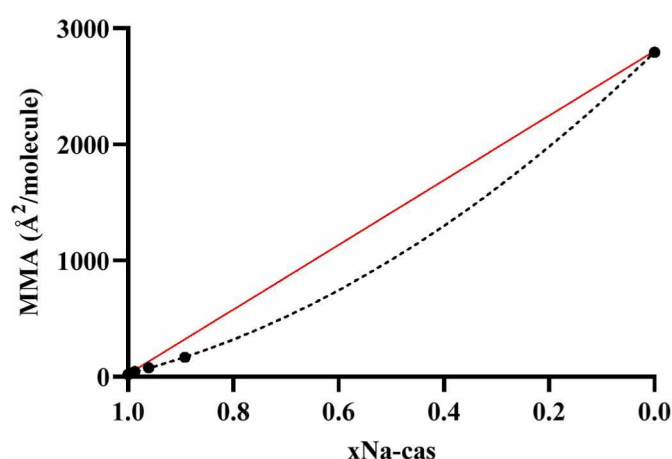


Fig. 7. Mean molecular area as a function of mole fraction of Na-cas at a surface pressure of 15 mN/m (Water subphase, 25 °C). The solid line represents ideal behavior as dictated by the additivity relation (Eq. (2.2)), dashed lines are included as a guide to the eye.

3.3.2. INTERFACIAL DILATATIONAL RHEOLOGY

The rheological properties of the interface were determined via dilatational rheology with a pendant drop tensiometer (sinusoidal deformations). Interfacial rheology is a very sensitive measure of interface structure, making it an ideal tool for studying competitive behavior. The instrument created a drop, then varied its volume by applying a sinusoidal deformation and measured the evolution of the SFT. It could be seen that for the same applied deformation (i.e. 5% of the drop area), the SFT of the protein-stabilized interface was affected more than that of the protein-HPC or pure HPC-stabilized interfaces. This system is more sensitive to area changes applied by the instrument. Dilatational elastic modulus E' and dilatational viscous modulus E'' were derived from this experiment using Eq. (2.4)-(2.5), and are presented at Fig. 8. For all samples, E' was significantly higher than E'' , indicating that at the investigated frequency (0.1 Hz), the interface had a predominantly elastic behavior. The elasticity of the protein-stabilized interface was high (27.74 ± 0.75 mN/m), while that of the HPC-stabilized interface was considerably lower (6.31 ± 0.11 mN/m). The elastic component of Na-cas was approximately four times higher than that of HPC and mixed systems, while its viscous component was about ten times higher. The presence of HPC had a very significant impact on the elastic properties of the interface (p -value < 0.01). The adsorption of HPC at the interface, even in small amounts, may reduce the protein-proteins interaction at the air-water interface. This would prevent them from forming such a cohesive film, and would explain the reduced surface dilatational elasticity.

Similar results were observed by Rouimi et al. (2005) who studied the interfacial properties of milk protein–surfactant systems. They reported that the presence of even a small amount of (in that case low molecular weight) surfactant at a protein-coated interface considerably reduced interfacial elasticity.

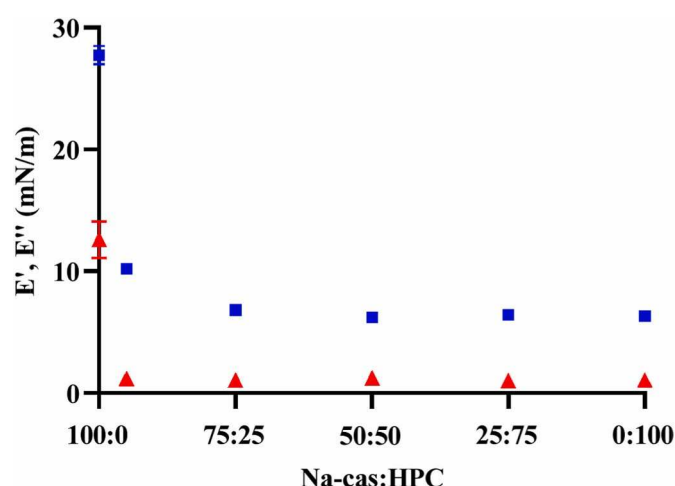


Fig. 8. Dilatational elastic modulus E' (■) and dilatational viscous modulus E'' (▲) of A/W interfaces stabilized with Na-cas/HPC mixed systems (25 °C, $\Delta A/A_0 = 5\%$; $\omega = 0.1$ Hz).

4. Conclusion

In the present work, the interactions between HPC and Na-cas at the A/W interface were investigated. The tensiometry results show a synergetic decrease in SFT at short adsorption times. This phenomenon was attributed to the presence of polysaccharide in the bulk phase, creating a driving force concentrating the proteins at the interface. A competitive behavior was observed at longer adsorption times, with a concentration dependent effect. The results show that HPC is able to migrate across the interface more rapidly than Na-cas, despite its higher molecular weight. The displacement of interfacial proteins is fast and occurs at low HPC concentration. Analysis of the π -A isotherms revealed that caseinates formed much more condensed films compared to HPC. The presence of HPC in the monolayer results in a marked increase in film area and maximum interface elasticity. The two components were found to be partially miscible within the monolayer under the experimental conditions. The competitive adsorption highlighted has a substantial effect on the rheological properties of the interface. The proteins have a significantly higher surface elasticity compared to HPC. Adsorption of even a small amount of HPC resulted in a significant decrease in surface elasticity to a value similar to that of HPC alone, further emphasizing the fact that HPC dominates the interface. These results provide important knowledge for applications using proteins in combination with HPC and involving interfaces, such as emulsions and foam products.

CRediT authorship contribution statement

Gilles Cremer: Investigation, Data curation, Methodology, Writing – original draft. **Vera Van Hoed:** Conceptualization, Writing – review & editing. **Sabine Danthine:** Methodology, Supervision. **Anne-Sophie Laveaux:** Conceptualization, Supervision. **Christophe Blecker:** Supervision, Methodology, Validation, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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