Hydroxypropylmethylcellulose surface activity at equilibrium and adsorption dynamics at the air–water and oil–water interfaces

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A B S T R A C T
The surface behaviour of hydroxypropylmethylcelluloses (HPMC) was studied at the air–water (A/W) and oil–water (O/W) interfaces. At the A/W interface, the π-C isotherms were sigmoidal and presented inflexions as the HPMC bulk concentration increases, related with different structural patterns adopted by the biopolymer segments. The behaviour of these biopolymers at the O/W interface resulted different. No inflections in the adsorption isotherm were observed, denoting the absence of any change in the structure of the adsorbed monolayer. The order in the interfacial activity was different at the two interfaces.

The dynamics of adsorption showed that the surface pressure (π) values and the rate of adsorption/penetration were lower at the O/W interface.

Analogously to π values, the surface dilatational modulus was smallest at the O/W interface, however at long-term adsorption strong viscoelastic films are formed at the O/W interface.

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1. Introduction

The composition and structure of adsorbed layer surrounding oil droplets in emulsions and air bubbles in foams are fundamental to the stability and behaviour of dispersed systems during their processing.

The adsorption of macromolecules at the A/W and O/W interface is different from the adsorption of low molecular weight surfactants. The formation of stabilizing interfacial layers requires, firstly, the diffusion of the polymer from the bulk to the interface and, secondly, its adsorption at the interface (Wollenweber, Makievski, Miller, & Daniels, 2000). Macromolecules adsorbed at the interface appear as train, loops and tails. (Graham & Philips, 1979; Nähringbauer, 1995; Perez, Carrera Sanchez, Pilosof, & Rodriguez Patino, 2008) which influence the surface properties of a macromolecule. A change in the conformation of the adsorbed macromolecule can cause a drastic effect both on the fraction of segments directly in contact with the surface (i.e., on the surface tension) and on the thickness of the adsorbed macromolecules layer (Nähringbauer, 1995). As an example, it could be mentioned that the properties of the interfacial layers, especially the ratio of train/loop and tail segments, and the elasticity of the layer, determine the emulsion stability, particularly the coalescence stability of the oil droplet (Wollenweber et al., 2000).

Williams and Prins (1996) compared the dilatational behaviour of two milk protein, β-lactoglobulin and β-casein, at both interfaces and they established a surprising similarity between the two types of interfaces for both proteins. This suggested that the interfacial structures of the molecules at the O/W and A/W interfaces can be similar. Wüstneck, Moser, and Muschiolik (1999) studying β-lactoglobulin adsorption layers at the A/W and O/W interfaces (sunflower or tetradecane oil) found that the concentration required for interfacial saturation was lower at the interface with sunflower oil. Interfacial dilatational elasticity and interfacial viscosity were largest at the air–water interface and smallest at the sunflower interface. The similarities and differences of the systems investigated were attributed to the adsorption behaviour and the solvation of different apolar and polar parts of the protein molecules in the neighbouring phase.

More recently, Rotureau, Leonard, Dellacherie, and Durand (2004) studied the adsorption of a polysaccharide, an amphiphilic derivative of dextran, at the A/W and O/W interfaces. They concluded that the kinetics of polymer adsorption at the dodecane–water interface is similar to that at the air–water interface.

An interesting group of tensioactive polysaccharides are the cellulose derivatives which have a strong tendency to accumulate at
the air–water and the oil–water interface (Nahriningbauer, 1995). Although, only four of them are used in the food area for their surface activity: methylcellulose (MC), carboxymethylcellulose (CMC), hydroxypropylcellulose (HPC) and hydroxypropylmethylcellulose (HPMC). Even, methylcellulose and hydroxypropylmethylcellulose appear to be more surface active than milk proteins (Arboleya & Wilde, 2005; Mezdour, Cuvelier, Cash, & Michon, 2007; Perez, Carrera Sanchez, Rodriguez Patino, & Pilosof, 2007).

Among other technological applications, hydroxypropylmethyl cellulose (HPMC) presents multiple applications in the food or pharmaceutical industry because it is non-toxic and possesses good mechanical properties. This polysaccharide is interesting for various pharmaceutical uses such as controlled drug release (Kita, Kaku, Kubota, & Dobashi, 1999). In the food industry, HPMC is used for the control of the texture and rheological properties of dispersions, as an emulsifier, for the modification of ice crystal formation and growth, and for its water binding capacity (Coffey, Bell, & Henderson, 1993). Topical food applications of HPMC are whipped toppings, mousses, baked goods, bakery products, fried foods, saucisssings, frozen desserts, reduced – or low-calorie foods, etc. This macromolecule is surface active with hydrophobic (methyl) and hydrophilic (hydroxypropyl) groups distributed along the cellulose backbone, thus it is adsorbed at interfaces lowering the surface tension (Ochoa-Machiste & Buckton, 1996; Wollweber et al., 2000). Besides all these HPMC attributes, this polysaccharide is known for exhibiting the interesting phenomenon of thermo-reversible gelation (Perez, Carrera Sanchez, Rodriguez Patino, & Pilosof, 2006; Sarkar, 1995; Yuguchi, Uraikawa, Kitamura, Ohno, & Kajiwara, 1995). The surface characteristic of hydroxypropylmethylcellulose (HPMC) have been widely studied (Arboleya & Wilde, 2005; Avranas & Tasopoulos, 2002; Nahriningbauer, 1995; Perez et al., 2008), but much less investigations have been focused on the behaviour of HPMC at the oil–water interface. Moreover, only a few papers focus on the comparison of the adsorption properties of particular compounds at the A/W and O/W interfaces (Beverung, Randke, & Blanch, 1999; Williams & Prins, 1996; Wüstneck et al., 1999).

This study was designed to study the equilibrium and dynamic surface characteristics of HPMCs at the O/W interface and compare them with the behaviour at the A/W interface. This work is part of an integral study undertaken to characterize the behaviour of HPMC at the O/W and A/W interfaces, alone and with other food ingredients such as proteins.

2. Materials and methods

2.1. Materials

Methocell E5LV, E15LV, E50LV and E4M (food grade) from the Dow Chemical Company were kindly supplied by Colorcon Argentina and used without purification. Table 1 shows some characteristic properties, such as methyl and hydroxypropyl content, methyl/hydroxypropyl ratio, molar substitution, the degree of substitution, the viscosity (20 °C) of 2 %wt solutions, and molecular weight. The moisture content of HPMC powders was 1.6%.

<table>
<thead>
<tr>
<th>HPMC</th>
<th>E4M</th>
<th>E50LV</th>
<th>E15LV</th>
<th>ESLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timethyl</td>
<td>28.0</td>
<td>29.1</td>
<td>29.2</td>
<td>29.5</td>
</tr>
<tr>
<td>Hydroxypropyl</td>
<td>10.2</td>
<td>9.2</td>
<td>9.3</td>
<td>9.7</td>
</tr>
<tr>
<td>Methyl/hydroxypropyl ratio</td>
<td>2.3</td>
<td>3.2</td>
<td>3.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Methyl substitution (DS)</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
</tr>
<tr>
<td>Hydroxypropyl substitution (MS)</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Total substitution (DS + MS)</td>
<td>2.13</td>
<td>2.13</td>
<td>2.13</td>
<td>2.13</td>
</tr>
<tr>
<td>Viscosity (cp), 2 %wt solution, 20 °C</td>
<td>4965</td>
<td>41</td>
<td>15</td>
<td>5.4</td>
</tr>
<tr>
<td>Molecular weight (Da)</td>
<td>90 000</td>
<td>18 000</td>
<td>6000</td>
<td>2000</td>
</tr>
</tbody>
</table>

Sample preparation. No aqueous solutions with a surface tension other than that accepted in the literature (72–73 mN/m at 20 °C) were used.

Commercial sunflower oil was used as the oil phase in this research without further purification in order to study the performance of HPMCs in a real interface (i.e. commercial oil widely used in food industry). In order to check differences in the interfacial behaviour of HPMCs that could be attributed to the heterogeneous composition of commercial oil, some adsorption measurements were performed using purified oil (Florisil 60–100 mesh Aldrich®).

The commercial sunflower oil and Florisil were allowed to interact for 24 h and then filtered (0.22 μm) the upper phase free of impurities. Sunflower oil contains triglycerides and both, saturated and unsaturated fatty acid. The alkyl chain lengths of these fatty acids vary between 14 and 22. Therefore, from surface science point of view, sunflower oil is a complex mixture (Wüstneck et al., 1999).

2.2. Surface pressure isotherm

Equilibrium surface tension (γsurf) was registered by the Wilhelmy plate method, using a roughened platinum plate attached to a Sigma 701 digital tensiometer (KSV, Finland) as described elsewhere (Rodríguez Niño & Rodriguez Patino, 1998). HPMCs solutions in an increased range of concentrations from 10−7–2% wt were allowed to age for 24 h at 4 °C, prior to each measurement, to achieve the HPMC adsorption. After ambient temperate, samples measurements were made at 20 °C within ±0.5 °C. The temperature was maintained constant by water circulating in a Heto thermostat. For measurements at the O/W interface, a 6–7 mm layer of oil was gently layered over the top of each aqueous HPMC solution, where the platinum plate was previously placed. The platinum plate was completely covered by the oil phase (Murray, Ventura, & Lallemant, 1998; Murray, 1997; Williams & Prins, 1996).

The reduction in surface tension (γ) was recorded continuously by a device connected to the tensiometer. Equilibrium was assumed when the tension did not change by more than 0.1 mN/m in 30 min. The final surface pressure (psurf) value was calculated as

\[
\gamma_{surf} = \gamma - \gamma_{0}
\]

where γ0 is the sub-phase interfacial tension (72 mN/m or 29.5 mN/ m for A/W or O/W interfaces, respectively) and γsurf is the interfacial tension of the HPMC aqueous solution at equilibrium. The experiments were repeated at least two times. It was found that ρsurf could be reproduced to ±0.5 mN/m.

2.3. Dynamic surface tension

Time-dependent surface pressure and surface viscoelastic parameters of adsorbed HPMC films at the A/W and O/W interfaces were determined by an automatic drop tensiometer (IT Concept, France) as described elsewhere (Álvarez Gomez, Ruiz Henestrosa, Carrera Sanchez, & Rodriguez Patino, 2008; Baeza, Carrera Sanchez, Pilosof, & Rodriguez Patino, 2005; Rodríguez Niño & Rodriguez Patino, 2002). The surface tension (γ) was calculated thorough the analysis of the drop profile (Labourdenne et al., 1994). The surface...
pressure is \( \pi = \gamma - \gamma \), where \( \gamma \) is the sub-phase interfacial tension and \( \gamma \) the interfacial tension of solution at each time \( (\theta) \). The average accuracy of the superficial tension is roughly 0.1 mN/m. However, the reproducibility of the results, for at least two measurements, was better than 1%.

From the adsorption dynamics measurements, the adsorption kinetics parameters can be obtained. The main features of the kinetics of adsorption of HPMC include (i) the diffusion of the biopolymer from the bulk onto the interface, (ii) adsorption (penetration) and interfacial unfolding, and (iii) aggregation (rearrangement) within the interfacial layer, even multilayer formation and interfacial gelation can also occur (MacRitchie, 1990; Nahringbauer, 1995; Perez et al., 2006; Perez et al., 2008; Wollenweber et al., 2000).

During the first step, at relatively low surface pressures when diffusion is the rate-determining step, a modified form of the Ward and Tordai equation can be used to correlate the change in surface pressure with time (Ward & Tordai, 1946):

\[
\pi = 2C_0KT(D_{\text{diff}}/3.14)^{1/2} \tag{1}
\]

where \( C_0 \) is the concentration in the aqueous phase, \( K \) the Boltzmann constant, \( T \) the absolute temperature, \( D_{\text{diff}} \) the diffusion coefficient, and \( \theta \) the adsorption time. If the diffusion at the interface controls the adsorption process, a plot of \( \pi \) against \( \theta^{1/2} \) will then be linear (de Feijter & Benjamins, 1987; MacRitchie, 1990; Perez et al., 2008; Xu & Damodaran, 1994) and the slope of this plot will be the diffusion rate \( (K_{\text{diff}}) \).

To analyze the rate of adsorption (penetration) and the rate of rearrangement of adsorbed biopolymer, the following semi-empirical first-order equation, proposed by Graham and Philips (1979), has been used:

\[
\ln\left(\frac{\pi_{180} - \pi_0}{\pi_{180} - \pi_0}\right) = -k_1 \cdot \theta \tag{2}
\]

where \( \pi_{180}, \pi_0, \) and \( \pi \theta \) are the surface pressures at 180 min of adsorption time, at time \( \theta = 0 \), and at any time, \( \theta \), respectively, and \( k_1 \) is the first-order rate constant.

### 2.4. Surface dilatational properties

The surface viscoelastic parameters surface dilatational modulus, \( E \), and its elastic, \( E_p \), and viscous, \( E_v \), components, were measured as a function of time, \( \theta \), at 10% deformation amplitude \( (\Delta A/A) \) and 0.1 Hz of angular frequency \( (\omega) \). Previously, the percentage area change had been determined to be in the linear region (data not shown). The method involved a periodic automated-controlled, sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume, at the desired amplitude. The surface dilatational modulus derived from the change in surface tension (dilatational stress), \( \gamma \) (equation (3)), resulting from a small change in surface area (dilatational strain), \( A \) (equation (4)), may be described by equation (5) (Lucassen & van den Temple, 1972):

\[
\gamma = \gamma_0 \sin(\omega \theta + \delta) \tag{3}
\]

\[
A = A_0 \sin(\omega \theta) \tag{4}
\]

\[
E = \frac{d\gamma}{dA/A} = \frac{d\pi}{dnA} \tag{5}
\]

where \( \gamma_0 \) and \( A_0 \) are the stress and strain amplitudes, respectively, and \( \delta \) is the phase angle between stress and strain.

The dilatational modulus is a complex quantity, which is composed of real and imaginary parts:

\[
E = (\gamma_0/A_0)(\cos \delta + i \sin \delta) = E_p + iE_v \tag{6}
\]

The real part of the dilatational modulus or storage component is the dilatational elasticity, \( E_p = |E| \cos \delta \). The imaginary part of the dilatational modulus or loss component is the surface dilatational viscosity, \( E_v = |E| \sin \delta \). The ratio \( (\gamma_0/A_0) \) is the absolute modulus, \( |E| \), a measure of the total unit material dilatational resistance to deformation (elastic + viscous). For a perfectly elastic material, the stress and strain are in phase \( (\delta = 0) \) and the imaginary term is zero. In the case of a perfectly viscous material \( \delta = 90^\circ \) and the real part is zero. The loss angle tangent can be defined by equation (7). Thus, if the film is purely elastic, the loss angle tangent is zero.

\[
\tan \delta = E_v/E_p \tag{7}
\]

The experiments in the automatic drop tensiometer, were carried out at 20 °C and the temperature of the system was maintained constant within ±0.1 °C by circulating water from a thermostat. The final concentrations of HPMC in the sub-phase were 1·10^{-2}wt and 1wt. The solutions were placed in the syringe and then in the compartment and were allowed to stand for 30 min to reach the desired constant temperature. Then a drop of HPMC solution was delivered to achieve macromolecule adsorption at the A/W or O/W interface. For the O/W interface measurements, the cuvette was full of oil. The syringe contained the HPMC solutions. The syringe broke the aqueous HPMCs solutions free into the oil volume.

### 3. Results and discussion

#### 3.1. Surface pressure isotherms for HPMCs at the A/W and O/W interfaces

The main properties of the cellulose derivatives studied are displayed in Table 1.

All of them belong to the same family of HPMC (serie E), with the same degree of substitution close to 2, that accounts for a high degree of hydrophobicity, necessary for surface activity (Perez et al., 2007). E4M posses the lowest % methyl substituent and the highest % hydroxypropyl substituent, which makes it the less hydrophobic HPMC, accounting for its lowest methyl/hydroxypropyl ratio (2.3). The other three HPMC studied, E5LV, E15LV and E50LV, have similar % substituents and methyl/hydroxypropyl ratio. E4M also has the higher molecular weight and viscosity.

The behaviour of these four commercial types of HPMC, was firstly measured by means of tensiometry. As for proteins, true equilibrium adsorption of HPSCs does not seem to be possible (no changes in \( \pi \) values upon time) (Rodríguez Niño & Rodríguez Patino, 1998; Perez et al., 2006). Therefore, we considered the surface pressure measured on solutions after 24 h of aging as the pseudo-equilibrium value.

The measurements made at the A/W and at O/W interfaces are shown comparatively in Fig. 1. A and B, respectively. At the A/W interface (Fig. 1A) a sigmoidal behaviour was observed, which is typical for surface active biopolymers and surfactants of low molecular weight. The surface pressure increased with HPMC concentration and tended to pseudo-equilibrium values. Similar results were achieved by Nahringbauer (1995), working with ethylhydroxyethylcellulose (EHEC) adsorbed at the air–water interface who found lower tension values for the more concentrated solutions and a critical concentration upon which no changes in the interfacial tension was discernible.

At lower HPMC bulk concentrations \((1·10^{-6} \text{ wt/wt})\) E4M showed surface activity, while concentrations two orders of magnitude higher were necessary to observe the same effect for E50LV, E15LV and E5LV. For bulk concentrations between \(1·10^{-6}\) and \(1·10^{-4}\) wt/wt,
E5LV showed a slightly higher surface pressure than E15LV, followed by E50LV. For bulk concentrations between 1-10^{-4} wt% and 1-10^{-1} wt% no discernible differences were observed in the surface pressure for all four HPMCs studied, the interface saturation state was achieved. Above 1-10^{-4} wt%, E4M showed higher values than the other HPMCs. According to these results, the order for the interfacial activity at the A/W interface would be: E4M > E5LV > E15LV > E50LV.

π-C isotherms at the air–water interface presented inflexions as the HPMC bulk concentration increased. This fact would be related with different structural patterns adopted by the polysaccharide segments at the A/W interface as the amount of such absorbed segments was higher. Perez et al. (2006) and Li, Xu, Xin, Cao, and Wu (2008) have presented evidence that the HPMCs monolayers are able to change their structural patterns as the surface biopolymer concentration increases: from structure I (a more expanded structure which implies the formation of trains) to structure II (a more condensed conformation with loop and tails formation) (Graham & Phillips, 1979). For the highest bulk concentration, a collapsed structure even with multilayer formation could be formed. The hydrophobicity and molecular flexibility of HPMC would cause more molecules to be adsorbed and packed into the interface, provoking a further increase in the surface pressure. As reported previously (Perez et al., 2008), E50LV only adopted structure I to finally collapse and E4M, structure I and II with multilayer formation at the collapsed structure.

At the O/W interface (Fig. 1B), the π-C isotherms were not sigmoidal and no inflexions in the adsorption isotherm were observed, suggesting that structural changes do not take place, at least in the concentration range studied. Moreover, the increase of HPMC bulk concentration had a very small effect. At concentrations higher than 1-10^{-4} %wt, the oil–water surface seemed to be saturated, since the surface pressure did not experiment appreciable changes with increasing bulk concentration. HPMC concentrations lower than 10^{-5} %wt lead to values affected by oil surface active compounds. At such low concentrations this effect can be magnified (Williams & Prins, 1996). In fact, low levels of (or weakly surface active) compounds in the sunflower oil were present as shown in Fig. 2, with effects of up to 10 mN/m.

It can be noticed that oil impurities adsorb rapidly and reach an equilibrium value at very short times (i.e. 2000s). This behaviour is characteristic of low molecular weight surfactants. Surface active components in sunflower oil may be a mixture of monoglycerides, free fatty acids and phospholipids. Accordingly to Wüstneck et al. (1999) such contaminants may be even essential for the interfacial behaviour of oil.

It is likely that HPMC at concentrations above 10^{-5} %wt displaced the low levels of impurities present, as shown by the high equilibrium surface pressure values (Fig. 1B) reached at 10^{-5} %wt by all the polysaccharides (around 20 mN/m), which remained almost constant when increasing HPMC bulk concentration. HPMC and impurities may compete for the interface, but HPMC dominates the surface pressure even at short adsorption times (Fig. 2), because of the following considerations: (a) unusual strong surface activity that leads to a strong competitive behaviour which displaces other competitive molecules (Perez et al., 2007; Perez, Carrera Sanchez, Pilosof, & Rodriguez Patino, 2009). Moreover, all HPMC studied exhibited higher π values than impurities, even at very low concentrations. It has been previously reported that HPMC dominates surface pressure in mixtures with proteins even when both components can saturate the interface (Martinez, Carrera Sanchez, Ruiz Henestrosa, Rodriguez Patino, & Pilosof, 2007; Perez et al., 2007). (b) Saturation of interface at very low bulk concentration. At the A/W or O/W interface saturation is attained at above 10^{-4} %wt (Fig. 1 A and B).
It has been reported that in mixtures of oil-soluble emulsifiers (monoglycerides, phospholipids) and proteins, the surface activity is determined by the protein as the protein saturate the monolayer (Rodriguez Patino, Rodriguez Niño, & Carrera Sanchez, 2003). A similar behaviour should be expected in mixtures of the impurities (i.e. monoglycerides, phospholipids and free fatty acids) and HPMCs, especially because HPMC is much more surface active than most proteins (Arboleya & Wilde, 2005; Mezdour et al., 2007; Perez et al., 2007). Murray (1997) claimed that the oil impurities present, did not generate substantial differences when studying the adsorption of bovine serum albumin (BSA) at the oil–water interface. Therefore they did not take special precautions to remove these impurities.

The behaviour of HPMCs at the O/W interface (Fig. 1B) was remarkably different from that shown at the A/W interface (Fig. 1A). It could obey to the different nature of the hydrophobic phase (oil) formed by triglycerides and fatty acids. The surface pressure achieved at each HPMC bulk concentration was lower at the O/W interface (Fig. 1B). Wüstneck et al. (1999) obtained the same trend achieved at each HPMC bulk concentration was lower at the O/W interface. Lower π values at the O/W interface, have been also reported by Rotureau et al. (2004) working with amphiphilic derivates of dextran and by Ganzevles, van Vliet, Cohen Stuart, and de Jongh (2007) in mixtures of β-lactoglobulin and pectins.

The better solvency offered by oil, for hydrophobic groups, compared with air, can explain the lower efficiency of HPMC to increase surface pressure at the O/W interface. Medrzycka and Zwierzykowski (2000), proposed that there exists more cohesion between carbon chains of surfactants molecules at the A/W interface. Such cohesions, known as trains and loops, provoke surface tension decrease. These cohesions would be absent, or present in a lower number, at the O/W interface.

The order for the interfacial activity at the O/W interface would be: E15LV > E5LV > E4M > E50LV. The HPMC E50LV, was the least efficient in decreasing surface pressure at both interfaces, but on the contrary to the performance at the air phase, the HPMC of lowest molecular weight (E51LV and E15LV) promoted the strongest decrease of surface tension at the O/W interface. The best performance at the oil phase of E51LV and E15LV may be explained by their high hydrophobicity (i.e. high methyl substitution) and low molecular weight which allows greater flexibility to anchor between the triglycerides of the oil phase (Hutchinson, 1948).

3.2. Adsorption dynamics of HPMCs at the A/W and O/W interface

In order to compare quantitatively the dynamics of adsorption of the HPMCs at the A/W and O/W interfaces, two HPMCs types were selected: E4M that posses molecular features that make it singular, i.e. the highest molecular weight, highest viscosity and also, the highest adsorption efficiency and surface activity at the A/W interface. Besides, this HPMC clearly displayed all the structural transitions mentioned before: structure I, structure II and the monolayer collapse (Perez et al., 2006). On the other hand, E50LV that represents the low viscosity HPMCs group analyzed in this research (Fig. 1A). It lacks of structural transitions upon adsorption at the A/W interface.

Fig. 3 shows the surface pressure (π) evolution upon time for E4M and E50LV at the A/W and O/W interfaces and its mixtures with low methoxyl and high methoxyl pectin at the A/W and O/W interfaces. This trend keeps good correspondence with that obtained from the π-C isotherms, where the equilibrium π values resulted lower at the O/W interface (Fig. 1B). As pointed out in the previous section, the reason for this finding would be the different nature of the hydrophobic phase. Murray (1997) studying the surface characteristic of β-lactoglobulin and bovine serum albumin (BSA) at both interfaces, concluded that the differences between the behaviour of these proteins were due to the better solvency of the oil phase for the more hydrophobic side chains of the amino acid residues, which in turn means lower surface activity.

Analysis of the A/W interface in Fig. 3, slight differences in the long-term pressure values can be noted with different polysaccharide concentrations, i.e. final π values for 1%wt of E4M bulk concentration at the A/W interface was 29 mN/m, whereas it was 26 mN/m for 1-10⁻² %wt. The same trend could be deduced at the O/W interface. However, the final π values from the dynamic studies resulted slightly lower than those of the equilibrium measurements, as the shortest times considered for dynamic measurements (up to 3 h) the final pseudo-equilibrium value would not be attained.
As the adsorption process started with a fast adsorption, except for HPMCs 10^{-2}\% wt at the O/W interface, the rate can be deduced that under these conditions, HPMCs adsorption at the A/W and also at O/W interface was not limited by the diffusion step. Other features, such as molecular size, unfolding, and conformation should also keep importance.

At the O/W interface and 1·10^{-2}\% wt bulk concentration (Fig. 3A) no discernible differences in the pseudo-equilibrium \(\pi\) values (16 mN/m) were observed between E4M and E50LV at \(t > 8000\) s. Differences at \(t < 8000\) s were registered between these celluloses since E4M promoted a higher increase of surface pressure. Beverung et al. (1999) sustained that the number of surface contacts plays a major role in surface pressure of a fluid interface. As it was previously reported (Perez et al., 2008) this cellulose presents the highest number of potentially adsorbed segments, since its average degree of polymerization is 4.5 times higher than E50LV, which is seen as a fast and continuous \(\pi\) values increasing.

E4M and E50LV did not show differences at 1\% wt bulk concentration (Fig. 3B) at the O/W interface, i.e. the shapes of curves and the obtained surface pressure. The \(\pi\) values registered for celluloses at 1\% wt resulted slightly higher than at 1·10^{-2}\% wt, at least within the time of the experiment.

At the A/W interface, differences in the adsorption behaviour were observed during the whole adsorption time and at both concentrations. The effect of increasing bulk concentration was more important at the A/W than at the O/W interface in accordance with the adsorption isotherms in Fig. 1.

As the rate of surface pressure change depends primarily on the rate of adsorption, Ward & Tordai equation (equation (1)) was used to describe these changes upon time. If the diffusion of the HPMCs at the A/W and O/W interfaces controls the adsorption process, a plot of \(\pi\) vs \(t^{1/2}\) will then be linear (de Feijter & Benjamins, 1987; MacRitchie, 1990; Xu & Damodaran, 1994) and the slope of this plot will be the diffusion rate constant.

It was found that the diffusion step for these polysaccharides (except for HPMCs 10^{-2}\% wt at the O/W interface) was too fast (\(\pi > 10\) mN/m), to be detected by the experimental technique used in this work, as deduced from the \(\pi\) vs \(t^{1/2}\) plots (data not shown). However, it was possible to obtain an estimation of the rate constant of diffusion (\(K_{\text{diff}}\)) from the slope of the first point and the origin of the coordinates.

Independently from the HPMC, \(K_{\text{diff}}^{\text{A/W}} > K_{\text{diff}}^{\text{O/W}}\) (Table 2), pointing out that the nature of the phase (air or oil) influenced the diffusion step. At 10^{-2}\% wt bulk concentration at the O/W interface the rate of diffusion is the lowest. This would be related to the presence of surface active compounds at the oil phase, that would compete at low HPMC concentration and at low of adsorption times (< 2000s) for the interface, decreasing \(K_{\text{diff}}\) because of its lower rate (Fig. 2).

After an initial diffusion of HPMCs at both interfaces, the rate of HPMCs adsorption is controlled by the penetration and rearrangement of the macromolecules at the interfaces (Rodríguez Niño & Rodríguez Patino, 2002; Perez et al., 2008). Malmsten and Lindman (1990) working with EHEC found that the adsorbed amount increases immediately after addition of the polymer solution, after which it levels off indicating the interface saturation.

In practice, a plot of equation (2) usually yields two or more linear regions. The initial slope is taken as a first-order rate constant of adsorption (penetration), \(K_{\text{ads}}\), and the second slope is taken as a first-order rate constant of aggregation (rearrangement), \(K_{\text{r}}\), occurring among a more or less constant number of adsorbed molecules. Because the interfacial concentration of adsorbed macromolecules is several times higher than that in the bulk phase, the molecular penetration and rearrangement steps are magnified processes happening at interface, especially for high molecular weight macromolecules (Perez et al., 2008). Figure 4 shows the fit of experimental data to this first-order kinetic equation for E4M and E50LV 1\% wt at the A/W and O/W interfaces. The main difference observed between the two interfaces is the appearance of a break in the linear plot at shorter times for the O/W interfaces. In comparison, at the A/W interface the rearrangement step seems to start around 10 000 s, when a high surface pressure is reached for E4M, but for E50LV the rearrangement step was not apparent within the period of the experiment (12 000 s). Baeza, Carrera Sánchez, Piñolos, and Rodriguez Patino (2004) obtained the same results when studying propyleneglycol alginate (PGA) adsorption at the A/W interface: only one first-order rate constant was obtained, related to the polysaccharide penetration at the interface. Table 2 summarizes the time at which the rearrangement step starts (or time at which ends the adsorption/penetration step) as well as the first-order rate constant of penetration (unfolding/adsorption), \(K_{\text{ads}}\). The occurrence of rearrangement of HPMC at lower surface pressures and adsorption times at the O/W interface could be ascribed to a steric constrain due to the presence of triglycerides molecules forming the oil phase. Similarly, the rate constants of adsorption were lower in the presence of the oil phase.

Table 2 shows that \(K_{\text{ads}}^{\text{A/W}} > K_{\text{ads}}^{\text{O/W}}\). Hutchinson (1948) suggested that oil molecules are present at the interface with the adsorbed surfactant molecules, therefore a competition exists at interfacial level between the non-polar portions of the surfactant molecule and the oil. As a consequence, the molecule penetration of HPMC would be sterically impeded due to the presence of the fatty acid molecules at the O/W interface.

Concerning to the bulk concentration effect, \(K_{\text{ads}}\) at the O/W interface slightly increased with bulk concentration (Table 2).

Rodriguez Patino, Rodriguez Niño, and Carrera Sánchez (1999) studying whey protein isolate adsorbed at the O/W interface, found that the adsorption and penetration were facilitated at higher concentrations. However, \(K_{\text{ads}}\) decreased or resulted equal when HPMC concentration increased at the A/W interface (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>HPMC (3% wt)</th>
<th>(K_{\text{ads}}^{\text{A/W}})</th>
<th>(K_{\text{ads}}^{\text{O/W}})</th>
<th>(t_{\text{final ads}}) (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(10^4) (mN m^{-1} s^{-1/2}) (LR)</td>
<td>(10^4) (s^{-1}) (LR)</td>
<td>A/W</td>
</tr>
<tr>
<td>E4M 10^{-2}</td>
<td>28.80 ± 0.10 (0.97)</td>
<td>7.00 ± 0.10 (0.98)</td>
<td>2.90 ± 0.10 (0.98)</td>
</tr>
<tr>
<td>E4M 1</td>
<td>88.10 ± 0.11 (0.98)</td>
<td>73.10 ± 0.05 (0.99)</td>
<td>2.00 ± 0.05 (0.99)</td>
</tr>
<tr>
<td>E50LV 10^{-2}</td>
<td>80.40 ± 0.05 (0.98)</td>
<td>24.0 ± 0.06 (0.99)</td>
<td>2.20 ± 0.06 (0.77)</td>
</tr>
<tr>
<td>E50LV 1</td>
<td>101.40 ± 0.07 (0.98)</td>
<td>55.50 ± 0.08 (0.98)</td>
<td>2.20 ± 0.05 (0.93)</td>
</tr>
</tbody>
</table>

\(K_{\text{ads}}\): Rate constant of penetration/adsorption/unfolding.

(LR): Linear regression coefficient.

\(^a\) Perez et al. (2008).

\(^b\) Mean ± SD of at least \(n = 2\).
At the O/W interface both HPMCs presented a continuous increase in $E_d$ value with adsorption time indicating a slow and a more gradual evolution of interfacial structure as compared to the A/W interface (Fig. 5A and C). The slow film structure evolution is also revealed by the evolution of the relative viscoelasticity of films ($\tan \delta$) in Fig. 5 B and D, mainly for E50LV.

$E_d$ values at 1%wt HPMC (Fig. 5A) were higher at the A/W interface over all the adsorption time, mainly for E4M. This cellulose showed the stronger elastic character, with a maximum value of 21 mN/m, which was attained almost immediately, reflecting the rapid adsorption and structuration of E4M monolayer at the A/W interface. The closer packing of macromolecules is the consequence of the existence of associations within the adsorbed molecules, as reflected by the significant increment in $E_d$ (Rodriguez Patino et al., 1999). As for $\pi$ values (Fig. 2A), $E_d$ were smallest at the O/W interface. As was claimed previously by Wüstneck et al. (1999) and Benjamins, Cagna, and Lucassen-Reyners (1996), the conditions to form an interfacial structure of high mechanical stability are best when conformation changes are not restricted, i.e. at the interface with air. The hydrophobic intramolecular interactions between HPMC molecules necessary to form an elastic film would be restricted by solvation of the hydrophobic groups in the oil phase. Williams and Prins (1996), also obtained lower elastic modulus at the O/W interface than at the A/W interface when comparing the behaviour of $\beta$-lactoglobulin and $\beta$-casein.

Time dependence of relative viscoelasticity ($\tan \delta$) for HPMCs films shown in Fig. 5 B for 1%wt HPMCs bulk concentration indicate that more viscoelastic films (lower $\tan \delta$) are formed at the A/W interface. The almost constant $\tan \delta$ indicated that $E_d$ and $\pi$ changed in the same magnitude upon time. $\tan \delta$ values well below 1, reached upon adsorption, indicated that the films had a gel-like structure, which involved the association of the hydrophobic methyl groups of HPMC (Kita et al., 1999), except for E50LV in the oil phase, where a viscoelastic film was formed only at long-term adsorption (8000 s).

Fig. 5C shows that at 1% wt bulk concentration, the solid character of films at the O/W interface increased slowly upon time as discussed previously. E4M formed more elastic films at the A/W interface as shown at the lowest concentration. However film viscoelasticity was lower at the A/W interface because of increasing values of the dilatational viscosity due to film collapse and multilayer formation (Perez et al., 2008). The elastic character of E50LV films (1%wt) in the absence of oil increased sharply after 6000 s of adsorption and reached values higher than those observed at the A/W interface (Fig. 4C). Accordingly, the relative viscoelasticity of the film increased (lower $\tan \delta$), reaching the values observed in the presence of air (Fig. 4D).

The evolution of surface dilatational modulus ($E$) with surface pressure for the adsorption of HPMCs at both interfaces is shown in Fig. 6. If the surface dilatational modulus is only due to the amount of HPMC adsorbed at the interfaces, all $E$ data should be normalized in a single master curve. Fig. 6 shows that this normalization was not possible for any bulk concentration at the two interfaces studied, indicating a non-ideal behaviour and reflecting the impact of macromolecule interactions.

As for other biopolymers, like proteins (Rodriguez Patino, Molina, Carrera, Rodriguez Nin˜o, & An˜on, 2003; Horne & Rodriguez Patino, 2003) and polysaccharides (Baeza et al., 2004), $E$ increased in most cases with interfacial pressure and this dependence reflects the existence of higher interactions within the adsorbed polysaccharide residues. The increase of $E$ with surface pressure, except for E50LV at the A/W interface, is consistent with the increase in interactions between adsorbed HPMC, which are higher at higher time and/or at higher concentration in solution (at higher $\pi$).

Fig. 7 shows the normalization of relative viscoelasticity of films ($\tan \delta = E_d/E_a$) with surface pressure. An almost linear decreased of $\tan \delta$ with surface pressure is apparent, except for E4M at 1%wt, $\tan \delta$ strongly decreased with increasing surface pressure, reflecting the increase of the relative elastic character of gelled films. The increase of $\tan \delta$ with $\pi$ for E4M at 1%wt may be attributed to the collapse and multilayer formation which increases the viscous contribution (Perez et al., 2008) as can be corroborated in Fig. 8 where the viscous component of the dilatational modulus is shown as a function of surface pressure.

Nevertheless strong differences in the behaviour of HPMC at the two interfaces is apparent from Figs. 6 and 7: (a) At the O/W
interface a “gel-like” structure film with strong viscoelasticity (i.e. tan δ = 0.2) is formed at surface pressures much lower than at the A/W interface (i.e. 18 mN/m against 25 mN/M), (b) At the A/W interface the gel-like structure is rapidly attained so that even at the beginning of adsorption low tan δ values are reached (values were always below 0.5). At the O/W interface the adsorption is slower (Table 2) so that the attainments of a gel-like structure.

However, at long-term adsorption, it can be concluded that HPMC interacts more efficiently at the O/W interface as strong viscoelastic films (Fig. 7) or more elastic films (Fig. 6) are formed at lower surface pressures. This result points out the role of the hydrophobic phase in structuring the film due to the participation of interacting triglycerides molecules with hydrophobic segments of HPMC in the structure of interfacial film.


