



Water interactions and microstructure of chitosan-methylcellulose composite films as affected by ionic concentration

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ABSTRACT

Edible films based on high molecular weight chitosan (CH) and methylcellulose (MC) were obtained by mixing different ratios (0:1, 0.5:1.5, 1.0:1.0, 1.5:0.5 and 1:0) of the biopolymers in two solvent conditions (0.95 and 6.85 mmol of ions per g polymer). In order to characterize the dry films, water sorption isotherms, water vapour permeability and film microstructure were evaluated. Water vapour permeability of CH-MC composite films was significantly affected by both the CH-MC ratio and the ionic concentration in the matrix. This can be attributed to the influence of ions on polymer chain packaging during the film formation and their role in the water uptake capacity of the films which affects the water transport properties.

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1. Introduction

Chitosan is a biodegradable cationic polymer with antimicrobial activity (Cuero, 1999; Jung & Kim, 1999; No, Park, Lee, & Meyers, 2001; Tharanathan & Kittur, 2003; Zheng & Zhu, 2003) and film-forming ability (Li, Dunn, Grandmaison, & Goosen, 1992). However, the uses of chitosan based films are limited because of their high water vapour permeability (Butler, Vergano, Testin, Bunn, & Wiles, 1996; Caner, Vergano, & Wiles, 1998) that can be improved by combining chitosan with other materials that soften its hydrophilic character (Park, Marsh, & Rhim, 2002; Vargas, Albors, Chiralt, & González-Martínez, 2009; Xu, Kim, Hanna, & Nag, 2004). To this end, Hoagland and Parris (1996) developed chitosan/pectin laminated films by interacting cationic groups of chitosan with the anionic groups of pectin. Xu et al. (2004), observed a decrease in water vapour transmission rates by combining chitosan with two thermally gelatinized corn starches. Pinotti, Garcia, Martino, and Zaritzky (2007) studied the mechanical properties and the microstructure of chitosan (CH) based films as affected by methylcellulose content (MC) and García, Pinotti, Martino, and Zaritzky (2004) evaluated the water vapour permeability of these CH-MC composite films, which had intermediate permeability values

compared to the films of pure components. However, to the best of our knowledge, there are no reported studies into the water sorption isotherms of such films and their relationship with their water barrier properties and film microstructure, which in turn are affected by the solvent conditions (ionic strength, pH, etc.) used to obtain the film-forming solutions of polymers.

The permeability of a film involves solubilisation and diffusion of water molecules through the film matrix. Biopolymers such as chitosan and methylcellulose are plasticized by water due to their inherent hydrophilic nature. The water vapour uptake of hydrocolloid films depends on both the chemical structure of the components and on their morphology. This is particularly significant for chitosan, since its conformation in solution greatly depends not only on structural parameters like the degree of acetylation and chain length but also on solution parameters, such as ionic strength, solvent, temperature, pH, dielectric constant of the solvent, etc. (Sorlier, Viton, & Domard, 2002). In this sense, Thongngam and McClements (2005) showed that the addition of NaCl to chitosan-sodium taurocholate systems weakened the electrostatic interactions between the bile acid and the chitosan, thereby reducing the driving force and favouring polymer binding.

The purposes of this study are to obtain composite edible films based on chitosan and methylcellulose and to evaluate the effect of the ratio of both polymers and the solvent conditions to obtain the film-forming solutions on the water sorption isotherms, microstructure and water vapour permeability of the dry films.

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2. Materials and methods

2.1. Materials

High molecular weight chitosan (CH), with a deacetylation degree of 82.7% (CAS Number 9012-76-4, Sigma–Aldrich, Batch 14624DB, Brookfield viscosity of 919 mPa s at 1 g/100 g in 1 g/100 g acetic acid solution); Methylcellulose, MC (50.6 kDa, Methocel A15 Food Grade, degree of substitution: 1.8, Dow Chemical Company, Midland, USA), 98% glacial acetic acid, NaOH, and Tween 80 (Pan-reac, Barcelona, Spain), were used to obtain the film-forming solutions (FFSs).

2.2. Preparation of film-forming solutions

Chitosan (2 g/100 g) was dispersed in an aqueous solution of glacial acetic acid (1 mL/100 g), and the pH was adjusted with NaOH (2 mol L⁻¹) to 3.8 (solvent I) or 5.2 (solvent II). Methylcellulose (2 g/100 g) was dispersed in a sodium acetate buffer solution (175 mmol L⁻¹) at pH 3.8 or 5.2. Concentration of buffer was calculated to obtain a similar ionic strength to that of chitosan solutions. Chitosan and methylcellulose solutions were mixed in different proportions to obtain the following CH-MC ratios: 0:1, 0.5:1.5, 1.0:1.0, 1.5:0.5 and 1:0. The ratio ions-polymer in solvent I and solvent II were 0.95 and 6.85 mmol/g polymer, respectively. Tween 80 at 0.1 mL/100 g was added to the aqueous solutions to improve wettability. A pH-metre C831 (Consort, Tumbout, Belgium) was used to determine the pH of the FFSs at 25 °C.

2.3. Preparation of films

Films were obtained by casting. FFSs were poured into a framed and levelled polytetrafluorethylene plate ($\varphi = 15$ cm) and were dried at room temperature for 48 h. Films were prepared by pouring the amount of FFS that will provide a surface density of polymer in the dry films of 56 g/m². Dry films were peeled off from the casting surface and preconditioned in desiccators at 5 °C and 58.5% RH prior to testing.

2.4. Film thickness

Film thickness was measured using a hand-held digital micrometre (Palmer -Comecta, Spain, ± 0.001 mm). The thickness of each film was measured five times and the mean values were used in water vapour permeability calculations.

2.5. Water sorption isotherms

For the adsorption experiments, film pieces of about 3 cm in diameter were transferred to vacuum chambers containing P₂O₅ to complete drying. Afterwards, film specimens, in triplicate, were placed at 5 °C in hermetic chambers containing oversaturated salt solutions with different water activity (a_w): LiCl (0.11), MgCl₂ (0.34), K₂CO₃ (0.43), Mg(NO₃)₂ (0.59), NaBr (0.64), KI (0.73), NaCl (0.76), KCl (0.88). Samples were weighed periodically till a constant value ($\Delta m \approx 0.0005$ g) was reached, where the equilibrium was assumed to be achieved (Spiess & Wolf, 1983). Finally, the equilibrium

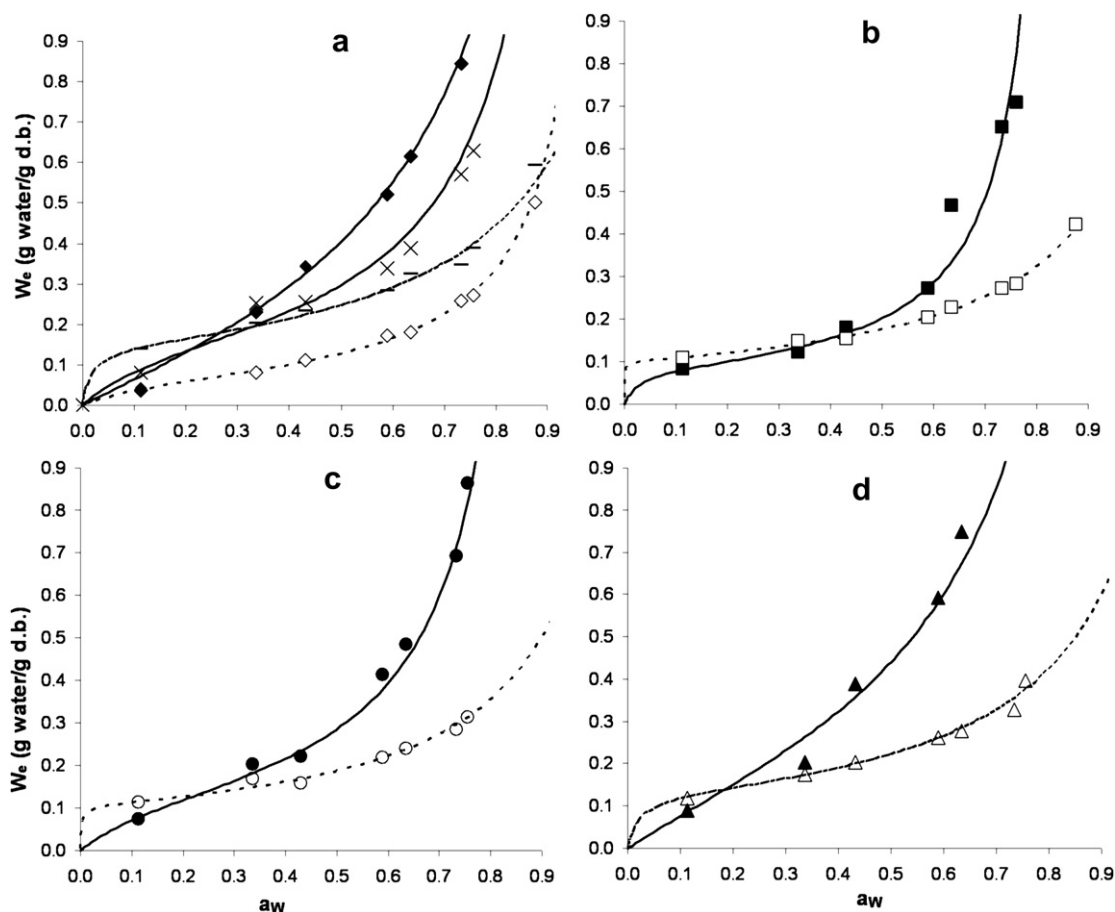


Fig. 1. Water sorption isotherms (experimental points and GAB fitted model) of pure films (a): \blacklozenge MC solvent I, \bullet MC solvent II, $-$ CH solvent I, \times CH solvent II, and composite films (b–d) obtained from solvent I (hollow symbols) and solvent II (solid symbols); (b) 0.5CH-1.5MC, (c) 1.0CH-1.0MC, (d) 1.5CH-0.5MC. CH: chitosan; MC: methylcellulose.

moisture content was determined using a vacuum oven at 70 °C for 24 h. Moisture sorption isotherms (equilibrium moisture content dry basis vs. water activity) were fitted by means of BET and GAB models (Eq. (1) and (2)) and model parameters were obtained.

$$\frac{a_w}{(1 - a_w) \cdot W_e} = \frac{1}{W_0 \cdot C} + \frac{C - 1}{W_0 \cdot C} \cdot a_w \quad (1)$$

$$\frac{a_w}{W_e} = \frac{1}{W_0 \cdot C \cdot K} + \frac{C - 2}{W_0 \cdot C} \cdot a_w + \frac{K \cdot (1 - C)}{W_0 \cdot C} \cdot a_w^2 \quad (2)$$

Where, W_e equilibrium moisture content on a dry basis (g water/g dry film) W_0 adsorbed monolayer moisture content (g water/g dry film)

C constant related to thermal effects

K factor related to the total heat of sorption of the multilayer

a_w water activity.

2.6. Water vapour permeability

Samples of dry films ($\phi = 7$ cm) were stored in desiccators at 58.5% relative humidity (RH) and 5 °C for at least 15 days prior to the evaluation of the water vapour transmission rate. Water vapour permeability (WVP) was measured, in quintuplicate, according to the “water method” of the ASTM E-96-95 (ASTM, 1995), using polymethylmethacrylate cups following the design proposed by Gennadios, Weller, and Gooding (1994). Deionised water was used inside the testing cup to achieve 100% RH on one side of the film, while an oversaturated magnesium nitrate solution was used to control the relative humidity on the other side of the film: 58.5%. During WVP testing, the side of the film in contact with the PTFE plate was placed in contact with the part of the test cup having the highest RH. This situation tries to simulate the case of a film applied on the wet surface of a fresh-cut vegetable or fruit. A fan placed on the top of the cup was used to promote convection. Water vapour transmission rate measurements (WVTR) were performed at 5 °C to reproduce the storage conditions of fresh-cut coated products. To calculate WVTR (Eq. (3)), the slopes of the steady state period of the curves of weight loss as a function of time were determined by linear regression. For each type of film, WVP measurements (Eq. (4)) were carried out in triplicate.

$$WVTR = \frac{J}{A} \quad (3)$$

$$WVP = \frac{WVTR}{P_{w1} - P_{w2}} \cdot L \quad (4)$$

Where

Table 1

BET and GAB parameters obtained from isotherms of films formulated from pure components (CH: chitosan or MC: methylcellulose) and CH-MC mixtures.

Film	Solvent	BET Parameters		GAB Parameters		
		W_0	C	W_0	C	K
MC		0.075	6.2	0.0747	6.8	0.982
0.5CH-1.5MC	I	0.0777	31.6	0.0995	2070.9	0.865
CH-MC		0.0854	58.5	0.1058	445.5	0.877
1.5CH-0.5MC		0.1063	71.1	0.1285	54.6	0.875
CH		0.1249	350.7	0.1464	66.3	0.839
MC		—	—	0.28	3.35	0.95
0.5CH-1.5MC	II	—	—	0.0844	29.0	1.182
CH-MC		—	—	0.1469	6.1	1.094
1.5CH-0.5MC		—	—	0.3149	2.6	0.981
CH		—	—	0.1684	6.8	1.008

W_0 : (g water/g dry solids); C , K : constants related to the heat of sorption of the monolayer and the multilayer, respectively.

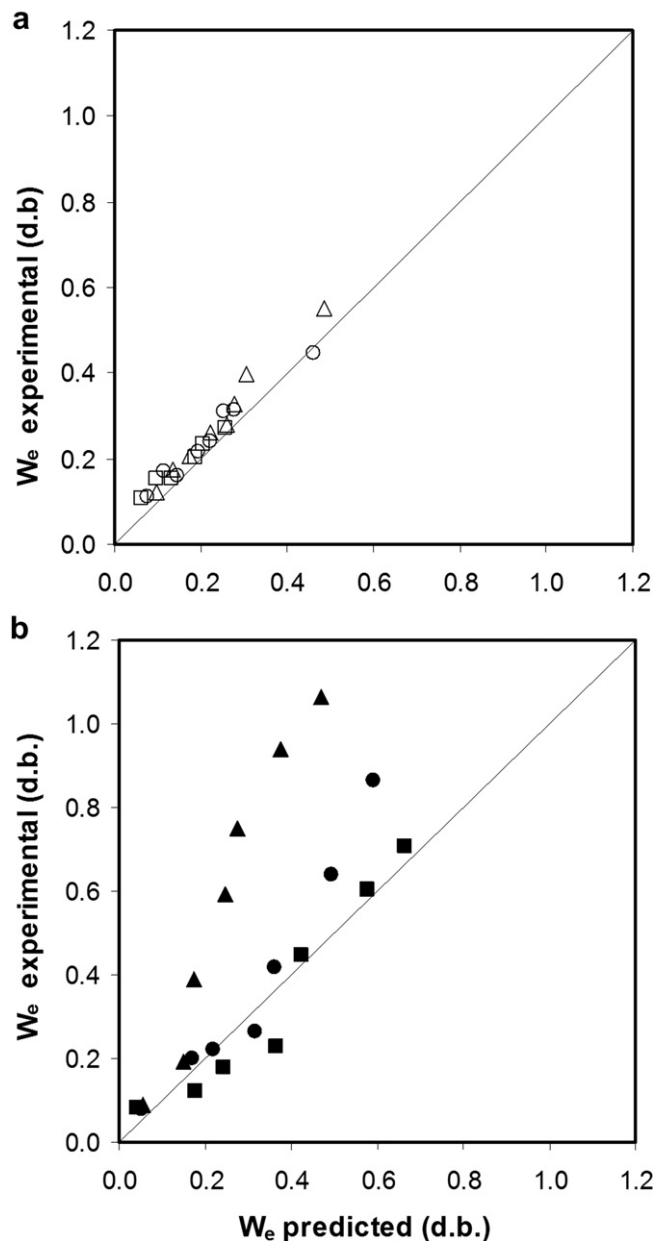


Fig. 2. Comparison between experimental values of equilibrium moisture content and those predicted by the linear model for the different composite films. (a) \square 0.5CH-1.5MC, \circ 1.0CH-1.0MC, \triangle 1.5CH-0.5MC, solvent I (b) \blacksquare 0.5CH-1.5MC, \bullet 1.0CH-1.0MC, \blacktriangle 1.5CH-0.5MC, solvent II. CH: chitosan; MC: methylcellulose.

WVTR water vapour transmission rate, $g\ h^{-1}\ m^{-2}$

J slope of the plotting of weight loss vs. time, $g\ h^{-1}$.

A area of the film, m^2

P_{w1} partial pressure of water vapour on the film's underside, Pa

P_{w2} partial pressure of water on the film's upper surface, Pa

L film thickness, m.

2.7. Scanning electron microscopy

Dry films were stored in a desiccator with P_2O_5 for at least 15 days. Microstructural analysis of cross-sections of the films was carried out using SEM technique in a JEOL JSM-5410 (Japan) electron microscope. Pieces of 5×1 mm were cut from films and mounted in copper stubs. Samples were gold coated and observed using an accelerating voltage of 10 kV.

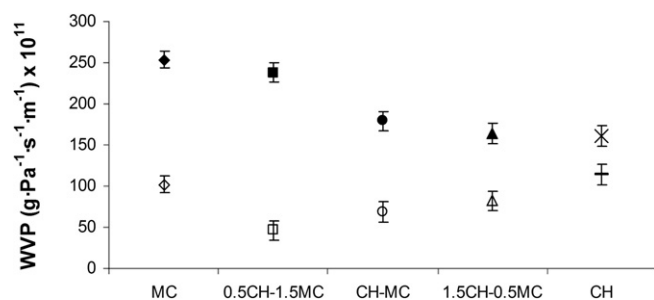


Fig. 3. Water vapour permeability at 5 °C and 100/59 RH gradient of pure films (◆ MC solvent I, ♦ MC solvent II, – CH solvent I, × CH solvent II) and CH-MC composite films obtained from solvent I (hollow symbols) and solvent II (solid symbols). Mean values and 95% LSD intervals. CH: chitosan; MC: methylcellulose.

2.8. Statistical analysis

Results were analysed by multifactor analysis of variance with 95% significance level using Statgraphics® Plus 5.1. Multiple comparisons were performed through 95% Least Significant Differences (LSD) intervals.

3. Results and discussion

3.1. Water sorption isotherms

The water sorption isotherms (WSI) at 5 °C of pure CH and MC films together with the composite films prepared by mixing CH and MC at different proportions and conditions are shown in Fig. 1a–d. The WSI of the films obtained with solvent I (pH = 3.8) conditions were sigmoid in shape, increasing slowly in line with a_w up to 0.45, beyond which a steep rise in moisture content was observed, owing to the solubilisation phenomenon. This sigmoid shape was also observed by other authors in pure CH films (Del Nobile, Buonocore, & Conte, 2004; Wiles, Vergano, Bunn, & Testin, 2000). According to Fernández Cervera et al. (2004) CH has three predominant adsorption sites: hydroxypropyl group, amine group and polymer chain end (composed of a hydroxyl group or aldehyde group).

For films obtained by using solvent II (pH = 5.2), a change in the sorption behaviour of the matrix was observed and the shapes of the isotherms were more typical of those systems rich in low molecular weight or ionic compounds, such as salts and sugars (Okos, 1985), and exhibited a greater equilibrium moisture content for intermediate and high water activity values, whereas at low water activity, the opposite trend was observed. This change in the WSI could be explained by taking into account the sorption effect of the greater amount of ions per g of polymer, which were incorporated to fit the pH of the FFSs at 5.2. The presence of ions increases the water sorption capacity of the matrix at intermediate and high a_w levels, as ions interact with water molecules and solubilisation phenomenon occur to a greater extent in low molecular charged species. At low water activity, the predominant adsorption on the active sites of the polymer network occurs and, in this sense, the more neutral character of the CH chains in solvent II (pH near isoelectric point) can explain the lower equilibrium water content in this case.

For pure films from solvent I, the CH films presented the highest water sorption capacity due to their greater hygroscopic nature. In solvent II, the opposite trend was observed due to the greater amount of ions added and their particular interactions with the polymer chains. Whereas the acetate groups are partially neutralized by the positive groups of the CH molecules, this neutralization does not occur in the non-ionic chain of MC. Therefore, pure MC from solvent II presented the greatest water adsorption capacity,

due to the presence of sodium and acetate ions, which are non-neutralized with polymer chain groups and so more available to interact with water molecules. The effect of charged groups in the polymer chains and the counterions has been studied by some authors (Berthold, Desbrières, Rinaudo, & Salmén, 1994; Jouon, Rinaudo, Milas, & Desbrières, 1995). A notable effect of dissociation degree of the chain groups and type of counterions was observed on the water sorption behaviour. For uncharged polymers such as CMC only tightly interacting water molecules are adsorbed and non-freezing water was detected at the equilibrium water contents in the complete RH range, which indicate the scarce mobility of the adsorbed water. Nevertheless, for charged polymers such as CH, freezing water was detected for equilibrium moisture contents corresponding to relative humidity below 100%, thus indicating that a fraction of water molecules are bonded to the ionic groups showing greater mobility. Water mobility degree will affect the WVP of the films.

Experimental sorption data were fitted to GAB and BET models and the results are reported in Table 1. To fit the GAB model, the second-degree polynomial equation was used for the regression analysis (Eq. (2)). Due to the high degree of mathematical correlation among the three GAB parameters (Schär & Rüegg, 1985) their physical meaning is not considered, although the model predicted values are used to plot water sorption isotherms (Fig. 1) over the whole a_w range. On the other hand, the BET equation constants (obtained for data with $a_w < 0.6$), which have a thermodynamic base, were used to interpret the interaction between the components with water molecules. Nevertheless, the fitting of BET model to the isotherm curves did not give coherent values of the parameters in solvent II, due to the hyperbolic shape of the isotherms, and so, they were not considered in this case. The monolayer moisture contents of the CH pure film from solvent I were higher than the values reported in the literature (Fernández Cervera et al., 2004), which can be attributed to the different temperature at which these values were determined (20 °C) and the different nature of the chitosan used (degree of acetylation and molecular weight). For CH-MC composite films obtained in solvent I BET parameters, W_0 and C , increased when the CH content increased, due to the greater hygroscopic nature of this polymer and to the stronger interactions established between water molecules and the substrate at low a_w values, where no solubilisation phenomenon occur.

In order to analyse interactions between MC and CH in the composite films, the equilibrium moisture content of these films at each a_w was compared with the value obtained by applying Eq. (5). In this equation, W_e was obtained from the equilibrium value of pure CH and MC films and its ratio in the dried film.

$$W_{est|a_w} = X_{CH} \cdot W_{eCH} + X_{MC} \cdot W_{eMC} \quad (5)$$

Where,

$W_{est|a_w}$ equilibrium moisture content of the composite film (d.b.) at a constant a_w .

X_{CH} mass fraction of chitosan in the composite dry film.

X_{MC} mass fraction of methylcellulose in the composite dry film.

W_{eCH} equilibrium moisture content of pure chitosan film (d.b) at a constant a_w .

W_{eMC} equilibrium moisture content of pure methylcellulose film (d.b) at a constant a_w .

Fig. 2 shows experimental versus predicted equilibrium moisture content for CH-MC composite films. For solvent I, predicted and experimental W_e values for composite films are located very close to the diagonal, although experimental values are slightly greater than predicted, which can be explained by the adsorption effects of the small amounts of ions. This seems to indicate that no interactions between film components could be assumed and that

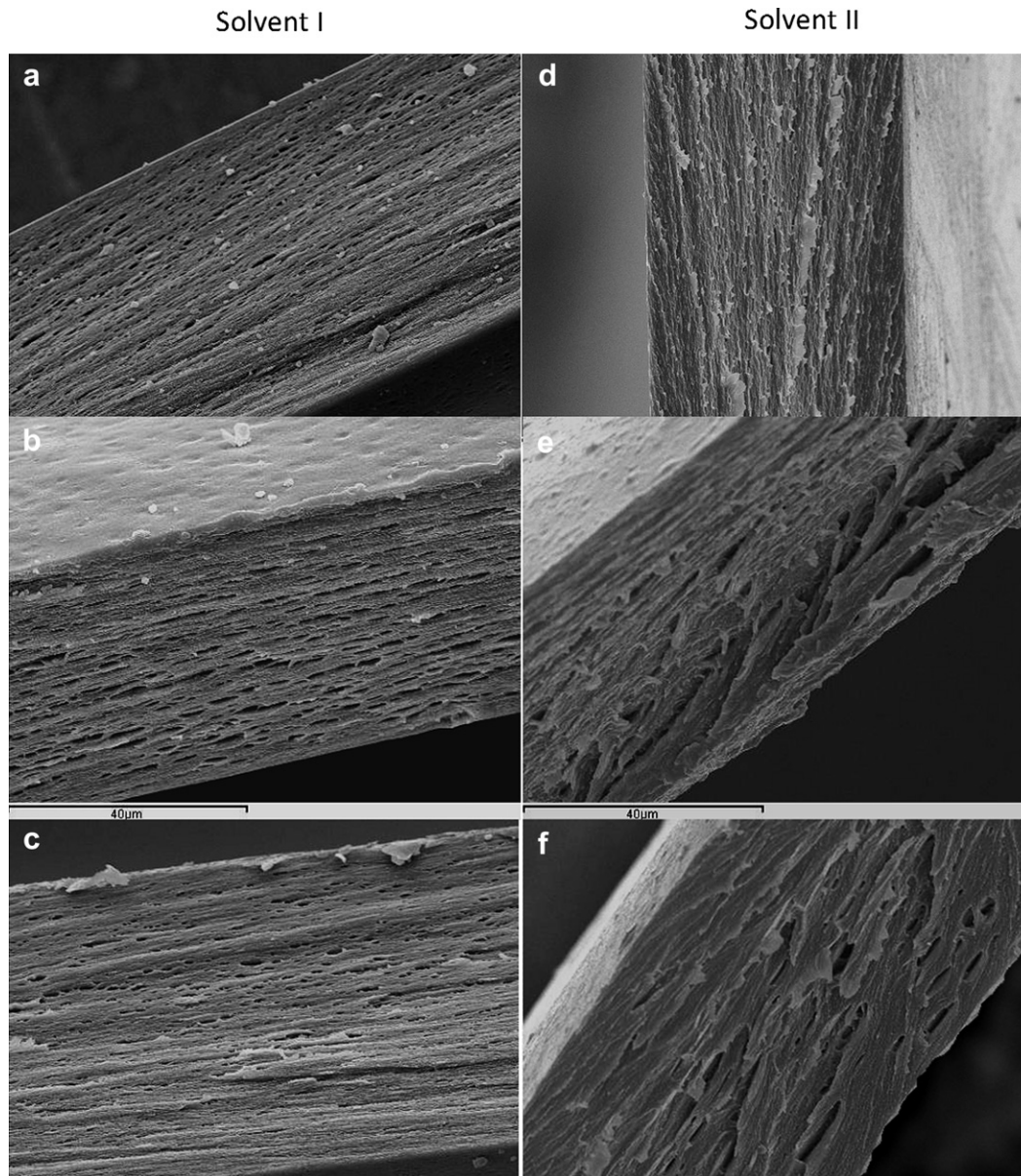


Fig. 4. SEM micrographs of the cross-sections of CH-MC composite films: (a, d) 0.5CH-1.5MC; (b, e) 1.0CH-1.0MC (c, f) 1.5CH-0.5MC obtained from solvents I (a,b,c) and II (d,e,f). Magnification is 1500 \times . CH: chitosan; MC: methylcellulose.

they are mostly in separate phases, as the observed behaviour can be explained by taking only into account the sorption behaviour of pure polymers and their mass fraction in the mixture.

When using solvent II, experimental values are greater than the predicted ones, especially for the film containing a greater proportion of CH. This indicates that the ions present play an important role in the water adsorption capacity of the film, modifying the molecular interactions in the matrix. In this sense, it is important to point out that the ratio between the amount of ions and the amount of chitosan changes in the different polymer mixtures, which can play an important role in the sorption behaviour of the composite.

3.2. Water vapour permeability

The relative humidity conditions used for the measurement of water vapour permeability (100/59) of the films were established

to simulate the environmental conditions when the films are applied as a coating for food stored at 5 °C. The average thickness of the films and standard deviation (σ) was 71 μm ($\sigma = 25$) for solvent I conditions and 127 μm ($\sigma = 29$) for films prepared with solvent II. Likewise, there was an observed tendency of the film thickness to increase in line with a greater ratio of CH in the matrix. The increase in the film thickness indicates that the packaging of polymer chains in the dried films is less compact when ionic concentration is greater due to the more folded macromolecular conformation in the initial solution, which made the chain aggregation more difficult when solvent is eliminated.

As seen in Fig. 3, films obtained by using solvent II presented higher WVP than those obtained with solvent I. As can be seen in the sorption isotherm curves, the water content of solvent II films is greater than that of solvent I films, at the relative humidity values corresponding to the WVP experiments (0.59–1.0 a_w range). This higher water content will plasticize the film structure, and, as

commented above, water bonded to ions or ionic groups have greater mobility (freezing water). These factors favour water molecule mobility and diffusion, thus contributing to increase WVP. Likewise, the ions hinder the polymer chain packaging due to their conformational effects in solution, giving rise to more folded tails which affects their arrangement in solid phase when the solvent is evaporated and which leads to more open microstructures, where the water transport is made easier. The greater film thickness obtained for solvent II films coincides with this effect. The promotion of WVP by the increase of the ionic concentration in the film is especially marked for pure MC films, probably due to the neutral character of the polymer which does not favour the bonding and adsorption of the ions on the chain, which are freer in the solvent of the FFSs, thus having a greater effect on the molecular conformation. In this sense, it is remarkable that the ionic concentration did not affect WVP to so great an extent in pure CH films which can be explained by the tight link and adsorption of ions on the chain's charged groups and their more limited availability in the solvent. In the CH-MC films with higher ionic concentration, the WVP decreased as the CH increased in the mixture, in agreement with the above-mentioned effect. The WVP values for pure CH films at the 100/59 RH gradient were in the range of those reported by Park and Zhao (2004) and Vargas et al. (2009).

3.3. Microstructure

Fig. 4 shows the SEM micrographs of composite films obtained from solvents I and II. As has been commented above, from the WVP data, films prepared with solvent I show a more compact multilayered structure where a more regular packaging of polymer chains can be observed. Nevertheless, in films prepared with solvent II a greater degree of polymer chain folding occurs which hinders the regular and lineal aggregation of the polymer chains. This leads to a more open matrix, which facilitates the water transport.

4. Conclusion

Water vapour permeability and water sorption capacity of CH-MC composite films were significantly affected by both the CH-MC ratio and the ionic concentration in the matrix. This is explained by the influence of ions on the macromolecular conformation in the FFSs and its effect on the polymer chain packaging during the film formation. Likewise, the ions also play an important role in the water uptake capacity of the film at intermediate and high a_w values, which contributes to a differing degree of matrix plasticization, thus affecting the water molecule mobility and consequently the water transport properties.

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