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Effect of concentration, temperature and plasticizer content on rheological properties of sodium caseinate and sodium caseinate/sorbitol solutions and glass transition of their films

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Abstract

The rheological properties of sodium caseinate film-forming solutions were studied in terms of concentration, temperature and plasticizer content. The shear stress–shear rate plot showed typical Newtonian behaviour in all of the studied systems. The effect of temperature on the viscosity was described by the Arrhenius equation, where the activation energy values obtained varied from 7.5 to 12.1 kJ mol^{-1} as the concentration changed from 10.5 to 13.0% (w/w), respectively. The viscosity increased with the protein concentration and followed a power law equation. The presence of plasticizer in the sodium caseinate film-forming solutions decreased the viscosity and the partial specific volume due to the protein dehydration and also induced a more ordered structure, as observed by circular dichroism spectroscopy. In consonance with the solution behaviour, the glass transition temperature, for solid films of sodium caseinate, decreased with the sorbitol content. Apparently, the hydrogen bonding formation between the sorbitol and the amino acid side chain decreased the protein-protein interaction, leading to a higher mobility of the protein chain.

Keywords: Sodium caseinate; Film-forming solution; Sorbitol; Viscosity

1. Introduction

Interest in the development of edible films and coatings for foods in recent years has been due to different aspects, such as consumer pressure in relation to health, food quality, convenience, increased purchasing power, and more proactive attitudes toward reducing the environmental impact of packaging wastes (Siew, Heilmann, Easteal, & Cooney, 1999). The technology, functionalities and potential uses of edible coatings and films have been reviewed (Gennadios, Weller, Mchugh, & Krochta, 1993; Kester, & Fennema, 1986; Krochta & De Mulder-Johnston, 1997; Miller & Krochta, 1997). Edible films and coatings are generally formed from a solution or dispersion of the film-forming agent, followed by any of the several methods available for separating it from the solvent. Additives (plasticizers), such as sorbitol, glycerol or poly(ethylene glycol), are in general used to enhance the film's flexibility and extensibility (Yang & Paulson, 2000).

Milk and whey proteins have been extensively studied as film-forming agents owing to their excellent nutritional value and their numerous functional properties, which are important for the formation of edible films (Mezgheni, D'Aprano, & Lacroix, 1998). The functionality of a protein is determined by its physical and chemical properties. The solubility of sodium caseinate in water, for example, is minimal in the pH range 3.8–4.0. The protein undergoes a conformational change following heat treatment at such pH (JahaniavaL, Kakuda, Abraham, & Marcone, 2000). For the same protein, Guo, Fox, Flynn, and Kindstedt (1996) reported that viscosity, relative hydrophobicity, and foaming and emulsifying capacity are reduced by heating up to 132 °C. In contrast, solubility and foam stability are increased by heating at the same temperature. Through

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densimetric investigations, Gekko and Timasheff (1981) have shown that the addition of certain proteins to the mixed solvent (glycerol-water) increases the chemical potential of the glycerol, and, in consequence, decreases the interaction between glycerol and proteins.

Despite considerable research on the rheological behaviour of caseinate emulsion gels (Chen, Dickinson, & Edwards, 1999), dispersions of high concentrations (Hayes, Southby, & Muller, 1968), and the development of biological films for food packaging, only a few studies have reported on the properties of film-forming solution (Siew et al., 1999). Thus, the purpose of this work was to determine the effect of temperature, concentration and plasticizer content on the rheological behaviour of aqueous film-forming solutions of sodium caseinate. Different aspects related to the conformational changes of sodium caseinate in solution and the corresponding behaviour of the glass transition temperature of the protein solid films were analysed.

2. Materials and methods

2.1. Materials

Commercial sodium caseinate (Alanate 180) was donated by the New Zealand Milk Products Company. The specified composition was 93.0% protein, 0.1% lactose, 1.0% lipid and 1.8% minerals. Aqueous solutions of sodium caseinate with concentrations of 10.0, 10.5, 11.0, 11.5, 12.0, 12.5 and 13.0% (w/w) were prepared by stirring for two hours at room temperature. The pH of the aqueous solutions was monitored and it was observed that the values remained constant at 6.5. The solutions were used immediately after preparation or up to 2 days at a maintained temperature of 4 °C. The viscosity of the solutions did not change during the mentioned period.

Solutions of sodium caseinate/sorbitol were prepared in the concentrations of 10.0, 12.0 and 13.0% (w/w) of sodium caseinate. The sorbitol concentration was varied in the range of 0.2–20 g for 100 g of solution.

The films for the TMA measurements were prepared from the caseinate/sorbitol homogeneous solutions by casting, at room temperature.

2.2. Rheological behaviour

Triplicate samples of sodium caseinate and sodium caseinate/sorbitol solutions were analysed at 17, 20, 25 and 30 °C in a Brookfield Rheometer (Mod. LVDV-IIICP) using CP-52 cone plate geometry [volume sample = 0.5 ml; cone angle = 3.0° and ϕ (diameter) = 24 mm]. The rheometer was connected to a thermostatted bath. The range of shear rate, 100–300 s^{-1}, was used because it covered all the concentrations and temperatures using the same cone plate geometry. Data analysis was performed using the Rheocalc32 software.

2.3. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed under denaturing conditions in a discontinuous gel system composed of 4 and 10% acrylamide stacking and running gel (Laemmli, 1970). Sodium caseinate extract was re-suspended in 50 mM Tris–HCl (pH 6.8), 1 mM EDTA, and 1 mM DTT, at a final concentration of 1mg ml^{-1}. Samples ranging from 0.5 to 10 μg were re-suspended in SDS sample buffer (25 mM Tris–HCl pH 6.8, 1% SDS, 5% v/v glycerol, 0.1 M DTT, 0.001% w/v bromophenol blue) and loaded into a vertical gel electrophoresis device (Gibco BRL), at 100 V for 5h. The gel was stained with Coomassie Blue and dried between cellophane sheets.

2.4. Partial specific volume

The partial specific volume was determined by the density values and the corresponding total volume of the solutions. A weighed quantity of sodium caseinate was added to a known mass of water and water–sorbitol solutions, and the total volume was obtained from the total weight and solution density. The density was determined directly in an Anton Paar densimeter, Mod. DMA 48.

2.5. Circular dichroism (CD)

For the CD measurements, solutions of sodium caseinate/sorbitol and sodium caseinate (1 mg ml^{-1}) were prepared in milipore quality water. All solutions were filtered using a Milipore filter (0.22 μm). Circular dichroism spectra were recorded on a Jobin Yvon Spex CD6 (Group Instruments S.A.), using a thermostatted (25 °C) cuvette of 1 mm pathlength. The results were expressed as molar ellipticity [θ] using Eq. (1):

$$[\theta] = 3300.\Delta\mathrm{Abs}/c.l \quad (1)$$

where ΔAbs is the observed difference in absorbance for the left and right circular components of the incident light, l is the pathlength (in cm), and c is the concentration in mol l^{-1}.

2.6. Glass transition temperature

The glass transition was determined by thermomechanical analysis (TMA) considering the variation of the linear thermal expansion coefficient (α) of the films, defined in Eq. (2):

$$\alpha = \frac{\mathrm{d}L}{\mathrm{d}T}\frac{1}{L_0} \quad (2)$$

where L_o is the original length of the sample and dL/dT is the slope of the TMA curve (Hatakeyama & Quinn, 1994; Reading & Haines, 1995). The slope of the TMA curves, and in consequence α, changes abruptly at the glass transition temperature of the films. The T_g values correspond to the temperature at which the extrapolations of the curves cross over each other (Hatakeyama & Quinn, 1994; Reading & Haines, 1995). The samples were heated at a rate of 10 °C min^{-1}, under a dry nitrogen flow-rate of 50 ml min^{-1}, from 25 to 220 °C. The samples were also dehydrated before testing, in order to avoid the plasticizing effect of the water content. The measurements were carried out on a Thermomechanical analyzer (TMA 50, Shimadzu) using a quartz staff.

3. Results and discussion

3.1. SDS-PAGE electrophoresis

SDS-PAGE electrophoresis traces for sodium caseinate solutions are shown in Fig. 1. SDS binds to hydrophobic portions of a protein, disrupting its folded structure and promoting an extended conformation in solution. From this point of view, the length of the SDS-protein complex can be considered as proportional to its molecular weight. The electrophoretical patterns of the sodium caseinate solutions studied in this work showed two main fractions of casein, which are in agreement with the main fractions α_{s1} and β present in the milk casein. These fractions correspond to a molecular weight of ca. 25 kDa. Two other weak signals, probably corresponding to α_{s2} and κ, were observed at the same molecular weight.

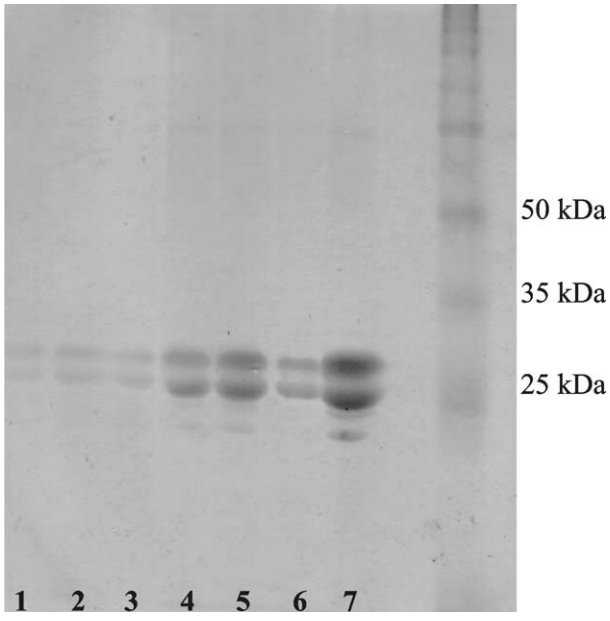

Fig. 1. SDS-PAGE gel of sodium caseinate. Columns 1–7 represent a gradient of concentrations.

3.2. Viscosity of sodium caseinate solutions

Fig. 2 shows the rheological behaviour for the 13% (w/w) sodium caseinate solutions with respect to Eq. (3):

$$\tau = \eta\ \dot{\gamma} \tag{3}$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), and η is the viscosity (Pa s). The plots of shear stress versus shear rate give straight lines characterizing Newtonian behaviour, in which the viscosity corresponds to the slope, showing that the viscosity increases with the sodium caseinate concentration increase (data not shown) and decrease with temperature (Fig. 2). For all the plots in Fig. 2, the linear coefficients considering Eq. (3), were equal to zero, confirming the Newtonian behaviour. For example, the linear regression fit for the solution of 13% sodium caseinate at 17 °C was $y = 0.10125x$ ($r = 0.9999$) with $\eta = 101.25$ mPa s. All the other solutions at different temperatures and concentrations showed similar behaviour (Newtonian behaviour) with the linear regression coefficient close to the unity ($r \cong 1$). The viscosity increase is in fact associated with the protein–water interactions, leading to a higher solvent immobilization than protein–protein interaction. Similar results have been obtained by Konstance and Strange (1991) for solutions of casein and caseinates in the presence of various salt types and pH.

The effect of the temperature on the solution viscosity has been described by many authors (Maskan, 1999; Özilgen & Bayindirli, 1992; Wang, Sun, Zeng, & Lu, 1999) in terms of the Arrhenius-type relationship [Eq. (4)]:

$$\eta = \eta_o \exp(E/RT) \tag{4}$$

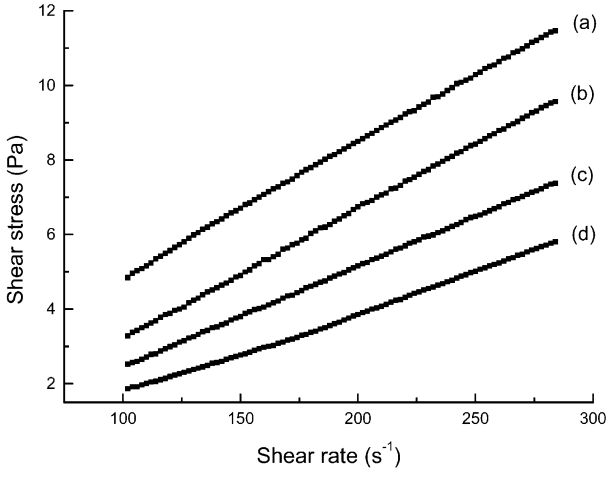

Fig. 2. Rheograms of solutions of sodium caseinate (13%) at temperatures of (a) 17, (b) 20, (c) 25 and (d) 30 °C.

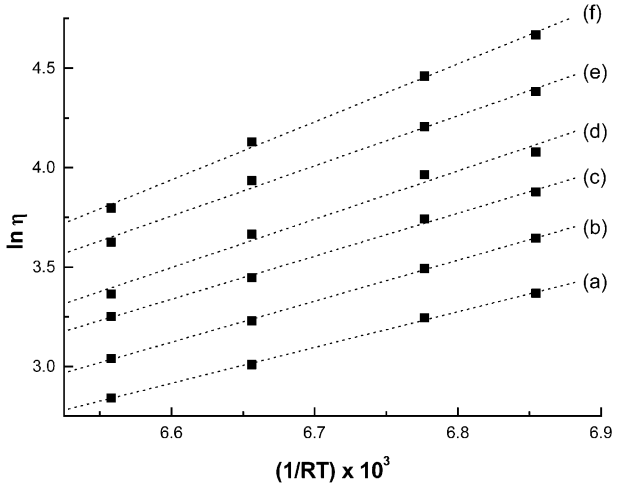

Fig. 3. Effect of temperature (Arrhenius plot) on the viscosity of sodium caseinate solutions at concentrations of (a) 10.5, (b) 11.0, (c) 11.5, (d) 12.0, (e) 12.5 and (f) 13.0%.

where η represents the viscosity, η_o the pre-exponential factor, E the activation energy, R the universal gas constant, and T the absolute temperature. From the slope of the plot of ln η versus $1/RT$ (Fig. 3), the E for the flow was determined for various sodium caseinate solutions (Table 1). The analysis of the behaviour depicted in Fig. 3 and the parameters in Table 1 suggests that the E of the flow increases with solution concentration. At the same time, the effect of the temperature on the decrease in viscosity is more accentuated at higher concentrations of sodium caseinate solutions.

The effect of concentration and temperature on the viscosity of sodium caseinate solution was also analysed with respect to the power law model represented by Eq. (5), where η_1 and A_1 are constants.

$$\eta = \eta_1(C)^{A_1} \tag{5}$$

The experimental results (plot of log η versus log C, where C is the concentration) at different temperatures are shown in Fig. 4. It may be observed that the viscosity increases with protein concentration at all the temperatures studied. Fig. 4 also shows that the viscosity of all the solutions decreases with increasing temperature.

Table 1
Parameters determined by considering Fig. 3 and Eq. (4) for sodium caseinate solutions

Concentration (%)	E (kJ mol^{-1})	η_0 (mPa s)	r
10.5	7.5	1.3×10^{-4}	0.999
11.0	8.8	2.8×10^{-5}	0.999
11.5	9.2	1.8×10^{-5}	0.998
12.0	10.0	3.7×10^{-6}	0.992
12.5	10.5	2.6×10^{-6}	0.997
13.0	12.1	2.3×10^{-7}	0.998

The parameters η_1 and A_1, determined from the intercepts and slopes from Fig. 4, are given in Table 2. The values of A_1 for sodium caseinate (4.4–6.0) are intermediate to the A_1 values for Bengal gran flour suspensions (7.0–7.7) (Bhattacharya, Bhat, & Raghuveer, 1992) and clarified banana juice (2.5) (Khalil, Ramakrishna, Nanjundaswamy, & Patwardhab, 1989). Apparently, the A_1 values are dependent on the type and composition of the solid present in the systems.

3.3. Effect of sorbitol on the viscosity of sodium caseinate solutions

The effect of sorbitol on the viscosity was studied in solutions with 10, 12 and 13% of sodium caseinate. The plots of shear stress versus shear rate of these solutions (not shown) suggested Newtonian behaviour, even when sorbitol was present. With the addition of sorbitol, the effect of the sorbitol content on viscosity (Fig. 5) was slight only in 10% (w/w) of sodium caseinate solutions, i.e. this effect was more accentuated in 12 and 13% (w/w) solutions. Apparently, the presence of sorbitol decreased the effective or hydrodynamic volume of the solutions, thus reducing the viscosity. The decrease in the molar volume for proteins such as ribonuclease A, β-lactoglobulin, lysozyme, bovine serum albumin, or

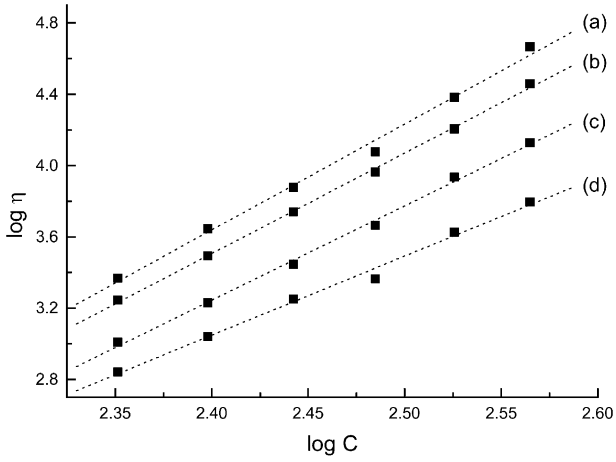

Fig. 4. Effect of concentration on the viscosity of sodium caseinate solutions following the power law equation [Eq. (4)]: (a) 17, (b) 20, (c) 25 and (d) 30 °C.

Table 2
Parameters determined considering the power law model [Eq. (5)] for sodium caseinate solutions

Temperature (°C)	Concentration (%)	η_1 (mPa s/%)	A_1	r
17	10.5–13.0	3.9×10^{-5}	6.0	0.997
20	10.5–13.0	2.2×10^{-5}	5.7	0.999
25	10.5–13.0	6.8×10^{-5}	5.3	0.998
30	10.5–13.0	5.5×10^{-4}	4.4	0.996

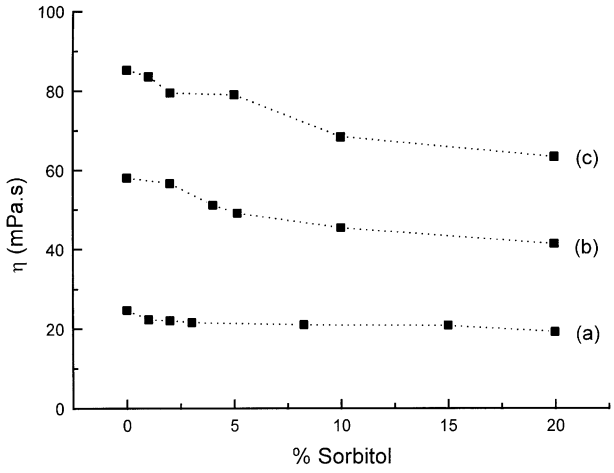

Fig. 5. Effect of sorbitol on the viscosity of sodium caseinate solutions at 20 °C. Concentrations (%): (a) 10, (b) 12 and (c) 13.

α-chemotrypsin in the presence of glycerol has been described by Gekko and Timasheff (1981). These authors did not observe conformational changes by circular dicroism, and the volume variation was attributed to the protein dehydration. On the other hand, the viscosity decrease in solutions of sodium caseinate in the presence of glycerol or poly(ethylene glycol) has been reported by Siew et al. (1999), and these authors attribute the decrease in the protein–protein interactions to the hydrogen bonding formation between the additives and the polypeptide chain of the caseinate and water molecules.

3.4. Circular dichroism

Circular dichroism (CD) spectroscopy was used to determine the changes in the secondary structure of the proteins, considering that the major elements, α-helix, β-sheet and coil, have characteristic CD spectra. The α-helix protein, particularly, has a spectrum that consists of an intense and positive band at 190 nm and negative bands at 208 and 220 nm.

Caseins are an unusual group of proteins, which are neither globular nor fibrous in nature. Despite the high content of hydrophobic amino acids, the casein shows an open and hydrated structure. In general, caseins are described as having a random structure with only small amounts of secondary structures, considering that they are characterized by a high content of proline residues.

Because sorbitol did not show any CD signals in the spectral wavelength range analysed, the CD spectrum observed in the water–sorbitol solutions must be due to the protein (sodium caseinate) alone. Fig. 6 shows the far UV-CD spectrum of sodium caseinate in the presence and absence of sorbitol in the solution. In the CD spectrum of sodium caseinate, the shape of the curve and the minimum ellipticity at ca. 203 nm was attributed to random coil structure. A formal random coil structure has a positive CD band near 215 nm, which is not observed for sodium caseinate in this region. The spectrum in Fig. 6 differs in intensity and shape, indicating the presence of a secondary structure. On the other hand, when the CD spectrum was obtained for the sodium caseinate in water-sorbitol solution, changes in the ellipticity values were observed in the wavelength range of 208–222 nm. The minimum around 203 nm, which was observed for the sodium caseinate in aqueous solution, changes to other minima around 208 and 222 nm. The ellipticity at 222 nm reflects the helical content, suggesting that the sodium caseinate in sorbitol–aqueous solution has a more ordered secondary structure and consequently a more compact structure. According to Subirade, Kelly, Guéguen, and Pézolet (1998) this effect is attributable to a combination of two factors: (i) the high dipole moment of the plasticizers (e. g. alcohols) which induces the disruption of the internal hydrogen bonds of the peptide groups through competition of the OH group (alcohol) and the peptide NH group in the attempt to bind the hydrogen to the amide C=O; and (ii) the low dieletric constant that is able to perturb the structure of the protein by reducing the hydrophobic effect.

With the increase of sorbitol content (0.8 g ml^{-1}), the bands at 190, 208 and 222 nm, which are correlated with the helical content of the protein, are more evident. This behaviour is in agreement with hydrodynamic and hydrostatic measurements, such as viscosity and partial specific volume (see Section 3.5), indicating that there is a conformational change in the protein structure which occurs in the presence of sorbitol and leads to a more compact structure.

3.5. Partial specific volume

The partial specific volume of a macromolecule can provide information about protein-solvent interactions

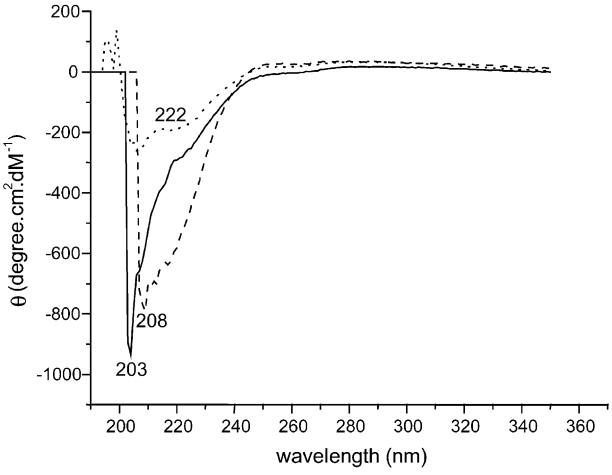

Fig. 6. Far UV-CD spectra of: pure sodium caseinate and sodium caseinate–sorbitol aqueous solutions (solid line) and with 0.3 (dashed line) and 0.8 (dotted line) g of sorbitol/ml.

and protein structure. Hence this volume was determined for the aqueous solutions of sodium caseinate containing sorbitol as an additive. The total volume for a binary solution is defined as shown in Eq. (6):

$$\overline{V} = n_1\overline{V}_1 + m_2\overline{v}_2 \quad (6)$$

where $\overline{V}$ is the total volume for a binary solution (ml), n_1 is the number of moles of the solvent, $\overline{V}_1$ is the partial molar volume for the solvent (ml mol^{-1}), m_2 is the mass of the sodium caseinate, and $\overline{v}_2$ is the partial specific volume (ml g^{-1}) which was determined from the slope of the plot of the solution volume ($\overline{V}$) versus the protein mass. The partial specific volumes were plotted against the mass of sorbitol present in the solution (Fig. 7). As shown, the partial specific volume of sodium caseinate was maximum in water, decreasing with the increase of the sorbitol concentration, and suggesting a change to a less expanded structure. The value of the sodium caseinate partial specific volume (0.732 ml g^{-1}) is in agreement with the value (0.731 ml g^{-1}) reported by Soloshenko, Sergeev, and Bezrukov (1984) for unfractionated casein. The presence of sorbitol in the solution must have disrupted the hydrogen bonding between the sodium caseinate and the water (solvent), decreasing the amount of immobilized water and, consequently, the partial specific volume. This behaviour is also in agreement with the decrease in viscosity discussed above. Similar behaviour has been observed by Kamiyama, Sadahide, Nogusa, and Gekko (1999) for the cytocrome C/sorbitol system.

3.6. Glass transition temperatures of films

Considering that the film-forming solutions' properties must be reflected in the behaviour of the solid film, the glass transition temperature, which can be interpreted as the range of temperatures at which segment motion of macromolecules becomes thermally activated, was analysed. The T_g of proteins increases with the chain rigidity and the intensity of both inter- and intramolecular interactions, including hindrance to internal rotation along the macromolecular chain. An effective plasticizer has to shield the inter- and intramolecular interactions, facilitating the molecular mobility and decreasing the internal friction in the biopolymer material.

The presence of sorbitol had a significant plasticizing effect on the sodium caseinate film (Fig. 8) and the observation of a single glass transition (in the range of studied temperature) reflects a compatibility between sorbitol and sodium caseinate. The observed behaviour was analysed in terms of the Fox and Gordon-Taylor models corresponding to Eqs. (7) and (8), respectively.

$$1/T_g = (w_1/T_{g1}) + (w_2/T_{g2}) \quad (7)$$

$$T_g = (w_1T_{g1} + kw_2T_{g2})/(w_1 + kw_2) \quad (8)$$

In these equations, w_1 and w_2 are the weight fractions of sorbitol and sodium caseinate respectively, and k is the constant related to the inverse of the plasticizing effect of sorbitol. Lower values of k indicated a higher plasticizer effect, and consequently a greater protein–plasticizer interaction. For the CS/S system, the value of k, determined from the experimental values in Eq. (8), was 0.72. This value was similar to those determined for the CS/water system ($k = 0.76$) by Kalichevsky, Blanshard, and Tokarczuk (1993). Both k values were higher than the values determined by the same authors for gluten ($k = 0.20$) and amylopectin ($k = 0.22$) with water as plasticizer. As shown in Fig. 8, the theoretical and experimental values of T_g are almost coincident, with better approximation using the Gordon–Taylor equation, suggesting an interaction between sodium caseinate and sorbitol in the studied composition range. The interaction and, in consequence, the plasticizing effect, could be attributed to the low molecular weight of sor-

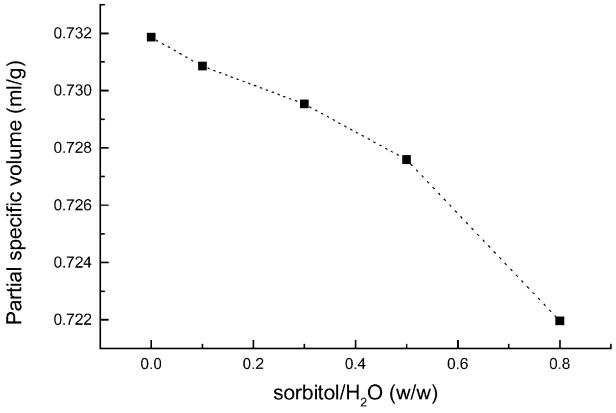

Fig. 7. Partial specific volume of sodium caseinate as a function of sorbitol concentration in solution.

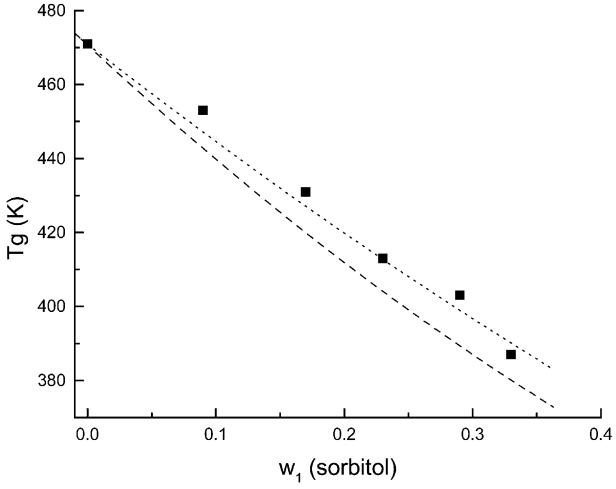

Fig. 8. Glass transition temperature of sodium caseinate and sodium caseinate–sorbitol films as a function of the mass of sorbitol: (square) experimental values and fitting, considering the (dashed) Fox and (dotted) Gordon–Taylor equations.

bitol and the presence of hydroxyl groups, leading to the formation of sodium caseinate-sorbitol interactions, despite the polymer–polymer interactions, increasing the intermolecular spacing as a result. The six hydroxyl groups of sorbitol might interact with lateral residues of sodium caseinate amino acids through hydrogen bonds. These interactions would decrease the partial specific volume of the protein (Fig. 7), allowing a greater backbone chain segmental mobility of the sodium caseinate and leading to lower values of glass transition temperatures, as shown in Fig. 8.

4. Conclusions

The rheological properties of sodium caseinate film-forming solutions, studied in terms of concentration, temperature and plasticizer content, show typical Newtonian behaviour. The effect of temperature on the viscosity is described by the Arrhenius equation. The viscosity increases with the protein concentration, following the power law model. The presence of plasticizer in the sodium caseinate solutions reduces the partial specific volume, leading the sodium caseinate to a more ordered structure, confirmed by CD spectroscopy. The properties of film-forming solutions should be taken into account, since they might reflect the solid film's behaviour. The decrease in the glass transition temperature observed for the solid films of sodium caseinate with the sorbitol addition are related to the properties of the film-forming solutions.

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