



# Influence of calcium and sodium chloride on caseinomacropeptide self-assembly and flow behaviour at neutral pH

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## ABSTRACT

The effect of adding NaCl or CaCl<sub>2</sub> on particle size distribution and flow behaviour of caseinomacropeptide (CMP) aqueous solutions was investigated over a wide range of concentrations (0–200 mmol L<sup>-1</sup>), temperatures (5–60 °C) and during 14 days of storage. In the absence of salts, CMP mainly presented a monomeric form at pH 7.0. If sodium or calcium chloride is added, hydrophobic associations of CMP is promoted due to screening of electric charges. Calcium chloride had a bigger impact than NaCl. Indeed, small variations in CaCl<sub>2</sub> concentration induced major changes in size distributions and increased the viscosity and the cloudiness of CMP solutions upon storage. The largest aggregates and highest viscosities were obtained at concentrated regime (CMP concentration > 8 g/100 g), high CaCl<sub>2</sub> concentration (> 1.2 mmol g<sup>-1</sup> CMP) and upon storage.

## 1. Introduction

Caseinomacropeptide (CMP) is the 64 C-terminal amino acid residue of κ-casein. It is formed by the Phe<sub>105</sub>-Met<sub>106</sub> peptide bond cleavage of κ-casein by chymosin (or pepsin) during cheese manufacture. The soluble CMP (fragment 106–169) is recovered in the sweet whey. CMP peptide chain is lack of cysteine and aromatic residues. The covalent bonds formation through the oxidation of sulfhydryl groups is not possible in CMP. Commercially available CMP consists of a aglyco- and glyco-fraction (Kreulß, Krause, & Kulozik, 2008). The terminal carbohydrate in the oligosaccharide chains of glycosylated CMP is often the N-acetylneuraminic acid (sialic acid) (Saito & Itoh, 1992).

Lacou, Léonil, and Gagnaire (2016) defined the self-assembly as a spontaneous and reversible reaction performed by hydrogen bonds, electrostatic and van der Waals interactions but the covalent bonds such as disulfide bonds are no involve. At neutral pH, the electrostatic repulsive forces of CMP dominate over the hydrophobic interactions being the monomer the predominant form. Lowering pH below 4.5, CMP can self-assemble by hydrophobic dimer formation, followed by electrostatic interactions, which finally lead to the development of a gel matrix (Farías, Martínez, & Pilosof, 2010). This process occurs at ambient temperature, but can be accelerated by heating (Martínez, Farías,

& Pilosof, 2011). CMP self-assembly has been investigated at pH 2.0–9.0 (Farías et al., 2010; Farías & Pilosof, 2016; Loria, Aragón, Torregiani, Pilosof, & Farías, 2018), but not in the presence of salts.

The presence of sodium and calcium ions can strongly influence the flow properties of protein suspensions (Thomar, Benyahia, Durand, & Nicolai, 2014). Also, they can be a vital parameter to control its assembly properties (Loveday et al., 2010; Wang et al., 2018). In a previous work (Loria et al., 2018), using both of viscosity and dynamic light scattering techniques, we characterized different concentration regime on CMP solutions (dilute and concentrated). Also, we analysed the effect of pH (5.0–9.0) and temperature (5–60 °C) on the flow properties and particle size distribution of CMP aqueous solutions. CMP solutions exhibited Newtonian flow dependence, particularly at pH values 5.0–6.0. The results showed the importance of reducing pH (between 5 and 6) and heating to control the viscosity of concentrated CMP solutions.

Salts are present in protein beverage formulations for different purposes (i.e., as flavouring agents, preservatives for coagulations, buffering agents, mineral fortification). The design of beverages high in protein and calcium is a great challenge, especially during processing, storage and temperature fluctuations (Westerik, Scholten, & Corredig, 2015). At this respect, the motivation of this study is related to the

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information searched in the literature about CMP-calcium salts interactions. The negatively-charged sialic acid forms complexes with calcium ion at neutral pH (Jaques, Brown, Barrett, Brey, & Weltner, 1977) and also at acidic pH (Villumsen et al., 2015). Besides, the parental CMP protein,  $\kappa$ -casein, is insensitive to calcium ions at neutral pH (Huppertz, Fox, & Kelly, 2018, pp. 49–92). Here, we report on an investigation about the effect of the presence of NaCl and CaCl<sub>2</sub> (0–200 mmol L<sup>-1</sup>), two common salts found in food formulations, on the self-assembly and flow behaviour of dilute and concentrated CMP solutions at neutral pH. Also, we explored the effect of temperature and storage on flow properties of CMP-salt solutions.

## 2. Materials and methods

### 2.1. Materials

CMP (protein content  $79.9 \pm 2.7$  g/100 g, the conversion factor was 7.07) was provided by Davisco Foods International (Le Sueur, MN, USA). The total N content was determined using Kjeldahl methods of AOAC (2005). Its calcium and sodium contents were 6820 and 9500 mg kg<sup>-1</sup> respectively, determined with an AAnalyst 200 PerkinElmer atomic absorption spectrometer (Shelton, CT, USA).

Analytical grade sodium and calcium chloride were supplied by Mallinckrodt (USA) and ultrapure water was used in the preparation of solutions. Sodium azide was added at 0.02 g/100 g for sample preservation.

### 2.2. Preparation of CMP solutions

CMP solutions (20 g/100 g) were diluted with ultrapure water and/or calcium or sodium chloride solution (0.5 M) to reach the desired salt concentrations (between 0 and 250 mmol L<sup>-1</sup>). Final CMP concentrations were 5 and 12 g/100 g. The required amount of 0.1 or 1 eq/L NaOH was added in order to raise the pH to 7.0. It is worth mentioning, the incorporated sodium ions concentrations at CMP-CaCl<sub>2</sub> solutions to raise the pH were lower than 1.25 mmol L<sup>-1</sup>. CMP is highly soluble in water and no precipitate was formed after adding salt solutions.

### 2.3. Particle size determination and CMP self assembly

Particle size was determined by dynamic light scattering (DLS) (Zetasizer Nano-Zs, Malvern Instruments, Worcestershire UK). Immediately after adjusting the pH to 7.0, samples were filtered using a 0.45, 0.22 and 0.02  $\mu$ m microfilter Whatman International Ltd. (Maidstone, England). The assay was performed in triplicate as reported previously (Fariás & Pilosof, 2016).

### 2.4. Steady shear rheological measurements

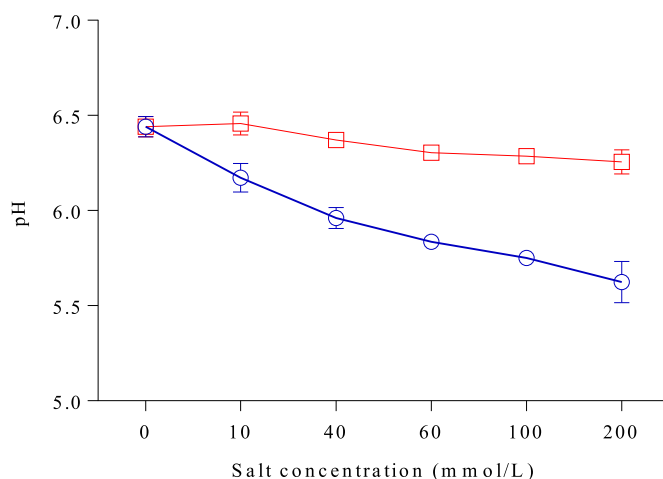
The steady shear rotational tests were conducted in a Physica MCR 301 controlled stress rheometer (Anton Paar, Germany). Flow curves assays were performed as reported Loria et al. (2018) at temperatures from 5 to 60 °C. At the end of 7 days of storage at 25 °C, the flow behaviour of samples was again analysed.

Shear stress ( $\tau$ ) and apparent viscosity ( $\eta$ ) were recorded as a function of shear rate ( $\dot{\gamma}$ ). Shear stress-shear rate data were fitted with the Herschel-Bulkley model (Ahmed & Ramaswamy, 2003). The assays were performed in triplicate.

### 2.5. Color and whiteness index

The color in units CIE L\*a\*b\* of 10 mL CMP-salt solution placed in transparent plastic vessel of length 3.8 cm and diameter 2.9 cm was determined using a portable colorimeter (HunterLab MiniScan EZ Color Spectrophotometer, Reston, VA, U.S.A.)

Color parameters lightness (L\*), redness (a\*), and yellowness (b\*)



**Fig. 1.** Effect of sodium (□) and calcium (○) chloride addition to CMP solution (5 g/100 g) on the pH. The pH was measured immediately after adding the salts (25 °C).

were determined. A standard white plate N° MESEZ0866 was used to calibrate. For whiteness calculations, the following equation (Paker & Matak, 2015) was used:

$$WI \text{ (Whiteness index)} = 100 - [(100-L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

The color of solutions after storage was determined at day 0, 7 and 14 using the method described on Dybowska and Fujio (1998) with modifications. Triplicate analysis of the samples was conducted.

### 2.6. Statistical analysis

Statistical analysis (ANOVA) was performed using GraphPad Prism 7 package for Windows, Version 7.0 (USA).

## 3. Results

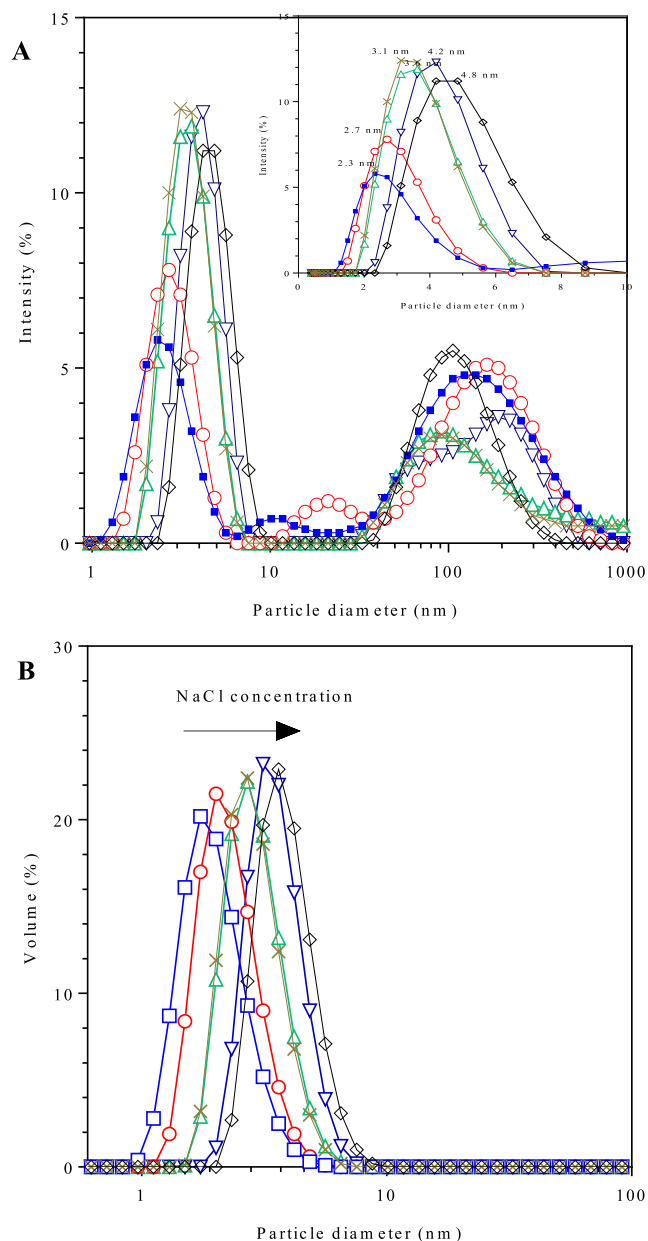
### 3.1. Effect of NaCl and CaCl<sub>2</sub> on the pH of CMP solutions

The addition of calcium chloride to CMP solutions (5 g/100 g) resulted in a decrease of pH from 6.4 in salt-free solution to 5.6 in presence of 200 mmol L<sup>-1</sup> CaCl<sub>2</sub>. However, when sodium chloride was added, the pH slightly decreased (Fig. 1). The decrease of pH with the addition of the calcium salt was probably due to calcium binding to CMP and protons liberation as has been reported previously to milk (Lin, Wong, Deeth, & Oh, 2018) and sodium caseinate solutions (Pitkowski, Nicolai, & Durand, 2009).

### 3.2. Effect of NaCl on CMP self-assembly

The intensity and volume size distributions of CMP solutions at pH 7.0 as affected by sodium chloride addition are shown in Fig. 2. The intensity size distribution on CMP solutions (5 g/100 g, pH 7.0, salt-free) presented three populations (Fig. 2 A). The  $d(H)$  of the predominant size peak was between 1 and 5 nm and the maximum value 2.3 nm, corresponding to the CMP monomer (average Mw about 7500 kDa) as it was previously reported by Fariás et al. (2010). Intensity size distribution is a common approach to present DLS data, nevertheless it can provide a misrepresentative view of multi-modal distributions (Smialowska, Matia-Merino, Ingham, & Carr, 2017).

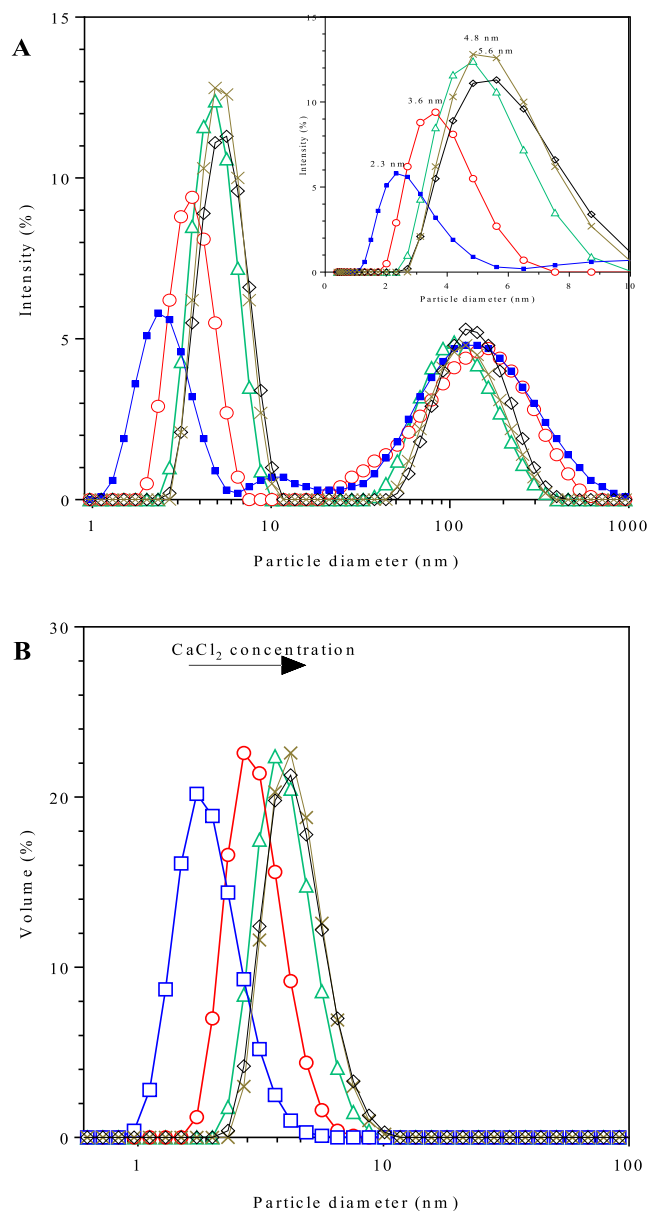
When sodium chloride was added, the  $d(H)$  of the predominant first peak shifted to higher sizes, from 2.3 nm (salt free solution) to 4.8 nm (200 mmol L<sup>-1</sup> NaCl) (Fig. 2 A) indicating an increasing self-assembly of CMP. Associated forms of 40–300 nm were former, but they were negligible as it can be seen in the volume size distribution (Fig. 2 B). These bigger forms can be attributed to CMP micelles (Loria et al., 2018).



**Fig. 2.** Intensity (A) and Volume (B) particle size distributions of CMP solutions (5 g/100 g) at pH 7.0 at 25 °C at different NaCl concentrations: 0 (■); 10 (○); 40 (△); 60 (×); 100 (▽) and 200 (◇) mmol L<sup>-1</sup>. An expanded view of the first peak using a linear scale is inserted in Figure A.

The zeta potential ( $\xi$ ) represents the level of repulsion between molecules in a specific environment. CMP has a strong negative charge,  $\xi = -24.12$  mV (Martinez, Pizones Ruiz-Henestrosa, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2013) in salt free solutions at pH 7.0. The electrostatic repulsion prevents the self-assembly of CMP (Farías et al., 2010) in this condition of pH and ionic strength. When electrostatic repulsion is screened by addition of NaCl, the CMP molecules can self-assemble. Mw estimation by the software of Zetasizer Nano-Zs equipment, allows the estimation of assembled form of CMP. In accordance with values shown in the insert of Fig. 2 A, CMP shifted from the monomeric (salt-free) to the tetrameric form (200 mmol L<sup>-1</sup> NaCl).

A similar behaviour has been reported in the literature for the association of sodium caseinate at pH 6.7 (HadjSadok, Pitkowski, Nicolai, Benyahia, & Moulai-Mostefa, 2008). At very low ionic strength (3 mmol L<sup>-1</sup>), sodium caseinate was mostly present in the form of individual molecules. When electrostatic repulsion was screened by NaCl



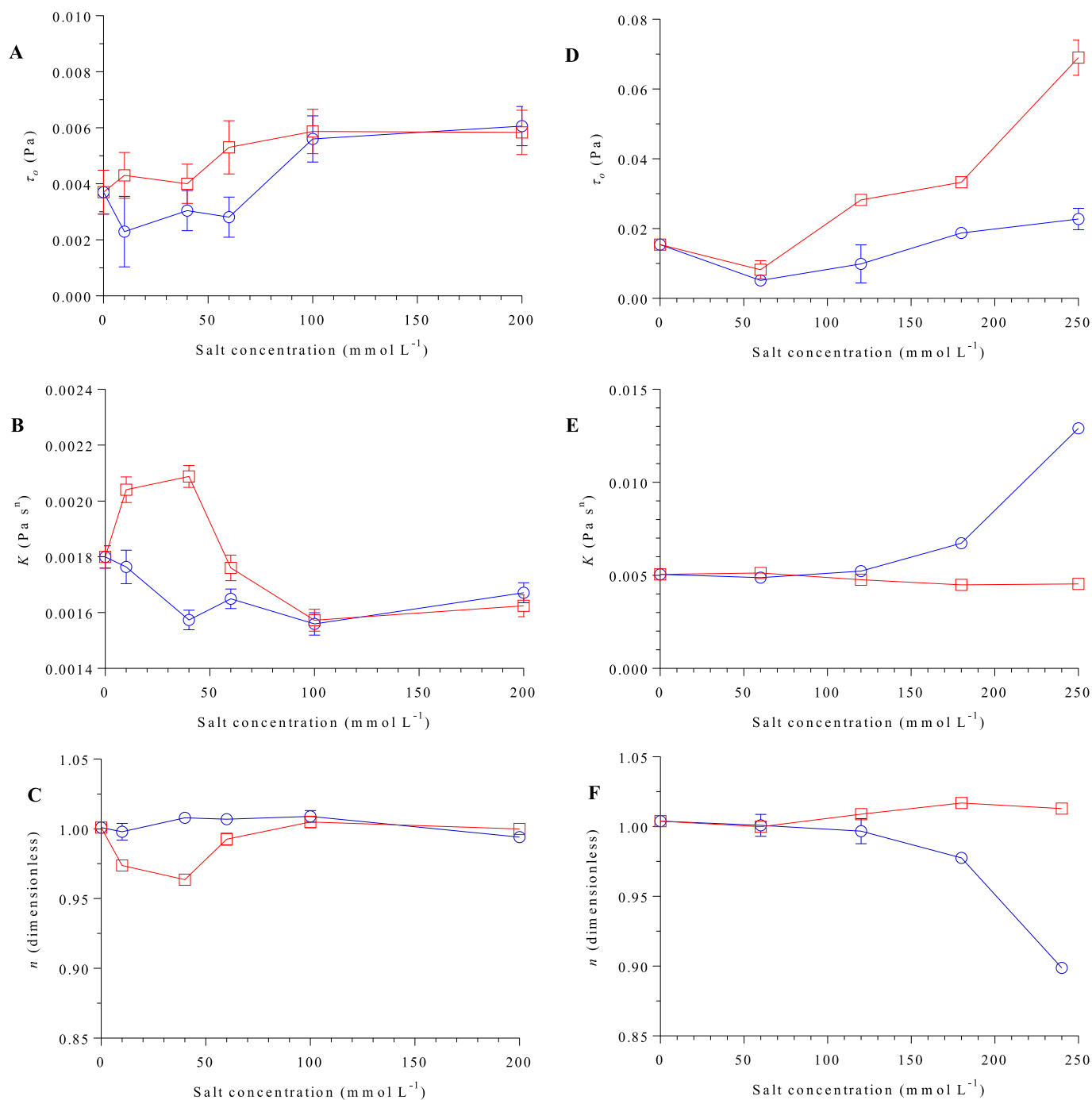
**Fig. 3.** Intensity (A) and Volume (B) particle size distributions of CMP solutions (5 g/100 g) at pH 7.0 at 25 °C at different CaCl<sub>2</sub> concentrations: 0 (■); 10 (○); 40 (△); 60 (×) and 200 (◇) mmol L<sup>-1</sup>. An expanded view of the first peak using a linear scale is inserted in Figure A.

addition ( $> 100$  mmol L<sup>-1</sup>), the casein molecules associated to form small aggregates.

### 3.3. Effect of CaCl<sub>2</sub> on CMP self-assembly

The particle size distribution of CMP solutions as affected by calcium chloride addition is shown in Fig. 3. The  $d(H)$  of the predominant lower size peak in the intensity size distributions exhibited significant changes as the calcium content increased (Fig. 3 A). The  $d(H)$  increased from 2.3 to 5.6 nm (60 mmol L<sup>-1</sup> calcium chloride). At around 60 mM, a critical CaCl<sub>2</sub> concentration was reached (Fig. 3 B), causing a maximum increase in the mean hydrodynamic size (5.6 nm). According to the software of Zetasizer Nano-Zs equipment, this size would correspond to the hexameric form of CMP.

As well the addition of CaCl<sub>2</sub> to sodium caseinate aqueous systems made the formation of micron sized aggregates (Pitkowski et al., 2009).



**Fig. 4.** Effect of sodium (□) and calcium (○) chloride addition to CMP solutions at 5 g/100 g (A, B and C) and 12 g/100 g (D, E y F) on Herschel-Bulkley model fitting parameters:  $\tau_0$  (A and D),  $K$  (B and E) and  $n$  (C and F). Temperature 25 °C, pH 7.0.

According to these authors, this effect was related to the specific binding of calcium by all casein fractions and the reduction of the electrostatic repulsion between molecules.

CaCl<sub>2</sub> producer higher ionic strength than NaCl, and would therefore reduce electrostatic repulsion and enhance self-assembly more effectively at the same molar concentration. However, comparing CMP association degrees in the presence of 60 mM CaCl<sub>2</sub> (ionic strength 180 mmol L<sup>-1</sup>, hexamers) and of 200 mmol L<sup>-1</sup> NaCl (ionic strength 200 mmol L<sup>-1</sup>, tetramers) shows that increasing ionic strength alone did not make greater the CMP association degree. The differences between CaCl<sub>2</sub> and NaCl effects reported here indicate that the nature of the cation has an important influence on the CMP self-assembly.

### 3.4. Effect of salts on CMP flow behaviour

At pH 7.0, the concentration dependence of the viscosity shows two different regimes (dilute and concentrated) being 8 g/100 g the overlap concentration (Loria et al., 2018). The shear dependence of dilute (5 g/100 g) and concentrate (12 g/100 g) CMP-salt solutions is well described by the Herschel-Bulkley model ( $R^2 > 0.998$ ) and the corresponding indexes are showed in Fig. 4. Salt-free CMP solution (5 g/100 g) exhibits Newtonian behaviour, showing the presence of a negligible yield stress ( $\tau_0 < 0.006$  Pa) (Fig. 4 A). When above 100 mmol L<sup>-1</sup> sodium or calcium chloride is added to a CMP solution,  $\tau_0$  index slightly increase. However, no differences were detected below 100 mmol L<sup>-1</sup> sodium or calcium chloride.

**Table 1**Flow activation energy ( $E_a$ ) and  $R^2$  for 5 g/100 g CMP-salts solutions at pH 7 obtained at apparent viscosity of  $100 \text{ s}^{-1}$  in a range of 5–60 °C.

	Salt-free *	40 mmol L <sup>-1</sup> NaCl	40 mmol L <sup>-1</sup> CaCl <sub>2</sub>	100 mmol L <sup>-1</sup> NaCl	100 mmol L <sup>-1</sup> CaCl <sub>2</sub>
$E_a$ (kJ/mol)	20.4 ± 0.9 <sup>a</sup>	20.5 ± 1.8 <sup>a</sup>	21.1 ± 0.7 <sup>a</sup>	19.5 ± 0.8 <sup>a</sup>	20.3 ± 0.1 <sup>a</sup>
$R^2$	0.998	0.996	0.999	0.953	0.999

Data are means ± standard deviations from two replicates. Different superscript letters indicate significant differences in the mean ( $p < 0.05$ ). \*From Loria et al. (2018).

A gradual decrease in  $K$  index with increasing calcium chloride concentration is observed when calcium chloride is added to a dilute CMP solution (Fig. 4 B). An increase in  $K$  index and a slight decrease in  $n$  index by increasing sodium chloride concentration were observed initially at low concentrations ( $< 40 \text{ mmol L}^{-1}$ ) followed by a decrease to values close to those noticed for calcium chloride addition (Fig. 4 C). As more sodium (up to  $100 \text{ mmol L}^{-1}$ ) or calcium chloride (up to  $40 \text{ mmol L}^{-1}$ ) were added, the  $K$  indexes essentially became independent of salt concentration.

When sodium chloride was added to a concentrated CMP solution,  $\tau_0$  values increased from 0.02 to 0.08 Pa, but they were not affected by CaCl<sub>2</sub> addition (Fig. 4 D). However, calcium addition strongly increased the  $K$  index of CMP solutions above 120–150 mmol L<sup>-1</sup> (Fig. 4 E). Concomitantly, the  $n$  index decreased (Fig. 4 F) Nevertheless,  $K$  and  $n$  indexes were not modified by sodium chloride addition.

Also the presence of NaCl or CaCl<sub>2</sub> can strongly modify the rheological properties of caseinate suspension (Pitkowski et al., 2009; Thomar et al., 2014). Concentrate sodium caseinate suspensions showed a tendency to increase viscosity when NaCl was added (Carr & Munro, 2004). However, adding a small quantity of CaCl<sub>2</sub> causes an increase of the viscosity, in spite of the fact that a large quantity can cause insolubility and precipitation of a fraction of the protein causing a decrease of the viscosity (Pitkowski et al., 2009; Thomar et al., 2014).

Cations as sodium or calcium can interact with carboxyl groups on Asp and Glu residues (abundant in CMP chain), C-terminal carboxylic groups or peptide carbonyls of chain (Loveday et al., 2010), glycosylated moieties and phosphorylated Thr. When salt (NaCl or CaCl<sub>2</sub>) is added to a dilute CMP solution, a decrease in viscosity is expected due to individual peptide chains assuming a smaller overall configuration. The chains have enough space between each other that they do not often come into close enough contact to interact. However, as polymer concentration increases (concentrated regime), the presence of CaCl<sub>2</sub> allows the formation of bridges between the different associate CMP forms. It well known that divalent positively-charged calcium acts as a bridge between negatively charged proteins (Westerik et al., 2015).

The viscosity of several polyelectrolytes (xanthan, carrageenan and gelan) has been measured in free and high salt solutions by Wyatt, Gunther, and Liberatore (2011). At low polymer concentrations (dilute regime), the zero shear rate viscosity decreases as much as 100-fold upon addition of a monovalent salt, namely NaCl. A viscosity increase has been observed for many different concentrated polyelectrolytes (chitosan, xanthan in potassium form, carrageenan, welan) solutions after NaCl addition (Wyatt et al., 2011). However,  $K$  and  $n$  indexes of concentrate CMP solutions did no change with increasing NaCl concentration (Fig. 4E and F).

The release into solution of a fraction of originally CMP-bound Ca<sup>2+</sup> (approximately 8.5 mM for 5 g/100 g CMP) by Na<sup>+</sup> competition could explain the increasing of  $K$  values by increasing NaCl concentration below  $60 \text{ mmol L}^{-1}$  in Fig. 4 B. According to Thomar et al. (2014), adding NaCl may be considered as equivalent to removing a fraction of caseinate-bound Ca<sup>2+</sup> suggesting a competition between Ca<sup>2+</sup> and Na<sup>+</sup> for the binding sites of peptide chain, which causes attractive interaction between small caseinate particles and an increase of the caseinate suspension viscosity.

### 3.5. Effect of temperature on viscosity of CMP-salts solutions

Viscosity of CMP-salts solutions decreases with increasing temperature, as salt-free CMP solutions (Loria et al., 2018). The temperature effect on the apparent viscosity,  $\eta_{100}$ , of dilute CMP-salt solutions (5 g/100 g) at a shear rate of  $100 \text{ s}^{-1}$  was modelled by an Arrhenius-type equation which could be expressed by

$$\ln \eta_{100} = \ln A + E_a/RT$$

where T was the Kelvin Temperature, A was a constant,  $E_a$  was the flow activation energy and R was the universal gas constant.

The  $E_a$  represents the temperature sensitivity of the apparent viscosity (Karataş & Arslan, 2016). According to Table 1, the presence of NaCl or CaCl<sub>2</sub> (40 or 100 mmol L<sup>-1</sup>) in CMP solutions did not have a significant effect on  $E_a$ . The magnitude of  $E_a$  was approximately  $20 \text{ kJ mol}^{-1}$ , similar to that reported in a previous work (Loria et al., 2018), that was also independent on concentration and pH. These finding confirmed that CMP spontaneously form micelles at pH  $> 4.5$  even in the presence of salts.

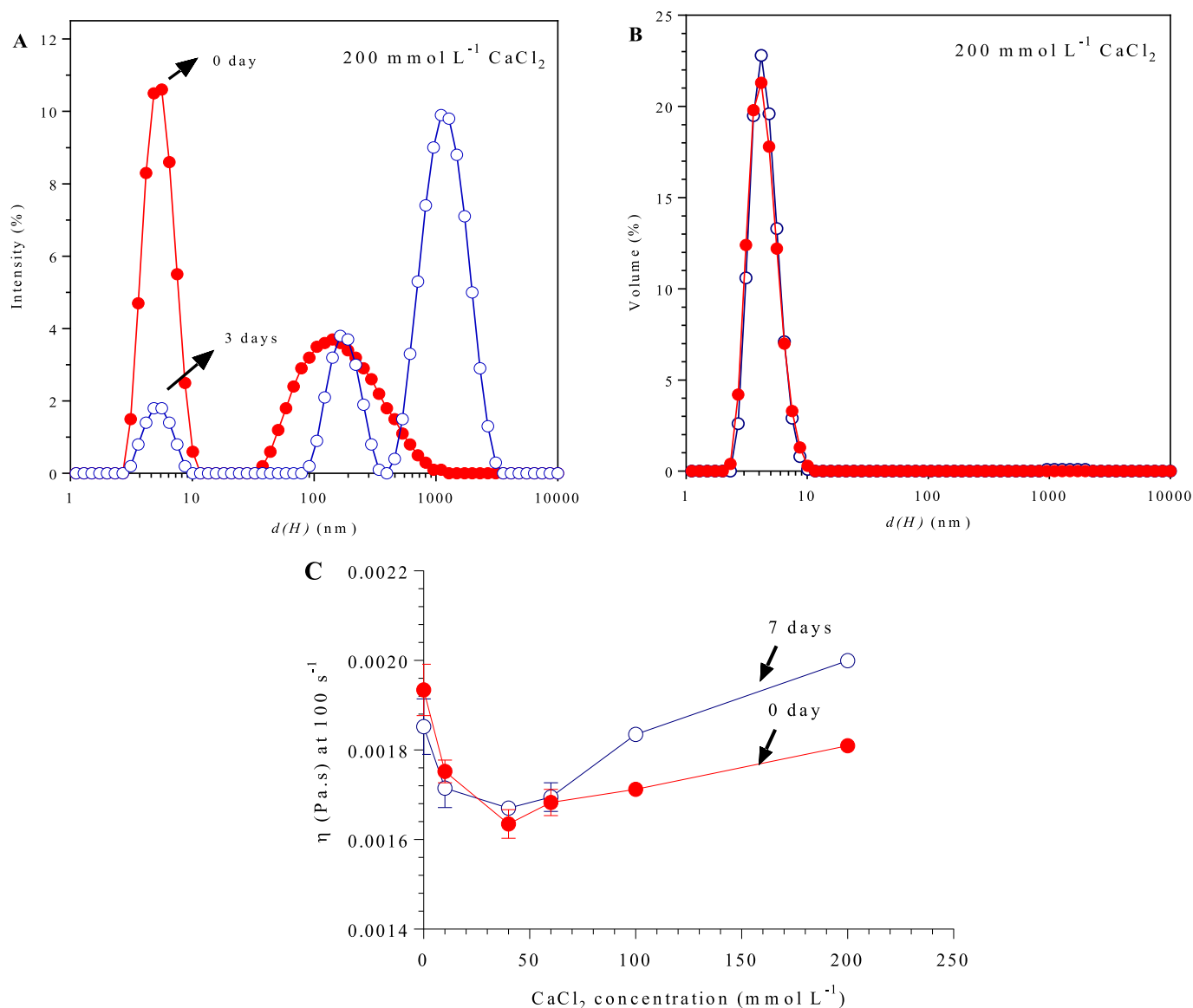
### 3.6. Effect of storage on CMP-salts solutions

It is of interest to know if salt induced CMP self-assembly at pH 7.0 leads to large associate structures as it occurs at acidic conditions (pH  $< 4.5$ ) (Farías et al., 2010; Martinez et al., 2011). Exploring self-association of concentrated regime of CMP solution can be challenging because it is not possible to measure by DLS technique. Therefore, the measurements were done on dilute solutions (5 g/100 g). Fig. 5 A shows the particle size intensity distribution of CMP in presence of  $200 \text{ mmol L}^{-1}$  CaCl<sub>2</sub> after 3 days at 25 °C. The predominant lower size peak decreased its intensity and associated forms of size higher than 1000 nm were formed over time. The number of these associated forms was practically negligible as shown by the volume size distribution plot (Fig. 5 B). Nevertheless, a very small volume peak near 1000 nm appeared, indicating the progress of CMP self-assembly and the formation of larger structures. On the other hand, CMP particle size distributions were stable over time at pH 7.0 in presence of NaCl (data not shown).

To further understand the time-dependent effect in more detail, the 5 g/100 g CMP-CaCl<sub>2</sub> solutions were stored 7 days at 25 °C and the flow properties were determined (Fig. 5 C). Accordingly with steady flow studies, the  $\eta_{100}$  increased at 7 days above 100 mM CaCl<sub>2</sub>.

Initially, the 12 g/100 g CMP solutions were transparent at pH 7.0 and 0–240 mmol L<sup>-1</sup> NaCl, whereas the samples with 100–240 mmol L<sup>-1</sup> CaCl<sub>2</sub> became cloudy (Fig. 6 A). The evolution of the whiteness index, WI, of 12 g/100 g concentrated CMP-salts solutions was used as an indicator for the progress of aggregate formation under storage. The results in Fig. 6 A, regarding the effects of NaCl, showed that the WI were stable under storage. The drastic increase for WI of CMP-CaCl<sub>2</sub> solutions over storage (Fig. 6 B) was in agreement with the particle size distribution changes of DLS studies (Fig. 5A and B). A higher WI is related to a greater degree of light scattering caused by an increase in the size of associated form of CMP in presence of CaCl<sub>2</sub>.

Fig. 6 C presents apparent viscosity at  $100 \text{ s}^{-1}$  of CMP solutions (12 g/100 g) and 0–250 mmol L<sup>-1</sup> NaCl. Clearly, the viscosity was not affected by the presence of NaCl or storage in agreement with WI index.



**Fig. 5.** Effect of storage time 0 (●) and 3 (○) days on intensity (A) and volume (B) particle size distributions of 5 g/100 g CMP solutions in presence of 200 mmol L<sup>-1</sup> CaCl<sub>2</sub> at pH 7.0 and 25 °C. C) Apparent viscosity at 100 s<sup>-1</sup> of 5 g/100 g CMP solutions increasing calcium chloride concentrations at 0 (●) and 7 (○) days of storage. Data are given as mean  $\pm$  standard error (temperature 25 °C).

On the other hand, Fig. 6 D shows that viscosity strongly increased over storage for CMP-CaCl<sub>2</sub> solutions.

In the presence of NaCl, CMP was able to associate probably via hydrophobic interactions (Farías et al., 2010) forming stable associate forms with a size below at 10 nm. The DLS measurements indicated that when the CaCl<sub>2</sub> concentration was above 0.8–1.2 mmol g<sup>-1</sup> CMP, the solution has enough calcium to reduce the electrostatic repulsion between the CMP associated forms allowing more growth. These larger structures with a radius of about a micron affected the transparency and the viscosity of the solutions under storage.

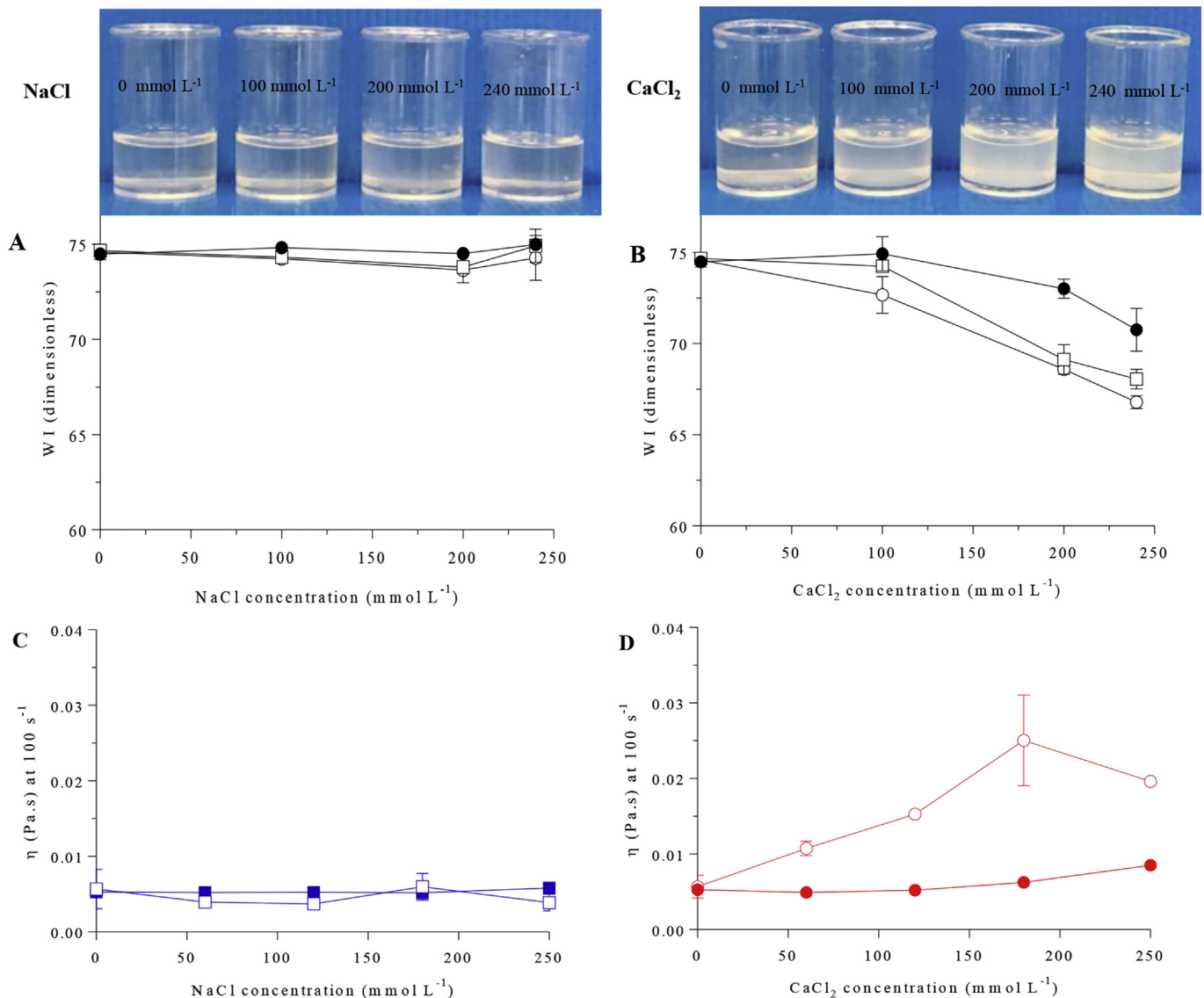
#### 4. Conclusion

In conclusion, the self-assembly of CMP is controlled by complicated mechanisms that are influenced by pH, presence and type of salt and concentration. CMP is present in the form of individual molecules at pH 7.0. If sodium or calcium chloride is added, the electrostatic charges are screened and the hydrophobic parts of the CMP molecules can associate. Calcium chloride has a bigger impact than NaCl. Indeed, small variations in CaCl<sub>2</sub> concentration induced major changes in size

distributions and increased the viscosity and the cloudiness of CMP solutions upon storage. CMP solutions allow the incorporation of large amounts of CaCl<sub>2</sub>, indicating that it can be an excellent alternative to increase calcium bioavailability in foods. However, the questions how the CMP associated forms bind calcium ions need to be examined in future research. The use of CMP may be a new tool towards the development of novel food and beverages and targeted calcium delivery vehicles with additional nutritional and bioactive benefits.

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**Fig. 6.** Appearance of suspensions and whiteness index (WI) on 12g/100g CMP solutions increasing sodium (A) and calcium (B) chloride concentrations immediately after adjusting to pH 7.0 (●) under storage: day 7 (□) and day 14 (○). C) Effect of storage time 0 (●) and 7 (○) days on apparent viscosity at 100 s<sup>-1</sup> on 12g/100g CMP solutions increasing sodium (C) and calcium (D) chloride concentrations. Data are given as mean ± standard error (temperature 25 °C).

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