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Dynamic surface pressure and dilatational viscoelasticity of sodium caseinate/xanthan gum mixtures at the oil–water interface

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ABSTRACT

The effect of xanthan gum (XG) on the surface dynamic properties (dynamic surface pressure and surface dilatational properties) of the sodium caseinate (CN) adsorbed film at the oil–water interface has been studied in this paper. The measurements were performed as a function of bulk protein concentration in the range of 0.001–1.0 wt% using an automatic pendant drop tensiometer. The XG concentration (0.05 wt%), temperature (25 °C), pH (7.0) were maintained constants. The results revealed a significant effect of XG on dynamic characteristics of CN adsorbed films. At short adsorption time, the diffusion (k_{diff}) of CN to the interface decreased due to the interactions with XG in the aqueous phase which could limit protein availability for the adsorption. The presence of XG led to a decrease in the rates of penetration (k_P) and rearrangement (k_R) of CN at the interface at long adsorption times. High concentrations of CN in the bulk solutions showed significant impact on the dilatational viscosity close to the interface, due either to diffusional relaxation or to rearrangement of the adsorbed primary layer and multilayers of protein molecules. Moreover, CN/XG mixtures presented higher dilatational elasticity at the bulk concentration of CN beyond 0.001 wt%.

1. Introduction

Biopolymers like proteins and polysaccharides are essential functional ingredients in the food industry. Being surface active, proteins can function as effective emulsifying agents, and the interfacial behaviour of adsorbed proteins play a crucial role in determining the stability of foams and emulsions (Baeza, Carrera, Rodríguez Patino, & Pilosof, 2005). Polysaccharides, due to their strong hydrophilic character, generally remain in the aqueous phase and affect the rheology characters of continuous phase through thickening, gelling or stabilizing (Dickinson, 2003; McClements, 2000). In practice, however, the mechanical stabilizing role of the added polysaccharide is complicated due to the protein–polysaccharide interactions. Especially, the protein–polysaccharide interactions induced at the interface are sufficiently strong to influence the viscoelastic properties of the adsorbed protein layer (Dickinson, 2008).

Sodium caseinate (CN) has been widely used in food industry owing to its excellent functional and nutritional properties. It is prepared from coagulated casein-micelles, which are subsequently washed and neutralized with NaOH. CN is composed of a mixture of four phosphoproteins: α_{s1}-, α_{s2}-, β- and κ-casein and is approximately 10 nm in diameter which is considerably smaller than casein-micelles (Fox, 2003). Compared with many other food proteins, CN molecules are highly disordered in solution and substantially hydrophobic (Surh, Decker, & McClements, 2006). These properties enhance their tendency to be adsorbed on the interface during emulsification and facilitate rapid establishment of a thick steric stabilizing layer, thereby protecting newly formed droplets against flocculation and coalescence (Hu, McClements, & Decker, 2003).

Xanthan gum (XG), an anionic bacterial polysaccharide, has been extensively studied and widely used in food products due to its specific physical (viscosity, pseudoplasticity) and chemical (water solubility, pH stability) properties (Kobori, Matsumoto, & Sugiyama, 2009). In fact, protein emulsions stabilized by casein or caseinate are extremely sensitive to destabilization by acidification and calcium ions (Dickinson, 2006). In contrast, the use of polysaccharides (xanthan, pectins, alginates, carrageenans, etc.) as stabilizing agents stimulates the formation of a thicker stabilizing layer which is capable of protecting droplets against aggregation over a wide range of unfavorable conditions (such as the addition of calcium salts) through different protein–polysaccharide interactions (Chanamai & McClements, 2002). However, in model oil-in-water emulsions, the addition of XG at fairly low concentrations has been proved to accelerate unfavorable creaming of emulsions,

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which is apparently due to the depletion flocculation of dispersed droplets (Cao, Dickinson, & Wedlock, 1990; Dickinson, Ma, & Povey, 1994).

Physicochemical properties of protein–polysaccharide mixtures have received considerable attention recently. The control of protein–polysaccharide macromolecular interactions is a key factor in the development of new functional dairy products, as well as in the formulation of food emulsion. There are many works concerning the interfacial behaviour of milk proteins (β-casein, caseinate, β-lactoglobulin, whey protein isolate, etc.) at the air–water or oil–water interface (Baeza et al., 2005; Benjamins & Lucassen-Reynders, 1998; Carrera & Rodríguez Patino, 2005; Perez, Carrara, Carrera, Santiago, & Rodríguez Patino, 2009). Furthermore, the research of the dynamics of adsorption of these proteins related to the presence of polysaccharide in model systems has drawn even much more attention. These studies reveal the way of protein–polysaccharide binding affinity (attraction or segregation) as well as the surface character of the polysaccharide, which is useful to manipulate the adsorption behaviour at liquid interfaces (Baeza et al., 2005). However, due to the complex mechanisms involved during the formation and stabilization of real food dispersions, more fundamental studies on real food systems especially for commercial food materials are required.

The addition of XG could have an impact on the dynamic properties of CN adsorbed films during the development of oil-in-water emulsion using caseinate. Although CN and XG solutions have been extensively studied, the interfacial behaviour of CN/XG mixtures is largely unknown. The aim of this work was to study the effects of XG on the dynamic surface pressure and dilatational viscoelasticity of the CN films adsorbed at the oil–water interface.

2. Materials and methods

2.1. Materials

CN (Alanate 180) with the following composition: 91.1 wt% protein (N × 6.38), 3.5 wt% ash, 4.0 wt% moisture, 1.1 wt% fat, and 0.1 wt% lactose was obtained from New Zealand Milk Products Inc. (Santa Rosa, CA). XG (Ticaxan Xanthan powder) with an average molecular weight of approximately 6 million was kindly provided by TIC Gums (Maryland, USA). Corn oil was purchased from local supermarket and purified with Florisil (60–100 mesh, PR, Sigma Aldrich). Phosphate buffer solutions (PBS) (50 mM, pH 7.0 ± 0.1) were made with distilled water further purified with a Milli-Q filtration unit (greater than 18.2 MΩ cm resistivity).

The absence of surface active contaminants in the aqueous PBS solution was checked by interfacial tension measurement before sample preparation. No aqueous solutions with a surface tension other than that accepted in the literature (72–73 mN/m at 25 °C) were used. The presence of surface active impurities in the XG solutions was checked by the measurement of the surface tension. The sample has a surface pressure of 1.0 ± 0.5 mN/m, confirming that the XG used in this study is a non-surface active polysaccharide which was consistent with previous reports (Carp, Bartholomai, Relkin, & Pilosof, 2001). All other reagents were of the highest purity commercially available.

2.2. Preparation of CN and XG solutions

CN solutions (0.001, 0.01, 0.1 and 1.0 wt%) or CN solutions (0.002, 0.02, 0.2 and 2.0 wt%) were prepared in 50 mM Milli-Q PBS buffer at pH 7.0 by stirring at room temperature at moderate shear-rate until the dried protein sample was completely dissolved. XG solution of 0.1 wt% was prepared by dissolving the powder in the same buffer solution and stirred for at least 12 h to ensure complete dissolution.

2.3. Preparation of CN/XG mixtures

All mixture solutions contained the same 0.05 wt% XG and different CN concentrations (0.001, 0.01, 0.1 and 1.0 wt%) at pH 7.0. The solutions were prepared by mixing the appropriate volume of each double concentrated biopolymer solution up to the required concentration and stirred for at least 90 min to ensure the complete formation of protein–polysaccharide mixtures. The CN and CN/XG mixed solutions were stored at 4 °C and adjusted to pH 7.0 before use if necessary.

2.4. Purification of the corn oil

The surface tension of commercial corn oil with water was found to decrease with time, suggesting the presence of impurities in the oil. Therefore, it was purified by percolating them through a column packed with Florisil to completely remove the higher levels of impurities as described elsewhere (Gaonkar, 1989). Corn oil was deemed acceptable only after the interfacial tension against the PBS buffer remained constant at a value of 24.5 ± 0.5 mN/m for 30 min. The interfacial tensions of the Milli-Q water and PBS buffer against air used in this study were 72.5 ± 0.2 and 72.8 ± 0.2 mN/m, respectively. All experiments were conducted at 25 °C.

2.5. Measurement of the dynamic surface properties

The CN or CN/XG mixtures adsorption at the oil–water interface was determined by monitoring the evolution of surface pressure (π) and surface dilatational parameters with time. The XG concentration was maintained constant at 0.05 wt% in all CN/XG mixtures. The effect of protein concentration (within the range of 0.001–1.0 wt%) on the dynamic surface properties of CN adsorbed films was analyzed.

Dynamic surface pressure (π) and surface dilatational measurements of CN and CN/XG mixtures adsorbed films at the oil–water interface were determined using an optical contact angle meter, OCA-20, with oscillating drop accessory ODG-20 (Dataphysics Instruments GmbH, Germany). Details of this apparatus are given elsewhere (Caseli, Masui, Furriel, Leone, & Zaniquelli, 2005). The method involved a periodic automated-controlled, sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume at the desired amplitude ($\Delta A/A$) and angular frequency (ω). The surface dilatational modulus (E) derived from the change in interfacial tension (σ), resulting from a small change in surface area, can be described by Eq. (1) (Lucassen & van den Tempel, 1972)

$$E = \mathrm{d}\sigma/(\mathrm{d}A/A) = -\mathrm{d}\pi/\mathrm{dln}A = E_\mathrm{d} + iE_\mathrm{v} \quad (1)$$

The dilatational modulus (E), a measure of the total material dilatational resistance to deformation, is a complex quantity and composed of real and imaginary parts. The real part of the dilatational modulus or storage component is the dilatational elasticity, $E_\mathrm{d} = |E| \cos\delta$. The imaginary part of the dilatational modulus or loss component is the surface dilatational viscosity, $E_\mathrm{v} = |E| \sin\delta$. The phase angle ($\delta$) between stress and strain is a measure of the relative film viscoelasticity. For a perfect elastic material, $\delta = 0°$ and in case of a perfect viscous material, $\delta = 90°$. The loss-angle tangent can be defined by Eq. (2).

$$\tan\delta = E_v/E_d \quad (2)$$

The experiments were carried out at 25 °C. CN and CN/XG solutions were placed in the syringe and allowed to stay for at least 30 min to reach the desired constant temperature. Then a drop was delivered and allowed to stand for 3 h to achieve protein adsorption

at the oil–water interface. The dynamic surface viscoelastic parameters (E, E_d, E_v and δ) were measured as function of adsorption time (θ), at 10% of deformation amplitude ($\Delta A/A$) and at 0.1 Hz of angular frequency (ω). The sinusoidal oscillation for surface dilatational measurements was started 30 s later after the formation of droplets.

Afterward, the drop was subjected to repeated measurements with five oscillation cycles followed by a time of 50 cycles without any oscillation up to the time required to complete adsorption. Measurements were made at least twice. The average standard accuracy of the surface pressure was roughly 0.1 mN/m. However, the reproducibility of the results was better than 0.5% and 5% for surface pressure and surface dilatational properties respectively.

3. Results and discussion

3.1. Effect of XG on the kinetics of CN formation at the oil–water interface

3.1.1. CN adsorbed films

Dynamic surface pressures (π) of CN adsorbed films at the oil–water interface with different protein concentrations in the bulk phase are presented in Fig. 1a. As a general rule, π values increased with adsorption time, a phenomenon that can be associated with the protein adsorption at the interface. Moreover, the π values increased with the increasing of protein concentration in the bulk which could be attributed to the higher surface activity of CN. This phenomenon is coincided with previous results reported in the literature for proteins (Rodríguez Niño, Carrera, Pizones Ruíz-Henestrosa, & Rodríguez Patino, 2005). However, non-zero values of the interfacial pressure were found at the moment of the interface formation even at 0.001 wt% CN, which was different from the adsorption of CN at the air–water interface (Benjamins & Lucassen-Reynders, 1998).

3.1.2. CN/XG mixtures adsorbed films

The effect of XG on the time evolution of π depended on the nature of the polysaccharide and protein concentration in the bulk phase (Fig. 1b). Although the π values increased with adsorption time and protein concentration in the bulk phase, the presence of XG in the aqueous phase had a significant effect on the CN interfacial characteristics and its ability to occupy the oil–water interface sites from the very beginning. The final values of π were lower for CN/XG mixtures compared to those of CN adsorbed films, especially at 0.001 wt% CN (Fig. 2b). It can be speculated that interactions between protein and polysaccharide reduce the availability of the hydrophobic binding sites on the protein, leading to a lower surface activity (Galazka, Dickinson, & Ledward, 2000).

3.2. Effect of XG on the kinetics of CN adsorption at the oil–water interface

From a kinetic point of view, protein adsorption to the air–water or oil–water interface has been described to occur in three main stages: (i) Diffusion from the bulk to the proximity of the interface; (ii) Actual molecular adsorption to the interface; (iii) Reorganization of the protein at the interface (Graham & Phillips, 1979; MacRitchie, 1978). While adsorption from dilute solution is commonly diffusion-controlled, it is not always the predominant or rate-limiting mechanism, reorganization upon unfolding can play an important role, especially for globular proteins (Miller, Policova, Sedev, & Neumann, 1993). The adsorption stage may also involve an energy barrier, the origin of which may depend on several factors, such as a change in protein conformation that would allow hydrophobic fragments to be more accessible, the reorientation of the protein to locate binding sites closer to the interface, as well as

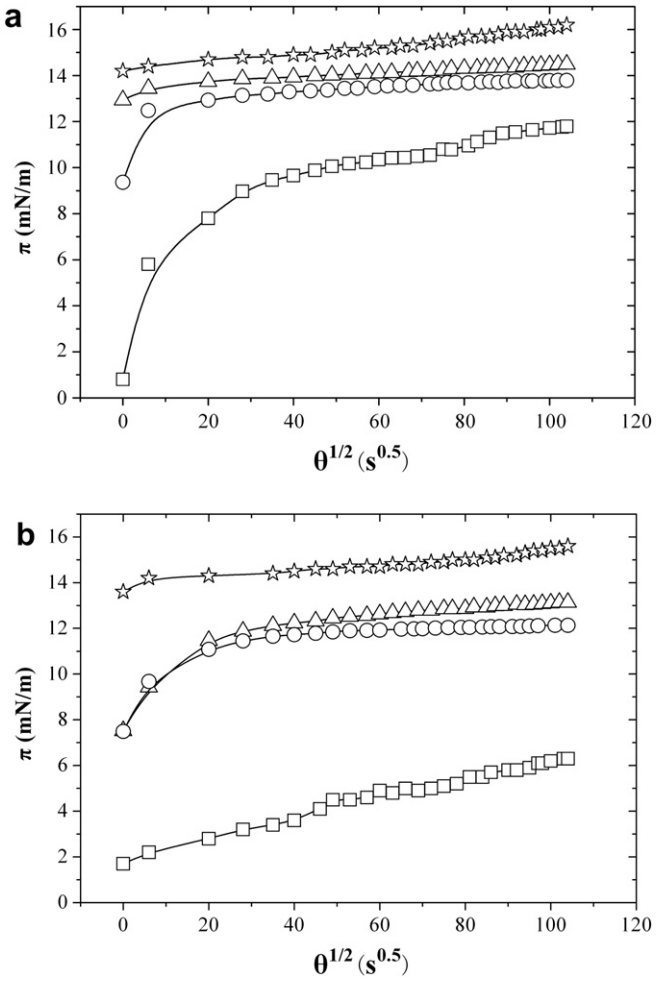

Fig. 1. The square root of time ($\theta^{1/2}$) dependence of surface pressure (π) for CN and CN/XG adsorbed films at the oil–water interface. (a) CN and (b) CN/XG mixtures (0.05 wt% XG). Symbols for CN concentration of the two systems: (☆) 1.0 wt%, (△) 0.1 wt%, (○) 0.01 wt%, and (□) 0.001 wt%. Temperature 25 °C, pH 7.0, and dissolved in 50 mM PBS buffer.

the exploration of available adsorption sites at the interface (Graham & Phillips, 1979; Murray, 2002). In the case of a protein/polysaccharide mixture, whether there is competitive or cooperative adsorption will depend on the concentration and surface activity of the adsorbed species, and on the nature of the interaction strength between two biopolymers (Murray, 2002).

3.2.1. Rheokinetics of protein adsorption at the oil–water interface

The kinetics of protein adsorption at the oil–water interface can be monitored by measuring changes in interfacial pressure (π). The change rate of surface concentration (Γ) can be expressed by Eq. (3) (MacRitchie, 1978):

$$d\Gamma/d\theta = (d\Gamma/d\pi)(d\pi/d\theta) \quad (3)$$

if $d\Gamma/d\pi$ is constant, $d\pi/d\theta$ can be used to evaluate the rate of protein adsorption. During the first step, at relatively low pressures when diffusion is the rate determining step, a modified form of the Ward and Tordai equation (Ward & Tordai, 1946) can be used to correlate the change in the interfacial pressure with time defined by Eq. (4).

$$\pi = 2C_0KT(D\theta/3.14)^{1/2} \quad (4)$$

where C_0 is the concentration in the bulk phase, K is the Boltzmann constant, T is the absolute temperature, and D is the diffusion

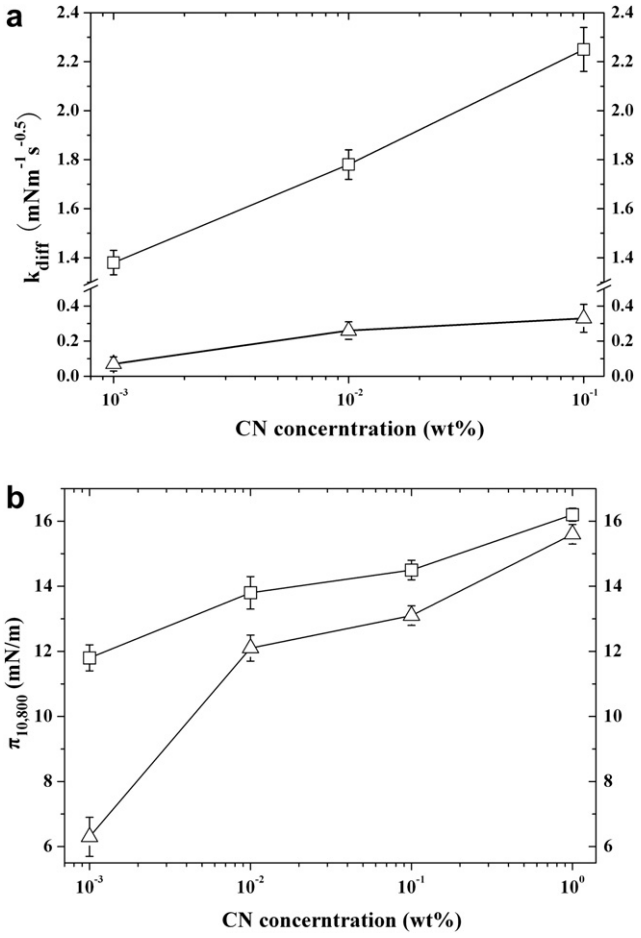

Fig. 2. Effect of CN concentration on (a) the apparent rate of diffusion to the interface (k_{diff}) and (b) the surface pressure at 10,800 s of adsorption time ($\pi_{10,800}$). Symbols: (□) single CN system, and (△) CN/XG mixtures (0.05 wt% XG). Temperature 25 °C, pH 7.0, and dissolved in 50 mM PBS buffer.

coefficient. If the diffusion of proteins at the interface controls the adsorption process, a plot of π against $\theta^{1/2}$ will then be linear (MacRitchie, 1978; Xu & Damodaran, 1994) and the slope of this plot will be the diffusion rate (k_{diff}).

The rates of penetration and rearrangements of adsorbed protein molecules have been analyzed by a first-order equation (Graham & Phillips, 1979):

$$\ln\left(\pi_f - \pi_\theta\right) \Big/ \left(\pi_f - \pi_0\right) = -k_i\theta \tag{5}$$

where π_f, π_0 and π_θ are the surface pressures at the final adsorption time of each step, at the initial time, and at any time respectively, and k_i is the first-order rate constant. In practice, a plot of Eq. (5) usually yields two or more linear regions. The initial slope corresponds to a first-order rate constant of penetration (k_P), while the second slope corresponds to rate constant of protein rearrangement (k_R).

The application of Eqs. (4) and (5) to the adsorption kinetics of milk and soy proteins to evaluate the rates of diffusion, penetration and rearrangement of protein at the oil–water interface or air–water interface have been discussed (Perez et al., 2009; Rodríguez Patino, Rodríguez Niño, & Carrera, 1999).

3.2.2. Diffusion of CN molecules to the oil–water interface

The initial adsorption of proteins at the interface is the most important step in emulsion formation. For milk proteins, the diffusion step is too fast to be detected with the experimental method used in this work. Thus, the jump in the surface pressure at the beginning of the adsorption shown in Fig. 2a is a qualitative measure of the diffusion of the protein to the interface (Rodríguez Niño et al., 2005). The results for 1.0 wt% CN (both with and without XG) are not included in Fig. 2a, because of over controlled diffusion step (with $\pi > 10$ mN/m at the first beginning). The same behaviour was also observed for the diffusion of milk protein and soybean globulins (7S and 11S) at high protein concentration (Alvarez Gómez & Rodríguez Patino, 2006).

For two systems studied here, the slope of the π against $\theta^{1/2}$ increased with the elevation of CN concentration in the bulk phase. The presence of XG obviously affected the diffusion step of CN. As a highly hydrophilic polysaccharide, XG is not considered to be surface active agents. In fact, XG did not cause an increase in surface pressure alone ($\pi = 1.0 \pm 0.5$) in this experiment (data not shown) which was consistent with previous reports (Carp et al., 2001). Due to its high molecular weight and anionic character, the existence of a finite thermodynamic incompatibility between CN and XG may lead to segregative phenomena in the bulk solution at neutral pH at certain concentration. This would improve the amount of adsorbed protein, and resulted into a higher surface pressure. However, the presence of 0.05 wt% XG in the aqueous phase produced an obvious decrease in k_{diff} value. This behaviour may probably be explained by protein diffusion resistance due to either the high viscosity of this gum or by the formation of CN–XG complex in the aqueous phase. Considered that XG and CN are negatively charged at neutral pH, a possible reason for the complex formation is that hydrophobic interactions would play a dominant role in binding XG to CN (Kobori et al., 2009). In brief, the formation of CN–XG complex could affect the protein adsorption rate, thereby leading to a low diffusion rate of CN to the oil–water interface (Ganzevles, Zinoviadou, van Vliet, Cohen Stuart, & de Jongh, 2006).

3.2.3. Penetration and rearrangements of CN molecules at the oil–water interface

After a rapid stage of protein diffusion to the interface (Fig. 1), the rate of CN adsorption is mostly controlled by the penetration (unfolding) and rearrangement of protein molecules at the interface. For all experiments, two linear regions in the plot of $\ln(\pi_f - \pi_\theta)/(\pi_f - \pi_0)$ against θ were found. The rate constants for the two processes involved in CN adsorption at the oil–water interface are shown in Table 1. Both k_P and k_R increased with protein concentration in two systems (an exception was for the protein concentration at 1.0 wt%). That is, penetration and rearrangement of the protein at the interface are facilitated at higher protein concentration in the bulk phase. However, beyond a critical value,

Table 1
Molecular penetration (k_P) and configuration rearrangement (k_R) parameters for dynamics adsorption of CN and CN/XG mixtures at the oil–water interface.

System	CN (CN:XG) (wt%)	$k_P \times 10^4$[a] (s^{-1})(LR)[b]	$k_R \times 10^4$[a] (s^{-1})(LR)[b]
CN	1.0	1.91 ± 0.05 (0.969)	14.26 ± 0.91 (0.920)
	10^{-1}	3.05 ± 0.06 (0.985)	20.37 ± 0.62 (0.992)
	10^{-2}	3.03 ± 0.11 (0.991)	11.96 ± 0.72 (0.952)
	10^{-3}	1.90 ± 0.09 (0.970)	8.50 ± 0.87 (0.983)
CN/XG	1.0:0.05	1.41 ± 0.03 (0.994)	12.65 ± 0.50 (0.974)
	10^{-1}:0.05	2.83 ± 0.03 (0.990)	9.81 ± 0.62 (0.955)
	10^{-2}:0.05	2.79 ± 0.10 (0.993)	9.78 ± 0.76 (0.985)
	10^{-3}:0.05	1.83 ± 0.06 (0.965)	8.47 ± 0.81 (0.967)

CN(CN:XG) (wt %): biopolymers ratio.
Temperature 25 °C, pH 7.0, and dissolved in 50 mM PBS buffer.
[a] Mean ± SD of at least $n = 2$.
[b] Linear regression coefficient.

the rate of penetration and rearrangement may be limited because of the steric hindrance effect.

In the presence of XG, decreases in both k_P and k_R were observed (Table 1), accompanied by the decrease in $\pi_{10,800}$ values (Fig. 2b). This could result from the existence of attractive interactions between the two polymers and/or from the protein aggregation in the presence of polysaccharide (Perez et al., 2009).

3.3. Effect of XG on the surface dilatational properties of CN adsorbed films

The evolution of E with π in the surface layer for the adsorption of CN and CN/XG mixed films is shown in Fig. 3a. The curve of E with π generally reflects the surface equation of state of the adsorbed material at the given interface, and it is a particularly sensitive tool for assessing non-ideal behaviour in the surface layer (Lucassen-Reynders, Lucassen, Garrett, Giles, & Hollway, 1975). If E is only due to the amount of macromolecule adsorbed at the oil–water interface, all E data should be normalized in a single master curve of E *vs* π (Rodríguez Patino et al., 1999). Fig. 3a shows that this state was not possible for the systems studied here. Thus, the E–π plot reflects the amount of protein adsorbed at the interface (giving high values of E as the surface pressure increase) and/or the degree of macromolecule interactions. It can be seen clearly in Fig. 3a that E increased with the interfacial pressure, and this dependence reflects the existence and the increase of interactions between film-forming components. E was higher at longer adsorption times, which was in agreement with the theory of Lucassen-Reynders (Lucassen-Reynders et al., 1975).

For the systems studied here, the slopes of the E–π plots were almost higher than 1.0 (represented by the dotted line in Fig. 3a, characteristic of the behaviour of an ideal gas) which implied a severely non-ideal behaviour for higher molecular interactions between film-forming components compared with those for an ideal behaviour (Lucassen-Reynders et al., 1975). However, the initial slope of the E *vs* π curve was only 0.32 at protein concentration of 0.001 wt% in the absence of XG. It showed a distinct behaviour as E only increased significantly at π above 9.0 mN/m, indicating that a higher interfacial protein concentration was needed to establish intermolecular interactions which give rise to a significant film structure (Martinez, Carrera, Rodríguez Patino, & Pilosof, 2009).

On the other hand, the E–π data for CN/XG mixtures were close to that of single CN and their slopes were higher than that of single CN except at 1.0 wt%, indicating that the presence of XG has a significant effect on the molecular structure and/or condensation (packing) of CN adsorbed segments at the oil–water interface. For 1.0 wt% CN, the slopes of CN and CN/XG films were 2.47 and 2.12, respectively. A slightly higher slope value for CN film may be due to the fact that, at higher CN concentration, the interactions between protein molecules are much stronger than protein–polysaccharide interactions in this experiment. Differences in the surface dilatational modulus are mainly attributed to looping of amino-acid residues at higher surface pressures and interactions between collapse residues, including multilayer formation at surface pressures higher than the equilibrium surface pressure (Carrera, Rodríguez Niño, Molina, Añón, & Rodríguez Patino, 2004).

The dynamic surface dilatational properties (E_d, and tanδ) are presented in Fig. 3b–c concerning the adsorbed films of CN and CN/XG mixtures at the oil–water interface as a function of the protein concentration in the bulk phase. The increase in E_d with time or the decrease of tanδ with π should be attributed to the adsorption of the protein at the interface (Martinez et al., 2009; Perez et al., 2009). As can be seen in Fig. 3b, because of its high flexibility, the E_d of CN was very low and showed little change with bulk protein concentration, which is quite different from the behaviour of globular protein molecule (Benjamins et al., 1998). This graph shows that the E_d decreased with the bulk protein concentration. The E_d of CN was 13.9 mN/m at 0.001 wt% CN, and dropped to 7.21 mN/m at 1.0 wt% CN. These results were in a fairly good

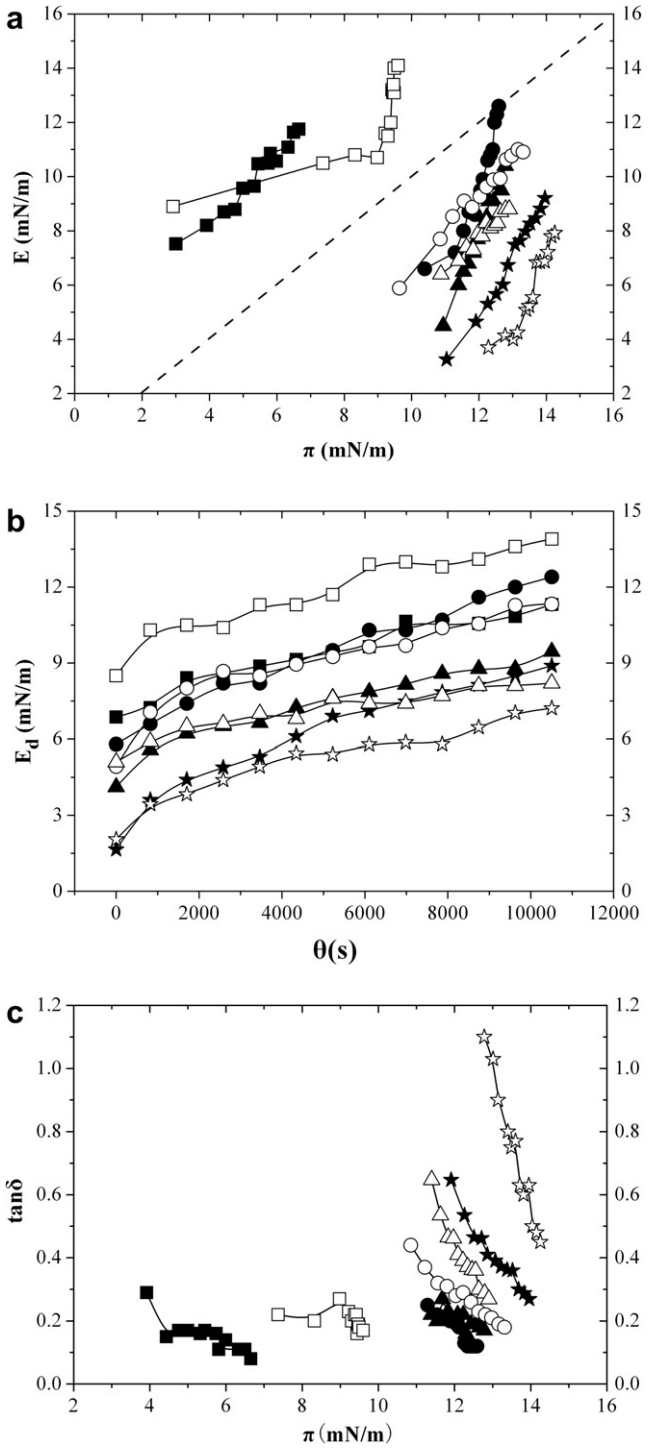

Fig. 3. (a) Surface dilatational modulus (E) and (c) phase angle tangent (tanδ) as a function of surface pressure (π) for systems with or without XG at the oil–water interface. (b) Time-dependent dilatational elasticity (E_d) for CN or CN/XG adsorbed films at the oil–water interface. Symbols for two systems: (☆) 1.0 wt%, (△) 0.1 wt%, (○) 0.01 wt%, and (□) 0.001 wt%. Open symbols: single CN system; closed symbols: CN/XG mixtures (0.05 wt% XG). Temperature 25 °C, pH 7.0, and dissolved in 50 mM PBS. Frequency: 0.1 Hz. Amplitude of compression/expansion cycle: 10%.

quantitative agreement with those found previously at the air–water interface (Benjamins et al., 1998). Graham and Phillips (1979) also found a maximum in the surface dilatational modulus at low protein concentrations, and they explained this phenomenon by the presence of a close packed structure in the interface.

Compared to single CN, addition of 0.05 wt% XG to CN aqueous solutions produced a significant increase in E_d especially at higher protein concentrations. It can be seen in Fig. 3b that at short adsorption time, the values of E_d for the mixtures were lower than those for single CN, which can be associated with the slow protein diffusion to the interface (Fig. 2a). These results confirmed that the formation of CN–XG complex and/or the high viscosity of XG produced a reduction of interactions between adsorbed CN residues at the interface (Perez et al., 2009). However, at long-term adsorption the values of E_d for the mixed system were higher than those for single CN. These results suggest that macromolecular interactions in the vicinity of the oil–water interface increased gradually which may result from the differences in the structure of interfacial film. This observation was consistent with the lower values of k_P and k_R of the mixture discussed in previous sections. That is, through a long time of molecular penetration and configurational rearrangement, a more dense interface layer can be formed which was proved once again by the effect of XG to tanδ (Fig. 3b). Interestingly, the CN/XG film showed a lower value of E_d than single CN at 0.001 wt% protein concentration, possibly due to the reduction of protein surface coverage as a result of protein–polysaccharide interactions which lead to the surface protein concentration lower than the critical value where the maximum modulus occurs.

The tanδ–π dependence for single CN and CN/XG mixtures as function of CN concentration is presented in Fig. 3c. An increase in the relative elasticity of the adsorbed films at higher π was observed over the adsorption period studied here for both of the systems. On the other hand, the films behaved as viscoelastic with tanδ higher than zero (Fig. 3c). It showed that as the protein concentration ranges from 1.0 wt% to 0.001 wt%, the relative dilatational viscosity of the surface decreased. Considering that CN is likely to be presented as a monolayer at the interface when the bulk concentration is low, it might be assumed that diffusion at the interface will be rather insignificant at such low concentrations. However, for the CN/XG films, the response of the interface was predominantly more elastic in nature at long adsorption time, which is characterized by the lower values of tanδ, indicating that the amount of relaxation occurring in the interface is rather limited and there is not much change in the conformation of the molecule as the surface area is changed, possibly due to the fact that the timescale for this type of rearrangement is much longer or shorter than that for a compression–dilation cycle (Williams & Prins, 1996).

4. Conclusions

In this research, we found that the dynamics of CN adsorbed films was modified by protein–polysaccharide interactions in solution and/or at the oil–water interface. The strength of interactions between CN and XG has an effect on the kinetics of adsorption of CN at the oil–water interface and depends on the state of interfacial saturation, the chemical structure of biopolymers, and their mixing ratio.

Due to the high viscosity of XG and/or the formation of CN–XG complex in the aqueous phase, CN/XG mixtures showed a decrease in adsorption rate. The existence of CN/XG interactions in the vicinity of the oil–water interface increased the surface dilatational elasticity at higher protein concentrations during the observation period. Finally, the CN/XG mixtures appeared to be more elastic compared to single CN adsorbed films at long adsorption time.

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