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Influence of sodium hexametaphosphate addition on the functional properties of milk protein concentrate solutions containing transglutaminase cross-linked proteins

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ABSTRACT

The functional properties of milk protein concentrate (MPC) powders are often hindered by their poor solubility. Calcium chelating salts have been shown to improve powder solubility, but generally their action contributes to higher viscosity due to disintegration of casein micelles and higher levels of serum-phase calcium. To help mitigate increases in viscosity associated with calcium chelation, transglutaminase (TGase), an enzyme that covalently crosslinks protein, was employed in an effort to stabilise the casein micelle structure. Sodium hexametaphosphate (SHMP) was added to control (C-MPC) and TGase crosslinked MPC (TG-MPC) dispersions at concentrations of 5, 12.5 and 25 mM prior to analysis. TG-MPC dispersions had lower viscosity than C-MPC dispersions across all SHMP concentrations studied. Crosslinking limited micelle dissociation on SHMP addition and led to greater retention of the white colour of the protein dispersions, while the turbidity of C-MPC dispersions decreased with increasing SHMP addition.

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1. Introduction

High protein dairy based powders, such as milk protein concentrate (MPC), milk protein isolate, micellar casein isolate and whey protein isolate, are increasingly being used as ingredients in value-added dairy products such as beverages, yoghurts and infant formulae. MPC powders are manufactured from skim milk using ultrafiltration, followed by diafiltration and possible evaporation, prior to spray drying to create high protein powders that are transported globally (Mistry, 2002; Sikand, Tong, Roy, Rodriguez-Saona, & Murray, 2011).

One of the greatest challenges encountered during the processing of milk protein concentrates is the high viscosity after ultrafiltration and evaporation. This high viscosity can be caused by the concentration of proteins and an increase in bound moisture due to denaturation/aggregation of protein during heat treatment (Henriques, Gomes, & Pereira, 2017). In a study by Ho et al. (2018), it was shown that high heat treatment temperatures resulted in a significant ($P < 0.05$) increase in viscosity from 36.3 to 74.8 mPa s. Consequently, such high protein systems are typically evaporated to relatively low total solids content, compared with skim milk, prior to drying (Bienvenue, Jiménez-Flores, & Singh, 2003; Rupp, Molitor, & Lucey, 2018; Vélez-Ruiz & Barbosa-Cánovas, 1998).

A common challenge in using MPC powders is their poor rehydration properties, which is primarily due to the close proximity of protein molecules and the hydrophobic nature of the casein protein constituent (Crowley, Desautel, Gazi, Kelly, Huppertz, & O'Mahony, 2015; de Kruif, Huppertz, Urban, & Petukhov, 2012; Holt, Carver, Ecroyd, & Thorn, 2013; Horne, 2006; Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2010). Several different formulation and technological approaches have been investigated to improve rehydration of MPCs, such as ultrasonication of liquid MPC, calcium depletion using ion

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exchange and addition of calcium chelating agents (Bhaskar, Singh, & Blazey, 2007; McCarthy, Kelly, Maher, & Fenelon, 2014; McCarthy, Power, Wijayanti, Kelly, Mao, & Fenelon, 2017).

The use of calcium chelating salts has been shown to improve the dissolution of MPC when added during the rehydration process (McCarthy et al., 2017); however, their addition to MPC systems can further contribute to viscosity, resulting in processing challenges during evaporation, spray drying or in end use applications. Calcium chelators work by sequestering calcium from the aqueous phase, causing a change in the electrostatic environment and depletion of calcium from within the casein micelle, through alteration of the calcium equilibrium. Calcium depletion causes increased hydration and subsequent swelling of casein micelles due to reduced structural rigidity (de Kort, Minor, Snoeren, van Hooijdonk, & van der Linden, 2011; McCarthy et al., 2017; Omoarukhe, On-Nom, Grandison, & Lewis, 2010; Power, Fenelon, O'Mahony, & McCarthy, 2019). Polyphosphate-based chelators, such as sodium hexametaphosphate (SHMP), have multiple calcium-binding sites, and therefore can simultaneously bind calcium in the serum phase and calcium from colloidal calcium phosphate (CCP) nano clusters within casein micelles. Through interactions with colloidal calcium, SHMP can also crosslink caseins via calcium phosphate complexes, thereby further increasing viscosity of MPC dispersions (De Kort, Minor, Snoeren, Van Hooijdonk, & Van Der Linden, 2009, 2011; Lucey & Horne, 2018; Mizuno & Lucey, 2007).

The enzyme transglutaminase (TGase) can be used to stabilise the casein micelle. Previous work by O'Sullivan, Kelly, and Fox (2002a,b) showed that TGase alters the heat stability of milk by crosslinking individual casein proteins and preventing dissociation of κ-casein from the micelles. Further research by Smiddy, Martin, Kelly, de Kruif, and Huppertz (2006) and Moon, Hong, Huppertz, Fox, and Kelly (2009) showed that casein micelles incubated with TGase had increased stability against micellar disruption by urea, sodium

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dodecyl sulphate or heating in the presence of ethanol, with stability increasing progressively with incubation time. Therefore, enzymatic crosslinking by TGase could be used to restrict increases in viscosity caused by greater hydration and micelle swelling in MPC samples treated with calcium chelators and also prevent destabilisation of the casein micelle. Thus, this study aimed to control micelle stability and maintain a lower viscosity of MPC dispersions in the presence calcium chelating agents by prior enzymatic crosslinking of casein proteins.

2. Materials and methods

2.1. Materials

Milk protein concentrate (MPC) powder (casein:whey protein ratio of 81:19) was obtained from a local dairy ingredient manufacturer and had protein, moisture, fat, lactose and ash content of 81.4% (w/w), 4.30% (w/w), 1.40% (w/w), 5.1% (w/w) and 7.8% (w/w), respectively. Transglutaminase enzyme preparations (Activa MP, Ajinomoto enzyme preparations) were sourced from Healy Group (Cookstown Industrial Estate, Tallaght, Co. Dublin, Ireland). Sodium hexametaphosphate (CAS number: 68915-31-1) was obtained from Sigma Aldrich (Vale Rd, Ballyraine Lower, Arklow, Co. Wicklow, Ireland).

2.2. Rehydration of milk protein concentrate powder and crosslinking of casein proteins

MPC powder was rehydrated (250 g sample at 10%, w/w, protein) as per the method outlined by Power et al. (2019). Sodium azide (0.02%, w/w) was added to MPC dispersions to prevent microbial growth. Proteins were covalently cross-linked using the enzyme

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transglutaminase (TGase) as described previously by Huppertz and de Kruif (2008). MPC dispersions (10%, w/w) were preheated to 30 °C and incubated with Activia TGase (0.5 g L^{-1}) for 24 h at pH 6.5. Following incubation, the enzyme was inactivated by heating at 80 °C for 5 min. A control dispersion was prepared and treated using the same procedure without the addition of TGase enzyme. Control MPC and enzymatically-crosslinked MPC dispersions are abbreviated to C-MPC and TG-MPC, respectively.

2.3. Rheological analysis

2.3.1. Viscosity of milk protein concentrate dispersions as a function of temperature

C-MPC and TG-MPC were divided into 50 mL aliquots and stored at 4, 20, 30 and 50 °C for 3 h prior to rheological analysis. Dispersions were analysed using a controlled-stress rheometer (AR-G2 Rheometer, TA Instruments, Crawley, UK) equipped with a concentric cylinder geometry. Sample aliquots were initially conditioned at a temperature of 4, 20, 30 or 50 °C and pre-sheared at 100 s^{-1} for 10 s, followed by a peak hold step at a shear rate of 300 s^{-1} for 5 min.

2.3.2. Viscosity of milk protein concentrate dispersions with sodium hexametaphosphate addition

Sodium hexametaphosphate (SHMP) was dissolved in 1 mL of water and the pH adjusted to 6.5 prior to addition to C-MPC and TG-MPC dispersions (17 mL; 10%, w/w, protein) to give final SHMP concentrations of 5, 12.5 or 25 mM. Following SHMP addition, samples were inverted ten times to ensure homogeneity prior to analysis using a controlled stress rheometer (AR-G2 Rheometer, TA Instruments, Crawley, UK) equipped with a concentric cylinder geometry. Rheological analysis consisted of a conditioning step

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142 performed at 20 °C and a pre-shear at 100 s^{-1} for 10 s, followed by a peak hold step at a shear
143 rate of 100 s^{-1} for 2 h.
144
145 *2.4. Particle size distribution of milk protein concentrate dispersions as a function of*
146 *temperature*
147
148 Particle size measurements were carried out using a Zetasizer nano (Malvern
149 Instruments, Worcestershire, UK) on both TG-MPC and C-MPC dispersions as a function of
150 temperature (i.e., 4, 20, 30 or 50 °C) after a 3 h storage period. Dispersions were diluted 1:50
151 with tempered deionised water prior to analysis. Sample analysis parameters were set at a
152 dispersant refractive index (RI) of 1.330 and viscosity of 0.8872 cp. The viscosity of
153 dispersions was measured in disposable cuvettes, and the samples were characterised as
154 protein using an RI of 1.45 and absorption value of 0.001. Experiments were carried out in
155 triplicate with a backscattering angle of 173°.
156 Size measurements were also carried out on C-MPC and TG-MPC dispersions with
157 added SHMP at concentrations of 0, 5, 12.5 and 25 mM using the Zetasizer nano as described
158 above. All measurements were carried out 1 h after SHMP addition at 20 °C.
159
160 *2.5. Zeta-potential analysis of milk protein concentrate dispersions*
161
162 The ζ-potential of C-MPC and TG-MPC dispersions was measured as a function of
163 pH using a Zetasizer (Malvern Instruments, Worcestershire, UK). Samples were diluted 1:10
164 using deionised water at 22 °C prior to pH adjustment using concentrated hydrochloric acid
165 or sodium hydroxide. Zeta potential analysis was performed using water as the dispersant,
166 with an RI of 1.330 and viscosity of 0.8872 cp. Dispersions were measured using disposable

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folding capillary cells (DTSI060/DTSI061). The protein was characterised using an RI of 1.45 and absorption value of 0.001. Measurements were performed in triplicate at 25 °C using the Smoluchowski model (Smoluchowski, 1917).

2.6. Colour analysis of control and cross-linked protein concentrate dispersions as a function of sodium hexametaphosphate concentration

The colour of C-MPC and TG-MPC dispersions containing 0, 5, 12.5 and 25 mM SHMP were measured using a Minolta Chroma Meter CR-400 colorimeter (Minolta Ltd., Milton Keynes, UK). Data was calculated using three parameters L^*, a^* and b^* to describe the colour spectrum of a sample within the three-dimensional visible colour range. The colorimeter was calibrated against a white standard prior to analysis. Measurements were taken three times and the mean calculated. Measurement data was displayed as L^* that represents a scale from black (0) to white (100), a^* that represents the green-red spectrum with a range from –60 (green) to +60 (red) and b^* that represents the blue-yellow spectrum, ranging from –60 (blue) to +60 (yellow), respectively (Mohammadi, Rafiee, Emam-Djomeh, & Keyhani, 2008).

Total colour difference (ΔE) was calculated using Equation 1 where L_o, a_o and b_o refer to the colour of C-MPC and TG-MPC dispersions without SHMP addition, while L, a and b denote the respective colour parameters of samples containing SHMP.

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \qquad \text{(Eq.1)}$$

2.7. Statistical data analysis

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All trials and measurements were carried out in triplicate. Rheological measurements were analysed using a paired T-test with a 95% confidence interval. Particle size and colour data was statistically analysed using one-way analysis of variance (ANOVA), with post hoc Tukey analysis. The level of significance was considered as $P < 0.05$. All statistical analysis was carried out using Minitab 17 (Minitab Inc, Coventry, United Kingdom).

3. **Results and discussion**

3.1. Rheological and particle size analysis of milk protein concentrate dispersions

The viscosity of C-MPC and TG-MPC dispersions decreased with increasing temperature. For example, the viscosity of C-MPC was significantly ($P < 0.05$) lower at 20 °C (11 mPa s) than at 4 °C (37 mPa s) (Fig. 1). The viscosity of TG-MPC dispersions showed a similar trend (i.e., 15 mPa s at 4 °C and 8 mPa s at 20 °C; Fig. 1), with the viscosity of C-MPC and TG-MPC dispersions decreasing progressively with increasing temperature; however, there was no significant ($P > 0.05$) difference in viscosity between non-crosslinked and crosslinked protein dispersions at 30 or 50 °C. A previous study by Ho et al. (2018) showed similar results to the present study where the viscosity of MPC increased with decreasing temperature from 55 to 25 °C. This decrease in viscosity could be attributed to a higher degree of flexibility within the casein micelles and reduced interactions between casein micelles, due to a decrease in intra- and intermolecular hydrophobic interactions, respectively. This is in agreement with particle size distribution profiles shown in Fig. 2, whereby the z-average diameter for C-MPC samples decreased with increasing temperature from 163 nm at 4 °C to 156 nm at 50 °C. Weakening of hydrophobic interactions within the

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casein micelle, results in the release of β-casein from the casein micelle into the serum phase at low temperature (< 20 °C), leading to increased micelle hydration and size.

Conversely, particle size values were lower for TG-MPC dispersions at 4 °C (158 nm) than at 50 °C (170 nm; Fig. 2). The contrasting trend observed for TG-MPC dispersions compared with C-MPC dispersions could potentially be due to the covalent bond network created by TGase-induced crosslinking of casein proteins restricting free movement of proteins (i.e., β-casein) within and out of the casein micelle at 4°C. Therefore, while hydrophobic bonds are weaker at 4 °C, swelling is prevented due to the more rigid cross-linked protein structure in the TGase treated samples. Casein proteins, both in micellar and non-micellar form, are the primary substrate for enzymatic crosslinking, which is due to the abundance of glutamic acid within casein. TGase catalyses cross-linking of peptide chains through the formation of an isopeptide bond between the γ-carboxyamide group of glutamine side chains and an amine donor of neighbouring lysine or glutamine residues depending on steric location (Jaros, Partschefeld, Henle, & Rohm, 2006; Moon et al., 2009; O'Sullivan et al., 2002a,b). Ercili-Cura et al. (2013) reported that gels produced from TGase modified milk had a significantly higher water holding capacity as a result of restricted particle movement due to crosslinking. Prior incubation of milk with TGase resulted in gels consisting of more fixed networks of small aggregates with defined pore sizes; hence preventing network contraction/rearrangement and subsequent syneresis of water. Potentially the high water holding capacity of TG-MPC dispersions could result in larger particle size values as a consequence of casein micelle swelling at increased temperatures (i.e. 50 °C: Fig. 2D).

3.2. Zeta-potential of milk protein concentrate dispersions

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The ζ-potential of C-MPC and TG-MPC dispersions as a function of pH are shown in Fig. 3. At an initial pH of 6.7, C-MPC had a ζ-potential of –18.8 mV while TG-MPC had a ζ-potential of –20.7 mV (Fig. 3). These values are in line with previous work carried out on skim milk at pH 6.7, which had a ζ-potential of −18 mV (Wade, Beattie, Rowlands, & Augustin, 1996). As expected, the ζ-potential of both C-MPC and TG-MPC dispersions became less negative with decreasing pH. Nogueira et al. (2019) reported similar results with ζ-potential values of approximately –20 mV at pH 6.0 for a cross-linked micellar casein system. However, in the present study, the ζ-potential at pH 5.5 for C-MPC and TG-MPC was –14.3 and –13.9 mV, respectively, compared with Nogueira et al. (2018) who reported a ζ-potential of –18 mV at pH 5, while at pH less than 5.5 in the present study, the ζ-potential was not measured due to extensive precipitation of casein. The fact that there were no significant differences in ζ-potential between C-MPC and TG-MPC dispersions showed that crosslinking did not alter the surface charge of the casein micelles. This is in line with previous work carried out by de Kruif, Tuinier, Holt, Timmins, and Rollema (2002) who showed, using small-angle neutron scattering (SANS), that crosslinking of casein micelles caused little or no restructuring of the casein micelle other than the formation of covalent linkages.

3.3. Particle size and colour analysis of milk protein concentrate dispersions containing sodium hexametaphosphate

C-MPC dispersions without (0 mM) and with 5 mM SHMP addition displayed narrow monomodal size distribution profiles, with particle size ranging from 68.1 to 459 nm (Fig. 4A). However, C-MPC dispersions containing 12.5 and 25 mM SHMP were significantly different, with a shift in profile towards a broader particle size distribution (Fig. 4A). The

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effect of SHMP addition on the size distribution profiles of MPC dispersions was also highlighted by the significant ($P < 0.05$) changes in polydispersity index (PdI) (Table 1), with PdI values of C-MPC dispersions increasing (0.09–0.41) with increasing SHMP content (Table 1). Particle size results for C-MPC dispersions correlated well with colour analysis (Table 2), with C-MPC dispersions containing 0 and 5 mM being relatively similar in terms of L^*-values (82.7 and 80.2, respectively) but with some differences observed in b^*-values, in conjunction with a ΔE value (i.e., 3.10; Table 2) denoting a visible change. A significantly ($P < 0.05$) lower L^*-value (denoting whiteness) was observed for C-MPC dispersions containing 12.5 and 25 mM SHMP (48.3 and 46.0, respectively), with respective ΔE values of 34.5 and 37.0 (Table 2; Fig. 4A inset). Considered collectively, these data provide evidence for the dissociation of casein micelles into primary casein particles (De Kort et al., 2009, 2011; Panouillé, Benyahia, Durand, & Nicolai, 2005; Pitkowski, Nicolai, & Durand, 2008). Strong polyphosphate-based calcium chelators, such as SHMP, cause partial disintegration of casein micelles by the depletion of calcium from the casein micelle, resulting in partial collapse of the micelle, releasing individual casein proteins/particles into the serum phase.

No differences were observed in particle size distribution profiles for TG-MPC dispersions containing 5 and 12.5 mM SHMP compared with the control. Previous studies (Myllärinen, Buchert, & Autio, 2007; O'Sullivan, Kelly, & Fox, 2002) showed similar results to those found in the current study, with TGase-treated casein micelles having increased resistance to dissociation during calcium depletion. Only when 25 mM SHMP was added to TG-MPC dispersions was a broadening of particle size distribution profile observed, with a primary peak between 78.8 and 825 nm, indicating increased casein micelle swelling and hydration, with a secondary smaller peak between 28.2 and 78.8 nm, indicating the presence of smaller casein micelle fragments (Fig. 4B). These primary casein particles have been shown to have a diameter of approximately 20 nm (De Kortet al., 2009, 2011; Huppertz et al.,

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2017; Panouillé et al., 2005; Pitkowski et al., 2008). This was also observed in PdI values for TG-MPC samples, with no significant ($P > 0.05$) difference in dispersions between 0 and 12.5 mM (0.13 to 0.14), while at 25 mM, the PdI value was significantly ($P < 0.05$) higher at 0.24 (Table 1). Similarly, the L^*-value for TG-MPC dispersions also decreased with increasing SHMP addition level (L^*-value decreased from 84.0 to 59.9; Table 2), albeit to a significantly ($P < 0.05$) lesser extent than observed for C-MPC dispersions. The resistance of TG-MPC dispersions to disintegration, and retention of whiteness, can be related to the strong isopeptide bond formed between amino acids during TGase incubation process. Therefore, casein micelles treated with TGase had improved micelle stability and as a result retained more light scattering ability in the presence of high concentrations of SHMP (see Fig. 4B inset).

3.4. Viscosity measurements of milk protein concentrate dispersions containing sodium hexametaphosphate

Viscosity profiles of C-MPC and TG-MPC dispersions with added SHMP at concentrations of 0, 5, 12.5 and 25 mM are shown in Fig. 5. At 5 mM SHMP addition both C-MPC and TG-MPC dispersions had higher viscosity with final values of 25 and 29 mPa s, respectively, compared with the respective samples without SHMP addition. Significantly ($P < 0.05$) higher viscosity was observed in C-MPC dispersions containing 12.5 and 25 mM SHMP, with final viscosities of 48 to 3217 mPa s and from 72.7 to 3838 mPa s, respectively (Fig. 5A). This higher viscosity can be attributed to chelation of calcium, which causes diffusion of calcium from the micelle into the serum phase and a reduction in the proportion of micellar CCP. The reduction in CCP causes increased electrostatic repulsion between casein proteins within the micelle, which together with the loss of the CCP-mediated casein

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314 protein cross-links, causes reduced structural integrity and increased swelling of the casein
315 micelles (De Kort et al., 2009, 2011; Holt, 1992).

316 TG-MPC dispersions displayed considerably less change in viscosity with addition of
317 SHMP (Fig. 5B). At higher addition levels of SHMP (i.e., 12.5 and 25 mM), TG-MPC
318 dispersions had significantly ($P < 0.05$) lower final viscosity values (162 and 991 mPa s,
319 respectively), compared with the corresponding C-MPC dispersions (3212 and 3838 mPa s,
320 respectively). The resistance of TG-MPC to increases in viscosity on the addition of SHMP
321 can be attributed to the impact of enzymatic crosslinking, which creates a secondary
322 structural organisation within the casein micelles, conferring increased resistance to micellar
323 disintegration.

324 TGase-mediated crosslinking could also potentially hinder the access of SHMP to
325 CCP within the casein micelle. Previous work carried out by Power et al. (2019) showed that
326 a change in ^{31}phosphate nuclear magnetic resonance signal occurred upon the addition of
327 SHMP to reconstituted MPC dispersions, indicative of a change in the structure of phosphate
328 prompted by an interaction between SHMP and phosphate-bound calcium associated with the
329 casein micelle. Chelation increases exposure of negatively-charged phosphate residues
330 through depletion of bound calcium, hence changing the charge on the phosphate residues
331 and its associated resonance signal (Holt, 1997; Walstra, 1990). Therefore, TGase-mediated
332 crosslinking may restrict the ability of SHMP to chelate calcium bound to phosphate residues
333 along the casein structure, creating a calcium-casein phosphate complex.

334

335 **4. Conclusion**

336

337 The chelation of calcium in milk protein concentrate dispersions significantly
338 modifies the structural integrity of casein micelles, leading to increased viscosity. In this

study, the use of transglutaminase to cross-link casein micelles prior to addition of sodium hexametaphosphate greatly reduced this viscosity development, with the effect being most pronounced at low temperature. Enzymatically crosslinking casein micelles also helped maintain the natural white colour of MPC even after SHMP addition. This may also be useful in other studies which use strategies such as ion-exchange to reduce the calcium content of micellar casein ingredients. Overall, this study provided new knowledge relating to the factors responsible for increased viscosity during calcium chelation, mainly casein micelle swelling and micelle dissociation, and demonstrated that enzymatic crosslinking is effective in controlling viscosity development in MPC systems with added calcium chelating salts.

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Figure legends

Fig. 1. Apparent viscosity of control (■) and transglutaminase cross-linked (□) milk protein concentrate dispersions (10%, w/w, protein) at 4, 20, 30 or 50 °C, measured at a shear rate of 100 s^{-1}. Values presented are the mean values ± SD.

Fig. 2. Particle size distribution profiles of control (—) and transglutaminase cross-linked (----) milk protein concentrate dispersions (10%, w/w, protein) measured at temperatures of: A, 4 °C; B, 20 °C; C, 30 °C; D, 50 °C.

Fig. 3. Zeta-potential of control (■) and transglutaminase cross-linked (◆) milk protein concentrate dispersions (10%, w/w, protein), as a function of pH. Values presented are the mean values ± SD.

Fig. 4. Particle size distribution profiles of control (A) and transglutaminase cross-linked (B) milk protein concentrate dispersions (10%, w/w, protein) containing 0 (——), 5 (— —), 12.5 (– – –) and 25 (·····) mM sodium hexametaphosphate. Insets: photographic image of control and transglutaminase cross-linked milk protein concentrate dispersions containing 0 (i), 5 (ii), 12.5 (iii) or 25 (iv) mM sodium hexametaphosphate.

Fig. 5. Viscosity profiles of control (A) and transglutaminase cross-linked (B) milk protein concentrate dispersions (10%, w/w, protein) containing 0 (——), 5 (·····), 12.5 (– – –) and 25 (– · – · ·) mM sodium hexametaphosphate, measured at a shear rate of 100 s^{-1} at 20 °C.

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Table 1

Particle size distribution parameters for control and transglutaminase cross-linked milk protein concentrate dispersions with added sodium hexametaphosphate. [a]

SHMP concentration (mM)	C-MPC		TG-MPC	
	Z-average (nm)	PdI (-)	Z-average (nm)	PdI (-)
0	180	0.09	182	0.13
5	180	0.10	182	0.12
12.5	138	0.34	200	0.14
25	988	0.41	211	0.24

[a] Abbreviations are: C-MPC, control milk protein concentrate; TG-MPC, transglutaminase cross-linked milk protein concentrate; SHMP, sodium hexametaphosphate; Z-average, intensity weighted mean hydrodynamic size of particles measured by dynamic light scattering; PdI, polydispersity index.

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Table 2

Colour chromaticity co-ordinates of control and transglutaminase cross-linked milk protein concentrate dispersions with added sodium hexametaphosphate. [a]

SHMP concentration (mM)	C-MPC				TG-MPC			
	L^*	a^*	b^*	ΔE	L^*	a^*	b^*	ΔE
0	82.7[a]	–4.20[c]	2.06[b]	-	84.0[a]	–4.14[a]	1.79[a]	-
5	80.2[a]	–4.67[c]	0.29[d]	3.10[a]	82.5[a]	–4.63[a]	1.04[b]	1.76[a]
12.5	48.3[b]	–1.65[b]	1.93[c]	34.5[b]	66.3[b]	–5.08[a]	-4.33[c]	18.8[b]
25	46.0[c]	–0.67[a]	3.84[a]	37.0[c]	59.9[c]	–4.26[a]	-4.45[c]	24.9[c]

[a] Abbreviations are: C-MPC, control milk protein concentrate; TG-MPC, transglutaminase cross-linked milk protein concentrate; SHMP, sodium hexametaphosphate; L^*, whiteness; a^*, green-red spectrum with a range from –60 (green) to +60 (red); b^*, blue-yellow spectrum with a range from –60 (blue) to +60 (yellow); ΔE, total colour difference. Values within a column not sharing a common superscript letter differ significantly ($P < 0.05$).

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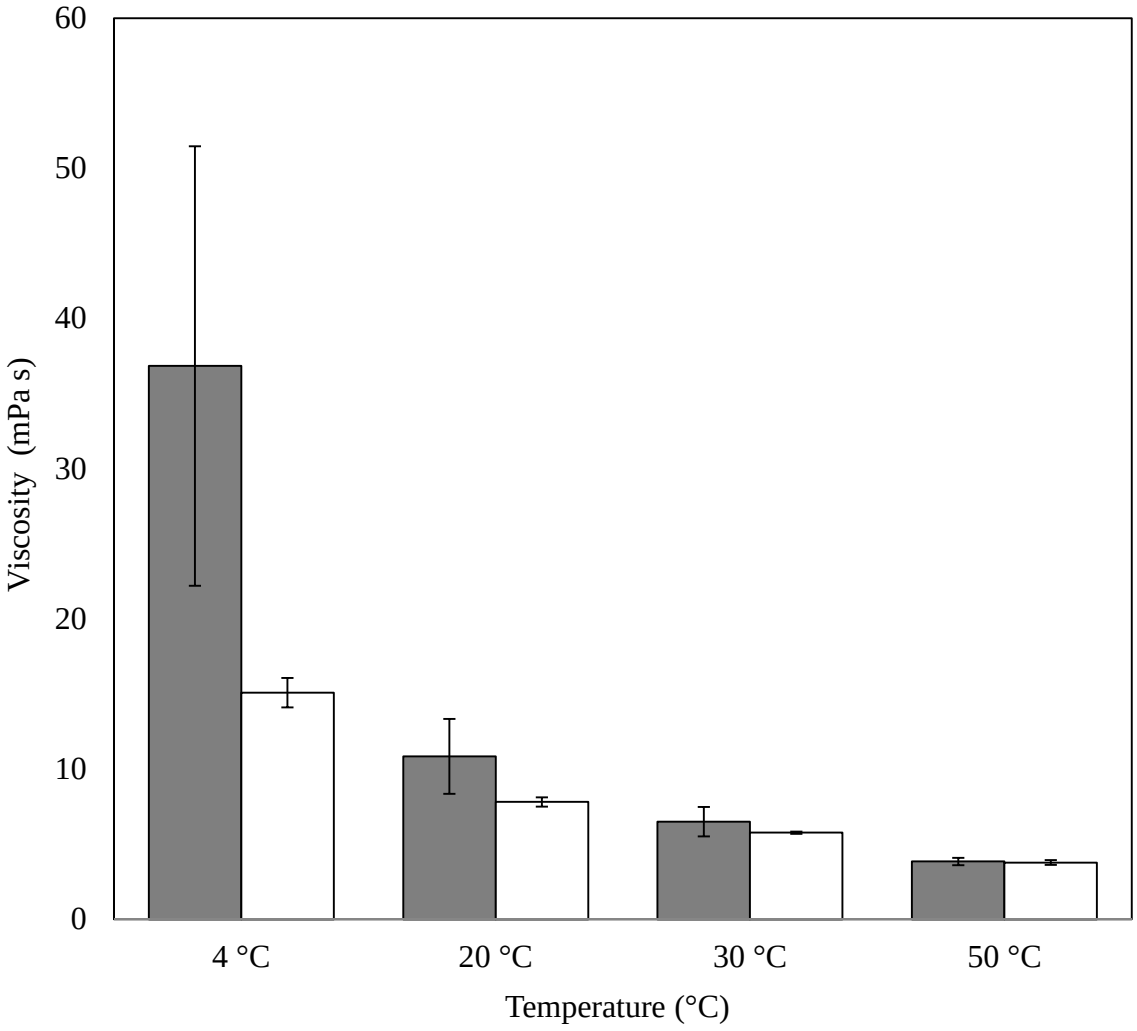


Figure 1

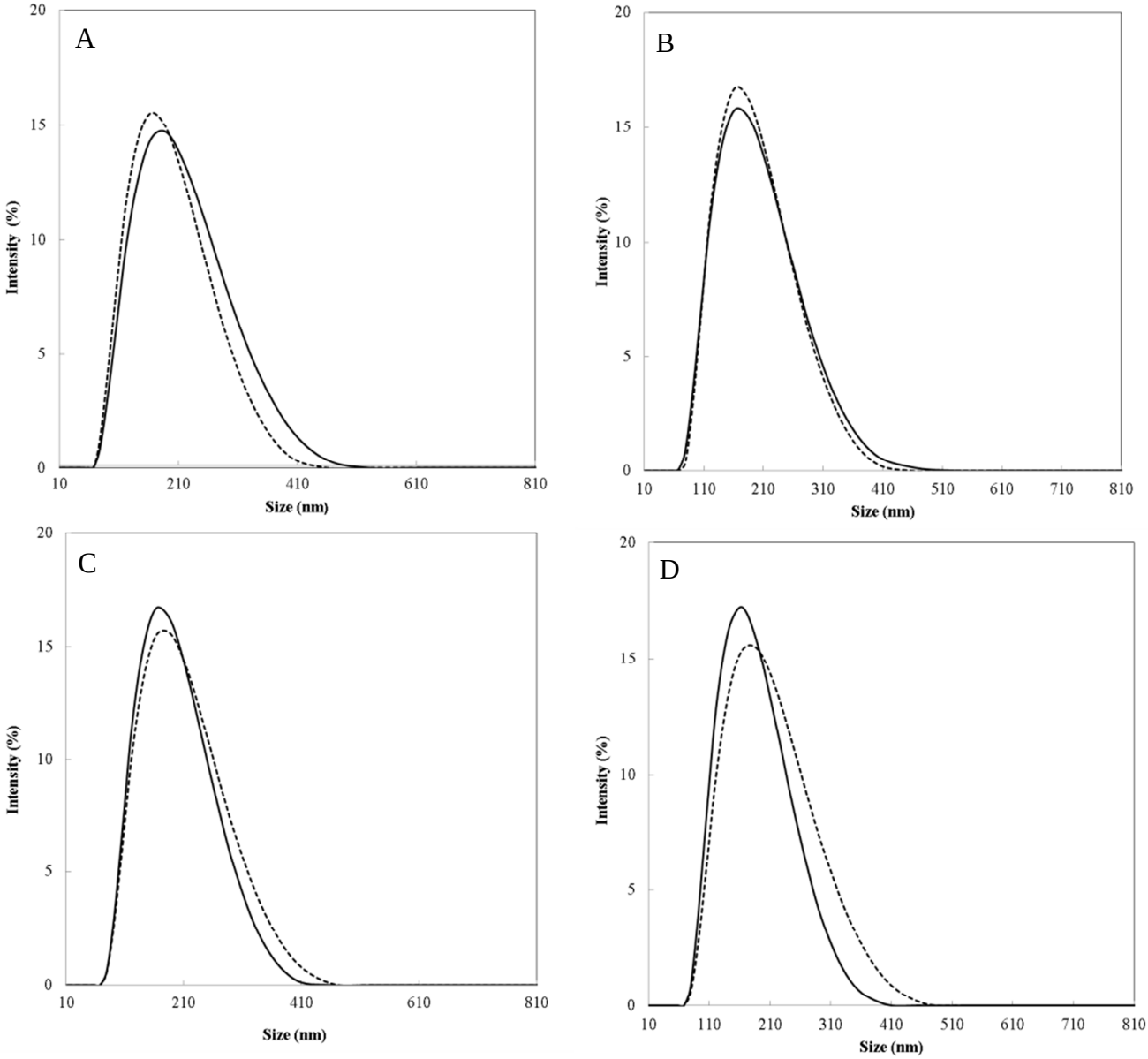

A
B
C
D
Intensity (%)
Size (nm)
0
5
10
15
20
10
110
210
310
410
510
610
710
810

Figure 2

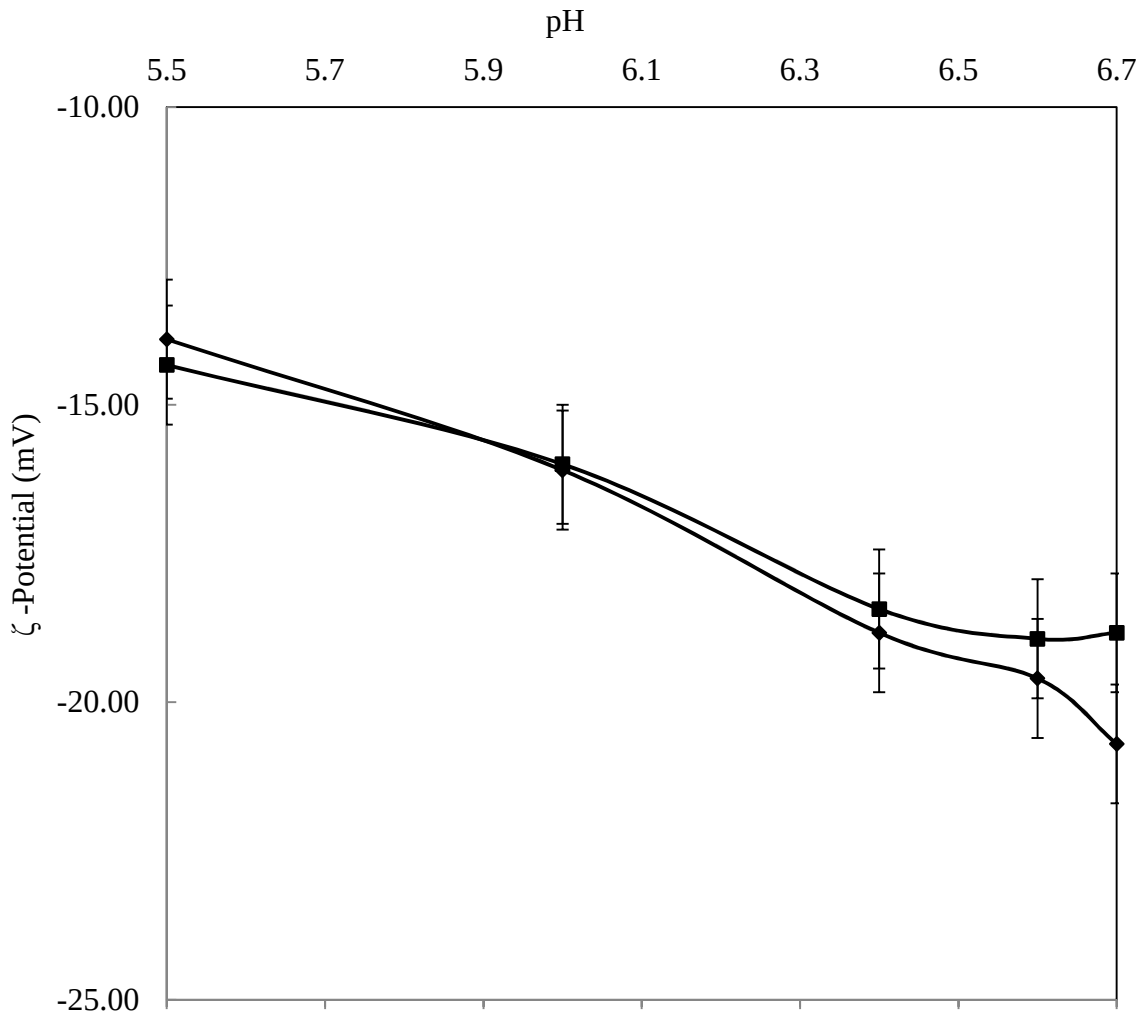


Figure 3

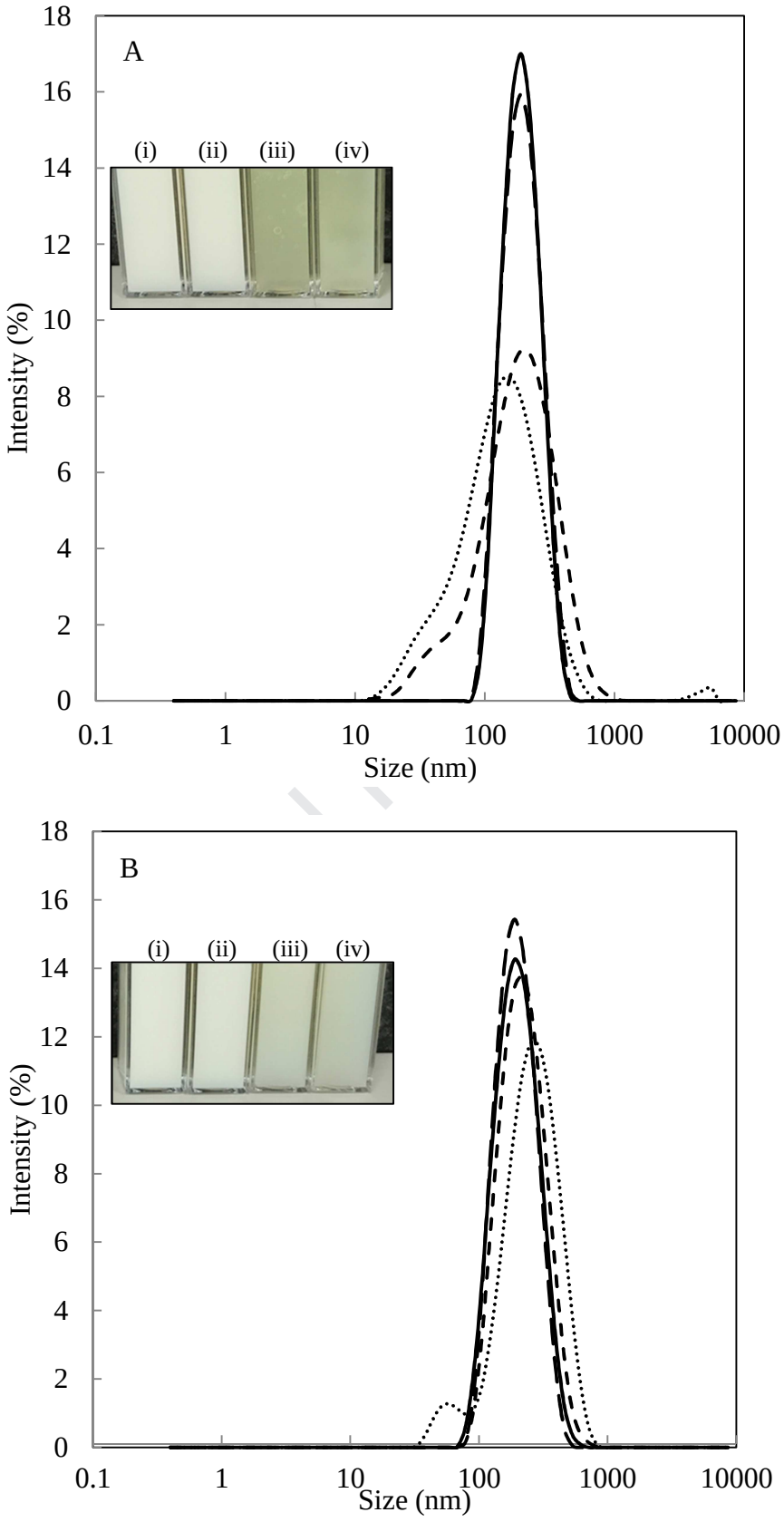
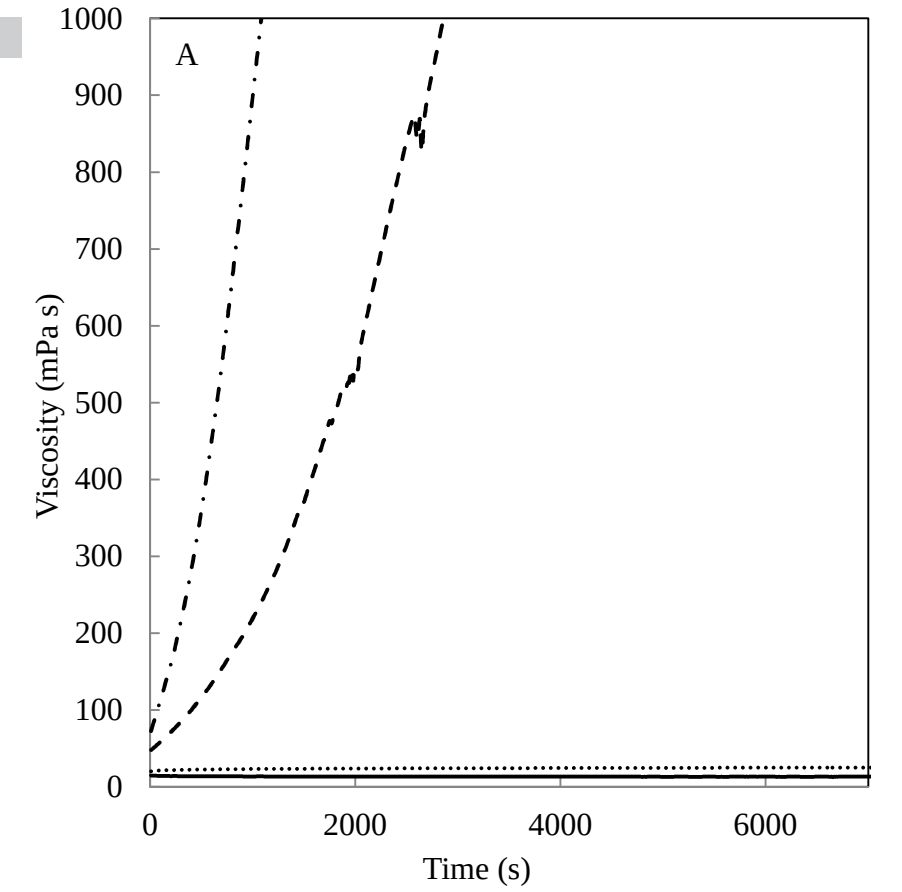

A
(i) (ii) (iii) (iv)
Intensity (%)
Size (nm)
0 2 4 6 8 10 12 14 16 18
0.1 1 10 100 1000 10000
B
(i) (ii) (iii) (iv)
Intensity (%)
Size (nm)
0 2 4 6 8 10 12 14 16 18
0.1 1 10 100 1000 10000

Figure 4


A
1000
900
800
700
600
500
400
300
200
100
0
Viscosity (mPa s)
0
2000
4000
6000
Time (s)

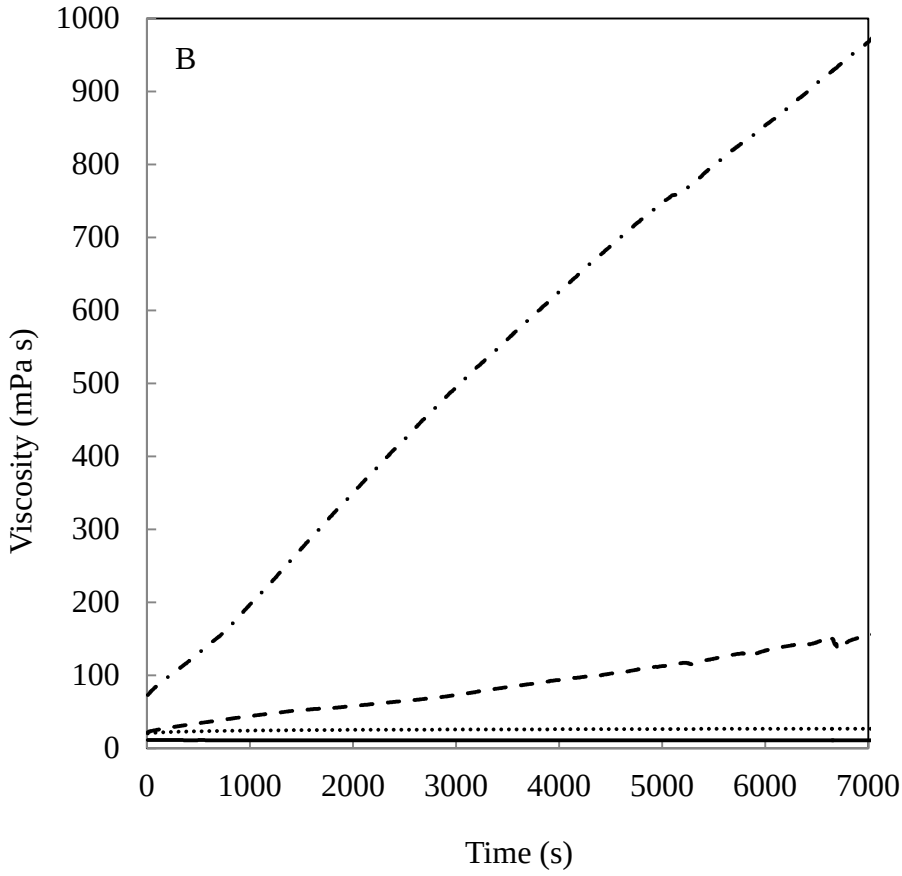

B
1000
900
800
700
600
500
400
300
200
100
0
Viscosity (mPa s)
0
1000
2000
3000
4000
5000
6000
7000
Time (s)

Figure 5